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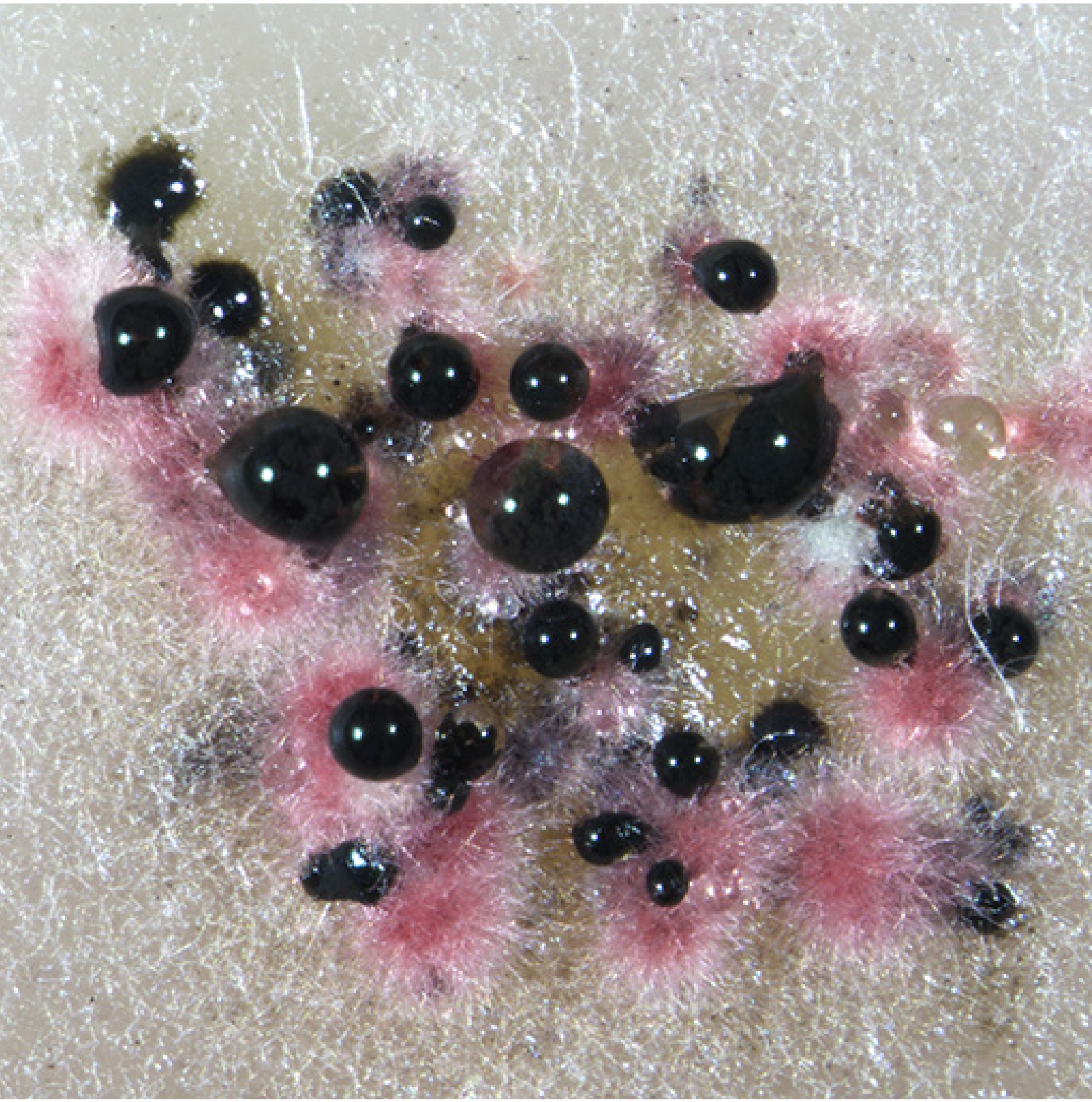

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Three new black *Elaphomyces* species (*Elaphomycetaceae, Eurotiales, Ascomycota*) from eastern North America with notes on selected European species

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hypogeous fungi
new taxa
sequestrate fungi

Abstract: We describe three new species of *Elaphomyces* from eastern North America. Of the three, *Elaphomyces loebiae* is the rarest, known only from North Carolina and South Carolina, and appears to associate primarily with ectomycorrhizal hardwoods but possibly also with conifers. *Elaphomyces cibulae* is widely distributed but disjunct from Florida, Mississippi, and North Carolina. *Elaphomyces cibulae* seems to primarily associate with *Quercus* species. *Elaphomyces mitchelliae* has the widest distribution of the three species, from Florida, Maryland, North Carolina, Virginia, and West Virginia, and appears to associate with either ectomycorrhizal hardwoods and/or conifers. In the course of comparing our new *Elaphomyces* species to previously described European species we discovered that *E. persoonii* var. *minor* is conspecific in all essential details with and thus a synonym of *E. cyanosporus*.

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INTRODUCTION

The hypogeous, sequestrate genus *Elaphomyces* (*Elaphomycetaceae, Eurotiales, Ascomycota*) forms ectomycorrhizal associations with roots of diverse gymnosperm and angiosperm trees and shrubs around the world (Miller & Miller 1984, Trappe *et al*. 2009, Castellano *et al*. 2016). Species of *Elaphomyces* release aromas that are detected by numerous mammal species that dig them up and utilize them as an important food, thereby dispersing the spores across the landscape (Boudier 1876, Fogel & Trappe 1978, Vogt *et al*. 1981, Genov 1982, Cork & Kenagy 1989, Vernes *et al*. 2004, Vernes & Poirier 2007). The leathery peridium is eaten by the animals and some of the powdery mass of hydrophobic spores within the fruiting bodies are ingested, some released to the air, and some left on the ground, logs, or stumps.

Elaphomyces species have been reported from every continent except Antarctica, and occur in diverse forest habitats ranging from temperate and subarctic conifer forests to lowland tropics (Corner & Hawker 1953, Trappe & Kimbrough 1972, Zhang & Minter 1989, Castellano *et al*. 2011, Reynolds 2011, Castellano *et al*. 2012a, c, 2016, Paz *et al*. 2012, Castellano & Stephens 2017). Recently Paz *et al*. (2017) revised the systematics of the European *Elaphomyces* species. They present all known species from Europe and provide an updated structure to the sections and subsections within *Elaphomyces*.

Our recent work on the genus indicates that eastern North America is likely the epicentre of *Elaphomyces* biodiversity with approximately 30 to 40 species, many of which are still undescribed (Castellano, unpubl. data). Historically in North America, most *Elaphomyces* spp. were assigned European names due to the difficulty in distinguishing species based on published descriptions that were often sparse in details (Trappe & Guzman 1971, Miller & Miller 1984, Luoma & Frenkel 1991, Gomez-Reyes *et al*. 2012). Most North American *Elaphomyces* with a black or dark coloured peridial surface were assigned the name *E. anthracinus*, and others with brown peridial surfaces were named *E. granulatus* or *E. muricatus,* depending on structure of the inner peridium. We now know that these species do not occur in North America. In eastern North America (from Québec south to the Gulf of Mexico), there are currently eight described *Elaphomyces* species, including *E. americanus, E. appalachiensis, E. bartlettii, E. macrosporus, E. oreoides, E. remickii, E. spinoreticulatus, E. verruculosus*, and *E. viridiseptum* (Linder 1939, Trappe & Kimbrough 1972, Zhang & Minter 1989, Castellano *et al*. 2012b, Castellano & Stephens 2017). The aim of the present study was thus to characterise several *Elaphomyces* spp. collected in the USA.

MATERIALS AND METHODS

Species of *Elaphomyces* typically develop below ground, so ascomata were collected by raking away the leaf and upper soil layers in suitable habitats, observing and excavating the area where animals had previously dug, or by looking for *Tolypocladium* species that fruit aboveground while parasitizing the ascomata of *Elaphomyces* species (Castellano *et al*. 2004). Occasionally, specimens had partially emerged from the soil in eroded or disturbed environments like road banks, campgrounds, or trail edges.

Descriptions of macro-morphological characters are based on fresh material. Colours are described in general terms based

on the observations of the authors and collectors. Tissues and spores from dried specimens were rehydrated and examined in 3 % KOH, Melzer's reagent, or cotton blue for study of microscopic characters. Neither tissues nor spores reacted distinctively to Melzer's reagent. Microscopic characters of ascoma tissues and spores were described from 3 % KOH mounts unless otherwise specified. Spore dimensions include ornamentation and are from twenty randomly selected ascospores measured from the holotype collections. Asci of *Elaphomyces* spp. generally disintegrate with maturation, so their features often cannot be recorded. Dried ascospores were mounted on aluminum pegs with double-sided tape and coated with gold for scanning electron microscopy (SEM) with a Quanta 600 FEG scanning electron microscope. Specimens are deposited in the following herbaria as noted for each collection: FLAS, OSC, RMS, ITCV, ISC (Index Herbariorum, referenced May 2017).

TAXONOMY

Elaphomyces cibulae Castellano, Trappe & D. Mitchell, ***sp. nov.*** MycoBank MB821236. Fig. 1.

Etymology: Named "*cibulae*" in honour of the late Dr Bill Cibula of Mississippi, enthusiastic collector of fungi in the Gulf States region of the southern USA.

Type: **USA**, *North Carolina*, Rutherford Co., near Gilkey, 25 Jul. 2012, *T. Elliott* (holotype OSC 149262).

Fresh *ascomata* up to 18 mm tall × 20 mm broad, subglobose to irregularly flattened, completely embedded in white to ivory mycelium and rhizomorphs to form a husk around individual ascomata that incorporates soil, ectomycorrhizae and debris; portions of mycelium staining brown when exposed, when dried off-white to pale brown. *Peridium* covered with mycelium and rhizomorphs to occlude observation of the peridial surface, this peridial surface carbonaceous, black to blue-black, smooth to the naked eye at first but close examination reveals a finely colliculate surface and the ascoma itself somewhat rugose when fresh, wrinkled to pleated when dried from inner peridial collapse. Colliculate surface comprised of flattened, multi-sided, irregularly shaped bumps up to 240 µm broad, ± 40 µm tall, contiguous with each other. Overall the fresh peridium ± 2 mm thick when mature, the carbonaceous outer layer ± 0.05 mm thick, black in section when fresh and dried, inner layer uneven, up to 2 mm thick, pale blue-white to blue-grey to grey, turning grey-green to blue where cut in some specimens, crisp and moist, off-white to grey when dried. *Gleba* when young filled with dense, white, cottony-membranous tissue that bears the asci, eventually forming a powdery, white spore mass containing web-like hyphae, spore mass turning pale grey-blue as spores mature among white capillitial hyphae, becoming dark blue to grey-blue to grey-blue-black or grey-black when fully mature. *Odour* not distinctive to cabbage-like. *Taste* not recorded.

Peridium two-layered with an additional ephemeral mycelial covering: outer layer 35–50 µm thick, carbonaceous, of dark brown, compact, septate, parallel hyphae, 2.5–3.5 µm broad, the outer ±20 µm portion of very dark brown hyphae, walls ± 1 µm broad; inner layer up to 2 mm thick when fresh, 400–700 µm thick when dried, of compact, uniform, hyaline to pale grey, fascicled hyphae (bundles of 5–10 hyphae), individual hyphae mostly 3.5–8(–9) µm broad, fascicles in a cross-hatched pattern, walls 1.5–3.5 µm thick, occasional disjunct amorphous areas of pale brown pigment diffused through the hyphae; outer mycelial covering composed of interwoven, much-branched, hyaline to pale green, slightly encrusted hyphae, 2–3 µm broad, walls <0.5 µm thick. *Gleba* of spores and hyaline, septate, extensively branched, compact, interwoven hyphae up to 4 µm broad, walls <0.5 µm thick. *Asci* more or less globose, distorted somewhat from maturing spores, hyaline, 29–33 µm broad at maturity, walls ± 1 µm thick, 8-spored. *Spores* globose, sometimes with a flattened side, (13–)14–15 µm broad (mean = 14.6 µm broad), walls ± 1 µm thick, in KOH yellow-brown to red-brown singly and in mass when mature, ornamented with rods, short ridges or a labyrinthine pattern, 1–2 µm tall.

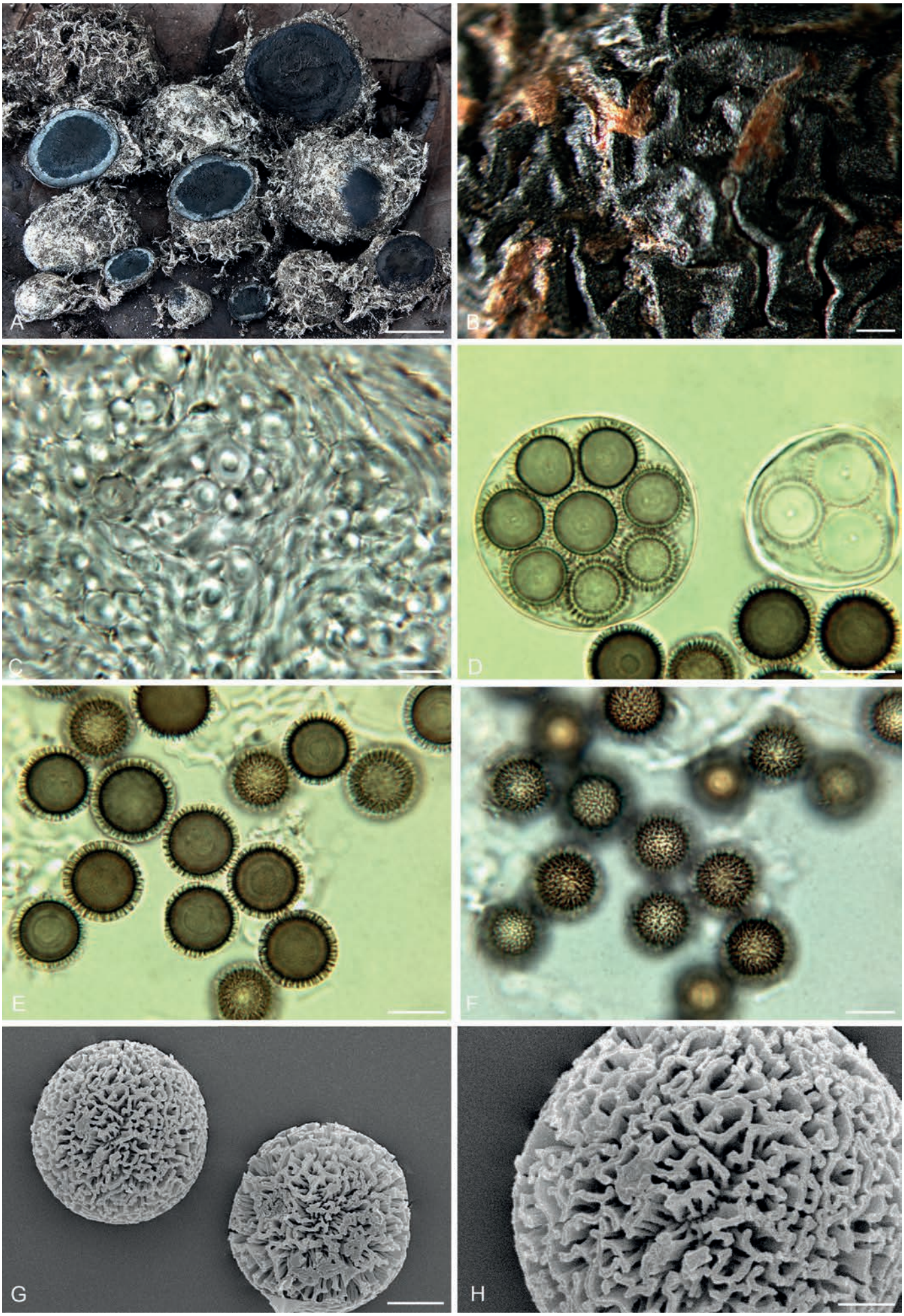
Distribution, habit, habitat, and season: Known from Florida, Mississippi, and North Carolina; hypogeous; under *Quercus virginiana*, *Quercus* sp. and possibly *Pinus* sp.; February, June through August.

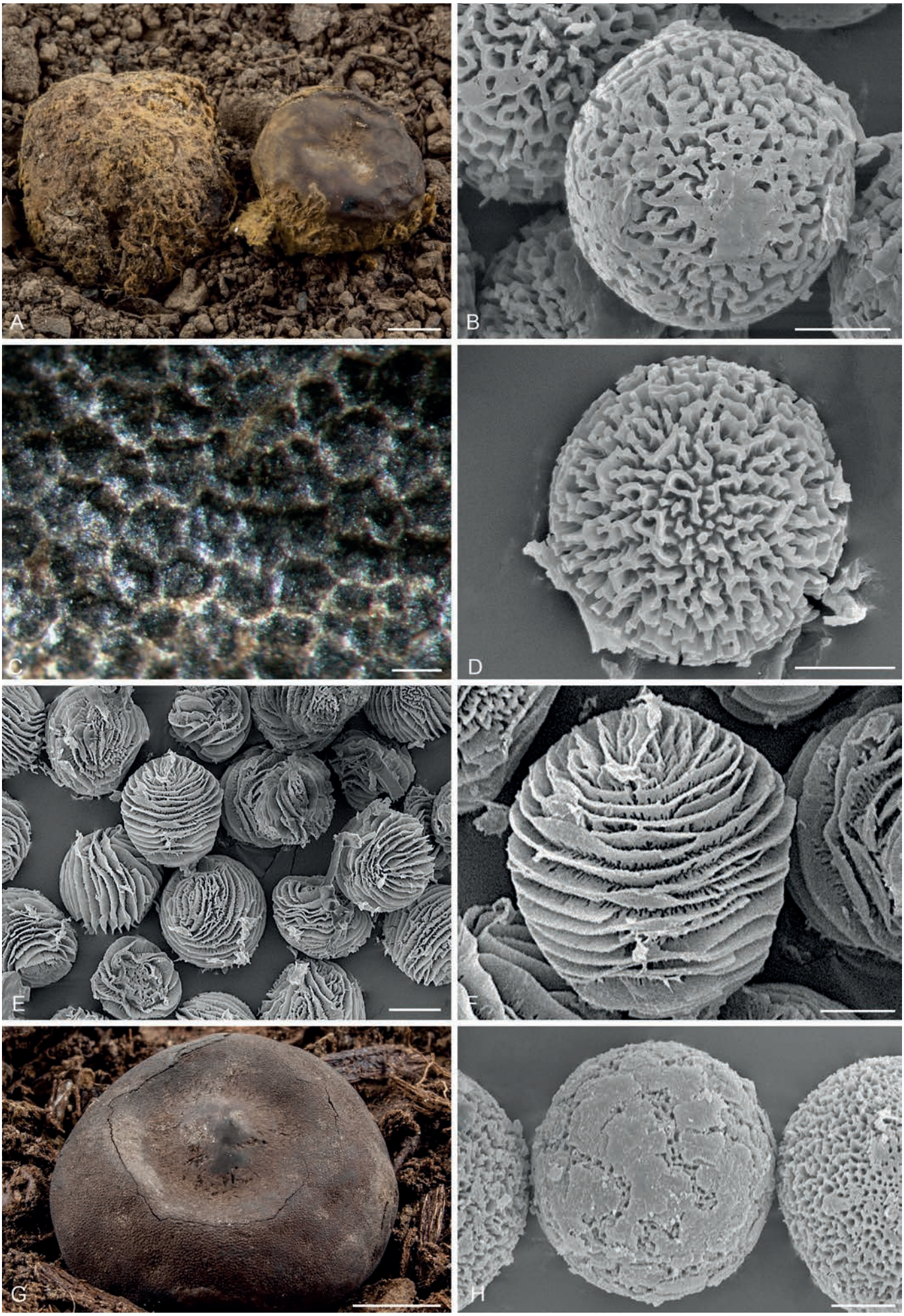
Additional collections examined: **USA**: *Florida*, Polk Co., along county road 54, just west of Champions Gate Blvd., north side of road next to small pond, 25 Feb. 2012, *M. Castellano,* OSC 148261; *Mississippi*, Hancock Co., Steenis Space Center, "old" Gainesville, near corner of Ambrose St. and Union St., under a very large, old-growth *Quercus virginiana* named "Jeremiah," 4 Jun. 1976, *J. Trappe 4601,* OSC 36196; same locality, 6 Aug. 2007, *M. Castellano,* OSC 150018.

Notes: We assign *Elaphomyces cibulae* to section *Malacodermei* on the basis of the thin, somewhat smooth, soft peridium and the small spores.

Castellano studied *Elaphomyces citrinus* collections in Torino (in OSC 149172) marked "Vittadini collection" and Kew (161174) marked "ex herb Berkeley from Vittadini." Castellano (unpubl. data) recorded data from OSC 149172 in part as follows: Dried specimens with peridial surface black, appearing wrinkled or wavy, covered by dark orange-yellow to orange-brown hyphae; spores globose, in KOH brown singly, slightly darker in mass, 15–16(–18) µm broad, mean = 15.8 µm including ornamentation that appears labyrinthine under light microscopy, in section appearing spiny to coarsely spiny; SEM reveals the spore ornamentation as anastomosed rods forming an irregular labyrinth with flattened apices (Fig. 2A–B). Dodge (1929) does not cite a type but reports it as common around Milan. Vittadini, a professor of medicine at the University of Milan, shared many of his truffle collections with contemporary mycologists in several European herbaria. How collections in his herbarium were dealt with after his death is uncertain but the

Fig. 1. *Elaphomyces cibulae*. **A.** Ascomata showing peridial surface, gleba, and peridium in section. **B.** Ascoma surface showing wrinkled nature of the peridium after drying and the finely colliculate surface. **C.** Peridial structure of the inner peridium showing bundled/clustered hyphae. **D.** Asci showing immature and mature spore development. **E.** Ascospore in cross-section showing height and pattern of the ornamentation. **F.** Ascospores in surface view. **G.** SEM micrograph of ascospores showing rod-like ornamentation. **H.** SEM micrograph of an ascospore showing the complex structure to the rod structure. A–F (OSC 149262 – holotype), G & H (OSC 36196). Bars: A = 10 mm, B = 500 µm, C–F = 10 µm, G = 5 µm, H = 2 µm.





Milan herbaria have no Vittadini collections. Mattirolo (1907) reconstituted many Vittadini collections at the Herbarium of the University of Turin (TO) with generous cooperation from other herbaria; the specimens are marked accordingly but with little or no information on their origin, associated habitat or specific collector(s). *Elaphomyces cibulae* as dried resembles *E. citrinus* in the black, carbonaceous outer peridium and in spore characters. The enveloping mycelium surrounding the ascomata of *E. citrinus* is orange-yellow to orange-brown coloured when dried, whereas *E. cibulae* has white to ivory mycelium when dried. Most strikingly are the blue tones of the inner peridium and gleba in fresh ascomata. These blue tones fade with time. In addition, the peridial layers of *E. cibulae* are much thicker than *E. citrinus*, and the spores of *E. cibulae* are slightly smaller than *E. citrinus*.

Castellano studied *Elaphomyces mutabilis* collections from TO marked "Originale de Vittadini – don. Dal Museo di Paris," and in Paris (PC 93923), marked "ex ipso, Mai 1845." Castellano (unpubl. data) recorded data from both collections above in part as follows: Dried peridial surface black, apparently smooth to the naked eye but under magnification revealing a fine pattern of multisided, contiguous, flattened bumps, 250–350 µm broad, completely and persistently covered by floccose, off-white mycelium, spores globose, in KOH brown singly, darker in mass, 14–15 µm broad, mean = 14.5 µm broad including ornamentation that appears as rods, spines, and short ridges under light microscopy, SEM reveals the spore ornamentation as spines or rods anastomosed into short, irregularly-shaped ridges that form an irregular labyrinth, apices flattened (Fig. 2C–D). Dodge (1929), Ceruti (1960), Montecchi & Sarasini (2000), and Rubio *et al*. (2006) all report that *E. mutabilis* when fresh has distinct blue tones to the inner peridium that fade to off-white or very pale blue over time and when dried. Dodge (1929) reports the type from "oak forests near Milan, July to November" but does not designate a collection as type. Castellano also studied PC 93924 marked as *E. mutabilis* var. *flocciger* Tul. & C. Tul., France, Nantes, leg. Menier (the likely type for this variety) and found it to be conspecific with *E. mutabilis*. Dried *E. cibulae* resembles *E. mutabilis* in the blue tones of the inner peridium and gleba when fresh and in the colour of the enveloping mycelium surrounding the ascoma when dried, the black, carbonaceous outer peridium and in spore characters. The inner peridium of *E. mutabilis* has larger sized hyphae and also scattered dark red-brown hyphal segments beneath the outer carbonaceous layer. These elements are lacking in *E. cibulae*. The carbonaceous, outer peridial layer in *E. mutabilis* is constructed of puzzle-like hyphal segments not the parallel hyphae found in *E. cibulae*.

Castellano studied an *Elaphomyces virgatosporus* Hollós collection from TO (also in OSC 149131) marked "Hungary, Nógrád Comite, Litke, 8 November 1904, Under *Fagus*." Castellano (unpubl. data) recorded data from this collection in part as follows: Peridial surface of black, variably sized and shaped, flattened warts, completely covered by dark brown mycelium, spores globose, in KOH brown to dark brown singly, darker in mass, (18–)19–22 µm broad, mean = 20.2 µm broad, including ornamentation that appears as long, continuous ridges in a pseudo-concentric pattern under light microscopy, SEM reveals the spore ornamentation as spines or rods anastomosed into long ridges in a pseudo-concentric pattern, apices flattened (Fig. 2E–F). We do not designate a type collection, as the type should reside in the Hungarian Natural History Museum at Budapest (BP), but collections are unavailable to study except on location. Dried *Elaphomyces cibulae* resembles *E. virgatosporus* in its black, carbonaceous outer peridium and spore size. The dried enveloping mycelium of *E. cibulae* is white to ivory colour, whereas *E. virgatosporus* has dark brown mycelium. The long-ridged spore ornamentation that forms a concentric pattern in *E. virgatosporus* is distinctly different from the irregularly shaped labyrinth ornamentation pattern of *E. cibulae* spores.

Elaphomyces loebiae Castellano & T.F. Elliott ***sp. nov.*** MycoBank MB821237. Fig. 3.

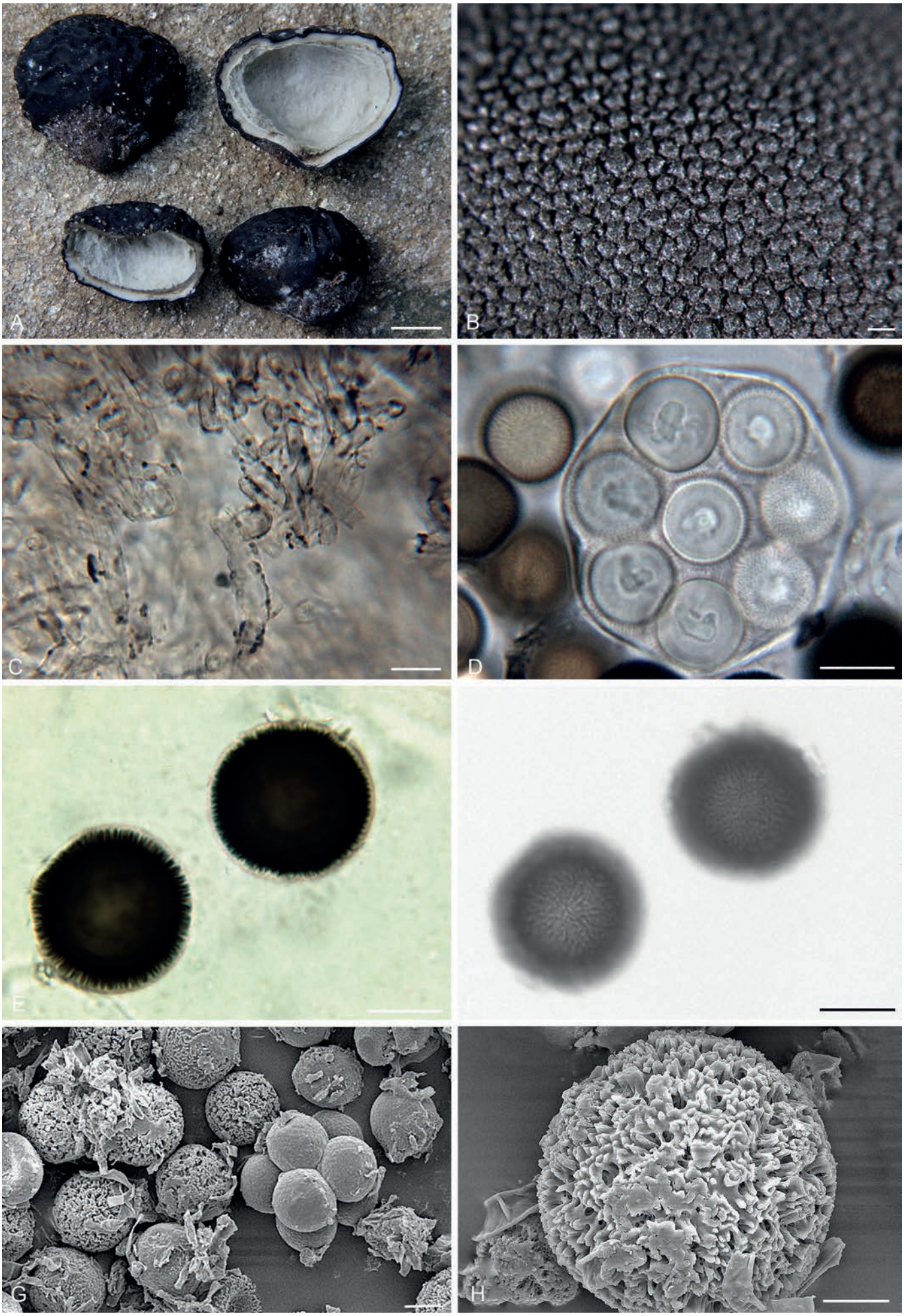
Etymology: Named "*loebiae*" in honour of Dr Susan C. Loeb, research ecologist with the US Forest Service and collector of the type specimens.

Type: **USA**, *North Carolina,* Haywood Co., Nantahala National Forest, Reinhart Gap, 15 Aug. 1996, *S. Loeb & F. Tainter 130* (holotype - OSC 149108).

Ascomata as dried subglobose or irregularly-shaped, up to 23 × 35 mm. *Fresh peridium* ± 1.1 mm thick, outer layer ± 300 µm thick, peridial surface black, brittle, uniformly papillate, papillae flattened or sometimes rounded, irregular in outline, contiguous, 140–260 µm broad at base; inner layer ± 800 µm thick, somewhat pale grey adjacent to epicutis then soon off-white and uniform in texture, specimens supplied for study had been thoroughly cleaned beforehand, so information on material covering specimens is lacking. *Gleba* composed of a dark brown to black, powdery spore mass. *Odour* not recorded. *Taste* not recorded.

Peridium as dried two-layered; outer layer ± 300 µm thick, of compact cells with dark brown to black walls ± 1 µm thick, in between papillae are parallel hyphae with dark brown to black walls < 0.5 µm thick; inner layer ± 800 µm thick, its outer pale grey portion ± 200 µm thick, of short, segmented, much branched, loosely interwoven hyphae 4–7 µm broad, walls 1–2 µm thick, encrusted with small dark granules, the inner off-white portion of hyaline, occasionally sinuous, to somewhat periclinal to interwoven or even interlaced hyphae, mostly ± 4(–8) µm broad, rarely up to 12 µm broad, walls 1–2 µm thick. *Gleba* of spores and hyaline, septate, sinuous hyphae, 1–2 µm broad, walls <0.5 µm thick. *Asci* globose, 45–55 µm broad, hyaline, walls ± 1 µm thick, 8-spored, arising from hyaline,

Fig. 2. Various *Elaphomyces* species from Europe. **A.** *Elaphomyces citrinus* ascomata showing peridial surface and adherent yellow toned mycelium (LE 162869). **B.** *Elaphomyces citrinus* SEM micrograph of ascospores showing the fine detail of the ornamentation (OSC 149172). **C.** *Elaphomyces mutabilis* ascomata showing wart structure and adherent off-white mycelium (OSC 149119). **D.** *Elaphomyces mutabilis* SEM micrograph of ascospores showing the fine detail of the ornamentation (OSC 149121). **E.** *Elaphomyces virgatosporus* SEM micrograph of ascospores showing spiral pattern of the ornamentation (OSC 149131). **F.** *Elaphomyces virgatosporus* SEM micrograph of ascospores showing the fine detail of the ornamentation (OSC 149131). **G.** *Elaphomyces anthracinus* ascoma showing peridial surface (W2008-1095). **H.** *Elaphomyces anthracinus* SEM micrograph of ascospores showing the ornamentation of irregular plates overlaying a fine reticulum (OSC 149163). Bars: A, G = 5 mm, B, D, F, H = 5 µm, C = 250 µm, E = 10 µm.



clustered, contorted, short segmented hyphae ± 6 µm broad. *Spores* globose, 21–22(–23) µm broad (mean = 21.7 µm), walls ± 1 µm thick, in KOH singly and in mass very dark red-brown to nearly black when mature, ornamentation opaque at maturity, consisting of rods or spines anastomosed at their apices to form a fine, irregular labyrinthine-like surface, 1–2 µm tall.

Distribution, habit, habitat, and season: Known only from North Carolina and South Carolina; hypogeous; under *Betula alleghaniensis, Fagus grandifolia*, *Picea rubens*, *Quercus rubra,* and *Tsuga canadensis*; March, May, July, and August.

Additional collections examined: **USA**, *North Carolina*, Haywood Co., Nantahala National Forest, Haywood Gap, 10 Jul. 1996, *S. Loeb & F. Tainter 106* (OSC 149107); Rutherford Co., Union Mills, along Painters Gap Rd., 22 Mar. 2007, *T. Elliott* (OSC 149237); *South Carolina*, Horry Co., Huntington State Park, 25 May 2012, *T. Elliott* (OSC 149109).

Notes: We assign *Elaphomyces loebiae* to section *Ceratogaster*, subsection *Sclerodermei* based on the absence of mycelial patches on the peridial surface.

Castellano studied an *Elaphomyces aculeatus* collection in TO (in FH 258126) marked "Originale di Vittadini dono al Museo di Paris." Castellano (unpubl. data) recorded data from this collection in part is as follows: Peridial surface appearing off-white to brown because of overlaying hyphae. Actual peridial surface is black, carbonaceous and warty, and the thick layer of overlying hyphae can be rubbed off to expose the apices of the warts and give the surface a black-spotted appearance against a brown background. Spores globose, in KOH brown to dark brown singly, slightly darker in mass, 21–24 µm broad, mean = 22.5 µm broad, including ornamentation that appears as irregular, short ridges under light microscopy, in section appearing warty in outline, SEM reveals the spore ornamentation as a fine, complete reticulum without overlying material (Fig. 4A–B). The somewhat smooth peridial surface and spore ornamentation of *E. loebiae* differs significantly from *E. aculeatus*. Castellano also studied a collection marked "*Elaphomyces rubescens*, 2 Aug. 1890, Eisenkaute, Herb Hesse" (OSC 158064) and found it to be conspecific with *E. aculeatus*.

Castellano studied an *Elaphomyces anthracinus* collection in TO (in OSC 149163) marked "Esemplare originale di Vittadini da Museo di Paris." Castellano (unpubl. data) recorded data from this collection in part as follows: Peridial surface black, appearing smooth to the naked eye, close examination reveals a distinct pattern of low, circular, rounded papillae, not warty or angular, ascoma base with a peduncle. Spores globose, in KOH brown to dark brown singly, slightly darker in mass, 20–24 µm broad, mean = 21.9 µm broad, including ornamentation that appears punctate or dimpled under light microscopy, in section appearing with some flattened sides in outline to the spore, SEM reveals the spore ornamentation as a fine, complete reticulum overlain with over-lapping, plate-like material partially or nearly completely (Fig. 2G–H). We agree with Dodge (1929) that *E. pyriformis* Vittad. (Castellano studied authentic Vittadini material in FH marked "ex ipso auctore") and *E. plumbeus* Hesse (Castellano studied authentic material in Marburg marked "Laurasen, Germany, April 1890") are conspecific. Dried *Elaphomyces loebiae* resembles dried *E. anthracinus* in its black, carbonaceous peridial surface covered with minute to small papillae and in having spores that are similarly sized, both approx. 21.5–22 µm broad with ornamentation. The spore ornamentation of *E. loebiae* is characterised by rods or spines anastomosed at their apices to form a fine, irregular labyrinth, whereas the spore ornamentation of *E. anthracinus* appears as a fine, complete reticulum overlain with plate-like material that is partially or nearly completely overlapping.

Elaphomyces loebiae resembles *E. cantabricus* in spore size and ornamentation and its black, carbonaceous peridial surface, but *E. cantabricus* has distinct sharp warts on the peridial surface in contrast to the papillae on the peridial surface of *E. loebiae*.

Elaphomyces loebiae resembles *E. spirosporus* in spore size, but the spore ornamentation of *E. spirosporus* appears as clearly spiraled ridges compared to the irregularly, labyrinthine-like spore ornamentation of *E. loebiae*.

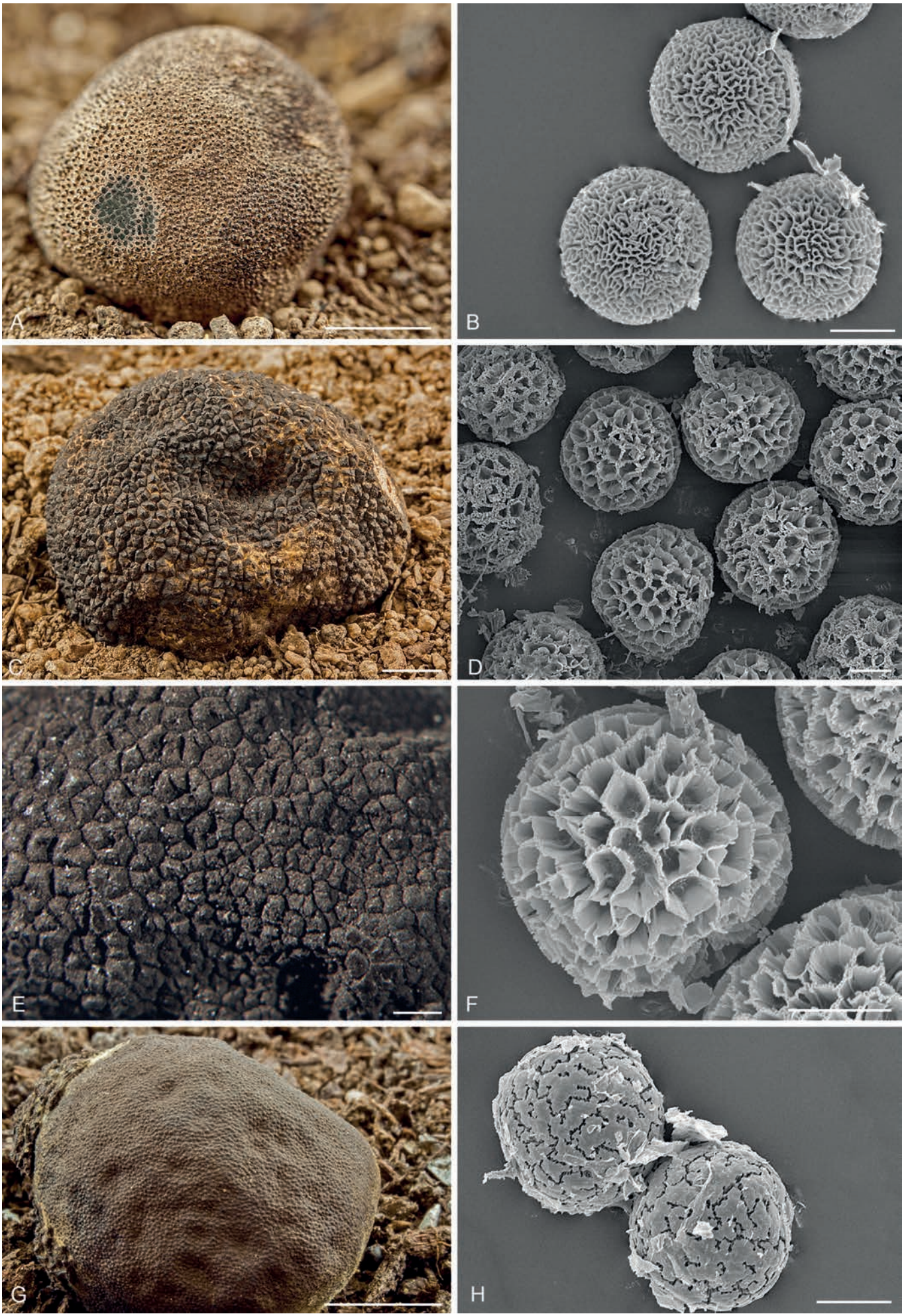
Elaphomyces mitchelliae Castellano & T.F. Elliott, ***sp. nov.*** MycoBank MB821238. Fig. 5.

Etymology: Named "*mitchelliae*" (*iae* – after) in honour and recognition of Donna Mitchell of West Virginia, accomplished collector of sequestrate taxa.

Type: **USA**, *Florida*, Alachua Co., along State Route 325, ± 1/2 mile north of junction with SR346, 10 Aug. 1985, *M. Castellano & S. Miller* (holotype - OSC 149206; isotype - RMS).

Dried ascomata subglobose to somewhat turbinate, 11–18 × 14–40 mm, completely embedded in a yellow to green-yellow mycelial mat which forms a husk around individual ascomata and incorporates much sand, ectomycorrhizae and debris, the mycelium is sparse on the upper part of the ascomata (found particularly in between the warts), more dense near base but above the stipitate basal projection; the hyphae with heavy deposits of amorphous yellow material which, along with the hyphae, turns orange to magenta with KOH and releases a red pigment, under magnification the mycelial covering immediately adjacent to the surface is actually brown and gives rise to the yellowish hyphae; KOH on ascoma surface instantly red, soon brown-black, ETOH instantly black (from show-through of peridium). *Peridium* 3–4 mm thick when fresh, 2.5–3 mm when dried, outer layer 500–900 µm thick when fresh, carbonaceous, black, brittle, with a surface of rounded to angled, irregularly shaped warts, individual warts constructed in a compound, stellate pattern, warts 250–300 µm tall; inner layer a composite of several layers, 2.5–3 mm thick when fresh, leathery, uniform to banded, pale brown to pale yellow-brown to dark grey-brown (near gleba), KOH on darkening but not distinctive; at the base of the ascoma the carbonaceous layer is thickened as a dense, black hyphal mass incorporating roots and debris, often forming

Fig. 3. *Elaphomyces loebiae*. **A.** Ascomata showing peridial surface, immature gleba, and peridium in section (OSC 149109). **B.** Ascoma showing small rounded warts on peridial surface. **C.** Inner layer (2nd) of the peridium with encrusted hyphae. **D.** Asci showing 8 immature spores. **E.** Ascospore in cross-section showing height and pattern of the ornamentation. **F.** Ascospores in surface view showing the ornamentation pattern. **G.** SEM micrograph of ascospores showing the labyrinthine pattern to the clumpy ornamentation. **H.** SEM micrograph showing the fine detail of the ornamentation. B–H (OSC 149108 – holotype). Bars: A = 10 mm, B = 500 µm, C = 10 µm, D = 15 µm, E–G = 10 µm, H = 5 µm.



a projection up to 11 mm long × 8 mm broad (usually 3 × 3 mm or less). *Gleba* stuffed with bright white mycelium when young, when mature developing a powdery spore mass that is dark green-blue, dark grey-blue to dark grey-brown, finally grey-black, with concolourous web-like hyphae. *Odour* not distinctive. *Taste* not recorded.

Peridium 7-layered, 1st layer carbonaceous, ± 200 µm thick, of golden yellow to dark brown to black, multi-sided plates or warts with slightly raised edges, many hyphae between plates and also on the centre of the plate where it has a depression, in profile the plates wart-like with depression between the warts; 2nd layer 200–230 µm thick, of dark brown, irregularly inflated cells up to 10 × 15 µm, walls 0.5 µm thick; 3rd layer 120–140 µm thick, of hyaline to pale tan, compact, interwoven hyphae, 4–5 µm broad, walls 0.5 µm thick; 4th layer 1–1.2 mm thick, of ectomycorrhizas that are enveloped with dark brown-black hyphae, this layer irregularly structured with more ectomycorrhizas found near the ascomata base and none found at top of ascomata; 5th layer 200–250 µm thick, hyaline to pale tan, loosely interwoven, parallel to somewhat bundled hyphae, 4–5 µm broad; 6th layer 800–900 µm thick, of hyaline to pale tan, distinctly bundled, cord-like, compactly parallel hyphae, 5–7 µm broad, bundles slightly wavy or sinuous; 7th layer 200–275 µm thick, of hyaline to pale tan, short-segmented, contorted, irregularly shaped, compact, interwoven hyphae or cells, 4–5(–9) µm broad. *Gleba* composed of spores and hyphae that are hyaline, septate, somewhat branched, sinuous, loosely interwoven, 3–5 µm broad, walls <0.5 µm thick. *Asci* globose, 65–70 µm broad, hyaline, walls 2–3 µm thick, 4- or 8-spored, arising from knots of short, irregularly curved or contorted clustered hyphae, up to 14 µm broad; occasionally forming asci up to 140 µm broad filled with 11 or 12 spores. *Spores* globose, (23–)24–27(–28) µm broad (mean = 25.5 µm broad); walls 2–3 µm thick, in KOH dark grey-brown to olive singly and in mass when mature, ornamentation a partial to complete reticulum, alveoli 4–5 sided, (3–)5 µm broad, ± 3 µm tall.

Distribution, habit, habitat, and season: Known from Florida, Maryland, North Carolina, Virginia, and West Virginia; hypogeous; under *Fagus grandifolia*, *Pinus serotina, P. taeda*, *Quercus alba, Q. coccinea, Q. laurifolia, Q. montana, Q. nigra, Q. rubra,* or *Q. virginiana*; February, April through May, September, and November.

Additional collections examined: **USA,** *Florida,* Alachua Co., Cross Creek, 17 Sep. 1980, *J. Trappe* 5951 (OSC 149202) and 5955 (OSC 40154; PERTH); Newman Lake, Owens-Illinois County Park, 3 May 2006, *M. Castellano* (OSC 149210; same locality, 11 Aug. 1985, *M. Castellano & S. Miller* (OSC 149209, RMS); same locality, 6 May 1987, *M. Castellano, D. Luoma, & T. O'Dell* (OSC 149103); same locality, 14 Jun. 2012, *M. Smith 602* (OSC 149216, FLAS); same locality, 25 Feb. 2012, *M. Castellano & M. Smith* (OSC 149215 & OSC 149213); same locality, 11 Aug. 2007, *M. Castellano* (OSC 149212); same locality, 23 Nov. 1981, *Col. E. Dickstein* (FLAS 55379); ±13 miles southeast of Gainesville, 5.3 miles north of Cross Creek, 24 Aug. 1979, *G. Benny, J. Kimbrough, L. Jacobs, & J. Gibson* (FLAS 52090, OSC 39528); Polk Co., Lake Kissimmee State Park, picnic area, 17 Aug. 2007, *M. Castellano* (OSC 149211); *Maryland,* Anne Arundel Co., Patuxent Research Refuge, Laurie-Bowie Rd., 20 Apr. 1966, *F.A. Uecker, O.K. Miller, & L. Stevens* (OSC 149887); *North Carolina*, Brunswick Co., Wilmington, across from Belk-Beery, 8 Sept. 1984, *S. Miller 806* (RMS, OSC 150029); Rutherford Co., Painters Gap Rd., 3 miles east of Cove Rd., 13 Jul. 2011, *T. Elliott* (OSC 149214); *Virginia*, Fairfax Co., no locality, 8 May 1926, *E.G. Artzberger* (OSC 149888); Accotink, 30 Jun. 1968, unknown collector (OSC 149101); West Virginia, Barbour Co. northwest portion of county, 18 Apr. 1992, *D. Mitchell* (OSC 149203).

Notes: We assign *Elaphomyces mitchelliae* to section *Asco-scleroderma* within *Elaphomyces* based on its distinct base.

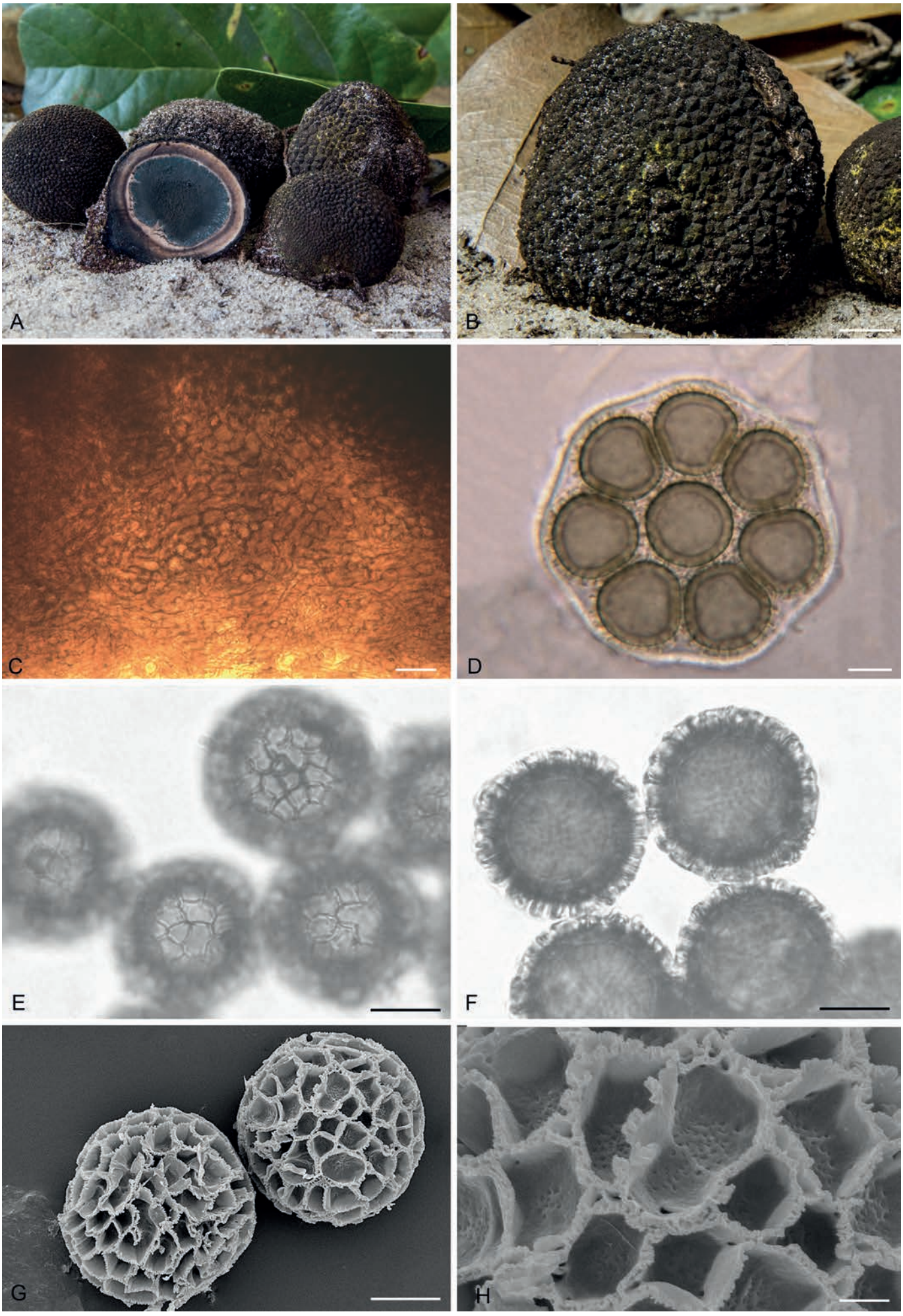
Samuelson *et al*. (1987) provide an ultrastructural study of spore ornamentation of the holotype collection of this species (as *E. persoonia*).

Castellano studied an *Elaphomyces cyanosporus* Tul. & C. Tul. collection in Kew (K161175). Tulasne (1841) lists specimens from Meudon, Clamart, and Chaville in the area surrounding Paris. Castellano could not locate any Tulasne material of this species in Paris (PC or FH). Castellano (unpubl. data) recorded data from K161175 in part as follows: Dried peridium ornamented with flat, coarse, irregularly shaped black warts, peridial surface black, base subturbinate, spores globose, in KOH dark brown singly, slightly darker in mass, 27–30 µm broad, mean = 28.0 µm broad, including ornamentation that is a complete reticulum with alveoli 3–4 µm broad × 2–3 µm tall under light microscopy, SEM reveals the spore ornamentation as a complete reticulum with coarse ridges (Fig. 4E–F). The spores of *E. cyanosporus* are slightly larger than *E. mitchelliae,* and the alveoli are smaller. In addition, the peridial surface of *E. cyanosporus* consists of flat, irregularly shaped warts, whereas the peridial surface of *E. mitchelliae* has distinct, rounded to angled warts in a compound stellate pattern.

Castellano studied an *Elaphomyces persoonii* var. *minor* Tul. & C. Tul. collection in Kew (K161168). The Tulasne brothers (1841) list specimens from Meudon, Clamart, and Chaville in the area surrounding Paris but do not designate a type. Castellano could not locate any Tulasne material of this species in Paris (PC or FH). Castellano (unpubl. data) recorded data from K161175 in part as follows: Peridial surface of black, flat, coarse, irregularly shaped warts, ascoma base subturbinate, spores globose, in KOH dark brown singly, slightly darker in mass, 27–30 µm broad, mean = 28.0 µm broad including ornamentation that is a complete reticulum, alveoli 3–4 µm broad × 2–3 µm tall under light microscopy, SEM reveals the spore ornamentation as a complete reticulum with coarse-looking ridges. *Elaphomyces persoonii* var. *minor* is conspecific in all essential details with *E. cyanosporus*.

Castellano studied an *Elaphomyces leveillei* Tul. & C. Tul. collection in Kew herbarium (K162150). Tulasne (1841) lists specimens from Meudon, Clamart, and Chaville in the area

Fig. 4. Additional *Elaphomyces* species from Europe. **A.** *Elaphomyces aculeatus* ascomata showing peridial surface and the black warts overlain by yellow-brown hyphae (LE 162850). **B.** *Elaphomyces aculeatus* SEM micrograph of ascospores showing the labyrinthine-like ornamentation (OSC 149159). **C.** *Elaphomyces persoonii* ascomata showing wart structure and adherent yellow toned mycelium (LE 162885). **D.** *Elaphomyces persoonii* SEM micrograph of ascospores showing reticulate ornamentation (OSC 149365). **E.** *Elaphomyces cyanosporus* ascomata showing wart structure. **F.** *Elaphomyces cyanosporus* SEM micrograph of ascospores showing the reticulate ornamentation (OSC 149176). **G.** *Elaphomyces leveillei* ascomata showing papillate peridial surface and some adherent yellow toned mycelium (W2000-805). **H.** *Elaphomyces leveillei* SEM micrograph of ascospores showing the ornamentation of irregular plates (OSC 149116). Bars: A, G = 1 cm, B, D, F, H = 10 µm, C = 5 mm, E = 500 µm.



surrounding Paris but does not designate a type. Castellano could not locate any Tulasne material of this species in Paris (PC). Castellano (unpubl. data) recorded data from K162150 in part as follows: Peridial surface of black, pusticulate (bumpy) to tuberculate, partially covered by pale tan, pale yellow-brown to yellow hyphae, ascoma base indented, spores globose, in KOH dark brown singly, slightly darker in mass, 26–28 µm broad, mean = 26.6 µm broad including ornamentation that appears pusticulate under light microscopy, in section appearing with flattening of spore outline at least on a portion of numerous spores, SEM reveals the spore ornamentation as spines or rods overlain with amorphous, small, irregular plates to form a discontinuous surface, plate surface slightly roughened (Fig. 4G–H). The spores of *E. leveillei* are similar in size (26–28 µm broad, mean = 26.6 µm broad) but have an ornamentation of irregularly shaped, discontinuous plates compared to the distinct reticulum of *E. mitchelliae*. In addition, the peridial surface of *E. leveillei* consists of bump-like features compared to the distinct rounded to angled warts in a compound stellate pattern of *E. mitchelliae*.

Castellano studied *Elaphomyces persoonii* Vittad. collections from TO - OSC 149124, Wien - W2008-1079 and Kew - K162166 marked as Vittadini. Castellano (unpubl. data) recorded data from Trappe 1470 in part as follows: Ascoma subturbinate to turbinate, peridial surface of large black warts, with dark brown hyphae seen between warts, spores globose, in KOH brown to dark brown singly, slightly darker in mass, 29–33(–35) µm broad, mean = 31.3 µm broad including ornamentation that is a complete reticulum, up to 5 µm tall, alveoli irregular, up to 5–7 µm across under light microscopy, SEM reveals the spore ornamentation as a complete reticulum with the digitate edges along the alveoli (Fig. 4C–D). The spores of *E. persoonii* are larger (29–33 µm broad, mean = 31.3 µm broad) and have taller alveoli walls (up to 5 µm tall) compared to the spores of *E. mitchelliae*. In addition, the peridial surface of *E. persoonii* has flat warts compared to the distinct rounded to angled warts in a compound stellate pattern of *E. mitchelliae.*

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Fig. 5. *Elaphomyces mitchelliae.* **A.** Ascomata showing peridial surface, gleba, and peridium in section (OSC 149215). **B.** Ascoma showing peridial warts with some adherent yellow mycelium (OSC 149213). **C.** Outer peridium showing the pale, stacked hyphae between the darker wart tissues. **D.** Asci showing 8 immature spores. **E.** Ascospores in surface view showing the ornamentation pattern. **F.** Ascospores in cross-sectional view showing the height and pattern of the ornamentation. **G.** SEM micrograph of ascospores showing the distinct reticulate pattern. **H.** SEM micrograph of ascospores showing the structure of the alveoli. C, E–F (OSC 40154); D (OSC 40154); G–H (OSC 149103). Bars: A = 20 mm, B = 5 mm, C = 30 µm, D–G = 10 µm, H = 2.5 µm.

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Epitypification and re-description of the zombie-ant fungus, *Ophiocordyceps unilateralis* (*Ophiocordycipitaceae*)

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Abstract: The type of *Ophiocordyceps unilateralis* (*Ophiocordycipitaceae*, *Hypocreales*, *Ascomycota*) is based on an immature specimen collected on an ant in Brazil. The host was identified initially as a leaf-cutting ant (*Atta cephalotes*, Attini, Myrmicinae). However, a critical examination of the original illustration reveals that the host is the golden carpenter ant, *Camponotus sericeiventris* (Camponotini, Formicinae). Because the holotype is no longer extant and the original diagnosis lacks critical taxonomic information – specifically, on ascus and ascospore morphology – a new type from Minas Gerais State of south-east Brazil is designated herein. A re-description of the fungus is provided and a new phylogenetic tree of the *O. unilateralis* clade is presented. It is predicted that many more species of zombie-ant fungi remain to be delimited within the *O. unilateralis* complex worldwide, on ants of the tribe Camponotini.

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INTRODUCTION

Ophiocordyceps unilateralis (*Ophiocordycipitaceae*: *Hypocrea-les*) is a fungal pathogen of ants belonging to the tribe Camponotini (Formicinae: Formicidae) with a pantropical distribution (Evans 2001). The fungus alters the behaviour of the ant host causing it to move and die away from the nest, often in an exposed position and, typically, clinging onto and biting into vegetation in a "death-grip" (Hughes *et al*. 2011). This host manipulation by *O. unilateralis* is a particularly spectacular and complex example of the extended-phenotype paradigm (Dawkins 1982, Andersen *et al*. 2009, Hughes 2013, Hughes *et al*. 2016), which duly garnered the epithet, the zombie-ant fungus (Evans *et al*. 2011a), and spawned considerable media coverage by the popular press and scientific magazines alike (Kaplan 2011, Costandi 2012, Boddy 2014, Pennisi 2014). In addition, it stimulated on-going research on the nature of the ant-fungal association, as well as on fungal phylogeny, that has generated a wealth of information (reviewed in Hughes *et al*. 2016). Significant advancement has been made in understanding the mechanisms involved at the molecular level: thus, manipulation of the ant brain by the fungus has been ascribed to two candidate metabolites – guanobutyric acid and sphingosine – previously implicated in neurological diseases and cancer (de Bekker *et al*. 2014). Using comparative genomics and a mixed transcriptomics approach, it has also been shown that genes unique to the fungus are up-regulated that encode for proteins known to cause neurological and behavioural changes (de Bekker *et al*. 2015, de Bekker *et al*. 2017).

Contemporary studies have tended to use the over-arching term *O. unilateralis sensu lato* for the zombie-ant fungus since it has long been suspected, but only recently established, that this is a species complex. In fact, morphological variations had been noted in collections from around the world from a very early stage (Petch 1924, 1931, 1933, 1935, 1937, Kobayasi 1941, Mains 1958, Evans 1974, 1982, Evans & Samson 1984), but it was concluded that "whilst it is tempting to separate geographic isolates (ecotypes), there is not enough evidence at the moment to conveniently divide the species into varietal units: more information is needed concerning host specificity and the range of variation in temperate, subtropical and tropical specimens" (Evans & Samson 1984). Some three decades later, Evans *et al*. (2011a) set out to uncover the taxonomic diversity of the newly-termed zombie or brain-manipulating fungus, based on an examination of fresh material collected on infected carpenter ants within a fragment of Atlantic rainforest in Brazil. Four *Camponotus* species were identified and, following a critical morphological comparison of the freshly-released (mature) ascospores – as well as of the germination process – and of the associated asexual morphs, four *Ophiocordyceps* species were delimited; leading to the supposition that "each species of the tribe Camponotini may be attacked by a distinct species of *Ophiocordyceps*" (Evans *et al*. 2011a), and "that there may be hundreds of species within the complex parasitising formicine ants worldwide" (Evans *et al*. 2011b). This hypothesis would appear to be holding true based on subsequent publications involving both morphological and molecular evidence, with six new species being described from Thailand (Luangsa-ard *et al*. 2011, Kobmoo *et al*. 2012, Kobmoo *et al*. 2015), one from Japan (Kepler *et al*. 2010), three from the Brazilian Amazon (Araújo *et al*. 2015) and another 14 in the pipeline (Araújo *et al*. 2018).

Significantly, however, only Kobayasi (1941) appears to have examined the type specimen – named as *Torrubia unilateralis*

on the Brazilian ant *Atta cephalotes* (Tulasne & Tulasne 1865) – and he noted that it "is now preserved in [the] Paris Entomological Museum [and] is immature". Unfortunately, repeated attempts to obtain the type for examination of the fungus and identification of the ant host were unsuccessful and it was concluded that the specimen was lost, leading to speculation that this may have gone missing during the Second World War (Evans *et al*. 2011a). From the latter study, and the confirmation that *O. unilateralis* represents a species complex, it became necessary to designate a new type, especially since *Ophiocordyceps* is the type genus of the recently-recognised family *Ophiocordycipitaceae* which is based on the placement of *O. unilateralis* within a Bayesian consensus tree (Sung *et al*. 2007). *Ophiocordyceps* is a highly diverse genus, with considerable pharmaceutical potential (Berenbaum & Eisner 2008, Paterson 2008, Molnár *et al*. 2010, Zhang *et al*. 2012) – species of which have also been identified recently as primary endosymbionts in certain insect hosts (Nishino *et al*. 2016, Gomez-Polo *et al*. 2017) – and thus *O. unilateralis* is central to our understanding of this medically-important group, as well as being considered as a keystone species for unravelling ecosystem functioning and biodiversity of fungi in tropical forests (Evans *et al*. 2011b).

In his diagnosis, Louis Tulasne described the unilateral position of the fleshy, hemispherical, fertile stroma on the stipe, but failed to provide details of the asci or ascospores, nor did these structures appear in the accompanying illustration by his brother, Charles (Tulasne & Tulasne 1865). This supports the statement of Kobayasi (1941) that the type was immature. Theoretically, the illustration could still stand as the holotype but, because there is no extant material, this would serve as the lectotype and a suitable epitype should be designated (Ariyawansa *et al*. 2014), not a neotype as Evans *et al*. (2011a) had originally and mistakenly proposed. The resultant search for a suitable epitype was based on the evidence from the illustration that the host representing the type is a *Camponotus* ant (Samson *et al*. 1982): specifically, the golden carpenter ant *Camponotus sericeiventris*, with its distinctive pronotal plate, and not the leafcutter *Atta cephalotes*, which is a myrmicine ant having no historical association with *O. unilateralis* (Evans & Samson 1984, Evans 2001). Cooke (1892) used the same Tulasne illustration to re-describe the so-called "one-sided ant club", with additional information that the fungus had been "collected by Trail in Brazil". This specimen is in the RBG Kew fungarium and was found by the English naturalist J.W.H. Trail in 1874 in the Brazilian Amazon, which was examined by Massee (1895) who reported it to be on the same ant species as the type. However, we consider that the type specimen of *O. unilateralis* was more likely to have originated in the Atlantic rainforest region of south-east Brazil – where several European naturalists were based in the 1860s – and from where the type of *Camponotus sericeiventris* was collected (Rio de Janeiro) during a series of French expeditions (Guérin-Menéville 1838); specimens from which were deposited in the Paris Entomological Museum, where the type of *O. unilateralis* was also deposited (Tulasne & Tulasne 1865).

Epitypification has been delayed until now because all the targeted collections of infected *C. sericeiventris* ants from Atlantic rainforest in south-east Brazil proved to be immature (Evans *et al*. 2011a). In fact, some newly-infected specimens were marked *in situ* – whilst others were harvested and incubated in the laboratory – to monitor progress, but none developed to maturity. The present paper is the result of the discovery of specimens with fertile stromata, from the same region of Brazil (Zona da Mata Mineira), enabling a full description of the species, as well as a phylogenetic analysis.

MATERIALS AND METHODS

Field collection

Collecting was concentrated in a vestige of secondary Atlantic rainforest near Viçosa, Minas Gerais, in the Zona da Mata Mineira of south-east Brazil – belonging to the Universidade Federal de Viçosa (UFV) – where *ad hoc* surveys for zombie-ant fungi had been carried out previously (Evans *et al*. 2011a, b). Although *Camponotus sericeiventris* is relatively common in this habitat, it is confined mainly to open, heavily-disturbed areas and the incidence of infected ants was found to be low. All the initial collections proved to be immature and it was decided to follow progress *in situ* by flagging specimens and monitoring development of the ascostromata through weekly observation. However, none of the five tagged specimens survived, due to predation and loss through heavy rain. Subsequently, additional specimens were bagged but were spoiled by run-off water following storms. Finally, several more immature specimens were harvested together with the vegetation, transferred to a humid chamber in a greenhouse at UFV – with an 8 h misting/16 h dry regime – and monitored. Asexual morphs developed successfully but, because ascostromatal development was slow, the specimens were overgrown by opportunistic fungi before maturation was complete. The taxonomy of the asexual morphs is based on these paratype specimens. The mature epitype was collected by one of us (VRH) from another forest reserve in the Zona da Mata Mineira, some 150 km from the main study site, in the municipality of Juiz de Fora. These specimens were deposited in the fungarium of the Universidade Federal de Viçosa (VIC).

DNA extraction and PCR

We used a BLAST search in the GenBank nucleotide database to ensure the quality of the sequences generated in this study. Sequences that were identified as species not closely related to the species treated in this study were discarded and interpreted to be from a contaminant. All the sequences included here passed the above quality control checks.

The molecular studies were conducted according to Araújo *et al*. (2018), described below. The DNA templates were obtained directly from two specimens of *O. unilateralis* infecting *Camponotus sericeiventris* from the type locality in Minas Gerais (Brazil) that were collected in the field and dried *in silica* to avoid overgrowth by opportunistic fungi. For DNA extraction, the ants were dissected and the fungal contents (mummified mycelium and hyphal bodies) within their abdomens were placed in 1.5 mL Eppendorf tubes with 100–200 µL of CTAB (2 % CTAB powder, 100 mM Tris pH8, 20 mM EDTA, 1.4 M NaCl) and ground mechanically; 400 µL of CTAB were then added and the tubes were incubated at 60 °C for 20 min and centrifuged for 10 min at 14 000 rpm. The supernatant (approx. 400 µL) was transferred to a new 1.5 mL Eppendorf tube, mixed with 500 µL of 24:1 chloroform: isoamyl-alcohol (Sigma) and mixed by inverting. The mix was then centrifuged for 20 min at 14 000 rpm and the

supernatant transferred to a new 1.5 mL Eppendorf tube and further cleaned using the GeneCleanIII kit (MP Biomedicals), following Araújo *et al*. (2018) modifications.

Four loci were used in the analyses, i.e. small subunit nuclear ribosomal DNA (SSU), large subunit nuclear ribosomal DNA (LSU), translation elongation factor 1-α (*tef1*) and the largest subunit of RNA polymerase II (*rpb1*). The final concatenated dataset consisted of 3 795 bp. The primers used were, SSU: NS1 (GTAGTCATATGCTTGTCTC) and NS4 (CTTCCGTCAATTCCTTTAAG) (White *et al*. 1990); LSU: LR0R (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAAACTTCG-3') (Vilgalys & Hester 1990); *tef1*: EF1-983F (5'-GCYCCYGGHCAYCGTGAYTTYAT-3') and EF1-2218R (5'- ATGACACCRACRGCRACRGTYTG-3') (Rehner & Buckley 2005); CRPB1: (5'-CCWGGYTTYATCAAGAARGT-3') (Castlebury *et al*. 2004) and RPB1Cr_oph: (5'-CTGVCCMGCRATGTCGTTGTCCAT-3') (Araújo *et al*. 2018).

To amplify the target loci, each 25 µL PCR amplification mix contained 4.5 µL of buffer E (Premix E – Epicentre) 0.5 µL of each forward and reverse primers (10 mM), 1 µL of DNA template, 0.1 Platinum Taq polymerase (Invitrogen) and 18.4 µL of ultra-pure distilled water (Gibco). The amplification reactions were placed in a Biometra T300 thermocycler under the following conditions: for SSU and LSU (1) 2 min at 94 °C, (2) 4 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min, followed by (3) 35 cycles of denaturation at 94 °C for 30 s, annealing at 50.5 °C for 1 min, and extension at 72 °C for 2 min and (4) 3 min at 72 °C. For *tef1* and *rpb1* (1) 2 min at 94 °C, (2) 10 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 1 min, and extension at 72 °C for 1 min, followed by (3) 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 1 min, and extension at 72 °C for 1 min and (4) 3 min at 72 °C. Each 25 µL amplification reaction was cleaned by adding 3.75 µL of Illustra ExoProStar enzymatic PCR clean up (1:1 mix of Exonuclease I and alkaline phosphatase, GE Healthcare Life Sciences), incubated at 37 °C for 1 h and 80 °C for 15 min in the thermocycler. The purified PCR products were sequenced by Sanger DNA sequencing [Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA, USA)] at the Genomics Core Facility service at Penn State University.

Phylogenetic analyses

The raw sequence reads were edited manually using Geneious v. 8.1.8 (Kearse *et al*. 2012). Individual gene alignments were generated by MUSCLE (Edgar 2004), implemented in Geneious v. 8.1.8 (Kearse *et al*. 2012). The alignment of every gene was improved manually, annotated and concatenated into a single combined dataset using Geneious v. 8.1.8 (Kearse *et al*. 2012). Ambiguously aligned regions were manually excluded from phylogenetic analysis and gaps were treated as missing data. The final alignment length was 3 795 bp – 1 071 for SSU, 961 for LSU, 1 011 for *tef1* and 752 for *rpb1*. A Maximum likelihood (ML) analysis was performed with RAxML v. 8.2.4 (Stamatakis 2006) on a concatenated dataset containing all four genes. The dataset consisted of eight data partitions. These included one each for SSU and LSU, and three for each of the three codon positions of the protein coding genes, *tef1* and *RPB1*. The GTRGAMMA model of nucleotide substitution was employed during the generation of 1 000 bootstrap (bs) replicates. The sequences generated for this study were deposited in GenBank (Table 1).

RESULTS

Taxonomy

Ophiocordyceps unilateralis (Tul.) Petch, *Trans. Br. Mycol. Soc*. **16**: 74.1933. *emend.* H.C. Evans, D.P. Hughes & Araújo. Figs 1–2. *Basionym*: *Torrubia unilateralis* Tul., *Sel. Fung. Carp.* **III**: 18. 1865. *Synonym*: *Cordyceps unilateralis* (Tul.) Sacc. *Syll. Fung*. **2**: 570. 1883.

Description on host: *External mycelium* sparse, pale brown; emerging from sutures on body and legs. *Clava* stromatal, solitary, arising from the dorsal pronotum; cylindrical, brown and hirsute at the base. *Ascostroma* produced unilaterally, almost encircling the clava; hemisphaerical, 1.5–1.7 × 0.8 µm, dark brown, with roughened surface due to prominent perithecial necks. *Ascomata* (*perithecia*) partially erumpent, flask-shaped, 200–250 × 140–160 µm. *Asci* 8-spored, hyaline, cylindrical, (90–)95–125 × 6–8(–9) µm, swollen centrally tapering to a distinct foot and apical cap region (5–6 × 4–5 µm). *Ascospores* multiserriate, hyaline, thin-walled, filiform, (70–)75–85 × 2–2.5 µm, 4–5-septate; curved, tapering at both ends.

Lectotype designated here: holotype **Brazil**, *"Atta cephalotes"*, Tulasne (1865) *Sel. Fung. Carp.* **III**, plate I, fig. 3–4, MBT379723.

Epitype designated here: **Brazil**, *Minas Gerais*, Juiz de Fora, Paraibuna river (700–800 m a.s.l.), on *Camponotus sericeiventris* (Camponotini: Formicinae: Formicidae), on shrub leaf, 10 Aug. 2014, *V.R. Halfeld*, I14-1369A (epitype VIC 44303, MBT379722).

Additional materials examined: **Brazil**, *Minas Gerais*, Viçosa, Mata do Paraíso (700 m a.s.l.), on *Camponotus sericeiventris*, on shrub leaf, 26 Apr. 2010, *H.C. Evans*, MAP-61 (paratype VIC 44354); 12 Aug. 2012, *H.C. Evans*, MP-426 (paratype VIC 44349); 7 Feb. 2013, *H.C. Evans*, MP-502 (paratype VIC 44350).

Asexual morph: The asexual morph of the epitype proved to be in poor condition and the diagnosis below is based on the paratype collections.

Apical region of the stromatal clava, smooth, pinkish-brown, tapering to an acute tip; covered by a loose to compact hymenium of scattered to dense phialides. *Phialides* of two types: with a prominent swollen base (10–12 × 3–3.5 µm), tapering abruptly to a thin neck region (12–15 × 0.5–1 µm), producing hyaline, guttulate, limoniform *conidia*, 6.5–8 × 2–2.5 µm, apically (= *Hirsutella* A-type, Evans & Samson 1984); with a cylindrical base (14–16 × 2.5–3 µm), tapering gradually to a long neck (45–50 µm), 1 µm at the tip, producing solitary, hyaline, cylindrical-fusoid *conidia*, 8–11 × 2.5–3 µm, with a rounded apex and truncate base (= *Hirsutella* B-type). *Hirsutella* B-type also produced separately in loose, brown sporodochia arising from the leg joints.

Notes: Other synonyms – *Torrubia formicivora*, *Cordyceps formicivora*, *C. ridleyi* and *C. subunilateralis* – have been listed by various authors (Petch 1933, Mains 1958, Evans & Samson 1984, Sung *et al*. 2007): however, because the ant hosts are not identified and the collecting localities of some are outside the geographic range of *Camponotus sericeiventris*, these can no longer be considered to be synonymous with *O. unilateralis s. str*. Examination of the types, as well as identification of the

Table 1. Specimen information and GenBank accession numbers for the sequences used in this study.

		GenBank Accession numbers[2]			
Species	**Voucher Information[1]**	**SSU**	**LSU**	***tef1***	***rpb1***
Ophiocordyceps acicularis	OSC 128580	DQ522543	DQ518757	DQ522326	DQ522371
Ophiocordyceps agriotidis	ARSEF 5692	DQ522540	DQ518754	DQ522322	DQ522368
Ophiocordyceps amazonica	HUA 186143	KJ917562	KJ917571	KM411989	KP212902
Ophiocordyceps annulata	CEM 303	KJ878915	KJ878881	KJ878962	KJ878995
Ophiocordyceps aphodii	ARSEF 5498	DQ522541	DQ518755	DQ522323	n/a
Ophiocordyceps araracuarensis	HUA 186135	KC610788	KC610769	KC610738	KF658665
Ophiocordyceps australis	HUA 186147	KC610784	KC610764	KC610734	KF658678
Ophiocordyceps blattarioides	HUA186093	KJ917559	KJ917570	KM411992	KP212910
Ophiocordyceps brunneipunctata	OSC 128576	DQ522542	DQ518756	DQ522324	DQ522369
Ophiocordyceps camponoti-atricipis	ATRI3	KX713666	n/a	KX713677	n/a
Ophiocordyceps camponoti-balzani	G104	KX713660	KX713593	KX713689	KX713703
	G143	KX713658	KX713595	KX713690	KX713705
Ophiocordyceps camponoti-bispinosi	BISPI2	KX713665	KX713588	n/a	KX713700
	OBIS	KX713639	KX713612	KX713694	KX713718
	OBIS3	KX713638	KX713614	KX713695	n/a
	OBIS4	KX713637	KX713615	KX713692	KX713720
Ophiocordyceps camponoti-leonardi	TL1	KJ201515	n/a	KJ201526	n/a
	C36	KJ201512	n/a	JN819013	n/a
Ophiocordyceps camponoti-rufipedis	G108	KX713659	KX713594	KX713679	KX713704
	G177	KX713657	KX713596	KX713680	n/a
Ophiocordyceps camponoti-saundersi	C19	n/a	n/a	JN819042	n/a
	C40	n/a	n/a	JN819044	n/a
Ophiocordyceps communis	NHJ 12582	EF468975	EF468830	EF468771	n/a
	NHJ 12581	EF468973	EF468831	EF468775	n/a
Ophiocordyceps curculionum	OSC 151910	KJ878918	KJ878885	n/a	KJ878999
Ophiocordyceps dipterigena	OSC 151911	KJ878919	KJ878886	KJ878966	KJ879000
Ophiocordyceps elongata	OSC 110989	n/a	EF468808	EF468748	EF468856
Ophiocordyceps evansii	HUA 186159	KC610796	KC610770	KC610736	KP212916
Ophiocordyceps formicarum	TNS F18565	KJ878921	KJ878888	KJ878968	KJ879002
Ophiocordyceps fulgoromorphila	HUA 186139	KC610794	KC610760	KC610729	KF658676
Ophiocordyceps gracilis	EFCC 3101	EF468955	EF468810	EF468750	EF468858
Ophiocordyceps halabalaensis	MY1308	n/a	n/a	GU797109	n/a
	MY5151	n/a	n/a	GU797110	n/a
Ophiocordyceps heteropoda	EFCC 10125	EF468957	EF468812	EF468752	EF468860
Ophiocordyceps irangiensis	OSC 128578	DQ522556	DQ518770	DQ522345	DQ522391
Ophiocordyceps kniphofioides	HUA 186148	KC610790	KF658679	KC610739	KF658667
Ophiocordyceps longissima	HMAS_199600	KJ878926	n/a	KJ878972	KJ879006
Ophiocordyceps lloydii	OSC 151913	KJ878924	KJ878891	KJ878970	KJ879004
Ophiocordyceps melolonthae	OSC 110993	DQ522548	DQ518762	DQ522331	DQ522376
Ophiocordyceps myrmecophila	CEM1710	KJ878928	KJ878894	KJ878974	KJ879008
Ophiocordyceps neovolkiana	OSC 151903	KJ878930	KJ878896	KJ878976	KJ879010
Ophiocordyceps nutans	OSC 110994	DQ522549	DQ518763	DQ522333	DQ522378
Ophiocordyceps polyrhachis-furcata	P39	KJ201504	n/a	JN819003	n/a
	P51	KJ201505	n/a	JN819000	n/a
Ophiocordyceps ponerinarum	HUA 186140	KC610789	KC610767	KC610740	KF658668
Ophiocordyceps pruinosa	NHJ 12994	EU369106	EU369041	EU369024	EU369063
Ophiocordyceps pulvinata	TNS-F 30044	GU904208	n/a	GU904209	GU904210

Table 1. (Ctd).

		GenBank Accession numbers[2]			
Species	**Voucher Information[1]**	**SSU**	**LSU**	***tef1***	***rpb1***
Ophiocordyceps purpureostromata	TNS F18430	KJ878931	KJ878897	KJ878977	KJ879011
Ophiocordyceps rami	MY6736	KM655823	n/a	KJ201532	n/a
Ophiocordyceps rhizoidea	NHJ 12522	EF468970	EF468825	EF468764	EF468873
Ophiocordyceps septa	C41	KJ201525	n/a	JN819037	
Ophiocordyceps sobolifera	TNS F18521	KJ878933	KJ878898	KJ878979	KJ879013
Ophiocordyceps sphecocephala	OSC 110998	DQ522551	DQ518765	DQ522336	DQ522381
Ophiocordyceps stylophora	OSC 111000	DQ522552	DQ518766	DQ522337	DQ522382
Ophiocordyceps tiputini	QCNE 186287	KC610792	KC610773	KC610745	KF658671
Ophiocordyceps unilateralis s. str.	VIC 44303	KX713628	KX713626	KX713675	KX713730
	VIC 44354	KX713627	n/a	KX713676	KX713731
Ophiocordyceps unilateralis var. *clavata*	INPA 274589	KX713652	KX713600	KX713681	KX713708
	INPA 274590	KX713651	n/a	KX713682	KX713709
Ophiocordyceps variabilis	OSC 111003	EF468985	EF468839	EF468779	EF468885
Ophiocordyceps yakusimensis	HMAS_199604	KJ878938	KJ878902	n/a	KJ879018
Stilbella buquetii	HMAS_199617	KJ878940	KJ878905	KJ878985	KJ879020
Tolypocladium capitatum	OSC 71233	AY489689	AY489721	AY489615	AY489649
Tolypocladium japonicum	OSC 110991	DQ522547	DQ518761	DQ522330	DQ522375
Tolypocladium ophioglossoides	OSC 106405	AY489691	AY489723	AY489618	AY489652

[1]ARSEF, USDA-ARS Collection of Entomopathogenic Fungal Cultures, Ithaca, NY; ATR, BISP, G and OBIS abbreviations from D.P. Hughes personal collection, Penn State University, PA, USA; C, P and TL abbreviations follow those of Kobmoo *et al*. (2015); CEM from J. W. Spatafora lab collection, Oregon State University, OR, USA; EFCC, Entomopathogenic Fungal Culture Collection, Chuncheon, South Korea; HMAS, Chinese Academy of Sciences, Beijing, China; HUA, Herbarium Antioquia University, Medellin, Colombia; INPA, Herbarium of National Institute of Amazonian Research, Manaus, Brazil; MY, J.J. Luangsa-ard personal collection, BIOTEC, Thailand; NHJ, Nigel Hywel-Jones personal collection; OSC, Oregon State University Herbarium, Corvallis, OR; TNS, National Museum of Science and Nature, Tsukuba, Japan.

[2]SSU: partial small subunit (18S) nrRNA gene; LSU: partial large subunit (28S) nrRNA gene; *tef1*: partial translation elongation factor 1-α gene; *rpb1*: partial fragment of the largest subunit of the RNA polymerase II gene.

Camponotus species involved, will be necessary to clarify their taxonomic status.

The characteristic that distinguishes *O. unilateralis* from all the other zombie-ant species described, thus far, is the presence of both the A- and B-type phialides within the same hymenium of the stromatal clava (Fig. 2). Cylindrical, pinkish brown synnemata may also arise separately from the body and legs forming both phialide types. In other species, only the A-type phialides are produced in a compact hymenium at the tip of stromatal clava or on separate synnemata. This was named much later as *Hirsutella formicarum* on specimens from Guyana (Petch 1935): however, the description matches that of the *Hirsutella* B-type (conidia 9–11 × 2 µm), rather than the significantly smaller, limoniform conidia described by Kobayasi (1941), as well as by Petch (1924) from Asian collections. This led Mains (1951) to question the validity of *H. formicarum*: "it hardly seems possible that these are all conidial stages of *Cordyceps unilateralis*". We can now begin to understand why there was this disparity in the asexual morphs collected on different and geographically-separated ant hosts, as highlighted by subsequent publications (Evans & Samson 1984, Rombach & Roberts 1989). Evans & Samson (1984) also illustrated the asexual morph collected on *C. sericeiventris* in Honduras and showed that both A- and B-phialides occurred together on the same synnema; although the taxonomic significance of this character was overlooked at the time. The majority of this Honduran collection (~70 specimens) comprised infected ants exhibiting both the A- and B-asexual morphs described herein. These were found around the buttress base of tropical forest trees whilst others were located in the classic death-grip on nearby shrubs. The latter specimens were reported to have only the A-asexual morph, with morphologically distinct phialides and conidia (Evans & Samson 1984). The explanation for this variability of the fungus within a single ant species may lie in the recently-confirmed classification of *C. sericeiventris* into five subspecies, three of which have a purely Mesoamerican distribution (Bolton *et al*. 2007). Evidently, therefore, pathogen-host specificity may be even more complex than envisaged previously, but this will only be clarified by more comprehensive collections of infected *C. sericeiventris* from the Neotropics, specifically from Central America. We are confident, however, that the epitype named here is on the ant, *C. sericeiventris sericeiventris* (Bolton *et al*. 2007), whilst it is possible that novel taxa of *Ophiocordyceps* remain to be discovered on the other five ant subspecies. In addition, fresh material with mature ascostromata is still needed in order to determine the mode of ascospore germination in *O. unilateralis s. str.*, an overlooked but significant taxonomic trait in these fungi (Evans *et al*. 2011a, b).

Phylogenetic relationships

The topology recovered in this study is in agreement with previous publications (Sung *et al*. 2007, Quandt *et al*. 2014, Sanjuan *et al*. 2015). The *Ophiocordyceps unilateralis s. lat.* clade

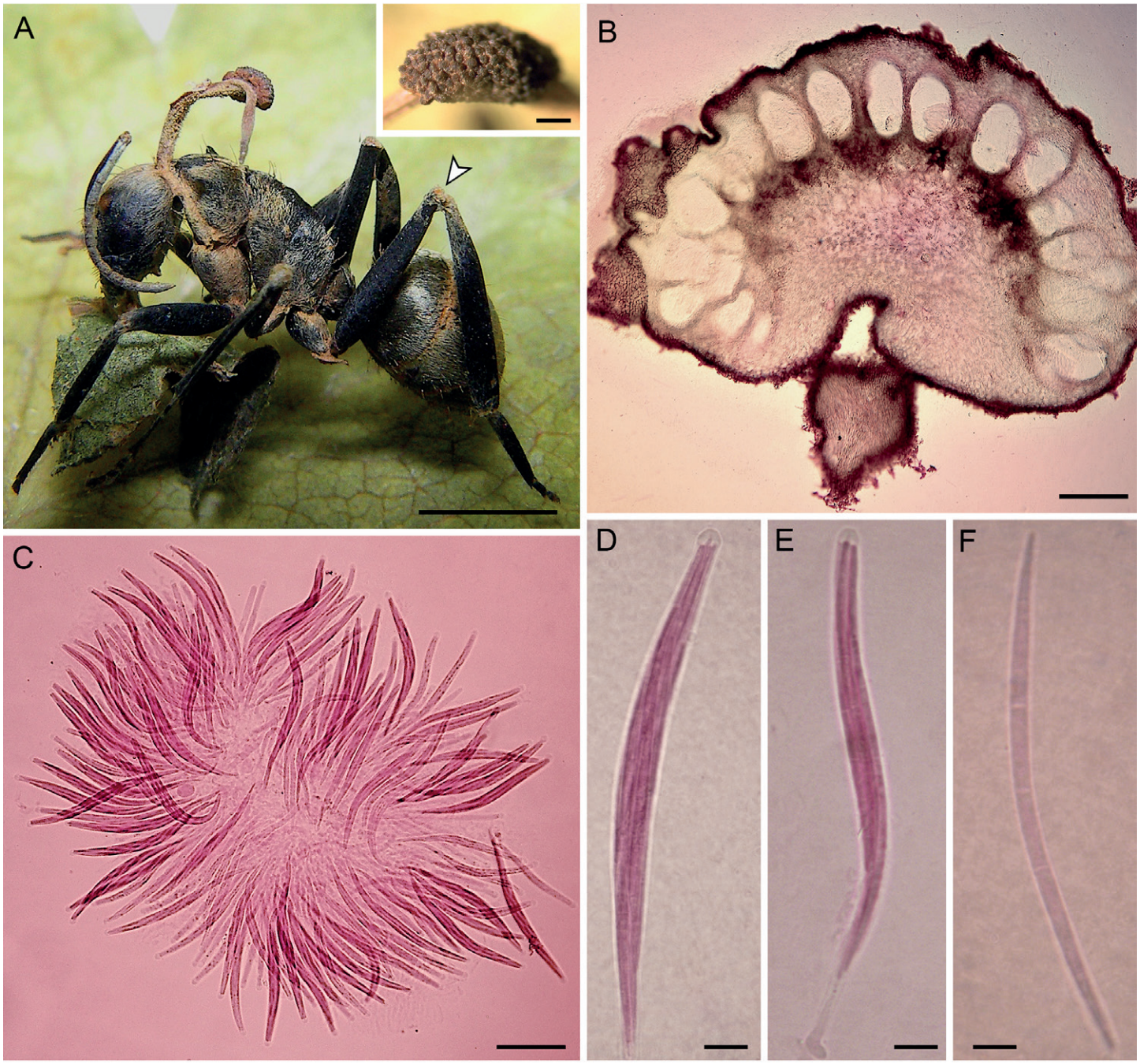

Fig. 1. *Ophiocordyceps unilateralis*, epitype (VIC 44303) on *Camponotus sericeiventris*. **A.** Golden carpenter ant biting into a leaf midrib, and the clava arising from the dorsal neck region with the unilateral ascostroma, arrow shows the sporodochium of the asexual morph (Bar = 3 mm); inset, showing details of the ascostroma (Bar = 0.8 mm). **B.** Section through the ascostroma, showing the crowded, partially erumpent ascomata (Bar = 200 µm). **C.** Asci en masse (Bar = 40 µm). **D–E.** Asci with the prominent apical cap and foot region (Bar = 10 µm). **F.** Ascospore (Bar = 8 µm).

was strongly supported (bs = 100 %). The proposed epitype – infecting *C. sericeiventris* – was strongly resolved, forming a sub-clade (bs = 75 %) with *O. camponoti-rufipedis*, which is a species native to the same geographic and ecological region as *O. unilateralis s. str.*, the Zona da Mata Mineira in the Atlantic rainforest of south-east Brazil.

DISCUSSION

Our phylogenetic results corroborate previous studies regarding the monophyly of *Ophiocordyceps unilateralis* core clade (bs = 100 %) (Araújo *et al*. 2015, 2018, Sanjuán *et al*. 2015). The clade shares numerous apomorphic traits, including: having ants of the tribe Camponotini as hosts; the ability to manipulate host behaviour resulting in biting into subaxial surfaces of leaves or twigs; producing multiple asexual morphs and; forming capillisporophores and capillispores during ascospore germination (Evans *et al*. 2011a, b). Besides the morphological evidence that characterises the epitype proposed herein, *Ophiocordyceps unilateralis s. str*., we also demonstrate that this species is unique at the molecular level. Our analysis shows that *O. unilateralis s. str*. sits within the New World clade (Fig. 3) sister to another species from the Atlantic rainforest, *O. camponoti-rufipedis* (bs = 75 %). However, within the New World subclade – composed of species from Atlantic and Amazon rainforests –

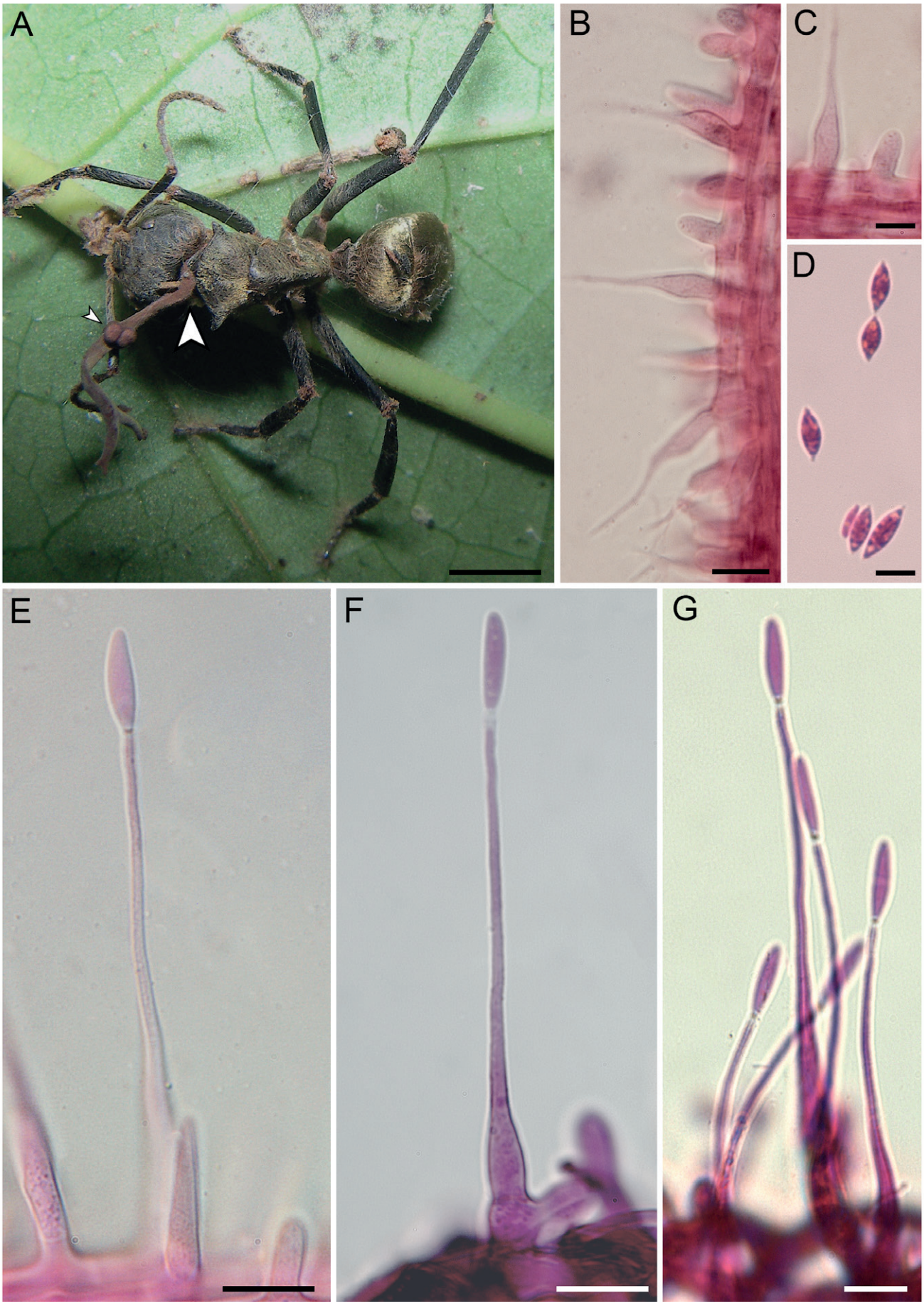

Fig. 2. Asexual morphs of *Ophiocordyceps unilateralis*, based on paratype (VIC 44350). **A.** *Camponotus sericeiventris* biting into midrib of shrub leaf, showing the clava emerging from the dorsal pronotum (large arrow) and the immature ascostromata forming laterally (small arrow) (Bar = 2.5 mm). **B–C.** Apical region of clava showing the A-phialides (Bar = 10 µm); **D**. Limoniform A-conidia (Bar = 7 µm). **E.** B-phialide from apical region of clava emerging from neck (Bar = 12 µm). **F–G.** B-phialides from sporodochium emerging from leg joint (Bars = 12 and 20 µm).

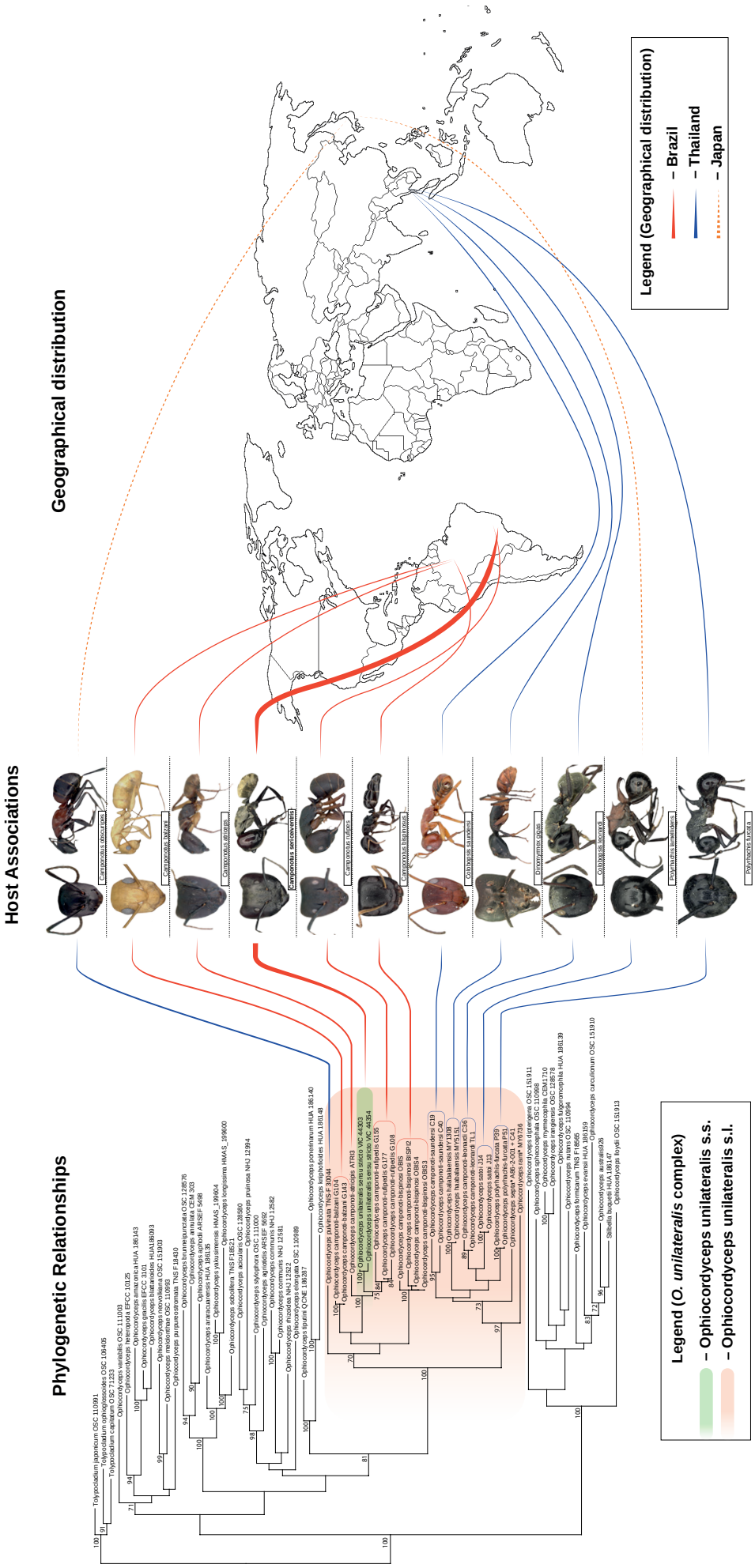

Fig. 3. Phylogeny, host association and geographic distribution of species within the *Ophiocordyceps unilateralis* complex. Phylogeny of *Ophiocordyceps* from the ML analysis obtained using RAxML to analyse a concatenated dataset of four loci (SSU, LSU, *tef1* and *rpb1*). The *O. unilateralis sensu lato* clade is highlighted in orange and the proposed epitype (*O. unilateralis sensu stricto*) line is bold. The host association and geographical distribution is also presented. Ant images from www.AntWeb.org and the photographers: April Nobile (*Camponotus obscuripes*, *Ca. balzani*, *Ca. atriceps*, *Ca. rufipes*, *Dinomyrmex gigas*), Will Ericson (*Ca. serviceventris*, *Polyrhachis lamellidens*), Estella Ortega (*Ca. bispinosus*), Michael Branstetter (*Colobopsis saundersi*), Zach Lieberman (*Co. leonardi*, *Po. furcata*).

there is no clustering of species according to the region. Further studies, including more species from different continents, are helping to resolve the relationships within this clade (Araújo *et al*. 2018).

With the selection and re-description of the epitype of *Ophiocordyceps unilateralis*, it is now possible to construct a more meaningful phylogenetic tree for the *O. unilateralis* clade. Previously, trees were constructed using a sequence of the fungus from an unidentified ant in the herbarium of the Oregon State University (OSC 128574) (Sung *et al*. 2007, Kepler *et al*. 2010, Araújo *et al*. 2015, Kobmoo *et al*. 2015). This will be critical as more new species are identified within the *O. unilateralis* complex and we begin to understand more about the intricacies of the pathogen-host relationship. None more so than within the type of *O. unilateralis* on *Camponotus sericeiventris*, in which the evidence from Honduran collections suggests that different subspecies of the ant occur within the same forest habitat and that this is reflected in different death positions of the infected ants, as well as in morphological variation within the fungal pathogen. In order to coexist, the ant subspecies must occupy different niches within this ecosystem and, therefore, the fungus may also have evolved at the subspecies level with different morphological (spore forms) and physiological (neurotoxins) traits to maximize infection.

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Fusarium oligoseptatum sp. nov., a mycosymbiont of the ambrosia beetle *Euwallacea validus* in the Eastern U.S. and typification of *F. ambrosium*

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Abstract: *Fusarium oligoseptatum* sp. nov. was isolated from the invasive Asian ambrosia beetle *Euwallacea validis* (Coleoptera, Scolytinae, Xyleborini) and from the galleries that females had constructed in dying *Ailanthus altissima* (tree-of-heaven) symptomatic for Verticillium wilt in south-central Pennsylvania, USA. This ambrosia fungus was cultivated by *Euwallacea validis* as the primary source of nutrition together with a second symbiont, *Raffaelea subfusca*. Female beetles transport their fungal symbionts within and from their natal galleries in paired pre-oral mycangia. *Fusarium oligoseptatum* was distinguished phenotypically from the 11 other known members of the Ambrosia *Fusarium* Clade (AFC) by uniquely producing mostly 1–2 septate clavate sporodochial conidia that were swollen apically. Phylogenetic analysis of multilocus DNA sequence data resolved *F. oligoseptatum* as a genealogically exclusive species-level lineage but evolutionary relationships with other members of the AFC were unresolved. Published studies have shown that *F. oligoseptatum* can be identified via phylogenetic analysis of multilocus DNA sequence data or a PCR multiplex assay employing species-specific oligonucleotide primers. In addition, to provide nomenclatural stability, an epitype was prepared from an authentic strain of *F. ambrosium* that was originally isolated from a gallery constructed in Chinese tea (*Camellia sinensis*) by *E. fornicatus* in India, together with its lectotypification based on a published illustration.

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INTRODUCTION

Ambrosia beetles (Coleoptera, Curculionidae: Scolytinae and Platypodinae) are obligate mutualistic mycetophagous insects that cultivate ambrosia fungi as a source of nutrition typically in dead but occasionally in healthy woody hosts (Hulcr & Stelinski 2017). Most ambrosia beetles studied to date carry specific symbiotic ambrosia fungi within their mycangia, which are disseminated by females when they leave their natal galleries to establish new colonies (Hulcr & Cognato 2010, Hulcr & Dunn 2011). Genera in the tribe Xyleborini (Scolytinae) are considered to be the most ecologically successful ambrosia beetles (Hulcr & Stelinski 2017). Several well-studied fungus-farming beetles, including representatives of several tribes, have recently caused significant mortality of trees. Notable examples include the invasive Asian ambrosia beetle *Xyleborus glabratus* and its nutritional symbiont *Raffaelea lauricola* on redbay (*Persea borbonia*) in the southeastern United States (Fraedrich *et al.* 2008), and *Platypus quercivorus* and its symbiont *Raffaelea quercivora* on Japanese oak (*Quercus serrata* and *Q. mongolica* var. *grosseserrata*) in Japan (Kubono & Ito 2002, Seo *et al.* 2012).

Compared to their beetle partners, relatively few fungal symbionts have been formally described. Most of the ambrosia fungi described to date are ascomycetous fungi in the *Ophiostomatales*, including members of *Afroraffaelea*, *Ceratocystiopsis*, *Dryadomyces* and *Raffaelea* (von Arx & Hennebert 1965, Upadhyay & Kendrick 1975, Gebhardt *et al.* 2005, Harrington *et al.* 2008, 2010, Alamouti *et al.* 2009, Dreaden *et al.* 2014, Bateman *et al.* 2016, Hulcr & Stelinski 2017). The *Microascales* also include multiple groups of ambrosia fungi, some of which are important and widespread: *Ambrosiella*, *Meredithiella*, and *Phialophoropsis* (Mayers *et al.* 2015). Less common are symbionts belonging to the *Polyporales* (Li *et al.* 2015, Kasson *et al.* 2016, Simmons *et al.* 2016), *Hypocreales* (i.e., *Geosmithia*) (Kolařik & Hulcr 2009, Kolařik & Kirkendall 2010), and *Saccharomycetales* (van der Walt 1972, Hulcr & Stelinski 2017).

In addition to the symbionts mentioned above, *Fusarium ambrosium* (*Hypocreales*, *Nectriaceae*) is cultivated by *Euwallacea fornicatus* (formerly *Xyleborus fornicatus*) as a source of nutrition (Gadd & Loos 1947, Norris & Baker 1967, Brayford 1987, Nirenberg 1990). The taxonomic history of *F. ambrosium*, however, is complicated because the species was originally misclassified and established in *Monacrosporium*, as *M. ambrosium*. This fungus

was isolated originally and described from galleries of the tea shot-hole borer, *E. fornicatus*, in *Camellia sinensis* (Chinese tea) and *Ricinus communis* (caster-oil tree) stems in Sri Lanka (Gadd & Loos 1947). Subsequently, *F. bugnicourtii* was described based on collections from galleries in Chinese tea in India, borer-damaged *Hevea brasiliensis* (rubber tree) and *Theoborma cacao* (cacao) in Sabah, Malaysia (Brayford 1987). Nirenberg (1990) synonymized *F. bugnicourtii* with *M. ambrosium* and recombined the latter as *F. ambrosium* based on nomenclatural priority. Brayford (1987) considered *F. bugnicourtii* to be conspecific with *F. tumidum* var. *coeruleum* (Bugnicourt 1939), but distinct from *F. tumidum*. Although the type of *F. tumidum* var. *coeruleum* based on a collection from *H. brasiliensis* appears to be phylogenetically distinct from *F. bugnicourtii*, the holotype of *F. bugnicourtii* selected by Brayford (IMI 296597 = NRRL 20438) is conspecific with *F. ambrosium* (Kasson *et al.* 2013).

Kasson *et al.* (2013) conducted an extensive multilocus molecular phylogenetic study on the ambrosial fusaria, based on isolates from beetles, their galleries, or from trees showing extensive borer damage and dieback. These included *Camellia sinensis*, *Persea americana* (avocado), *Ailanthus altissima* (tree-of-heaven), *Acer negundo* (box elder), and *Hevea brasiliensis* from natural and cultivated ecosystems, and avocado in the United States, Israel and Australia. Seven different *Fusarium* species lineages were reported to be associated with *Euwallacea* ambrosia beetles within the Ambrosia *Fusarium* Clade (AFC) and one other species (i.e., *Fusarium* sp. AF-9) with *Xyleborus ferrugineus* in Costa Rica. The monophyletic AFC is nested within Clade 3 of the *F. solani* species complex (FSSC; O'Donnell 2000), which contains 60 plus phylogenetic species based on genealogical concordance phylogenetic species recognition (GCPSR; Taylor *et al*. 2000). The AFC comprises two strongly supported clades: the four species within Clade A typically produce curved fusiform septate macroconidia, which are typical of *Fusarium*, whereas nine of the 10 species within Clade B produce clavate macroconidia (Kasson *et al*. 2013, Aoki *et al.* unpubl.), described as 'dolphin-shaped' by Brayford (1987). O'Donnell *et al.* (2015) conducted a multilocus phylogenetic analysis of the AFC and *Euwallacea* and found evidence of repeated host shifts rather than strict co-evolution of this mutualism.

Freeman *et al.* (2013) described a new species, *F. euwallaceae*, based on isolates corresponding to the ambrosia species symbiotic with the *Euwallacea* sp. #1 *sensu* O'Donnell *et al.* (2015), which causes serious damage to avocado production in Israel and California, USA (Mendel *et al.* 2012, Eskalen *et al.* 2013). *Fusarium euwallaceae* is closely related morphologically to *F. ambrosium*, but it can be distinguished from the latter by the abundant production of bluish to brownish sporodochial conidia that form greenish masses on PDA after 1 mo in culture, together with hyaline conidia. To date only three of the 16 species within the AFC have been described formally (Kasson *et al.* 2013, O'Donnell *et al.* 2015, Na *et al*. 2018). Similar to *E. validus*, *Euwallacea* sp. #1 also carries additional symbiotic fungi, *Graphium euwallaceae* and *Paracremonium pembeum* (Freeman *et al.* 2016, Lynch *et al.* 2015). Recently, PCR multiplexes were developed to discriminate *Fusarium* symbionts of invasive *Euwallacea* ambrosia beetles that inflict damage on numerous tree species throughout the United States, including *F. euwallaceae* and *F. kuroshium* along with four unnamed AFC species-level lineages: AF-3, AF-4, AF-6 and AF-8 (Short *et al.* 2017). One of the undescribed species, which was informally referred to as *Fusarium* sp. AF-4, is cultivated by the ambrosia beetle *E. validus* primarily in Verticillium wilt-stressed and dying stands of *A. altissima*, as well as from Verticillium wilt-stressed *Acer pensylvanicum* (striped maple), *Aralia spinosa* (devils walkingstick) and *Rhus typhina* (staghorn sumac) in south-central Pennsylvania, USA (Schall & Davis 2009, Kasson *et al.* 2013, 2015). In the present study, this species is described as *F. oligoseptatum sp. nov.* based on a comparison with *F. ambrosium* (AF-1) and *F. euwallaceae* (AF-2) (Kasson *et al.* 2013, Freeman *et al.* 2013). In addition, because type material for *F. ambrosium* was not designated (Gadd & Loos 1947), or appears to have been lost (Nirenberg 1990), a line-drawing of a clavate conidium of the species from Gadd & Loos (1947) was selected as the lectotype. Furthermore, an epitype was prepared from an authentic strain of this species to stabilize its taxonomy, according to the International Code of Nomenclature for algae, fungi and plants (ICN, the Melbourne Code; McNeill *et al.* 2012).

MATERIALS AND METHODS

Fungal isolates and type specimens

Fusarium strains examined in this study (Table 1) are stored in the Agriculture Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research (NCAUR), U.S. Department of Agriculture in Peoria, Illinois, USA. These strains were originally isolated from *Euwallacea* ambrosia beetles and their galleries, or from host trees showing extensive borer damage (Kasson *et al.* 2013). The Pennsylvanian strains of *F. oligoseptatum* were isolated from *E. validus* ambrosia beetles that had colonized *A. altissima*. Beetles were surface disinfested for 15 s in 70 % ethanol and then washed three times in sterile deionized water. Whole beetles or their heads were macerated using sterile Tenbroek homogenizers (Pyrex, Corning, NY), or pellet pestles (Fisher Scientific, Hampton, NH), suspensions were diluted 1:10 and 1:100, and then spread evenly over half-strength Potato Dextrose Agar (PDA, BD-Difco™, Thermo Fisher Scientific, Waltham, MA) amended with 100 ppm streptomycin sulfate (Sigma-Aldrich, St. Louis, MO) as described in Kasson *et al.* (2013). Other related ambrosia fusaria or close relatives within the *F. solani* species complex (O'Donnell *et al*. 2008) were obtained from culture collections (Table 1). Isolates used in this study are available upon request from NRRL (http://nrrl.ncaur.usda.gov/cgi-bin/usda/), NARO Genebank, Microorganisms Section (MAFF), Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan (http://www.gene.affrc.go.jp/about-micro_en.php), and the Westerdijk Institute (formerly CBS-KNAW Fungal Biodiversity Center), Utrecht, the Netherlands (http://www.westerdijkinstitute.nl/). Isolates of four novel Taiwanese AFC species discovered very recently, i.e. AF-13 to AF-16 (Na *et al*. 2018), were not included in this study.

Holotype and epitype specimens newly prepared from the selected strains were deposited in BPI, US National Fungus Collection (https://nt.ars-grin.gov/fungaldatabases/specimens/specimens.cfm).

Incidence of *Fusarium oligoseptatum* and other fungi from *Euwallacea validus* mycangia across recently confirmed tree hosts

Mycangial fungal communities were characterized as previously described by Kasson *et al.* (2013) for adult female

Table 1. Strains of Ambrosia *Fusarium* Clade (AFC) species examined in present study. Bold text is used to identify ex-holotype of *Fusarium oligoseptatum* and ex-epitype of *F. ambrosium*.

Species	AFC clade #	NRRL strain # [a]	Equivalent nos. in other collections [b]	Host beetle [c]	Host plant	Origin	Country	Date	Collector [d]	Remark
F. oligoseptatum	AF-4	62578	FRC S-2576	*Euwallacea validus*	*Ailanthus altissima*	Dauphin Co., Pennsylvania	USA	30-Jan-10	M. Kasson Bh17	Studied only phylogenetically
	AF-4	**62579**	**FRC S-2581 = MAFF 246283 = CBS 143241**	***Euwallacea validus***	***Ailanthus altissima***	**Dauphin Co., Pennsylvania**	**USA**	**30-Jan-10**	**M. Kasson Bh24**	**Ex-HOLOTYPE**
	AF-4	62580	FRC S-2594 = MAFF 246284 = CBS 143242	*Euwallacea validus*	*Ailanthus altissima*	Franklin Co., Pennsylvania	USA	9-Mar-10	M. Kasson Ch19	
	AF-4	62581	FRC S-2616 = MAFF 246285 = CBS 143243	*Euwallacea validus*	*Ailanthus altissima*	Huntington Co., Pennsylvania	USA	27-Feb-10	M. Kasson Dh24	
	AF-4	62582	FRC S-2627 = MAFF 246286 = CBS 143244	*Euwallacea validus*	*Ailanthus altissima*	Mifflin Co., Pennsylvania	USA	1-Jul-09	M. Kasson Eh11	Degenerated strain
F. ambrosium	AF-1	20438	IMI 296597 = MAFF 246291	*Euwallacea fornicatus*	*Camellia sinensis*	Chinchona, Maharashtra	India	17-Jul-85	anonymous	Ex-holotype of *F. bugnicourtii*
	AF-1	22345	BBA 65389 = MAFF 246288	*Euwallacea fornicatus*	*Camellia sinensis*	Upari Tea Institute	India	9-May-90	V. Agnihothrudu	
	AF-1	**22346**	**BBA 65390 = CBS 571.94 = MAFF 246287**	***Euwallacea fornicatus***	***Camellia sinensis***	**Upari Tea Institute**	**India**	**9-May-90**	**V. Agnihothrudu**	**Ex-EPITYPE**
	AF-1	36510	BBA 65390 = MAFF 246289	*Euwallacea fornicatus*	*Camellia sinensis*	Upari Tea Institute	India	9-May-90	V. Agnihothrudu	Duplicate of NRRL 22346
	AF-1	46583	IMI 339338 = MAFF 246290	*Euwallacea fornicatus*	*Camellia sinensis*	High Forest Estate, Anamallais, Coimbatore District, Tamil Nadu	India	26-Mar-90	anonymous	Received as *F. bugnicourtii*

Table 1. (Continued).

Species	AFC clade #	NRRL strain # [a]	Equivalent nos. in other collections [b]	Host beetle [c]	Host plant	Origin	Country	Date	Collector [d]	Remark
	AF-1	62605		*Euwallacea fornicatus*	*Camellia sinensis*	Tea Research Institute of Sri Lanka, St. Coombs, Talawakelle	Sri Lanka	Mar-12	S. Freeman 31.14	
F. euwallaceae	AF-2	54727	MAFF 243816 = CBS 135859	*Euwallacea* sp. #1	*Persea americana*	Volcani	Israel	17-Feb-10	S. Freeman 5-4	
	AF-2	62626		*Euwallacea* sp. #1	*Persea americana*	California	USA		A. Eskalen 1854	
Fusarium sp.	AF-3	62606		*Euwallacea interjectus*	*Acer negundo*	Gainesville, Florida	USA		J.A. Smith PL1499	
	AF-3	62628		*Euwallacea interjectus*	*Acer negundo*	Gainesville, Florida	USA		J.A. Smith 1190	
	AF-5	22231	IMI 110107	unknown	*Hevea brasiliensis*	Agriculture Research Centre Tuaran, Sabah, Borneo	Malaysia	19-Nov-64		Received as *F. bugnicourtii*
	AF-5	46518	FRC S-2075	unknown	*Hevea brasiliensis*		Malaysia			
	AF-6	62590		*Euwallacea* sp. #2	*Persea americana*	Miami, Florida	USA		R.C. Ploetz AF9	
	AF-6	62591		*Euwallacea* sp. #2	*Persea americana*	Miami, Florida	USA		R.C. Ploetz AF10	
	AF-7	62610		*Euwallacea* sp. #3	*Persea americana*	Queensland	Australia		A.D.W. Geering 1	
	AF-7	62611		*Euwallacea* sp. #3	*Persea americana*	Queensland	Australia		A.D.W. Geering 2	
	AF-8	62584		*Euwallacea* sp. #2	*Persea americana*	Miami, Florida	USA		R.C. Ploetz Amb2	
	AF-8	62585		*Euwallacea* sp. #2	*Persea americana*	Miami, Florida	USA		R.C. Ploetz AF4	
	AF-9	22643	ATCC 44215	*Xyleborus ferrigineus*			Costa Rica		E.B. Smalley	
	AF-9	66088		unknown	*Delonix regia*	Florida	USA			
	AF-10	62941	IMI 351954	unknown			Singapore			
	AF-11	62943		*Euwallacea* sp. #4	*Camellia sinensis*		Sri Lanka		P. Liyanage	
	AF-11	62944		*Euwallacea* sp. #4	*Camellia sinensis*		Sri Lanka		P. Liyanage	
F. kuroshium	AF-12	62945		*Euwallacea* sp. #5	*Platanus racemosa*	San Diego, CA	USA		A. Eskalen	
	AF-12	62946		*Euwallacea* sp. #5	*Platanus racemosa*	San Diego, CA	USA		A. Eskalen	

Table 1. (Continued).

Species	AFC clade #	NRRL strain #[a]	Equivalent nos. in other collections[b]	Host beetle [c]	Host plant	Origin	Country	Date	Collector[d]	Remark

[a] NRRL: ARS Culture Collection, NCAUR-ARS-USDA, Peoria, IL, USA.

[b] ATCC: American Type Culture Collection, Manassas, VA, USA; BBA: Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Mikrobiologie (currently Julius-Kühn-Institut), Berlin, Germany; CBS: Westerdijk Institute (formerly CBS-KNAW Fungal Biodiversity Center), Utrecht, the Netherlands; FRC: Fusarium Research Center, The Pennsylvania State University, State College, PA, USA; IMI: CABI Biosciences, UK Centre, Egham, Surrey, UK; MAFF: NARO Genebank, Microorganisms Section, Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan.

[c] The five unnamed *Euwallacea* spp. are distinguished by #1–#5 (O'Donnell *et al*. 2015). AF-12 was later described as *F. kuroshium* (Na *et al*. 2018).

[d] V. Agnihothrudu, Upari Tea Institute, India; A. Eskalen, Department of Plant Pathology and Microbiology, University of California, Riverside, CA, USA; S. Freeman, Department of Plant Pathology and Weed Research, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel; A. Geering, University of Queensland, Brisbane, Australia; M. Kasson, Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA; R. C. Ploetz, University of Florida, Homestead, FL, USA; J. A. Smith, University of Florida, Gainesville, FL, USA.

beetles that harbor paired pre-oral mycangia. In addition to *F. oligoseptatum*, described in this paper, previous studies revealed that *E. validus* harbors a second less abundant symbiont, *Raffaelea subfusca* (Kasson *et al.* 2013). To determine if these trends held across other tree hosts and geographic locations, *E. validus* from *Ailanthus* and 16 other confirmed tree hosts across seven states were sampled. When available, a minimum of 10 adult females were included. Log-extracted females were processed as previously described (Kasson *et al.* 2013). Following serial dilution plating of head macerates, individual fungal colony forming units (CFUs) were quantified by morphotype and representatives of each morphotype retained for molecular characterization using the ITS barcoding gene. Unlike the other fungal morphotypes, representative fusaria were subjected to an AF-3 / *Fusarium oligoseptatum* (AF-4) multiplex PCR recently developed by Short *et al.* (2017) to discriminate known *Fusarium* symbionts of *E. interjectus* and *E. validus* in the eastern U.S.

When comparing the CFUs recovered from individual beetle heads between the primary symbionts of *E. validus*, *F. oligoseptatum* and *R. subfusca*, a chi-squared test was performed across all tree species. To examine if there were differences in the relative amount of colony forming units (CFUs) recovered from individual beetle heads between the primary symbionts of *E. validus*, *F. oligoseptatum* and *R. subfusca* within individual species, a second chi-squared test was performed for each individual species. Results of the tests were deemed significant if $p < 0.05$.

Molecular systematics and biology

Methods for culturing mycelium, DNA extraction, PCR amplification, DNA sequencing and phylogenetic analyses followed published protocols (Kasson *et al.* 2013, O'Donnell *et al*. 2015). DNA sequence data included in this study were deposited in GenBank as JQ038007–JQ038034.

Phenotypic characterization

Strains were grown on PDA and synthetic low-nutrient agar (SNA; Nirenberg 1990, Nirenberg & O'Donnell 1998) in the dark, under continuous black light (Black light blue fluorescent tubes, FL8BL-B 8W/08, Panasonic, Osaka, Japan), or under an ambient daylight photoperiod. Strains were cultured on PDA in 9 cm Petri dishes at 20 °C in the dark to characterize colony color, odor and morphology. Kornerup & Wanscher (1978) was used as the color standard. PDA cultures were also used for determining mycelial growth rates in the dark at eight temperatures (5–40 °C) at 5 °C increments (Aoki *et al.* 2015). Culture plates were examined at 1 and 4 d post inoculation, and radial growth was calculated as arithmetic mean values per day by measuring 16 radii around the colony. Measurements of growth rate at different temperatures were replicated twice, and the data averaged for each strain. Cultures on SNA were used for examination of microscopic characters as described by Aoki *et al.* (2015). Conidia and conidiophores were examined in water mounts after culturing on SNA under continuous black light. Phenotypic characters were compared with data from the related AFC species, *F. ambrosium* (published as *Monacrosporium ambrosium*; Gadd & Loos 1947), *F. bugnicourtii* (synonymized as *F. ambrosium*; Brayford 1987, Nirenberg 1990), and *F. euwallaceae* (Freeman

et al. 2013). To compare the number of conidial septa in strains of *F. oligoseptatum* and *F. ambrosium*, they were incubated on SNA at 25 °C under continuous black light for one to two weeks and the number septa in the clavate sporodochial conidia were counted.

RESULTS

Incidence of *Fusarium oligoseptatum* and other fungi from *Euwallacea validus* mycangia across recently confirmed tree hosts

Mycangial communities were characterized from adult female beetles extracted from sixteen native host trees and *Ailanthus* (Fig. 1). Overall, *F. oligoseptatum* and *Raffaelea subfusca* comprised 84 % of all fungal CFUs from female heads across all plant hosts with *F. oligoseptatum* yielding significantly more CFU's (10 992) compared to *R. subfusca* (7 014; $p < 0.0001$). The remainder included miscellaneous yeasts and other fungi including *Paracremonium* sp. and a putatively novel *Graphium* sp. (Freeman *et al.* 2016, Lynch *et al.* 2016), as well as a variety of singleton taxa that were not further characterized. Incidence of the two primary symbionts from the heads of female *E. validus* was compared across and within plant hosts. Overall, significant differences were detected across hosts indicating that the relative proportion of the two symbionts varied across hosts with a majority of beetles from a majority of plant hosts yielding higher counts of *F. oligoseptatum* (Fig. 1). Of these, beetles from 11 of the 16 plant hosts, including *Ailanthus*, had significantly higher total CFU counts of *F. oligoseptatum* compared to *R. subfusca*. Only five species had a mean percent incidence of *F. oligoseptatum* below 50 %: *Fraxinus americana* (white ash), *Pinus virginiana* (Virginia pine), *Populus grandidentata* (bigtooth aspen), *Quercus montana* (chestnut oak), and *Amelanchier arborea* (serviceberry) (Fig. 1). Of these, white ash, Virginia pine, and chestnut oak had significantly higher total CFU counts of *R. subfusca* compared to *F. oligoseptatum* (Fig. 1).

Molecular phylogenetics

A 4914 bp 31-taxon 4-locus dataset was constructed that included the internal transcribed spacer region and domains D1 and D2 of the nuclear ribosomal large subunit (ITS+LSU rDNA: 1004 bp alignment, 26 parsimony informative characters (PIC)), and portions of translation elongation factor 1-α (*TEF1*: 687 bp alignment, 53 PIC), DNA-directed RNA polymerase II largest (*RPB1*: 1588 bp alignment, 164 PIC) and second largest subunit (*RPB2*: 1635 bp alignment, 165 PIC) from 12 AFC species. Molecular phylogenetic analyses were conducted using maximum parsimony (MP) with PAUP v. 4.0b10 (Swofford 2003) and maximum likelihood (ML) with GARLI 2.01 (Zwickl 2006). Sequences of *Fusarium neocosmosporiellum* (≡ *Neocosmospora vasinfecta*; Geiser *et al.* 2013) NRRL 22468 and 43467 were used to root the phylogenies based on more inclusive analyses (O'Donnell *et al.* 2013). *Fusarium oligoseptatum* (AF-4) was poorly supported (MP and ML bootstrap = 63–56 %) as sister to a clade that included *F. euwallaceae* (AF-2) from Israel and California and *Fusarium* sp. (AF-3) from Florida. With the exception of *F. ambrosium*, the other AFC species represented by two or more strains received moderate to strong monophyly bootstrap support. The putative triparential hybrid strain *F. ambrosium* NRRL 62605 from Sri Lanka (Kasson *et al*. 2013), however, did not form a genealogically exclusive group with *F. ambrosium* from India. As reflected by poor bootstrap support along the backbone on the phylogeny, relationships among the species were generally unresolved (Fig. 2). The analyses did support monophyly of the AFC and the early diverging subclades designated Clade A and B (Fig. 2).

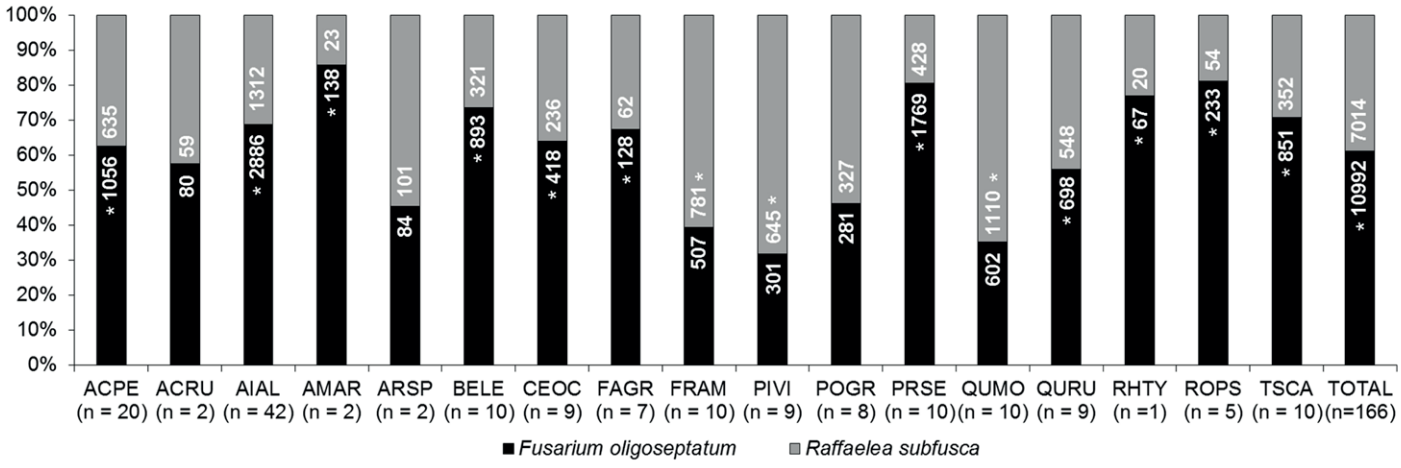

Fig. 1. Total number of *F. oligoseptatum* and *Raffaelea subfusca* CFUs recovered from macerated heads of adult female *E. validus* from 17 tree hosts. A significant difference between the two fungal CFUs within a specific host is indicated with an asterisk on the side corresponding to the higher count. Host plant IDs are based on USDA PLANTS Database (https://plants.usda.gov/java/) abbreviations, which are derived from the first two letters of the genus and species of the Latin binomial. Abbreviations are as follows: ACPE: *Acer pensylvanicum*, ACRU: *Acer rubrum*, AIAL: *Ailanthus altissima*, AMAR: *Amelanchier arborea*, ARSP: *Aralia spinosa*, BELE: *Betula lenta*, CEOC: *Celtis occidentalis*, FAGR: *Fagus grandifolia*, FRAM: *Fraxinus americana*, PIVI: *Pinus virginiana*, POGR: *Populus grandidentata*, PRSE: *Prunus serotina*, QUMO: *Quercus montana*, QURU: *Quercus rubra*, RHTY: *Rhus typhina*, ROPS: *Robinia pseudoacacia*, and TSCA: *Tsuga canadensis*.

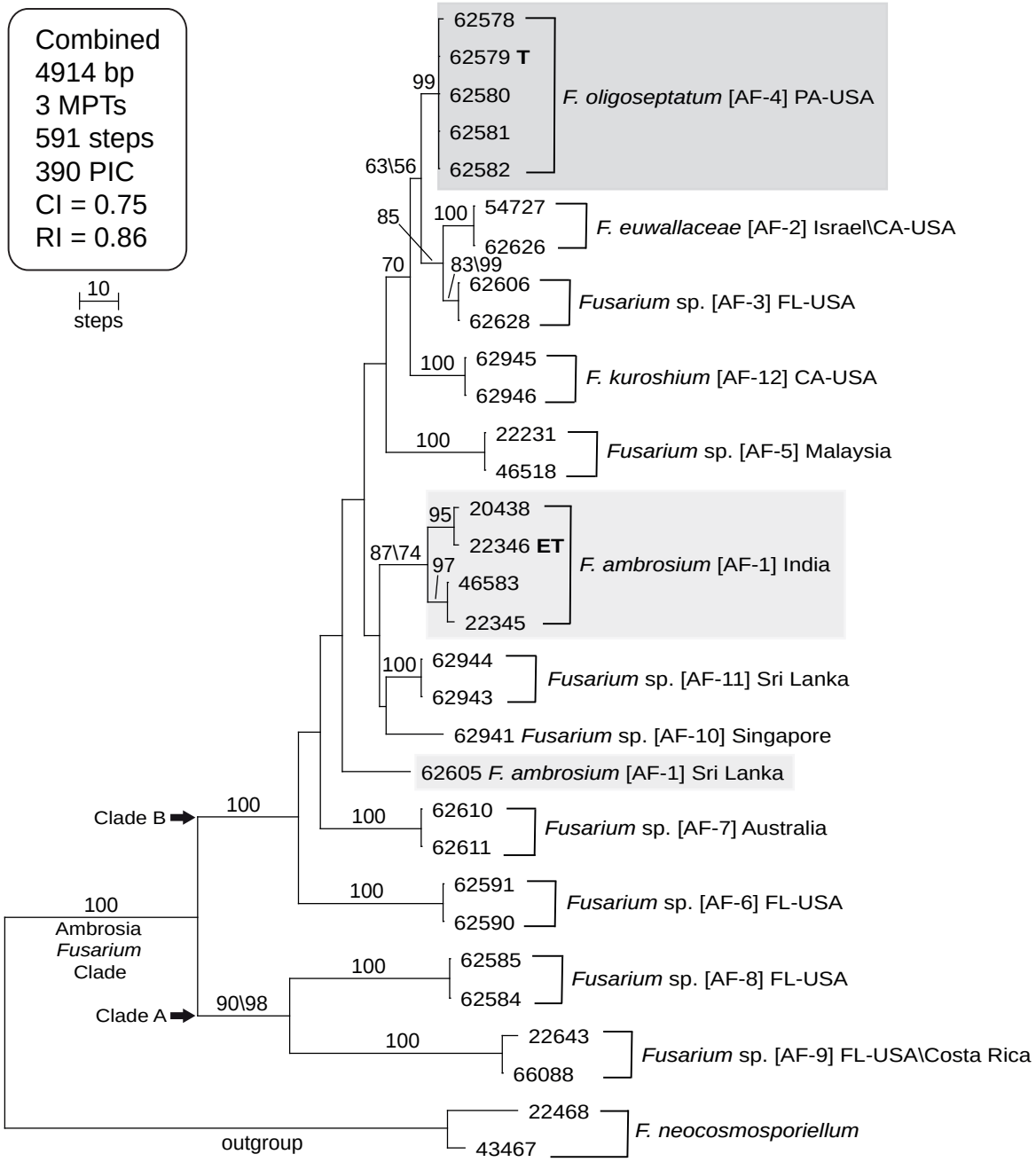

Fig. 2. One of three most-parsimonious trees (MPTs) inferred from a combined 4-gene dataset (ITS + 28S rDNA, *RPB1*, *RPB2* and *TEF1*) comprising 4914 bp of aligned DNA sequence data. The phylogram was rooted on sequences of *Fusarium neocosmosporiellum* NRRL 22468 and 43367 based on more inclusive analyses (O'Donnell *et al*. 2013). The 12 species within the Ambrosia *Fusarium* Clade (AFC) are identified as AF-1 through AF-12 using an ad hoc nomenclature (Kasson *et al*. 2013). Two early diverging monophyletic sister clades are identified as Clade A and B. Numbers above nodes represent maximum parsimony (MP) and maximum likelihood (ML) bootstrap based on 1000 pseudoreplicates of the data (MP-BS\ML-BS). The ML-BS value is only shown when it differed by ≥5 % of the MP-BS value. Note that 10 of the 11 AFC species with two or more strains received strong monophyly bootstrap support. However, *Fusarium ambrosium* highlighted in light gray was resolved as non-monophyletic because the interspecific hybrid strain NRRL 62605 from Sri Lanka did not group with the other strains of this species. The five strains of *F. oligoseptatum*, formally described herein, are identified using dark gray highlight. CI, consistency index; ET, ex-epitype; PIC, parsimony informative characters; RI, retention index; T, ex-holotype.

TAXONOMY

Fusarium oligoseptatum T. Aoki, M.T. Kasson, S. Freeman, Geiser & O'Donnell, ***sp. nov.*** MycoBank MB822305. Figs 3–5.

Etymology: oligo- + septatum, based on frequent production of sporodochial conidia with only 1–2 septa.

Diagnosis: Distinguished from *F. ambrosium* and *F. euwallaceae* by three times as many 0–2-septate sporodochial conidia (i.e., ≥75 % compared with values less than 25% in *F. ambrosium* and *F. euwallaceae*).

Type: **USA**, *Pennsylvania:* Dauphin Co., a dried specimen from a culture of NRRL 62579, isolated from a live female ambrosia beetle, *Euwallacea validus*, extracted from a gallery in a tree-

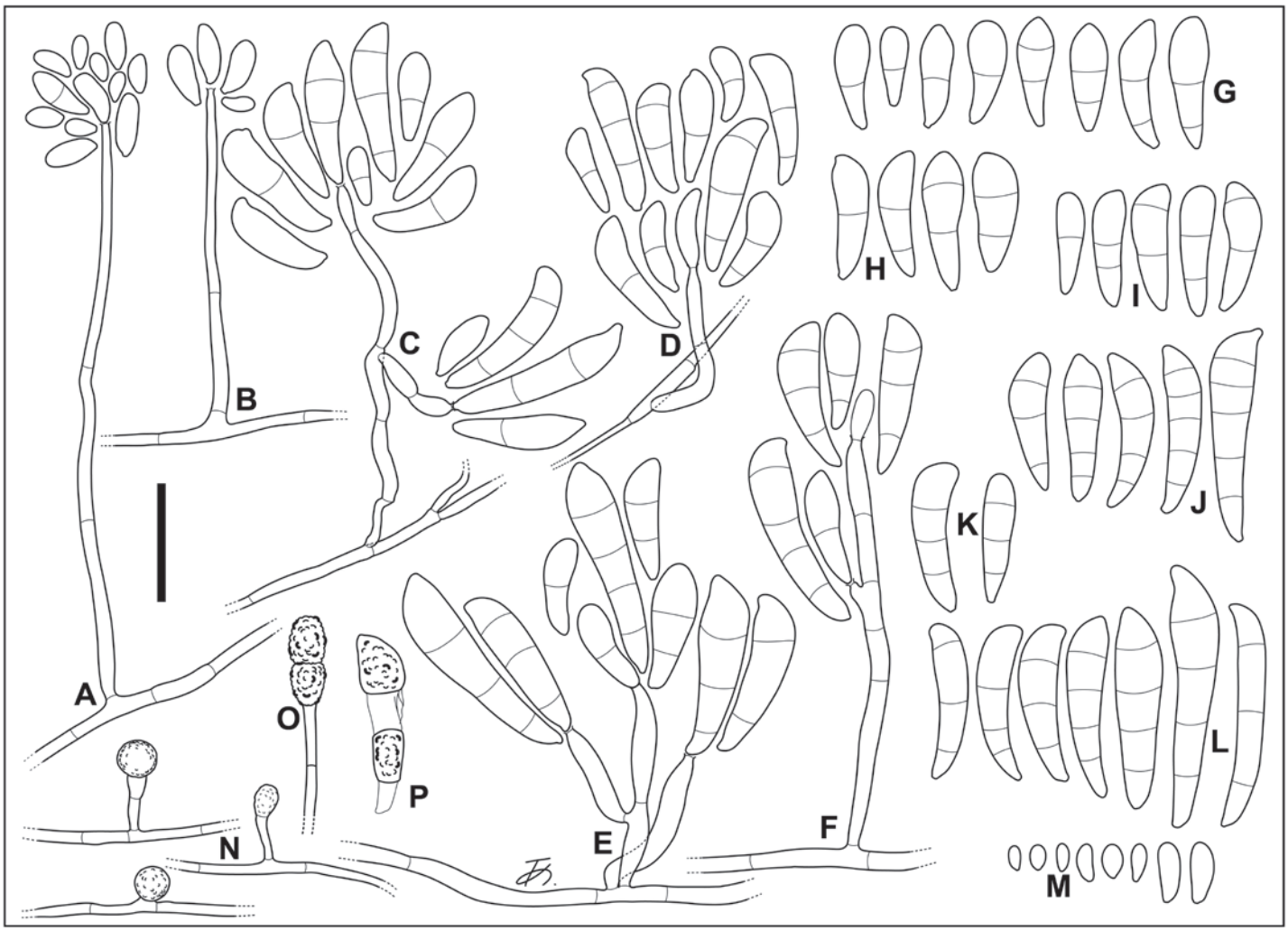

Fig. 3. *Fusarium oligoseptatum* cultured on SNA under black light. **A, B.** Tall and short aerial conidiophores forming mostly 0-septate conidia. **C, D.** Short aerial conidiophores forming conidia with relatively few septa. **E, F.** Sporodochial conidiophores forming clavate conidia with different septation. **G–L.** Septate conidia formed on sporodochial conidiophores, with some large clavate conidia (G–I: Short clavate conidia with 1–2 septa). **M.** 0-septate conidia formed on aerial conidiophores. **N–P.** Chlamydospores in hyphae (N, O) and in conidia (P). A–D, G, J, N from NRRL 62579 (ex-holotype); E, H, L, P from NRRL 62580; F, I, K, M, O from NRRL 62581. Bar = 25 µm.

of-heaven, *Ailanthus altissima*, 30 Jan. 2010, *Matthew T. Kasson (Kasson Bh24)* (BPI 910525 – holotype, designated in this study; NRRL 62579 = FRC S-2581 = MAFF 246283 = CBS 143241 – ex-holotype cultures).

Additional strains examined: **USA**: *Pennsylvania*: Franklin Co., isolated from a live *E. validus* female infesting an *A. altissima* tree, 9 Mar. 2010, *Matthew T. Kasson (Kasson Ch19)* (NRRL 62580 = FRC S-2594 = MAFF 246284 = CBS 143242); *Pennsylvania:* Huntingdon Co., isolated from a live *E. validus* female infesting an *A. altissima* tree, 27 Feb. 2010, *Matthew T. Kasson (Kasson Dh24)* (NRRL 62581 = FRC S-2616 = MAFF 246285 = CBS 143243; *Pennsylvania:* Mifflin Co., isolated from a live *E. validus* female infesting an *A. altissima* tree, 1 July 2009, *Matthew T. Kasson (Kasson Eh11)* (NRRL 62582 = FRC S-2627 = MAFF 246286 = CBS 143244; morphologically degenerated strain).

Description: *Colonies* on PDA showing radial mycelial growth rates of 2.2–3.6 mm/d at 20 °C and 3.3–4.6 mm/d at 25 °C in the dark. Colony color on PDA white (1A1) to yellowish-white (4A2) or orange white (5A2) in the dark, white (1A1) to yellowish-white (3–4A2) or pale yellow (3–4A3) under black light. Aerial mycelium white (1A1), sparsely formed or floccose in the dark, more abundantly formed and covering entire surface of colonies under black light. Colony margin entire to undulate. Reverse pigmentation absent or yellowish-white (3–4A2) or pale yellow (3–4A3) in the dark and under black light. *Exudates* absent. *Odor* absent, or slightly moldy or sweet in some strains. *Hyphae* on SNA 1.5–7.5 µm wide. *Chlamydospores* present but formation delayed in hyphae and in septate sporodochial conidia, mostly subglobose to round ellipsoidal, intercalary or terminal, mostly single, sometimes in chains, ordinary hyaline to very pale-yellow, wall smooth or often minutely roughened, 6–23.5 × 4.5–9 µm. *Sclerotia* absent. *Sporulation* on SNA and PDA generally rapid and abundant under black light, delayed in the dark, sometimes less sporulation on PDA in the dark; light-colored on SNA and PDA under black light or under daylight; sporodochia formed sparsely on SNA, rare on PDA. *Aerial conidiophores* formed abundantly on SNA under black light, less frequently in the dark, erect, short or tall and narrow, mostly unbranched, rarely branched sparsely, up to 130 µm long, 3–5.5 µm wide at base, thin-walled, forming monophialides integrated in the apices. *Phialides* on aerial conidiophores simple, subcylindrical to subulate, tapering towards apex, often with a minute collarette at the tip, 10–62.5 × 2.5–5.5 µm. *Aerial conidia* mostly (1) elliptical, oblong-elliptical,

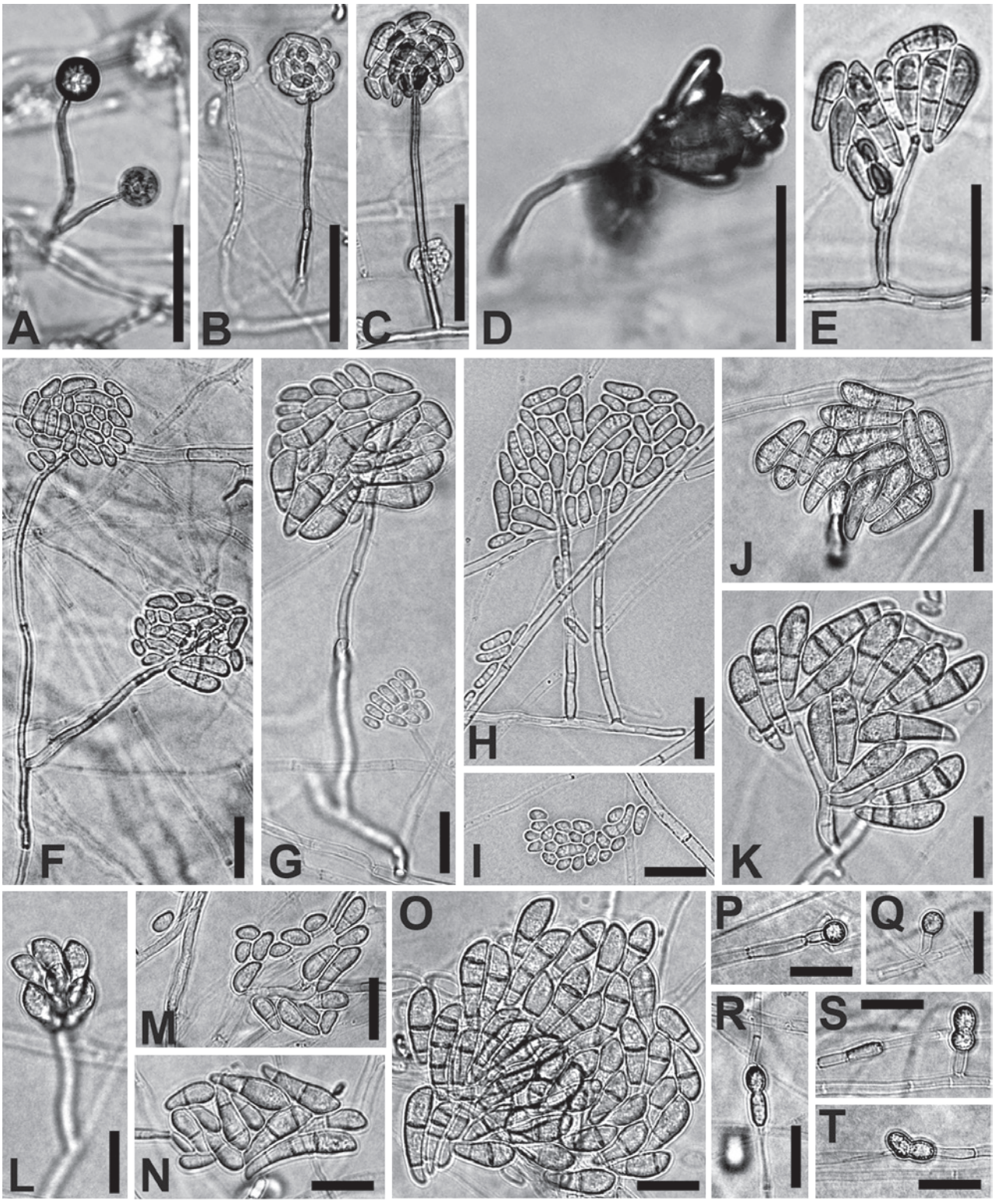

Fig. 4. *Fusarium oligoseptatum* cultured on SNA under black light. **A–C, F–H.** Aerial conidiophores forming 0–1(–2)-septate conidia. **D, E, J–L.** Sporodochial conidiophores forming clavate conidia, often swollen apically with 1–4 septa. **I.** Round to obovate 0-septate aerial conidia. **M–O.** Mostly clavate conidia formed on sporodochial conidiophores, including some 0-septate that are oblong or short-clavate. **P–T.** Chlamydospores formed in hyphae. A–I, K, M, N, Q–S from NRRL 62579 (ex-holotype); J, O, P, T from NRRL 62580; L from NRRL 62581. (A–E: Aerial view without a cover slip; F–T: Mounted in water with a cover slip). Bars: A–E = 50 µm, F–T = 20 µm.

fusiform-elliptical to short clavate, occasionally reniform, some obovate to subglobose, 0–1(–2)-septate; 0-septate on SNA in the dark: 3–13 × 2–5.5 µm in total range, 5.3–8.5 × 2.8–3.9 µm on average [ex type (NRRL 62579): 3.5–12 × 2–4 µm in total range, 6.9±2.0 × 2.8±0.5 µm on average ± S.D.]; 0-septate on SNA under black light: 3–17 × 2–6.5 µm in total range, 6.0–9.0 × 2.8–3.8 µm on average [ex type (NRRL 62579): 4–17 × 2.5–6.5 µm in total range, 9.0±2.8 × 3.8±0.9 µm on average ± S.D.]; 1-septate on SNA under black light: 7.5–26 × 2.5–8 µm in total range, 14.5–15.3 × 4.6–4.9 µm on average [ex type: 10.5–21.5 × 2.5–6 µm in total range, 15.1±2.7 × 4.6±0.7 µm on average ± S.D.]; sometimes with (2) larger, falcate to clavate, or curved cylindrical,

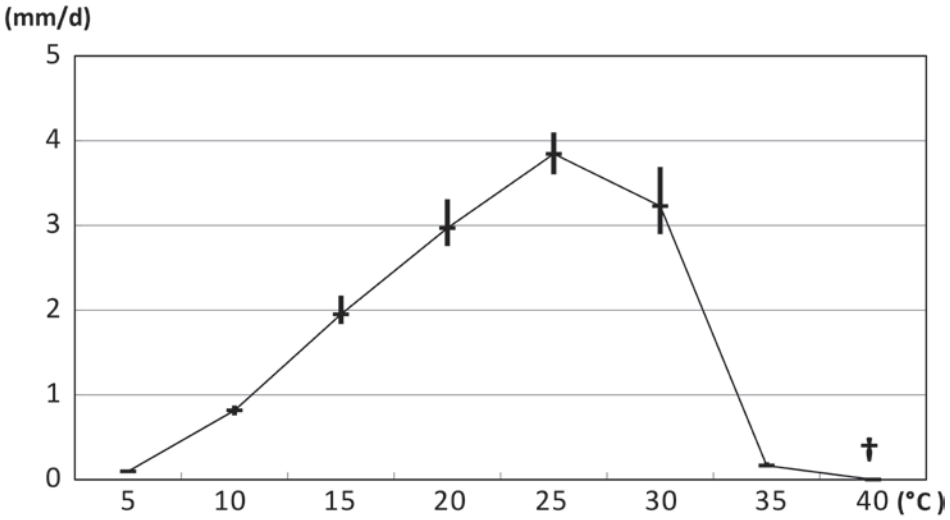

Fig. 5. Radial mycelial growth rate of *Fusarium oligoseptatum* per day on PDA cultured at eight different temperatures. Thick horizontal and vertical bars indicate means and total ranges, respectively, of the 4 isolates analyzed. All isolates failed to grow and died at 40 °C.

(1–)2(–3)-septate conidia, morphologically continuous with falcate sporodochial conidia. *Sporodochial conidiophores* generally shorter and thicker than aerial conidiophores, unbranched or sometimes sparsely branched, contorted, forming monophialides integrated apically, 20–145 × 3–6 µm, or sometimes adelophialides. *Sporodochial phialides* simple, subulate, lanceolate or subcylindrical, often with a conspicuous collarette at the tip, 9.5–44 × 2.5–5.5 µm. *Sporodochial conidia* hyaline, mostly falcate to long clavate, sometimes curved cylindrical, often swollen slightly or conspicuously in their upper part, tapering towards base, often with a rounded or papillate apical cell, and an indistinct foot-like or rounded basal cell, (0–)1–3(–5)-septate; swollen conidia sometimes 'dolphin-like' (Brayford 1987) or comma-shaped when 1- to 2-septate, formed on SNA frequently under black light, less frequently in the dark, very rarely formed on PDA under black light; 1-septate on SNA under black light: 11–32.5 × 4–10 µm in total range, 18.2–20.1 × 6.1–7.2 µm on average [ex type: 13.5–30 × 4.5–8.5 µm in total range, 19.3±3.9 × 6.2±1.0 µm on average ± S.D.]; 2-septate on SNA under black light: 15.5–39.5 × 5.5–12 µm in total range, 24.8–26.5 × 7.4–8.0 µm on average [ex type: 22–34 × 5.5–12 µm in total range, 26.1±3.2 × 7.4±1.2 µm on average ± S.D.]; 3-septate on SNA under black light: 20–60 × 5.5–12.5 µm in total range, 31.4–35.7 × 8.3–8.7 µm on average [ex type: 23–60 × 5.5–12.5 µm in total range, 35.7±7.2 × 8.7±1.3 µm on average ± S.D.]; 4-septate on SNA under black light: 28.5–67.5 × 7–11 µm in total range, 38.7–47.9 × 8.8–8.9 µm on average [ex type: 28.5–67.5 × 7–10 µm in total range, 47.9 × 8.9 µm on average]. Together with multiseptate sporodochial conidia, often forming (0-)1(-2)-septate, oblong to naviculate or short-clavate, straight or curved conidia with a rounded apex and truncate base.

Clade-based diagnosis: Distinguished by phylogenetic analysis of multilocus DNA sequence data (Kasson *et al*. 2013, O'Donnell *et al*. 2015).

Substrates or hosts: All ex-holotype and authentic strains were isolated from *E. validus* in the galleries of *A. altissima* in Pennsylvania (PA), USA. *Fusarium oligoseptatum* has also been confirmed using multilocus sequence typing from Ohio (OH), Virginia (VA) and Maryland (MD) (O'Donnell *et al*. 2015), and from Tennessee (TN) and (West Virginia) WV using the AF-3 / *F. oligoseptatum* (AF-4) multiplex PCR assay (Short *et al.* 2017). Currently known from 16 additional plant hosts, all of which have been confirmed molecularly as *F. oligoseptatum* using AF-3 / *F. oligoseptatum* (AF-4) multiplex PCR (Short et al. 2017): *Acer pensylvanicum* (PA, USA), *Acer rubrum* (PA, USA), *Amelanchier arborea* (VA, USA), *Aralia spinosa* (PA, USA), *Betula lenta* (PA, USA), *Celtis occidentalis* (WV, USA), *Fagus grandifolia* (OH, USA), *Fraxinus americana* (WV, USA), *Populus grandidentata* (PA, USA), *Prunus serotina* (GA, USA), *Quercus montana* (PA, USA), *Quercus rubra* (PA, USA), *Rhus typhina* (PA, USA), *Robinia pseudoacacia* (PA, USA), *Tsuga canadensis* (OH, USA), and *Pinus virginiana* (VA, USA).

Distribution: Presently confirmed from GA (Georgia), MD, OH, PA, TN, VA, and WV, USA.

Notes: Morphological data on sporodochial conidia was based mainly on NRRL 62579, 62580 and 62581. Strain NRRL 62582 appears degenerated and produced only 1-septate sporodochial conidia after 1 mo on SNA under continuous black light. Strains of this species were all isolated from female *E. validus* ambrosia beetles infesting *A. altissima* that were collected in different counties in Pennsylvania, USA. The most distinctive morphological feature of this fungus is the frequent production of sporodochial conidia with 1–2 septa (Table 2, Figs. 3E–I, 4E, J–O). This species formed sporodochial conidia with more than two septa, but the percentage of 0–2-septate conidia (76.5–81 %) was much higher than observed in *F. ambrosium* (3.7–24.5 %) and *F. euwallaceae* (Freeman *et al.* 2013), where more than 75 % of the conidia were 3–5-septate. Cultures appear whitish to yellowish-white when aerial mycelium is sparse on PDA.

Fusarium ambrosium (Gadd & Loos) Agnihothr. & Nirenberg, *Stud. Mycol.* **32**: 98. 1990. MycoBank MB130225. Figs 6–8.
Basionym: *Monacrosporium ambrosium* Gadd & Loos, *Trans. Brit. Mycol. Soc.* **31**(1 & 2): 13. 1947. MB288427.
Synonyms: *Dactylella ambrosia* (Gadd & Loos) K.Q. Zhang, Xing Z. Liu & L. Cao, *Mycosystema* **7**: 112. 1995. MB447506.
Neocosmospora ambrosia (Gadd & Loos) L. Lombard & Crous, *Stud. Mycol.* **80**: 227. 2015. MB810957.
Fusarium bugnicourtii Brayford, *Trans. Brit. Mycol. Soc.* **89** (3): 350. 1987. MB133337.

Type: **India**, Upari Tea Institute, a dried specimen from culture of NRRL 22346, isolated from a gallery of *Euwallacea fornicatus* infesting a tea tree, *Camellia sinensis*, 9 May 1990, *V. Agnihothrudu*

Table 2. Percentage of clavate sporodochial conidia of *Fusarium oligoseptatum* and *F. ambrosium* with different numbers of septa cultured on SNA under black light at 25 °C.

	Percentage of conidia with different numbers of septa								
Species/strain	**0-septate**	**1-septate**	**2-septate**	**3-septate**	**4-septate**	**5-septate**	**6-septate**	**7-septate**	**Total number of conidia counted**
F. oligoseptatum[a]									
NRRL 62579 (ex-holotype)	**9.5**	**41.0**	**30.5**	**15.2**	**1.9**	**1.9**	**0**	**0**	**105**
NRRL 62580	12.1	46.8	20.6	16.8	3.7	0	0	0	107
NRRL 62581	3.8	47.1	25.5	18.9	3.8	0.9	0	0	106
F. ambrosium									
NRRL 20438	0	3.4	5.0	44.5	38.7	6.7	1.7	0	119
NRRL 22345	0	5.9	7.8	25.5	23.5	29.5	4.9	2.9	102
NRRL 22346 (ex-epitype)	**0**	**0**	**3.7**	**40.2**	**46.8**	**8.4**	**0.9**	**0**	**107**
NRRL 36510	0	0.9	12.3	30.2	43.4	13.2	0	0	106
NRRL 46583	0	4.4	5.3	28.1	44.6	12.3	5.3	0	114
NRRL 62605	1.0	4.9	18.6	52.0	18.6	4.9	0	0	102

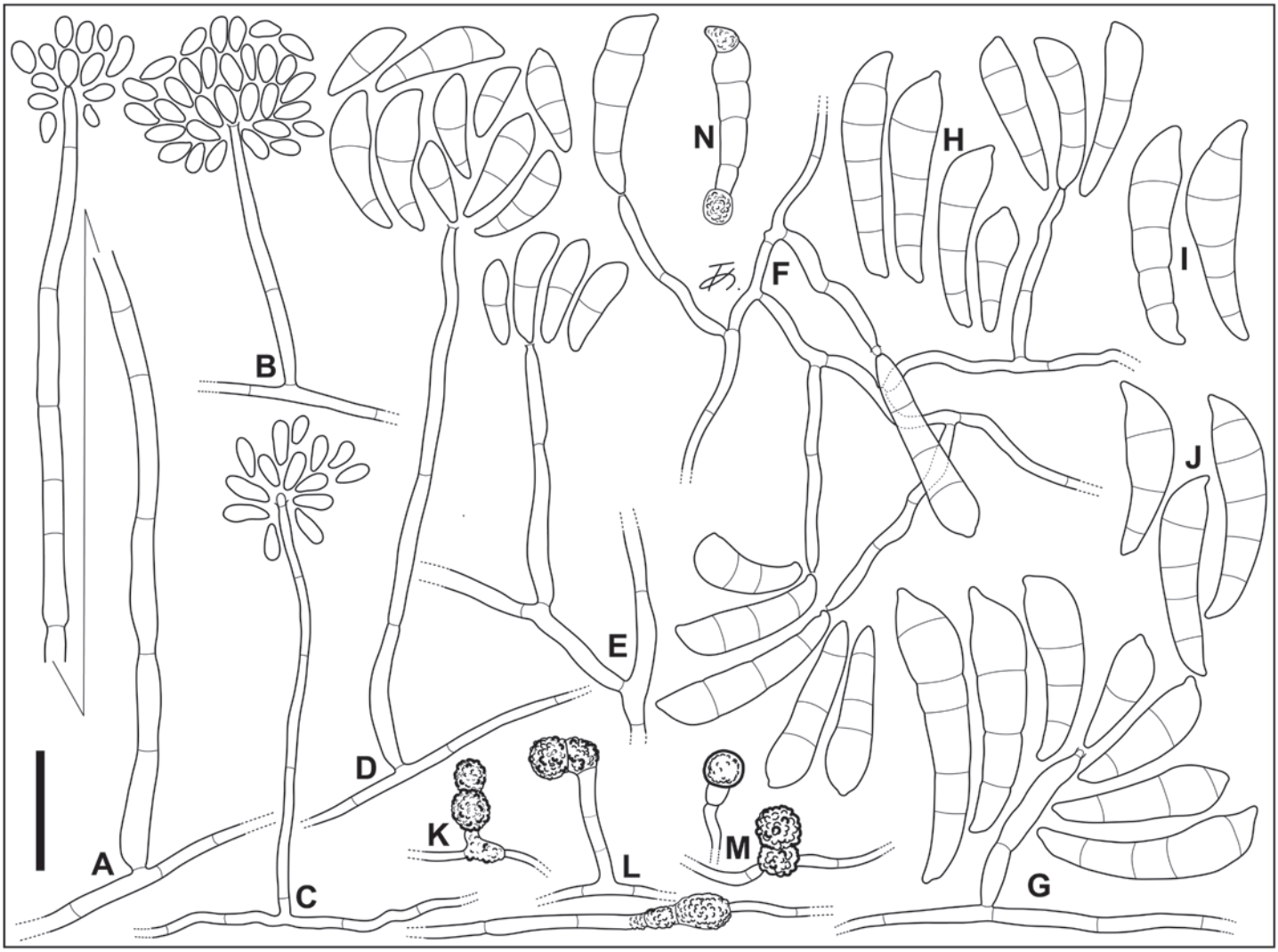

Fig. 6. *Fusarium ambrosium* cultured on SNA under black light. **A–C.** Tall and short aerial conidiophores forming 0-septate conidia. **D, E.** Short and tall aerial conidiophores forming septate conidia. **F, G.** Sporodochial conidiophores forming large clavate conidia. **H–J.** Clavate multiseptate septate conidia. **K–N.** Chlamydospores in hyphae (K–M) and conidium (N). A, E, F, L from NRRL 62605; B, D, H, K, N from NRRL 20438; C, G, M from NRRL 22346 (ex-epitype); I from NRRL 22345; J from NRRL 46583. Bar = 25 µm.

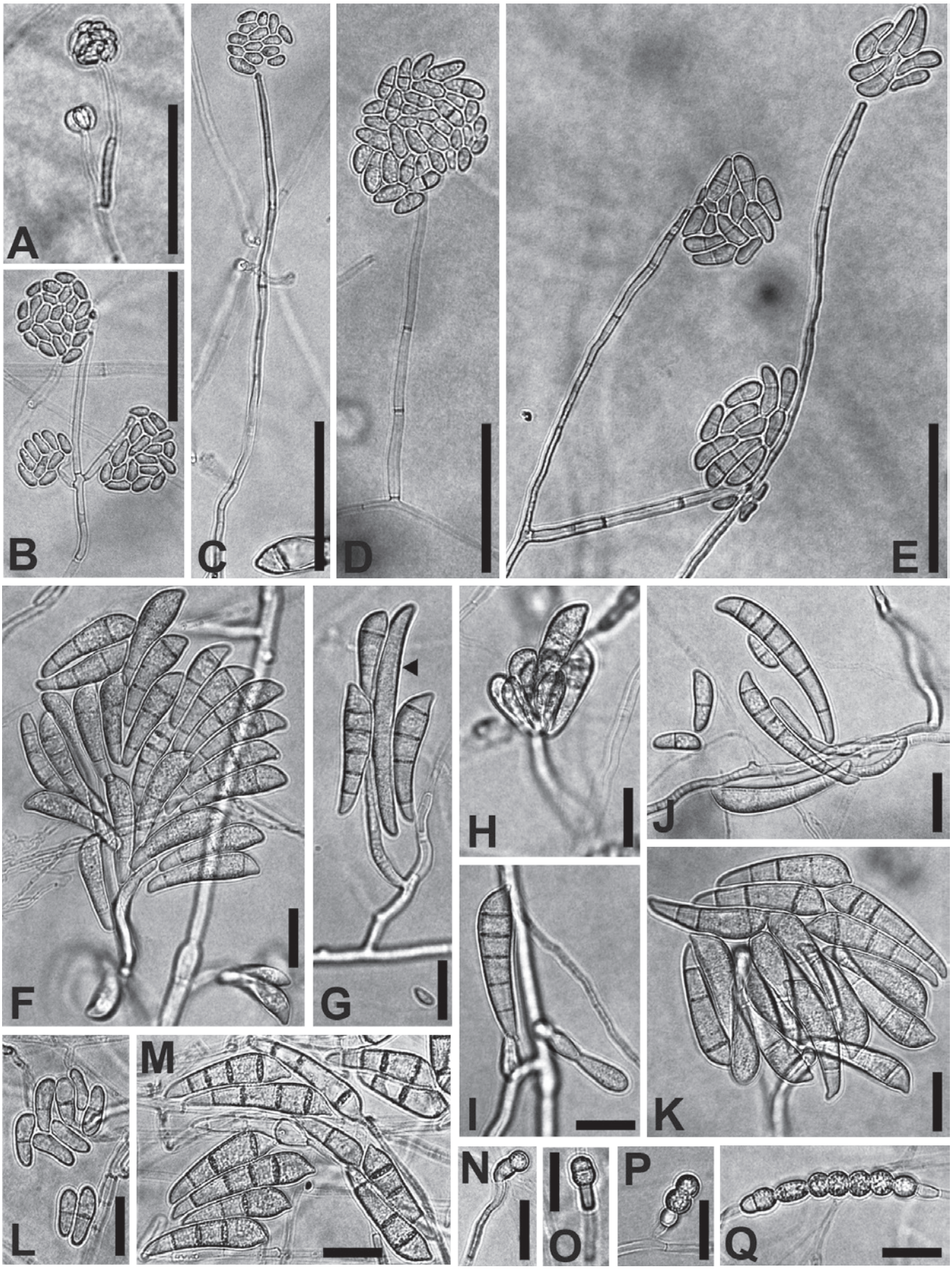

Fig. 7. *Fusarium ambrosium* cultured on SNA under black light. **A–E.** Aerial conidiophores forming 0–1(–2)-septate conidia. **F–K.** Sporodochial conidiophores forming mostly multiseptate clavate conidia, swollen apically with (2–)3–5(–6)-septa; (Arrowhead in G:) crescent-shaped conidium without septa. **L.** 0–1-septate conidia formed on aerial conidiophores. **M.** Clavate conidia formed on sporodochial conidiophores. **N–Q.** Chlamydospores formed in hyphae. A, L, M, O from NRRL 62605; B, C, E, I, K, N from NRRL 22346 (ex-epitype); D, F–H, J, P, Q from NRRL 20438. (A–E: Aerial view without a cover slip; F–Q: Mounted in water with a cover slip.) Bars: A–E = 50 µm, F–Q = 20 µm.

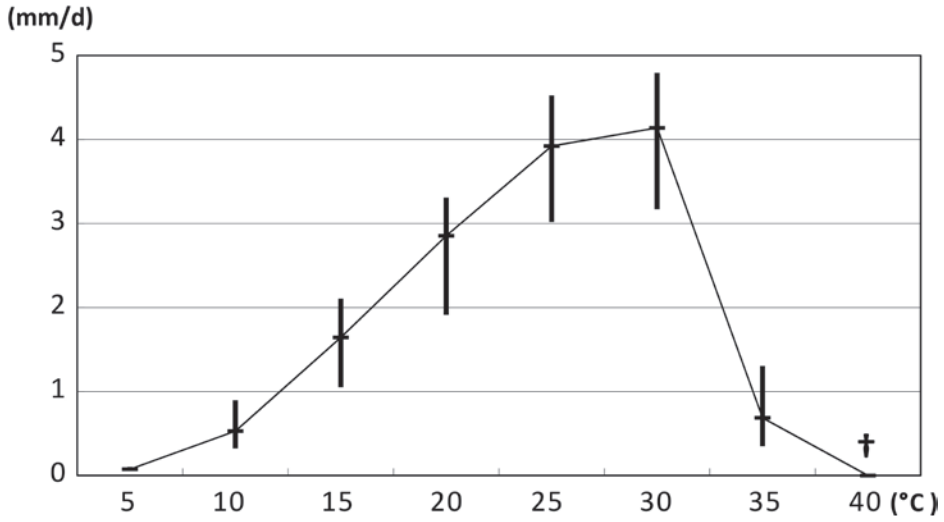

Fig. 8. Radial mycelial growth rate of *Fusarium ambrosium* per day on PDA cultured at eight different temperatures. Thick horizontal and vertical bars indicate means and total ranges, respectively, of the 6 isolates analyzed. All isolates failed to grow and died at 40 °C.

(BPI 910524 – epitype MBT 378232, designated in this study; NRRL 22346 = BBA 65390 = CBS 571.94 = MAFF 246287 – ex-epitype cultures). **Sri Lanka**, an illustration of a mature conidium of the fungus taken from a gallery of *Euwallacea fornicatus* infesting a tea tree, *Camellia sinensis*, C.H. Gadd & C.A. Loos (*Trans. Brit. Mycol. Soc.* **31** (1 & 2): 16, Text-fig. 5 (1987) – lectotype, MBT379562, designated in this study; the original description by Gadd & Loos (1947) did not designate type material of *M. ambrosium* and it was not found in the IMI, K, and BPI herbaria).

Additional strains examined: **India**, *Maharashtra*: Chinchona, isolated from a gallery of *E. fornicatus* infesting a *C. sinensis* tree, 17 Jul. 1985, (*unknown collector)* (NRRL 20438 = IMI 296597 = MAFF 246291, preserved as the ex-holotype strain of *F. bugnicourtii*); *(State name of India not recorded)*: Upari Tea Institute, isolated from a gallery of *E. fornicatus* infesting *C. sinensis* tree, 9 May 1990, *V. Agnihothrudu* (NRRL 22345 = BBA 65389 = MAFF 246288; NRRL 36510 = BBA 65390 = MAFF 246289 as a duplicate of NRRL 22346); *Tamil Nadu*: Coimbatore District, Anamallais, High Forest Estate, isolated from a gallery of *E. fornicatus* infesting stem of *C. sinensis* tree, 26 Mar. 1990, *(unknown collector)* (NRRL 46583 = IMI 339338 = MAFF 246290, preserved as *F. bugnicourtii*). **Sri Lanka**, *Talawakelle*, St. Coombs, Tea Research Institute of Sri Lanka, isolated from a gallery of *E. fornicatus* infesting *C. sinensis* tree, Mar. 2012, *S. Freeman* (NRRL 62605).

Description: *Colonies* on PDA showing radial mycelial growth rates of 1.9–3.3 mm/d at 20 °C and 3.0–4.5 mm/d at 25 °C in the dark. Colony color on PDA white (1A1) to yellowish-white (4A2) in the dark, white (1A1) to yellowish-white (3–4A2) or pale yellow (4A3) under black light. Aerial mycelium white (1A1) sparse to floccose in the dark, more abundantly formed and often covering entire surface of colonies under black light. Colony margin entire to undulate. Reverse pigmentation absent or yellowish-white (3–4A2) or pale yellow (4A3) in the dark and under black light. Olive (3E–F5–8) to olive-brown (4E–F5–8) spots in some strains when sporodochia formed on PDA. *Exudates* absent. *Odor* absent, or slightly moldy or sweet in some strains. *Hyphae* on SNA 1.5–9.5 µm wide. *Chlamydospores* present in hyphae and in septate sporodochial conidia, mostly subglobose to round ellipsoidal, intercalary or terminal, single or often in chains, hyaline or slightly pale yellow, smooth to often minutely rough-walled, 5–31.3 × 4.5–13 µm. *Sclerotia* absent. *Sporulation* on SNA and PDA generally rapid and abundant under black light, less abundant in some strains, delayed or sometimes less production in the dark; generally light-colored on SNA and PDA under black light or under daylight; sporodochia formed sparsely on SNA, and rarely on PDA under daylight; olive (3E–F5–8) to olive brown (4E–F5–8) when produced in mass. *Aerial conidiophores* formed abundantly on SNA under black light, erect, short or tall, mostly narrow but rarely thicker, mostly unbranched, rarely branched sparsely, up to 320 µm long, 2.5–7 µm wide at base, thin-walled, forming monophialides integrated in the apices. *Phialides* on aerial conidiophores simple, subcylindrical to subulate, tapering towards apex, often with a minute collarette at the tip, 15–66 × 2.5–4 µm. *Aerial conidia* typically (1) elliptical, oblong-elliptical, fusiform-elliptical to short clavate, occasionally reniform, some obovate, 0–1-septate; 0-septate on SNA in the dark: 3.5–14 × 1.5–7 µm in total range, 8.4–9.4 × 2.8–3.3 µm on average [ex epitype (NRRL 22346): 3.5–14 × 2–5.5 µm in total range, 9.4±2.4 × 3.2±0.6 µm on average ± S.D.]; 0-septate on SNA under black light: 3.5–22 × 2–7.5 µm in total range, 7.8–10.8 × 3.5–4.5 µm on average [ex epitype (NRRL 22346): 4.5–18.5 × 2–7.5 µm in total range, 10.2±2.4 × 4.1±1.1 µm on average ± S.D.]; 1-septate on SNA in the dark: 8–26 × 2.5–6.5 µm in total range, 13.6–16.3 × 3.7–4.9 µm on average [ex epitype: 12–19 × 3.5–5.5 µm in total range, 15.3±1.9 × 4.8±0.4 µm on average ± S.D.]; 1-septate on SNA under black light: 8.5–37 × 2.5–10.5 µm in total range, 15.3–20.1 × 5.4–6.6 µm on average [ex epitype: 10.5–36 × 2.5–10.5 µm in total range, 19.3±5.1 × 6.2±1.5 µm on average ± S.D.]; sometimes with (2) larger, falcate to clavate, or curved clavate, (1–)2(–3)-septate conidia, morphologically continuous with falcate sporodochial conidia. *Sporodochial conidiophores* generally short, unbranched or rarely sparsely branched, contorted, monophialides integrated apically, 20–61.3 × 3.5–5 µm. *Sporodochial phialides* simple, subulate, lanceolate or subcylindrical, often with a conspicuous collarette at the tip, 13–60 × 3–6 µm. *Sporodochial conidia* hyaline, mostly falcate to long clavate, sometimes curved cylindrical, mostly swollen in the upper part, tapering towards base, often with a round or papillate apical cell, and a distinct or indistinct foot-like, or rounded basal cell, swollen conidia often appear "dolphin-like" (Brayford 1987), (0–)2–5(–7)-septate, formed on SNA under black light, less frequently in the dark, sometimes formed on PDA under black light, rarely in the dark; 2-septate on SNA under black light: 15–61.5 × 3–12 µm in total range, 24.7–32.2 × 6.9–9.1 µm on average [ex-epitype: 15–61.5 × 4–10 µm in total range, 28.3±7.8 × 7.8±1.2 µm on average ± S.D.]; 3-septate on SNA under black light: 21–57.5 × 3.5–13 µm in total range, 34.1–40.4

× 8.3–10.0 µm on average [ex epitype: 30–57 × 7.5–12.5 µm in total range, 40.4±4.9 × 8.8±1.1 µm on average ± S.D.]; 4-septate on SNA under black light: 25.5–78.5 × 6–12.5 µm in total range, 40.7–45.6 × 8.8–10.4 µm on average [ex epitype: 33–78.5 × 7.5–11.5 µm in total range, 45.3±6.1 × 8.8±0.9 µm on average ± S.D.]; 5-septate on SNA under black light: 30–64 × 7–12.5 µm in total range, 42.9–52.1 × 8.8–10.3 µm on average [ex epitype: 37–51.5 × 7.5–12 µm in total range, 45.6±3.7 × 8.8±1.1 µm on average ± S.D.]. Together with multiseptate sporodochial conidia, often forming (0-)1(-2)-septate, oblong to naviculate or short-clavate, straight or curved conidia, with a rounded apex and a truncate base.

Notes: Strain NRRL 62605 did not form conidia on SNA or PDA in the dark. Therefore, the description of conidia in the dark was based on the three other strains examined in this study. All of the strains studied were isolated from galleries of *E. fornicatus* infesting *C. sinensis* trees in India and Sri Lanka. *Fusarium ambrosium* was originally isolated from *E.* (*Xyleborus*) *fornicatus* galleries in stems of Chinese tea and caster-oil trees in Sri Lanka, and was described as a new species of *Monacrosporium*, i.e., *M. ambrosium* by Gadd & Loos (1947). Forty years later, it was redescribed by Brayford (1987) as *F. bugnicourtii* based on collections from beetle galleries in Chinese tea in India and borer-damaged *Hevea brasiliensis* and *Theoborma cacao* in Malaysia. Brayford (1987) considered *F. bugnicourtii* to be conspecific with *F. tumidum* var. *coeruleum* (Bugnicourt 1939) isolated from *H. brasiliensis*, but distinct from *F. tumidum*. *Fusarium bugnicourtii* was recognized as conspecific with Gadd and Loos' species, *M. ambrosium* from the shot-hole borer on tea, and synonymized under the new combination, *F. ambrosium* based on its nomenclatural priority (Nirenberg 1990). Because type material of *M. ambrosium* (≡ *F. ambrosium*) was not designated in the original description by Gadd & Loos (1947) and not found in the IMI, K, and BPI herbaria, we selected a line-drawing (illustration) of a conidium from Gadd & Loos (1947) as the lectotype, according to Art. 9.2 and 9.3 of the ICNafp (McNeill et al. 2012). To supplement the lectotype, BPI 910524, a dried culture of NRRL 22346 (= BBA 65390 = CBS 571.94), isolated from a gallery of *E. fornicatus* infesting *C. sinensis* in India by V. Agnihothrudu, was selected as the epitype according to Art. 9.8 of the code. Because an authentic strain of *F. ambrosium*, IMI 296597 (= NRRL 20438), isolated from *E.* (*Xyleborus*) *fornicatus* galleries in tea tree from India in 1985, was designated as the holotype of *F. bugnicourtii* by Brayford (1987), this material was not selected for the epitype, per Art. 52.1 and 52.2 of the ICN (McNeill *et al.* 2012). Although NRRL 62605 was isolated from an *E. fornicatus* gallery in Sri Lanka from the same host and type locality, it was not selected as the epitype because it appears to be an interspecific hybrid that contains alleles from what appear to be two other AFC species (Kasson *et al.* 2013). The present epitypification was prepared to stabilize the taxonomy of this species.

Fusarium ambrosium is most similar morphologically to *F. euwallaceae* (Freeman *et al.* 2013, Kasson *et al.* 2013). *Fusarium ambrosium* and *F. euwallaceae* produce very similar falcate to clavate, septate sporodochial conidia that are swollen in their upper half, together with ovoid to ellipsoid, 0-septate aerial conidia. The conidial sizes and number of septa of these two fusaria are almost identical (Table 2, Figs 6, 7; Freeman *et al.* 2013). However, *F. ambrosium* and *F. euwallaceae* can be distinguished by the production of hyaline or olive to olive-brown conidia when old in the former and bluish to greenish conidia in the latter when produced in mass on PDA after 1 mo (Freeman *et al.* 2013). Production of sporodochial conidia in *F. euwallaceae* is easily observed on SNA and PDA in the dark and under black light, but in *F. ambrosium* it is often delayed or limited without black light illumination, even if cultured on SNA.

A preliminary morphological comparison of the sporodochial conidia for 10 of the 12 AFC species has been conducted (T. Aoki *et al.* unpubl.). Ten of the species produced clavate sporodochial conidia that were swollen apically, and two, including AF-6 associated with *Euwallacea* sp. #2 in avocado in the Miami-Dade area of southern Florida, USA and AF-9 from *Xyleborus ferrugineus* in Costa Rica and *Delonix regia* (royal poinciana) in southern Florida only produced curved fusiform, septate sporodochial conidia in culture, as commonly observed in typical members of the *F. solani* species complex. Three novel AFC species that were reported recently (O'Donnell *et al.* 2015, Na *et al*. 2018), including AF-10 from Singapore, AF-11 from Sri Lanka, and AF-12 (= *F. kuroshium*) from San Diego County, California, produced clavate sporodochial conidia in culture.

The available data suggests that most of the AFC species might possess diagnostic phenotypic characters. For example, AF-3 ex *Euwallacea interjectus* infesting *Acer negundo* in Gainesville, Florida produced sporodochial conidia that were variable in size and shape; AF-5 from Malaysia ex *Hevea brasiliensis* produced the shortest sporodochial conidia when comparing those with the same number of septa produced by the members of the AFC; AF-7 from *Euwallacea* sp. #3 ex *Persea americana* in Australia produced sporodochial conidia that were densely and/or obliquely septate; AF-8 from *Euwallacea* sp. #2 ex avocado in the Miami-Dade area of southern Florida appeared to be unique in that it is the only AFC species that produced swollen clavate and curved fusiform sporodochial conidia (Kasson *et al.* 2013); AF-10 from Singapore produced clavate sporodochial conidia that were narrower than conidia of other AFC species with the same number of septa; AF-11 ex *E. fornicatus* in Chinese tea from Sri Lanka produced sporodochial conidia that were frequently pointed and curved or hooked to one side; *F. kuroshium* AF-12 ex *Euwallacea* sp. #5 from *Platanus racemosa* (California sycamore) and several other woody hosts in San Diego County, California was distinguished by the production of clavate sporodochial conidia together with crescent- or comma-shaped conidia (T. Aoki *et al.* unpubl.). By way of contrast, AF-6 ex *Euwallacea* sp. #2 in avocado in the Miami-Dade area of Florida and AF-9 from Costa Rica and the Miami-Dade area of Florida only produced curved cylindrical multiseptate conidia and elliptical to oblong aerial conidia (Kasson *et al.* 2013). However, the sporodochial conidia formed by AF-9 are atypical of the *F. solani* species complex because they were pointed at both ends, terminating in a short apical beak and a conspicuous foot-like basal cell. Detailed studies will be required to assess whether AF-6 and AF-9 possess morphological characters that distinguish them from other members of the *F. solani* species complex.

DISCUSSION

The AFC symbiont cultivated by the ambrosia beetle *Euwallacea validus* in *Ailanthus altissima* in eastern U.S. is formally described herein as *Fusarium oligoseptatum*. Sampling across 7 eastern U.S. states and 17 tree hosts confirmed that *F. oligoseptatum* is the

primary symbiont of *E. validus* and dominant, regardless of plant host with few exceptions. This species can be distinguished from the 11 other known AFC species by producing significantly more 0–2-septate clavate sporodochial conidia that are swollen apically and via multilocus molecular phylogenetics where it was strongly supported as a genealogically exclusive species-level lineage in the analyses reported here and in previous studies (Kasson *et al*. 2013, O'Donnell *et al*. 2015, Na *et al*. 2018). *Fusarium oligoseptatum* was strongly supported as a reciprocally monophyletic sister to *F. euwallaceae* + *Fusarium* sp. (AF-3) in Kasson *et al*. (2013), but the sister group relationship of *F. oligoseptatum* was unresolved in analyses that included the closely related *F. kuroshium* (AF-12) from San Diego, California (O'Donnell *et al*. 2015, and present study). Efforts to develop a robust hypothesis of evolutionary relationships among these four AFC species, which are estimated to have shared a most recent common ancestor approximately 1.6 Mya (O'Donnell *et al*. 2015), might benefit from the comparative phylogenomic analyses that are currently underway (Stajich *et al*., pers. comm.).

Herein, an epitype of *F. ambrosium* was designated based on material originally isolated from a gallery of *E. fornicatus* infesting Chinese tea in India to provide nomenclatural stability for this species. AFC species have been collected in eight different countries, including Sri Lanka (*F. ambrosium* AF-1 and *Fusarium* sp. AF-11), India (*F. ambrosium* AF-1), Malaysia (*Fusarium* sp. AF-5), Singapore (*Fusarium* sp. AF-10), Australia (*Fusarium* sp. AF-7), Israel (*F. euwallaceae* AF-2), Costa Rica (*Fusarium* sp. AF-9) and the United States (*F. euwallaceae* AF-2, *Fusarium* sp. AF-3, *F. oligoseptatum* AF-4, *Fusarium* spp. AF-6, AF-8, AF-9 and *F. kuroshium* AF-12)(Brayford 1987, Nirenberg 1990, Freeman *et al.* 2013, Kasson *et al.* 2013, O'Donnell *et al.* 2015, Short *et al.* 2017, Na *et al*. 2018). To date only three species within the AFC have been described formally, i.e., *F. ambrosium* (AF-1; Gadd & Loos 1947, Nirenberg 1990), *F. euwallaceae* (AF-2; Freeman *et al.* 2013) and *F. oligoseptatum* (AF-4; in this study). Although nine of the AFC species are currently unnamed, the prospects for naming them are excellent because most of them appear to possess unique phenotypic/morphological features. Delimitations of such features may, in time, help to uncover the mechanisms underlying the production of clavate conidia, a posited adaptation for the *Euwallacea* – *Fusarium* symbiosis (Kasson *et al.* 2013). Indeed, analogous adaptations in agaricalean fungi (i.e., gongylidia) farmed by higher and occasionally lower attine ants (Schultz & Brady 2008, Masiulionis *et al.* 2014) also appear to exhibit variation among closely related lineages. However, quality of the substrate, pH, and temperature have also been shown to affect the growth and size of gongylidia in some higher attine ant cultivars when cultivated under lab conditions (Powell & Stradling 1986).

It remains unclear whether *F. ambrosium, F. euwallaceae*, or *F. oligoseptatum* are each farmed by a single *Euwallacea* species, including within their native range, where evidence of hybridization and co-cultivation with other closely related AFC members have been reported (O'Donnell *et al.* 2015). However, it has been shown that *F. euwallaceae* from avocado is obligately required for the survival and development of *Euwallacea* sp. #1 sensu O'Donnell *et al.* (2015) currently occurring in Israel, whereas *F. ambrosium* does not support development of this beetle species (Freeman *et al*. 2012). Likewise, specificity exists for *F. ambrosium* and its beetle host. Future studies focused on vector specificity could help clarify the threats these beetle-fungus consortia pose to our native ecosystems. This is especially important given that some AFC members such as *F. euwallaceae* have caused significant damage to orchard, landscape and forest trees and threaten avocado production worldwide (Mendel *et al.* 2012, Eskalen *et al.* 2013, Kasson *et al.* 2013), while other AFC members such as *F. oligoseptatum* appear to be quite innocuous when challenged against numerous plant species (Berger 2017).

The FSSC includes over 60 species (Zhang *et al.* 2006, O'Donnell *et al*. 2008, Short *et al.* 2013), a majority of which lack formal Latin binomials thus making it difficult to link specific plant diseases with specific phylogenetic species within the FSSC, including the AFC (Montecchio *et al*. 2015). The designation of formal Latin binomials for a majority of phylogenetic species within the FSSC coupled with recent abolishment of the dual system of fungal nomenclature will likely reduce confusion surrounding molecular identification of taxa within this large species complex. Nevertheless, the use of multilocus phylogenetic studies will remain the gold standard to discriminate closely related members in the FSSC.

Another avenue to further resolve these closely related phylogenetic species is to examine functional differences among closely related AFC. A recent study by Kasson *et al.* (2016) assessed the enzyme activity and wood degrading capacity of *F. oligoseptatum* and *R. subfusca*, the two known symbionts of *E. validus* in the eastern U.S. Polyphenol oxidase production was detected from *F. oligoseptatum* but not *R. subfusca*. An earlier study by Norris (1980) on AFC member *Fusarium* sp. AF-9 revealed this fungus was capable of degrading lignin. Further enzymatic studies among closely related AFC may compliment morphological and phylogenetic studies within the *Euwallacea* – *Fusarium* mutualism, revealing significant differences in enzyme activity. This is particularly important given recent studies by Aylward *et al.* (2015) that showed a diverse but consistent set of enzymes present in gongylidia, which are essential for initial degradation of plant substrates in the leaf-cutter ant-*Leucoagaricus* mutualism.

The results of this study suggest that many of the unnamed AFC species like *F. oligoseptatum* possess unique phenotypic/morphological features, which will facilitate formal description of these economically important pathogens. Phenotypic/morphological studies on the four additional AFC species from Tawain (Na *et al*. 2018) are, therefore, also fully expected. Our ongoing research is focused on advancing the systematics of the AFC to promote accurate communication within the global scientific community.

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DISCLAMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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Phylogeny and taxonomy of the genus *Tubakia s. lat.*

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Abstract: The genus *Tubakia* is revised on the basis of morphological and phylogenetic data. The phylogenetic affinity of *Tubakia* to the family *Melanconiellaceae* (*Diaporthales*) was recently postulated, but new analyses based on sequences retrieved from material of the type species of *Tubakia*, *T. dryina*, support a family of its own, viz. *Tubakiaceae* fam. nov. Our phylogenetic analyses revealed the heterogeneity of *Tubakia s. lat.* which is divided into several genera, viz., *Tubakia s. str.*, *Apiognomonioides* gen. nov. (type species: *Apiognomonioides supraseptata*), *Involutiscutellula* gen. nov. (type species: *Involutiscutellula rubra*), *Oblongisporothyrium* gen. nov. (type species: *Oblongisporothyrium castanopsidis*), *Paratubakia* gen. nov. (type species: *Paratubakia subglobosa*), *Racheliella* gen. nov. (type species: *Racheliella wingfieldiana* sp. nov.), *Saprothyrium* gen. nov. (type species: *Saprothyrium thailandense*) and *Sphaerosporithyrium* gen. nov. (type species: *Sphaerosporithyrium mexicanum* sp. nov.). *Greeneria saprophytica* is phylogenetically closely allied to *Racheliella wingfieldiana* and is therefore reallocated to *Racheliella*. Particular emphasis is laid on a revision and phylogenetic analyses of *Tubakia* species described from Japan and North America. Almost all North American collections of this genus were previously referred to as *T. dryina s. lat.*, which is, however, a heterogeneous complex. Several new North American species have recently been described. The new species *Sphaerosporithyrium mexicanum*, *Tubakia melnikiana* and *T. sierrafriensis*, causing leaf spots on several oak species found in the North-Central Mexican state Aguascalientes and the North-Eastern Mexican state Nuevo León, are described, illustrated, and compared with similar species. Several additional new species are introduced, including *Tubakia californica* based on Californian collections on various species of the genera *Chrysolepis*, *Notholithocarpus* and *Quercus*, and *T. dryinoides*, *T. oblongispora*, *T. paradryinoides*, and *Paratubakia subglobosoides* described on the basis of Japanese collections. *Tubakia suttoniana* nom. nov., based on *Dicarpella dryina*, is a species closely allied to *T. californica* and currently only known from Europe. *Tubakia dryina*, type species of *Tubakia*, is epitypified, and the phylogenetic position and circumscription of *Tubakia* are clarified. A revised, supplemented key to the species of *Tubakia* and allied genera on the basis of conidiomata is provided.

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Dedicated to Vadim Alexandrovich Mel'nik (*16 March 1937, †10 April 2017).

INTRODUCTION

Tubakia species are endophytes in leaves and twigs of many tree species, but can also cause conspicuous leaf symptoms as plant pathogens (Cohen 1999, Gonthier *et al.* 2006, Hashizume *et al*. 2008, Sieber 2007). Obvious leaf spots are dotted with minute punctiform conidiomata (pycnothyria) composed of convex scutella with radiating threads of cells fixed to the substratum by a central columella, mostly surrounded by a sheath of small fertile cells that give rise to one-celled, phialidic conidiogenous cells. The conidia are globose, subglobose, ellipsoid, broad ellipsoid-obovoid to subcylindrical or somewhat irregular in shape, aseptate, hyaline, subhyaline to pigmented. The conidiomata and the conidia are distinct and readily allow identification to generic level. Other fungal fruiting structures including crustose-pycnidial conidiomata on overwintering twigs that open by lateral to irregular dehiscence or sporodochia formed on leaf veins (Holdenrieder & Kowalski 1989, Harrington

et al. 2012) may also be formed and have been described for *T. dryina* and *T. iowensis*. Some species produce a second type of much smaller conidia (microconidia), either in "normal" pycnothyria or in separate, mostly smaller pycnothyria. Reports of the occurrence and sporulation of *Tubakia dryina* as a saprobe on necrotic tissue caused by other fungi (Munkvold & Neely 1990) are unconfirmed and need further study and clarification.

Tubakia was previously assigned to the *Diaporthales* within *Ascomycota* (Yokoyama & Tubaki 1971, Yun & Rossman 2011). In a more detailed study on its phylogenetic affinity and position in the hierarchical system of the *Ascomycota*, Senanayake *et al*. (2017) placed *Tubakia* in the newly introduced family *Melanconiellaceae*. The sexual morph of "*T. dryina*" has been referred to as *Dicarpella dryina* (Belisario 1991). However, *D. dryina* is not the type species of *Dicarpella*, and at least one species, *Dicarpella quercifolia* was linked to *Mastigosporella hyalina* (≡ *Harknessia hyalina*; Barr 1979, Nag Raj & Di Cosmo 1981, Rossman *et al*. 2015). *Dicarpella quercifolia*, together with *D. georgiana*, were subsequently reallocated to the genus *Wuestneiopsis* (Reid & Dawsett 1990). The relation and synonymy of *Dicarpella* and *Tubakia* are, however, unclear. Clarification requires phylogenetic data for *D. bina*, the type species of this genus, which is only known from the type collection.

Saccardo (1913) introduced the genus *Actinopelte* for *A. japonica*, a scutellate fungus found in Japan on *Castanea crenata* (= *C. pubinervis*). Saccardo (*l.c.*) confused the large conidia of this species with asci, which was clarified and corrected by Theissen (1913: 509) who provided a detailed discussion, description, and illustration (Theissen 1913: 508, fig. VI) of *A. japonica*. Höhnel (1925) revisited *Actinopelte*, added a new species, *A. americana,* and introduced the new combination *A. dryina*, based on *Leptothyrium dryinum*. Yokoyama & Tubaki (1971) discussed the history of this genus in detail, published results of comprehensive examinations of Japanese collections *in vivo* and *in vitro*, and described *A. castanopsidis*, *A. rubra*, and *A. subglobosa* based on Japanese collections. Since Saccardo's *Actinopelte* turned out to be illegitimate (Art. 53.1, later homonym of *Actinopelte* Stitzenb. 1861), Sutton (1973) introduced the replacement name *Tubakia* and reallocated all species recognised and treated in Yokoyama & Tubaki (1971) to this genus. Three additional *Tubakia* species have subsequently been described: *T. seoraksanensis* (Yun & Rossman 2011), *T. iowensis* (Harrington *et al*. 2012), and *T. chinensis* (Braun *et al*. 2014). So far, all European collections in the genus *Tubakia* have been assigned to a single species, *T. dryina*, whereas in Asia this genus has a much higher degree of species diversity. Additional undescribed species from Asia were predicted, and were detected in the course of the present studies. Braun *et al*. (2014) described and illustrated *T. chinensis* on *Castanea henryi*, a species with very large pycnothyria (135–200 µm diam), and globose to subglobose conidia. Based on its very small scutella, Braun *et al*. (2014) described and illustrated a second *Tubakia* species on *Castanea henryi* in China, tentatively referred to as *Tubakia* sp. The recently introduced *Tubakia thailandensis* (Senanayake *et al*. 2017) is morphologically very close to the Chinese collection on *Castanea henryi* and could be conspecific. According to Harrington *et al*. (2012), Japanese specimens referred to as *T. dryina* in Yokoyama & Tubaki (1971) do not belong to *T. dryina s. str*. The phylogeny of the Asian species of *Tubakia* has not yet been determined. Boroń & Grad (2017) examined numerous Polish strains referred to as *T. dryina* and found two different ITS groups designated as haplotypes. The diversity of *Tubakia* species in North America is not well known, although it is likely to contain more than just the single species listed in various publications as *T. dryina*. North American collections assigned to *T. dryina* are undoubtedly heterogeneous, i.e., *T. dryina* in North America is a complex of cryptic species (Harrington *et al*. 2012). An important contribution to resolve problems around the diversity of the *T. dryina* complex in North America has recently been published by Harrington & McNew (2018), including several new species based on US collections. Several *Tubakia* samples recently collected in Mexico on endemic oaks appear to be morphologically similar to *T. dryina* but are sufficiently distinct to suggest that they are undescribed species. A *Tubakia* causing serious oak diseases in California also appears to represent an additional undescribed species. Therefore, the present study presents a comprehensive examination and revision of *Tubakia* spp. in North America and worldwide, based on *in vivo* and *in vitro* morphological analyses as well as phylogenetic data. This work has been accomplished in collaboration by an international group of mycologists and phytopathologists from Asia, Europe and North America.

MATERIALS AND METHODS

Isolates

Isolates included in this study were obtained from symptomatic leaves of diverse hosts, and identified as species of *Tubakia* based on primarily the presence of pycnothyrial conidiomata and aseptate conidia. In addition, several isolates were obtained from the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS culture collection), in Utrecht, The Netherlands, the CDFA Plant Pathogen Collections (CDFA), Sacramento, USA, and from the working collection of Pedro Crous (CPC), housed at the Westerdijk Institute. Japanese isolates were obtained from NBRC (National Institute of Technology and Evaluation, Culture Collection Division, Chiba, Japan) and examined by C. Nakashima. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing pine needle agar (PNA) (Smith *et al*. 1996), 2 % malt extract agar (MEA), potato-dextrose agar (PDA), and oatmeal agar (OA) (Crous *et al.* 2009b), and incubated at 22 °C under continuous near-ultraviolet light to promote sporulation. Descriptions of culture characteristics on Czapek agar and potato sucrose agar refer to Yokoyama & Tubaki (1971). The cultures included in this study are listed in Table 1.

DNA extraction, amplification (PCR) and phylogeny

Fungal mycelium of strains (Table 1) was harvested with a sterile scalpel and the genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturers' protocols. Four partial nuclear genes were subjected to PCR amplification and sequencing: 28S nrRNA gene (LSU), internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrDNA operon, beta-tubulin (*tub2*) and translation elongation factor 1-alpha (*tef1*) using the primers listed in Table 2. The PCR amplifications were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The PCR mixtures consisted of 1 µL genomic DNA, 1× NH4 reaction buffer (Bioline, Luckenwalde, Germany), 2–5 mM $MgCl_2$ (ITS: 2.5 mM, LSU: 5 mM, *rpb2* and

Table 1. Collection details and GenBank accession numbers of *Tubakia* and tubakia-like isolates considered in this study.

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
Apiognomonioides supraseptata	CBS 632.92[Ex-type of *Apiognomonia supraseptata*] = ATCC 58737 = TMI 70024	S. Kaneko	*Quercus glauca*	Japan	MG976447	MG976448	–	–	–
Involutiscutellula rubra	CBS 192.71[Ex-isotype of *Actinopelte rubra*] = A684 = IFO 9271 = MUCC2302 = NBRC 9271	T. Yokoyama, 7 Jun. 1969	*Quercus phillyraeoides*, dead leaf	Japan	MG591899	MG591993	MG592086	MG592180	MG976476
	MUCC2303 = NBRC 9272	T. Yokoyama, 7 Jun. 1969	*Quercus phillyraeoides*, dead leaf	Japan	MG591900	MG591994	MG592087	MG592181	MG976477
	MUCC2304[Ex-type of *Tubakia rubra*] = NBRC 9273 = ATCC 22473 = IMI 157597	T. Yokoyama, 28 Oct. 1969	*Quercus phillyraeoides*, living leaf	Japan	MG591901	MG591995	MG592088	–	MG976478
	MUCC2305 = NBRC 9274	T. Yokoyama, 27 Jan. 1970	*Quercus phillyraeoides*	Japan	MG591902	MG591996	MG592089	MG592182	MG976479
	MUCC2306 = NBRC 9275	T. Yokoyama, 27 Jan. 1970	*Quercus phillyraeoides*	Japan	MG591903	MG591997	MG592090	–	MG976480
	MUCC2307 = NBRC 9276	T. Yokoyama, 14 Apr. 1970	*Quercus phillyraeoides*	Japan	MG591904	MG591998	MG592091	MG592183	MG976481
	MUCC2308 = NBRC 9277	T. Yokoyama, 29 May 1970	*Quercus phillyraeoides*	Japan	MG591905	MG591999	MG592092	MG592184	MG976482
	MUCC2309 = NBRC 9371	T. Yokoyama, 2 Aug. 1970	*Quercus phillyraeoides*	Japan	MG591906	MG592000	MG592093	MG592185	MG976483
Melanconis groenlandica	CBS 116540[Ex-type of *Myrothecium groenlandicum*] = UPSC 3407	M. Bohn, Jul. 1991	*Betula nana*, twigs	Greenland	KU878552	KU878553	KU878554	KU878555	–
Oblongisporothyrium castanopsidis	CBS 124732 = MUCC2288 = NBRC 9262	T. Yokoyama, 26 Mar. 1970	*Castanopsis cuspidata*	Japan	MG591849	MG591942	MG592037	MG592131	MG976453
	CBS 189.71[Ex-type of *Actinopelte castanopsidis*] = A686 = ATCC 22470 = IFO 9263 = IMI 157598 = MUCC2289 = NBRC 9263	T. Yokoyama, 27 Jan. 1970	*Castanopsis cuspidata*, dead leaf	Japan	MG591850	MG591943	MG592038	MG592132	MG976454
Paratubakia subglobosa	CBS 124733 = MUCC2311 = NBRC 9344	T. Yokoyama, 27 Feb. 1970	*Quercus glauca*	Japan	MG591913	MG592008	MG592102	MG592194	MG976489
	CBS 193.71[Ex-type of *Actinopelte subglobosa*] = A685 = ATCC 22474 = IFO 8931 = IMI 157596 = MUCC2310 = NBRC 8931	T. Yokoyama, 30 Jan. 1968	*Quercus glauca*, dead leaf	Japan	MG591914	MG592009	MG592103	MG592195	MG976490
Paratubakia subglobosoides	MUCC2293[Ex-type of *Paratubakia subglobosoides*] = NBRC 9343	T. Yokoyama, 27 Feb. 1970	*Quercus glauca*	Japan	MG591915	MG592010	MG592104	MG592196	MG976491
Racheliella saprophytica	MFLUCC 12–0298[Ex-type of *Greeneria saprophytica*] = NTCL052-1	N. Tangthirasunun, 21 Mar. 2012	*Syzygium cumini*, dead leaves	Thailand	KJ021933	KJ021935	–	–	–

Table 1. (Continued).

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
Racheliella wingfieldiana	CBS 143669[Ex-type of *Racheliella wingfieldiana*] = CPC 13806	P.W. Crous, 6 Mar. 2007	*Syzigium guineense*	South Africa	MG591911	MG592006	MG592100	MG592192	MG976487
Saprothyrium thailandense	MFLUCC 12–0303[Ex-type of *Tubakia thailandensis*]	K. Wisitrassameewong, 2 May 2012	Decaying leaf	Thailand	MF190163	MF190110	–	–	–
Sphaerosporithyrium mexicanum	CPC 31361	O. Moreno-Rico, Apr. 2016	*Quercus eduardi*	Mexico	MG591894	MG591988	MG592081	MG592175	–
	CPC 32258 = CFNL 2941 = id 11	J. G. Marmolejo, 9 Nov. 2012	*Quercus eduardi*	Mexico	MG591895	MG591989	MG592082	MG592176	–
	CPC 33021[Ex-type of *Tubakia mexicana*] = CFNL 2945	O. Moreno-Rico, 19 Jan. 2017	*Quercus eduardi*	Mexico	MG591896	MG591990	MG592083	MG592177	MG976473
Tubakia americana	A1105	M. Guthmiller, 23 Sep. 2011	*Quercus macrocarpa*, leaf	USA: Wisconsin	TCH	–	TCH	–	–
	A1190	–	*Quercus* sp.	USA: Missouri	TCH	–	–	–	–
	A1201	D. McNew, 5 Aug. 2012	*Quercus bicolor*, leaf	USA: Missouri	–	–	TCH	–	–
	A693	D. McNew, 19 May 2009	*Quercus macrocarpa*, acorn cup	USA: Iowa	TCH	–	TCH	–	–
	A791	–	*Quercus robur*	USA: Iowa	TCH	–	TCH	–	–
	A822	–	*Quercus macrocarpa*, acorn	USA: Iowa	TCH	–	–	–	–
	A829	–	*Quercus macrocarpa*	USA: Iowa	TCH	–	–	–	–
	A844	–	*Quercus bicolor*	USA: Iowa	TCH	–	–	–	–
	A845	–	*Quercus bicolor*	USA: Iowa	TCH	–	TCH	–	–
	CBS 129014 = A749 = BH3-L2	D. McNew, 10 Jun. 2009	*Quercus macrocarpa*, leaf spot	USA: Iowa	MG591873	MG591966	MG592058	MG592152	MG976449
	CBS 137350 = A1007	D. McNew, 6 Apr. 2011	*Quercus rubra*, twig	USA: Iowa	TCH	–	TCH	–	–
	CBS 137353 = A1158	D. McNew, 12 Oct. 2011	*Quercus macrocarpa*, leaf spot	USA: Iowa	MG605064	–	MG603571	–	–
	no culture (NY E&E FC286 ex McClatchie[Lectotype of *Tubakia americana*])	J.B. Ellis & B.M. Everhart, Aug. 1883	*Quercus coccinea*, leaf spot	USA: New Jersey	MG605063	–	–	–	–
Tubakia californica	CPC 31496 = CDFA#993	S. Rooney-Latham, 3 Apr. 2012	*Quercus agrifolia*	USA: California	MG591829	MG591922	MG592017	MG592111	MG976450
	CPC 31497 = CDFA#1007	S. Rooney-Latham, 27 Apr. 2012	*Quercus agrifolia*	USA: California	MG591830	MG591923	MG592018	MG592112	–

Table 1. (Continued).

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
	CPC 31498 = CDFA#1029	S. Rooney-Latham, 23 May 2012	*Quercus agrifolia*	USA: California	MG591831	MG591924	MG592019	MG592113	–
	CPC 31499 = CDFA#1076	S. Rooney-Latham, 18 Jul. 2012	*Quercus wislizeni*	USA: California	MG591832	MG591925	MG592020	MG592114	–
	CPC 31502 = CDFA#1103	S. Rooney-Latham, 9 Aug. 2012	*Quercus wislizeni*	USA: California	MG591833	MG591926	MG592021	MG592115	–
	CPC 31504 = CDFA#1105	S. Rooney-Latham, 9 Aug. 2012	*Quercus kelloggii*	USA: California	MG591834	MG591927	MG592022	MG592116	–
	CBS 143670[Ex-type of *Tubakia californica*] = CPC 31505 = CDFA#1428	S. Rooney-Latham, 19 Sep. 2014	*Quercus kelloggii*	USA: California	MG591835	MG591928	MG592023	MG592117	MG976451
	CPC 31506 = CDFA#1430	S. Rooney-Latham, 19 Sep. 2014	*Quercus kelloggii*	USA: California	MG591836	MG591929	MG592024	MG592118	–
	CPC 31507 = CDFA#1431	S. Rooney-Latham, 19 Sep. 2014	*Quercus kelloggii*	USA: California	MG591837	MG591930	MG592025	MG592119	–
	CPC 31508 = CDFA#1434	S. Rooney-Latham, 19 Sep. 2014	*Quercus kelloggii*	USA: California	MG591838	MG591931	MG592026	MG592120	–
	CPC 31509 = CDFA#1407	S. Rooney-Latham, 28 Sep. 2014	*Quercus kelloggii*	USA: California	MG591839	MG591932	MG592027	MG592121	–
	CPC 31510 = CDFA#1408	S. Rooney-Latham, 28 Sep. 2014	*Quercus kelloggii*	USA: California	MG591840	MG591933	MG592028	MG592122	–
	CPC 31512 = CDFA#1448	S. Rooney-Latham, 12 Oct. 2014	*Quercus wislizeni*	USA: California	MG591841	MG591934	MG592029	MG592123	–
	CPC 31513 = CDFA#1455	S. Rooney-Latham, 12 Oct. 2014	*Quercus kelloggii*	USA: California	MG591842	MG591935	MG592030	MG592124	–
	CPC 31514 = CDFA#1477	S. Rooney-Latham, 30 Oct. 2014	*Lithocarpus densiflorus*	USA: California	MG591843	MG591936	MG592031	MG592125	–
	CPC 31515 = CDFA#1542	S. Rooney-Latham, 26 Feb. 2015	*Lithocarpus densiflorus*	USA: California	MG591844	MG591937	MG592032	MG592126	–
	CPC 31516 = CDFA#1800	S. Rooney-Latham, 10 Dec. 2015	*Quercus agrifolia*	USA: California	MG591845	MG591938	MG592033	MG592127	–
	CPC 31517 = CDFA#1782	S. Rooney-Latham, 12 Jan. 2016	*Chrysolepis chrysophylla*	USA: California	MG591846	MG591939	MG592034	MG592128	–
	CPC 32250 = CFNL 2934 = id 02	J. G. Marmolejo, 6 Oct. 2012	*Quercus canbyi*	Mexico	MG591847	MG591940	MG592035	MG592129	MG976452
	CPC 32251 = CFNL 2935 = id 03	J. G. Marmolejo, 6 Oct. 2012	*Quercus canbyi*	Mexico	MG591848	MG591941	MG592036	MG592130	–
Tubakia dryina	A658	–	*Quercus alba*	USA: Iowa	TCH	TCH	–	–	–
	A789	–	*Quercus alba*	USA: Iowa	TCH	TCH	–	–	–

Table 1. (Continued).

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
	A841	–	*Quercus robur*	Germany	TCH	–	–	–	–
	CBS 112097[Ex-epitype of *Tubakia dryina*] = A680	S. Mutto-Accardi, –	*Quercus robur*	Italy	MG591851	MG591944	MG592039	MG592133	MG976455
	CBS 114386 = A681	R. Afford, 11 Nov. 2003	*Quercus robur*, leaves with lesions	New Zealand	MG591852	MG591945	MG592040	MG592134	–
	CBS 114912	–	*Quercus* sp., green leaf	Netherlands	MG591853	MG591946	MG592041	MG592135	MG976456
	CBS 114915	–	*Quercus* sp., green leaf	Netherlands	MG591854	MG591947	MG592042	MG592136	–
	CBS 114919	–	*Quercus* sp., green leaf	Netherlands	MG591855	MG591948	MG592043	MG592137	–
	CBS 114920	–	*Quercus* sp., green leaf	Netherlands	MG591856	MG591949	MG592044	MG592138	–
	CBS 115007	–	*Quercus robur*, green leaf	Netherlands	MG591857	MG591950	MG592045	MG592139	–
	CBS 115009	–	*Quercus robur*, green leaf	Netherlands	MG591858	MG591951	MG592046	MG592140	–
	CBS 115012	–	*Quercus robur*, green leaf	Netherlands	MG591859	MG591952	MG592047	MG592141	–
	CBS 115014	–	*Quercus robur*, green leaf	Netherlands	MG591860	MG591953	MG592048	MG592142	–
	CBS 115096	–	*Quercus robur*, green leaf	Netherlands	MG591861	MG591954	MG592049	MG592143	–
	CBS 115097	–	*Quercus robur*, green leaf	Netherlands	MG591862	MG591955	–	–	–
	CBS 115098	–	*Quercus* sp., dead leaf	Netherlands	MG591863	MG591956	–	–	–
	CBS 115100	–	*Quercus* sp., dead leaf	Netherlands	MG591864	MG591957	MG592050	MG592144	–
	CBS 115102	–	*Quercus robur*, green leaf	Netherlands	MG591865	MG591958	MG592051	MG592145	–
	CBS 115306	–	*Quercus robur*, dead leaf	Netherlands	MG591866	MG591959	MG592052	MG592146	–
	CBS 115308	–	*Quercus robur*, dead leaf	Netherlands	MG591867	MG591960	MG592053	MG592147	–
	CBS 115971	–	–	Netherlands	MG591868	MG591961	MG592054	MG592148	–
	CBS 116070	–	–	Netherlands	MG591869	MG591962	MG592055	MG592149	–
	CBS 129016 = A876 = WO 60	D. McNew, 23 Mar. 2010	*Quercus alba*, twig	USA: Iowa	MG591870	MG591963	MG592056	MG592150	MG976457
	CBS 129018 = A996 = WO 165	D. McNew, 2 Aug. 2010	*Quercus macrocarpa*, leaf spots	USA: Iowa	MG591871	MG591964	MG592057	MG592151	–

Table 1. (Continued).

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
	CBS 213.66 = IFO 9101	H.A. van der Aa, 26 Jun. 1966	*Quercus robur*, leaf spot in young leaf	Netherlands	MG591872	MG591965	–	–	–
	EMS9	–	*Pinus tabulaeformis*, endophyte	China	AY546028	–	–	–	–
	mh1548.5	–	*Liquidambar styraciflua*, seedling upper stem	USA: North Carolina	GQ996086	–	–	–	–
Tubakia dryinoides	1-52	X.H. Wu, –	*Lindera glauca*, endophyte	China	JF502454	–	–	–	–
	2007LN001	–	*Quercus* sp.	China	FJ598616	–	–	–	–
	CBS 115970	–	–	Netherlands	AY853242	–	–	–	–
	CBS 329.75 = 847 B	M. Morelet, 14 May 1974	*Quercus* sp., fruits	France	MG591874	MG591967	MG592059	MG592153	MG976458
	CBS 335.86 = A682	H.A. van der Aa, –	*Cynips kollari*, gall on *Quercus pedunculata*	France	MG591875	–	MG592060	MG592154	–
	MUCC2290 = NBRC 9265 = ATCC 22471 = CBS 190.71	T. Yokoyama, 11 Nov. 1968	*Castanea crenata*, leaf	Japan	MG591876	MG591968	MG592061	MG592155	MG976459
	MUCC2291 = NBRC 9266	T. Yokoyama, 8 Oct. 1969	*Castanea crenata*	Japan	MG591877	MG591969	MG592062	MG592156	MG976460
	MUCC2292[Ex-type of *Tubakia dryinoides*] = NBRC 9267	T. Yokoyama, 8 Oct. 1969	*Quercus phillyraeoides*	Japan	MG591878	MG591970	MG592063	MG592157	MG976461
	O7-1141	–	Endophyte	China	FJ025349	–	–	–	–
Tubakia hallii	A663	–	*Quercus stellata*	USA: Missouri	TCH	TCH	–	–	–
	A664	–	*Quercus stellata*	USA: Missouri	TCH	TCH	–	–	–
	A906	–	*Quercus macrocarpa*	USA: Iowa	TCH	–	TCH	–	–
	A940	–	*Quercus macrocarpa*	USA: Missouri	TCH	–	TCH	–	–
	A969	–	*Quercus stellata*	USA: Missouri	TCH	–	TCH	–	–
	A1000	–	*Quercus stellata*	USA: Kansas	TCH	–	TCH	–	–
	A1102	M. Guthmiller, 23 Sep. 2011	*Quercus macrocarpa*	USA: Wisconsin	TCH	–	TCH	–	–
	A1188	–	*Quercus macrocarpa*	USA: Minnesota	TCH	–	–	–	–
	A1197	–	*Quercus macrocarpa*	USA: Wisconsin	TCH	–	–	–	–

Table 1. (Continued).

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
	CBS 129013[Ex-type of *Tubakia hallii*] = A666 = MDC 144-FB1	S. Kim, 2 Sep. 2009	*Quercus stellata*, leaf spot	USA: Missouri	MG591880	MG591972	MG592065	MG592159	MG976462
	CBS 129015 = A762 = W014	–, 27 Aug. 2009	*Quercus stellata*, leaf spot and vein	USA: Arkansas	MG591881	MG591973	MG592066	MG592160	–
	CBS 137348 = A949	D. McNew, 25 Aug. 2010	*Quercus macrocarpa*	USA: Iowa	TCH	–	–	–	–
	CPC 23753	–	*Quercus* sp., dead leaf	Iran	MG591884	MG591976	MG592069	MG592163	MG976463
Tubakia iowensis	A569	–	*Quercus macrocarpa*	USA: Iowa	TCH	TCH	–	–	–
	A573	–	*Quercus macrocarpa*, petiole	USA: Iowa	JF704196	JF704185	–	–	–
	A668	–	*Quercus macrocarpa*, endophyte	USA: Iowa	TCH	TCH	–	–	–
	A947	–	*Quercus macrocarpa*	USA: Nebraska	TCH	–	–	–	–
	A999	–	*Quercus macrocarpa*	USA: Kansas	–	–	TCH	–	–
	A1086	–	*Quercus macrocarpa*	USA: Minnesota	–	–	TCH	–	–
	A1088	–	*Quercus macrocarpa*	USA: Minnesota	TCH	–	TCH	–	–
	A1089	–	*Quercus macrocarpa*	USA: Wisconsin	TCH	–	TCH	–	–
	A1104	–	*Quercus macrocarpa*	USA: Wisconsin	TCH	–	TCH	–	–
	A1200	–	*Quercus bicolor*	USA: Iowa	TCH	–	–	–	–
	A1217	–	*Quercus macrocarpa*	USA: South Dakota	TCH	–	–	–	–
	A1219	–	*Quercus macrocarpa*	USA: Minnesota	TCH	–	–	–	–
	CBS 129012[Ex-type of *Tubakia iowensis*] = A607 = BH2-L(2)	T. Harrington, 21 Aug. 2008	*Quercus macrocarpa*, hanging leaf tissue	USA: Iowa	MG591879	MG591971	MG592064	MG592158	–
	CBS 129017 = A995 = WO164	K. Scanlon, Oct. 2010	*Quercus macrocarpa*, leaves	USA: Wisconsin	MG591882	MG591974	MG592067	MG592161	–
	CBS 129019 = A1003 = WO169 = BH2	D. McNew, 16 Nov. 2010	*Quercus macrocarpa*, petioles	USA: Iowa	MG591883	MG591975	MG592068	MG592162	–
Tubakia japonica	CBS 191.71 = A872 = IFO 9340 = MUCC2299 = NBRC 9340	T. Yokoyama, 2 Sep. 1970	*Castanea crenata*, leaf	Japan	MG591885	MG591977	MG592070	MG592164	MG976464
	MUCC2296[Ex-epitype of *Tubakia japonica*] = NBRC 9268 = ATCC 22472	K. Uchida, 13 Aug. 1969	*Castanea crenata*	Japan	MG591886	MG591978	MG592071	MG592165	MG976465

Table 1. (Continued).

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
	MUCC2297 = NBRC 9269	Y. Kobayashi, 17 Sep. 1969	*Castanea crenata*	Japan	–	MG591979	MG592072	MG592166	MG976466
	MUCC2298 = NBRC 9270	Y. Kobayashi, 17 Sep. 1969	*Castanea crenata*	Japan	–	MG591980	MG592073	MG592167	MG976467
	MUCC2300 = NBRC 9341	T. Yokoyama, 2 Sep. 1970	*Castanea crenata*	Japan	MG591887	MG591981	MG592074	MG592168	MG976468
	MUCC2301 = NBRC 9342	T. Yokoyama, 30 Oct. 1970	*Castanea crenata*	Japan	MG591888	MG591982	MG592075	MG592169	MG976469
Tubakia liquidambaris	CBS 139744 = A771	D. McNew, 27 Aug. 2009	*Liquidambar styraciflua*, leaf spot	USA: Arkansas	MG605068	MG605077	MG603578	–	–
	CBS 139745 = A830	D. McNew, 27 Aug. 2009	*Liquidambar styraciflua*	USA: Arkansas	TCH	–	TCH	–	–
Tubakia macnabbii	A1001	–	*Quercus* sp.	USA: Kansas	TCH	–	TCH	–	–
	A1039	–	*Quercus* sp.	–	TCH	–	TCH	–	–
	A1177	S. Latham, 27 Apr. 2012	*Quercus agrifolia*, twig	USA: California	TCH	–	–	–	–
	A1192	–	*Quercus rubra*	USA: New Hampshire	TCH	–	TCH	–	–
	A1193	–	*Quercus rubra*	USA: New Hampshire	TCH	–	–	–	–
	A608	–	*Quercus rubra*	USA: Iowa	TCH	TCH	–	–	–
	A655	–	*Quercus macrocarpa*	USA: Iowa	TCH	TCH	–	–	–
	A766	–	*Castanea dentata*	USA: Iowa	TCH	TCH	–	–	–
	A805	–	*Quercus velutina*	USA: Arkansas	TCH	–	TCH	–	–
	A806	–	*Quercus marilandica*	USA: Arkansas	TCH	–	TCH	–	–
	A809	–	*Quercus* sp.	USA: Arkansas	TCH	–	TCH	–	–
	A853	–	*Quercus nigra*	USA: Florida	TCH	–	TCH	–	–
	A859	–	*Quercus imbricaria*	USA: Missouri	TCH	–	TCH	–	–
	A929	D. McNew, 28 Jul. 2010	*Quercus rubra*, leaf	USA: Illinois	TCH	–	TCH	–	–
	A954	T. Harrington, 27 Aug. 2010	*Quercus macrocarpa*, leaf	USA: Iowa	TCH	–	TCH	–	–
	CBS 137346 = A810	D. McNew, 27 Aug. 2009	*Quercus muehlenbergii*, leaf vein	USA: Arkansas	MG605071	–	TCH	–	–

Table 1. (Continued).

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
	CBS 137347 = A852	T. Schubert, Nov. 2009	*Castanea dentata* x *mollissima* (Dunstan), leaf spot	USA: Florida	MG605070	–	TCH	–	–
	CBS 137349 = A989[Ex-type of *Tubakia macnabbii*]	D. Brandt, Sep. 2010	*Quercus palustris*, leaf spot	USA: Missouri	MG605069	–	MG603579	–	–
Tubakia melnikiana	CPC 32249 = CFNL 2933 = id 01	J. G. Marmolejo, 6 Oct. 2012	*Quercus canbyi*	Mexico	MG591889	MG591983	MG592076	MG592170	MG976470
	CPC 32252 = CFNL 2936 = id 04	J. G. Marmolejo, 29 Oct. 2012	*Quercus canbyi*	Mexico	MG591890	MG591984	MG592077	MG592171	MG976471
	CPC 32253 = id 05	J. G. Marmolejo, 29 Oct. 2012	*Quercus canbyi*	Mexico	MG591891	MG591985	MG592078	MG592172	–
	CPC 32254 = CFNL 2938 = id 06	J. G. Marmolejo, 29 Oct. 2012	*Quercus laeta* (= *Q. prinopsis*)	Mexico	MG591892	MG591986	MG592079	MG592173	–
	CPC 32255[Ex-type of *Tubakia melnikiana*] = CFNL 2939 = id 07	J. G. Marmolejo, 29 Oct. 2012	*Quercus canbyi*	Mexico	MG591893	MG591987	MG592080	MG592174	MG976472
Tubakia oblongispora	MUCC2295[Ex-type of *Tubakia oblongispora*] = NBRC 9885	T. Yokoyama, 11 Jun. 1972	*Quercus serrata*	Japan	MG591897	MG591991	MG592084	MG592178	MG976474
Tubakia paradryinoides	MUCC2294[Ex-type of *Tubakia paradryinoides*] = NBRC 9884	T. Kobayashi & K. Sasaki, 12 Oct. 1972	*Quercus acutissima*	Japan	MG591898	MG591992	MG592085	MG592179	MG976475
Tubakia seoraksanensis	CBS 127490[Ex-type of *Tubakia seoraksanensis*] = SA3V = A785	Hye Young Yun, 31 Aug. 2009	*Quercus mongolica*, living leaves	South Korea	MG591907	KP260499	MG592094	MG592186	–
	CBS 127491[Ex-isotype of *Tubakia seoraksanensis*] = SA3S = A786	Hye Young Yun, 31 Aug. 2009	*Quercus mongolica*, living leaves	South Korea	HM991735	KP260500	MG592095	MG592187	MG976484
	CBS 127492[Ex-isotype of *Tubakia seoraksanensis*] = SA4S = A787	Hye Young Yun, 31 Aug. 2009	*Quercus mongolica*, living leaves	South Korea	MG591908	MG592001	MG592096	MG592188	MG976485
	CBS 127493[Ex-isotype of *Tubakia seoraksanensis*] = SA4V = A788	Hye Young Yun, 31 Aug. 2009	*Quercus mongolica*, living leaves	South Korea	HM991737	MG592002	–	–	–
	CPC 26552	Ying Zhang, 23 Jul. 2014	*Quercus mongolica*	China	MG591909	MG592003	MG592097	MG592189	–
	CPC 26553	Ying Zhang, 23 Jul. 2014	*Quercus mongolica*	China	–	MG592004	MG592098	MG592190	–
Tubakia sierrafriensis	CPC 33020[Ex-type of *Tubakia sierrafriensis*] = CFNL 2944	O. Moreno-Rico, 19 Jan. 2017	*Quercus eduardi*	Mexico	MG591910	MG592005	MG592099	MG592191	MG976486
Tubakia sp. 1	CBS 115011	–	*Quercus robur*, green leaf	Netherlands	MG591912	MG592007	MG592101	MG592193	MG976488
Tubakia sp. E	A901	–	*Quercus* sp.	USA: Iowa	TCH	–	–	–	–
	NY7452a	K.S. Jefferson-George, –	*Quercus rubra*	USA: New York	HM855225	–	–	–	–
	NY7452b	K.S. Jefferson-George, –	*Quercus rubra*	USA: New York	HM855226	–	–	–	–

Table 1. (Continued).

Species name	Culture accession number(s)[1]	Collector and Collection date	Substrate (including host)	Country	GenBank accession number[2]				
					ITS	LSU	*tef1*	*tub2*	*rpb2*
Tubakia suttoniana	CBS 114911	–	*Quercus* sp., green leaf	Netherlands	MG591916	MG592011	MG592105	MG592197	–
	CBS 115006	–	*Quercus robur*, green leaf	Netherlands	MG591917	MG592012	MG592106	MG592198	–
	CBS 115300	–	*Quercus robur*, green leaf	Netherlands	MG591918	MG592013	MG592107	MG592199	–
	CBS 229.77	H.J. Boesewinkel, Nov. 1975	*Quercus cerris*, branch and trunk canker	New Zealand	MG591919	MG592014	MG592108	MG592200	MG976492
	CBS 387.89	Centro di Sperimentazione Agr. Forest., Roma, Italia, –	*Quercus rubra*, dead leaf	Italy	MG591920	MG592015	MG592109	MG592201	–
	CBS 639.93[Ex-isotype of *Dicarpella dryina*]	A. Belisario, Feb. 1989	*Quercus rubra*, overwintered fallen leaves	Italy	MG591921	MG592016	MG592110	MG592202	MG976493
Tubakia tiffanyae	A1035	–	*Quercus rubra*	USA: Iowa	TCH	–	TCH	–	–
	A1108	–	*Quercus rubra*	USA: Iowa	TCH	–	TCH	–	–
	A627	–	*Quercus rubra*	USA: Iowa	TCH	TCH	–	–	–
	A792	–	*Quercus imbricaria*	USA: Iowa	TCH	–	TCH	–	–
	A800	–	*Quercus rubra*	USA: Iowa	TCH	TCH	TCH	–	–
	A802	–	*Quercus rubra*	USA: Iowa	TCH	–	TCH	–	–
	A808	–	*Quercus rubra*	USA: Iowa	TCH	–	TCH	–	–
	CBS 137345[Ex-type of *Tubakia tiffanyae*] = A803	T. Harrington, 5 Sep. 2009	*Quercus rubra*, leaf spot	USA: Iowa	MG605081	–	MG603581	–	–
	CBS 137351 = A1042	–, 29 Jul. 2011	*Quercus rubra*, leaf spot	USA: Iowa	MG605082	–	TCH	–	–
	CBS 137352 = A1052	D. McNew, 27 Jul. 2011	*Quercus imbricaria*, leaf	USA: Iowa	TCH	–	TCH	–	–

[1] ATCC: American Type Culture Collection, Virginia, USA; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CDFA: CDFA Plant Pathogen Collections, 3294 Meadowview Road, Sacramento, CA 95832, USA; CFNL: Herbarium and culture collection at the Faculty of Forestry Sciences, University of Nuevo León, México; CPC: Culture collection of Pedro Crous, housed at CBS; IFO: Institute for Fermentation, Osaka, Yodogawa-ku, Osaka, Japan (collection transferred to NBRC); IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, United Kingdom; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCC (Japan): Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan; NBRC: NITE Biological Resource Center, Department of Biotechnology, National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan, TMI: Tottori Mycological Institute, Japan Kinoko Research Center Foundation, Tottori, Japan.

[2] ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: large subunit (28S) of the nrRNA gene operon; *tef1*: partial translation elongation factor 1-alpha gene; *tub2*: partial beta-tubulin gene; *rpb2*: partial DNA-directed RNA polymerase II second largest subunit gene. TCH refers to sequences obtained directly from Tom C. Harrington.

Table 2. List of primers used for amplification and sequencing in this study.

Name[1]	Sequence (5' – 3')	Orientation	Reference
ITS			
ITS4	TCC TCC GCT TAT TGA TAT GC	Reverse	White *et al*. (1990)
ITS5	GGA AGT AAA AGT CGT AAC AAG G	Forward	White *et al*. (1990)
LSU			
LR0R	GTA CCC GCT GAA CTT AAG C	Forward	Rehner & Samuels (1994)
LR5	TCC TGA GGG AAA CTT CG	Reverse	Vilgalys & Hester (1990)
rpb2			
RPB2-5F2	GGG GWG AYC AGA AGA AGG C	Forward	Sung *et al*. (2007)
RPB2-7cR	CCC ATR GCT TGY TTR CCC AT	Reverse	Liu *et al*. (1999)
tef1			
EF-2	GGA RGT ACC AGT SAT CAT GTT	Reverse	O'Donnell *et al*. (1998)
EF1-728F	CAT CGA GAA GTT CGA GAA GG	Forward	Carbone & Kohn (1999)
tub2			
Bt2a	GGT AAC CAA ATC GGT GCT GCT TTC	Forward	Glass & Donaldson (1995)
Bt2b	ACC CTC AGT GTA GTG ACC CTT GGC	Reverse	Glass & Donaldson (1995)

[1] ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: large subunit (28S) of the nrRNA gene operon; *rpb2*: partial DNA-directed RNA polymerase II second largest subunit gene; *tef1*: partial translation elongation factor 1-alpha gene; *tub2*: partial beta-tubulin gene.

tef1: 2 mM, *tub2*: 3 mM), 40–60 µM of dNTPs (ITS: 56 µM, LSU: 60 µM, *rpb2*, *tef1* and *tub2*: 40 µM), 5–5.5 % dimethyl sulfoxide (DMSO; ITS, LSU, and *tub2*: 5 %, *rpb2* and *tef1*: 5.5 %), 0.2 µM of each primer and 0.5 U *Taq* DNA polymerase (Bioline) in a total volume of 12.5 µL. The PCR cycling conditions for ITS, LSU, *tef1* and *tub2* were: initial denaturation (94 °C, 5 min); 35 cycles amplification (denaturation 94 °C for 30 s; annealing 48 °C for 50 s; extension 72 °C for 90 s), and final extension (72 °C, 7 min). The PCR cycling conditions for *rpb2* were: initial denaturation (95 °C, 5 min); 5 cycles amplification (denaturation 94 °C for 45 s, annealing 60 °C for 45 s, extension 72 °C for 2 min); 30 cycles amplification (denaturation 94 °C for 45 s, annealing 54 °C for 45 s, extension 72 °C for 2 min), and final extension (72 °C, 10 min). The resulting fragments were sequenced in both directions using the respective PCR primers and the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA, USA). DNA sequencing amplicons were purified through Sephadex G-50 Superfine columns (Sigma-Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were analysed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA, USA). The DNA sequences generated were analysed and consensus sequences were computed using SeqMan Pro v. 13 (DNASTAR, Madison, WI, USA). All novel sequences generated in this study were deposited in GenBank (Table 1).

The generated sequences for each gene were subjected to megablast searches (Zhang *et al*. 2000) to identify closely related sequences in the NCBIs GenBank nucleotide database. For the LSU and *rpb2* alignments, subsets of sequences from the alignments of Senanayake *et al*. (2017) and Fan *et al*. (2018) were used as backbones. For the *tef1* alignment, *tef1* sequences from Harrington & McNew (2018) were downloaded from GenBank or kindly supplied by the authors when not available in a public database. Loci were aligned with the online version of MAFFT v. 7 (Katoh & Standley 2013) after which the alignments were manually checked and improved where necessary using MEGA v. 7 (Kumar *et al.* 2016). Isolates missing both *tef1* and *tub2* sequences were excluded from the concatenated ITS/*tef1*/*tub2* alignments.

The phylogenetic methods used in this study included Bayesian analyses performed with MrBayes v. 3.2.6 (Ronquist *et al*. 2012) and Maximum Parsimony analyses performed with PAUP v. 4.0b10 (Swofford 2003) as explained in Videira *et al*. (2017). For the Bayesian analyses, trees were sampled every 100 generations, the temperature parameter was set to 0.25 and the stop value to 0.01. The Bayesian analyses were applied to the separate overview LSU and *rpb2* alignments as well as the concatenated ITS/*tef1*/*tub2* alignments and the expanded *tef1* alignment after MrModelTest v. 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model settings for each data partition.

All resulting trees were printed with Geneious v. 11.0.3 (http://www.geneious.com, Kearse *et al.* 2012) and the layout of the trees was done in Adobe Illustrator v. CC 2017. The alignments and respective phylogenetic trees were deposited in TreeBASE, study number 21897.

Morphology

All fungal structures were examined by means of light microscopy, using an Olympus BX50 or Zeiss Axio imager A1 microscope. Shear's liquid or distilled water and lactic acid were used as mounting media, and aniline blue (cotton blue) was used to stain colourless structures (columella, conidiogenous

structures, and conidia). If possible, measurements of 30 conidia and other structures were made at a magnification of ×1000, and the 95 % confidence intervals were determined (extreme values in parentheses). Cultures were studied on MEA, OA, PDA, and PNA. Colony colours were rated using the charts of Rayner (1970).

RESULTS

Phylogeny

LSU phylogeny

The alignment contained 127 isolates representing a large majority of families known from sequence data belonging to *Diaporthales*. A strain of *Phialemonium atrogriseum* (CBS 981.70, GenBank HQ231984.1; *Sordariales*) was used as outgroup. The final alignment contained a total of 704 characters used for the phylogenetic analyses, including alignment gaps. MrModelTest recommended that the Bayesian analysis should use dirichlet base frequencies and the GTR+I+G model. The Bayesian analyses generated 153 402 trees from which 115 052 trees were sampled after 25 % of the trees were discarded as burn-in. The posterior probability values (PP) were calculated from the 115 052 trees (Fig. 1; first value: PP >0.74 shown). The alignment contained a total of 208 unique site patterns. The Maximum Parsimony (MP) analyses generated the maximum of 1 000 equally most parsimonious trees and the bootstrap support values (MP-BS) were mapped on the Bayesian tree as the second value (Fig. 1; MP-BS >74 % shown). From the analysed characters, 481 were constant, 63 were variable and parsimony-uninformative and 160 were parsimony-informative. A strict consensus tree was calculated from the equally most parsimonious trees and the branches were mapped with a thicker line on the Bayesian tree (Fig. 1; Length = 790, CI = 0.376, RI = 0.841, RC = 0.316). The parsimony phylogeny showed the same terminal family clades as those presented in the Bayesian phylogeny (Fig. 1); however, the order of the clades was different, for example, in the parsimony analysis *Apoharknessiaceae* was the sister clade to *Tubakia* and not *Melanconiellaceae* (data not shown, available in TreeBASE).

Twenty-one families are represented in the phylogenetic tree (Fig. 1). The majority of families were supported by high posterior probability or bootstrap support values. However, *Stilbosporaceae* was only fully supported by the Bayesian analysis whilst *Harknessiaceae* did not receive significant support from either the Bayesian or the maximum parsimony analysis. *Cryphonectriaceae* and *Melanconiellaceae* each received moderate Bayesian posterior probability support but no maximum parsimony bootstrap support. The clade containing the *Tubakia* sequences was fully supported in both the Bayesian and maximum parsimony analyses and a novel family is proposed for this genus below. The node connecting *Melanconiellaceae* and *Tubakiaceae* was only fully supported in the Bayesian analysis but not supported in the maximum parsimony analysis. A sequence of the ex-type culture of "*Greeneria" saprophytica* is also included in this clade; this species was described by Tangthirasunun *et al*. (2014) but no *Tubakia* sequences were included in their phylogenetic tree. Our phylogenetic tree shows that this species has to be excluded from *Greeneria* and is better accommodated in *Tubakiaceae*. Supported by the isolated position in the LSU tree within the *Tubakiaceae* but outside of *Tubakia s. str*. and distant from all other genera of the family, this species warrants a genus of its own. Phylogenetic signals of LSU are low within *Diaporthales* in general and its families. Therefore, phylogenetic analyses using *rpb2* sequences were also performed to distinguish whether the family *Tubakiaceae* contains a single genus, *Tubakia*, or several genera, but even the overall topology of the LSU tree suggests the heterogeneity of *Tubakia s. lat.*

rpb2 *phylogeny*

The alignment contained 130 isolates representing a large majority of families known from sequence data belonging to *Diaporthales*. A strain of *Sordaria fimicola* (CBS 723.96, GenBank DQ368647.1; *Sordariales*) was used as outgroup. The final alignment contained a total of 785 characters used for the phylogenetic analyses, including alignment gaps. MrModelTest recommended that the Bayesian analysis should use dirichlet base frequencies and the GTR+I+G model. The Bayesian analyses generated 6 742 trees from which 5 058 trees were sampled after 25 % of the trees were discarded as burn-in. The posterior probability values (PP) were calculated from the 5 058 trees (Fig. 2; first value: PP >0.74 shown). The alignment contained a total of 473 unique site patterns. The Maximum Parsimony (MP) analyses generated the maximum of 20 equally most parsimonious trees and the bootstrap support values (MP-BS) were mapped on the Bayesian tree as the second value (Fig. 2; MP-BS >74 % shown). From the analysed characters, 313 were constant, 28 were variable and parsimony-uninformative and 444 were parsimony-informative. A strict consensus tree was calculated from the equally most parsimonious trees and the branches were mapped with a thicker line on the Bayesian tree (Fig. 2; Length = 3 934, CI = 0.237, RI = 0.792, RC = 0.188). The parsimony phylogeny showed the same overall topology as that presented in the Bayesian phylogeny (Fig. 2); however, the order of the genera in *Gnomoniaceae* differed slightly between the analyses (data not shown, available in TreeBASE).

Twelve families are represented in the phylogenetic tree (Fig. 2). The majority of families was supported by high posterior probability and bootstrap support values. However, *Melanconiellaceae* and *Tubakiaceae* were both only fully or highly supported by the Bayesian analysis while the node connecting *Melanconiellaceae* and *Tubakiaceae* was only fully supported in the Bayesian analysis and supported with 90 % in the maximum parsimony analysis. The *rpb2* sequences demonstrate a clear substructure in *Tubakia s. lat.* with highly supported lineages, and therefore several novel genera are introduced in the Taxonomy section below to accommodate those lineages.

*Combined ITS/*tef1*/*tub2 *phylogeny – all species*

It was not possible to generate all loci for all of the included isolates, mainly due to the fact that some loci failed to amplify for some isolates, even though several attempts were made to obtain a product suitable for sequencing. To reduce the amount of missing data in the alignment, such isolates were excluded from the analyses. The alignment contained 95 isolates representing *Tubakia* and allied taxa, and a strain of *Melanconis groenlandica* (CBS 116540; GenBank KU878552.1, KU878554.1 and KU878555.1, respectively ITS/*tef1*/*tub2*) was used as outgroup. The final alignment contained a total of 1 740 characters used for the phylogenetic analyses, including alignment gaps. The Maximum Parsimony (MP) analyses generated the maximum of 1 000 equally most parsimonious

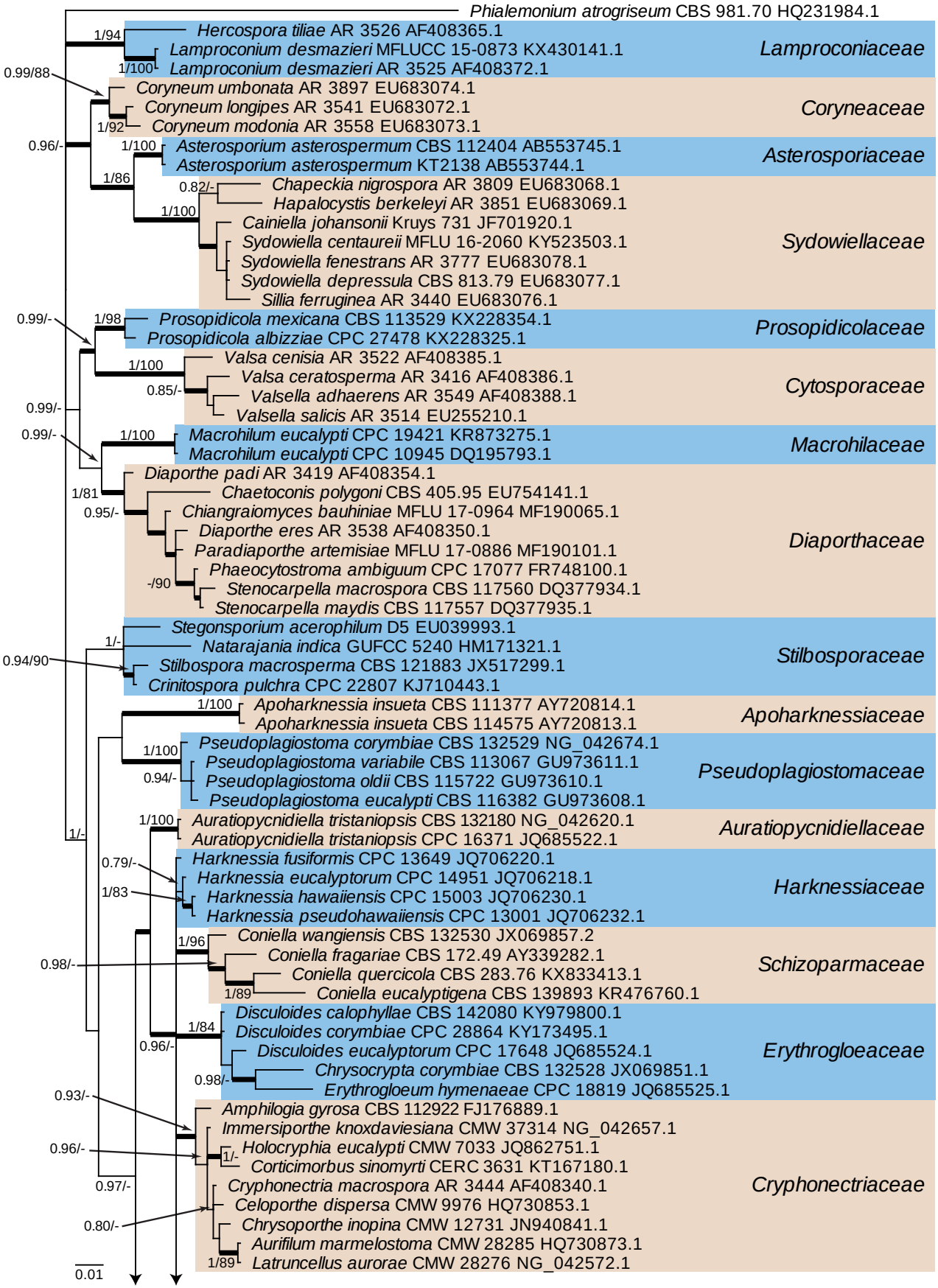

Fig. 1. Consensus phylogram (50 % majority rule) from a Bayesian analysis of the LSU sequence alignment. Bayesian posterior probabilities (PP) >0.74 and maximum parsimony bootstrap support values (MP-BS) >74 % are shown at the nodes (PP/MP-BS) and thickened lines represent those branches present in the strict consensus maximum parsimony tree. The scale bar represents the expected changes per site. Families are indicated with coloured blocks to the right of the tree and the genera in *Tubakiaceae* are highlighted on the tree. Culture/specimen and GenBank accession numbers are indicated behind the species names. The tree is rooted to *Phialemonium atrogriseum* (GenBank HQ231984.1) and the novel family and genera are indicated in **bold** face.

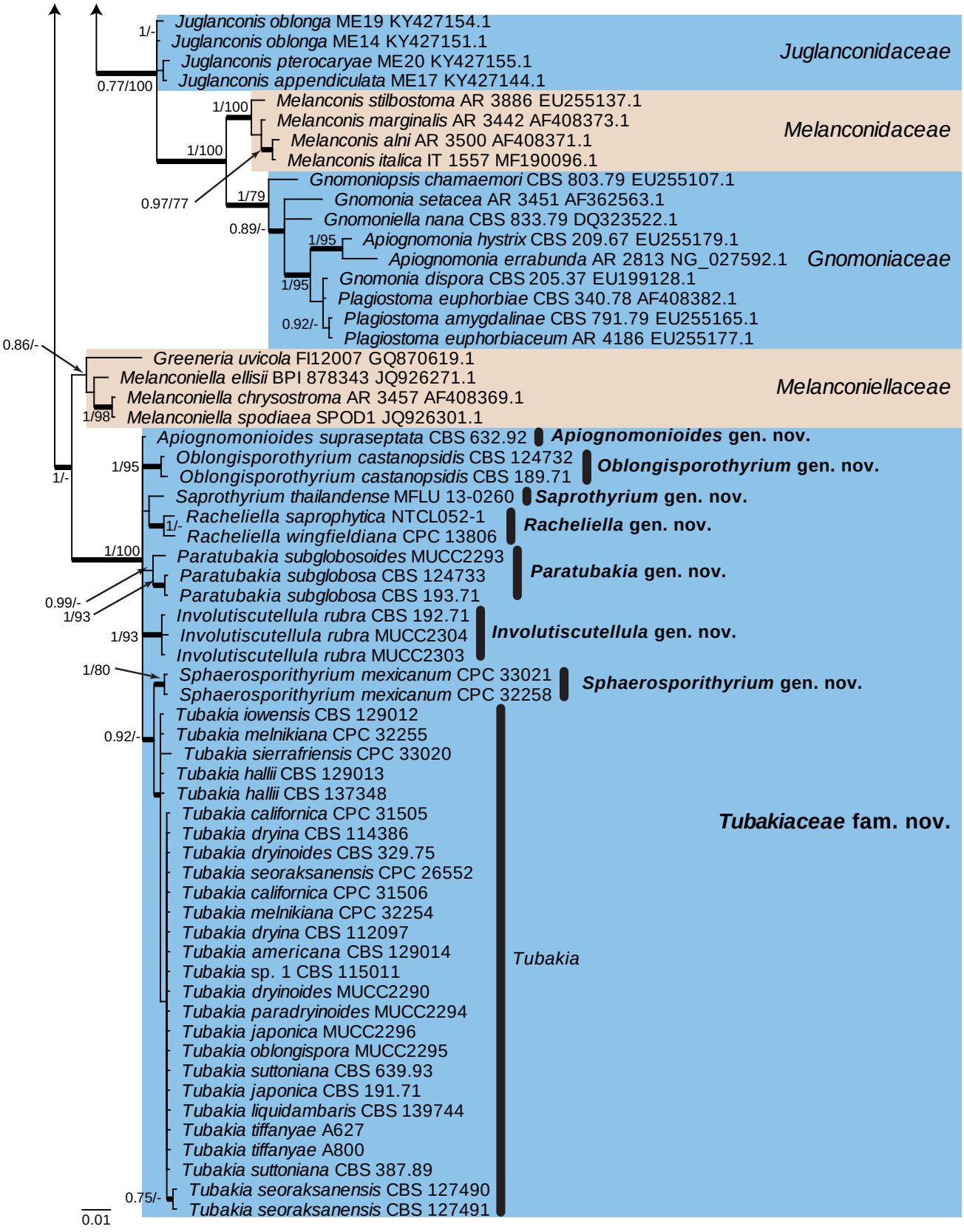

Fig. 1. (Continued).

trees, the first of which is shown in Fig. 3 (Length = 2 660, CI = 0.665, RI = 0.916, RC = 0.609), and the bootstrap support values (MP-BS) were mapped on the tree as the second value (MP-BS >74 % shown). From the analysed characters, 734 were constant, 184 were variable and parsimony-uninformative and 822 were parsimony-informative. A strict consensus tree was calculated from the equally most parsimonious trees and the strict consensus branches were mapped with a thicker line on the presented phylogenetic tree (Fig. 3). MrModelTest recommended that the Bayesian analysis should use dirichlet base frequencies for the *tef1* and *tub2* data partitions and equal base frequencies for the ITS partition. The SYM+I+G model was

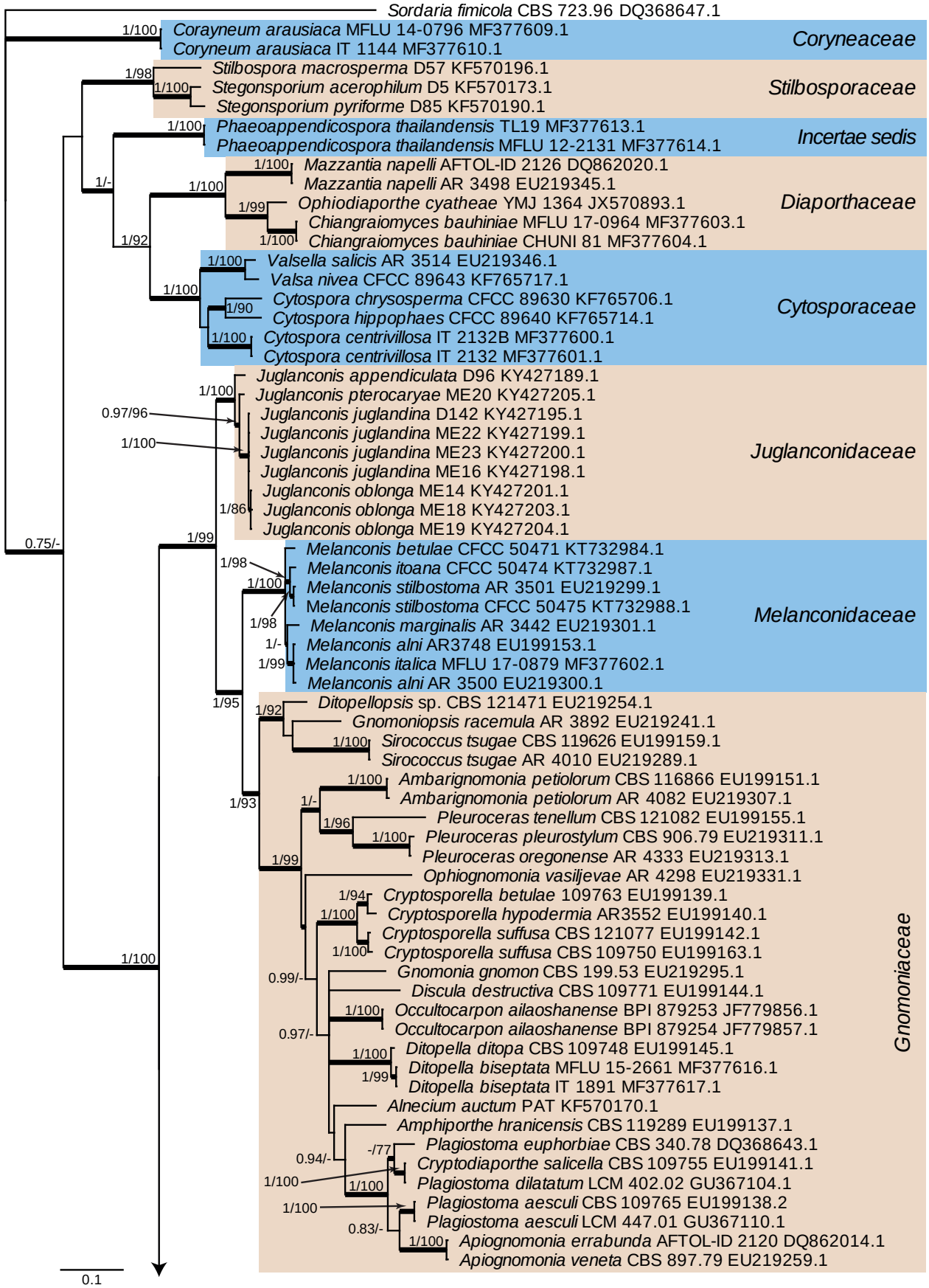

Fig. 2. Consensus phylogram (50 % majority rule) from a Bayesian analysis of the *rpb2* sequence alignment. Bayesian posterior probabilities (PP) >0.74 and maximum parsimony bootstrap support values (MP-BS) >74 % are shown at the nodes (PP/MP-BS) and thickened lines represent those branches present in the strict consensus maximum parsimony tree. The scale bar represents the expected changes per site. Families are indicated with coloured blocks to the right of the tree and the genera in *Tubakiaceae* are highlighted on the tree. Culture/specimen and GenBank accession numbers are indicated behind the species names. The tree is rooted to *Sordaria fimicola* (GenBank DQ368647.1) and the novel family and genera are indicated in **bold** face.

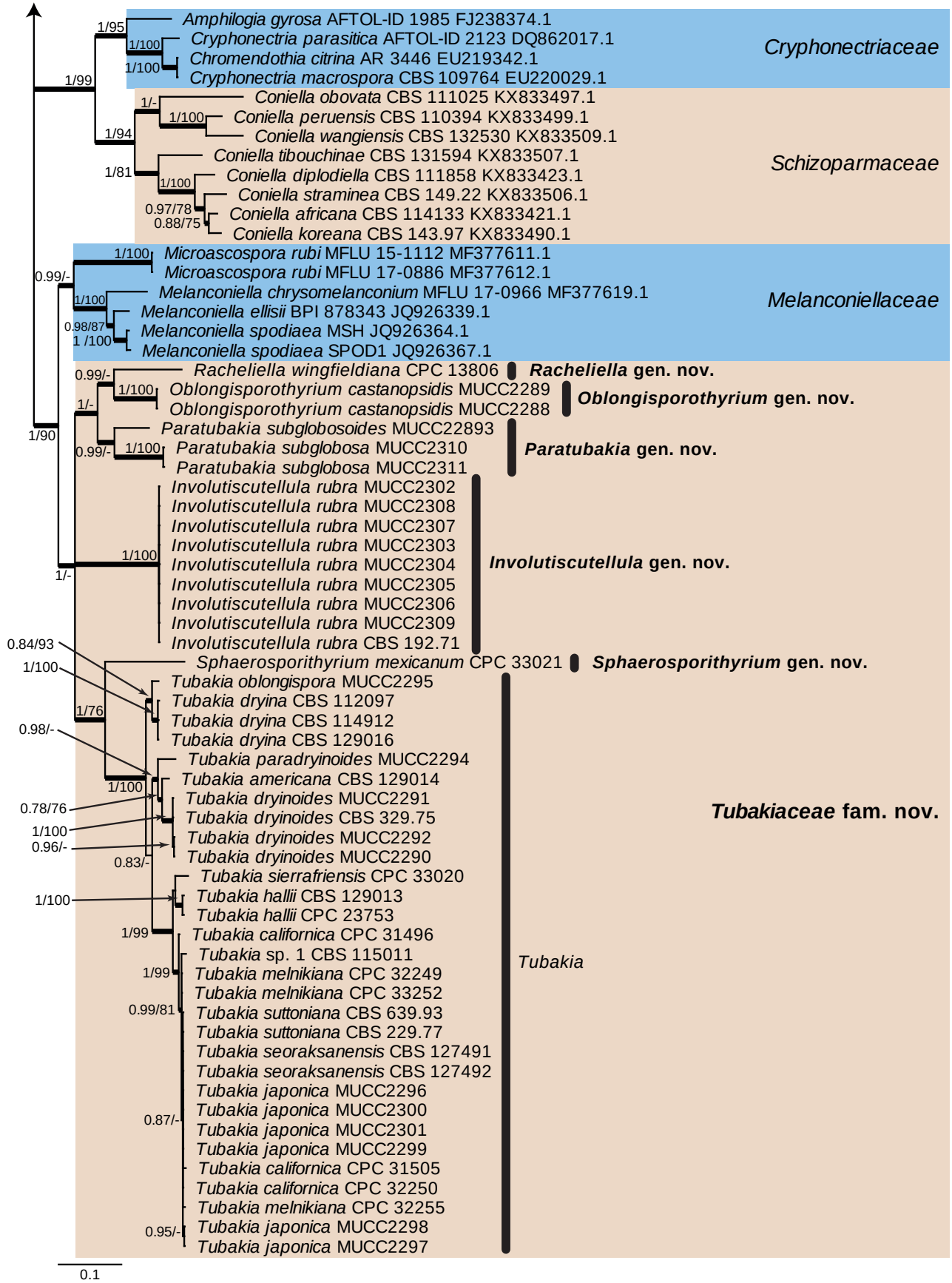

Fig. 2. (Continued).

proposed for ITS and HKY+I+G for *tef1* and *tub2*. The Bayesian analyses generated 12 702 trees from which 9 528 trees were sampled after 25 % of the trees were discarded as burn-in. The posterior probability values (PP) were calculated from the 9 528 trees (Fig. 3; first value: PP >0.74 shown). The alignment contained a total of 925 unique site patterns (ITS: 229, *tef1*: *423*, *tub2*: 273). The Bayesian phylogeny supported the same terminal clades as those presented in the parsimony phylogeny (Fig. 3), with some rearrangements in the order of the clades (data not shown, available in TreeBASE).

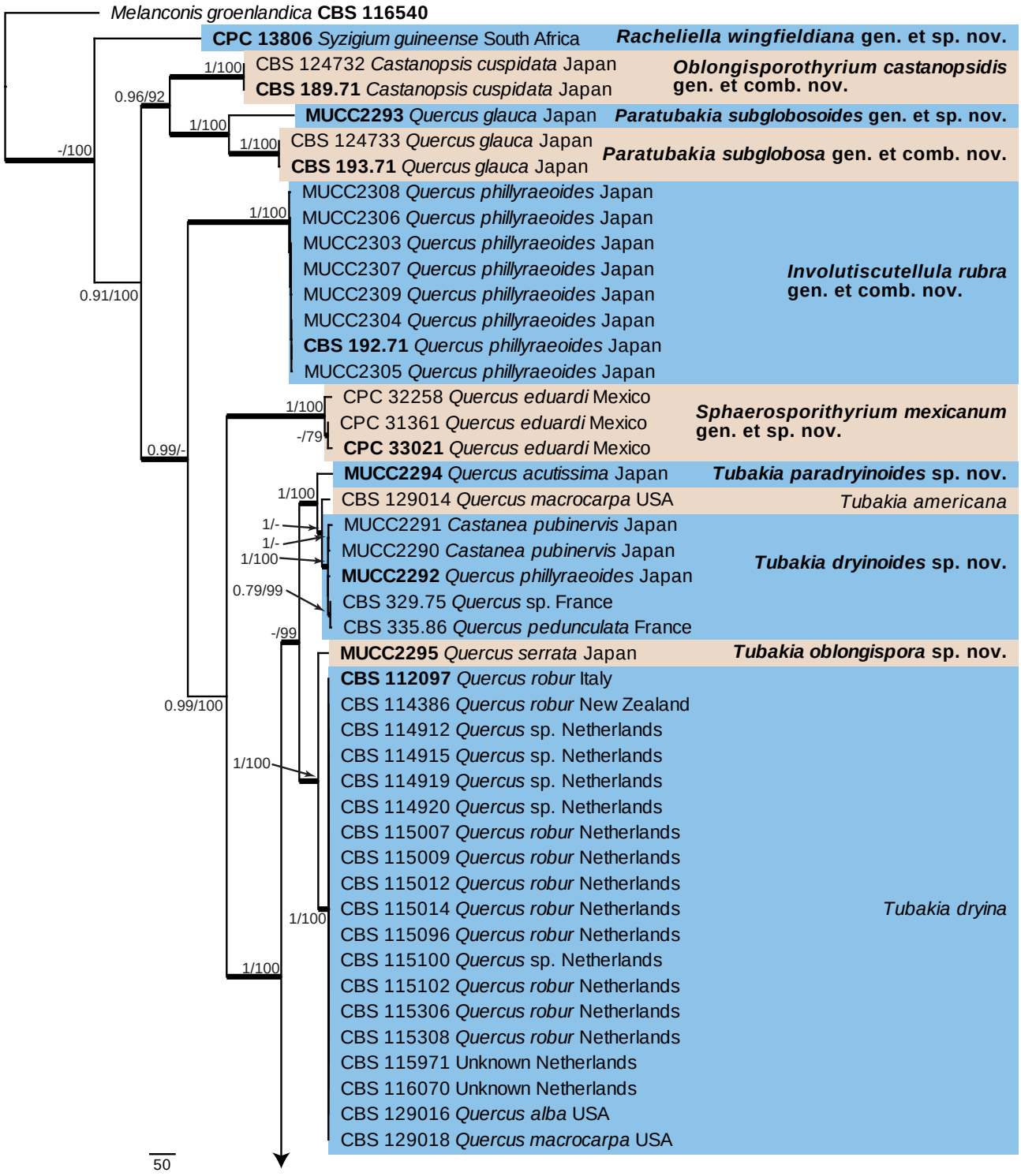

Fig. 3. The first of 1 000 equally most parsimonious trees obtained from the combined ITS/*tef1*/*tub2* alignment. Bayesian posterior probabilities (PP) >0.74 and maximum parsimony bootstrap support values (MP-BS) >74 % are shown at the nodes (PP/MP-BS) and thickened lines represent those branches present in the strict consensus maximum parsimony tree. The scale bar represents the number of changes per site. Species and species complexes are indicated with coloured blocks to the right of the tree. Culture numbers, host and country of origin are indicated for each strain. The tree is rooted to *Melanconis groenlandica* (culture CBS 116540) and taxonomic novelties and ex-type cultures are indicated in **bold** face.

Eighteen species lineages/clades are represented in the phylogenetic tree (Fig. 3). The majority of species were supported by high posterior probability or bootstrap support values, especially in the basal part of the tree (Fig. 3, part 1). However, the bottom half of the second part of the phylogeny was not as well resolved as the basal part of the phylogeny, with most of the phylogenetic signal coming from the *tub2* sequences.

Overall, the same species clades/lineages are observed in the individual gene trees, although the order or basal organisation sometimes changed between the different loci (data not shown). ITS was the least successful in resolving species clades (for example *T. paradryinoides*/*dryinoides* and the *T. suttoniana* complex). In addition, two isolates of *T. japonica* (MUCC2297, MUCC2298) and one of *T. seoraksanensis* (CPC 26553) clustered in the *T. dryina* clade. The *tef1* phylogeny could not resolve

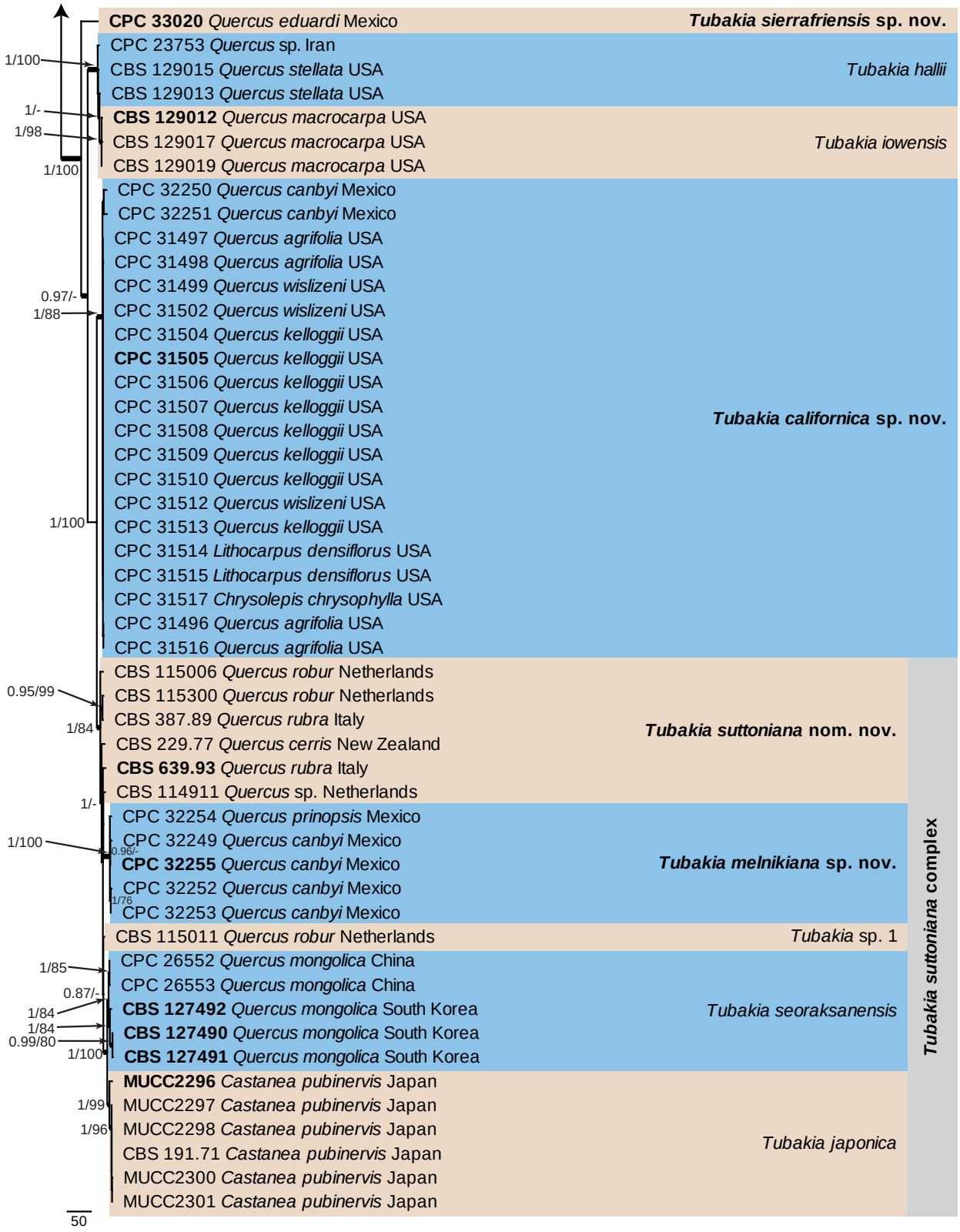

Fig. 3. (Continued).

T. paradryinoides/dryinoides and isolates of *T. suttoniana* did not cluster together in a monophyletic lineage. The *tub2* phylogeny provided the best resolution for *T. oblongispora* and *T. paradryinoides*. However, two isolates of *T. rubra* (MUCC2304, MUCC2306) clustered in the *T. dryinoides* clade and one isolate of *T. californica* (CPC 32250) clustered in the *T. melnikiana* clade. Given the overlap in hosts and geographical distribution between the different species, exchange of genetic material between the different species cannot be ruled out.

*Combined ITS/*tef1*/*tub2 *phylogeny* – T. suttoniana *complex*

A subset combined alignment was subjected to Bayesian and parsimony analyses to better resolve the species in the *T. suttoniana* complex. The alignment contained 48 isolates and a strain of *Tubakia castanopsidis* (CBS 189.71; GenBank accession numbers listed in Table 1) was used as outgroup. The final alignment contained a total of 1 542 characters used for the phylogenetic analyses, including alignment gaps. The Maximum Parsimony (MP) analyses generated four equally most

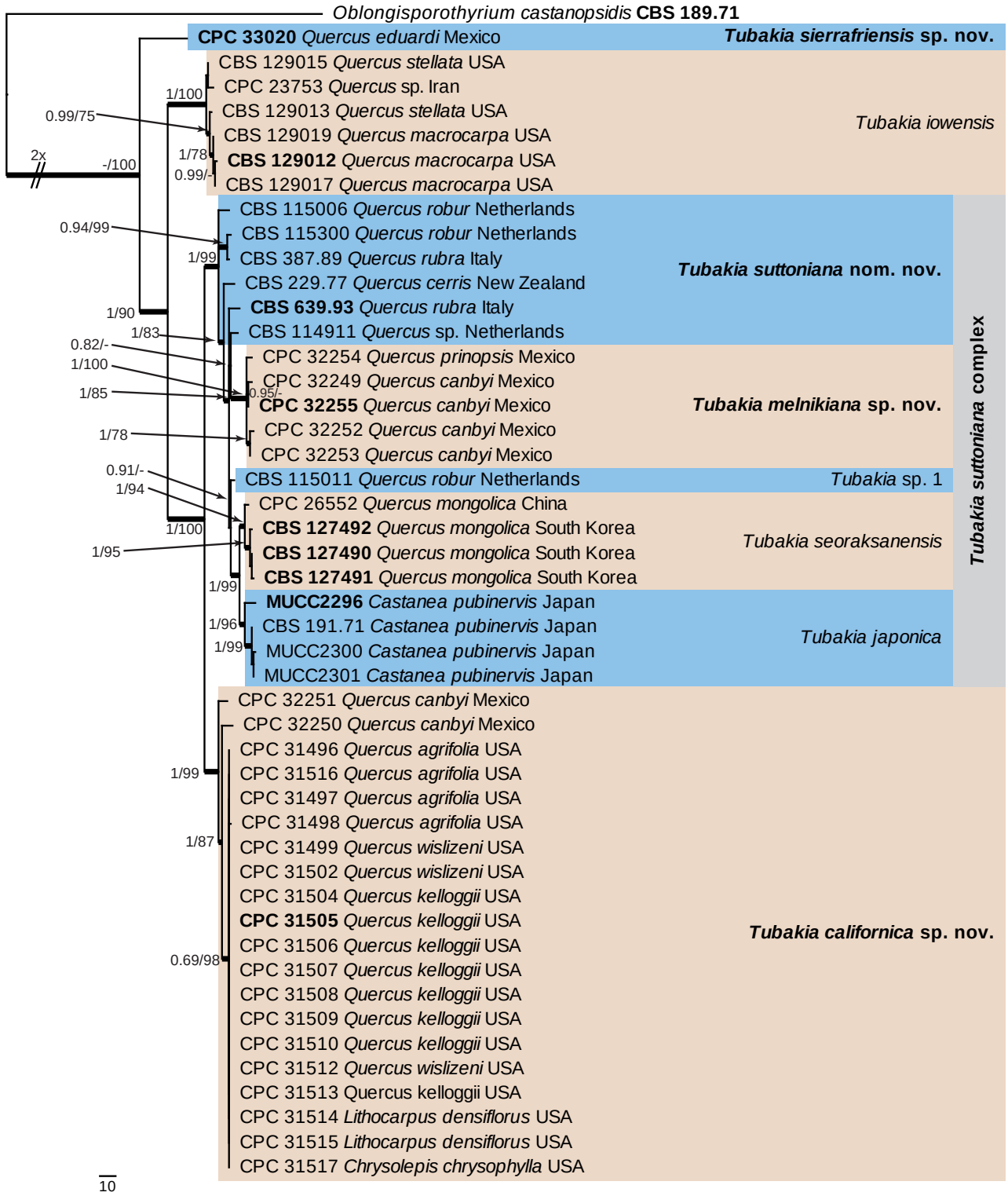

Fig. 4. The first of four equally most parsimonious trees obtained from the combined ITS/*tef1*/*tub2* alignment focused on the *T. suttoniana* complex and closely related species. Bayesian posterior probabilities (PP) >0.74 and maximum parsimony bootstrap support values (MP-BS) >74 % are shown at the nodes (PP/MP-BS) and thickened lines represent those branches present in the strict consensus maximum parsimony tree. The scale bar represents the number of changes per site. Species and species complexes are indicated with coloured blocks to the right of the tree. Culture numbers, host and country of origin are indicated for each strain. The tree is rooted to *Oblongisporothyrium castanopsidis* (culture CBS 189.71) and taxonomic novelties and ex-type cultures are indicated in **bold** face. The length of the most basal branch was halfed to facilitate layout.

parsimonious trees, the first of which is shown in Fig. 4 (Length = 553, CI = 0.915, RI = 0.947, RC = 0.867), and the bootstrap support values (MP-BS) were mapped on the tree as the second value (MP-BS >74 % shown). From the analysed characters, 1 097 were constant, 322 were variable and parsimony-uninformative and 123 were parsimony-informative. A strict consensus tree was calculated from the equally most parsimonious trees and the strict consensus branches were mapped with a thicker line on the presented phylogenetic tree (Fig. 4). MrModelTest recommended that the Bayesian analysis should use dirichlet base frequencies for the *tef1* and *tub2* data partitions and equal base frequencies for the ITS partition. The SYM+I+G model was

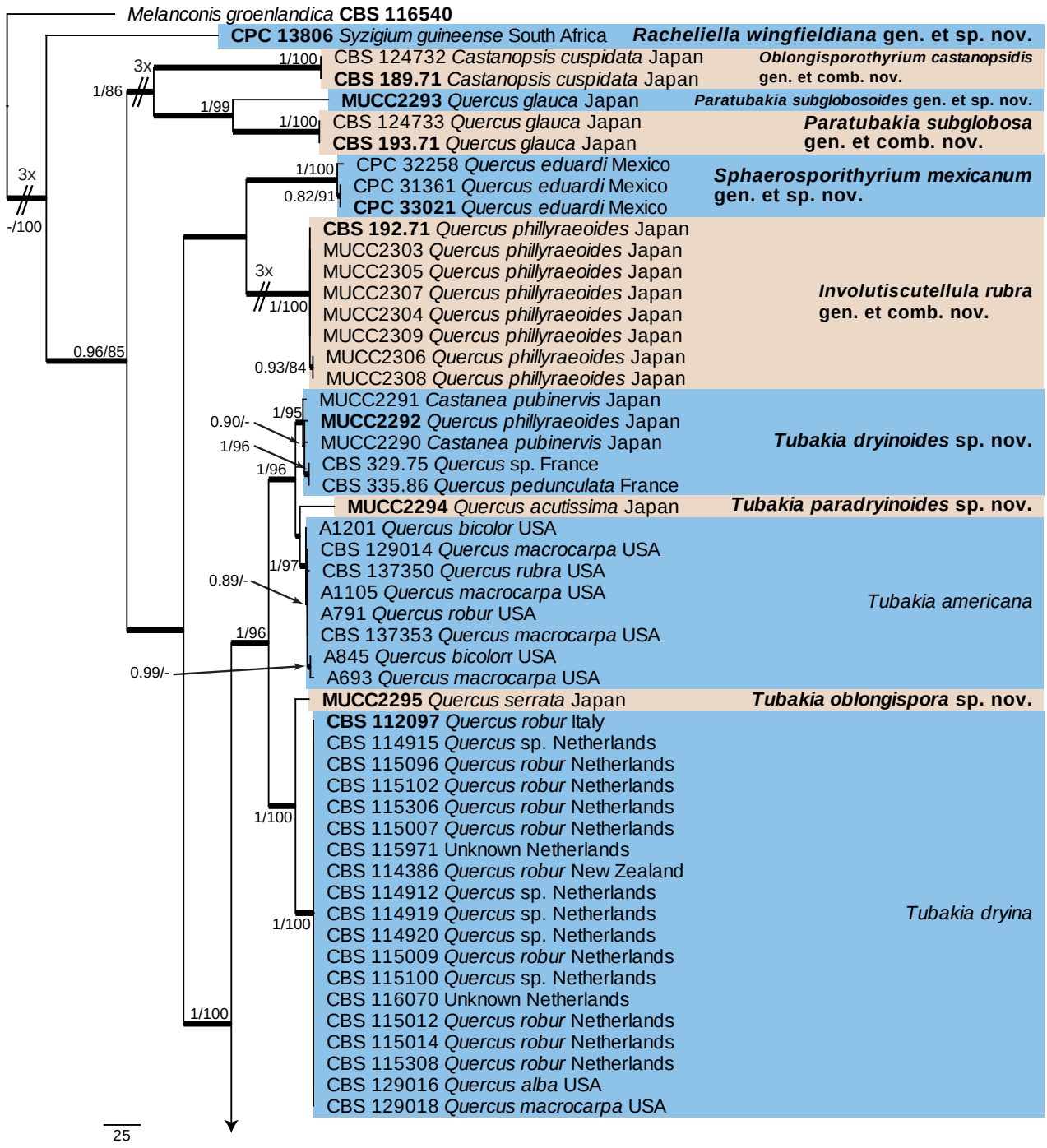

Fig. 5. The first of 1 000 equally most parsimonious trees obtained from the expanded *tef1* alignment of species in the *Tubakiaceae*. Bayesian posterior probabilities (PP) >0.74 and maximum parsimony bootstrap support values (MP-BS) >74 % are shown at the nodes (PP/MP-BS) and thickened lines represent those branches present in the strict consensus maximum parsimony tree. The scale bar represents the number of changes per site. Species and species complexes are indicated with coloured blocks to the right of the tree. Culture numbers, host and country of origin are indicated for each strain. The tree is rooted to *Melanconis groenlandica* (culture CBS 116450) and taxonomic novelties and ex-type cultures are indicated in **bold** face. The lengths of some of the most basal branches were shortened to facilitate layout.

proposed for ITS and HKY+I+G for *tef1* and *tub2*. The Bayesian analyses generated 2 602 trees from which 1 952 trees were sampled after 25 % of the trees were discarded as burn-in. The posterior probability values (PP) were calculated from the 1 952 trees (Fig. 4; first value: PP >0.74 shown). The alignment contained a total of 310 unique site patterns (ITS: 63, *tef1*: *149*, *tub2*: 98). The Bayesian phylogeny supported the same terminal clades as those presented in the parsimony phylogeny (Fig. 4; data not shown, available in TreeBASE) and the same species as described above for Fig. 3.

tef1 *phylogeny*

A *tef1* phylogeny was also generated (Fig. 5) to enable a more direct comparison of the data in the present paper with the recently published data from Harrington & McNew (2018). The alignment contained 136 isolates and a strain of *Melanconis groenlandica* (CBS 116540; GenBank accession numbers listed in Table 1) was used as outgroup. The final alignment contained a total of 592 characters used for the phylogenetic analyses, including alignment gaps. The Maximum Parsimony (MP) analyses generated the maximum of 1 000 equally most

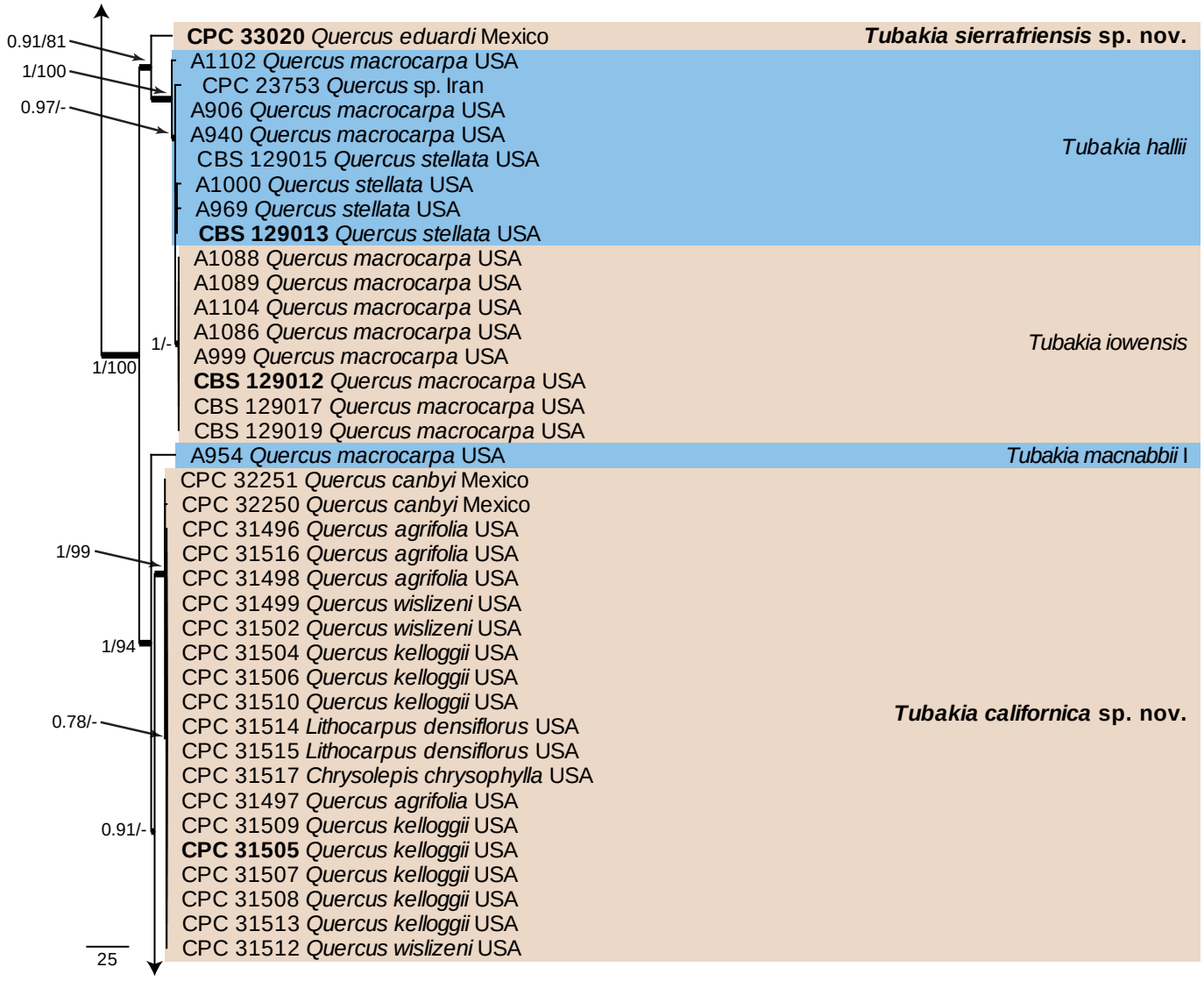

Fig. 5. (Continued).

parsimonious trees, the first of which is shown in Fig. 5 (Length = 1 356, CI = 0.620, RI = 0.919, RC = 0.570), and the bootstrap support values (MP-BS) were mapped on the tree as the second value (MP-BS >74 % shown). From the analysed characters, 179 were constant, 39 were variable and parsimony-uninformative and 374 were parsimony-informative. A strict consensus tree was calculated from the equally most parsimonious trees and the strict consensus branches were mapped with a thicker line on the presented phylogenetic tree (Fig. 5). MrModelTest recommended that the Bayesian analysis should use dirichlet base frequencies and the HKY+I+G model. The Bayesian analyses generated 19 002 trees from which 14 252 trees were sampled after 25 % of the trees were discarded as burn-in. The posterior probability values (PP) were calculated from the 14 252 trees (Fig. 5; first value: PP >0.74 shown). The alignment contained 400 unique site patterns. The Bayesian phylogeny showed the same terminal clades as those presented in the parsimony phylogeny with some rearrangements in the backbone of the tree (Fig. 5; data not shown, available in TreeBASE). This *tef1* phylogeny highlights the close relation between *T. hallii* and *T. iowensis* and the broad concept currently being applied to *T. macnabbii* and *T. suttoniana*.

TAXONOMY

Tubakiaceae U. Braun, J.Z. Groenew. & Crous, ***fam. nov.*** MycoBank MB823660.

Type genus: *Tubakia* B. Sutton.

Classification: *Ascomycota, Pezizomycotina, Sordariomycetes, Sordariomycetidae, Diaporthales.*

Saprobic, endophytes in leaves and twigs and plant pathogens causing leaf spots and twig dieback. *Asexual morphs*: sporodochia, crustose to pustulate pycnidioid stromatic conidiomata and superficial scutellate pycnothyria. *Conidiogenous cells* monophialidic, colourless, often with collarettes. *Conidia* formed singly, mostly globose to broad ellipsoid-obovoid, aseptate, hyaline to pigmented, often with basal frill or truncate peg-like hilum. *Sexual morphs*: apiognomonia- and dicarpellum-like, diaporthaloid, dark, rostrate, ostiolate perithecial ascomata, with dark stromatic layers, polyascal; *asci* unitunicate, 8-spored; *ascospores* aseptate or with a single septum near the apex, hyaline.

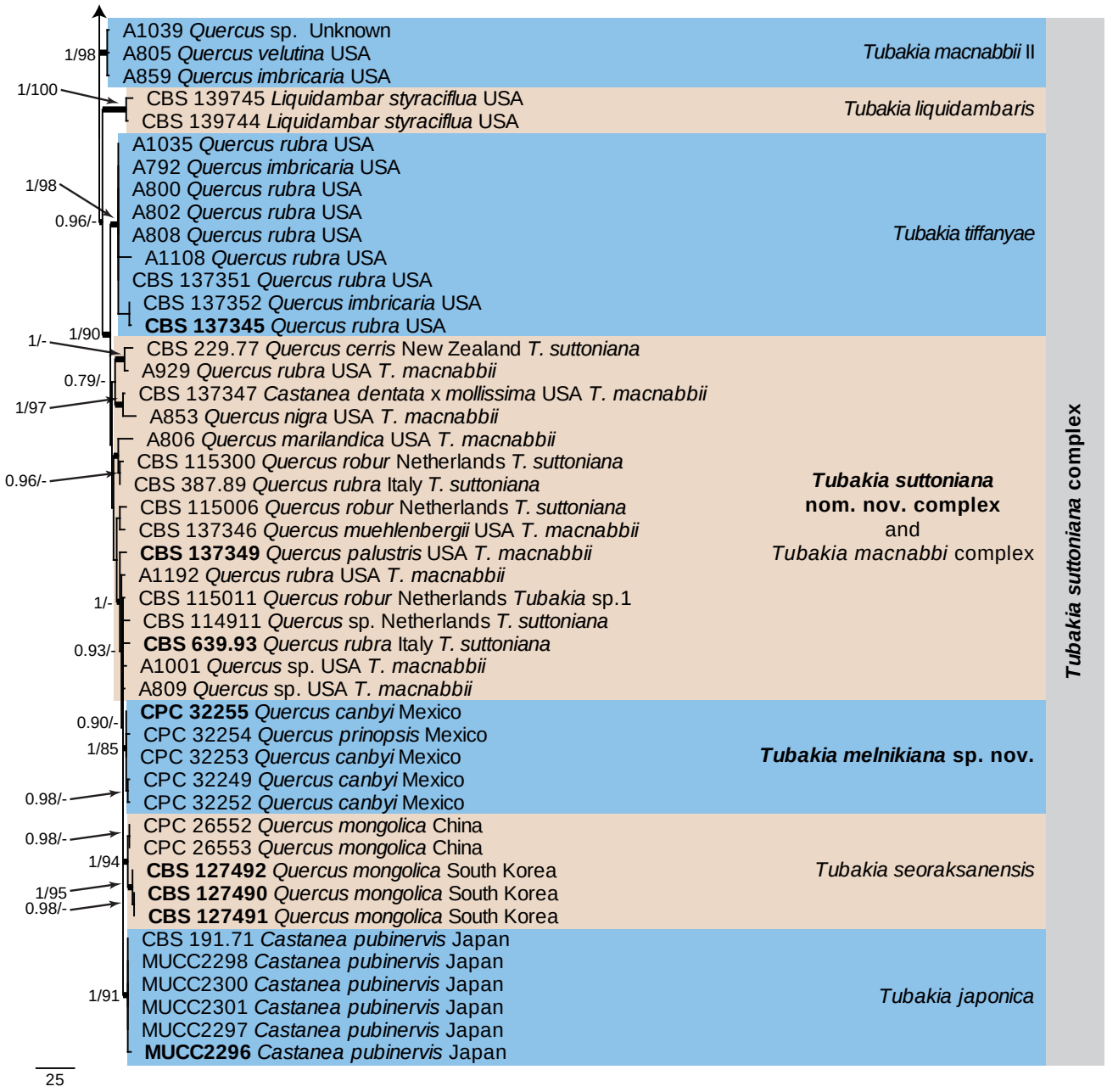

Fig. 5. (Continued).

Notes: The clade containing all former *Tubakia* species was highly supported in both Bayesian and maximum parsimony analyses (Fig. 1; PP = 1, MP-BS = 99 %). In addition, its sister relation changed between the two analyses; in the Bayesian analysis, it was sister to *Melanconiellaceae* and in the maximum parsimony analysis it was sister to *Apoharknessiaceae*. The branch separating the *Tubakiaceae* clade from its closest sister clade was longer than the branches separating several other families in the phylogeny, e.g. *Melanconidaceae* / *Gnomoniaceae* and *Harknessiaceae* / *Schizoparmaceae* (Fig. 1). Analyses of LSU and above all *rpb2* data and corresponding trees clearly indicate the heterogeneity of the genus *Tubakia s. lat*. Some lineages are included that warrant the division of *Tubakia s. lat*. into several genera.

Apiognomonioides U. Braun, J.Z. Groenew. & Crous, ***gen. nov.*** MycoBank MB824479.

Etymology: Composed of the name of the genus *Apiognomonia* and -oides (resembling), referring to the similarity between the new genus and the latter one.

Type species: *Apiognomonioides supraseptata* (Kaneko & Kobayashi) U. Braun, J.Z. Groenew. & Crous (≡ *Apiognomonia supraseptata* Kaneko & Kobayashi).

Genus of *Tubakiaceae*. *Sexual morph* reseming *Apiognomonia*, but ascospores with a single septum near the apex. *Perithecia* immersed, globose to depressed, with a central to rarely eccentric beak, erumpent, perithecial wall 2–3 cell layers thick, composed of dark brown, flattened cells and a hyaline innermost layer; *asci* numerous, clavate to cylindrical-clavate, with an apical ring at the thickened apex, unitunicate, 8-spored; *ascospores* irregularly biseriate, ellipsoid, 1-septate near the apex, slightly constricted at the septum, hyaline, thin-walled.

Notes: Kaneko & Kobayashi (1984) introduced the name *Apiognomonia supraseptata* on the basis of ascomata formed on leaves of *Quercus glauca* incubated in a humid petri dish. Conidia were not formed. An ex-type strain (ATTC 58737) was used to retrieve a LSU rDNA sequence (GenBank AF277127, Zhang & Blackwell 2001) which was used by Harrington & McNew (2018) as basis to transfer *A. supraseptata* to *Tubakia*. They classified the mycelial colonies in culture to be tubakia-like. According to Kaneko & Kobayashi (1984), *Apiognomonia* has two-celled ascospores in which the top cell is larger than the bottom cell, but in *A. supraseptata* the top cell is smaller. Harrington & McNew (2018) postulated that *A. supraseptata* represents the only clearly demonstrated sexual morph of *Tubakia*. The allocation of *Apiognomonia supraseptata* to *Tubakiaceae* is reasonable and could be confirmed in our own analyses (Fig. 1). *Apiognomonia supraseptata* is not included in the *rpb2* tree due to lack of sequence data, but in the LSU (Fig. 1) and ITS trees (not shown) this species clusters distantly from all other lineages outside of the *Tubakia s. str*. clade, i.e., this species cannot be maintained in *Tubakia s. str*. Owing to its isolated position in the LSU tree within the *Tubakiaceae*, but distant from all other genera of this family, *Apiognomonia supraseptata* is best accommodated in a genus of its own. *A. supraseptata* and the sexual morph of *Tubakia suttoniana* have various morphological characters in common (rostrate perithecia, unitunicate 8-spored asci, colourless conidia), but *A. supraseptata* differs in forming uniseptate ascospores with a septum near the apex.

Apiognomonioides supraseptata (Kaneko & Kobayashi) U. Braun, J.Z. Groenew. & Crous, ***comb. nov.*** MycoBank MB824480.
Basionym: *Apiognomonia supraseptata* Kaneko & Kobayashi, *Trans. Mycol. Soc. Japan* **25**: 11. 1984.
Synonym: *Tubakia supraseptata* (Kaneko & Kobayashi) T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi. org/10.1007/s10482-017-1001-9 [16]. 2017.

Illustrations: Kaneko & Kobayashi (1984: 13, figs 1–10).

Description in vivo: *Perithecia* formed in lesions on leaves after incubation in a humid petri dish, black, immersed, globose or depressed at the base, 130–220 µm diam, 80–180 µm high, with a central to rarely eccentric beak, erumpent, mostly hypophyllous, to 300 µm long and 50–75 µm wide at the base, perithecial wall 2–3 cell layers thick, composed of dark brown, flattened cells and a hyaline innermost layer. *Asci* numerous, clavate to cylindrical-clavate, tapering towards the base, with an apical ring at the thickened apex, unitunicate, 50–70 × 10–12.5 µm, 8-spored. *Ascospores* irregularly biseriate, ellipsoid, 1-septate near the apex, slightly constricted at the septum, 11–15 × 4.5–6 µm, hyaline, thin-walled.

In vitro: On PSA rapidly growing at 25 °C, attaining 35–45 mm diam after 10 d, more or less zonate, wooly, white, reverse white to pale luteous, colony colour deeper at temperatures higher than 27 °C; aerial mycelium composed of branched, hyaline, septate hyphae, 1.5–2 µm wide; immersed hyphae 2–5 µm wide; development of perithecia in culture 14 d after incubation at 20 °C with continuous fluorescent light, scattered or in concentric circles, somewhat immersed (ascomata formed in culture 250–280 µm diam; asci 65–78 × 10–12.5 µm, basal part more elongated than on the host, ascospores 11.5–15 × 4.5–6.5 µm).

Type: **Japan**, Tottori Pref., Tottori City, Ohchidani Park, on leaves of *Quercus glauca* [incubated in a humid petri dish for 28 d after collection], 11 Feb. 1982, S. Kaneko (TMI 7647 – holotype; TFM : FPT5447; ATTC 58737 = CBS 632.92 = TMI cult. 70024 – ex-type strains).

Involutiscutellula U. Braun & C. Nakash., ***gen. nov.*** MycoBank MB824481.

Etymology: Composed of "involutus" (involute), "scutellulum" (referring to the pycnothyrial scutella), and -ula (diminutive) = minute scutellum with involute margin.

Type species: *Actinopelte rubra* T. Yokoy. & Tubaki [≡ *Involutiscutellula rubra* (T. Yokoy. & Tubaki) U. Braun & C. Nakash.].

Genus of *Tubakiaceae*. Living as endophytes in leaves. *Colonies in vitro* finally turning reddish brown. *Mycelium* internal, hyaline, and external, pigmented; hyphae in culture finally turning reddish brown. *Asexual morphs* forming crustose conidiomata on shed leaves (litter) and superficial pycnothyria on symptomless leaves, occasionally on reddish discolorations. *Pycnothyria* subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella; *scutellum* convex to flattened, membranous, compact, neither loose nor splitting, outline regular, circular-subcircular, margin continuous, more or less undulate, distinctly involute; *conidiophores* reduced to conidiogenous cells, conical, cylindrical, ampulliform, pale orange yellowish to yellowish brown, arising from the underside of the scutella, around the columella, conidiogenous cells phialidic; *conidia* formed singly, globose, subglobose to broad ellipsoid-obovoid, smooth; microconidia oblong-bacilliform, cylindrical, straight to curved-sigmoid, very narrow, only 1 µm wide.

Notes: In all of the phylogenetic trees, especially the LSU and *rpb2* phylogenies, *Actinopelte rubra* takes an isolated basal position and forms a single species lineage that warrants a classification as a genus of its own (Figs 1, 2). *A. rubra* is morphologically readily distinguishable from all other tubakia-like species by colonies and hyphae turning reddish brown with age and distinctive pycnothyria with continuous, more or less undulate, distinctly involute margin and very narrow oblong-bacilliform to cylindrical microconidia.

Involutiscutellula rubra (T. Yokoy. & Tubaki) U. Braun & C. Nakash., ***comb. nov.*** MycoBank MB824482. Fig. 6.
Basionym: *Actinopelte rubra* T. Yokoy. & Tubaki, *Res. Commun. Inst. Ferment. Osaka* **5**: 47. 1971.
Synonym: *Tubakia rubra* (T. Yokoy. & Tubaki) B. Sutton, *Trans. Brit. Mycol. Soc.* **60**: 165. 1973.

Illustrations: Yokoyama & Tubaki (1971: 65, pl. 1F, 67, pl. 2D; 69, pl. 3D; 72, pl. 6A–G; 76, pl. 10A–H).

Description in vivo: Living as endophyte in leaves, forming crustose conidiomata on the surface of shed leaves (litter), usually without distinct symptoms (lesions), sometimes causing a reddish tinge on the leaf surface. *Mycelium* internal and external, forming hyaline, branched intra- and intercellular external hyphae, external hyphae observed on the upper leaf surface, pale brown, branched. *Conidiomata* (*pycnothyria*) amphigenous,

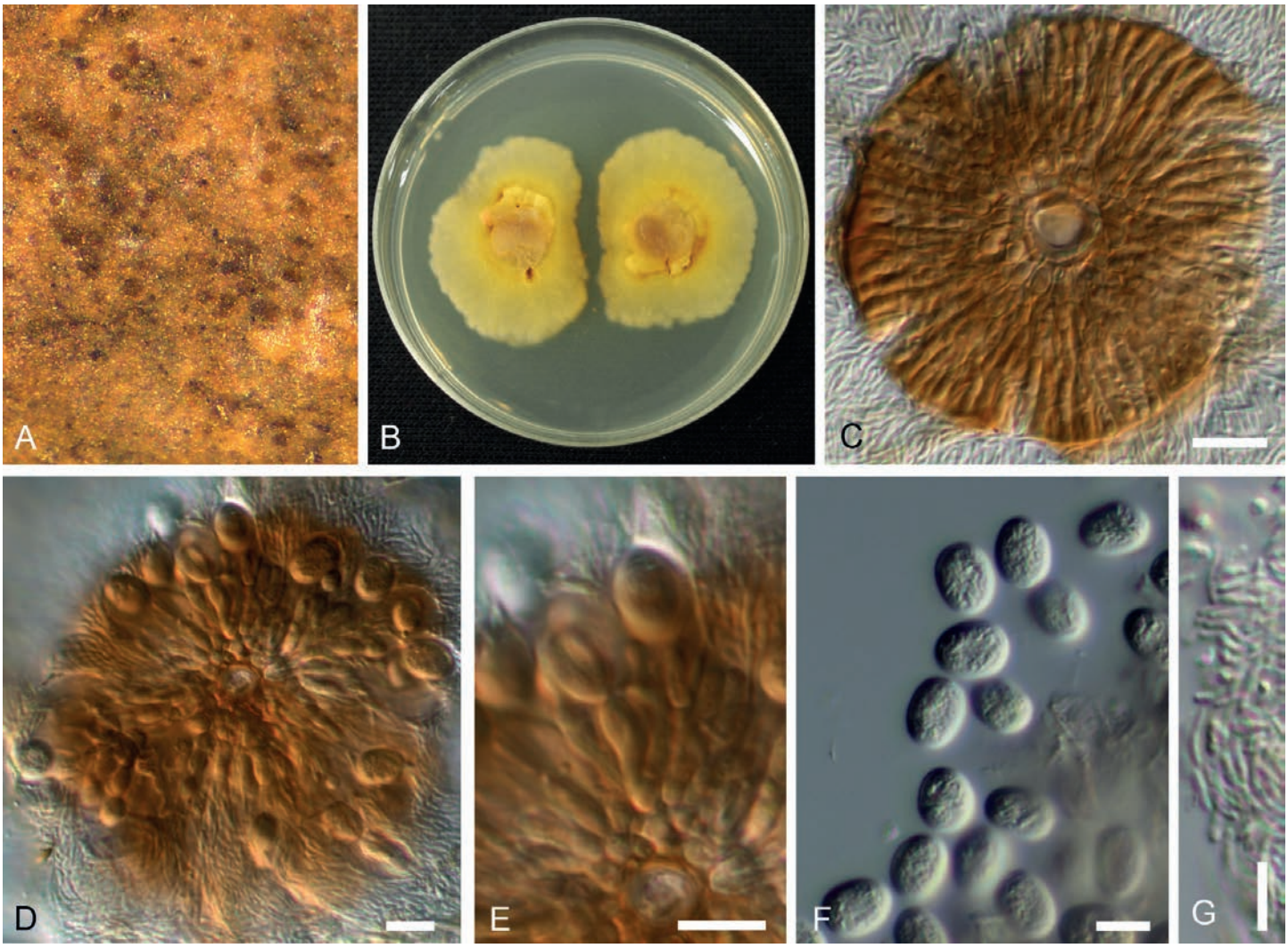

Fig. 6. *Involutiscutellula rubra* (NBRC H-11622 – holotype). **A.** Pycnothyria on the leaf surface. **B.** Culture on MEA (NBRC 9273 – ex-type culture). **C.** Scutellum. **D.** Central columella. **E.** Conidiophores. **F.** Conidia. **G.** Microconidia. Bars = 10 µm.

scattered to gregarious, occasionally confluent, punctiform, superficial, easily removable, subcircular in outline, 60–120 µm diam, ochraceous to orange brown (stereomicroscopy), scutellate, fixed to the leaf surface by a central columella. *Scutella* convex to flat, membranous, dense, compact, neither loose nor splitting, outline regular, circular-subcircular, margin continuous, more or less undulate, distinctly involute, with a central hyaline disc, 6–12 µm diam, surrounded by oblong hyphal cells, rarely branched, 2–3 µm wide, giving rise to radiating hyphal strands, cells 5–10 × 2.5–3 µm, pale brown to medium dark brown, thick-walled (–1 µm), smooth, rarely bifurcating, ultimate branchlets with rounded tips. *Central columella* composed of a central cell surrounded by pseudoparenchymatous cells or distinct large or small cells, 12–30 µm diam. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, around the columella, radiating, orientation outward and downward, conical, cylindrical, ampulliform, larger at the base and attenuated towards a narrow tip, delicate, about 7–20 × 2–4.5 µm, neck about 1 µm wide, hyaline to pale brown, thin-walled, smooth. *Conidia* solitary, globose, subglobose to broad ellipsoid-obovoid, 10–14 × 6–10 µm, length/width ratio 1–1.9, conidiogenesis phialidic, apex and base rounded, wall thin, at first hyaline, later pale orange yellowish to yellowish brown, with inconspicuous to conspicuous basal hilum, up to 1 µm, occasionally truncate when conspicuous. *Microconidia* oblong-bacilliform, cylindrical, straight to curved-sigmoid, 6–10 × 1 µm, formed in common pycnothyria or in separate conidiomata.

In vitro: On MEA with optimal growth at 20 °C, attaining 20–25 mm after 14 d, margin scalloped, creamy yellow, ochraceous, yellowish brown, finally reddish brown; aerial mycelium poorly developed, concolorous; immersed hyphae rapidly growing, white, creamy to ochraceous, later reddish [sporulation on MEA not observed after 14 d; according to Yokoyama & Tubaki (1971) sporulation at the centre or spread, forming pale yellowish brown, viscid, yeast-like masses]. On potato sucrose agar rapidly growing, ochraceous to yellowish brown, later reddish brown to deep reddish fuscous; aerial mycelium moderately developed, compact, viscid; immersed hyphae rapidly growing, pale ochraceous to reddish brown; reverse concolorous; sporulation abundant, above all in the centre, forming yeast-like conidial masses. On OA moderately growing, creamy to pale ochraceous, later reddish brown, smooth, viscid; aerial mycelium poorly developed; immersed hyphae moderately developed, ochraceous to reddish brown; reverse concolorous; sporulation abundant, evenly scattered, forming yeast-like conidial masses. On Czapek agar growth lacking or only restricted, reddish brown (from Yokoyama & Tubaki 1971).

Type: **Japan,** Kyoto Pref., Kyoto, on *Quercus phillyraeoides*, 28 Oct. 1969, *T. Yokoyama 44102801* (NBRC H-11622 – holotype;

NBRC 9273 = ATCC 22473 = IMI 157597 = MUCC2304 – ex-type cultures).

Additional collections examined: **Japan,** Kyoto Pref., Kyoto, on *Quercus phillyraeoides*, 7 Jun. 1969, *T. Yokoyama*, NBRC H-11620; NBRC 9271 = MUCC2302 = CBS 192.71 and NBRC H-11621; NBRC 9272 = MUCC2303; Shiga Pref., Ootsu, on *Quercus phillyraeoides*, 27 Jan. 1970, *T. Yokoyama*, NBRC H-11623; NBRC 9274 = MUCC2305 and NBRC H-1624; NBRC 9275 = MUCC2306; Ootsu, on *Quercus phillyraeoides*, 14 Apr. 1970, *T. Yokoyama*, NBRC H-11625; NBRC 9276 = MUCC2307 and NBRC H-1624; NBRC 9275 = MUCC2306; Kagoshima Pref., Yaku Is., on *Quercus phillyraeoides*, 29 May 1970, *T. Yokoyama*, NBRC 9277 = MUCC2308; *Mie Pref.*; Miyagawa, on *Quercus phillyraeoides*, 2 Aug. 1970, *T. Yokoyama*, NBRC 9371 = MUCC2309.

Host range and distribution: On *Quercus* (*phillyraeoides*, *serrata*), *Fagaceae*, Asia (Japan, Korea).

Notes: Records from Korea on *Quercus serrata* date from Lee *et al.* (1991) and Cho & Shin (2004).

Oblongisporothyrium U. Braun & C. Naksh., ***gen. nov.*** MycoBank MB824483.

Etymology: Composed of "oblongisporo-" (oblong spores) and "-thyrium" (referring to the conidioma, i.e., pycnothyrium).

Type species: *Actinopelte castanopsidis* T. Yokoy. & Tubaki [≡ *Oblongisporothyrium castanopsidis* (T. Yokoy. & Tubaki) U. Braun & C. Nakash.].

Genus of *Tubakiaceae*. Living as endophyte in leaves. *Mycelium* internal, hyaline, and external, pigmented. *Asexual morphs* forming crustose conidiomata on the surface of leaf litter and superficial pycnothyria on brown, necrotic areas on leaves. *Pycnothyria* usually circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella; *scutellum* convex to flattened, often recurved at the edge, membranous, dense, compact when young, later loose at the margin, outline regular, circular to subcircular, composed of hyphal strands, mostly branched, thick-walled, pigmented, margin often recurved at the edge, ultimate tips of the hyphal strands obtuse to rounded; *conidiophores* reduced to conidiogenous cells, obclavate, hyaline to pigmented, arising from small, colourless fertile cells around the central pycnothyrial columella; *conidiogenous cells* phialidic, sometimes forming indistinct periclinal thickenings or annellations; *conidia* formed singly, oblong to oblong-ellipsoid, wall thin to somewhat thickened, hyaline, smooth; microconidia not observed.

Notes: In all of the phylogenetic trees, especially the LSU and *rpb2* phylogenies (Figs 1, 2), *Actinopelte castanopsidis* clusters outside and basal of the *Tubakia s. str*. clade, i.e., this species does not belong to the *Tubakia* core species. Hence, this species has to be excluded from *Tubakia s. str*. *Actinopelte castanopsidis* is allied to *Paratubakia* species [on *Quercus* (*Cyclobalanopsis*) *glauca*] (Figs 2, 3, 5), but forms a separate single species lineage, suggesting a genus of its own. This species shares scutella with margins somewhat curved inwardly, hyaline to pigmented conidiogenous cells, and colourless conidia with *Paratubakia subglobosa*, type species of *Paratubakia*, but differs in having oblong conidia (vs. globose to subglobose in *T. subglobosa*).

Oblongisporothyrium castanopsidis (T. Yokoy. & Tubaki) U. Braun & C. Nakash., ***comb. nov.*** MycoBank MB824484. Fig. 7.
Basionym: *Actinopelte castanopsidis* T. Yokoy. & Tubaki, *Res. Commun. Inst. Ferment. Osaka* **5**: 50. 1971.
Synonym: *Tubakia castanopsidis* (T. Yokoy. & Tubaki) B. Sutton, *Trans. Brit. Mycol. Soc.* **60**: 165. 1973.

Illustrations: Yokoyama & Tubaki (1971: 65, pl. 1H, 67, pl. 2F; 69, pl. 3F; 73, pl. 7A–D).

Description in vivo: Living as endophyte in leaves, forming crustose conidiomata on the surface of leaf litter. *Mycelium* internal and external, forming hyaline, branched intra- and intercellular hyphae, external hyphae observed on the lower leaf surface, pale brown, branched. *Conidiomata* (*pycnothyria*) epiphyllous, rarely hypophyllous, on brown, necrotic areas, scattered, sometimes gregarious, occasionally confluent, punctiform, dark brown to blackish, superficial, easily removable, circular to subcircular when view from above, 100–170 µm diam, scutellate, fixed to the leaf surface by a central columella. *Scutella* convex to flattened, often recurved at the edge, membranous, dense, compact when young, later loose at the margin, outline regular, circular to subcircular, with a central hyaline to subhyaline disc, 4–8 µm diam, scutellum more or less uniformly pigmented, pale brown to brown, central cells subcircular or angular-irregular in outline, giving rise to radiating strands of oblong hyphal cells, 1–3 times bifurcating, 8–12 × 3–5 µm, smooth, septate, walls thickened, ultimate branchlets with obtuse to rounded tips. *Central columella* below the scutellum composed of a central cell surrounded by small, hyaline, fertile cells, forming a pseudoparenchymatous sheath, 25–50 µm diam. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, from parenchymatous cells around the upper part of the columella, radiating, orientation outward and downward, obclavate, enlarged at the base and attenuated towards a narrow tip, 1 µm wide, delicate, 11–20 × 2.5–6 µm, hyaline to pale brown, thin-walled, smooth, apex obtuse to truncate, conidiogenesis phialidic, sometimes forming indistinct periclinal thickenings or annellations. *Conidia* solitary, oblong to oblong-ellipsoid, 14–17 × 7–9.5 µm, length/width ratio 1.6–2.2, apex rounded, base rounded, often with distinct frill, wall thin to somewhat thickened, hyaline, smooth. *Microconidia* not observed.

In vitro: On MEA with optimal growth at 20 °C, attaining 25–30 mm after 14 d, margin scalloped, dingy white to clay-coloured, more or less viscid; aerial mycelium effuse, floccose, white; immersed hyphae rapidly growing, moderately sporulating after 14 d, sporodochial conidiomata [abundantly formed in concentric ring] scattered, blackish brown, forming white to creamy mucous masses of released conidia. Conidia originating from sporodochia ellipsoid-ovoid, 10–15 × 7–10 µm, smooth, hyaline, later pale olivaceous brown. On potato sucrose agar moderately growing, white to clay-coloured, zonate, cartilaginous; aerial mycelium poorly developed, white; immersed hyphae moderately growing; reverse concolorous; sporulation abundant, creamy-white, forming yeast-like masses. On OA very rapidly growing, white to pale clay-coloured; aerial mycelium lacking or poorly developed, white; immersed hyphae abundant, pale ochraceous to clay-coloured; reverse concolorous; sporulation abundant, forming numerous punctiform, blackish brown conidiomata that release creamy, viscid, mucous masses of conidia. On Czapek agar without any

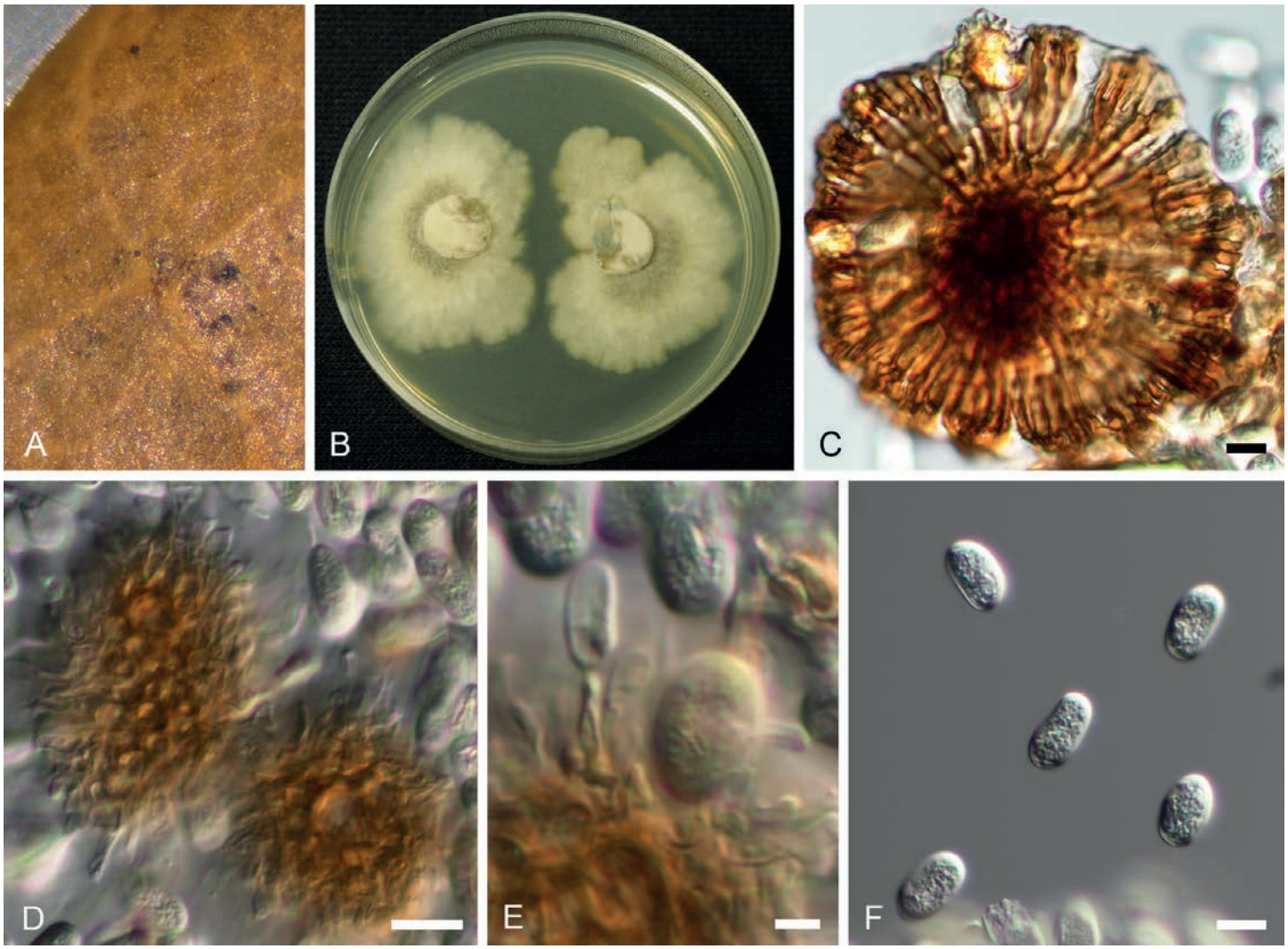

Fig. 7. *Oblongisporothyrium castanopsidis* (NBRC H-11631 – holotype). **A.** Pycnothyria on the surface of leaf litter. **B.** Culture on MEA (NBRC 9263 – ex-type culture). **C.** Scutellum. **D.** Central columellas. **E.** Conidiophores. **F.** Conidia. Bars = 10 µm.

colonies or, if any, strongly restricted (from Yokoyama & Tubaki 1971).

Type: **Japan**, Shiga Pref., Ootsu, on *Castanopsis cuspidata*, 27 Jan. 1970, *T. Yokoyama* (NBRC H-11631 – holotype; NBRC 9263 = ATCC 22470 = CBS 189.71 = IMI 157598 = MUCC2289 – ex-type cultures).

Additional collection examined: **Japan**, Ootsu, on *Castanopsis cuspidata*, 26 Mar. 1970, *T. Yokoyama*, NBRC 9262 = CBS 124732 = MUCC2288.

Hosts range and distribution: on *Castanopsis cuspidata*, *Fagaceae*, Asia (Japan).

Notes: Morphologically, *Actinopelte castanopsidis* belongs to a group of tubakia-like species characterised by having obtuse to rounded outer tips of the scutellum strands, and resembles *T. sierrafriensis* from which it is readily distinguishable by its uniformly oblong-ellipsoid, colourless conidia. *Tubakia oblongispora* is another morphologically similar species but differs in having narrower conidia, 12–20 × 4.5–7.5 µm, with a length/width ratio of 1.8–3.8, and much longer conidiogenous cells, 13–35 × 2–5.5 µm. Phylogenetically, *Actinopelte castanopsidis* (≡ *Tubakia castanopsidis*) is quite distantly related to the two morphologically similar species (Fig. 2).

Paratubakia U. Braun & C. Nakash., ***gen. nov.*** MycoBank MB824485.

Etymology: Composed of "para-" (next to, near) and *Tubakia* (referring to the similarity with the latter genus).

Type species: *Actinopelte subglobosa* T. Yokoy. & Tubaki [≡ *Paratubakia subglobosa* (T. Yokoy. & Tubaki) U. Braun & C. Nakash.].

Genus of *Tubakiaceae*. Living as endophyte in leaves. *Mycelium* internal, hyaline, and external, pigmented. *Asexual morphs* forming crustose conidiomata on shed leaves (litter) and superficial pycnothyria on leaf spots of living leaves. *Pycnothyria* usually circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella; *scutellum* convex to flattened, often recurved at the edge, membranous, dense to looser towards the periphery, outline irregular, sometimes splitting with age, subcircular, composed of hyphal strands, mostly branched, thick-walled, pigmented, ultimate tips of the hyphal strands obtuse to pointed; *conidiophores* reduced to conidiogenous cells, subcylindrical, oblong-obclavate, hyaline to pale olivaceous brown or pale brown, arising from small, colourless fertile cells around the central pycnothyrial columella, *conidiogenous cells* phialidic; *conidia* formed singly, globose, subglobose to broad

ellipsoid, wall thin, hyaline to pale yellowish ochraceous, smooth to faintly rough; microconidia not observed.

Notes: *Actinopelte subglobosa* (≡ *Tubakia subglobosa*) and a new closely allied Japanese species cluster together, but outside of the *Tubakia s. str*. clade, i.e., these species do not belong to the *Tubakia* core species, so that they have to be excluded from *Tubakia s. str*. This lineage is fully supported in the Bayesian trees based on *rpb2* and LSU (Figs 1, 2) and requires a genus of its own, which is described as *Paratubakia*. *Actinopelte castanopsidis* is allied to *Paratubakia*, but forms a separate single species lineage, treated as separate genus, *Oblongisporothyrium gen. nov*. The type species of the latter genus shares scutella with margins somewhat curved inwardly and colourless conidia with *Paratubakia subglobosa*, type species of *Paratubakia*, but differs in having oblong conidia (*vs*. globose to subglobose in *T. subglobosa*).

Paratubakia subglobosa (T. Yokoy. & Tubaki) U. Braun & C. Nakash. ***comb. nov.*** MycoBank MB824486. Fig. 8.
Basionym: *Actinopelte subglobosa* T. Yokoy. & Tubaki, *Res. Commun. Inst. Ferment. Osaka* **5**: 49. 1971.
Synonym: *Tubakia subglobosa* (T. Yokoy. & Tubaki) B. Sutton, *Trans. Brit. Mycol. Soc.* **60**: 165. 1973.

Illustrations: Yokoyama & Tubaki (1971: 65, pl. 1G, 67, pl. 2E; 69, pl. 3E; 73, pl. 7E–H; 76, pl. 10A–H).

Description in vivo: Living as endophyte in leaves, forming crustose conidiomata on the surface of shed leaves (litter); *leaf spots* formed on living and fallen leaves, irregularly shaped, with distinct margin. *Mycelium* internal and external, forming hyaline, branched intra- and intercellular hyphae, external hyphae observed on the lower leaf surface, pale brown, branched. *Conidiomata* (*pycnothyria*) epiphyllous, rarely hypophyllous, scattered to more or less gregarious, punctiform, superficial, easily removable, subcircular in outline when viewed from above, 50–150 µm diam, almost black, brown to dark brown (stereomicroscopy), scutellate, fixed to the leaf surface by a central columella. *Scutella* convex to flattened, margin often recurved, membranous, dense to loser around the periphery, sometimes splitting with age, outline irregular, subcircular, with a central hyaline disc, 6–10 µm diam, surrounded by small cells, subcircular to angular in outline, 3–6 µm diam, composed of radiating threads of oblong hyphal strands, cells 7–22 × 3.5–6.5 µm, pale to medium brown, fuscous, thick walled (–1 µm), smooth, simple or 1–3 times bifurcating, septate, ultimate branchlets with obtuse to pointed tips. *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, about 25–30 µm wide, surrounded by small, thin

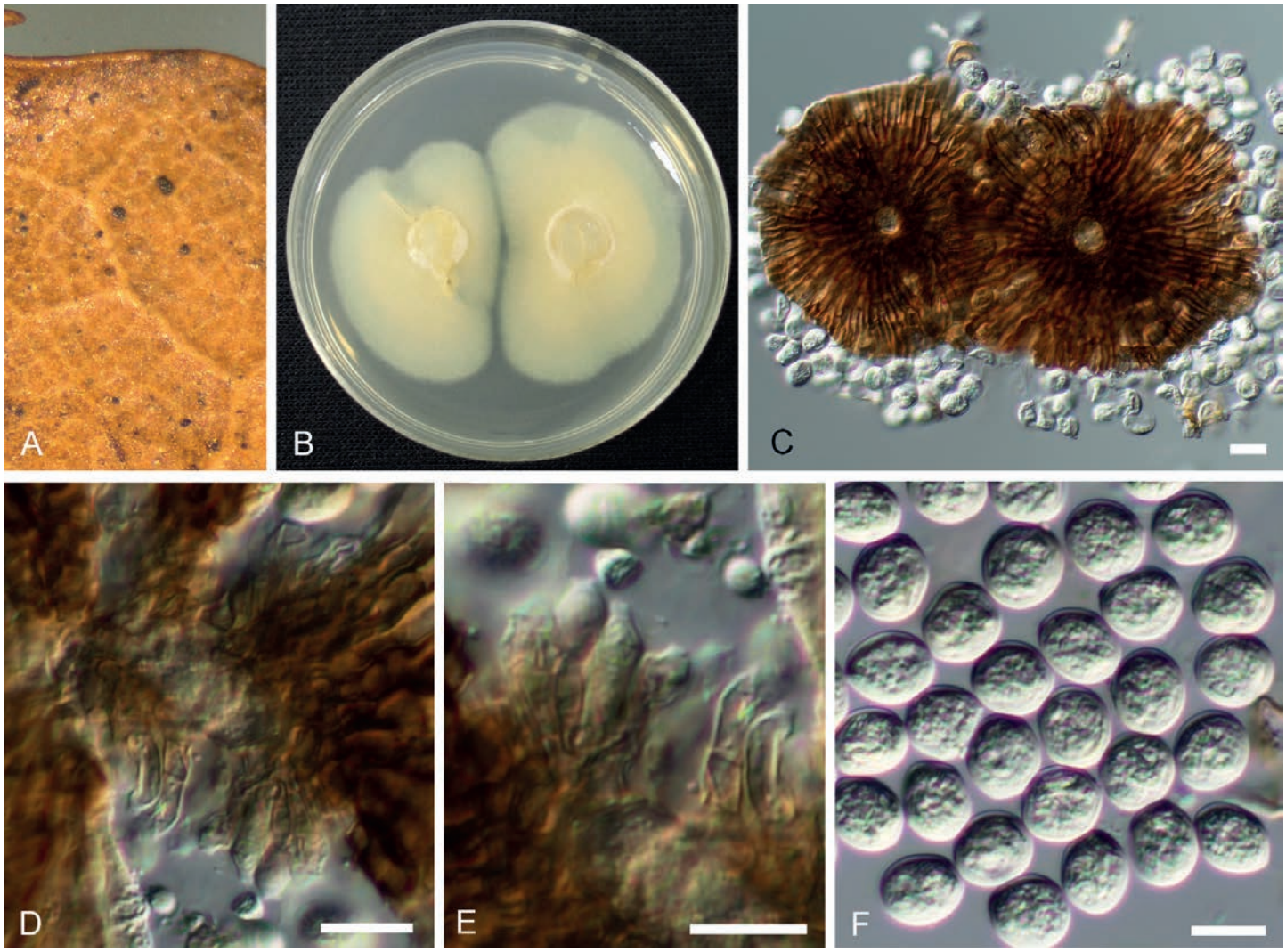

Fig. 8. *Paratubakia subglobosa* (NBRC H-11629 – holotype). **A.** Pycnothyria on the surface of leaf litter. **B.** Culture on MEA (NBRC 8931 – ex-type culture). **C.** Scutella. **D.** Central columella. **E.** Conidiophores. **F.** Conidia. Bars = 10 µm.

walled, pseudoparenchymatous cells [according to Yokoyama & Tubaki (1971) composed of a single cell, 8–10 µm wide, usually surrounded by smaller, spherical, hyaline, fertile cells, 5–10 µm diam, that form a pseudoparenchymatous sheath]. *Conidiophores* reduced to conidiogenous cells, delicate, arising from the underside of the scutella, from parenchymatous cells around the columella, radiating, orientation outward and downward, 8–12 × 2–3 µm, subcylindrical, oblong-obclavate, attenuated towards a narrow tip, 0.5–1 µm diam, hyaline to pale olivaceous brown, thin-walled, smooth. *Conidia* solitary, globose to subglobose, 10–13 × 8–11 µm, length/width ratio 0.9–1.4, wall thin, up to 1 µm wide, hyaline to pale yellowish ochraceous, conidiogenesis phialidic, smooth, with inconspicuous to conspicuous basal hilum, occasionally somewhat peg-like and truncate when conspicuous. *Microconidia* not observed.

In vitro: On MEA with optimal growth at 20 °C, attaining 30–35 mm after 14 d, margin smooth, creamy white, pale ochraceous to pale tan, later cinnamon with reddish tint in zonate circles, viscid; aerial mycelium effuse, finely floccose, varying from thin to thick within concentric zonation, whitish to pale greyish brown; immersed hyphae rapidly growing, well-developed, pale ochraceous to pale greyish brown [sporulation not observed]. On potato sucrose agar moderately growing, white, clay-coloured, blackish brown, viscous, zonate; aerial mycelium effuse, finely floccose; immersed hyphae moderately developed, concolorous; reverse greyish fuscous. On OA rapidly growing, clay-coloured, olive brown to dark brown, with reddish tint, viscid, zonate; aerial mycelium effuse, finely floccose, concolorous; immersed hyphae rapidly growing, concolorous; reverse concolorous, sporulation abundant, evenly scattered. On Czapek agar growth lacking or very reduced (from Yokoyama & Tubaki 1971).

Type: **Japan**, Kyoto Pref., Kyoto, on *Quercus glauca*, 30 Jan. 1968, *T. Yokoyama* (NBRC H-11629 – holotype; NBRC 8931 = ATCC 22474 = CBS 193.71 = IMI 157596 = MUCC2310 – ex-type cultures).

Additional collection examined: **Japan**, Shiga Pref., Ootsu, on *Quercus glauca*, 27 Feb. 1970, *T. Yokoyama*, NBRC 9344 = MUCC2311 = CBS 124733.

Hosts range and distribution: On *Quercus glauca*, *Fagaceae*, Asia (Japan).

Notes: *Paratubakia subglobosa* and *P. subglobosoides* sp. nov. are characterised by having obtuse to acute scutellum tips, resembling scutella of *Tubakia dryina* and *T. iowensis*, but they are morphologically readily distinguishable from the latter two species by their globose, subglobose to broad ellipsoid conidia. Furthermore, they are phylogenetically clearly distinct from and not closely allied to *T. dryina* (Figs 1–3, 5), i.e., they cluster outside of the *Tubakia s. str*. clade.

Paratubakia subglobosoides C. Nakash., ***sp. nov.*** MycoBank MB823668. Fig. 9.

Etymology: Composed of the epithet of the comparable species, *Paratubakia subglobosa,* and -oides (similar to).

Description in vivo: Living as endophyte in leaves, forming crustose conidiomata on the surface of leaf litter. *Mycelium* internal and external, forming hyaline, branched intra- and intercellular hyphae, external hyphae observed on the lower leaf surface, pale brown, branched. *Conidiomata* (*pycnothyria*) epiphyllous, scattered to gregarious, punctiform, superficial, easily removable, subcircular in outline when viewed from above, 80–130 µm diam, blackish (stereomicroscopy), scutellate, fixed to the leaf surface by a central columella. *Scutella* convex to flattened, membranous, dense to looser around the periphery, sometimes splitting with age, outline irregular, subcircular, with a central hyaline disc, 5–8 µm diam, surrounded by small cells, subcircular to angular in outline, 2–3 µm diam, composed of radiating threads of oblong hyphal strands, often uprising radially from scutella, cells 5–25 × 2–5 µm, pale to medium brown, fuscous, thick-walled (–1 µm), smooth, simple or 1–3 times bifurcating, septate, ultimate branchlets with pointed tips. *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, about 25–33 µm wide, surrounded by large, thin walled, brown cells, 4–8 µm diam. *Conidiophores* reduced to conidiogenous cells, delicate, arising from the underside of the scutella, from large brown cells around the columella, radiating, orientation outward and downward, subcylindrical, oblong-obclavate, attenuated towards a narrow tip, 0.5–1 µm diam, 8–18 × 2–4 µm, pale olivaceous brown to pale brown, thin-walled, smooth. *Conidia* solitary, subglobose to ellipsoid, 10–12.5 × 5.5–10 µm, length/width ratio 1.27–2, wall thin, to 1 µm wide, hyaline to pale yellowish ochraceous, smooth to faintly rough, conidiogenesis phialidic, with inconspicuous to conspicuous basal hilum, occasionally somewhat peg-like and truncate when conspicuous. *Microconidia* not observed.

In vitro: On MEA with optimal growth at 20 °C, attaining 38–40 mm after 14 d, margin indefinite, at first pale olivaceous, reverse pale olivaceous brown. On the dried culture prepared from the culture NBRC 9343, abundant sporulation was observed.

Type: **Japan**, Shiga Pref., Ootsu, on *Quercus glauca*, 27 Feb. 1970, *T. Yokoyama* (NBRC H-11619 – holotype; NBRC 9343 = MUCC2293 – ex-type culture).

Hosts range and distribution: On *Quercus glauca*, *Fagaceae*, Asia (Japan).

Notes: *Paratubakia subglobosoides* is phylogenetically (Figs 1–3, 5) closely allied to *P. subglobosa* and the pycnothyria formed by the two species are morphologically similar. However, the conidia in *P. subglobosa* are globose-subglobose, with a smaller length/width ratio of 0.9–1.4, and this species forms distinct leaf spots. Furthermore, the culture characteristics of the two species on MEA are quite different, and they are genetically distinct (Figs 1–3, 5).

Racheliella Crous & U. Braun, ***gen. nov.*** MycoBank MB824487.

Etymology: Named for Rachel Wingfield, the first grandchild of Michael J. Wingfield and Brenda D. Wingfield, born 27 January 2018.

Genus of *Tubakiaceae*. Saprobic and plant pathogenic (possibly endophytic) on *Syzygium* spp. (*Myrtaceae*). *Mycelium* internal, forming branched intra- and intercellular hyphae. *Asexual morphs* forming stromatic (crustose) conidiomata on shed leaves (litter) or superficial pycnothyria on leaf spots of living leaves.

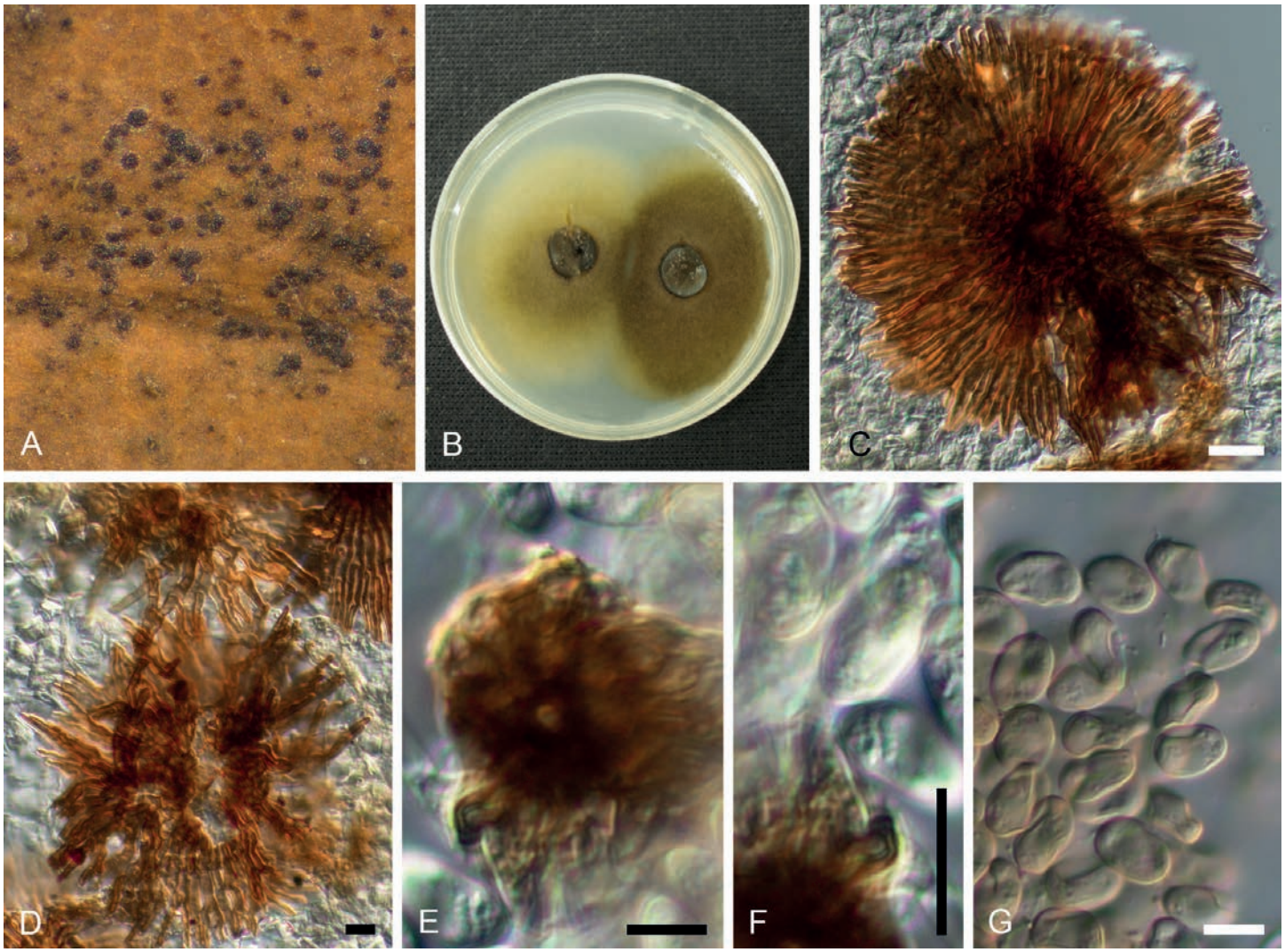

Fig. 9. *Paratubakia subglobosoides* (NBRC H-11619 – holotype). **A.** Pycnothyria on the surface of leaf litter. **B.** Culture on MEA (NBRC 8931 – ex-type culture). **C.** Scutellum. **D.** Hyphal strands uprising radially from scutellum. **E.** Central columella. **F.** Conidiophore. **G.** Conidia. Bars = 10 µm.

Pycnothyria amphigenous, scattered, separate to gregarious, punctiform, blackish, circular or subcircular, superficial; *scutella* convex to flattened, membranous, dense at the centre, looser at the edge, with radiating hyphal strands, bifurcating, ultimate branchlets subcylindrical to attenuated towards the tips, obtuse to pointed; central columella below the scutellum delicate, easily collapsing and loose; *conidiophores* reduced to conidiogenous cells or with a supporting cell, orientation outward-downward, subcylindrical to ampulliform, hyaline, thin-walled, smooth, apex truncate, phialidic, with distinct periclinal thickenings or minute percurrent proliferation; *conidia* solitary, ellipsoid to obovoid, hyaline to subhyaline, guttulate, smooth, apex obtuse, base with conspicuous basal hilum. *Stromatic* (*crustose*) *conidiomata* on lead leaves, superficial to semi-immersed, pigmented, wall pseudoparenchymatous, cells of *textura angularis*; *conidiophores* compactly arranged above the pseudoparenchymatous tissue layer, relatively long, branched, septate, hyaline, phialidic with a flared, conspicuous collarette with a serrate margin; *conidia* solitary, fusiform to oval or obovoid, aseptate, pale brown to olivaceous brown.

Type species: *Racheliella wingfieldiana* Crous & U. Braun.

Notes: A new tubakia-like species found in South Africa on *Syzygium guineense* clusters together with *Greeneria saprophytica*, described from Thailand on old shed leaves of *Syzygium cumini* (Fig. 1). The two taxa are undoubtedly closely allied but unfortunately no culture or DNA of *Greeneria saprophytica* was available to generate more sequence data for comparison. However, based on ITS, these two species are 464/553 (84 %) similar. They cluster outside of *Tubakia s. str.* and distant from all other recognised genera of *Tubakiaceae*, suggesting that they pertain to a genus of their own. The pycnothyria of the type species are reminiscent of conidiomata of other genera of *Tubakiaceae*. Colourless conidia are in line with other tubakioid genera excluded from *Tubakia s. str*. The stromatic conidiomata formed by *Greeneria saprophytica* clearly differ from crustose conidiomata of all other tubakioid genera in having branched, septate conidiophores with phialidic conidiogenous cells provided with flared, conspicuous collarettes with serrate margin.

Racheliella saprophytica (N. Tangthirasunun *et al.*) Crous & U. Braun, ***comb. nov.*** MycoBank MB824498.
Basionym: *Greeneria saprophytica* N. Tangthirasunun *et al.*, *Phytotaxa* **184**(5): 277. 2014.

Illustrations: Tangthirasunun *et al.* (2014: 279, fig. 2, 280, fig. 3).

Description in vivo: *Saprobic* on dead leaves. *Conidiomata* stromatic (crustose), 140–175 µm diam, superficial to semi-

immersed, separate, dark olivaceous to black; wall 3–7 layered, 30–44 µm wide, pale to moderately dark brown, with a well-developed pseudoparenchymatous layer of cells of *textura angularis* at the base that becomes thinner at the margin of conidiomata. *Conidiophores* 24–44 × 3–4 µm, septate, branched, compactly arranged above the pseudoparenchymatous tissue layer, hyaline, cylindrical, tapered towards the apex, smooth. *Conidiogenous cells* enteroblastic, phialidic with a flared, conspicuous collarette with a serrate margin, rarely percurrently proliferating, discrete or integrated, cylindrical to vase-like, tapered towards the apex. *Conidia* fusiform to oval or obovoid, with a rounded apex and narrowly truncate to pointed base, 9–15 × 5–6 µm, length/width ratio 2.2:1, aseptate, pale brown to olivaceous brown, thick-walled, smooth-walled, with one to many oil droplets.

In vitro: Colonies on PDA medium to dark brown from above and reverse, with medium to dense surface mycelium, flat, irregular to rhizoidal, with undulate to lobate margin, attaining 25 mm diam in 7 d at 27 °C; sporulating after 14 d.

Types: **Thailand**, Chiang Rai, Mae Fah Luang University campus, on dead leaves of *Syzygium cumini*, 21 Mar. 2012, *N. Tangthirasunun* (MFLU 13-0255 – holotype; PDD 105206 – isotype; MFLUCC 12-0298 = NTCL 052-1 = ICMP 20057 – ex-type cultures).

Host range and distribution: Only known from the type collection.

Notes: The position of *Greeneria saprophytica* in the consensus tree published in Tangthirasunun *et al.* (2014) clearly suggested that this species and *G. uvicola*, the type species of *Greeneria*, are not congeneric. *Tubakia* species were not included in the phylogenetic analyses which led to an allocation of the new species collected in Thailand on dead leaves of *Syzygium cumini* to *Greeneria*. In the current analyses, the sequences retrieved from the ex-type culture of *G. saprophytica* cluster within the *Tubakiaceae*, but outside of the *Tubakia s. str*. clade, i.e., this species does not belong to the latter genus (Fig. 1). A collection of "*Tubakia* sp." found in South Africa on *Syzygium guineensis* is closely allied and clusters together with *G. saprophytica* and the two species require a genus of their own. At first glance, *Greeneria saprophytica* seems to be morphologically quite distinct from species of *Tubakia s. lat*. However, the conidiomata of the latter species are reminiscent of those of *Tubakia californica*, a species that does not form any pycnothyria but only stromatic conidiomata, usually (50–)80–200(–220) µm diam, composed of basal stromatic layers and phialidic conidiogenous cells. The size of the conidiomata in *Greeneria saprophytica* and *Tubakia californica* is comparable. Aseptate pigmented conidia are common in *Tubakia s. lat*. A clear difference between *Greeneria saprophytica* and all species hitherto assigned to *Tubakia* lies in the characters of the conidiophores, which are longer, septate and sometimes branched in *G. saprophytica* [*vs*. shorter, unbranched, usually aseptate (conidiophores reduced to conidiogenous cells) in species of all other genera of *Tubakiaceae*]. In addition, the conidiogenous cells have flared collarettes with serrate margins. *G. saprophytica* is phylogenetically allied to *Tubakia thailandensis*, which differs in forming small pycnothyria, aseptate, unbranched conidiophores, and subglobose, colourless conidia. Stromatic conidiomata are not developed. *G. saprophytica* found in Thailand on *Syzygium cumini* was assigned to *Greeneria* (*Melanconiellaceae*) owing to morphological similarities of the conidiomata, but since this species pertains to the *Tubakiaceae* it has to be excluded from *Greeneria* and is reallocated together with a new species on *Syzygium* from South Africa to the new genus *Racheliella*.

Racheliella wingfieldiana Crous & U. Braun, ***sp. nov.*** MycoBank MB824490. Fig. 10.

Etymology: Named for the Wingfield family, Michael J. Wingfield (South African mycologist and forest pathologist), his wife

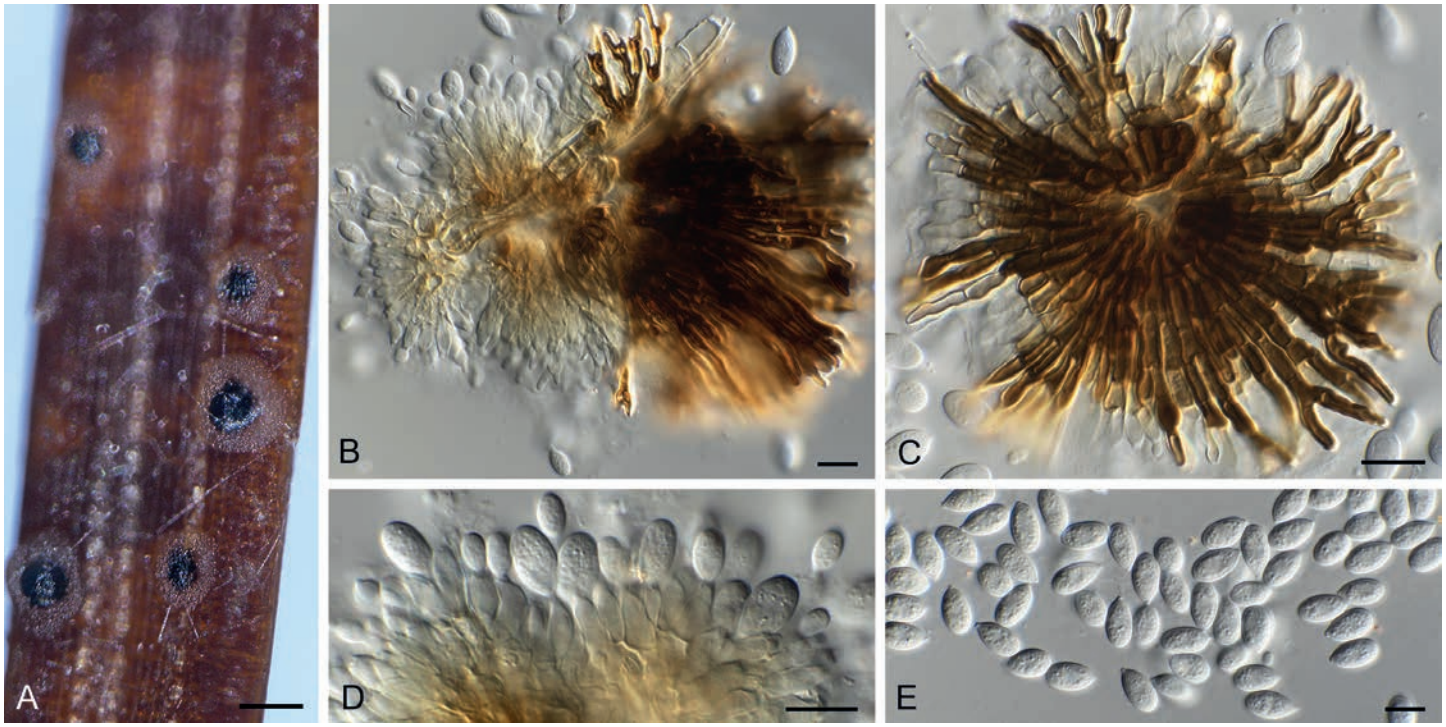

Fig. 10. *Racheliella wingfieldiana* (CBS H-23399 – holotype). **A.** Pycnothyria forming in culture on PNA. **B, C.** Pycnothyria in culture (from PNA). **D.** Conidiogenous cells giving rise to conidia. **E.** Conidia. Bars = 150 mm (A), 10 mm (B–E).

Brenda D. Wingfield (South African fungal geneticist), Beverley Wingfield (daughter and fanatical cyclist), and Anthony and Jane Wingfield, the parents of Rachel Wingfield.

Description in vivo: Occurring on leaves, forming distinct leaf lesions, amphigenous, shape and size variable. *Mycelium* internal, forming branched intra- and intercellular hyphae. *Conidiomata* (*pycnothyria*) amphigenous, scattered on unaffected portions of leaves, separate to gregarious on leaf spots, punctiform, blackish, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella, 80–130 µm diam. *Scutella* convex sometimes more flattened, membranous, dense at the centre, looser at the edge, with a central hyaline or pale disc, giving rise to radiating hyphal strands, cells 5–10 × 2–5 µm, dark brown, thick-walled (–1 µm), smooth, simple or 1–3 times bifurcating, ultimate branchlets subcylindrical to attenuated towards the tips, obtuse to pointed. *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, about 20–30 µm wide, surrounded by large brown cells. *Conidiophores* reduced to conidiogenous cells or with a supporting cell, arising from the underside of the scutella, around the columella, radiating, orientation outward-downward, subcylindrical to ampulliform, 6–10 × 3–4 µm, hyaline, thin-walled, smooth, apex truncate, phialidic, with distinct periclinal thickenings or minute percurrent proliferation. *Conidia* solitary, ellipsoid to obovoid, (11–)12–14(–15) × (6.5–)7(–7.5) µm, hyaline to subhyaline, guttulate, smooth, apex obtuse, base with conspicuous hilum (frill), peg-like and truncate, 1 µm diam.

In vitro: Colonies flat, spreading, with sparse aerial mycelium and feathery, lobed margins, reaching 60 mm diam after 2 wk. On MEA, PDA and OA surface isabelline with cinnamon spore masses, reverse isabelline. On PDA, surface olivaceous to isabelline, reverse honey to isabelline.

Type: **South Africa**, Eastern Cape Province, Haga Haga, on leaves of *Syzygium guineense*, 6 Mar. 2007, *M.J. Wingfield* (CBS H-23399 – holotype; CBS 143669 = CPC 13806 – ex-holotype culture).

Host range and distribution: Only known from the type collection.

Saprothyrium U. Braun, Crous & J.Z. Groenew., ***gen. nov.*** MycoBank MB824491.

Etymology: Composed of "sapro-" (saprobic) and -thyrium (referring to the conidiomata [pycnothyrium]).

Type species: *Tubakia thailandensis* Senan. *et al.* [≡ *Saprothyrium thailandense* (Senan. et al.) U. Braun, Crous & J.Z. Groenew.].

Genus of *Tubakiaceae*. Foliicolous, saprobic (perhaps also living as endophyte). Mycelium internal. *Asexual morph* forming superficial pycnothyria on dead leaves; pycnothyria usually circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella; scutellum convex, membranous, dense, outline irregular, composed of hyphal strands, arising from a small central disc, cells thick-walled, pigmented, ultimate tips of the hyphal strands obtuse; conidiophores reduced to conidiogenous cells, small, arising below the scutellum, phialidic, with a minute collarette and wide periclinal thickening; conidia formed singly, globose, subglobose, wall thickened, hyaline, smooth; microconidia not observed.

Notes: *Tubakia thailandensis* was not included in the phylogenetic analyses used to generate Fig. 2 as only ITS and LSU sequence data are available for it. Based on a megablast search against the ITS sequences used in the combined alignment, the closest match was *T. dryinoides* with 89 % (404/452). It is also not included in the RPB2 tree, but its position in the LSU tree and a comparision with other species included in the LSU as well as the RPB2 tree clearly indicate that *T. thailandensis* clusters within the *Tubakiaceae*, but outside of the *Tubakia* clade, i.e., this species has to be excluded from *Tubakia s. str.* It forms a separate lineage in the LSU tree close to *Racheliella*, which justifies the classification as genus of it own. Pycnothyria of *Saprothyrium* are well characterised by having obtuse outer tips of the scutellum strands combined with globose-subglobose colourless conidia, and shares these traits with the genus *Sphaerosporithyrium*, which is, however, phylogenetically distant, clustering in basal position to *Tubakia s. str.* Colourless globose-subglobose conidia formed in pycnothyria distinguish *Saprothyrium* from all species of *Tubakia s. str*. in terms of morphology.

Saprothyrium thailandense (Senan. *et al.*) U. Braun, Crous & J.Z. Groenew., ***comb. nov.*** MycoBank MB824499.
Basionym: *Tubakia thailandensis* Senan. *et al.*, *Stud. Mycol.* **86**: 281. 2017.

Illustration: Senanayake *et al*. (2017: 280, fig. 34).

Description in vivo: Saprobic on dead leaves. *Conidiomata* 40–50 µm high, 50–75 µm wide, pycnothyria with radiate scutella, scattered to gregarious, superficial on the substratum. *Scutella* convex, brown to dark brown, thick-walled cells, radiating from a central point, ultimate tips obtuse, not pointed. *Conidiophores* short, forming under the developing scutella. *Conidiogenous cells* 5–10 µm high, 2–4 µm wide, phialidic, with a minute collarette and wide periclinal thickening. *Conidia* globose to subglobose, 10–12.5 × 7.5–8.5 µm (on average 11.3 × 8.1 µm, n = 20), smooth, hyaline, thick-walled.

In vitro: Mycelium on PDA white when young, dark green, light grey to black from above and reverse when aged, with medium mycelium, flat, rhizoid to irregular form, lobate margin, and attaining a diam of 46 mm in 7 d at 27 °C.

Type: **Thailand**: *Chiang Rai*: Doi Mae Salong, on dead leaf, *K. Wisitrassameewong*, 2 May 2012, NTCL059 (MFLU 13–0260 – holotype; MFLUCC 12–0303 – ex-type culture).

Notes: *Saprothyrium thailandense* is morphologically close to "*Tubakia* sp." on *Castanea henryi* in China described and illustrated in Braun *et al*. (2014). The relation between *S. thailandense*, collected as saprobic fungus on dead leaves in Thailand, and *Tubakia* sp., found in China on distinct leaf spots on *Castanea henryi*, is unclear. It is also unclear if the leaf spots associated with "*Tubakia* sp." in China are truly caused by this tubakia-like fungus. The leaves were covered with lesions of several other fungi, including *Tubakia chinensis*, i.e., it is possible that it represents an endophyte or saprobic species forming conidiomata on necrotic tissue caused by other fungi. Culture and sequence data are not available for the Chinese *Tubakia*, although urgently necessary for a comparison of the two taxa.

Sphaerosporithyrium U. Braun, Crous, O. Moreno-Rico & Marm., ***gen. nov.*** MycoBank MB824492.

Etymology: Composed of "sphaero-" (globose), "spori-" (spore) and "-thyrium (referring to pycnothyrium, the special conidioma type).

Type species: *Sphaerosporithyrium mexicanum* O. Moreno-Rico, U. Braun & Marm.

Genus of *Tubakiaceae*. Living as endophyte in leaves and causing leaf spots. *Mycelium* internal. *Asexual morph* forming superficial pycnothyria on leaf spots of living leaves. *Pycnothyria* usually circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella; *scutellum* flattened-convex, membranous, compact, dense, uniformly pigmented, pale to medium dark brown, or with a darker central zone, outline regular, subcircular, composed of hyphal strands, mostly branched, thick-walled, pigmented, ultimate tips of the hyphal strands obtuse or even truncate; *conidiophores* usually reduced to conidiogenous cells, conical, ampulliform-cuspidate, subcylindrical, delicate, not very conspicuous, arising from the underside of the scutella, around the upper portion of the columella, radiating, conidiogenous cells phialidic; *conidia* formed singly, globose, subglobose to broad ellipsoid-ovoid, thin-walled, hyaline or with a very pale greenish to olivaceous tinge, smooth; microconidia not observed.

Notes: A new undescribed tubakia-like species was collected in Mexico on *Quercus eduardi*. Sequences retrieved from the new Mexican species cluster in all phylogenetic trees close to but always outside of the *Tubakia s. str*. clade (Figs 1–3, 5), suggesting that this species cannot be assigned to the latter genus, i.e., it warrants a genus of its own. Owing to the conidial shape and colour, the new genus *Sphaerosporithyrium* is morphologically comparable with *Paratubakia*. Pycnothyria formed by species of the latter genus differ, however, in having pointed tips of hyphal scutellum strands. Colourless globose-subglobose conidia clearly distinguish *Sphaerosporithyrium* from all species of *Tubakia s. str*.

Sphaerosporithyrium mexicanum O. Moreno-Rico, U. Braun & Marm., ***sp. nov.*** MycoBank MB823664. Fig. 11.

Etymology: Named after Mexico, the country where this species was collected.

Description in vivo: *Leaf spots* amphigenous, subcircular to angular-irregular, 2–10 mm diam or confluent and larger, developing to large blotches, 10–40 mm diam, pale to medium dark brown, centre sometimes paler, dingy greyish white, margin indefinite or with darker brown to reddish brown margin or marginal line, sometimes zonate. *Conidiomata* (*pycnothyria*) amphigenous, more abundant on the upper leaf surface, scattered to gregarious, punctiform, superficial, easily removable, circular to subcircular in outline, 80–120 µm diam,

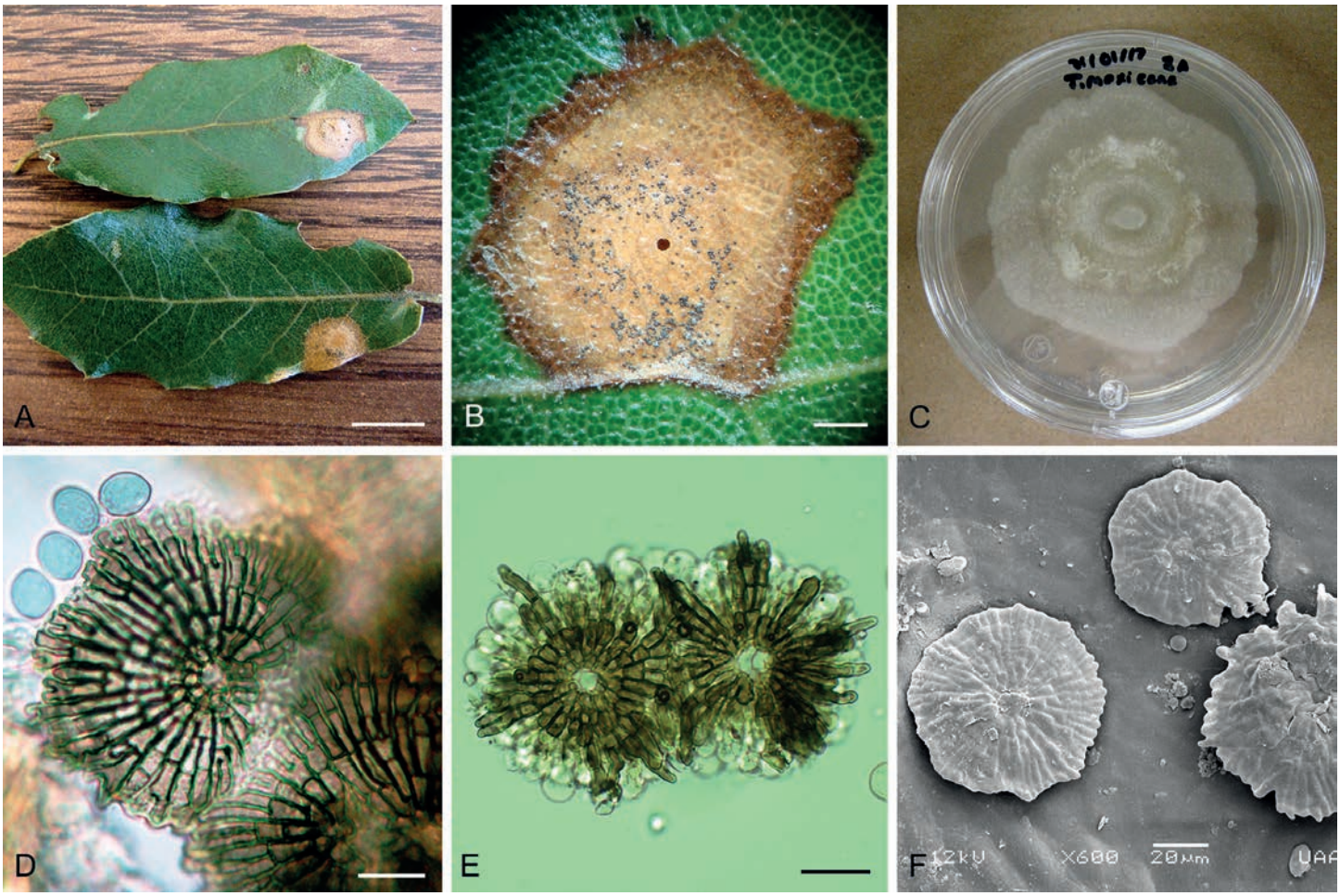

Fig. 11. *Sphaerosporithyrium mexicanum* (CFNL 2945 – holotype). **A.** Leaf spots on *Quercus eduardi*. **B.** Close-up of a leaf spot. **C.** Culture on MEA. **D, E.** Two pycnothyria and conidia. **F.** Three pycnothyria (SEM picture). Bars = 1 cm (A), 0.5 cm (B), 20 µm (D, E).

dark brown to blackish (stereomicroscopy), scutellate, fixed to the leaf surface by a central columella. *Scutella* convex to flattened-convex, membranous, compact, dense, outline regular, with a central colourless or pale disc, 8–20 µm diam, scutellum uniformly pigmented (microscopy) pale to medium dark brown, or with a darker central zone, central cells subcircular or angular-irregular in outline, 3–8 µm diam, giving rise to radiating threads of hyphal cells, 1–3 times bifurcating, cells 6–30 × 2–6 µm, walls to 1.5 µm thick, tips of the threads simple or 1–2 times forked, branchlets short, ultimate tips consistently obtuse or even truncate; central columella below the scutellum delicate, easily collapsing and loose, short, composed of thin-walled, colourless or pale fertile cells, circular to somewhat angular-irregular in outline, 2–6 µm diam. *Conidiophores* reduced to conidiogenous cells, rarely 1–2-septate, arising from the underside of the scutella, around the upper portion of the columella, radiating, conical, ampulliform-cuspidate, subcylindrical, delicate, not very conspicuous, about 6–14 × 3–5 µm, hyaline, thin-walled, smooth. *Conidia* solitary, globose, subglobose to broad ellipsoid-ovoid, small conidia 7–10 × 5–8 µm, larger fully developed conidia 9–16(–19) × (7–)9–12 µm, length width ratio 1.0–1.5(–1.6), aseptate, apex rounded, base rounded or with a minute truncate hilum or even with a small peg-like base, thin-walled, hyaline or with a very pale greenish to olivaceous tinge, smooth. *Microconidia* not observed *in vivo*.

In vitro: On MEA at 22 °C colonies attaining 67–69 mm diam after 16 d, margin undulate, with concentric rings of aerial mycelium, center white, reverse colourless, without sporulation.

Type: **Mexico,** Aguascalientes, San José de Gracia, Las Manzanitas, 22°11’31.3”N, 102°36’50.4”W, 2 612 m alt, on *Quercus eduardi*, 19 Jan. 2017, *O. Moreno-Rico* (CFNL 2945 – holotype; CFNL 2945 = CPC 33021 – ex-type cultures).

Additional collections examined: **Mexico**, Nuevo León, Iturbide, Bosque Escuela, 24°42’23.9’’N, 99°51’44.2’’W, 1 618 m alt, on *Quercus eduardi*, 4 Nov. 2016, *J. Marmolejo*, HAL 3180 F; CFNL 2942; *ibid*., 24°42’23.9’’N, 99°51’44’’W, 1 618 m alt, on *Q. eduardi*, 10 Nov. 2016, *J. Marmolejo*, HAL 3183 F; CFNL 2941 = CPC 32258.

Notes: *Sphaerosporithyrium mexicanum* is well-characterised by a combination of globose, subglobose to broad ellipsoid-ovoid conidia and pycnothyria with obtuse to truncate tips of the radiating scutellum threads. The conidial shape is reminiscent of *Paratubakia subglobosa* conidia, but the latter species, known from Asia on *Quercus glauca* (≡ *Cyclobalanopsis glauca*), differs in having scutella with pointed tips of radiating hyphal threads. The status of *S. mexicana* as species in its own right is fully supported in the phylogenetic trees (Figs 1–3, 5).

Tubakia B. Sutton, *Trans. Brit. Mycol. Soc.* **60**: 164. 1973, *emend*. *Basionym*: *Actinopelte* Sacc., *Ann. Mycol*. **11**: 315. 1913, non Sitzenb., 1861.

Type species: *Actinopelte japonica* Sacc. [≡ *Tubakia japonica* (Sacc.) B. Sutton].

Genus of *Tubakiaceae*. Living as endophytes in leaves and twigs, and pathogenic, forming distinct leaf lesions. *Asexual morphs* forming sporodochial or crustose to pustulate, pycnidioid, stromatic, unilocal conidiomata on petioles and twigs, erumpent, dehiscing by irregular fissures, and superficial pycnothyria on living, faded or shed leaves, without distinct symptoms on living leaves or forming distinct leaf spots. *Pycnidia* usually circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella; *scutellum* composed of loose to dense hyphal strands, mostly branched, thick-walled, pigmented, margin compact or outer portions of the radiating hyphal strands looser to free, tips rounded, truncate or pointed, margin usually not recurved; *conidiophores* reduced to conidiogenous cells, usually subcylindrical-conical, lageniform, hyaline to pale brown, arising from small, colourless fertile cells around the upper part of the central pycnothyrial columella, conidiogenous cells phialidic, percurrently proliferating, sometimes forming indistinct periclinal thickenings or annellations (collarettes); *conidia* formed singly, globose to broad ellipsoid-obovoid, sometimes subcylindrical or somewhat irregular, wall thin to somewhat thickened, smooth to faintly rough, hyaline to pigmented, apex rounded, base rounded to attenuated, sometimes with distinct frill or peg-like basal hilum. *Sexual morph*: Diaporthaloid, dicarpella-like, forming dark stromatic pseudoparenchymatic layers with pigmented, dark perithecia on fallen overwintered leaves, rostrate, beak short, usually lateral-eccentric, slightly protuberant, ostiolate, ostiole periphysate, peridium variable in thickness, paler than stromatic layers, polyascal; *asci* unitunicate, 8-spored, oblong-ellipsoid, stalk short to oblong, ascal apex with two refractive conoid structures, asci deliquescing at maturity; *paraphyses* lacking; *ascospores* more or less uniseriate, becoming irregularly biseriate, one-celled, hyaline, ellipsoid to fusiform, often inequilateral or slightly *curved,* wall finely ornamented, content granular-guttulate. Putative sexual morph: *Dicarpella dryina* (see *Tubakia suttoniana*).

Notes: In its traditional circumscription, *Tubakia s. lat.* comprises a wide range of species with characteristic superficial conidiomata composed of radiating scutella, a basal columella fixing the scutellum to the leaf surface, and colourless phialidic conidiogenous cells arising from fertile cells around the upper part of the columella bearing solitary, one-celled, colourless to pigmented conidia. However, results of phylogenetic analyses revealed the heterogeneity of *Tubakia s. lat*. and led to a new circumscription of *Tubakia s. str*. (*emend*.) which forms a separate well-supported clade within the family *Tubakiaceae* evident in the *rpb2* and LSU trees (Figs 1, 2). Species clustering outside of the *Tubakia* clade, belonging to other lineages, are excluded and assigned to other (new) genera. *Tubakia* species may form different types of asexual morphs. Pycnothyria with typical scutella are characteristic, diagnostic and commonly formed. In addition, sporodochial conidiomata composed of clusters of conidiogenous cells may be developed, e.g. on leaf veins formed by *T. iowensis*. Crustose or pustulate pycnidioid conidiomata may be formed by several *Tubakia* species, including *T. californica*, *T. dryina*, and *T. iowensis*. In *T. californica* they are the only hitherto known fructification. They are mostly formed on petioles and leaf blades (often on and close to veins) of old necrotic leaves, either shed (litter) or still attached as in the case of *T. californica*. The classification of these conidiomata caused some uncertainty and confusion. Holdenrieder & Kowalski (1989) designed these conidiomata as “pycnidial” although they clearly described non-ostiolate conidiomata dehiscing by irregular rupture. Harrington *et al*. (2012) used the general term “conidioma” and added “pycnothyrium” in brackets. These conidiomata cannot be referred to as pycnidia owing to lacking

ostioles, but due to stromatic wall layers and a dehiscence by irregular fissures they should rather be classified as stromatic.

Tubakia americana (Höhn.) T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi.org/10.1007/s10482-017-1001-9 [14]. 2017.
Basionym: *Actinopelte americana* Höhn., *Mitt. Bot. Inst. Techn. Hochsch. Wien* **2**(3): 68. 1925.

Illustrations: Harrington & McNew (2018, figs 2, 4).

Description in vivo: Leaf and twig endophyte, also causing *leaf spots*, amphigenous, subcircular to angular-irregular, 1–18 mm diam, brown, with narrow darker margin, dark violet, brown to blackish or along necrotic leaf veins. *Conidiomata* (*pycnothyria*) amphigenous, mainly hypophyllous, superficial, scattered to gregarious, dark brown to blackish, 40–115 µm diam, about 30 µm high, circular in outline, scutellate, fixed to the leaf surface by a central columella. *Scutella* somewhat convex, centre with a colourless or pale disc, about 5–30 µm diam, surrounding small cells, about 2.5–3 µm diam, giving rise to radiating threads of hyphal cells, simple to often 1–4 times bifurcating, cells 5–20 µm long and 2–5 µm wide, wall about 1 µm thick, ends of the radiating threads simple or furcate, tips obtuse; central *columella* below the scutellum parenchymatic, colourless or pale, about 20 µm high and 25–30 µm broad; *Conidiophores* reduced to conidiogenous cells, delicate, formed around the upper part of the columella. *Conidia* solitary, broad ellipsoid(-ovoid), 9–13(–14) × 6–8.5 µm, aseptate, subhyaline or pale to medium olivaceous brown, wall thin (0.5–1 µm), smooth or almost so. *Microconidia* sometimes produced from same pycnothyrium as macroconidia or from smaller pycnothyria, hyaline, fusiform, curved, aseptate, (3.5–)4–7 × 2–3 µm. *Crustose conidiomata* forming in winter or spring, erumpent on twigs or inside of acorn caps, black, irregular in shape, 150–500 µm diam, covering layer composed of thick-walled cells, *textura angularis*, dehiscing marginally or by irregular fissures; conidiophores lining inside of crustose conidiomata; conidia thick-walled, hyaline to light brown, obovoid to ellipsoidal, aseptate, 9.5–14 × 5.5–7.5 µm.

In vitro: On MYEA with optimal growth at 25 °C, attaining a diam of 56 to 64 mm after 7 d, initially with a distinct ring of dense aerial mycelium, later developing concentric rings of fluffy white to grey aerial mycelium with wet conidial masses that are initially hyaline, becoming olive green then black, coalescing into large areas. *Conidia* in culture abundant, thick-walled, smooth to finely varicose, hyaline to dark brown, obovoid to ellipsoidal, aseptate, 9–15(–16.5) x (4–)4.5–7.5(–8) µm.

Type: **USA**, New Jersey, Newfield, on *Quercus coccinea*, Aug. 1883 [Ellis & Everh., Fungi Columb. 286] (NY – *lectotype*, designated by Harrington & McNew 2017; *isolectotypes* – Ellis & Everh., Fungi Columb. 286 [e.g. BPI 390073, 390075, BRU 1082, CUP, ISC 453285, NY, WIS-F-120]; *topotypes* [from 1881 and 1894] – Ellis & Everh., N. Amer. Fungi 732, 3168 [e.g., CUP-A-33522, 33530; ILLS 45680, 45682, 73344; MU 246655]).

Host range and distribution: on *Quercus* (*bicolor, coccinea*, *macrocarpa*, *robur*, *rubra*), *Fagaceae*, North America (USA, Illinois, Iowa, Missouri, Wisconsin).

Notes: Höhnel (1925) described *Actinopelte americana* based on a North American collection on *Quercus coccinea* and distinguished his new species from *T. dryina* by having smaller conidia. Type material of this species has been examined and shown to be morphologically rather similar to *T. dryina*, but distinguishable by having obtuse (non-pointed) ultimate tips of radiating hyphal scutellum threads. Attempts to locate the particular specimen of Fungi Columb. 286 that Höhnel had examined failed. Therefore, Harrington & McNew (2018) designated a duplicate from the McClatchie Herbarium in NY as lectotype, which, however, contains pycnothyria of two *Tubakia* spp. associated with different necrotic spots. One of these *Tubakia* species has relatively small conidia, as described by Höhnel (1925) for *A. americana*, and the tips of the radiating scutellum strands are blunt (Harrington & McNew 2018, fig 2a, b). The second type of pycnothyria on the lectotype material has scutella with acute tips and larger conidia (Harrington & McNew 2018, fig 2c), rather matching the wide concept of *T. macnabbii* (Harrington & McNew 2018). According to Harrington & McNew (2018), other duplicates of Fungi Columb. 286 in NY and ISC each contained leaves with two *Tubakia* species. In other duplicates, e.g., BPI 390073, 390075, only the small-spored *Tubakia* with obtuse tips of scutellum strands has been observed. The lectotypification proposed by Harrington & McNew (2018) is reasonable, follows and maintains Höhnel's original description.

Harrington & McNew (2018) managed to extract DNA form the lectotype of *A. americana* and a generated sequence matched that of *Tubakia* sp. A (Harrington & McNew 2016; Harrington *et al*. 2012), which they have commonly isolated as a twig and leaf endophyte in *Q. macrocarpa* and other *Quercus* spp. in Iowa, often also associated with leaf spots and necrotic veins (Harrington & McNew 2018, fig 4).

Tubakia americana is phylogenetically (Figs 3, 5) closely related to *T. dryina*, and both species produce crustose pycnothyria on twigs (Harrington *et al.* 2012), but the two species are readily distinguishable by differences in the pycnothyrial scutella (tips of the scutellum strands obtuse in *T. americana* and pointed in *T. dryina*). In culture, the mycelium of *T. americana* is much lighter in colour than the dark mycelium of *T. dryina* (Harrington *et al.* 2012). Species of *Quercus* sect. *Quercus* (*Q. bicolor, Q. macrocarpa* and *Q. robur*) seem to be the primary hosts of *T. americana* in Iowa (Harrington *et al.* 2012). The "*T. americana*" ITS cluster published in Harrington *et al*. (2012) is divided in two groups – the upper one composed of North American sequences represents *T. americana*, and the lower one is composed of allied cryptic taxa from Eurasia. The Asian taxon in this group is described herein as *T. dryinoides*. The European sequences based on isolates from France might represent another cryptic species, but due to insufficient sampling and lacking ecological and morphological data we prefer to maintain them in *T. dryinoides*, at least tentatively. ITS sequences retrieved from leaves of *Quercus robur* and *Fagus sylvatica* in Poland (haplotype 2 in Boroń & Grad 2017) seem to belong to this cryptic European taxon and suggest that it might be rather common.

Tubakia californica S. Rooney-Latham & U. Braun, ***sp. nov.*** MycoBank MB823661. Figs 12–14.

Etymology: Named after California, the origin of the type collection and numerous additional isolates.

Description in vivo: Living as endophyte in leaves and twigs, causing necrotic leaf lesions and twig dieback. Symptoms and development on *Quercus kelloggii*: Deciduous leaves of affected trees do not

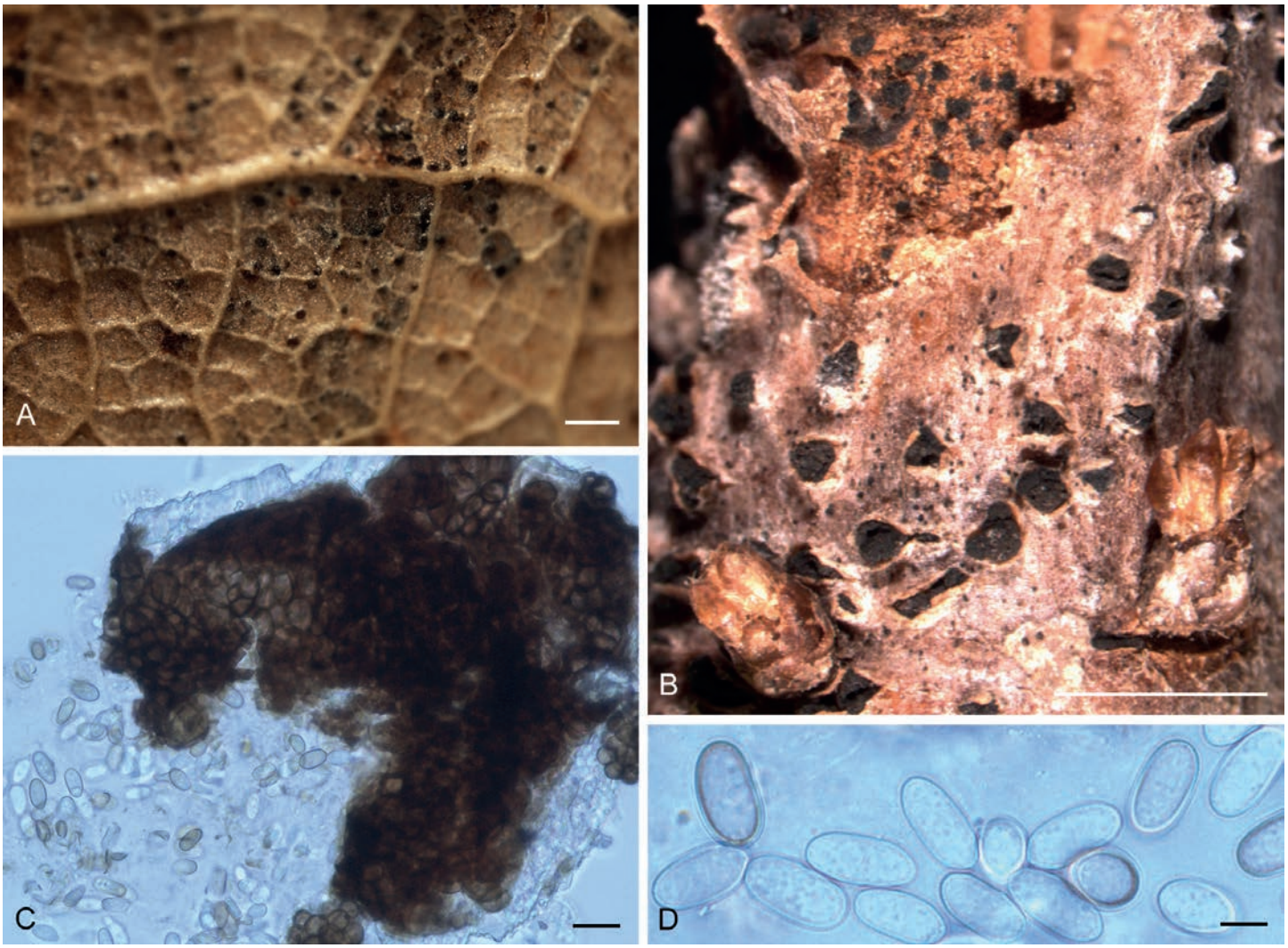

Fig. 12. *Tubakia californica* (on *Quercus kellogii*, non-preserved sample). **A.** Conidiomata on a necrotic leaf (close-up). **B.** Old conidiomata on a petiole (close-up). **C.** Crushed conidiomata and conidia. **D.** Conidia. Bars = 1 mm (A, B), 20 µm (C), 5 µm (D).

fully defoliate in the fall. Symptomatic trees are primarily mature trees, approximately 25 years and older; dry, brown leaves from the previous seasons' growth remain attached on branches in the spring; symptom progression in the late summer and fall is variable depending on location; in the California foothills where the disease is prominent, the new distal growth appears healthy until late August when small, brown lesions develop on the leaves, predominantly on the abaxial side; irregular, brown lesions with chlorotic borders may also occur near the leaf margins; lesions enlarge over time, and by early September, the associated lateral leaf veins become brownish black, by late September, more than half of the leaf area on affected leaves is brown and discoloration of the veins extends to the midrib; in October, many of the affected leaves become dry and uniformly brown and most leaf veins turn black; small, black, embedded crustose *Tubakia* conidiomata with conidia develop in late September on symptomatic petioles and leaves, often associated with the veins, but also on the leaf blade. *Conidiomata* crustose, pycnidioid, stromatic, scattered to loosely aggregated, subepidermal, erumpent, at first "closed" (covered by a black stromatic layer), protruding portion hemispherical to depressed-convex (hourglass-shaped), dehiscing by irregular fissures, finally exposed, widely opened, subcircular to somewhat irregular in outline, usually (50–)80–200(–220) µm diam, wall about 10–20 µm thick, black, crustose covering layer composed of large cells, mostly 4–10 µm diam, with thin to thickened walls, 0.5–1 µm wide, pale to medium dark brown, dark brown in mass, rounded, oblong to angular-irregular in outline (*textura globulosa* to *angularis*), basal layer paler; conidiogenous cell lining the base of the conidiomata, colourless, thin-walled, attenuated towards the tip, about 10 × 3–4 µm, but early collapsing and lapsing (hence detailed observations and measurements not possible); *conidia* solitary, broad ellipsoid, ellipsoid-ovoid to short and broad subcylindrical, straight, rarely slightly curved or somewhat irregular in shape, both ends rounded to subtruncate, basal frills or truncate peg-like bases not observed, 8–15 × 4.5–7 µm, length/width ratio 1.4–2.3 (on average 1.8), at first subhyaline to pale greenish, later greenish, pale olivaceous to brownish, wall thin, to about 0.5 µm, smooth or almost so (light microscopy), microconidia not observed (pycnothyria with free radiating scutella not developed).

In vitro: Optimal growth at 25–30°C on MEA, colonies attaining 60 mm after 7 d, dingy white to pale yellow with regular margin, becoming yellowish grey with concentric rings in reverse, conidial formation not observed. On PDA, colonies initially creamy white, becoming greyish beige and forming olivaceous brown concentric rings in reverse, margin irregular to scalloped. Conidiomata brownish black, forming in concentric rings; conidia broadly ellipsoid, ellipsoid-ovoid, straight, initially subhyaline, later becoming olivaceous brown, 10–14 × 7–9 µm (average 11.9 × 8.0), length/width ratio 1.2–1.9 (average 1.5).

Fig. 13. *Tubakia californica*, symptoms on infected California black oak (*Quercus kelloggii*) trees in Tuolumne County, CA. **A.** Early spring growth showing healthy new leaves and infected brown leaves still hanging from the previous season. **B.** Current season foliage with angular lesions developing near the leaf margin and progressing along the leaf veins. **C.** Symptomatic branch showing necrotic lesions on the new growth as well as old, infected brown leaves that did not abscise and presumably served as the primary inoculum source. **D.** Progression of foliar symptoms from healthy leaves (top left) to leaves with small black veins, to leaves with more prominent black veins along the midrib and larger necrotic areas to completely brown leaves. Bar (C, D) = 10 cm.

Type: **USA**, California, Tuolumne County, Groveland, 956 m alt., on *Quercus kelloggii*, 19 Sep. 2014, J. Haas [dried culture, plated and dried 2017] (BPI 910537 – holotype; CBS 143670 = CPC 31505 = CDFA#1428, ex-holotype culture).

Additional cultures examined: See Table 1.

Additional material examined: **USA**, California, Tuolumne County, Groveland, on *Quercus kelloggii*, 5 May 2017, *J. Haas* (HAL 3212 F – paratype).

Hosts range and distribution: On *Chrysolepis chrysophylla* (≡ *Castanopsis chrysophylla*), *Notholithocarpus densiflorus* (≡ *Lithocarpus densiflorus*), *Quercus* (*agrifolia*, *kelloggii*, *wislizeni*), *Fagaceae*, North America (USA, California).

Notes: *Tubakia californica* is an endophyte found in California on several oak species and two additional fagaceous host species. It causes symptoms and lesions on the affected tree species, crustose, stromatic, pycnidioid conidiomata may be formed, but characteristic leaf spots spread over the whole leaf blade (comparable with those formed by *T. dryina* and most other *Tubakia* spp.) and pycnothyria are not developed or have at least not yet been observed. The leaf lesions caused by *T. californica* are rather reminiscent of those formed by *T. iowensis*. In October 2014, *Tubakia* was cultured from *Q. kelloggii* trees in

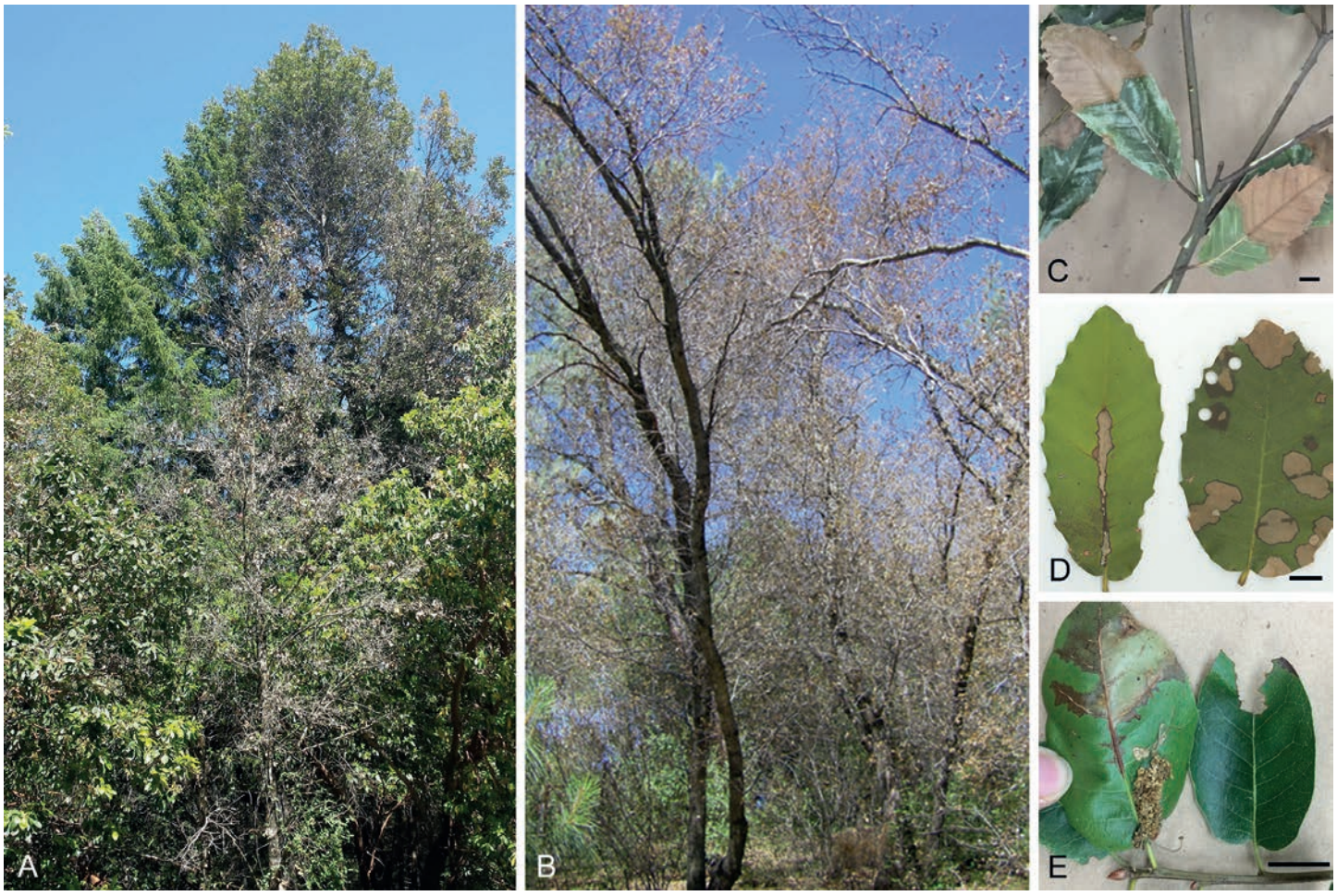

Fig. 14. *Tubakia californica*, symptoms on other *Fagaceae* hosts. **A.** Tanoak (*Notholithocarpus densiflorus*) tree in Humboldt Co., CA with significant dieback of the lower two-thirds of the crown foliage. **B.** Canopy loss and dieback on an interior live oak (*Quercus wislizeni*) tree in Shasta Co., CA. **C.** Tanoak leaf with marginal necrosis. **D.** Tanoak leaf with necrotic spots and dark midrib. **E.** Interior live oak leaf with necrotic veins. Bars (C–E) = 1 cm.

California, El Dorado Co., with foliar symptoms similar to those seen in Groveland. Between 2012 and 2017, this fungus was also isolated from similar leaf lesions and twig dieback of other *Fagaceae* species throughout California including the counties of Contra Costa, Del Norte, Humboldt, Marin, San Luis Obispo, and Shasta. Overall, symptoms seem to develop later in the season in the Sierra Nevada foothill locations (Tuolumne Co. and El Dorado Co.) than in the other California counties. The elevations of the Groveland and the El Dorado Co. sites are 956 m and about 850 m respectively, while most of the other locations were at or near sea level. In addition to colder winters, both foothill locations leaf out later in the season, which may explain the difference in the onset of disease symptoms to the valley and coastal locations. Overall, disease severity was greater on trees in low areas and on branches in the lower portion of the canopy (unpublished observations).

Owing to lacking pycnothyria, a phylogenetic approach was required to evaluate the Californian taxon. Analyses of the sequence data and their position in the phylogenetic trees clearly favour a separate species. The *T. californica* clade is well supported in both combined analyses (Fig. 3: PP = 1, MP-BS 88 %; Fig. 4: PP = 1, MP-BS 99 %). Two cultures isolated from *Tubakia* on *Quercus canbyi* in Mexico (CPC 32250 and 32251) belong to this clade and are currently only tentatively assigned to *T. californica* since they are not identical to Californian strains. Whether they are conspecific with *T. californica* or if they represent a closely allied Mexican species cannot yet be determined without a larger sampling of Mexican collections. Conidiomata of *T. californica* are macroscopically not easily discernible on old necrotic leaves since several macroscopically similar coelomycetes may develop together with the crustose conidiomata of the new species. In an examined collection on *Quercus kelloggii* (HAL 3212 F), conidiomata of *T. californica* developed on leaves together with acervular conidiomata of *Apiognomonia errabunda* as dominating fungus. The conidia of the latter species are similar in length but narrower (about 6–17 × 3–5 µm), and consistently colourless.

Tubakia chinensis U. Braun, S. Bien & Hantsch, *Schlechtendalia* **28**: 23. 2014.

Illustration: Braun *et al*. (2014: 24, fig. 1).

Description in vivo: *Leaf spots* amphigenous, subcircular to angular-irregular, 0.5–5 mm diam, at first dingy greenish to greyish green, later brownish to dingy greyish brown, finally grey to greyish white, with narrow darker margin, brown to reddish brown, finally darker, often slightly raised or limited by veins. *Conidiomata* (*pycnothyria*) epiphyllous, small lesions with a single pycnothyrium, larger ones with up to 18 pycnothyria, punctiform, black, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella. *Scutella* convex, 135–200 µm diam, membranous, somewhat translucent (conidia more or less

visible beneath the scutellum when viewed from above), with a central hyaline or pale disc, 15–20 µm diam, giving rise to radiating hyphae, cells (5–)8–15(–20) × 3–8 µm, medium brown, thick-walled (–1 µm), smooth, usually 1–3 times bifurcating, either only at the periphery or deeply cleft, ultimate branchlets with obtuse or often pointed tips. *Conidiophores* reduced to conidiogenous cells, arising from the underside of scutella around the upper part of the columella, radiating downward and towards margin, subcylindrical, subclavate, ampulliform, mostly attenuated towards the tip, straight to slightly curved, 10–20 × 4–8 µm, hyaline or subhyaline, thin-walled, smooth, with a single terminal locus, monophialidic. *Conidia* solitary, globose, subglobose or broad ellipsoid-obovoid, (20–)25–40 × 20–30 µm, length/width ratio 1.1–1.4, wall 0.7–1.2 µm wide, hyaline or pale, smooth, cell content pale brownish, sometimes somewhat granular, apex and base broadly rounded, with inconspicuous to conspicuous basal hilum, somewhat peg-like when conspicuous, about 4 µm wide and 1 µm high, with delicate frill. *Microconidia* not observed.

Type: **China**, Jiangxi Province, Xingangshan, subtropical forest site of the BEF-China Project, 29.1250°N, 117.9085°E, on living leaves of *Castanea henryi*, 8 Sep. 2013, S. Bien (HAL 2674 F – holotype).

Host range and distribution: on *Castanea henryi*, *Fagaceae*, Asia (China, Jiangxi Province).

Notes: *Tubakia japonica* (Yokoyama & Tubaki 1971) and *T. seoraksanensis* (Yun & Rossman 2011) form a group of *Tubakia* species with rather large conidia (length on average > 15 µm) to which *T. chinensis* belongs, but it can be easily differentiated by traits of the scutella and conidia. *Tubakia japonica* has much larger, colourless conidia, 40–55 × 35–45 µm, and forms microconidia, 5–7 × 1.5–2 µm (Yokoyama & Tubaki 1971), and *T. seoraksanensis* differs in having smaller scutella, 90–160 × 90–130 µm, narrower conidiogenous cells, 14–22 × 3–5 µm, and much smaller conidia, 13–25 × 10–15 µm. The size of mature conidia of *T. chinensis* does not overlap with those of *T. japonica* and *T. seoraksanensis*. Cultures and data of sequence analyses are not yet available for *T. chinensis*, i.e., the phylogenetic affinity of this species is still unknown. However, *T. japonica* and *T. seoraksanensis* are phylogenetically very closely related (Figs 3–5).

Tubakia dryina (Sacc.) B. Sutton, *Trans. Brit. Mycol. Soc.* **60**: 165. 1973, *emend*. (*s. str*.).
Basionym: *Leptothyrium dryinum* Sacc., *Michelia* **1**: 200. 1878.
Synonym: *Actinopelte dryina* (Sacc.) Höhn., *Mitt. Bot. Inst. Techn. Hochsch. Wien* **2**(3): 69. 1925.

Literature: Jones & Holcomb (1978 – cytology and ontogeny), Glawe & Crane (1987 – comprehensive treatment), Holdenrieder & Kowalski (1989 – pathogenicity and fructification), Taylor & Clark (1996 – infection and development of pycnothyria), Taylor (2001 – ultrastructure of pycnothyria), Harrington *et al.* (2012: 88–89 – comprehensive survey, taxonomy).

Illustrations: Glawe & Crane (1987: 106, figs 1–3, 107, figs 4–11, 108, figs 12–17), El-Gholl *et al.* (1996: 2, fig. 2), Harrington *et al.* (2012: 88, fig. 3).

Description in vivo: Living as endophyte in leaves and twigs, forming crustose to pustulate pycnidial conidiomata on twigs, erumpent, and pathogenic, forming distinct *leaf lesions*, amphigenous, shape and size variable, subcircular to angular-irregular, 2–15 mm diam, yellowish ochraceous, straw-coloured to brown, finally sometimes dingy greyish brown, margin indefinite or with narrow darker border, dark purplish violet, brown, reddish brown to blackish, occasionally with a diffuse halo, sometimes forming large ochraceous to brown necroses, to 60 mm diam. *Mycelium* internal, forming branched intra- and intercellular hyphae. *Conidiomata* (*pycnothyria*) amphigenous, scattered to gregarious, punctiform, blackish, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella, (40–)60–120(–140) µm diam. *Scutella* convex, sometimes more flattened, membranous, dense, compact, later sometimes less compact, looser, with a central hyaline or pale disc, 5–20 µm diam, surrounded by small cells, subcircular to angular in outline, 3–8 µm diam, giving rise to radiating hyphal strands, cells 6–30(–35) × 2–6(–7) µm, pale to medium dark brown, thick-walled (–1 µm), smooth, simple or 1–3 times bifurcating, ultimate branchlets with obtuse to mostly pointed tips (uniformly pointed or at least a large portion of tips being acute). *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, about 10–20 µm wide, surrounded by small, thin-walled, colourless or pale (pseudoparenchymatous) cells. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, around the columella, radiating, orientation outward-downward, conical, ampulliform-cuspidate, delicate, about 8–18 × 2–5(–6) µm, hyaline, thin-walled, smooth, apex obtuse to truncate, conidiogenesis phialidic, percurrently proliferating, sometimes forming indistinct periclinal thickenings or annellations (collarettes). *Conidia* solitary, broad ellipsoid-obovoid, (7–)9–16(–18) × (5–)6–10(–10.5) µm, length/width ratio 1.1–1.6(–2.2), wall thin, to 1 µm wide, at first hyaline, subhyaline, later pale olivaceous, olivaceous brown to brownish, smooth, old conidia occasionally faintly rough-walled, apex and base broadly rounded, with inconspicuous to conspicuous basal hilum (frill), occasionally somewhat peg-like and truncate when conspicuous. *Microconidia* formed in some collections, fusiform, mostly straight, 4–9 × 1.5–4 µm, hyaline, thin-walled, smooth. Crustose to pustulate *pycnidioid conidiomata* may be formed on twigs, black, circular, oblong to somewhat irregular in outline; pustulate *conidiomata* 0.2–1 mm diam, scattered to gregarious, wall to about 20 µm thick, composed of *textura angularis*, cells about 3–4 µm diam, at the base *textura angularis* or *globosa*, cells paler and thinner, forming a pulvinate conidiogenous layer, non-ostiolate, opening by irregular dehiscence; conidiogenous cells usually arising from the basal pulvinate layer, lageniform, 8–34 × 4–6 µm, straight to curved, tips of the neck 1.5–2(–2.5) µm wide, conidia developed in crustose conidiomata ellipsoid-obovoid, oblong or even slightly asymmetrical, 10–16 × 6–7.5(–8.5) µm, wall somewhat thickened, smooth to faintly rough, at first colourless, later somewhat pigmented, pale brown.

In vitro: on MEA with optimal growth at 25 °C, attaining 60–66 mm diam after 7 d, margin scalloped, at first creamy white, forming concentric rings of aerial hyphae, reverse in the middle dark grey, yellow to medium brown towards the rim at 10 d; sporodochial conidiomata abundantly formed at 10 d, in concentric rings, conidial mass dark grey to blackish, sporodochial conidia formed in culture, ellipsoid-ovoid, 10–16 × 5.5–8.5 µm, with somewhat thickened walls, finely rough, at first colourless, later somewhat pigmented.

Type: **Italy**, Veneto, Treviso, Bosco Montello, on *Quercus robur*, Sep. 1875 [Sacc., Mycoth. Ven. 555] (PAD, MBT379573 – *lectotype* designated here). *Isolectotypes*: Sacc., Mycoth. Ven. 555 (e.g. BPI 391920, CUP, HAL, ILLS 420). **Italy**, *Veneto*, Treviso, Fagaré Forest, on *Quercus robur*, undated, *S. Mutto-Accardi* (CBS H-23357, MBT379616 – epitype designated here; CBS 112097 – ex-epitype culture).

Host range and distribution: on *Fagus sylvatica*, *Quercus* (*alba*, *macrocarpa*, *robur*), *Fagaceae*, Europe (Germany, Italy, Netherlands, Poland, Romania, Russia, UK), North America (USA, Iowa, Louisiana), and New Zealand.

Notes: *Tubakia dryina*, originally described as leaf-spotting fungus on *Quercus robur* in Italy, was previously circumscribed and applied in a very broad sense comprising collections from Asia, Europe, and North America mainly on *Quercus* spp., but also on some additional fagaceous genera, e.g. on *Castanea* and *Fagus* spp., and in North America on host plants belonging to numerous other plant families (Glawe & Crane 1987, Yokoyama & Tubaki 1971, etc.). Numerous collections with leaf spots and pycnothyria morphologically indistinguishable from *T. dryina* have been examined: **Mexico**, Nuevo León, Linares, Vivero Facultad de Ciencias Forestales, 24°47'47.9''N, 99°32'30.9''W. 380 m alt, on *Quercus shumardii*, 9 Nov. 2016, *J. Marmolejo*, HAL 3181 F, 3182 F; CFNL 2940. **USA** (collections arranged according to host names and with herbarium accession numbers of BPI and ILLS – details of the particular collection available via: http://mycoportal.org/portal/collections/index.php): *Acer saccharum*, ILLS 29734, *Acer* sp., BPI 391880, *Arbutus menziesii*, BPI 391882, *Castanea dentata*, BPI 391884, *C. sativa*, BPI 390033, 390034, 391883, 391885, *Cercis canadensis*, ILLS 25737, *Eucalyptus* sp., BPI 391886, *Fraxinus americana*, ILLS 29744, *F. nigra*, ILLS 5562, *F. pennsylvanica*, BPI 391887, *F. profunda*, ILLS 29738, *Nyssa sylvatica*, BPI 391889–391891, *Quercus alba*, BPI 863075, *Q. bicolor*, ILLS 16125, *Q. borealis*, BPI 391905, 391906, *Q. coccinea*, BPI 390074, 391907, *Q. ellipsoidalis*, BPI 391908–391912, *Q. falcata*, ILLS 32849, *Q. macrocarpa*, BPI 291913–391914, *Q. marilandica*, ILLS 29755, 30075, 32836, 32841, *Q. nigra*, BPI 391918, 840864, *Q. phelos*, BPI 391921, 391922, *Q. rubra*, BPI 391917, 391928, 391929, 390077–390081, 390084, 390085, 391925–391927, 803135, 863076, *Q. shumardii*, ILLS 117552, *Q. stellata*, BPI 391930, *Q. velutina*, BPI 390087, 390088, 391931, 391933–391936, 863077, *Q. virginiana*, BPI 391932, *Toxicodendron radicans* [≡ *Rhus radicans*], ILLS 30072, *Ulmus alata*, ILLS 29740. First molecular examinations and analyses of *T. dryina s. lat*. carried out by Harrington *et al.* (2012) suggested that this species represents a strongly heterogeneous complex comprising several cryptic species. Some of them have recently been described by Harrington & McNew (2018), and additional ones are introduced in the present work. Host range and distribution of *T. dryina s. str.* is insufficiently known and probably largely unrevealed due to its basically endophytic life strategy. Documented collections usually refer to material with distinct leaf spots and developed pycnothyria. Nevertheless, *T. dryina* seems to be a primarily European species with *Quercus robur* as principal host plant on which this fungus has probably been introduced to other regions of the world, including proven cases in New Zealand and North America (USA, Harrington & McNew 2018). In the eastern USA, *T. dryina s. str*. occurs also on *Quercus alba* and *Q. macrocarpa* (Harrington *et al.* 2012, Harrington & McNew 2018). Therefore, it is currently not possible to finally decide if *T. dryina* being a native North American species or an introduced neomycete. However, most North American *Tubakia* collections previously referred to as *T. dryina* pertain to other species, including *T. americana*, *T. hallii*, *T. iowensis*, *T. liquidambaris*, *T. macnabbii*, and *T. tiffanyae*, but *Tubakia* on numerous *Quercus* spp. and on numerous non-fagaceous hosts in North America has not yet been properly examined, i.e., identifications based on cultures and sequence analyses are still lacking. Heredia (1993) reported "*T. dryina*" from Mexico on *Quercus germana* and *Q. sartorii*. These records are doubtful and belong very probably to other *Tubakia* species (current analyses of *Tubakia* in Mexico revealed several endemic species – see *Sphaerosporithyrium mexicanum, Tubakia melnikiana and T. sierrafriensis*).

Collections of "*T. dryina*" from Asia do not belong to this species. Harrington *et al*. (2012) emphasised that Japanese specimens referred to as *T. dryina* in Yokoyama & Tubaki (1971) do not agree with the concept of *T. dryina s. str*., which could be confirmed in the course of our own re-examinations of the collections concerned (see *T. dryinoides*). Zahedi *et al*. (2011) reported and illustrated "*Tubakia dryina*" on *Quercus castaneifolia* from North Iran (Guilan Province), which is, however, quite distinct from true *T. dryina s. str*. (see notes under *Tubakia* sp. [Excluded, doubtful and insufficiently known species]). The identity of "*T. dryina s. lat*." on *Quercus hartwissiana* in Turkey (Huseyinov & Selçuk 2001) is unclear as well.

Boroń & Grad (2017) examined *T. dryina* in Poland and analysed the variation of ITS data of numerous isolates. Haplotype 1 in Boroń & Grad (2017) is representative for *T. dryina s. str*. The published results and analyses confirm *T. dryina* as common and widespread species on *Quercus robur* and *Fagus sylvatica*, at least in Poland. In a single case, they found this species on *Tilia cordata* (*Tiliaceae*), which needs, however, further research and confirmation. Haplotype 2 probably belongs to European sequences of *T. dryinoides* (*s. lat*.).

The conidiogenesis of *Tubakia dryina* was examined in detail by Jones & Holcomb (1978) and described to be phialidic, which was later confirmed in Glawe & Crane (1987). Taylor & Clarke (1996) described the formation of internal mycelium and conidial germination, and Taylor (2001) dealt with the ultrastructure of pycnothyria. Holdenrieder & Kowalski (1989) emphasized that *T. dryina* may live as endophyte in healthy leaves and twigs, and Boddy & Rayner (1984) isolated this fungus from living and dead twigs in the UK.

Tubakia dryinoides C. Nakash., ***sp. nov*.** MycoBank MB823662. Fig. 15.

Etymology: Named after the morphological and phylogenetic affinity to *Tubakia dryina*.

Description in vivo: Living as endophyte in leaves, forming distinct leaf lesions, amphigenous, shape and size variable, subcircular to angular-irregular, 2–5 mm diam, purplish brown to dingy greyish brown, margin indefinite or with narrow darker border, dark purplish violet, reddish brown to blackish, occasionally with a diffuse halo. *Mycelium* internal, forming branched intra- and intercellular hyphae. *Conidiomata* (*pycnothyria*) amphigenous, scattered on unaffected portions of leaves, gregarious on leaf spots, punctiform, greyish to blackish, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella, 50–145 µm

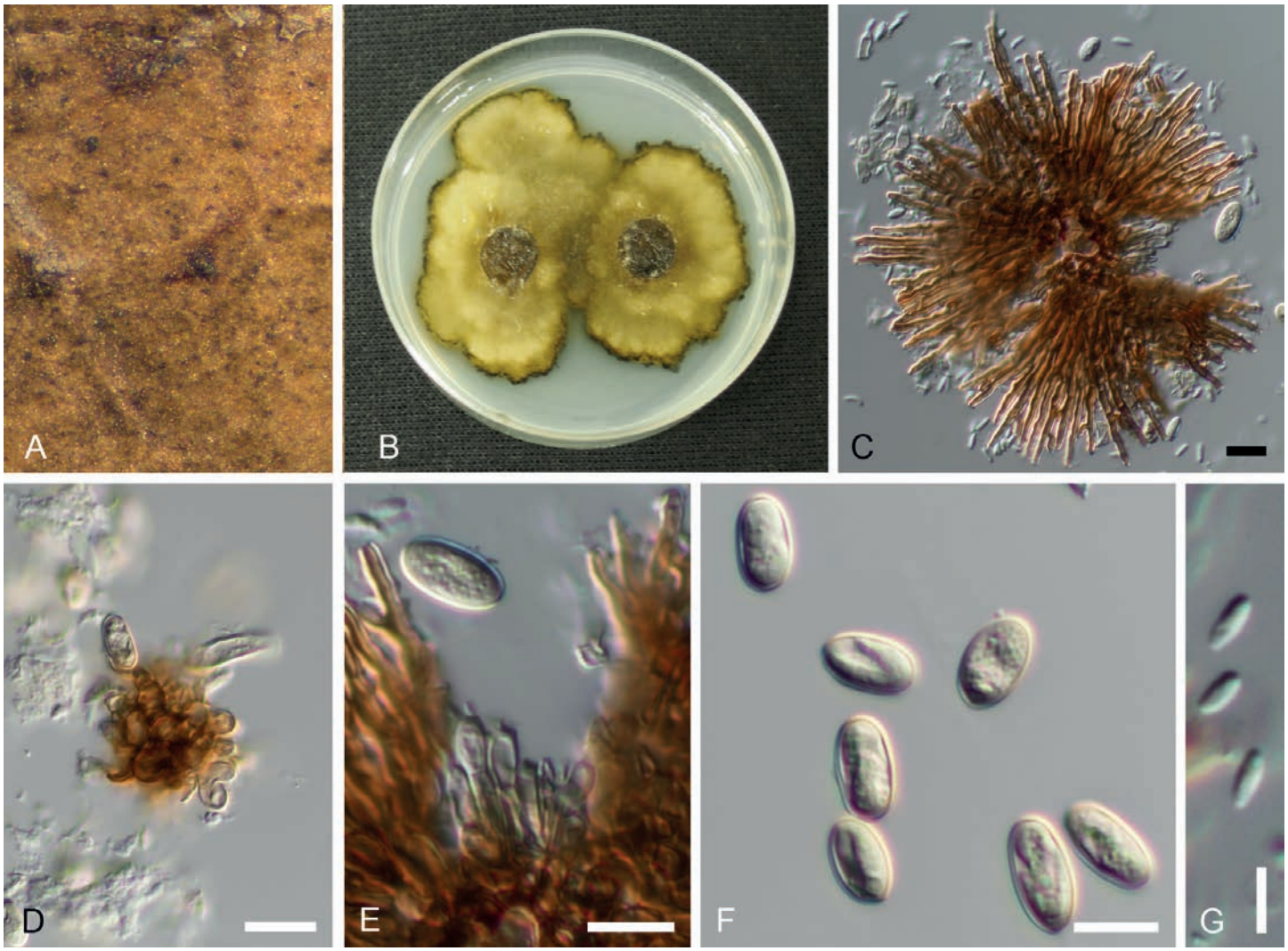

Fig. 15. *Tubakia dryinoides* (NBRC H-11618 – holotype). **A.** Pycnothyria on the surface of leaf litter. **B.** Culture on MEA (NBRC 9267 – ex-type culture). **C.** Scutellum. **D.** Central columella. **E.** Conidiophores. **F.** Conidia. **G.** Microconidia. Bars = 10 µm.

diam. *Scutella convex* sometimes more flattened, membranous, dense at the centre, looser at the edge, with a central hyaline or pale disc, 4–6 µm diam, surrounded by cells subcircular to angular in outline and 2–6 µm diam, giving rise to radiating hyphal strands, cells 7–25 × 2–5 µm, pale to dark brown, thick-walled (–1 µm), smooth, simple or 1–3 times bifurcating, ultimate branchlets with obtuse to mostly pointed tips. *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, about 13–37 µm wide, surrounded by large brown cells. Conidiophores reduced to conidiogenous cells, arising from the underside of the scutella, around the columella, radiating, orientation outward-downward, cylindrical, conical, delicate, about 8–10 × 2–7 µm, hyaline, thin-walled, smooth, apex obtuse to truncate, conidiogenesis phialidic, sometimes forming indistinct periclinal thickenings. *Conidia* solitary, ellipsoid to obovoid, 8.5–14.5 × 5.5–8.5(–10) µm, length/width ratio 1.3–2.3, wall thin, up to 1 µm, hyaline to subhyaline, smooth, apex and base broadly rounded, with inconspicuous to conspicuous basal hilum (frill), occasionally somewhat peg-like and truncate when conspicuous. *Microconidia* narrowly ellipsoid-ovoid, fusiform, mostly straight, 3–10 × 1.5–2.5 µm, hyaline, thin-walled, smooth.

In vitro: on MEA with optimal growth at 20° C, attaining 30–40 mm diam after 14 d, margin scalloped, at first creamy white, forming concentric rings of olivaceous mycelium, reverse greyish white, with olivaceous edge. Conidial formation not observed.

Type: **Japan**, Osaka, Suita, on *Quercus phillyraeoides*, 8 Oct. 1969, *T. Yokoyama* (NBRC H-11618 – holotype; NBRC 9267 = MUCC2292 – ex-type cultures).

Hosts range and distribution: On *Castanea crenata* and *Quercus phillyraeoides*, *Fagaceae*, Asia (Japan) [(?) on *Fagus sylvatica* and *Quercus robur*, Europe (France, Poland)].

Additional collections examined: **Japan**, Osaka, Ikeda, on *Castanea crenata*, 11 Nov. 1968, *T. Yokoyama*, NBRC H-11616; NBRC 9265 = MUCC2290; Minoo, on *Castanea crenata*, 8 Oct. 1969, *T. Yokoyama*, NBRC H-11617; cultures NBRC 9266 = MUCC2291.

Notes: *Tubakia dryinoides* is morphologically similar to *T. dryina*, at least in terms of characters of the pycnothyria, and was referred to as *T. dryina* in Yokoyama & Tubaki (1971). In contrast to genuine *T. dryina* collections, the conidia in *T. dryinoides* remain hyaline or subhyaline, at least until germination (see Yokoyama & Tubaki 1971). The phylogenetic analyses corroborated a close affinity of the Japanese collections to *T. dryina* but clearly suggested a separate species (Figs 3–5), which confirms the doubts upon the correct assignment of Japanese collections to *T. dryina* by Harrington *et al*. (2012). *Tubakia dryinoides* belongs to a cluster undoubtedly

composed of several species, including *T. paradryinoides*, which is genetically clearly distinct from *T. dryinoides* and morphologically easily distinguishable by its much larger conidia, 14–21 × 10–15 µm. *Tubakia americana* (Figs 3–5) is another closely allied species. Harrington & McNew (2018) included ITS sequences belonging to *T. dryinoides* in their *T. americana* "clade", which is, however, heterogeneous and divided into at least two clades representing *T. americana* and *T. dryinoides*. Several Chinese strains, isolated from *Quercus* sp. (GenBank FJ598616), *Linderia glauca* (GenBank JF502454) and from an unknown host as endophyte (GenBank FJ025349), all unpublished, belong to this clade and may be *T. dryinoides*. The identity of European strains (CBS 329.75 and CPC 33586, *Quercus* spp., France) belonging to this cluster is still unclear, but may indicate the presence of an additional cryptic European species. The current sampling is, however, not sufficient for a final conclusion. Above all, the morphology of pycnothyria and the colour of conidia of the European taxon are unknown (conidia colourless in *T. dryinoides*). Therefore, the collections concerned are tentatively maintained in *T. dryinoides*. ITS sequences retrieved from leaves of *Quercus robur* and *Fagus sylvatica* in Poland ("*T. dryina*" haplotype 2 in Boroń & Grad 2016) seem to belong to this cryptic European taxon and suggest that it might be rather common, but pycnothyria and the conidial colour of the Polish collections were not described.

Tubakia hallii T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi.org/10.1007/s10482-017-1001-9 [10]. 2017.

Illustrations: Harrington & McNew (2018, figs 1a–c).

Description in vivo: *Conidiomata* (pycnothyria) superficial, hypophyllous or epiphyllous on necrotic interveinal spots and along necrotic mid and lateral leaf veins. *Scutella* 45–155 µm diam, radiate, composed of a series of dark brown, thick-walled cells originating from a central cell, ending in blunt to acute tips. *Sporodochia* superficial with only a few radiating dark hyphae, mainly hypophyllous, on necrotic interveinal tissues and along leaf veins. *Conidiophores* reduced to conidiogenous cells, on underside of scutella or on top of sporodochia. *Conidia* hyaline, turning light brown with age, smooth to slightly varicose, aseptate, obovoid to ovoid, 9.5–14.5(–16) × 7.5–10(–11) µm (mean 12.4 × 8.7 µm). *Microconidia* not seen on leaves.

In vitro: On MYEA with optimal growth between 25–30 °C, attaining 50–70 mm diam after 7 d, initially white with dense aerial mycelium, smooth to scalloped at edge, turning cream to light grey at 10 d, developing concentric rings of dense mycelium, underside yellow, becoming golden yellow to brown at 10 d, sometimes with dark cell masses on surface or subsurface. *Conidiophores* rare to abundant, short, hyaline, aggregated (sporodochia) on agar surface, producing conidia in dark brown to black, wet masses. *Conidia* hyaline, becoming light brown, thick-walled, aseptate, ellipsoidal to obovate, 12.5–16.5(–17) × 5.3–7.5 µm (mean 14.5 × 6.2 µm). *Microconidia* not seen on agar media but produced from scutella developing on autoclaved pieces of leaves of *Q. macrocarpa* placed on MEA, hyaline, aseptate, fusiform, 3.5–7.5 × 1.0–2.5 µm.

Type: **USA**, Missouri, Kirbyville, on leaf of *Quercus stellata*, 2 Sep 2008, *D. Brandt* (ISC 453286 – holotype; CBS 129013 = A666 – ex-type strains).

Host range and distribution: On *Quercus* (*alba, bicolor*, *macrocarpa*, *muehlenbergii*, *stellata*), *Fagaceae*, North America (USA, Arkansas, Iowa, Kansas, Minnesota, Missouri, Wisconsin).

Notes: *Tubakia hallii* has recently been introduced by Harrington & McNew (2018) for collections previously referred to as *Tubakia* sp. B (Harrington *et al.* 2012, Harrington & McNew 2016). *Tubakia hallii* is morphologically and genetically close to *T. iowensis* (Figs 3, 5), but the analysis of a combined dataset of ITS and *tef1* sequences (Harrington & McNew 2018) showed that the two species form monophyletic sister groups, and they are morphologically differentiated. *Tubakia iowensis* causes characteristic oak blight characterised by forming necroses of leaf veins (Harrington *et al*. 2012) and has a restricted host range and distribution, whereas *T. hallii* is associated with leaf spots and necrotic veins, appears to have a broader host range (*Quercus alba, Q. bicolor*, *Q. macrocarpa*, *Q. muehlenbergii*, and *Q. stellata*) and geographic distribution, and crustose pycnothyria have not been observed on the petioles of overwintering leaves. Pycnothyria may be larger in *T. hallii* (up to 155 µm) compared to *T. iowensis* (up to 110 µm), but the sizes are variable and overlapping. In culture on MYEA, the production of conidia in *T. hallii* is not uncommon, but rare in *T. iowensis* (Harrington & McNew 2016).

Tubakia iowensis T.C. Harr. & D. McNew, *Mycologia* **104**: 86. 2012.

Illustrations: Harrington *et al.* (2012: 82, fig. 1A–N, 87, fig. 2A–L).

Description in vivo: Causing a late-season disease on bur oak (*Quercus macrocarpa*), named bur oak blight, first symptom on leaves visible as small purple to brown spots on veins, hypophyllous, occasionally forming small necrotic spots on the leaf blade, lesions later expanding along the veins and coalescing, portions of the leaf blade sometimes becoming chlorotic-necrotic and die off; severe infections may cause early leaf dieback and defoliation as well as branch dieback in later stages; some leaves with black pustulate conidiomata at the base of the petioles, remaining green or becoming necrotic, but usually remaining attached to the twigs. *Mycelium* internal, forming branched intra- and intercellular hyphae. *Conidiomata* (*pycnothyria*) amphigenous, scattered to gregarious on and along leaf veins (midveins and major lateral veins), punctiform, blackish, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella, 40–120(–160) µm diam. *Scutella* convex, membranous, dense to less compact, looser, with a small central pale disc, 5–15(–20) µm diam, surrounded by small cells, subcircular to angular in outline, (3–)4–8(–10) µm diam, giving rise to radiating hyphal strands, cells 5–20(–30) × 2–8 µm, pale to medium dark brown, thick-walled (–1 µm), smooth, simple or 1–3 times bifurcating, ultimate branchlets with obtuse to often pointed tips. *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, about 10–20 µm wide, surrounded by small, thin-walled, colourless or pale (pseudoparenchymatous) fertile cells. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, around the columella, radiating, subcylindrical to usually conical-ampulliform, delicate, 5–10(–15) × 2–5 µm, hyaline, thin-walled, smooth, apex obtuse to truncate, conidiogenesis phialidic. *Conidia* solitary, broad ellipsoid-obovoid to subglobose, 9–17 ×

6.5–10.5 µm, length/width ratio 1.1–2.4, wall thin, to about 1 µm wide, at first hyaline, subhyaline, later somewhat pigmented, olivaceous to brown, smooth, older conidia may be faintly rough-walled, apex and base broadly rounded, with inconspicuous to conspicuous basal hilum (frill), occasionally somewhat peg-like and truncate when conspicuous, about 1 µm wide. *Microconidia* formed in some collections, narrowly fusiform, often curved, 4–8.5 × 1–2 µm, hyaline, thin-walled, smooth. *Sporodochial conidiomata*, composed of conidiophore clusters, hypophyllous, on veins, irregular, occasionally with small, poorly developed scutella. *Crustose conidiomata* may be formed on overwintered leaves, at the base of petioles, and twigs, subepidermal, black, irregular, 15–500 µm diam, occasionally confluent, opening by irregular dehiscence; conidia developed in crustose conidiomata ellipsoid-obovoid, 9.5–14 × 6.5–8.5 µm, wall somewhat thickened, smooth to faintly roughened, at first colourless, later pigmented.

In vitro: On MEA with optimal growth at 25 °C, attaining 50–56 mm diam after 7 d, margin scalloped, at first white, felt-like, light grey at 10 d, forming concentric rings of aerial hyphae, reverse yellow at 10 d, with dark grey, dense "tissue" in concentric rings; conidia formed in culture ellipsoid-obovoid, 9–15.5(–18.5) × 5–8(–8.5) µm, with somewhat thickened walls, smooth to finely rough, at first colourless, later somewhat pigmented.

Type: **USA**, Iowa, Ames, Brookside Park, on *Quercus macrocarpa*, 21 Aug. 2008, *T. Harrington* (ISC 448599 – holotype); BPI 881219 – isotype.

Additional collection examined: **USA**, Iowa, Ames, Brookside Park, on *Quercus macrocarpa*, 21 Aug. 2008, *T. Harrington*, BPI 881221.

Additional cultures examined: See Table 1.

Host range and distribution: On *Quercus* (*macrocarpa, stellata*), *Fagaceae*, North America (USA, Arkansas, Iowa, Missouri, Wisconsin) [? *Quercus* sp., Asia, Iran].

Notes: *Tubakia iowensis* is a species belonging to the *T. dryina* complex and is morphologically similar to *T. dryina s. str*. Harrington *et al.* (2012) published results of detailed examinations of this species, described it as new species and pointed out core differences between the new species and *T. dryina s. str*. Although the pycnothyria of *T. iowensis* are difficult to differentiate from *T. dryina* conidiomata, the symptoms of bur oak blight are quite different. Characteristic circular to angular-irregular leaf spots dispersed on the whole leaf blade are not formed with *T. iowensis*, as they are for *T. dryina*; the lesions are rather confined to and spread along the veins. Severe infections may lead to early leaf dieback and defoliation, and, finally, branch dieback. Fusiform microconidia formed in pycnothyria are narrower, only 1–2 µm wide (vs. 1.5–2.5 µm wide in *T. dryina*). The status of *T. iowensis* as a separate species has been confirmed by sequence analyses; it is fully to highly supported in both the Bayesian and maximum parsimony analyses (Fig. 3). The Mexican isolate presenting *T. sierrafriensis* on *Quercus eduardi* is related to *T. iowensis*/*californica*/*suttoniana* complex as well, but these species are genetically separated (Figs 3–5) and morphologically quite different: they have pycnothyrial scutella with obtuse to sometimes even truncate tips of radiating cell threads and clear differences in conidial shape. A sequence retrieved from a *Tubakia* culture (CPC 23753) isolated from dead leaves of *Quercus* sp. in Iran clusters in the *T. iowensis* clade. This unusual finding requires further research based on a broader sampling of Iranian collections.

Tubakia japonica (Sacc.) B. Sutton, *Trans. Brit. Mycol. Soc.* **60**: 165. 1973. Fig. 16.
Basionym: *Actinopelte japonica* Sacc., *Ann. Mycol*. **11**: 312. 1913.

Illustrations: Yokoyama & Tubaki (1971: 65, pl. 1A–D, 67, pl. 2A; 69, pl. 3A; 70, pl. 4A–H; 74, pl. 8A–B).

Description in vivo: Living as endophytes in leaves, forming crustose to pustulate conidiomata on the surface of shed leaves (litter), and plant pathogenic, forming distinct leaf lesions. *Leaf spots* amphigenous, circular, subcircular to angular-irregular, 2–6 mm diam or oblong, ochraceous to pale brown, finally greyish white, margin distinct, reddish brown to fuscous. *Conidiomata* (*pycnothyria*) amphigenous, scattered to gregarious, occasionally confluent, punctiform, superficial, easily removable, circular to subcircular in outline, 155–260(–310) µm diam when mature, yellowish brown to blackish brown or almost black (stereomicroscopy), scutellate, fixed to the leaf surface by a central columella. *Scutella* convex to flattened, membranous, dense when young, later loose at the margin, outline regular, circular-subcircular, with a central colourless to pale brown disc, 8–15 µm diam, scutellum more or less uniformly pigmented, brown, central cells subcircular or angular-irregular in outline, 3–10 µm diam, giving rise to radiating threads of oblong hyphal cells, 7–35(–120) × 2.5–6 µm, septate, pale brown to brown, thick-walled (–1 µm), smooth, simple or one to two times bifurcating, tips of the threads simple to forked, ultimate tips obtuse to pointed. *Central columella* delicate, easily collapsing, loose, ephemeral, cylindrical, 15–60(–80) µm wide (in culture to 200 µm diam), central cells surrounded by smaller, hyaline to pale brown fertile cells that form a pseudoparenchymatous sheath. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, from parenchymatous cells around the upper part of the columella, radiating, orientation outward and downward, delicate, enlarged at the base and attenuated towards a narrow tip, cylindrical, conical to ampulliform, 7–28 × 4–12 µm, neck about 2–3 µm wide, hyaline to pale brown, thin-walled, smooth, apex obtuse to truncate, conidiogenesis phialidic, forming indistinct periclinal thickenings, sometimes percurrently proliferating, forming annellations. *Conidia* solitary, globose, subglobose to broad ellipsoid, large, 30–55 × 21–43 µm, length/width ratio 0.8–1.5, apex rounded, base rounded, often with distinct frill, wall somewhat thickened, hyaline or with a pale ochraceous tinge, smooth, wall 1 µm thick. *Microconidia* bacilliform, botuliform or narrowly navicular, 5–10 × 1–2 µm, formed in smaller conidiomata, 60–80 µm diam and 15–30 µm high.

In vitro: On MEA with optimal growth at 20 °C, attaining 35–40 mm after 14 d, margin scalloped, ivory white, velvety and flattened on the surface of colony, reverse pale greyish (conidial formation not observed). On potato sucrose agar rapidly growing, pale yellowish brown to chestnut-brown; aerial mycelium compact, silky, white to pale yellow; immersed hyphae rapidly growing; reverse yellowish brown to blackish brown; sporulation rare. On OA rapidly growing, ochraceous to pale yellowish brown; aerial mycelium compact, finely floccose, white to cream; immersed hyphae rapidly growing, reverse concolorous. On Czapek agar growth only restricted, greyish

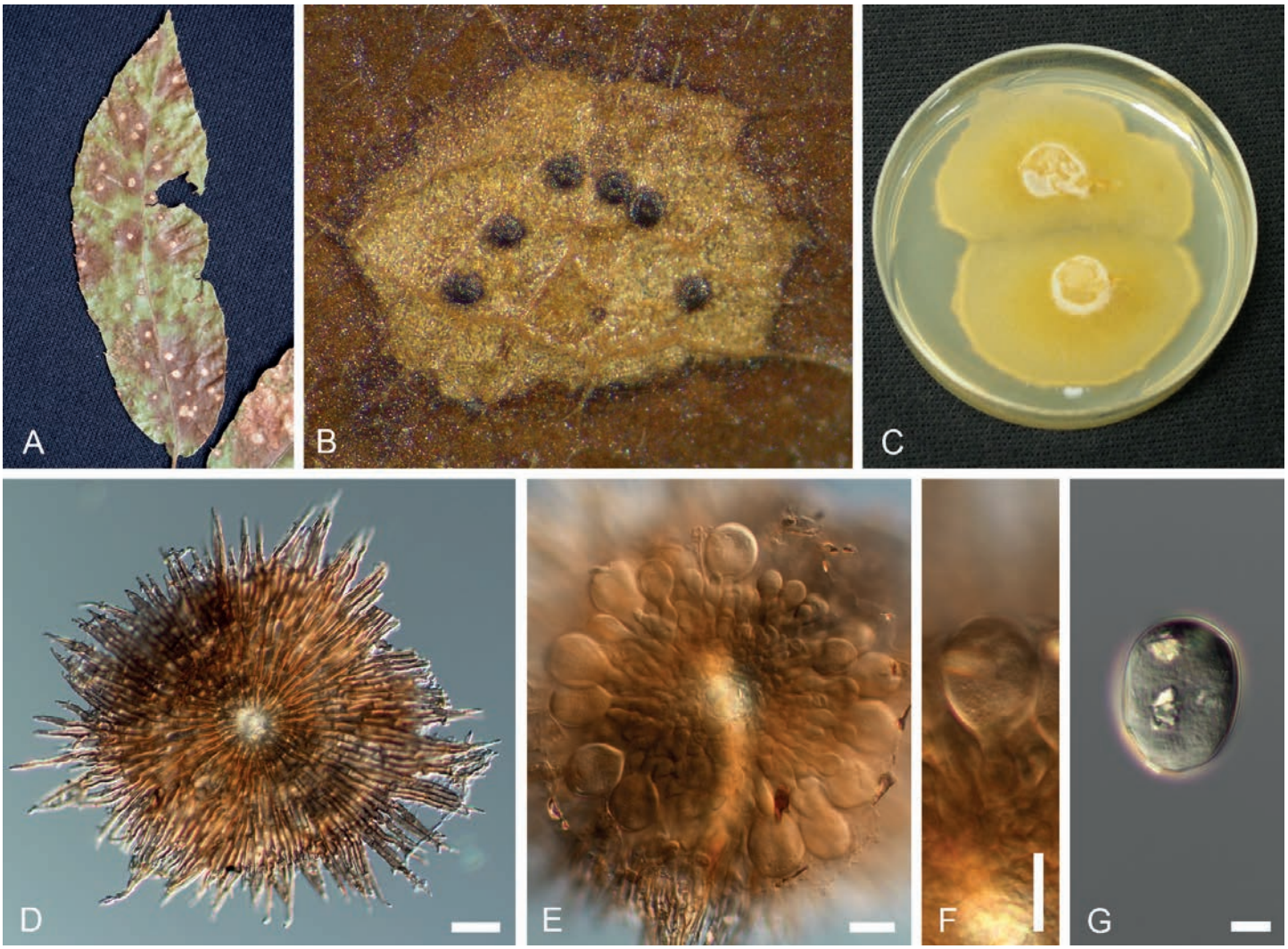

Fig. 16. *Tubakia japonica* (NBRC H-11611 – epitype). **A.** Symptoms on *Castanea crenata*. **B.** Pycnothyria on the surface of leaf spot. **C.** Culture on MEA (NBRC 9268 – ex-epitype culture). **D.** Scutellum. **E.** Central columella. **F.** Conidiophore. **G.** Conidium. Bars = 10 µm.

brown to pale blackish brown; aerial mycelium very poorly developed; immersed hyphae scarcely developed, reverse concolorous.

Types: **Japan**, Gifu Pref. (Prov. Mino), Kawauye-mura, on *Castanea crenata* (= *C. vesca* var. *japonica*), Oct. 1910, *K. Hara* (PAD – holotype; FH 888612 – isotype). *Topotypes*: **Japan**, Gifu Pref., Kawauye-mura, on *Castanea crenata*, Aug. 1920, *K. Hara* [Syd., Fungi Exot. Exs. 526] (e.g. BPI 391948, CUP, FH, HBG, K, MICH, MIN, PH, S). *Epitype* (designated here, MycoBank, MBT379574): **Japan**, Ibaraki Pref., Chiyoda, on *Castanea crenata*, 13 Aug. 1969, *K. Uchida* (NBRC H-11611; NBRC 9268 = MUCC2296 = ATCC 22472 – ex-epitype cultures).

Additional collection examined: **Japan**, Ibaraki Pref., Kasama, on *Castanea crenata* (= *C. pubinervis*), 17 Sep. 1969, *Y. Kobayashi*, NBRC H-11612; NBRC 9269 = MUCC2297; Kasama, on *Castanea crenata,* 17 Sep. 1969, *Y. Kobayashi*, NBRC H-11883; NBRC 9270 = MUCC2298; Shiga Pref., Ootsu, on *Castanea crenata,* 2 Sep. 1970, *T. Yokoyama*, NBRC H-11613; NBRC 9340 = MUCC2299 = CBS 191.71; Ootsu, on *Castanea crenata,* 30 Oct. 1970, *T. Yokoyama*, NBRC H-11615; NBRC 9342 = MUCC2301; Shiga Pref., Ootsu, on *Castanea crenata,* 2 Sep. 1970, *T. Yokoyama*, NBRC9341 = MUCC2300.

Host range and distribution: On *Castanea* (*crenata*, *mollissima*, *Castanea* sp.), *Quercus* (*acutissima*, *aliena*), *Fagaceae*, Asia (China, Japan, Korea).

Notes: *Tubakia japonica* is characterised by very large conidia, larger than those of any other *Tubakia* spp., and differs from any other species in forming narrow, mostly bacilliform microconidia (1–2 µm wide) that develop in special small conidiomata. The Chinese report of *T. japonica* on *Castanea mollissima* goes back to Chen (2002), and Korean records of this species on *Castanea crenata*, *Quercus acutissima* and *Q. aliena* refer to Lee *et al*. (1991) and Cho & Shin (2004). Phylogenetically *T. japonica* is closely allied to *T. seoraksanensis* (Figs 3–5), but differs from this species in having much smaller conidia and host range.

Tubakia liquidambaris (Tehon & Stout) T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi.org/10.1007/s10482-017-1001-9 [16]. 2017.
Basionym: *Leptothyriella liquidambaris* Tehon, *Mycologia* **21**: 192. 1929.

Description in vivo: *Leaf spots* amphigenous, subcircular, 1–6 mm diam, brown. *Pycnothyria* mainly epiphyllous, scattered to gregarious, 60–140 µm diam, morphologically barely distinguishable from pycnothyria of *T. dryina*. *Conidia* 8.5–14 × 6–9 µm, hyaline, subhyaline, later slightly pigmented.

In vitro: On MYEA similar to cultures of *T. macnabbii*, but with slower growth, a flat surface, and abundant production of conidia formed from sporodochia in concentric rings (CBS 139744 = A771 and CBS 139745 = A830, both from ISC 453303).

Type: **USA**, Illinois, Pulaski County, Olmsteadt, necrotic leaf spots on *Liquidambar styraciflua*, 9 Aug. 1922, *P. A. Young 4985* (ILLS 1445 – holotype).

Host range and distribution: on *Liquidambar styraciflua*, *Altingiaceae*, North America (USA, Arkansas, Florida, Illinois, Maryland, Mississippi, North Carolina, Oklahoma).

Notes: Pycnothyria of *Leptothyriella liquidambaris* are morphologically indistinguishable from those of *Tubakia dryina*. Therefore, it is not surprising that *L. liquidambaris* was previously usually considered a synonym of the latter species. However, based on culture characteristics and results of molecular sequence analyses, Harrington & McNew (2018) demonstrated that *Tubakia* on *Liquidambar styraciflua* represents a species of its own (Fig. 5).

Tubakia macnabbii T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi.org/10.1007/s10482-017-1001-9 [12]. 2017.

Illustrations: Harrington & McNew (2018, figs 1d–g).

Description in vivo: *Conidiomata* (pycnothyria) superficial, hypophyllous or epiphyllous, on necrotic spots and along mid and lateral leaf veins, scutella radiate, (45–)65–135(–178) µm diam, composed of a series of dark brown, thick-walled cells radiating from a central cell, ending in a blunt to acute point. *Sporodochia* with no scutella or poorly developed scutella, epiphyllous or hypophyllous on necrotic veins and tissue, light to dark brown due to mass of conidia. *Conidiophores* on underside of scutella or on sporodochia. *Conidia* from radiate pycnothyria and sporodochia hyaline, turning light brown with age, smooth to slightly varicose, aseptate, obovoid to ellipsoidal, 9.5–14.5 × (6–)7–9(–10) µm (mean 11.8 × 8.2 µm). *Microconidia* sterile, hyaline, aseptate, fusiform, 3.5–9(–11.5) × 1–3 µm, from small, radiate pycnothyria, alone or along with macroconidia. *Crustose conidiomata* hypophyllous or epiphyllous on necrotic mid and lateral veins of late-season leaves, leaves of the current year and overwintering leaves still hanging from trees, black, pulvinate, irregularly shaped, 0.7–1.5 mm diam, single or grouped, covered with thick-walled cells, breaking open with swelling when wet. *Conidia* hyaline to light brown, aseptate, ellipsoidal to obovate or irregular in shape, (8–)10–15(–19) × (6–)6.5–8(–9) µm (mean 13 × 7.1).

In vitro: On MYEA with optimal growth at 25 °C, diam. 50–65 mm after 7 d, creamy-white aerial mycelium, smooth to scalloped at edge, may develop concentric rings of dense mycelium, underside golden yellow to slightly darker with age, sometimes with dark cell masses on surface or subsurface. *Conidiophores* rare to abundant, short, hyaline, sometimes aggregated (sporodochia) on agar surface. *Conidia* hyaline to dark brown, thick-walled, smooth to slightly varicose, aseptate, obovoid to ellipsoidal, 8.5–15 × 5.5–10 µm.

Type: **USA**, Missouri, Jackson County, on leaves of *Quercus palustris*, Sep 2010, *D. Brandt* (ISC 453290 – holotype; CBS 137349 = A989 – ex-type strains).

Host range and distribution: On *Castanea* sp., *Quercus* (*alba*, *hemisphaerica*, *imbricaria*, *kelloggii*, *laurifolia*, *macrocarpa*, *marilandica*, *muehlenbergii*, *nigra*, *palustris*, *rubra*, *stellata, velutina*, *virginiana*), North America (USA, Arkansas, Florida, Illinois, Iowa, Kansas, Louisiana, Maryland, Minnesota, Missouri, New Hampshire, Ohio, Oklahoma, Wisconsin).

Notes: According to Harrington & McNew (2018), *Tubakia macnabbii*, previously referred to as *Tubakia* sp. D (Harrington and McNew 2016), was the most commonly encountered *Tubakia* species in the eastern USA, where it appears to be indigenous and widespread on oaks belonging to sect. *Lobatae*, but this species was also found on *Castanea* spp. in Florida and Iowa. Harrington & McNew (2018) assigned *Tubakia* specimens from California on leaves and twigs of *Quercus agrifolia*, *Q. wislizeni*, *Q. kelloggii*, and *Lithocarpus densiflorus*, collected by S. Latham, to *T. macnabbii*, which belong, however, to *T. californica* (see discussion under the latter species). CBS 639.93, ex-type strain of *Dicarpella dryina*, isolated from leaves of *Quercus rubra* collected in a nursery in Italy, was also included in *T. macnabbii*, but has to be excluded (see discussion under *T. suttoniana*). Harrington & McNew (2018) questioned that the sexual morph described by Belisario (1991) actually pertains to *Tubakia*, although the ex-type strain of *D. dryina* clusters in the *Tubakia* clade and was assigned by them to *T. macnabbii*.

Harrington & McNew (2018) distinguished *Tubakia macnabbii* from *T. dryina* by the production of crustose conidiomata on vein and leaf tissue, rather than twigs (Harrington *et al.* 2012), larger conidia formed in crustose conidiomata of *T. macnabbii*, and cultures lacking the concentric rings typical of *T. dryina*. The conidia of *T. macnabbii* from radiate scutella are slightly smaller than those of *T. hallii* and *T. iowensis*, which usually occur on oaks of *Quercus* sect. *Quercus*. Unlike *T. iowensis*, *T. macnabbii* readily produces conidia in culture. *Tubakia californica*, another species belonging to the *T. macnabbii* complex, forms quite distinct symptoms, and radiate pycnothyria are lacking. *Tubakia tiffanyae* is another closely allied species on red oaks, which differs from *T. macnabbii* by somewhat larger conidia and characteristically circular leaf spots. *Tubakia suttoniana*, although insufficiently known, seems to be morphologically distinguished from *T. macnabbii* by having obtuse tips of radiating scutellum strands and short cylindrical to subclavate microconidia with rounded apex and broadly truncate base.

Tubakia macnabbii, as currently circumscribed and confined to North American collections, represents a genetically heterogeneous complex of cryptic taxa that needs further research (Fig. 5).

Tubakia melnikiana Marm. & U. Braun, ***sp. nov.*** MycoBank MB823663. Fig. 17.

Etymology: Dedicated to the recently deceased Russian mycologist, Vadim A. Mel'nik (*1937, †2017).

Description in vivo: *Leaf spots* amphigenous, subcircular to angular-irregular, often spread along veins, 2–20 mm diam, sometimes expanded, occupying large leaf portions, to 40 mm diam, pale to medium dark brown, finally sometimes paler brown, ochraceous, straw-coloured or greyish, margin indefinite or with a narrow darker border, dark purplish violet, brown to blackish. *Mycelium* internal, forming branched intra- and intercellular hyphae. *Conidiomata* (*pycnothyria*) amphigenous, scattered to

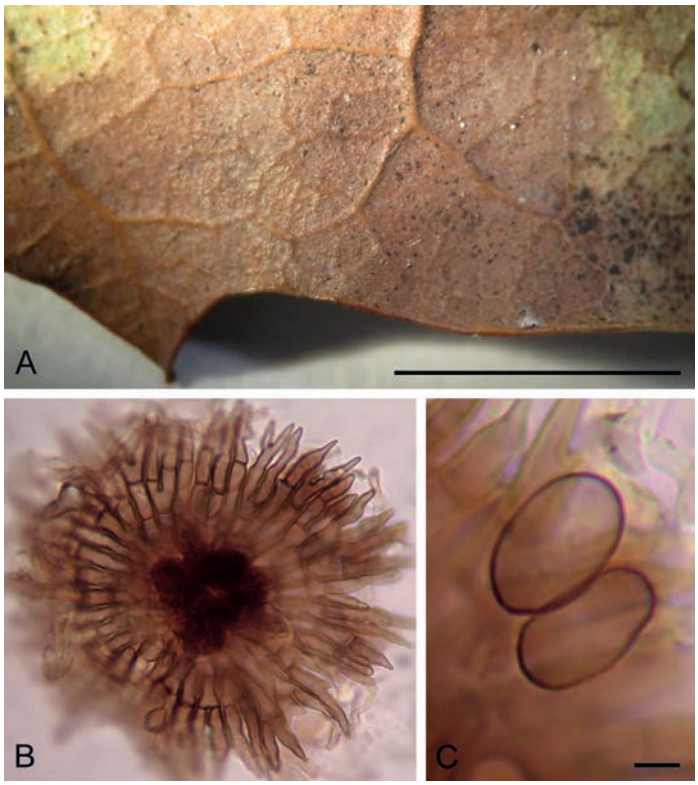

Fig. 17. *Tubakia melnikiana* (HAL 3179 F – holotype). **A.** Necrotic leaf lesion with pycnothyria. **B.** Pycnothyrium. **C.** Conidia. Bars = 1 cm (A), 20 µm (B), 5 µm (C).

gregarious, punctiform, blackish, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella, 80–100(–140) µm diam. *Scutella* convex, sometimes more flattened, membranous, dense, compact, later sometimes less compact, looser, with a central hyaline or pale disc, 5–15 µm diam, surrounded by small cells, subcircular to angular in outline, 3–8 µm diam, giving rise to radiating hyphal strands, cells oblong, 6–30(–35) × 2–6 µm, pale to medium dark brown, thick-walled (–1 µm), smooth, simple or 1–2(–3) times bifurcating, ultimate branchlets with obtuse to pointed tips. *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, surrounded by small, thin-walled, colourless (pseudoparenchymatous) cells. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, around the columella, radiating, orientation outward-downward, conical, ampulliform-cuspidate, delicate, about 10–18 × 2–5(–6) µm, hyaline, thin-walled, smooth, apex obtuse to truncate, conidiogenesis phialidic, percurrent proliferations not observed. *Conidia* solitary, subglobose, broad ellipsoid-obovoid, occasionally subcylindrical, (7–)9–16 × 5–9 µm, length/width ratio 1.2–2.2, wall thin, up to 1 µm wide, at first hyaline, subhyaline, later pale olivaceous to olivaceous brown, smooth or almost so, apex rounded, base rounded or with truncate basal hilum, occasionally somewhat peg-like. *Microconidia* occasionally formed narrowly ellipsoid(-subcylindrical) to broad ellipsoid-ovoid, apex obtuse, rounded, base round to short obconically truncate, straight, 4–8 × 2–4 µm, hyaline, thin-walled, smooth.

In vitro: On MEA at 22°C colonies attaining 60–70 mm diam after 12 d, margin scalloped, at first white, felted, later cream, forming concentric rings, older cultures becoming light brown. Conidia formed in older cultures ovoid to broad ellipsoid, 10–16 × 7–10 µm, with slightly thickened walls, at first colourless, later becoming light brown.

Type: **Mexico,** Nuevo León, Iturbide, Bosque Escuela, 24°42’30’’N, 99°51’44.6’’W, 1 620 m alt, on *Quercus canbyi*, 29 Oct. 2016, *J. Marmolejo* (HAL 3179 F – holotype; CFNL 2939 = CPC 32255 – ex-type cultures).

Additional collections examined: **Mexico**, Nuevo León, Iturbide, Bosque Escuela, 24°42’30.1’’N, 99°51’48.2’’W, 1 620 m alt, on *Quercus canbyi*, 6 Oct. 2016, *J. Marmolejo*, HAL 3174 F, 3175 F; CFNL 2933 = CPC 32249, CFNL 2934 = CPC 32250; ibid., 24°42’30.1’’N, 99°51’44.7’’W, 1 617 m alt, on *Quercus canbyi*, 6 Oct. 2016, *J. Marmolejo*, HAL 3176 F; CFNL 2935 = CPC 32251; ibid., 24°42’30.7’’N, 99°47.3’’W, 1614 m alt, on *Quercus canbyi*, 29 Oct. 2016, *J. Marmolejo*, HAL 3177 F; CFNL 2936 = CPC 32252; ibid., 24°42’30.2’’N, 99°51’47.8’’W, 1 619 m alt, on *Quercus laeta* (= *Q. prinopsis*), 29 Oct. 2016, *J. Marmolejo*, HAL 3178 F; CFNL 2938 = CPC 32254; ibid., 24°42’23.9’’N, 99°51’44’’W, 1 620 m alt, on *Quercus eduardi*, 10 Nov. 2016, *J. Marmolejo*, HAL 3184 F; CFNL 2943.

Host range and distribution: On *Quercus canbyi, Q. eduardi,* and *Q. laeta* (= *Q. prinopsis*), *Fagaceae*, North America, Mexico.

Notes: *Tubakia melnikiana* is morphologically close to and confusable with *T. dryina* and only distinguishable from typical collections of the latter species by having obtuse to acute ultimate tips of the radiating pycnothyrial hyphal strands and narrowly ellipsoid (-subcylindrical) to broad ellipsoid-ovoid microconidia with obtuse to rounded apex and round to short obconically truncate base. The differences in the scutella of the two species are only gradual, and microconidia are only occasionally formed. However, *T. melnikiana* and *T. dryina* are genetically clearly distinct and not closely allied (Figs 3–5). *Tubakia melnikiana* is closer to *T. suttoniana*. The *T. melnikiana* clade is fully supported by both the Bayesian and maximum parsimony analyses (Figs 3, 4).

Tubakia oblongispora C. Nakash., ***sp. nov.*** MycoBank MB623665. Fig. 18.

Etymology: Epithet referring to the more oblong conidia in comparison with *T. dryina*.

Description in vivo: Living as endophyte in leaves, forming crustose conidiomata on the surface of shed leaves (litter). *Mycelium* internal and external, forming hyaline, branched intra- and intercellular hyphae, external hyphae observed on the lower leaf surface, pale brown, branched. *Conidiomata* (*pycnothyria*) amphigenous, scattered to gregarious, punctiform, blackish grey to blackish, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella, 63–150 µm diam. *Scutella* convex to campanulate, sometimes more flattened, membranous, dense, compact, later sometimes less compact, with a central hyaline or pale brown disc, 5–10 µm diam, surrounded by small cells, subcircular to angular in outline, 3–5 µm diam, giving rise to radiating hyphal strands, cells 16–40 × 2–5.5 µm, subhyaline to brown, thick-walled (–1 µm), smooth, simple or 1–2 times bifurcating, ultimate branchlets with obtuse tips. *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, about 10–20 µm wide, surrounded by small, thin-walled, pale to brown cells. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, around the columella, radiating, orientation outward-downward, conical, ampulliform-cuspidate, delicate,

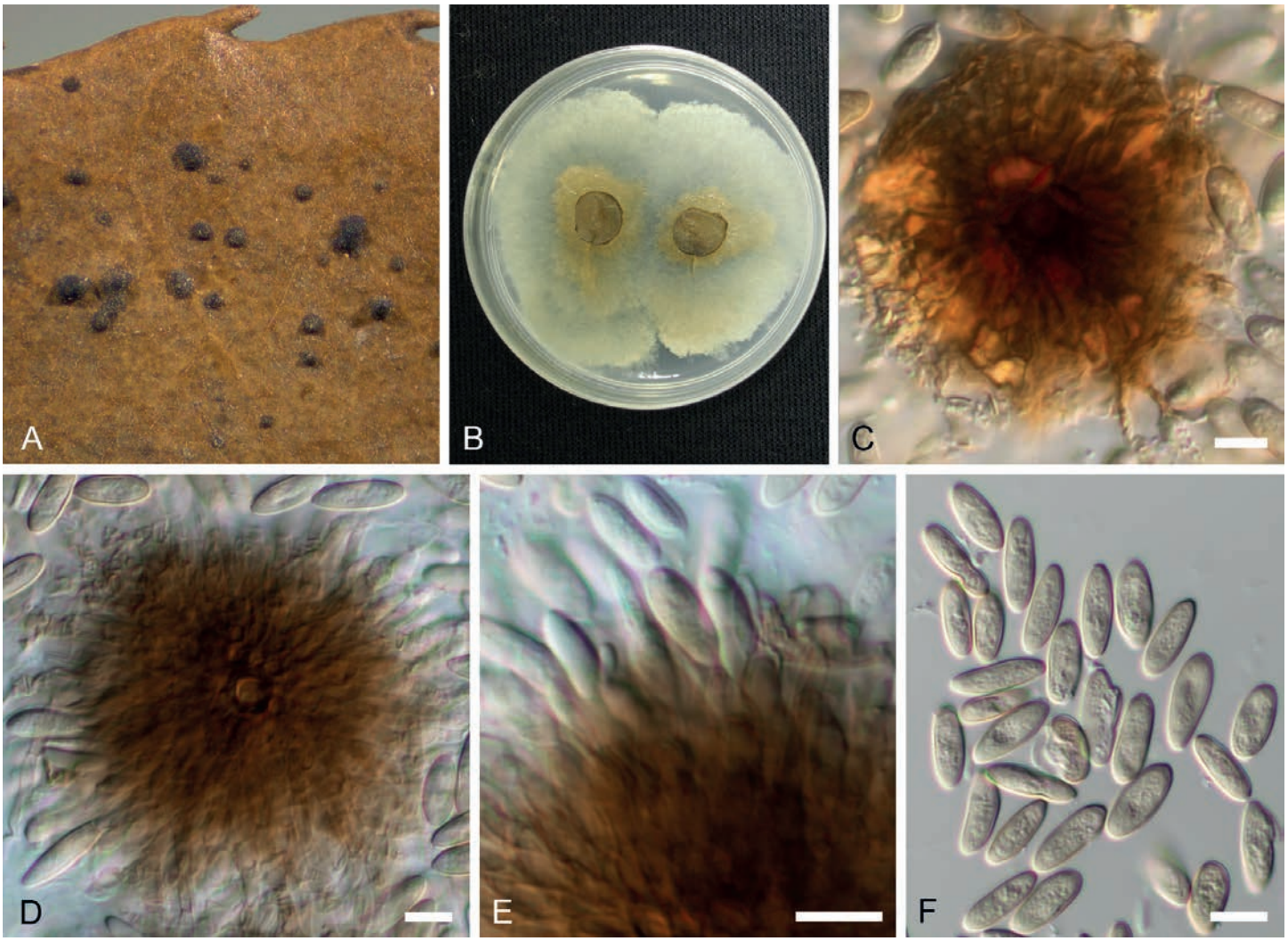

Fig. 18. *Tubakia oblongispora* (NBRC H-11881 – holotype). **A.** Pycnothyria on the surface of leaf litter. **B.** Colonies on MEA (NBRC 9885 – ex-type culture). **C.** Scutellum. **D.** Central columella. **E.** Conidiophores. **F.** Conidia. Bars = 10 µm.

13–35 × 2–5.5 µm, subhyaline to brown, thin-walled, smooth, apex obtuse to conically truncate, conidiogenesis phialidic, proliferating percurrently, forming periclinal thickenings or collarettes. *Conidia* solitary, ellipsoid-obovoid, fusiform, oblong, straight to slightly curved, 12–20 × 4.5–7.5 µm, length/width ratio 1.8–3.8, wall thin, to 1 µm wide, subhyaline to pale brown, smooth, apex broadly rounded, base obconically truncate, with inconspicuous to conspicuous basal hilum. *Microconidia* not observed.

In vitro: On MEA with optimal growth at 20 °C, attaining 20–25 mm diam after 14 d, margin scalloped, at first ivory white, velvety and flattened on the surface of colony, reverse pale grey. Conidial formation not observed *in vitro*.

Type: **Japan**, Osaka, Mt. Yamato-Katsuragi San, on *Quercus serrata*, 11 Jun. 1972, *T. Yokoyama* (NBRC H-11881 – holotype; NBRC 9885 = MUCC2295 – ex-type cultures).

Hosts range and distribution: On *Quercus serrata*, *Fagaceae*, Asia (Japan).

Notes: *Tubakia oblongispora* is phylogenetically closely allied to *T. dryina* but morphologically quite distinct (see diagnosis; Figs 3, 5). It belongs to a group of *Tubakia* species with obtuse, non-acute tips of the ultimate branchlets of radiating scutellum strands. Among species of this morphological group, *T. oblongispora* is comparable with *Oblongisporothyrium castanopsidis*, which differs, however, in having hyaline, broader conidia, 11–20 × 7–9.5 µm, with a length/width ratio of 1.6–2.2.

Tubakia paradryinoides C. Nakash., ***sp. nov.*** MycoBank MB823666. Fig. 19.

Etymology: Composed of para- (similar to) and the name of the comparable species, *Tubakia dryinoides*.

Description in vivo: Living as endophyte in leaves, forming crustose conidiomata on the surface of leaves, and pathogenic, causing *leaf spots*, amphigenous, subcircular to irregular, 5–25 mm diam, yellowish ochraceous, straw-coloured to greyish brown, margin distinct. *Mycelium* internal, forming hyaline, branched intra- and intercellular hyphae, external hyphae not observed. *Conidiomata* (*pycnothyria*) amphigenous, mainly epiphyllous, scattered to gregarious, punctiform, yellowish brown to blackish, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella, 50–155 µm diam. *Scutella* convex, sometimes more flattened, membranous, dense at centre, looser towards the margin, with a central hyaline or pale brown disc, 5–12 µm diam, surrounded by small pale brown cells, subcircular to angular in outline, 3–6 µm diam, giving rise to

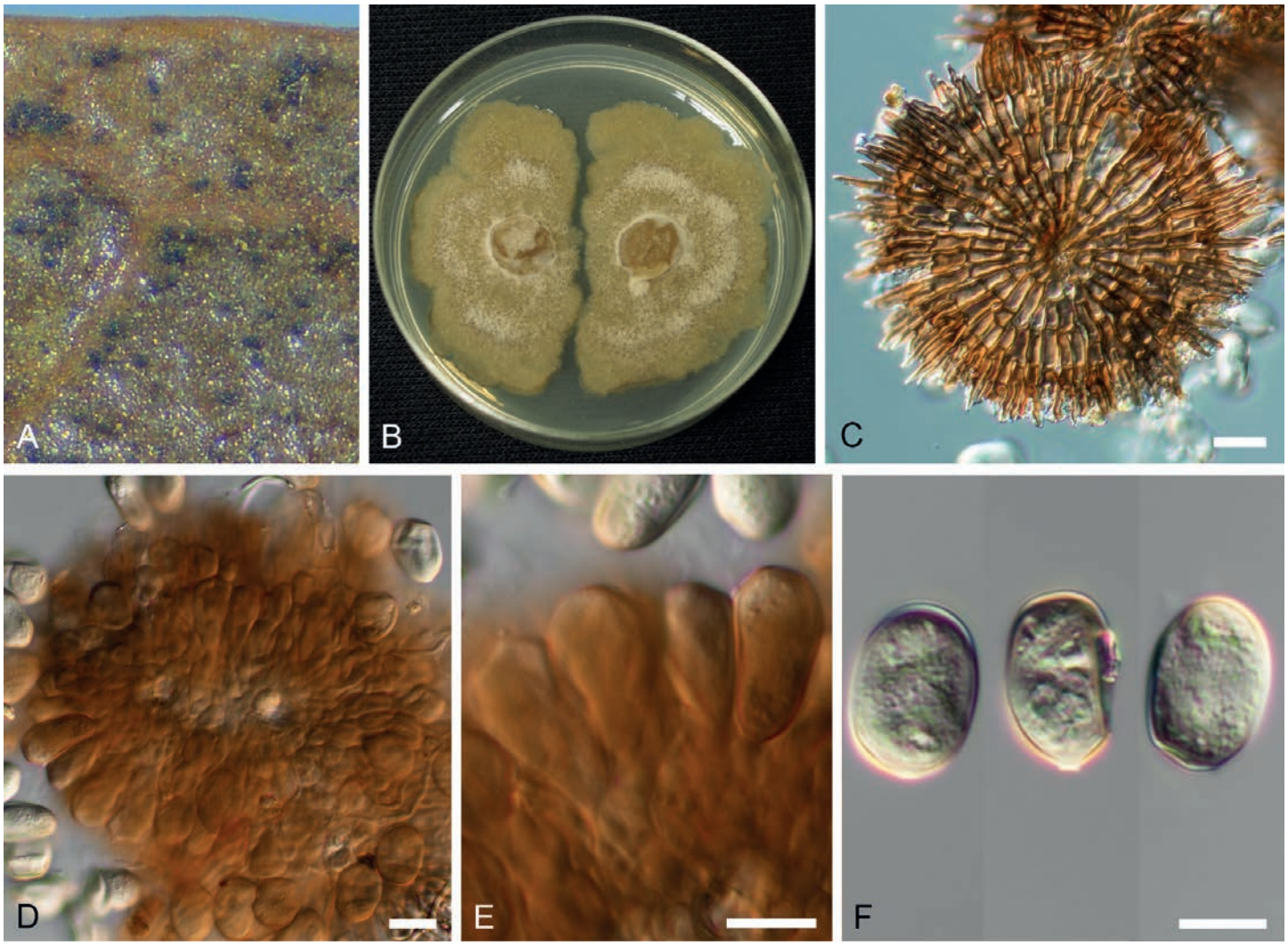

Fig. 19. *Tubakia paradryinoides* (TFM:FPH 3923 – holotype). **A.** Pycnothyria on the surface of leaf spot. **B.** Culture on MEA (NBRC 9884 – ex-type culture). **C.** Scutellum. **D.** Central columella. **E.** Conidiophores. **F.** Conidia. Bars = 10 µm.

radiating hyphal strands, cells 7–30(–37) × 3–6 µm, pale brown to medium dark brown, thick-walled (–1 µm), smooth, simple or 1–3 times bifurcating, ultimate branchlets with pointed tips. *Central columella* below the scutellum delicate, easily collapsing and loose, ephemeral, about 18–27 µm wide, surrounded by small, thin-walled, colourless or pale pseudoparenchymatous cells. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, around the columella, radiating, orientation outward-downward, conical to ampulliform, 10–20 × 4–8 µm, subhyaline to pale brown, thin-walled, smooth, apex obtuse to truncate, conidiogenesis phialidic, sometimes forming indistinct periclinal thickenings. *Conidia* solitary, broad ellipsoid-obovoid, 14–21 × 10–15 µm, length/width ratio 1.1–1.8, wall thin, up to 1 µm wide, hyaline to subhyaline, faintly rough-walled, apex and base broadly rounded, with inconspicuous to conspicuous basal hilum, 2–2.5 µm diam. *Microconidia* not observed.

In vitro: On MEA with optimal growth at 20 °C, attaining 35–40 mm after 14 d, margin scalloped, straw coloured, forming concentric ring of aerial hyphae, reverse in straw coloured, forming a dark brown concentric ring. Conidial formation not observed.

Type: **Japan,** Ibaraki Pref., Hokota, on *Quercus acutissima*, 12 Oct. 1972, *T. Kobayashi & K. Sasaki* (TFM:FPH 3923 – holotype; NBRC 9884 = MUCC2294 – ex-type cultures).

Hosts range and distribution: On *Quercus acutissima*, *Fagaceae*, Asia (Japan).

Notes: *Tubakia paradryinoides* is phylogenetically very closely allied to *T. dryina* and more distant from *T. dryinoides* and *T. oblongispora* (Figs 3, 5), which is also reflected in the morphological characters of pycnothyria of the species involved. The pycnothyrial scutella of these species are characterised by having hyphal strands with acute ultimate tips. However, *T. paradryinoides* is easily distinguishable from *T. dryina* and *T. dryinoides* by its hyaline to subhyaline, much larger conidia, 14–21 × 10–15 µm (*vs*. at first hyaline, subhyaline, but later pale olivaceous, olivaceous brown to brownish and (7–)9–16(–18) × (5–)6–10(–10.5) µm in *T. dryina*, and 8.6–14.7 × 5.5–8.5 (–10) µm in *T. dryinoides*). The independence of *T. paradryinoides* as a distinct species is mainly supported by its unique *tub2* sequence which influences its position in the phylogenetic tree (data not shown, see Fig. 3). Also see the notes under *T. dryinoides*; this assemblage of sequences could comprise several closely allied lineages that might represent additional cryptic species.

Tubakia seoraksanensis H.Y. Yun, *Mycotaxon* **115**: 371. 2011.

Illustrations: Yun & Rossman (2011: 372, fig. 1A–F).

Description in vivo: Causing *leaf spots* on Mongolian oak (*Quercus mongolica*), amphigenous, mainly epiphyllous, circular to broad elliptical, 2–10 mm diam, sometimes confluent, spread over the leaf blade, or forming necrotic lesions along the midrib or veins, pale brown, with regular to irregular margin, darker brown. *Conidiomata* (*pycnothyria*) amphigenous, mainly epiphyllous, mostly associated with leaf spots, superficial, scattered to gregarious, sometimes confluent, above all at veins, brown to dark brown, 90–160 µm diam, circular in outline, scutellate, fixed to the leaf surface by a central columella, easily removable. *Scutella* somewhat convex, membranous, dense to less compact, looser, centre with a colourless or pale disc, consisting of a single hyaline cell, surrounded by small cells, subcircular to angular in outline, giving rise to radiating hyphal strands, cells 4–6 µm wide, brown to dark brown, thick-walled (to about 1 µm), smooth, simple or 1–3 times bifurcating, ultimate branchlets with acute, cornuted tips. *Central columella* below the scutellum delicate, easily collapsing and ephemeral. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, around the upper part of the columella, radiating downward and towards the margin, cylindrical, conical-clavate, fusiform, delicate, 14–22 × 3–5 µm, thin-walled, smooth, hyaline to pigmented, narrowed towards a thin point at neck, conidiogenesis phialidic. *Conidia* solitary, subglobose, ellipsoid to broad ellipsoid, 13–25 × 10–15 µm, length/width ratio 1.15–1.56, wall thin or finally slightly thickened, hyaline, later sometimes pale yellowish brown, smooth, apex broadly rounded, with a conspicuous basal hilum and prominent frill. *Microconidia* not observed.

In vitro: On MEA reaching 32–44 mm diam after 10 d at 25 °C in the dark, low velutinous to fuzzy, margin uneven, whitish to pale yellow, centre darker, becoming paler towards the margin, olive brown, light olive brown to yellow, margin white, reverse wrinkled, non-sporulating; *hyphae* branched, septate, 3.2–4.8 µm wide, hyaline to slightly brownish in mass, some hyphae with short coils on side branches.

Type: **South Korea,** Gangwon, Seorakasan National Park, Seorak-dong, Sokcho-si, Gangwon-do, on *Quercus mongolica*, *Fagaceae*, 31 Aug. 2009, H.Y. Yun (BPI 880799 – holotype; CBS 127490–127493 – ex-type cultures).

Host range and distribution: Only known from the type collection.

Notes: This species belongs to a group of relatively large-spored *Tubakia* spp., but differs from *T. japonica* and *T. chinensis* in having smaller conidia, not overlapping in size. *T. seoraksanensis* belongs to the *T. suttoniana* complex and is closely related to *T. japonica*, but well supported as a separate species (Fig. 3: PP = 1.0, MP-BS = 84 %; Figs 4, 5: PP = 1.0, MP-BS = 94 %).

Tubakia sierrafriensis O. Moreno-Rico & U. Braun, ***sp. nov.*** MycoBank MB823667. Figs 20, 21.

Etymology: Named after Sierra Fría, mountain in Aguascalientes, México, the origin of the type collection.

Description in vivo: *Leaf spots* amphigenous, usually 1–3 per leaf, subcircular to angular-irregular, 2–15 × 2–10 mm, usually ochraceous brown to medium dark brown, margin indefinite or with narrow darker brown margin or marginal line, occasionally somewhat raised, sometimes zonate, with slightly paler centre surrounded by a darker marginal line and broad brown halo. *Conidiomata* (*pycnothyria*) amphigenous, effuse, loose to aggregated, dense, sometimes in concentric rings, punctiform, superficial, easily removable, circular to subcircular in outline, (40–)50–120(–135) µm diam, dark brown to blackish (stereomicroscopy), scutellate, fixed to the leaf surface by a central columella. *Scutella* convex, membranous, loose to compact, outline regular to somewhat irregular, medium to medium dark brown (stereomicroscopy), with a central colourless or pale disc, 5–15 µm diam or sometimes even lacking, scutellum uniformly pigmented (microscopy), olivaceous to medium brown, or with a darker central zone, 20–60 µm diam, and paler periphery, central cells angular-irregular to almost rounded in outline, 2–10 µm diam, or oblong, 10–15 × 2–7 µm, giving rise to radiating threads of hyphal cells, simple to often 1–3 times bifurcating, cells 4–25 × 2–5 µm, walls about 1 µm thick, tips of the threads simple or once to two times forked, branchlets short, ultimate tips consistently obtuse or sometimes even truncate; central *columella* below the scutellum delicate, easily collapsing and loose, short and about 25–30 µm wide, composed of thin-walled, colourless or pale fertile cells. *Conidiophores* reduced to conidiogenous cells, arising from the underside of the scutella, from fertile cells around the upper part of the columella, radiating, conical, ampulliform-cuspidate, delicate, not very conspicuous, about 5–15 × 2–4 µm, hyaline, thin-walled, smooth. *Conidia* solitary, polymorphous, broad ellipsoid-obovoid, short subcylindrical, some conidia even subglobose or irregular in shape, occasionally with oblique base, straight to occasionally somewhat curved, 9–18 × 5–9 µm (length/width ratio 1.3–2.7, average 1.95), aseptate, apex rounded, base rounded to attenuated and then with a truncate hilum or even with a small peg-like base, thin-walled, hyaline, finally very pale olivaceous, smooth. *Microconidia* occasionally present *in vivo*, ellipsoid-fusiform, straight, rarely slightly curved, 5–10 × 2–3.5 µm, thin-walled, hyaline, smooth.

In vitro: On MEA at 22 °C colonies attaining 80–82 mm diam after 20 d, margin undulate, at first white, with concentric rings of aerial mycelium, center green olive with brown hyphal stripes, reverse still colourless after 20 d, without sporulation.

Type: **México,** Aguascalientes, San José de Gracia, Laguna Seca, 22°10’40.61’’N, 102°38’36.48’’W, 2.662 m alt, on *Quercus eduardi*, 19 Jan. 2017, *O. Moreno-Rico* (CFNL 2944 – holotype; CFNL 2944 = CPC 33020 – ex-type culture).

Additional collections examined: *ibid*., on *Quercus eduardi*, 21 Apr. 2016, *O. Moreno-Rico*, HAL 3163 F; 22 Nov. 2016, *O. Moreno-Rico*, HAL 3173 F.

Hosts range and distribution: On *Quercus eduardi*, *Fagaceae*, North America (Mexico).

Notes: *Tubakia sierrafriensis* belongs to a group of *Tubakia* species that are morphologically distinguished from *T. dryina* by having rounded-obtuse to truncate tips of the outer ends of the radiating scutellum threads, and is comparable to *Oblongisporothyrium castanopsidis* from which it differs in having smaller scutella [(40–)50–120(–135) µm diam, *vs*. 100–150 µm diam in *O. castanopsidis*] and polymorphous conidia, broad ellipsoid-obovoid, short subcylindrical, some conidia even subglobose or irregular in shape, occasionally with oblique base, at first hyaline, finally very pale olivaceous (vs. uniformly oblong-

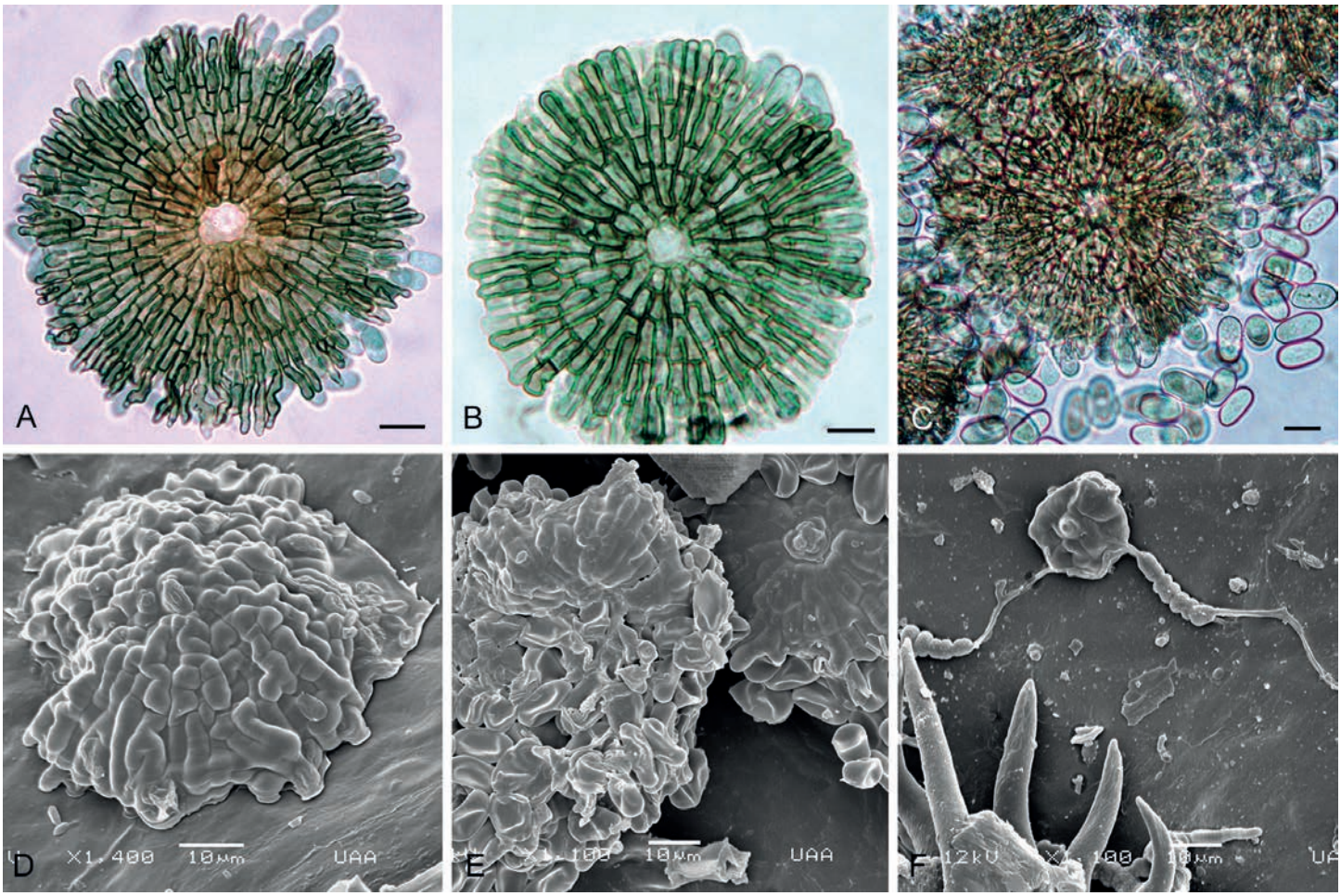

Fig. 20. *Tubakia sierrafriensis* (CFNL 2944 – holotype). **A, B.** Pycnothyria. **C.** Pycnothyria (crushing preparation) with conidia. **D.** Pycnothyria (SEM picture). **E.** Pycnothyria and conidia (SEM picture). **F.** Two pycnothyria with superficial hyphae (SEM picture). Bars = 10 µm.

ellipsoid, 12–13 × 7–8 µm, and colourless). *Tubakia sierrafriensis* is phylogenetically distinct from the other species included in the phylogenetic analyses (Figs 3–5).

Tubakia suttoniana U. Braun & Crous, ***nom. nov.*** MycoBank MB823669.
Basionym: *Dicarpella dryina* Belisario & M.E. Barr, *Mycotaxon* **41**: 154. 1991, non *Tubakia dryina* (Sacc.) B. Sutton, 1973.

Etymology: Named after the British mycologist B.C. Sutton, who introduced the name *Tubakia*.

Illustrations: Belisario (1990: 54–56, figs 1–3, 5–8; 1991: 149, figs 1–3, 151, figs 4–9).

Description in vivo: *Leaf spots* on living green leaves, numerous, scattered over the entire leaf blade, relatively small, angular-irregular, brown. *Conidiomata* (*pycnothyria*) on necrotic spots, morphologically close to pycnothyria of *T. dryina* but outer tips of radiating scutellum strands obtuse, not acute, and microconidia short cylindrical to subclavate, apex rounded, but base broadly truncate, mature macroconidia broad ellipsoid-obovoid, brown, 10.5–16 × 7.5–10 µm, on average 12.8 × 8.3 µm. *Sexual morph*: Formed on fallen overwintered leaves; *perithecia* scattered to gregarious, brownish to black, immersed, together with a stroma occupying the entire leaf thickness when mature, globose to slightly flattened, about 190–260 µm broad (diam) and 220–290 µm deep, sometimes oblique or horizontal; rostrate, *beak* short, usually lateral-eccentric, slightly protuberant through the upper leaf surface, rarely hypophyllous, 80–90 µm long and 15–20 µm wide at the base, apex rounded; ostiolate, ostiole periphysate, communicating with the cavity at maturity; stromatic pseudoparenchymatic layers centrally 30–40 µm thick, around the beak 40–50 µm thick, composed of several layers of dark, compact cells, rounded to ellipsoid, 8.5–14.5 µm diam; *peridium* variable in thickness, composed of a few layers of thin-walled, paler, compressed cells, tightly connected with the stroma; *asci* unitunicate, 8-spored, oblong-ellipsoid, 29–52.5 × 5.5–10.5 µm, formed in a spore-bearing part, peripheral, often with short stalk, or oblong, to 45 µm, ascal apex with two refractive conoid structures, asci deliquescing at maturity; paraphyses lacking; *ascospores* more or less uniseriate, becoming irregularly biseriate, 10–16 × 4.5–6.5 µm, one-celled, hyaline, ellipsoid to fusiform, often inequilateral or slightly curved, wall finely ornamented, content granular-guttulate.

In vitro: Colony covering dish in 2 wk at 25 °C with moderate aerial mycelium and feathery margins. On MEA surface smoke grey, reverse smoke grey with concentric circles of olivaceous grey. On PDA surface smoke grey, reverse smoke grey with olivaceous grey margin. On OA surface smoke grey with patches of olivaceous grey.

Types: **Italy,** Tuscany, Grosseto, farm nursery, on overwintered leaves of *Quercus rubra*, Feb. 1989, *A. Belisario* (ROHB – holotype; ROPV – isotype; ROPV, CBS 639.93 – ex-isotype cultures).

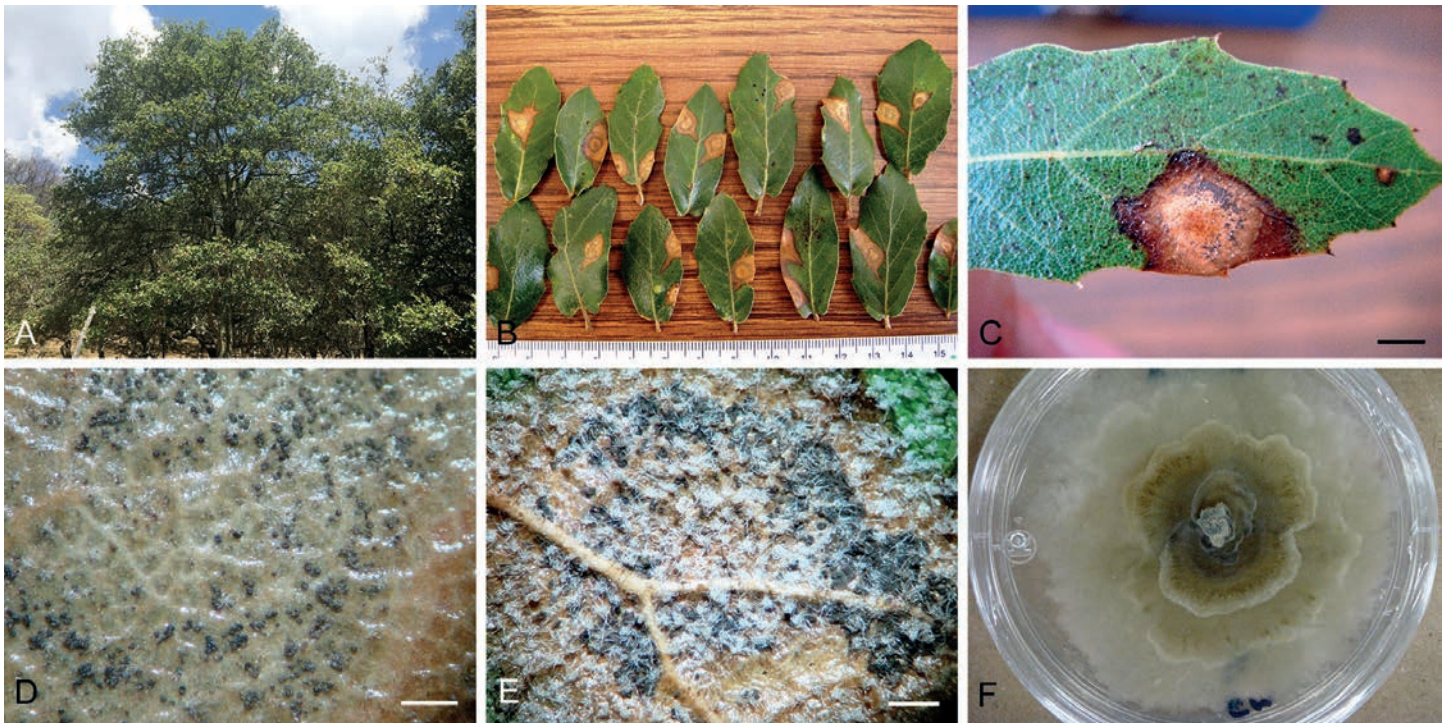

Fig. 21. *Tubakia sierrafriensis* (CFNL 2944 – holotype). **A.** Affected oak tree (*Q. eduardi*). **B.** Symptoms (leaf spots) on leaves of *Quercus eduardi*. **C.** Leaf with leaf spot. **D.** Close-up of an epiphyllous leaf spot with pycnothyria. **E.** Close-up of a hypophyllous leaf spot with pycnothyria. **F.** Culture on MEA. Bars = 0.5 cm (C), 1 cm (D, E), 20 µm.

Hosts range and distribution: On *Quercus rubra*, Europe (Italy).

Notes: *Dicarpella dryina* was introduced as sexual morph of "*Tubakia dryina*" (Belisario 1991). Harrington *et al*. (2012) questioned that the two morphs belong together and speculated that *D. dryina* might instead be the sexual morph of *Actinopelte americana* or another related cryptic American *Tubakia* species. However, sequences retrieved from the ex-type strain of *D. dryina* cluster in the *Tubakia* clade and were assigned to *T. macnabbii* in Harrington & McNew (2018). Belisario (1990) emphasised that a comparison of cultures derived from single ascospores and the original cultures obtained from naturally infected tissue and conidia did not show any differences, i.e., the ex-type cultures represent single ascospore cultures. Taking this into account, there are currently no objective reasons to doubt that *D. dryina* represents a sexual morph belonging to *Tubakia*. Unfortunately, Belisario (1989, 1990) did not describe the pycnothyria associated with *D. dryina* in detail, but her figures (Belisario 1991: 149, figs 1–3) suggest that the terminal tips of radiating scutellum strands are obtuse-rounded and not acute as in *T. dryina*. The microconidia are cylindrical to short clavate with rounded apex and broadly truncate base *vs*. ellipsoid-ovoid to fusiform, attenuated towards a narrow base as those occurring in *T. dryina*. This confirms that the asexual morph of *D. dryina* does not coincide with *T. dryina*, and these differences do not support the conspecificity of *D. dryina* and *T. dryina* as discussed in Harrington *et al*. (2012). Furthermore, *D. dryina* (as *T. suttoniana*) and *T. dryina* are clearly confirmed as separate species in the phylogenetic trees (Figs 3, 5).

Harrington & McNew (2018) *de facto* reduced *Dicarpella dryina* to synonymy with the new species *Tubakia macnabbii* since the ex-type culture of *D. dryina* (CBS 639.93) was cited and included in the protologue of *T. macnabbii*. The latter species, based on type material from Missouri on *Quercus palustris*, is undoubtedly a common North American species with wide host range (Harrington & McNew 2018). The taxonomic conclusion to include *Tubakia* collections from Italy on *Quercus rubra* in *T. macnabbii* was just based on phylogenetic analyses. However, the resolution of the "*T. macnabbii* clade" in their ITS tree is insufficient, and their *tef1* tree reflects a higher degree of variation, suggesting a complex of several cryptic taxa (also see Fig. 5 in the present study). Harrington & McNew (2018) supposed that the North America *T. macnabbi* occurring on various hosts of *Quercus* sect. *Lobatae* had been introduced to Europe (Italy), but the cluster including the sequence retrieved from the ex-type strain of *Dicarpella dryina* encompasses several sequences obtained from strains isolated from European collections (Netherlands) on the European oak *Quercus robur* and one strain on *Q. cerris* in New Zealand. Furthermore, Belisario's (1990) micrographs of "*T. dryina*" on *Q. rubra* show pycnothyria with obtuse tips of radiating scutellum strands and short cylindrical to subclavate microconidia with rounded apex but broadly truncate base, which is not in agreement with morphological characters of *T. macnabbii*. Therefore, we prefer to keep *D. dryina* as separate species.

Although the ITS sequence is not distinct from sequences of the newly described *Tubakia* from California (*T. californica* sp. nov.), *T. suttoniana* clusters separately from *T. californica* in the *tef1* (Fig. 5), *tub2* (not shown, see TreeBASE) and combined trees (Figs 3, 4). Based on their ITS tree, Harrington & McNew (2018) assigned a strain isolated from twigs of *Quercus agrifolia* in California, 27 April 2012, *S. Latham* (A1177) to *T. macnabbii*. However, this strain was also included in our own analyses (CPC 31497 = CDFA#1007) and clearly belongs to *T. californica*, which further demonstrates the heterogeneity of *T. macnabbii*.

As outlined above, in the phylogenetic trees, *T. suttoniana* belongs to an assemblage of poorly resolved *Tubakia* strains from Europe (Italy, Netherlands, isolated from *Quercus robur*, *Q. rubra*

and *Quercus* sp.) and New Zealand (isolated from *Quercus cerris*) that neither form a distinct cluster nor a clade, but rather reflect different lineages which might be different species. "*Tubakia dryina*" formed on leaf spots on *Quercus cerris* (Belisario 1993) and isolated as endophyte from buds and shoots of *Q. cerris* (Gennario *et al*. 2001) was also reported from Italy. *Tubakia macnabbii* is also part of this complex. The sample numbers are currently too small to resolve this assemblage of some taxa and more isolates are needed. *Tubakia suttoniana* represents a cryptic species morphologically and is phylogenetically distinct from *T. dryina*, but currently only known with certainty from its type material. In addition, it forms a complex of closely related species which also includes *T. japonica*, *T. melnikiana*, *T. seoraksanensis* and "*Tubakia* sp. nov. II" (Figs 3–5).

According to Art. 55.1, the name *D. dryina* is legitimate and applicable as basionym although published under the illegitimate genus name *Dicarpella* Syd. & P. Syd. (younger homonym, Art. 53.1). In the type paragraph, Belisario (1990) cited two different collections, one from Grosseto, Tuscany, the other one from Rome, and specified ROHB as herbarium in which the holotype was deposited [as "Belisario (RHOB)"], but without clear indication which of the two cited collections represents the genuine holotype. Due to this imprecise, confusing citation, the name *D. dryina* would be invalid according to Art. 40.1, 40.6. However, on page 148 it was mentioned that she (Belisario) first found this fungus in the Grosseto farm nursery in February 1989. Later the same fungus was collected on dead leaves in a nursery in Rome. The reference to "Belisario" may be accepted as reference to the first collection from "Grosseto" as holotype so that one can consider *D. dryina* to be validly published.

Tubakia tiffanyae T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi.org/10.1007/s10482-017-1001-9 [13]. 2017.

Illustrations: Harrington & McNew (2018, figs 1h–m).

Description in vivo: *Conidiomata* (pycnothyria) superficial, hypophyllous or epiphyllous, on circular leaf spots or along necrotic leaf veins. *Scutella* radiate, (40–)60–150 µm diam, composed of a series of dark brown, thick-walled cells originating from a central cell, ending in blunt to acute tips. *Sporodochia* with no scutella or poorly developed scutella epiphyllous or hypophyllous on necrotic vein tissue, light to dark brown due to masses of conidia. *Conidiophores* on underside of scutella or on sporodochia. *Conidia* hyaline, turning light brown with age, smooth to slightly varicose, aseptate, obovoid to ovoid (9–)10–15.5 × (8–)8.5–11.5(–12) µm (mean 12.9 × 9.6 µm). *Microconidia* sterile, hyaline, aseptate, fusiform 3–8 × 1–2.5 µm may develop from small pycnothyria, alone or along with macroconidia. *Crustose conidiomata* developing in late summer to fall on underside of necrotic veins and found on overwintering leaves still hanging from twigs, erumpent, brown to black, irregularly shaped, 80–580 µm diam, single to grouped, covered with dark, thick-walled cells that break open in fissures due to swelling when wet. *Conidia* hyaline to brown, ellipsoidal to obovate to irregularly shaped, aseptate, (11–)13–18(–20) × (6–)7–9.5 µm (mean 15.2 × 7.9 µm).

In vitro: On MYEA with optimal growth at 25 C, 40–58 mm diam after 7 d, creamy white aerial mycelium, smooth to scalloped at edge, developing concentric rings of dense mycelium, underside golden yellow to slightly darker with age, sometimes with dark cell masses on surface or subsurface. *Conidiophores* rare to abundant, short, hyaline, sometimes aggregated (sporodochia) on agar surface. *Conidia* hyaline to dark brown, thick-walled, smooth to slightly varicose, aseptate, obovoid to ellipsoidal, 10–16 × 7–11 µm.

Type: **USA**, Iowa, Ames, N42° 04' 4'' W93° 65' 22'', on leaf of *Quercus rubra*, 5 Sep. 2009, *T. Harrington* (ISC 453296 – holotype; ex-type strains CBS 137345 = A803).

Host range and distribution: On *Quercus* (*ellipsoidalis, imbricaria*, *rubra*), *Fagaceae*, North America (USA, Iowa, Minnesota).

Notes: This species was recently introduced by Harrington & McNew (2018) for *Tubakia* sp. C (Harrington & McNew 2016) which is closely allied to *T. macnabbii* but genetically separated (Fig. 5) and morphologically distinguished by having wider conidia and causing characteristic circular leaf spots with light-coloured centres (see Harrington & McNew 2018: fig 1h) in addition to the vein necrosis.

Excluded, doubtful and insufficiently known species

Actinopelte acnisti (Syd.) Toro, *Bol. Acad. Ci. Fís.* **2**(7): 217. 1935, *nom. inval*. (Art. 36.1, c).
Basionym: *Calopeltis acnisti* Syd., *Ann. Mycol.* **23**: 393. 1915.

Notes: *Calopeltis* is a recognised genus belonging to the *Microthyriaceae*. Syntypes of this species are distributed in several herbaria and include "Syd., Fungi Exot. Exs. 689" (BPI 645025–6425029, CUP, ILL 10525–10528, LSU 156932, MICH 13880, S-F10919,10920, 194870, 194872, 194873).

Actinopelte psychotriae I. Hino & Katum., *Bull. Fac. Agric. Yamaguchi Univ.* **15**: 506. 1964.

Notes: This species, described by Hino & Katumoto (1964) on leaves of *Psychotria serpens* from Japan, is unrelated to *Tubakia*. In the original description and illustration (Hino & Katumoto 1964: 506, fig. 1), this species was described to form pycnothyria with scattered globose ostiolate locules and small "pycnospores" (6–8 × 2.5–3 µm), which is quite distinct from pycnothyria of *Tubakia*.

Actinopelte stellata M.L. Farr, *Mycopathol. Mycol. Appl.* **31**: 63. 1967.
Synonym: *Tubakia stellata* (M.L. Farr) T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi.org/10.1007/s10482-017-1001-9 [16]. 2017.

Notes: The generic affinity of this species is unclear. Cultures and results of molecular sequence analyses are not yet available. This species is only known from its type collection (**Brazil**, *Para*, Belem, on leaves of *Byrsonima coriacea*, 3 Feb. 1963, *F.C. Albuquerque*, BPI 391941) and has not yet been revised and reassessed. However, the described and illustrated characteristics of *A. stellata* do not fit into the generic concept of *Tubakia*. Farr (1967) described a basal membrane, unknown in true *Tubakia* species, a one-celled columella, and conidiogenous cells arising from the underside of the scutellum. In *Tubakia* spp., the columella is composed of several cells or a single cell surrounded by small, fertile, parenchymatous cells giving rise to conidiogenous cells,

above all around the point of attachment of the columella at the scutellum and peripherally. *Tubakia* species are usually confined to fagaceous hosts in the northern hemisphere. The reallocation of *A. stellata* to *Tubakia* by Harrington & McNew (2018) was just a formal act, neither supported by culture and sequence data nor accompanied by a critical discussion of the morphology of this species.

Actinothyrium gloeosporioides Tehon, *Mycologia* **16**: 136. 1924.
Synonym: *Tubakia gloeosporioides* (Tehon) T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi.org/10.1007/s10482-017-1001-9 [15]. 2017.

Holotype: **USA**: Illinois, Franklin County, Christopher, on *Sassafras albidum*, *Lauraceae*, 20 Jul. 1922, *P. A. Young 3311* (ILLS 2972).

Notes: Harrington & McNew (2018) examined type material of *A. gloeosporioides* and additional specimens on *Sassafras albidum* from Illinois (ILLS 2972, 3671, 29748, 17547) and New Jersey (as *Leptothyrium dryinum* f. *sassafras*). The pycnothyria were 45–132 µm diam (reported as 50–95 µm diam by Tehon 1924) with conidia 9.5–12.5 × 7–8.5 µm (reported as 11–12 × 6–7.5 µm in Tehon 1924), which are similar to pycnothyria and conidia of *T. dryina s. lat.* Harrington & McNew (2018) compared it with *T. macnabbii* and commented that the two species are indistinguishable without cultures or DNA. They introduced the new combination *T. gloeosporioides* just based on the assumption that the latter taxon represents a species of its own since it was described on a lauraceous host. However, it should be noted that an ITS sequence retrieved from leaves of *Lindera glauca* (*Lauraceae*) in China clusters with *T. dryinoides*. Hence, the status and true affinity of *A. gloeosporioides* remain unclear and unresolved pending cultures and results of molecular sequence analyses.

Leptothyrium castaneicola Ellis & Everh., *J. Mycol*. **4**: 137. 1888.

Type: **USA,** New Jersey, on *Castanea sativa* [*vesca*], 20 Oct. 1888, without collector (NY 927812 – holotype).

Notes: This species has usually been considered a synonym of *Tubakia dryina*. Type material has been re-examined. Pycnothyria formed on leaf spots on *Castanea sativa* are morphologically barely distinguishable from those of *T. dryina* and allied North American species with pointed tips of radiating scutellum strands. This species may be a synonym of one of the North American *Tubakia* species, but it can also not be excluded that an additional cryptic species on sweet chestnut being involved, but cultures and sequence data are not yet available to be able to answer this question.

Pirostoma nyssae Tehon, *Mycologia* **16**: 137. 1924.
Synonym: *Tubakia nyssae* (Tehon) T.C. Harr. & McNew, *Antonie van Leeuwenhoek J. Microbiol. Serol.* doi.org/10.1007/s10482-017-1001-9 [16]. 2017.

Holotype: **USA,** Illinois, Johnson County, Tunnel Hill, on *Nyssa sylvatica*, 25 Jul. 1922, *P. A. Young 3665* (ILLS 2940).

Notes: Collections of *Pirostoma nyssae* have been examined (*Nyssa sylvatica*, BPI 391889–391891). Pycnothyria (65–110 µm diam, tips of the radiating scutellum strands pointed) are barely distinguishable from those of *T. dryina*, so that it is not surprising that *P. nyssae* has previously usually been considered a synonym of the latter species. Harrington & McNew (2018) compared *P. nyssae* rather with *T. macnabbii*, which is, however, genetically totally heterogeneous and undoubtedly composed of several cryptic taxa. Without any cultures and sequence data, the status and affinity of *P. nyssae* remain quite unclear. The reallocation to *Tubakia* was just based on the occurrence of this species on *Nyssa sylvatica* (*Nyssaceae*), i.e., on a non-fagaceous host, which is, however, insufficient for a final conclusion and reassessment.

Tubakia sp. (see Braun *et al*. 2014: 25)

Leaf spots amphigenous, subcircular to angular-irregular, 1–7 mm diam, sometimes oblong, up to 10 µm, medium brown on the upper leaf surface, paler below, finally with pale centre, pale brownish to ochraceous, margin indefinite or narrow and somewhat darker, occasionally surrounded by a narrow diffuse halo, yellowish to yellow-green. *Conidiomata* (*pycnothyria*) epiphyllous, up to about 15 per leaf spot, punctiform, scutellate, blackish. *Scutella* convex, 40–80 µm diam, membranous, barely translucent, with a central paler disc, 8–15 µm diam, giving rise to radiating hyphae, cells 4–15 × 2–5 µm, peripheral cells mostly somewhat broadening towards the margin, medium brown, thick-walled (–1 µm), smooth, up to three times bifurcating, either only at the periphery or deeply cleft, peripheral bifurcations mostly shallow, branchlets with obtuse to truncate tips. *Central columella* below the scutellum delicate, easily collapsing and ephemeral. *Conidiophores* reduced to conidiogenous cells, arising from the underside of scutella around the columella, radiating downward and towards margin. *Conidia* solitary, globose to subglobose, 9–11 × 7–9 µm, length/width ratio 1–1.2, wall thin, 0.3–0.8 µm wide, hyaline or subhyaline, very pale greenish or faintly olivaceous, smooth, apex and base broadly rounded, basal hilum inconspicuous or with minute, not very conspicuous, delicate frill or peg. *Microconidia* not observed.

Material examined: **China**, Jiangxi Province, Xingangshan, subtropical forest site of the BEF-China Project, 29.1250° N, 117.9085° E, on living leaves of *Castanea henryi*, *Fagaceae*, 8 Sep. 2013, *S. Bien*, HAL 2675 F.

Notes: *Tubakia* sp. from *Castanea henryi* in China belongs to an undescribed species. Cultures and results of sequence analyses are not available, and the material deposited at HAL is too meagre for a formal description of a new species. The Chinese fungus on *Castanea henryi* is morphologically close to, and possibly identical with, *Saprothyrium thailandensis*, but unproven due to lack of cultures and sequence data for comparison. The ecology of the Chinese collection on *Castanea henryi* is unclear. The pycnothyria were found on leaf spots of living leaves, but lesions of several fungi, including *Tubakia chinensis*, were developed. Thus, it cannot be excluded that the Chinese *Tubakia* on *Castanea henryi* with small pycnothyria was formed on necrotic spots caused by other fungi.

Tubakia sp. (see Zahedi *et al*. 2011: 67, fig. 3).

Notes: Zahedi *et al*. (2011) reported and illustrated "*Tubakia dryina*" on *Quercus castaneifolia* from North Iran (Guilan Province). This fungus is quite distinct from true *T. dryina* (*s. str*.) and readily distinguishable by its small pycnothyria, about 60 µm diam, blunt tips of radiating scutellum strands, and broad

ellipsoid-ovoid, colourless or pale conidia, about 7–10 × 5–7 µm. Shape and size of the pycnothyria are reminiscent of those of *Tubakia* sp. on *Castanea henryi* in China (Braun *et al*. 2014: 25) and *Saprothyrium thailandense*, which differ, however, in having globose to subglobose conidia. There is no *Tubakia* species with comparable pycnothyria. Therefore, the collection on *Q. castaneifolia* from Iran represents probably an undescribed species, but without cultures and sequence data the generic affinity remains unclear.

Key to species of *Tubakia* and allied genera (*Tubakiaceae*) based on conidiomatal characters

1. Pycnothyria with characteristically radiating scutella not developed; only acervuloid, crustose, pycnidioid, stromatic black conidiomata formed, (50–)80–200(–220) µm diam; on fagaceous hosts in California or *Syzygium cumini* (*Myrtaceae*) in Thailand 2
1* Pycnothyria developed; sporodochial and crustose, pycnidioid, stromatic conidiomata may be developed in addition to pycnothyria or may be absent 3

2. Conidiomata crustose, pycnidioid, stromatic, black, dehiscing by irregular fissures, formed on petioles and leaf blades of necrotic, brown leaves, often close to veins, dry, brown leaves with conidioma from the previous seasons' growth remain attached to many branches of affected trees until spring; conidiophores aseptate, unbranched; conidia broad ellipsoid, ellipsoid-ovoid to short and broad subcylindrical, rarely irregular in shape, 8–15 × 4.5–7 µm, length/width ratio 1.4–2.3 (on average 1.8), thin-walled, at first subhyaline to pale greenish, later greenish, pale olivaceous to brownish; on *Chrysolepis chrysophylla* (≡ *Castanopsis chrysophylla*), *Notholithocarpus densiflorus* (≡ *Lithocarpus densiflorus*), and various *Quercus* spp., North America, California (and perhaps Mexico) ..... *Tubakia californica*
2* Conidiomata acervuloid, basistromatic; conidiophores septate, branched; 9–15 × 5–6 µm, light brown to olivaceous brown, thick-walled; on dead leaves of *Syzygium cumini*, Thailand ..... *Racheliella saprophytica*

3. Conidia large, length on average > 25 µm 4
3* Conidia smaller, length on average < 25 µm 5

4. Conidia very large, 40–55 × 35–45 µm; microconidia present, 5–10 × 1–2 µm, formed in smaller conidiomata; on *Castanea crenata*, *C. mollissima* and *Quercus acutissima*, *Q. aliena*, Asia (China, Japan, Korea) ..... *Tubakia japonica*
4* Conidia (20–)25–40 × 20–30 µm; microconidia not formed on *Castanea henryi* ..... *Tubakia chinensis*

5(3) Scutella relatively small, 60–100 µm diam, margin continuous, compact, more or less undulate and distinctly involute; conidia broad ellipsoid-ovoid, 12–15 × 10–13 µm, at first hyaline, later pale yellowish brown to light orange yellow; microconidia formed, bacilliform, 8–10 × 1 µm; on *Quercus phillyreoides*, *Q. serrata*, Japan, Korea ..... *Involutiscutellula rubra*
5* Scutella larger, up to 160 µm diam and/or conidia narrower (width < 10 µm), margin of the scutellum different, loose, not distinctly involved, tips either pointed or obtuse-truncate; microconidia either much broader or not developed 6

6. Outer tips of the bifurcating hyphal scutellum strands obtuse, rounded or even more or less truncate, but not pointed 7
6* Outer tips of the bifurcating hyphal scutellum strands always or predominantly pointed (or at least all or most ultimate branchlets distinctly attenuated towards an obtuse to pointed tip) 15

7. Conidia globose or subglobose to broad ellipsoid-obovoid 8
7* Conidia not consistently globose-subglobose, either consistently broad ellipsoid-obovoid or oblong to oblong-ellipsoid or polymorphous, broad ellipsoid-obovoid, short subcylindrical, some conidia even subglobose or irregular in shape, occasionally with oblique base 10

8. Scutella larger, 80–120 µm diam; conidia globose, subglobose to broad ellipsoid-obovoid, small conidia 7–10 × 5–8 µm, larger fully developed conidia 9–16(–19) × (7–)9–12 µm, length/width ratio 1.0–1.5(–1.6), on average 1.27, hyaline to pale greenish or olivaceous; on *Quercus eduardi*, Mexico ..... *Sphaerosporithyrium mexicanum*
8* Scutella small, 40–80 µm diam; conidia subglobose, mature conidia narrower, 7–9 µm, hyaline or subhyaline 9

9. Isolated as saprobic fungus from an unidentified leaf in Thailand ..... *Saprothyrium thailandense*
9* On leaf spots on *Castanea henryi*, China ..... *Tubakia* sp.

10(7*) Conidia polymorphous, broad ellipsoid-obovoid, short subcylindrical, some conidia even subglobose or irregular in shape, occasionally with oblique base, 9–18 × 5–9 µm, at first hyaline, finally very pale olivaceous; on *Quercus eduardi*, Mexico ..... *Tubakia sierrafriensis*
10* Conidia uniform, not polymorphous, not or barely curved; in Africa, Asia or Europe 11

11. Conidia hyaline 12
11* Conidia pigmented, at least when mature 13

12. Pycnothyria large, 100–170 µm diam; conidiophores 11–20 µm long; conidia 7–9.5 µm wide; on *Castanopsis cuspidata* (*Fagaceae*), Japan ..... *Oblongisporothyrium castanopsidis*
12* Pyconothyria smaller, 80–130 µm diam; conidiophores 6–10 µm long; conidia (6.5–)7(–7.5) µm wide; on *Syzygium guineense* (*Myrtaceae*) ..... *Racheliella wingfieldiana*

13(11*) Conidia long and narrow, 12–20 × 4.5–7.5 µm, length/width ratio 1.8–3.8, subhyaline to pale brown; conidiogenous cells long, 13–35 × 2–5.5 µm; on *Quercus serrata*, Japan ..... *Tubakia oblongispora*
13* Conidia broader, 9–16 × 6–10 µm, length/width ratio below 2.3 14

14. Conidia 9–14 × 6–8.5 µm, on average 11.1 × 6.9 µm; microconidia fusiform, attenuated towards apex and base; on oaks in North America ..... *Tubakia americana*
14* Conidia somewhat larger, 10.5–16 × 7.5–10 µm, on average 12.8 × 8.3 µm; microconidia short cylindrical to subclavate, apex rounded, but base broadly truncate; on *Quercus rubra*, Europe (Italy) ..... *Tubakia suttoniana*

15(6*) Conidia large, 13–25 × 10–15 µm 16
15* Conidia narrower, (7–)9–16(–18) × (5–)6–10(–10.5) µm, or subglobose, 10–13 × 9–11 µm 17

16. Scutella 90–160 × 90–130 µm; conidiogenous cells 3–5 µm wide; on *Quercus mongolica*, Korea ..... *Tubakia seoraksanensis*
16* Scutella 55–155 µm diam; conidiogenous cells 4–8 µm wide; on *Quercus acutissima*, Japan ..... *Tubakia paradryinoides*

17(15*) Conidia globose or subglobose, 10–13 × 9–11 µm (width on average > 9 µm), length/width ratio 0.9–1.4, hyaline to pale yellowish ochraceous; on leaf spots on *Quercus glauca*, Japan ..... *Paratubakia subglobosa*
17* Conidia mostly broad ellipsoid-obovoid, (7–)9–16(–18) × 5.5–10(–11) µm (width on average < 9 µm), length/width ratio (1.1–)1.2–2.3 18

18. Ends of the radiating hyphal strands of the scutella often attenuated towards the tips, which are obtuse to pointed 19
18* Ends of the radiating hyphal strands of the scutella usually with pointed ultimate tips 20

19. Conidia hyaline to yellowish ochraceous, 10–12.5 × 5.5–10 µm; on leaf litter of *Quercus glauca*, Japan, leaf spots lacking ..... *Paratubakia subglobosoides*
19* Conidia hyaline or subhyaline, (11–)12–14(–15) × (6.5–)7(–7.5) µm; on *Syzygium guineense*, South Africa, causing leaf spots ..... *Racheliella wingfieldiana*

20(18*) Conidia hyaline to subhyaline, not pigmented; on *Quercus phillyreoides* and *Castanea pubinervis*, Japan ..... *Tubakia dryinoides*
20* Conidia at first hyaline to subhyaline, later distinctly pigmented 21

21. Microconidia narrowly fusiform, often curved, 4–8.5 × 1–2 µm wide; causing bur oak blight characterised by forming lesions rather confined to and spread along veins, severe infections may lead to early leaf dieback and defoliation, and, finally, branch dieback; on *Quercus macrocarpa* and *Q. stellata*, North America, USA ..... *Tubakia iowensis*
21* Microconidia wider, 4–9 × 1.5–4 µm, usually straight, or not formed on leaves; causing definite leaf spots spread over the entire leaf surface, pronounced dieback symptoms and premature defoliation not developed (*T. dryina* complex in terms of morphology) 22

22. Leaf spots formed on *Liquidambar styraciflua* in North America; pycnothyria as in *T. dryina* ..... *Tubakia liquidambaris*
22* On fagaceous hosts 23

23. On *Quercus canbyi*, Mexico; ultimate tips of the hyphal strands of the scutella obtuse to pointed when mature; microconidia narrowly ellipsoid (-subcylindrical) to broad ellipsoid-ovoid, apex obtuse, rounded, base round to short obconically truncate; sporodochia and crustose conidiomata not observed ..... *Tubakia melnikiana*
23* On fagaceous hosts, Europe and North America; ultimate tips of the hyphal strands of the scutella consistently or predominantly pointed; microconidia narrowly ellipsoid-ovoid, fusiform, attenuated towards both ends; forming sporodochia and/or crustose conidiomata 24

24. Sporodochia not formed; crustose conidiomata formed on twigs; on *Fagus* and *Quercus* spp., Europe, North America, and introduced in New Zealand ..... *Tubakia dryina*
24* Sporodochia and/or crustose conidiomata formed on leaves (on leaf spots or along veins), North American species 25

25. Leaf spots characteristically circular with light-coloured centre; sporodochia formed on necrotic veins; crustose conidiomata in late summer to fall on underside of necrotic veins and found on overwintering leaves still hanging from twigs ..... *Tubakia tiffanyae*

25* Leaf spots variable, not characteristically circular; sporodochia formed on leaf spots and along veins; crustose conidiomata either lacking or formed on leaf tissue before overwintering 26

26. Sporodochia formed on leaf lesions and along leaf veins; crustose conidiomata lacking; microconidia not developed on leaves (microconidia formed on autoclaved pieces of leaves of *Q. macrocarpa* placed on MEA) ..... *Tubakia hallii*

26* Sporodochia and crustose conidiomata formed on leaves (fusiform microconidia, 3.5–9(–11.5) × 1–3 µm, formed in small, radiate pycnothyria, alone or along with macroconidia) ..... *Tubakia macnabbii*

DISCUSSION

The genus *Tubakia* was assigned to the *Diaporthales* (Yokoyama & Tubaki 1971, Yun & Rossman 2011, http://www.mycobank.org/ and http://www.indexfungorum.org/names/names.asp) primarily based on the description of *Dicarpella dryina* as the putative sexual morph of *Tubakia dryina*. However, the latter species is not the type species of *Tubakia*, and *D. dryina* is also not the type species of *Dicarpella*, which requires a more detailed discussion. Phylogenetic analyses of these species were previously not available. During the course of the present studies on *Tubakia* spp., ex-type cultures of *Tubakia japonica*, type species of *Tubakia*, and *Dicarpella dryina* could be included in phylogenetic analyses and proved to be congeneric. However, *Dicarpella dryina* is not conspecific with *Tubakia dryina,* as previously assumed and postulated in Harrington *et al*. (2012), and phylogenetically represents a separate species belonging to an assemblage of related *Tubakia* species, including *T. californica*, *T. dryinoides*, *T. japonica*, *T. macnabbii*, *T. melnikiana*, and *T. seoraksanensis*. All recognised species of *Tubakia*, except for *T. chinensis*, are known *in vitro* and have been included in the present molecular sequence analyses, which revealed that *Tubakia s. lat.*, as previously circumscribed, represents a complex of cryptic genera. The question that remains is whether the genera *Dicarpella* and *Tubakia s. str.* are congeneric. A simple answer to this question is not yet possible since it requires phylogenetic data for *Dicarpella bina*, the type species of *Dicarpella*, which is hitherto only known from its type collection (Sognov *et al*. 2008). Based on the morphological similarity between *D. bina* and *D. dryina*, it might be possible that the two genera are, indeed, congeneric. *Dicarpella bina* was originally described from living leaves of *Quercus agrifolia* in California. *Quercus agrifolia* is also a common host of *Tubakia californica*. On the other hand, it can also not be excluded that the type species of *Dicarpella* might be more distantly related to *Tubakia* as currently suspected or even unrelated. A final conclusion is still pending and further cultures and sequence data for *D. bina* are needed. In any case, the older name *Dicarpella* would not threaten the younger name *Tubakia* in case that the two genera were congeneric, since *Dicarpella* Syd. & P. Syd., 1913 (nom. illeg.) being a younger homonym of *Dicarpella* Sitzenb., 1861, i.e., *Tubakia* would be the correct name in any case.

Barr (1978) assigned *Dicarpella* to the *Pseudovalsaceae* subfam. *Pseudovalsoideae* tribe *Ditopelleae*, and later included *Dicarpella quercifolia* with *Mastigosporella hyalina* as putative asexual morph (Barr 1979). A similar unnamed asexual morph was found in *D. georgiana*, which was later described as *M. nyssae* (Nag Raj & Di Cosmo 1981). *Mastigosporella hyalina* (≡ *Harknessia hyalina*) was the reason for previous reports that *Dicarpella* spp. might be associated with *Harknessia* spp. as asexual morphs (Cannon 2001). Monod (1983) retained *Dicarpella georgiana* in *Gnomoniella*, recognised *D. bina* and *D. quercifolia* in *Dicarpella*, and added *D. liquidambaris-styracifluae* and *D. orientalis*. Rossman *et al*. (2007) cited *Harknessia* spp. under diaporthalean fungi of uncertain position. However, true *Harknessia* spp. are not closely allied to former *Dicarpella* spp. with *Mastigosporella* asexual morphs and have been placed in a family of its own, viz. *Harknessiaceae* (Crous *et al*. 2012). Reid & Dowsett (1990) examined *Dicarpella* in detail, excluded *D. georgiana* and *D. quercifolia*, both associated with asexual morphs belong to *Mastigosporella*, and reallocated them to the new genus, *Wuestneiopsis*. Rossman *et al*. (2015) recommended to protect the name *Mastigosporella* and to reduce *Wuestneiopsis* to its synonymy. Thus, the illegitimate name *Dicarpella* currently comprises *D. bina*, *D. liquidambaris-styracifluae*, and *D. orientalis*, but to date none of them has been phylogenetically clarified. The illegitimate name *Dicarpella* was in need of a new genus name, but since the relation between *Dicarpella* and *Tubakia* remains unproven and unclear, any corresponding nomenclatural change would be premature.

Another question concerns the affiliation of *Tubakia* in the hierarchical system of the *Ascomycota*. The placement within the *Diaporthales* is clearly resolved. *Tubakia* is currently usually listed as diaporthalean genus of unclear family affinity (Yokoyama & Tubaki 1971, Yun & Rossman 2011, http://www.mycobank.org/ and http://www.indexfungorum.org/names/names.asp). *Dicarpella* was usually assigned to the *Melanconidaceae s. lat.*, comprising up to almost 30 genera (Eriksson *et al*. 2001, Lumbsch & Huhndorf 2007, Maharachchikumbura *et al*. 2015, 2016). This affiliation is, however, disputable (see discussion above). Most genera allocated to the *Melanconidaceae s. lat.* have not yet been phylogenetically examined, i.e., the affinity of the genera concerned remains unclear and unproven. *Melanconis*, the type genus of the family *Melanconidaceae*, has been phylogenetically examined and the independent status of the latter family has been confirmed (Castlebury *et al*. 2002, Du *et al*. 2017). Castlebury *et al*. (2002) emphasised that the family *Melanconidaceae* should rather be confined to the genus *Melanconis*, and recommended to exclude the numerous other previously included genera, including *Melanconiella* (Voglmayr *et al.* 2012). Voglmayr *et al*. (2017) introduced the new family *Juglanconidaceae* for the new genus *Juglanconis*, which splits the genus *Melanconis* and *Melanconidaceae s. lat*. The present phylogenetic analyses indicate a relation of *Tubakia* to *Melanconiella* spp., possibly in sister positions, depending on the kind of analyses employed. Voglmayr *et al*. (2012) confirmed *Melanconiella* as a separate genus, clearly distinct from the morphologically similar genus *Melanconis* and not affiliated to the *Melanconidaceae*, and stressed that this genus does not belong to any established dothidealean family. Morphological traits of *Melanconiella* spp., including the characters of associated asexual morphs, do not favour *Melanconiella* spp. and *Tubakia* being placed in the same family. In contrast to *Tubakia*, the bark-inhabiting *Melanconiella* spp. are characterised by forming ectostromatic discs with a central column, perithecia with lateral non-rostrate ostioles, broad band-like paraphyses, consistently bicellular ascospores,

sometimes with blunt appendages or a thin gelatinous sheath, and they are associated with acervular discosporina- and melanconium-like asexual morphs (Voglmayr *et al.* 2012). On the basis of phylogenetic analyses of dothidealean genera and their relations to putative families, Senanayake *et al.* (2017) validated the invalidly published family *Melanconiellaceae* and assigned *Tubakia* to this family, although it was not based on sequence data of the type species. The present phylogenetic analyses, including sequence data of the type species of *Tubakia* confirm that *Tubakia* warrants a family of its own, viz., *Tubakiaceae* fam. nov.

On the basis of ITS and LSU sequences retrieved from an ex-type strain, Harrington & McNew (2018) reallocated *Apiognomonia supraseptata* (Kaneko & Kobayashi 1984), a sexual morph described from Japan without any asexual morphs, to *Tubakia*. *Apiognomonia supraseptata* differs from true *Apiognomonia* spp. in having ascospores with a septum near the apex. *Apiognomonia supraseptata* and *D. dryina*, the only two sexual morphs associated with *Tubakia*, have various characters in common, viz., rostrate perithecia of similar size, unitunicate 8-spored asci, and colourless ascospores of similar size. The only basic difference is in the septation of the ascospores which are aseptate in *D. dryina* and 1-septate near the apex in *A. supraseptata*. Our own phylogenetic analyses confirm the inclusion of *A. supraseptata* in the *Tubakiaceae*. However, this allocation raises the question whether the inclusion of the latter species in *Tubakia*, as proposed by Harrington & McNew (2018), was reasonable, or if this result might be an indication of a separation of the *Tubakiaceae* cluster into two or several genera as the LSU phylogeny (Fig. 1) already indicated some heterogeneity. Therefore, the LSU phylogeny was also supplemented with *rpb2* sequence data (Fig. 2) and proved that the *Tubakiaceae* cluster represents an assemblage of several cryptic genera, including *Tubakia s. str.* that forms a separate, well-supported clade. *Tubakia castanopsidis*, *T. rubra*, *T. subglobosa*, and *T. thailandensis* do not belong in the *Tubakia s. str*. clade and cluster outside, i.e., they have to be excluded from *Tubakia s. str*. and reallocated to other genera. The *Tubakia* clade is large, comprising numerous species, and is based on multigene sequence data. Several lineages, often only represented by a single species, are evident in the portions of excluded taxa in the phylogenetic trees, and led to the introduction of the new genera *Apiognomonioides* (type species: *Apiognomonia supraseptata), Involutiscutellula* (type species: *Actinopelte rubra*), *Oblongisporothyrium* (type species: *Actinopelte castanopsidis*), *Paratubakia* (type species: *Actinopelte subglobosa*), *Racheliella* (type species: *R. wingfieldiana*), *Saprothyrium* (type species: *Tubakia thailandensis*) and *Sphaerosporithyrium* (type species *S. mexicanum*). *Racheliella wingfieldiana* and *Greeneria saprophytica* share *Syzygium* (*Myrtaceae*) as host genus and are phylogenetically closely allied, suggesting the allocation of the latter species to the new genus *Racheliella.* The available phylogenetic data for *Apiognomonia supraseptata* and *Tubakia thailandensis*, belonging in *Tubakiaceae*, suggest that the two species require separate (new) genera. Interestingly, almost all excluded taxa and new genera are confined to east and southeast Asia (Japan, Thailand). This region seems to be a hot spot of the generic diversity of *Tubakiaceae*, whereas Europe and above all North America exhibit a higher diversity of species of the genus *Tubakia s. str*. Although the new genera segregated from *Tubakia s. lat.* are basically phylogenetically established, there are also morphological peculiarities that distinguish these genera from *Tubakia s. str.*, e.g., *Involutiscutellula* differs from all other species of tubakia-like genera in having small pycnothyria with compact scutella provided with continuous, more or less undulate and distinctly involute margin. *Paratubakia* spp., confined to *Quercus* (*Cyclobalanopsis*) *glauca*, are characterised by having more or less globose-subglobose and usually hyaline conidia.

Within *Tubakia s. str.*, *T. dryina* and *T. iowensis*, are clearly confirmed as species in the phylogenetic trees, although the two species possess very similar pycnothyria. On the other hand, there are differences in the habit and biology of these species. Although *T. japonica* and *T. seoraksanensis* seem to be phylogenetically closely allied, and morphologically similar with larger spores and similar pycnothyrial scutella, they are morphologically and genetically distinct and represent two distinct species. The new Mexican species *Sphaerosporithyrium mexicanum* and *T. sierrafriensis* are also phylogenetically supported as new, separate species and are clearly distinct in the trees. The position of Californian *Tubakia* sequences supports a separate, undescribed species belonging to the *T. suttoniana* complex in the phylogenetic trees. This species, described as *T. californica*, is an endophyte, forming conidia in pustulate conidiomata, but pycnothyria are lacking. Two strains isolated from leaves of *Quercus canbyi* in Mexico are close and tentatively included. Another *Tubakia* on *Quercus canbyi* and *Q. laeta* in Mexico, described as *T. melnikiana*, is morphologically only gradually distinguished from *T. dryina*, but phylogenetically clearly separated and belongs to the *T. suttoniana* complex. The inference by Harrington *et al*. (2012) that Japanese collections referred to as *T. dryina s. lat.* are not conspecific with the genuine *T. dryina s. str.* was confirmed in the course of the present examinations. *Tubakia dryinoides*, *T. paradryinoides*, *T. oblongispora*, and *Paratubakia subglobosoides* were proven to belong to genetically and morphologically distinct new species by analysing herbarium material, including corresponding cultures, and phylogenetic data of the collections concerned. *Greeneria saprophytica*, described from Thailand on *Syzygium cumini*, turned out to cluster within the *Tubakiaceae*, close to a new South African species. The morphology of this species is not in conflict with the current concept of taxa of the *Tubakiaceae*, but it clustered outside of the *Tubakia s. str*. clade. Owing to its phylogenetic position and morphological peculiarities, *G. saprophytica* cannot be assigned to *Tubakia*, but rather requires a genus of its own, viz., *Racheliella,* with *R. wingfieldiana* sp. nov. as type species.

Harrington & McNew (2018) recently revised the North American *T. dryina* complex, described several new species previously classified as *Tubakia* sp. (Harrington *et al.* 2012, Harrington & McNew 2016), and introduced several new combinations, including *T. americana* and *T. liquidambaris*. *Tubakia hallii*, *T. liquidambaris*, *T. tiffanyae* are well-supported recognised species confirmed in our own analyses. *Tubakia americana* is, however, heterogeneous in the circumscription of Harrington & McNew (2018) and comprises *T. americana s. str.* (on American oaks) and *T. dryinoides.* The clade representing the latter species contains Asian and European sequences. The European ones might reflect an additional cryptic species, but the current sampling is not sufficient for a final conclusion, and morphological characters of conidiomata of the European taxon are not yet known. *Tubakia macnabbii* turned out to be genetically quite heterogeneous and can currently only be considered as a complex compound species (*s. lat*.) that we currently confine to North American collections on oaks, but excluding Californian collections that form a clade of its own

described herein as *T. californica*. *Tubakia gloeosporioides* (≡ *Actinothyrium gloeosporioides*) and *T. nyssae* (≡ *Pirostoma nyssae*) are two additional combinations introduced by Harrington & McNew (2018), but cultures and sequence data are not yet available for the two taxa, and the morphological characters of the pycnothyria formed on leaves of *Nyssa* and *Sassafras* are not distinguishable from those of *T. dryina s. lat*. Therefore, we prefer to assign these species to the list of "Excluded, doubtful and insufficiently known species", at least tentatively until the affinity of the two taxa can be elucidated by means of cultures and sequence data.

Thus, the number of species pertaining to the family *Tubakiaceae* (*Tubakia s. lat.* in its previous circumscription) increased from eight in 2016 to the current 26 accepted here. However, examination of the individual gene trees has shown that there is some movement of isolates between species clades for ITS and *tub2* (see results above), indicating some degree of gene transfer between species, which could be explained by the overlap in host species. More isolates need to be subjected to multi-gene phylogenies or other molecular analyses to understand the underlying processes and frequency at which these transfers happen.

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Postia caesia complex (*Polyporales, Basidiomycota*) in temperate Northern Hemisphere

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Abstract: Taxonomy of the *Postia caesia* complex is revised based on morphology and two genetic markers, ITS and *tef1*. In total, we recognize 24 species, multiplying the known species diversity in the complex. We provide descriptions for 20 temperate Northern Hemisphere taxa. Identity of the core species, *P. caesia*, is re-established, and a neotype from the type locality is selected. Four new combinations are proposed, and 10 new species are described: *P. arbuti, P. auricoma, P. bifaria, P. comata, P. cyanescens, P. glauca, P. livens, P. magna, P. populi*, and *P. yanae*.

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INTRODUCTION

The *Postia caesia* species complex contains closely related brown-rot polypore species with blue-tinted basidiocarps making them easy to recognize. Distinct blue colors are rather rare among fungi, and among polypores only species of *Skeletocutis nivea* coll. develop a similar blue-tinted pore surface. For a long time, all blue-tinted *Postia* spp. went under the name *Postia caesia* (=*Oligoporus caesius*), described from conifers in Europe.

David (1974, 1980) first showed through mating tests and morphological analyses that two other species are present in Europe besides *P. caesia*, describing *P. luteocaesia* and *P. subcaesia* as new species. Jahn (1979) noted that David's *P. subcaesia* comes in many forms. He then introduced *P. subcaesia* "f. *minor*", which Niemelä and Vampola later described as *Postia alni* (Niemelä *et al.* 2001). Lastly, Pieri & Rivoire (2005) introduced the fifth European species, *P. mediterraneocaesia*.

Outside the Northern temperate area, a few further species have been included in the complex. Ryvarden (1983) noted that Patouillard's *Polyporus caesioflavus* from Ecuador is closely related but separate from *P. caesia*. Ryvarden (1988a) described *Oligoporus africanus* from Burundi. Corner (1989) introduced two new species from Malaysian Borneo, *Tyromyces amyloideus* and *T. coeruleivirens*, confirmed by Hattori (2005) to belong to the *Postia caesia* species complex. Papp (2015) provided combinations of the above-mentioned species to *Postia*. In New Zealand, Rajchenberg (1995) noted that *Postia atrostrigosa* is a relative of *Postia caesia*.

The above-mentioned authors, however, never reviewed the availability of older names for European taxa, and no revision of the species complex in Europe or elsewhere has been attempted. Yao *et al.* (2005) showed that three ITS-sequence-based groups were present in England, but they could not connect those groups to existing names. Ortiz-Santana *et al.* (2013) and Pildain & Rajchenberg (2013) presented genus-level phylogenies that included representatives of the *Postia caesia* complex. Both concluded that the *Postia caesia* complex belongs to the genus *Postia*, but did not touch upon species concepts.

In this study we have sampled specimens of the *Postia caesia* species complex originating from Europe, Siberia, East Asia and North America from a molecular and morphological perspective. The material is extensive, covering 146 localities from 20 countries. Our aim is to revise the species concepts within the northern temperate area. To establish a firm nomenclatural basis for species concepts in this group, we have conducted type studies of all known species names from the temperate Northern Hemisphere.

MATERIAL AND METHODS

Morphology

We studied types and specimens from the herbaria BPI, CFMR, CUP, H, K, LE, LY, MJ and O as well as from private herbarium of the author JV. Herbarium acronyms are given according to Index Herbariorum (2017). Sequenced specimens are marked with an asterisk (*).

Due to small morphological differences between *P. caesia* and its relatives, all specimens were examined following the same routine. Number of pores per mm was measured with a stereomicroscope targeting areas with regular pore form. When studying hyphal structure and measuring hyphae, the part of the basidiome cut may influence the outcome. Thus we studied

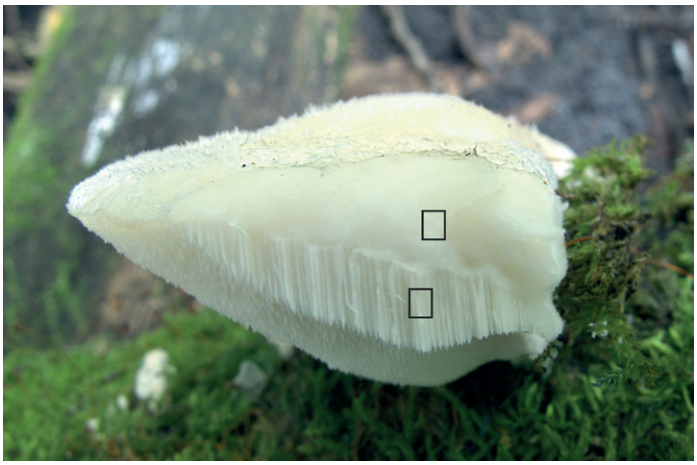

Fig. 1. Cross-section of a *Postia livens* basidiome (Miettinen 16714) showing which parts should be studied under microscope for comparable results.

context cut from its lower or middle part, and trama from the middle (Fig. 1).

All microscopical structures were measured with Leica microscopes using Cotton Blue in lactic acid (CB, Merck 1275), with ×1250 magnification and phase contrast illumination. At least 20 hyphae from the context and hymenophoral trama, as well as 10 basidia and 30 basidiospores were measured per each specimen reported in Supplements 2 and 3. For presenting variation of hyphal width and basidiospores, the 20 % and 5 % extreme tails are given in parentheses, respectively (hyphal width variation is larger than spore size variation). Additionally, Melzer's reagent (IKI) and 5 % KOH were used for microscopy. In KOH and to lesser extent also in Melzer's reagent the hyphal walls swell inward, and our descriptions of hyphal wall thickness and width are not valid for these reagents.

Sketches were made using a drawing tube with the exception of spores that were drawn with free hand after real measured spores. The sketches were then imported to CorelDRAW 2017 and redrawn to vector graphics on Wacom DTK-2700 drawing board. Spore statistics were produced with R v. 3.2.2 (R Core Team 2013).

Variation between juvenile, well-developed and senescent specimens may be significant and should be taken into account when reading the descriptions. Young basidiomes have typically thinner-walled hyphae, while senescent and overwintered specimens tend to produce longer basidia and more thick-walled, larger and sometimes slightly sigmoid spores. Our descriptions have generally excluded such variation and refer to normal, well-developed specimens.

We define matt here as a surface which is felt-like or finely hairy under the dissecting microscope (i.e. hyphae are not agglutinated). What matters is the distinction of hairy or pubescent (visible hairs of about a millimeter or longer as in *P. subcaesia*) versus glabrous (no hairs at all) or matt (projecting hairs visible only with a lens, as in *P. populi*). When describing basidiome size, small refers to about 1–3 cm wide caps, and medium about 4–8 cm). When describing basidiome thickness, we define thin as 2–5 mm and thick as 15 mm and above.

DNA extraction

DNA extractions and PCR products were prepared and sequencing undertaken with one of the following methods:

1. Essentially as described in Ortiz-Santana *et al.* (2013). For *tef1* primers 983F and 1567R were used (Matheny *et al.* 2007). In the case of critical samples, the concentration of NaCl in the extraction buffer was lowered from 0.7 M to 0.5 M which reduced the extraction of contaminating polysaccharides from fungal material. Also, the weight of processed tissue was reduced from 20–200 mg to 1–2 mg which enabled in many cases to obtain acceptable DNA also from tissues with moderate yeast contamination. 35 cycles PCR was then used for both ITS and TEF amplification.
2. With Phire Animal Tissue Direct PCR Kit (ThermoFisher), using the following PCR protocols: ITS primers ITS5-LR22 98 °C 5 min, (98 °C 5 s, 50 °C 30 s, 72 °C 20 s) ×40, 72 °C 1 min, 4 °C forever; TEF primers 983.2F-1567R 98 °C 5 min, (98 °C 5 s, 66 °C 20 s, 72 °C 20 s) ×8, (98 °C 5 s, 53 °C 40 s, 72 °C 20 s) ×36, 72 °C 1 min, 4 °C forever. Primer sequence 983.2F (modified after Matheny *et al*. 2007): GCHYCHGGNCAYCGTGAYTTYAT. PCR products were sent to Finnish Institute for Molecular Medicine (FIMM) or Macrogen for sequencing.

Phylogenetic analyses

We sequenced nuclear ribosomal DNA internal transcribed spacer (ITS) from 100 samples, large subunit (nLSU, 28S) from 9 samples and translation elongation factor 1-α (*tef1*) from 44 samples. The resulting sequences are available in INSDC under the accession numbers MG137026–MG137169. We also utilized three ITS sequences provided by Viktor Papp as well as 63 ITS and 28S sequences from the INSDC (Suppl. 1), based on BLAST searches and Ortiz-Santana *et al*. (2013), Pildain & Rajchenberg (2013), Shen *et al.* (2014), and Justo *et al.* (2017).

We constructed four datasets for analyses:

1. The 28S-ITS-dataset includes sequences of 34 species in *Dacryobolaceae* combining 34 ITS and 26 28S sequences. The purpose of this dataset is to assess whether *Postia caesia* coll. form a monophyletic group within *Postia*. Total alignment length is 1393 bp (454 bp ITS, 939 28S) with 205 (113 and 93) parsimony informative characters. The tree was rooted with *Dacryobolus karstenii* following Justo *et al.* (2017).
2. The ITS-dataset includes all available ITS sequences of the *Postia caesia* complex (139), excluding bad-quality sequences. The purpose of this dataset was to assess species number and limits. Alignment length is 572 bp with 66 parsimony informative characters. Rooted with *Postia auricoma* based on the 28S-ITS-dataset analysis.
3. The *tef1*-dataset includes 44 *tef1* sequences of the *Postia caesia* complex, to complement the ITS-dataset in species delimitation. Alignment length is 583 bp with 112 parsimony informative characters, midpoint rooting.
4. The ITS-*tef1* dataset combines sequences from the ITS and *tef1* datasets in a joint analysis for the 41 specimens with both ITS and *tef1* sequences. Total alignment length is 1154 bp with 150 parsimony informative characters

MAFFT online v. 7.310 was used for aligning sequences with the strategy E-INS-I (http://mafft.cbrc.jp, Katoh & Standley 2013). Resulting alignments were refined and characters with unclear homology excluded manually using PhyDE v. 0.9971 (Müller *et al*. 2010). Numbers of characters were calculated in MEGA6 (Tamura *et al*. 2013).

Phylogenies were constructed with MrBayes v. 3.2.3 and 3.2.4 (Ronquist *et al.* 2012). The 28S-ITS-dataset was partitioned to 28S and ITS. Nucleotide substitution models were chosen with jmodeltest v. 2.1.10 based on AIC (Darriba *et al*. 2012): GTR+I+G for the full ITS-dataset, for the ITS partition of the ITS-*tef1*-dataset, and 28S partition of the 28S-ITS-dataset; SYM+I+G for the *tef1*-datasets and for the ITS partition of the 28S-ITS-dataset. Analyses were run with eight chains in three parallel runs, temp=0.1 for 10 million generations sampling a tree every 2000 generations (5 million generations for *tef1*-dataset). All runs had converged to below 0.01 average standard deviation of split frequencies by the end of the run. A burn-in of 25 % was used before computing the consensus tree.

Also maximum likelihood analyses were conducted for each of the datasets with RAxML v. 8.1.3 (Stamatakis 2014), using similar partitioning and GTR+G for all partitions. A hundred parallel analyses were run to find the highest likelihood tree, and 1000 bootstrap replicates to calculate bootstrap support values. Trees of the Bayesian and maximum likelihood analyses were identical in all well-supported nodes, so below we only report bootstrap support values of the maximum likelihood analyses mapped to Bayesian consensus trees.

The phylogenetic analyses were conducted at the CSC – IT Center for Science (https://www.csc.fi) multi-core computing environment. The alignments and phylograms have been deposited in TreeBASE (S22087).

Maps and distribution terms

Distribution maps for each species (Suppl. 4) were drawn with R v. 3.2.2 with the help of package sp (Pebesma & Bivand 2005) and Natural Earth (http://www.naturalearthdata.com). We use definitions of Hämet-Ahti (1984) for boreal zone subdivision when discussing species ranges.

RESULTS

Phylogenetic analysis of the 28S-ITS-dataset confirms that the *Postia caesia* complex forms a distinct lineage of closely related species within the genus *Postia* (Fig. 2). The closest, but still distant, relatives of the *P. caesia* complex in our analysis were *P. lactea* (the type species of *Postia*) and *P. venata.* In the absence of close relatives and small interspecific variation, no firm conclusions can be reached about the earliest diverging species and branching order within the *P. caesia* complex.

Our ITS-dataset separates 19 species with good support (clades with posterior probability≥0.9): *P. auricoma, P. subvirids, P. arbuti, P. magna, P. coeruleivirens, P. subcasesia, P. bifaria, P. glauca, P. caesia, P. livens, P. yanae, P. cyanescens, P. alni*, and *P. populi* (Fig. 3). In addition, one variable clade that includes *P. luteocaesia* and *P. simulans*, receives fair support (PP=0.85), and one morphologically defined species, *P. caesiosimulans*,

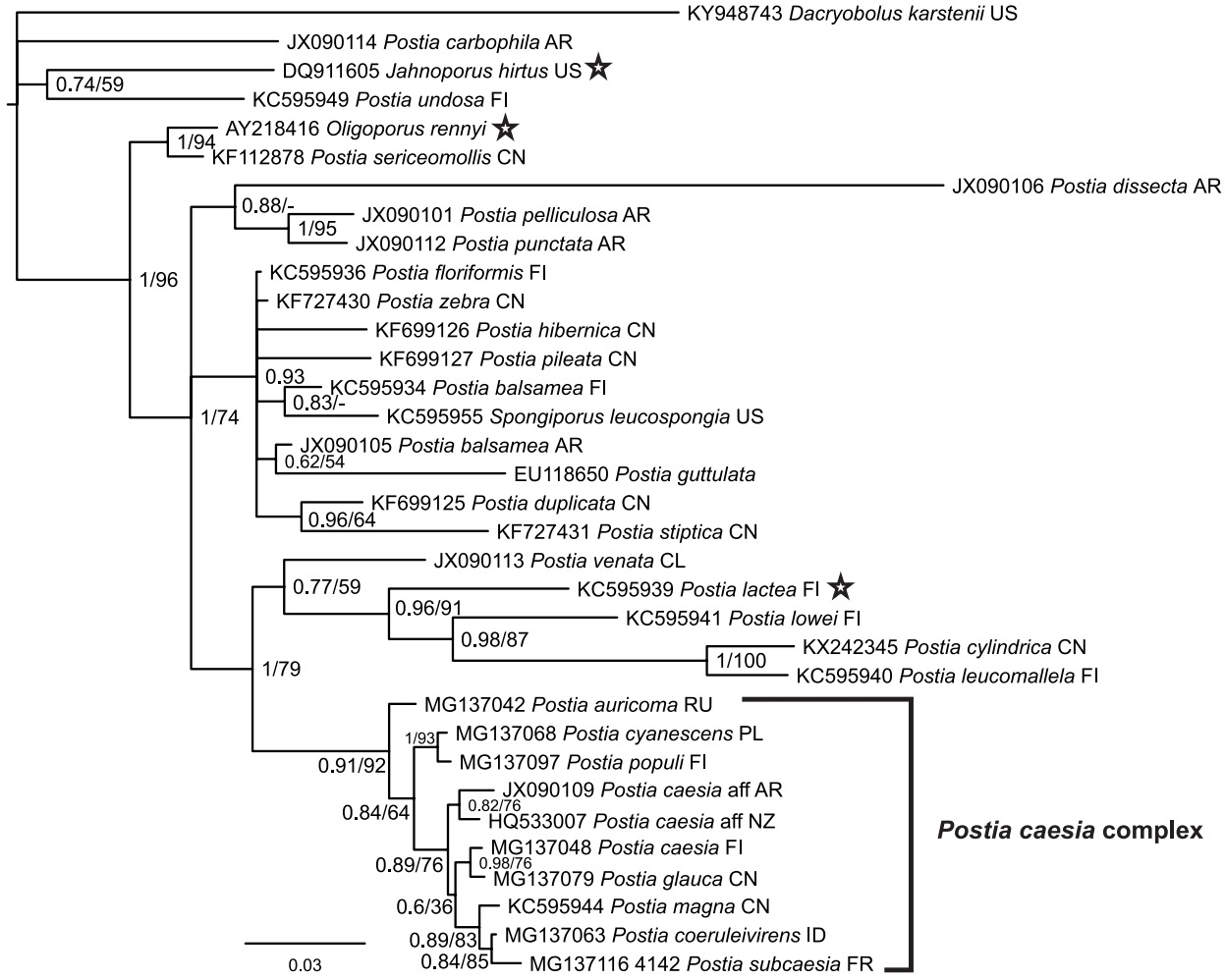

Fig. 2. Phylogeny of the *Dacryobolaceae* around *Postia caesia* complex. Bayesian consensus tree based on 28S and ITS sequences. Numbers up to one denote posterior probabilities, above one bootstrap support values of a maximum likelihood analysis. Genus types are marked with stars. Two-letter codes after species names denote country of origin (ISO 3166).

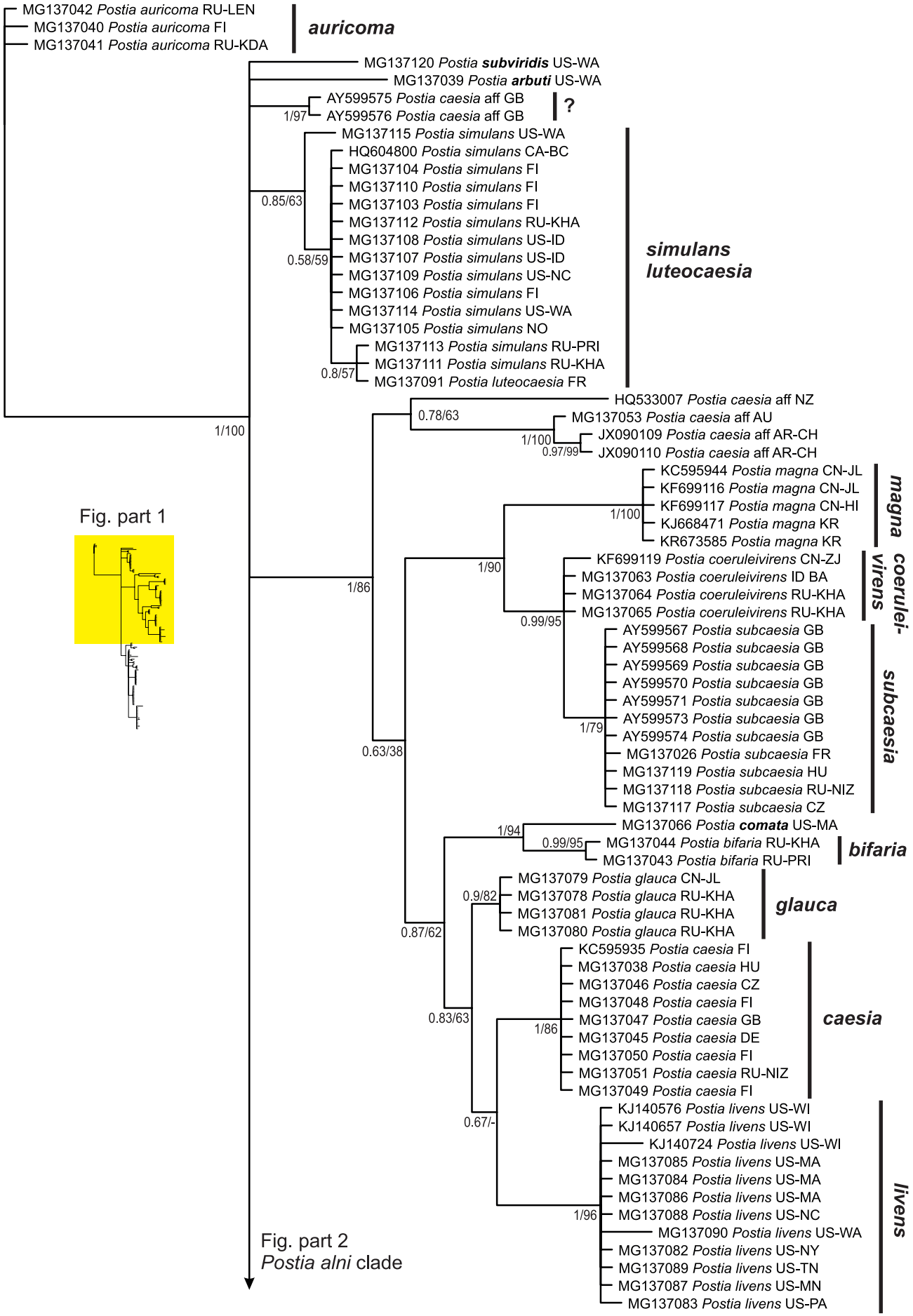

Fig. 3. *Postia caesia* complex ITS phylogeny. Bayesian consensus tree, where numbers up to one denote posterior probabilities and above one bootstrap support values of a maximum likelihood analysis. Two- and three-letter codes after species names denote country and province of origin (ISO 3166, IATA for Argentina, GB/T 2260 for China).

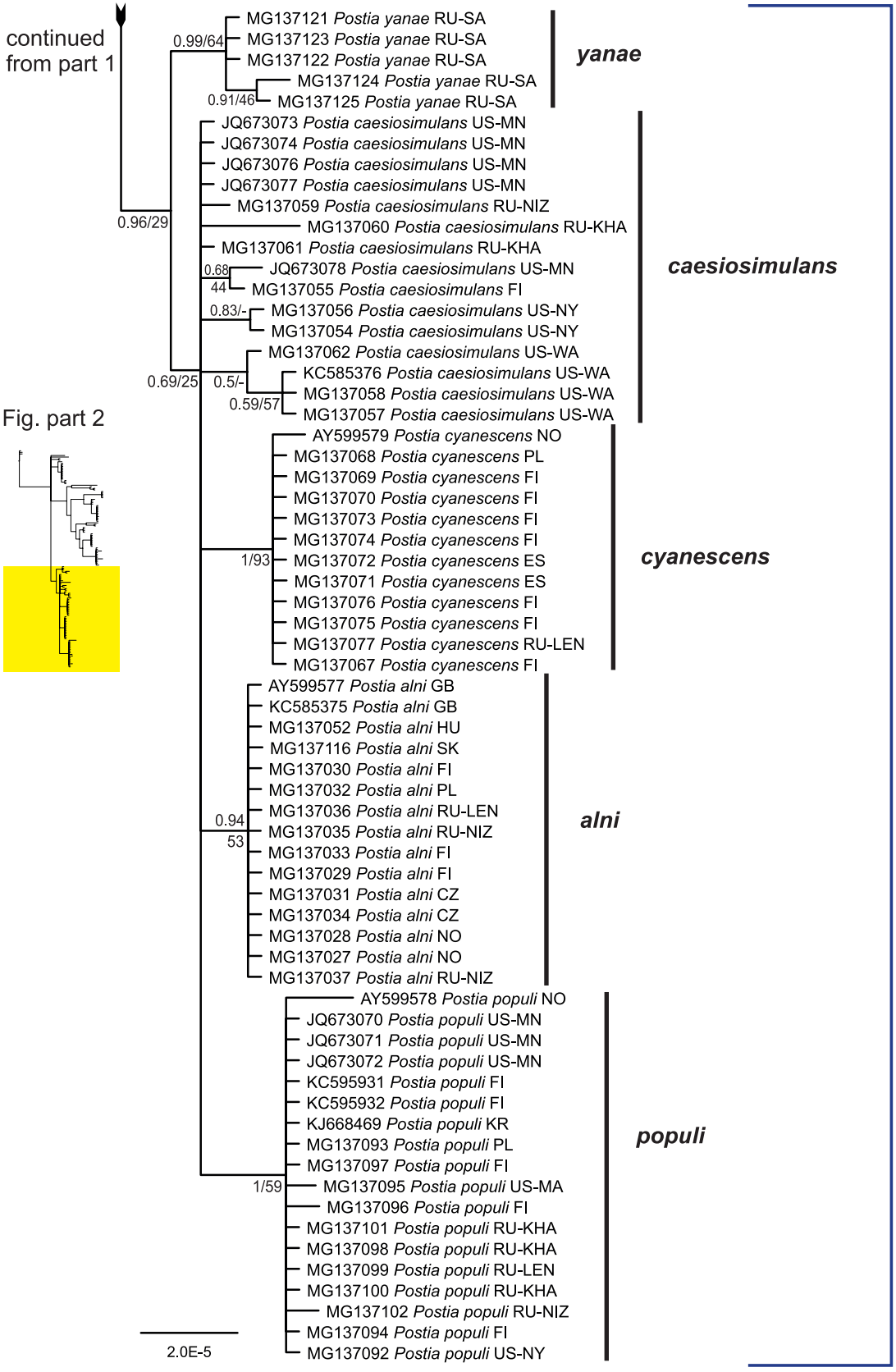

Fig. 3. (Continued).

isn't supported against its closest relatives. These two difficult-to-reconcile cases are discussed below.

The *tef1*-dataset, with a more limited sampling, shows excellent support (PP≥0.95) for 14 species/clades: *P. bifaria, P. magna, P. livens-subcaesia, P. caesia, P. mediterraneocaesia, P. glauca, P. gossypina, P. arbuti, P. simulans, P. yanae, P. subviridis, P. populi, P. alni*, and *P. cyanescens* (Fig. 4). In addition, *P. caesiosimulans* receives lower support (PP=0.73). Two species, *P. mediterraneocaesia* and *P. gossypina*, are only represented by a *tef1*-sequence in this paper.

The delimitation of terminal clades or species is congruent in the ITS- and *tef1*-analysis except for two cases: First, the *tef1*-

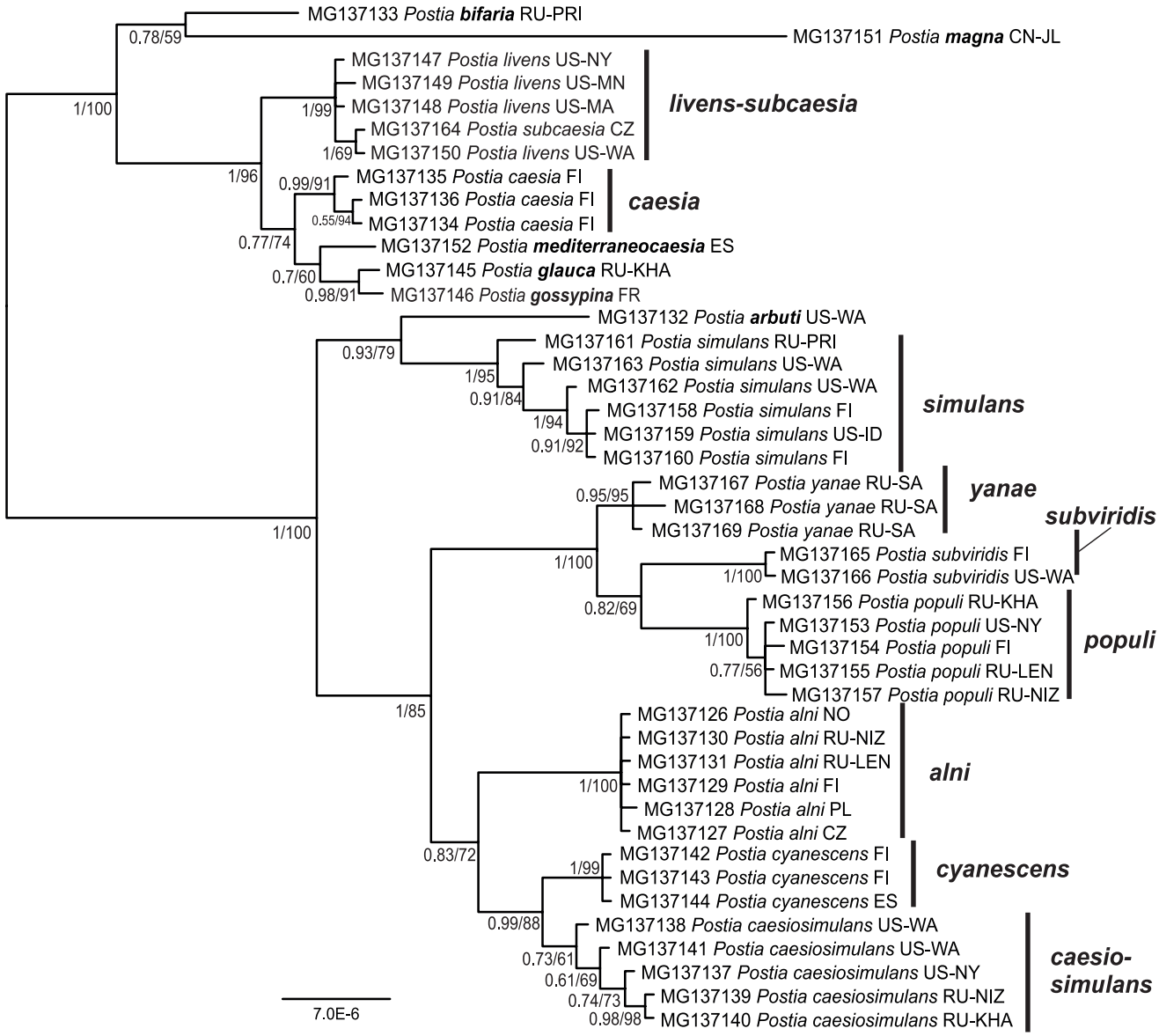

Fig. 4. *Postia caesia* complex *tef1* phylogeny. Bayesian consensus tree, where numbers up to one denote posterior probabilities and above one bootstrap support values of a maximum likelihood analysis. Two- and three-letter codes after species names denote country and province of origin (ISO 3166, IATA for Argentina, GB/T 2260 for China).

dataset does not separate between *P. livens* and *P. subcaesia* (Fig. 4), while the ITS-dataset clearly does (Fig. 3). Second, sequences of *P. caesiosimulans* form as a clade only in the *tef1*-analysis (Fig. 4).

The concatenated ITS-*tef1*-datset includes only specimens, for which both ITS and *tef1* were sequenced (Fig. 5). It resolves 13 species with excellent support (PP≥0.99), while failing to treat *P. caesiosimulans* as a distinct clade from the well-supported *P. cyanescens*. The ITS-*tef1*-analysis supports distinction of *P. livens* from *P. subcaesia*, a conflict between analysis of ITS (separate clades) and *tef1* (one clade).

Combining results of these three datasets, we have sequence data available for altogether 24 species in the *Postia caesia* complex, including 2–3 Southern Hemisphere species we will not treat further here. Also one putative species represented by two ITS sequences from England (AY599575, AY599576) belonging to the difficult *P. alni* clade (Fig. 3) had to be left out of this treatment due to absence of *tef1*-sequences. Combined with morphological evidence, this allows us to treat 20 species in detail below. Our concepts of two of these species, *P. caesiosimulans* and *P. simulans*, may represent species complexes, i.e. contain further species than we are able to uncover here. Although genetic differences are small in many cases, all the 20 species we treat are supported phylogenetically with one exception: *P. luteocaesia*, an already existing, morphologically distinct species discussed below.

Comparing genetic markers, *tef1* shows generally larger differences than ITS between species. Without *tef1*-sequences revision of the *P. alni* clade (Fig. 3) would have been difficult: ITS sequences vary only between 1 and 6 bp between species in this clade, whereas *tef1* variation is between 9 and 32 bp. An extreme case is *P. yanae*, which differs from *P. caesiosimulans* by only 1 bp in ITS but by 25 bp in t*ef1*. Combining the two genetic markers with ecological and morphological characters we have reached a reasonable solution for splitting the *P. alni* clade into four homogenous (*P. alni, P. cyanescens, P. populi* and *P. yanae*) and one variable species (*P. caesiosimulans*), while leaving the above-mentioned English ITS-sequenced material untreated. It is evident that more work has to be done around *P. caesiosimulans* to fully understand species limits. As

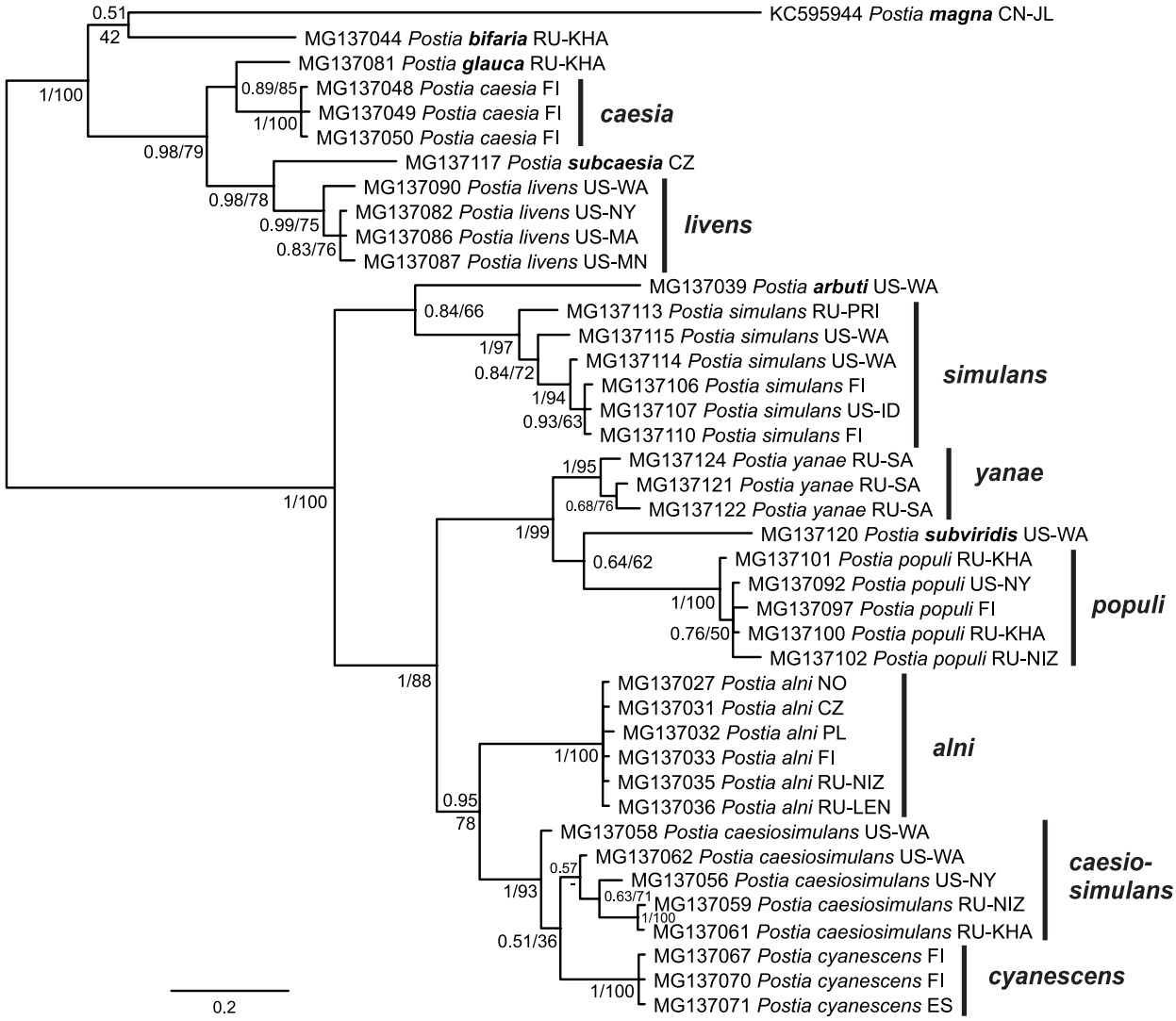

Fig. 5. *Postia caesia* complex *tef1*-ITS phylogeny for a concatenated alignment. Bayesian consensus tree, where numbers up to one denote posterior probabilities and above one bootstrap support values of a maximum likelihood analysis. Two- and three-letter codes after species names denote country and province of origin (ISO 3166, IATA for Argentina, GB/T 2260 for China).

this species is already described, the best interim solution is to apply the name to a wide set of specimens while considering that we have only limited phylogenetic support for this wide concept.

Another puzzle lies in the *P. simulans* clade, which includes two previously described species, yellow-colored *P. luteocaesia* and white *P. simulans*. Here we have a situation, in which ITS and *tef1* data show enough variation (up to 3 and 6 bp respectively) that the clade probably contains several species. It would appear that East Asian *P. simulans* is distinct from European and North American material (1 bp ITS, 6 bp *tef1* difference), but its ITS sequence is identical with *P. luteocaesia* (no *tef1* available for the latter). East Asian specimens are white in color in contrast to the European *P. luteocaesia*. One Western North American specimen is also distinct both in ITS and *tef1* (differing by 2 and 4 bp respectively against *P. simulans*). Microscopically spore variation within this group is negligible but tramal hyphae vary more, potentially offering useful characters for future species delimitation. For now our data does not allow dividing this clade to phylogenetically well-defined species, and our pragmatic solution is to recognize two morphological species, *P. luteocaesia* for yellow European specimens and *P. simulans* for all the white-colored specimens.

Morphological differences between species in the *P. caesia* complex are generally small, but we have managed to find reliable characters to separate nearly all species and relate them to the phylogenetic results. Of importance are basidiome color and size, pore size, upper surface hairiness, hyphal width and wall thickness, and spore size. In addition, the host species greatly helps in identifying many (but not all) species. The differences are too small to construct any sensible dichotomous identification key, but we have constructed comparative tables that summarize the main characters between species for identification purposes (Tables 1–3).

Taxonomy

Postia caesia complex

Basidiocarps pileate to effused-reflexed, white, whitish or rarely yellow, with bluish tints at least deep in tubes, small to medium sized, caps projecting up to 40 mm, 5–100 mm wide, 3–40 mm thick. Margin sharp to blunt. Consistency soft, fragile when dry. Pores rounded angular, regular but when old merging together, more rarely sinuous, mouths even to serrate, 4–8 per mm. Section: context white (cream-colored in old herbarium

Table 1. Morphological comparison of European members of the *Postia caesia* complex. Data for *P. subviridis* are based on European material only, and *P. simulans* on European and North American material (excluding East Asia).

	Species	Distribution/ host	Upper surface	Pores per mm	Tramal hyphae (measurements include 60 % of variation around median diameter)	Basidiospores (variation of specimen means)	Other
Narrow-spored species (W<1.4 µm)	*alni*	Europe; temperate / hemiboreal; deciduous trees (but not *Populus*)	smooth to pubescent, brown when old	5–6	slightly to moderately thick-walled, 2.9–3.6 µm (walls 0.2–0.8 µm)	L=4.85-5.3 µm, W=1.16-1.22 µm, Q=3.98-4.34	
	caesiosimulans	holarctic; temperate; known from ***Corylus*** in Europe	smooth	5–7	slightly to **distinctly thick-walled,** often densely arranged, 2.9–3.8 µm (walls up to 1 µm)	L=4.47-5.14 µm, W=1.14-1.27 µm, Q=3.79-4.05	Q mostly <4, context hyphae wide
	cyanescens	Europe; boreal, temperate to mediterranean mountains; ***Picea, Abies***	smooth to matt	5–6	thin- to moderately thick-walled, +-parallel, 2.9–3.7 µm	L=4.89-5.64 µm, W=1.23-1.45 µm, Q=3.38-4.49	rarely W>1.4 µm, hyphae not collapsing or colored
	populi	holarctic; temperate / boreal; almost exclusively ***Populus***	smooth	5–7	**thick-walled**, densely arranged, 2.7–3.3 µm (walls regularly >1 µm)	L=4.58-5.07 µm, W=1.12-1.24 µm, Q=3.86-4.37	undulating cap margin, Q mostly >4, context hyphae narrow
	subcaesia	Europe; temperate; deciduous trees	**pubescent**	4–6	thin- to slightly thick-walled, **3.1–4.1 µm**	L=4.29-5.08 µm, W=1.18-1.29 µm, Q=3.61-3.94	**big basidiome, thick context, wide context hyphae** (4.2–6.6 µm)
	subviridis	Holarctic; temperate / boreal, **conifers**	smooth	6–8	thin- to slightly thick-walled, 2.5–3.0 µm	L=4.92 µm, **W=1.10 µm**, Q=4.46	
Wide-spored species (W>1.4 µm)	*caesia*	Europe; temperate / hemiboreal; prefers conifers	**pubescent**, often blue	**4–5**	thin- to slightly thick-walled, loosely arranged, 2.8–3.6 µm	L=4.45-4.94 µm, W=1.42-1.64 µm, Q=2.95-3.36	**greenish-bluish when bruised, colored (amyloid) hyphae**
	gossypina	Europe; temperate	smooth to matt	4–6	thin- to slightly thick-walled, **2.3–3.0 µm**	**L=4.41-4.52 µm**, W=1.41-1.46 µm, Q=3.1-3.13	
	mediterraneocaesia	Europe; warm temperate - mediterranean; various hosts	smooth	5–6	**thick-walled, twisted, 2.3–3.2 µm**	L=4.45-5.47 µm, W=1.43-1.55 µm, Q=3.11-3.53	**dry habitat, thin branches, basidiocarps pendant**
	simulans	holarctic; temperate / boreal; preferrably conifers	smooth to pubescent, often blue	**5–7**	thin- to slightly thick-walled, **collapsing**, 2.8–3.8 µm	L=4.89-5.93 µm, W=1.44-1.55 µm, Q=3.17-4.12	
Yellow species	*auricoma*	Eurasia; **boreal / temperate**; *Larix* and *Pinus*	matt to pubescent, sometimes blue	4–6	slightly to distinctly thick-walled, loosely arranged, **3.1–4.1 µm**	L=5.03-5.05 µm, W=1.62-1.69 µm, Q=2.98-3.12	deep green when bruised
	luteocaesia	Europe; **mediterranean,** *Pinus*	matt to pubescent	**3–5**	thin- to moderately thick-walled, **2.7–3.2 µm**	L=4.89-5.26 µm, W=1.65-1.7 µm, Q=2.96-3.13	deep green when bruised

Table 2. Morphological comparison of East Asian members of the *Postia caesia* complex. Description of *P. simulans* is based on East Asian material only.

	Species	Distribution/ host	Upper surface	Pores per mm	Tramal hyphae (measurements include 60 % of variation around median diameter)	Basidiospores (variation of specimen means)	Other
Narrow-spored species (W<1.3 µm)	*bifaria*	East Asia; **cold temperate**; **conifers**	pubescent	6–8	thin- to slightly thick-walled, **collapsing,** 2.5–3.8 µm	**L=4.09-4.1 µm, W=1.11-1.16 µm**, Q=3.53-3.69	
	caesiosimulans	holarctic; temperate; *Abies* in East Asia, various hosts elsewhere	smooth	5–7	slightly to distinctly thick-walled, often densely arranged, 2.9–3.8 µm (walls up to 1 µm)	L=4.47-5.14 µm, W=1.14-1.27 µm, Q=3.79-4.05	Q mostly <4
	coeruleivirens	East Asia; warm temperate - tropical; **deciduous trees**	**pubescent**	6–8	thin- to moderately thick-walled, 2.4–3.4 µm (walls up to 0.8 µm)	**L=3.69-4.32 µm, W=1-1.16 µm,** Q=3.59-3.72	
	glauca	East Asia; **cold temperate; conifers**	smooth to pubescent	5–8	thin- to slightly thick-walled, loosely arranged, 2.6–3.3 µm	**L=4.45-4.9 µm, W=1.27-1.28 µm**, Q=3.48-3.86	**colored (amyloid) hyphae**
	magna	East Asia; temperate; deciduous trees	**pubescent**	**4–5**	slightly thick-walled, 2.2–3.3 µm	**L=3.97 µm**, W=1.13 µm, Q=3.51	**big basidiomes, thick context, wide context hyphae (4.2–6.0 µm)**
	populi	holarctic; temperate / boreal; deciduous trees, prefers ***Populus***	smooth	5–7	**thick-walled**, densely arranged, 2.7–3.3 µm (walls regularly >1 µm)	L=4.58-5.07 µm, W=1.12-1.24 µm, Q=3.86-4.37	undulating cap margin, Q mostly >4
Wide-spored species (W>1.3 µm)	*simulans*	holarctic; **temperate** / boreal; prefers conifers	smooth, brown when old	5–7	slightly to distinctly thick-walled, **2.7–3.2 µm (walls 0.2-1 µm thick)**	L=5.17-5.65 µm, W=1.34-1.53 µm, Q=3.69-4	**spores often slightly fusiform**
	yanae	**Siberia; boreal; *Larix* and *Pinus***	smooth, white to ochraceous	5–7	thin- to slightly thick-walled, **winding, collapsing, 2.2–2.9 µm**	L=4.74-5.36 µm, W=1.35-1.45 µm, Q=3.39-3.81	small basidiomes on branches, dry habitat
Yellow species	*auricoma*	Eurasia; **boreal / temperate**; *Larix* and *Pinus*	matt to pubescent, sometimes blue	4–6	slightly to distinctly thick-walled, loosely arranged, 3.1–4.1 µm	L=5.03-5.05 µm, W=1.62-1.69 µm, Q=2.98-3.12	deep green when bruised

specimens), tube layer white or yellow, discoloring bluish gray upon drying or when old. *Hyphal system* monomitic, clamps always present. Hyphae thin- to thick-walled, CB– to CB(+), weakly amyloid in masses. Context hyphae loosely arranged, some of them in bundles, thin- to slightly thick-walled, often developing refractive (sclerified) content leaving visible only a capillary, irregular cytoplasm. *Tramal hyphae* loosely to rather tightly packed, narrower than in context, subparallel to interwoven (particularly older parts). Free-floating oily matter present in slides. *Amorphous aggregates* (Fig. 7) develop

Table 3. Morphological comparison of North American members of the *Postia caesia* complex. Description of *P. simulans* is based on European and North American material (excluding East Asia).

	Species	Distribution/ host	Upper surface	Pores per mm	Tramal hyphae (measurements include 60 % of variation around median diameter)	Basidiospores (variation of specimen means)	Other
Narrow-spored species (W<1.4 µm)	*arbuti*	North America (NW); temperate; ***Arbutus***	smooth	**6–8**	thick-walled, densely arranged, 2.4–3.1 µm (walls up to 1 µm)	L=4.54-4.58 µm, W=1.13-1.15 µm, Q=3.95-4.05	
	caesiosimulans	holarctic; temperate; **Fagus, deciduous trees**, rarely conifers	smooth	5–7	slightly to distinctly thick-walled, often densely arranged, 2.9–3.8 µm (walls up to 1 µm)	L=4.47-5.14 µm, W=1.14-1.27 µm, Q=3.79-4.05	Q mostly <4; few western conifer specimens W>1.4 µm; context usually thin
	comata	North America (**NE**); temperate; **conifers**	**pubescent**	4–6	**thick-walled**, 2.8–3.8 µm (walls up to 1 µm)	**L=4.34-4.39 µm,** W=1.2-1.21 µm, Q=3.59-3.66	
	livens	North America; temperate; conifers and deciduous trees	**matt to pubescent**	**4–6**	**thin- to moderately** thick-walled, 2.9–4.0 µm (walls up to 0.5–0.8 µm)	L=4.30-5.25 µm, W=1.2-1.37 µm, Q=3.31-4.17	**thick context, often big basidiomes**
	populi	holarctic; temperate / boreal; deciduous trees, prefers ***Populus***	smooth	5–7	**thick-walled**, densely arranged, 2.7–3.3 µm (**walls regularly >1 µm**)	L=4.58-5.07 µm, W=1.12-1.24 µm, Q=3.86-4.37	undulating cap margin, Q mostly >4
	subviridis	North America (W); temperate; **conifers**	smooth	**6–8**	thick-walled, **2.5–3.1 µm** (walls up to 1 µm)	L=4.07-4.92 µm, **W=1.1-1.16 µm**, Q=3.51-4.47	
Wide-spored species (W≥1.4 µm)	*simulans*	holarctic; temperate / boreal; prefers conifers	smooth to pubescent, often blue	5–7	**thin- to slightly thick-walled,** rarely thick-walled, **often collapsing**, (2.2)2.8–3.8 µm	L=4.87-5.93 µm, W=1.4-1.55 µm, Q=3.17-4.12	

slowly in CB, and upon long exposure form needle-like crystals. Hyphal walls in all parts swell strongly inwards in KOH and to lesser degree in IKI. *Hymenium*: Cystidioles sometimes present, poorly differentiated. *Basidia* shortly clavate, slightly tapering to the apical part, often with a slight medial constriction, mostly terminal but occasionally pleural. *Basidiospores* allantoid, usually with slightly thickened walls, 4–7×1–2 µm, CB(+), hyaline to greyish, inamyloid to weakly amyloid.

Ecology: Brown-rotters on conifers and deciduous trees.

Distribution: Found in all forested continents. In the tropics mostly found in the mountains. Holarctic taxa: *P. caesiosimulans*, *P. populi*, *P. simulans, P. subviridis*. European taxa: *P. alni*, *P. auricoma*, *P. caesia*, *P. cyanescens*, *P. gossypina, P. luteocaesia sensu typi*, *P. mediterraneocaesia, P. subcaesia*. Temperate Asian taxa: *P. auricoma, P. bifaria*, *P. coeruleivirens*, *P. glauca*, *P. magna, P. yanae*. North American taxa: *P. arbuti*, *P. comata*, *P. livens*. Tropical and Southern Hemisphere taxa not treated here: *P. africana, P. amyloidea, P. atrostrigosa,* and *P. caesioflava*.

Remarks: The blue color, slightly thick-walled, curved, weakly cyanophilous and greyish spores and amorphous aggregates (Fig. 7) characterize the complex within *Postia*. Similar amorphous bodies are also found in slides of *Rhodonia placenta* (=*Postia placenta*) and to lesser extent in *Postia balsamea*, but we know of no other polypore species with this character. We have noticed that KOH stains the bottom of the tubes bluish or greenish in fresh specimens even when blue color is absent otherwise. Other than this use, we strongly recommend against using KOH when identifying species in this group, since hyphae and their walls swell rendering important characters useless.

Context hyphae are often sclerified i.e. their walls appear to thicken inwards, but this takes place so that the original wall is still distinct while the refracting, irregular "inner wall" dominates hyphal content; these hyphae appear to collapse just as easily as non-sclerified ones, so it is unclear if this phenomenon is really caused by growth of hyphal walls inwards. In any case it looks like the space for cytoplasm shrinks considerably (Fig. 6 under *P. populi*). In want of a better term and deeper understanding we use the term "sclerified" here.

The genus name *Cyanosporus* McGinty (=*Postia* subg. *Cyanosporus* (McGinty) Papp) has sometimes been used for *Postia caesia* complex (Papp 2015; Pieri and Rivoire 1998). McGinty is a pseudonym of C. G. Lloyd, who used it to ridicule his fellow mycologists who in his opinion were creating too many names. *Cyanosporus* is such a case, never intended to be taken seriously, as stated by Stevenson & Cash (1936) in their compendium of new fungal names published by Lloyd. Also Donk (1960) rejected the name for this reason. The code states that a name is valid only if it is accepted by the author, and thus *Cyanosporus* should be considered invalid (ICN art. 36.1). If *Cyanosporus* is deemed invalid, as we do, also *Postia* subg. *Cyanosporus* should be viewed invalid. Furthermore, *Cyanosporus* cannot be resurrected either due to *Cyanospora* Heald & F.A. Wolf 1910.

Quite aside the nomenclatural situation around *Cyanosporus* we see no reason to split the *Postia caesia* complex to a separate genus from *Postia*, either on morphological or phylogenetic grounds. The complex is distinct morphologically, but nevertheless very similar to other *Postia* spp. Separating the *P. caesia* complex to its own genus would create a cascade of splitting that would end up with many morphologically unrecognizable *Postia*-like genera.

The type species of *Postia*, *P. lactea* (=*P. tephroleuca* sensu auct.), and its close relative, *P. grata*, can look confusingly similar to the *P. caesia* complex when the blue color hasn't emerged yet. In such a case the formation of amorphous aggregates in microscopical slides of Cotton Blue is the best separating character.

Postia alni Niemelä & Vampola, *Karstenia* **41**: 7. 2001. Figs 6–9.

Holotype: **Slovakia**, Bratislava, Svätý Jur, *Alnus glutinosa*, 12 Oct. 1995, *Vampola** (H 7019137, studied).

Basidiocarps conchate to flabelliform, rarely effused-reflexed, small or rarely medium-sized polypores, mostly thin; margin sharp. Upper surface first cream colored, almost glabrous to matt, then pubescent, radially striate, ochraceous to brownish, often with bluish-greyish hues. Tubes white to cream-colored, in older and dry specimens with light bluish-greyish tint; *pores* 4–6(–7) per mm. *Section*: Context 1–4 mm thick, tubes 1–6 mm long. *Context hyphae* thin- or only slightly thick-walled, (2.4–)3.9–5.5(–7.4) µm in diam. *Tramal hyphae* with slightly to distinctly thickened walls (0.2–0.8 µm thick), (2.0–)2.9–3.6(–4.3) µm in diam. *Basidia* 10–14.8(–16) × 3.3–4.2 µm. *Basidiospores* (4.1–)4.3–6.1(–6.8) × (1.0–)1.1–1.3(–1.5) µm, L=5.05 µm, W=1.20 µm, Q=4.22.

Distribution and ecology: Europe, temperate to southern boreal, common; hardwood logs and thick fallen branches (*Acer*, *Alnus*, *Betula*, *Carpinus*, *Corylus*, *Fagus*, *Prunus*, *Quercus*, *Ulmus*), most common in riversides and coastal areas, herb-rich forests in the north.

Specimens examined: **Czech Republic**, Jihočeský kraj, Chlum u Třeboně, Bukové Kopce Nat. Res., *Fagus sylvatica*, 18 Sep. 2010, *Vampola** (MJ 27/10); Jihočeský kraj, Český Krumlov, Žofín Nat. Res., *F. sylvatica*, 16.IX.2012 *Vampola** (MJ 17/12). **Denmark**, Lolland, Faursted, *F. sylvatica*, 4 Oct. 2007 *Schigel 5425* (H). **Finland**, Uusimaa, Helsinki, Veräjämäki, *Alnus incana*, 19 Oct. 2011, *Miettinen 14918.2** (H), *Sorbus aucuparia*, 12 Dec. 2015, *Miettinen 19883* (H); Uusimaa, Helsinki, Pornaistenniemi, *A. incana*, 24 Sep. 2012, *Miettinen 15741* (H); Uusimaa, Kirkkonummi, Sundsberget, *Prunus padus*, 24 Oct. 2012, *Miettinen 15830** (H); Etelä-Häme, Hämeenlinna, Lammi, *Betula* sp., 21 Sep. 2016, *Niemelä 9233** (H); *S. aucuparia*, 14 Sep. 2015, *Miettinen 19386* (H); Satakunta, Ylöjärvi, Viljakkala, *Alnus glutinosa*, 2 Oct. 2011, *Niemelä 8843* (H). **Germany**, Schleswig-Holstein, Sachsenwald, *F.*

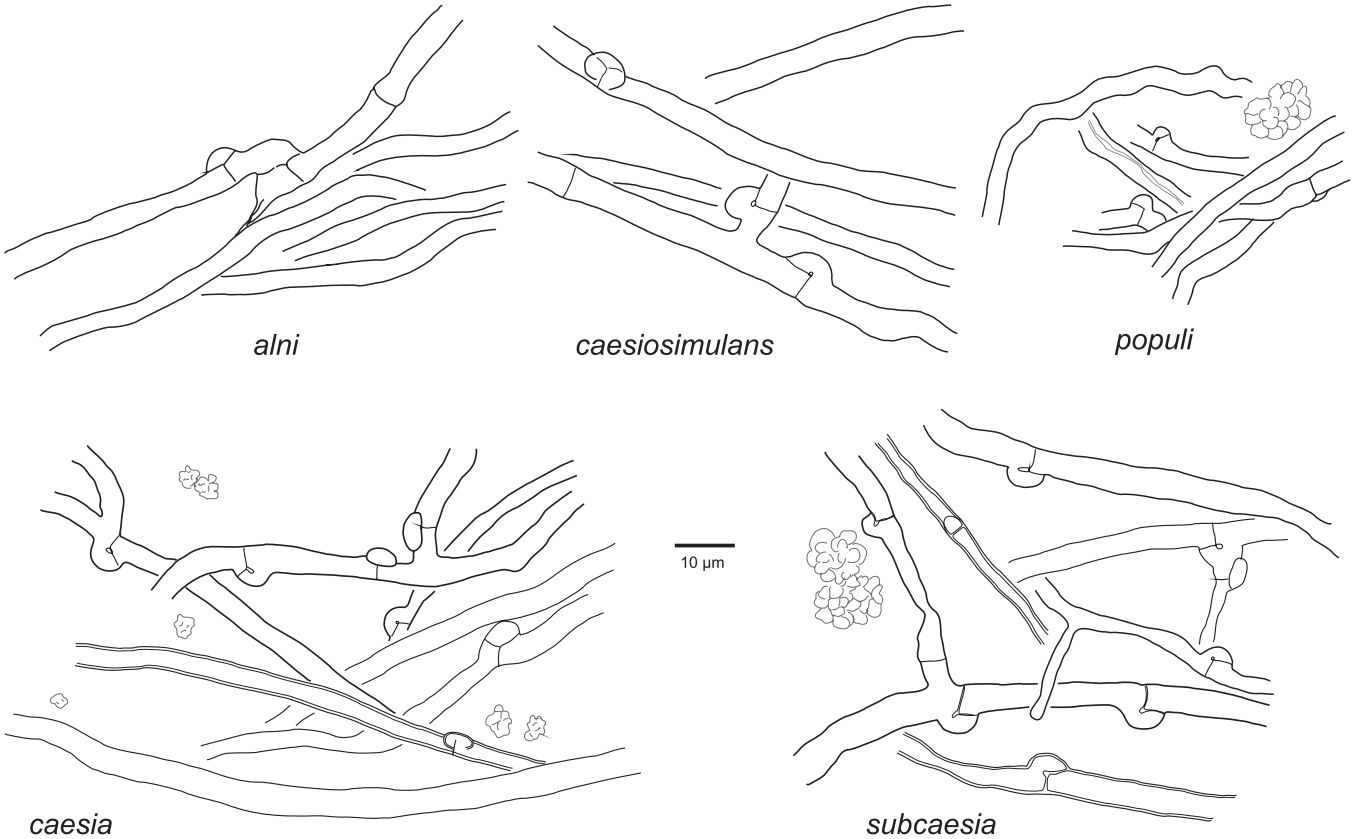

Fig. 6. Context hyphae in the *Postia caesia* complex. All drawings are from holo-, iso- or neotypes, except *P. caesiosimulans* from epitype and *P. subcaesia* from Legon 14 Oct. 1995.

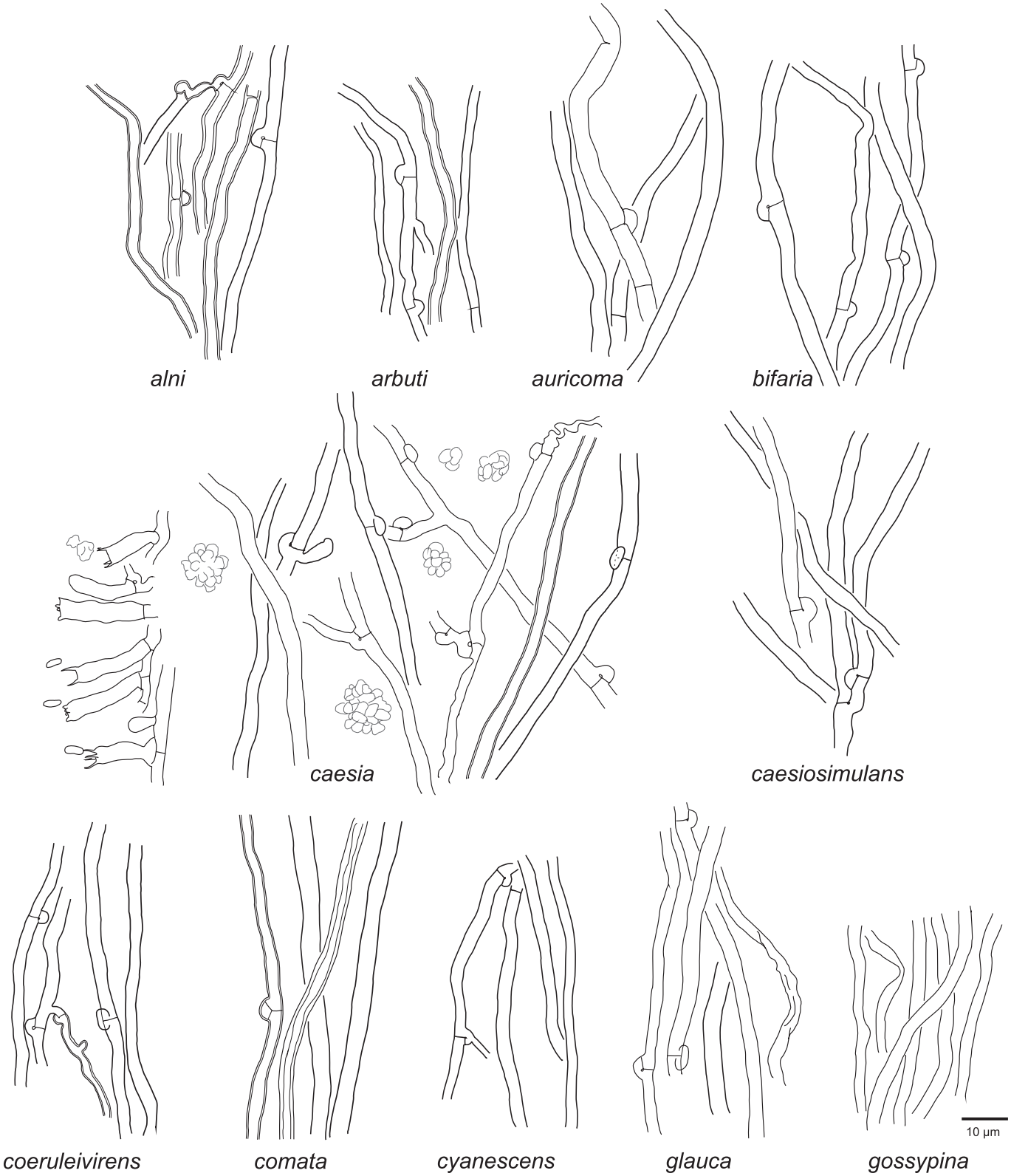

Fig. 7. Tramal hyphae and hymenial cells in the *Postia caesia* complex. All drawings are from holo-, iso-, lecto- or neotypes, except *P. caesiosimulans* and *P. simulans* from epitypes, *P. coeruleivirens* from Spirin 5301 and *P. subcaesia* from Legon 14 Oct. 1995. Drawings of *P. caesia* and *P. populi* depict amorphous aggregates that are characteristic to the complex.

sylvatica, 13 Oct. 1907, *Jaap* (Fungi Selecti Exsiccati 927, H). **Norway**, Akershus, Enebakk, Omberg, *Corylus avellana*, 1 Sep. 2016, *Nordén** (H); Akershus, Nesodden, Roerskogen, *Quercus robur*, 18 Sep. 2014, *Larsson** (O 248173). **Poland**, Podlasie, Hajnówka, Białowieża, *Carpinus betulus*, 16 Sep. 2012, *Niemelä 8933** (H). **Russia,** Leningrad Reg., Boksitogorsk Dist., Goryun, *Salix* sp., 23 Sep. 2011, *Spirin 4602** (H); Leningrad Reg., Volkhov Dist., Zagubie, *Acer platanoides*, 3 Oct. 2010, *Spirin 3640* (H); Nizhny Novgorod Reg., Lukoyanov Dist., Razino, *Ulmus glabra*, 18. Aug. 2015, *Spirin 9502** (H); Nizhny Novgorod Reg., Panzelka, *Betula pubescens* (?), 15 Aug. 2006, *Spirin 2548** (H); Sverdlovsk Reg., Pervouralsk, Khomutovka, *P. padus*, 26 Aug. 2002, *Kotiranta 19807* (H). **Slovakia**, Bratislava (holotype, see above).

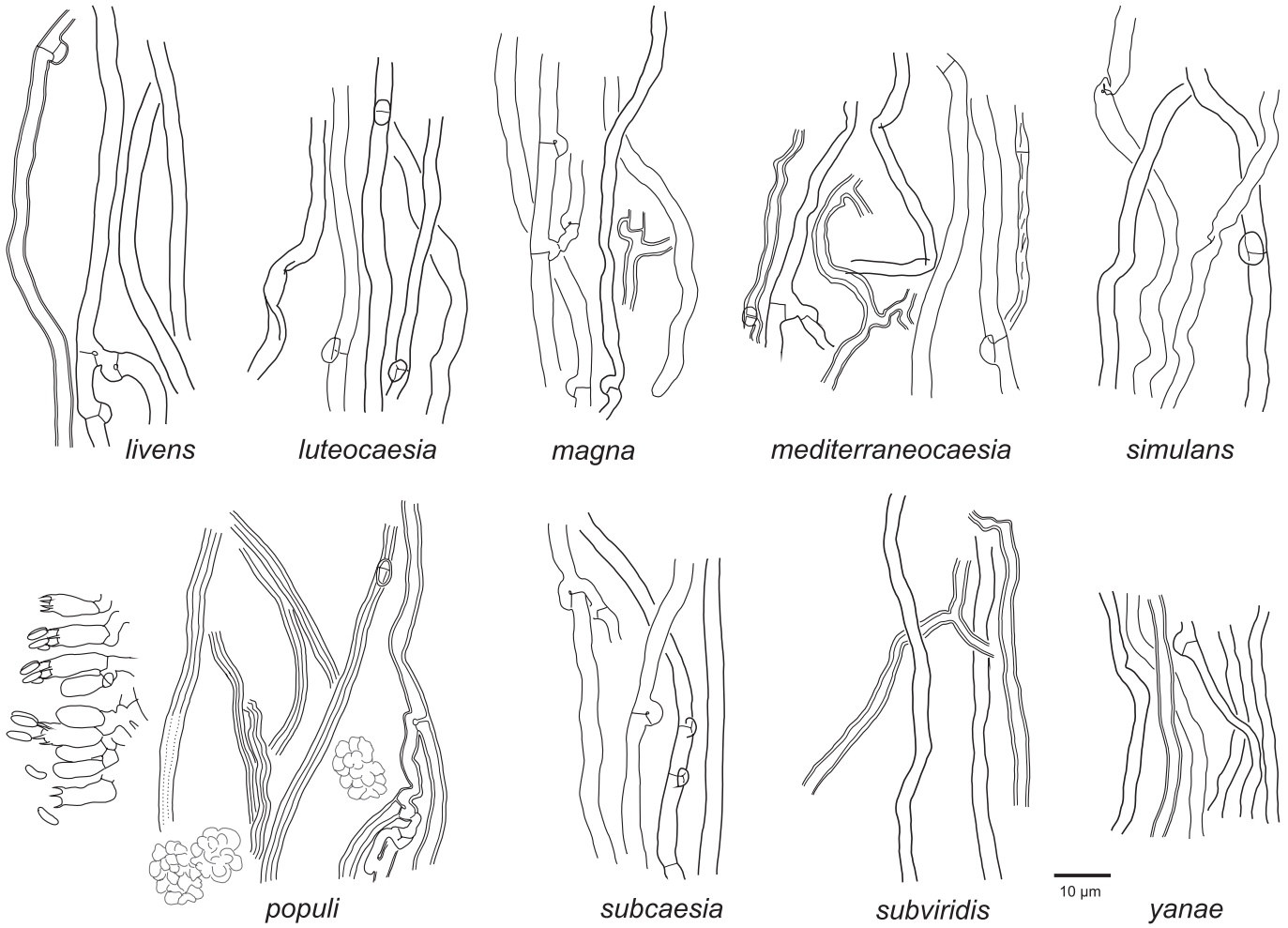

Fig. 7. (Continued).

Remarks: *Postia alni* belongs to the narrow-spored group within the *P. caesia* complex (Table 1). In North Europe it has been mixed with *P. populi*, and indeed some of the paratypes of *P. alni* actually represent *P. populi. Postia populi* differs in having tightly arranged, slightly narrower and more thick-walled tramal hyphae. Useful aids in identification are also the undulating margin of many *P. populi* specimens, absent in *P. alni*, and the deep brown cap color of mature specimens of *P. alni*. *Postia populi* occurs almost exclusively on *Populus tremula* in Europe, while *P. alni* prefers other deciduous trees. *Postia populi* is a northerly species in Europe; however, their distribution areas overlap, and the two species can be found growing side by side in the same forest.

Another very similar though much rarer species in Europe is *P. caesiosimulans*, which has been detected so far only a few times growing on *Corylus* in the hemiboreal zone. We have not been able to find consistent microscopical differences between the two species, although spore Q-values are generally higher in *P. alni*. Cap surface of *P. alni* is typically pubescent and turns brown when old, whereas cap of *P. caesiosimulans* is matt to glabrous and remains light-colored even when old. When young, specimens of the two species are indistinguishable morphologically.

Temperate *P. subcaesia* produces thicker and softer basidiocarps than *P. alni*, its hyphae are wider and tramal hyphae possess thinner walls. Papp (2014) provided an illustrated comparison of *P. alni* and *P. subcaesia*.

Postia arbuti Spirin, ***sp. nov.*** MycoBank MB823896. Figs 7, 8.

Holotype: **USA**, Washington: Jefferson Co., Port Townsend, Fort Worden, 48.13778° N 122.7673° W, alt. 74 m, *Arbutus menziesii*, 9 Oct. 2014, *Spirin 8327** (H 7008651).

Etymology: After the host, *Arbutus menziesii.*

Basidiocarps conchate, pendant to effused-reflexed, small, thin; margin sharp. Upper surface white to pale cream colored, almost glabrous to matt. Tubes white to cream-colored, in older and dry specimens with light bluish-greyish tint; *pores* 6–8 per mm, angular to sinuous. *Section*: context 0.5–2 mm thick, tubes 0.5–2 mm long. *Context hyphae* thin-walled but sclerified more distinctly than in other species, (2.3–)3.2–4.6(–5.4) µm. *Tramal hyphae* with slightly to distinctly thickened (up to 1 µm thick) walls, densely packed, (1.8–)2.4–3.1(4.0) µm. *Basidia* (9.7–)11–17(–19.2) × 3.3–4.2 µm. *Basidiospores* (4.0–)4.1–5.1(–5.2) × 1.0–1.2(–1.3) µm, L=4.56 µm, W=1.14 µm, Q=4.00.

Distribution and ecology: North America (North-West), temperate; so far collected only from fallen dry branches of madrona (*Arbutus menziesii*).

Specimens examined: **USA**, Washington, Jefferson Co., Port Townsend, Fort Worden, *Arbutus menziesii*, 9 Oct. 2014, *Spirin 8318* (H), *8327** (holotype, see above).

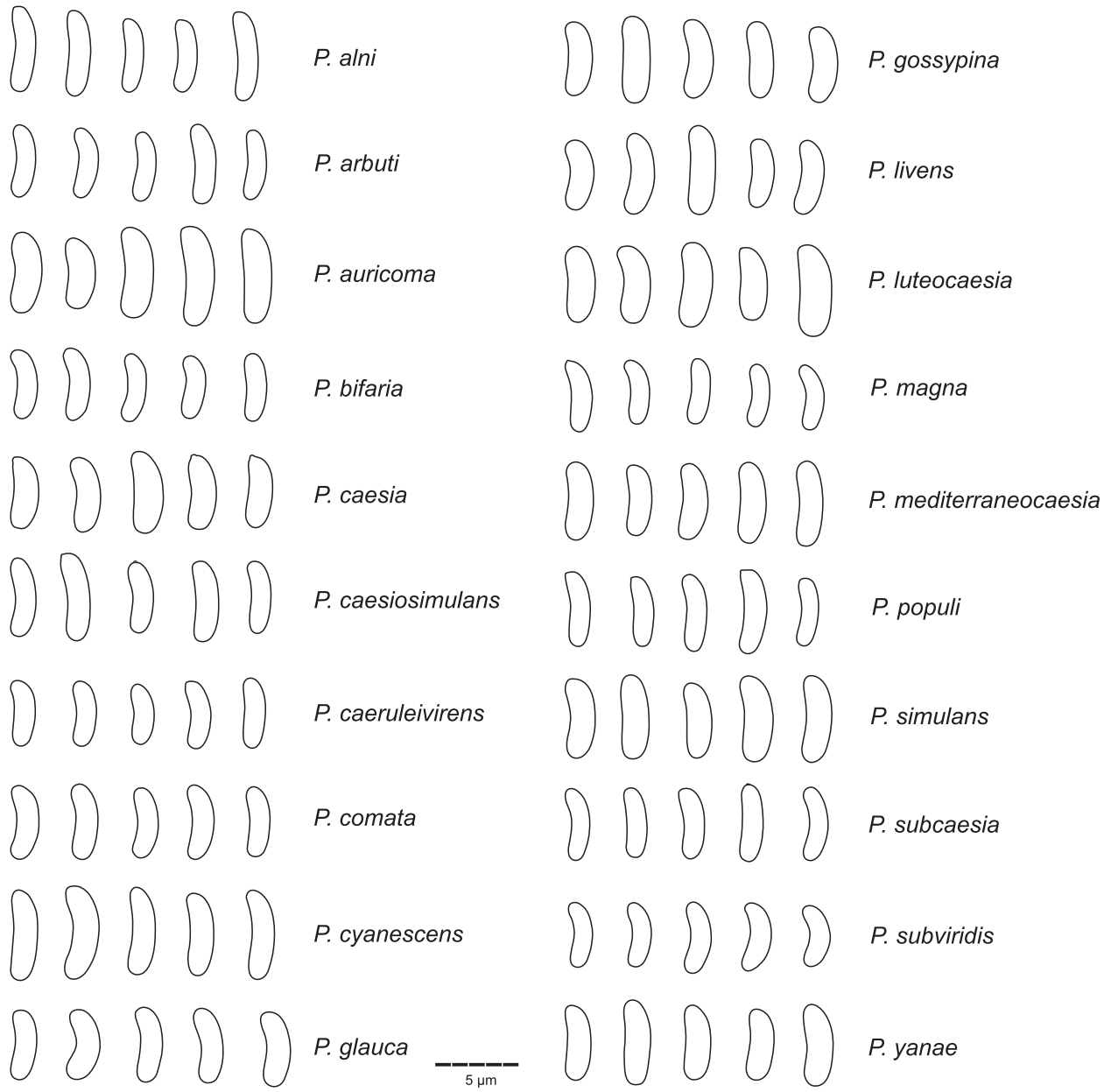

Fig. 8. Basidiospores of species in the *Postia caesia* complex. All drawings are from holo-, iso-, lecto- or neotypes, except *P. caesiosimulans* and *P. simulans* from epitypes, *P. coeruleivirens* from Spirin 5301 and *P. subcaesia* from Legon 14 Oct. 1995; in addition two rightmost spores were drawn from Miettinen 14828 for *P. livens* and from Rivoire 1903 for *P. mediterraneocaesia*.

Fig. 9. *Postia alni*. **A.** Basidiomes with the distinct brownish upper surface (Miettinen 15830). **B.** Paler basidiomes (Niemelä 9233).

Fig. 10. *Postia auricoma*. **A.** Niemelä 8315. **B.** Spirin 4598.

Remarks: *Postia arbuti* is morphologically virtually indistinguishable from *P. populi*. Genetically it is clearly a separate species. For now the only reliable non-molecular characters we can point to are the host species in *Ericaceae* and slightly smaller pores.

Postia auricoma Spirin & Niemelä, ***sp. nov.*** MycoBank MB823897. Figs 7, 8, 10.

Holotype: **Finland**, Pohjois-Savo, Enonkoski, Kolovesi National Park, Vaajasalo, 62.23° N 28.87° E, alt. 90 m, old-growth pine forest, rocky hilltop, on a fallen *Pinus sylvestris*, wood still hard, 26 Sep. 2006, *Niemelä 8310** (H 6014002).

Etymology: *Auricoma* (Lat.), with golden yellow head, refers to the yellow surface.

Basidiocarps conchate, small. Upper surface first while to cream colored, then yellowish to bright yellow, in older basidiocarps pale to dark ochraceous, matt to pubescent. Pore surface first bright yellow, quickly turning green when bruised, then with ochraceous tints; *pores* 4–6 per mm. *Section*: Context 2–3 mm thick, white to pale cream-colored, tubes 2–4 mm long, concolorous with or slightly paler than pore surface. *Context hyphae* slightly thick-walled, (3.8–)4.2–5.2(–6.2) µm. *Tramal hyphae* slightly to distinctly thick-walled (walls 0.2–1 µm thick), some hyphal segments with strongly amyloid (greenish in IKI) and cyanophilous content, (2.0–)3.1–4.0(–4.5) µm. *Basidia* (11.8–)14–20(–24) × 3.8–5.3 µm. *Basidiospores* (4.2–)4.4–5.6(–6.0)×(1.4–)1.5–1.8(–2.0) µm, L=5.04 µm, W=1.65 µm, Q=3.06.

Distribution and ecology: Eurasia, temperate to boreal, rare; fallen logs of *Pinus sylvestris* and *Larix gmelinii*.

Specimens examined: **Finland**, Etelä-Savo, Kouvola, Repovesi NP, 16 Sep. 2004, *Niemelä 7887* (H); Pohjois-Savo, Enonkoski, Kolovesi NP, *Pinus sylvestris,* 26 Sep. 2006, *Niemelä 8310* (holotype, see above), *8315* (H). **Poland**, Podlasie, Hajnówka, Białowieża, *P. sylvestris*, 4 Oct. 2010, *Niemelä 8760* (H). **Russia**, Irkutsk Reg., Irkutsk, Talzi, 20 Aug. 2000, *Kotiranta 17047* (H); Leningrad Reg., Boksitogorsk Dist., Vozhani, *P. sylvestris*, 22 Sep. 2011, *Spirin 4586** (H), Goryun, *P. sylvestris*, 23 Sep. 2011, *Spirin 4598, 4608* (H).

Remarks: *Postia auricoma* is a bright yellow species restricted to *Pinus sylvestris* and *Larix gmelinii* in the north temperate and boreal zones of Eurasia. Up to now, it has been mixed up with the morphologically similar *P. luteocaesia,* occurring in the Mediterranean on southern pine species. However, DNA data show that these species are not closely related. One more yellow-colored species in the *Postia caesia* complex is the South American *P. caesioflava*.

European collections derive from old-growth forests or their vicinity. The Siberian collection came from a young manage forest. Three of the five European known localities are well-preserved old-growth forests. The fourth locality (*Spirin 4586*) is a recent clear-cut but with old, rotten pine logs persisting from the time before the forest was logged, indicating that even that site had an old-growth forest history; also some indicator species of high conservation value such as *Crustoderma dryinum* were present in the site, and proportion of old-growth forests in the surrounding landscape was relatively high. The fifth (*Niemelä 7887*) was collected in a trivial site but in a national park where old-growth forest species are present. Thus it may be that *P. auricoma* is dependent on old-growth forests in Europe.

Postia bifaria Spirin, ***sp. nov.*** MycoBank MB823898. Figs 7, 8.

Holotype: **Russia**, Primorie: Krasnoarmeiskii Dist., Valinku, 48.2159° N 136.6902° E, alt. 1420 m, *Picea ajanensis*, 27 Aug. 2013, *Spirin 6402** (H 7008646).

Etymology: *Bifarius* (Lat.), two-faced, refers to color contrast between upper and lower surfaces of the basidiocarp.

Basidiocarps conchate, small or medium-sized. Upper surface first light grey, then with ochraceous hues, strigose. Tubes white to cream-colored, with light ochraceous tings in older and dry specimens; *pores* 6–8 per mm. *Section*: Context 2–4 mm thick, tubes 2–3 mm long. Context hyphae thin- to slightly (up to 0.5 µm) thick-walled, (3.0–)4.0–7.0(–8.0) µm. *Tramal hyphae* thin- or slightly thick-walled, easily collapsing, (2.0–)2.5–3.8(–4.2) µm. *Basidia* 9.8–14.8×3.4–4.5 µm. *Basidiospores* (3.6–)3.7–4.4(–5.2) × 1.0–1.2(–1.3) µm, L=4.1 µm, W=1.14 µm, Q=3.60.

Distribution and ecology: East Asia, cold temperate mountains, rather rare; fallen conifer logs (*Pinus, Picea, Larix*).

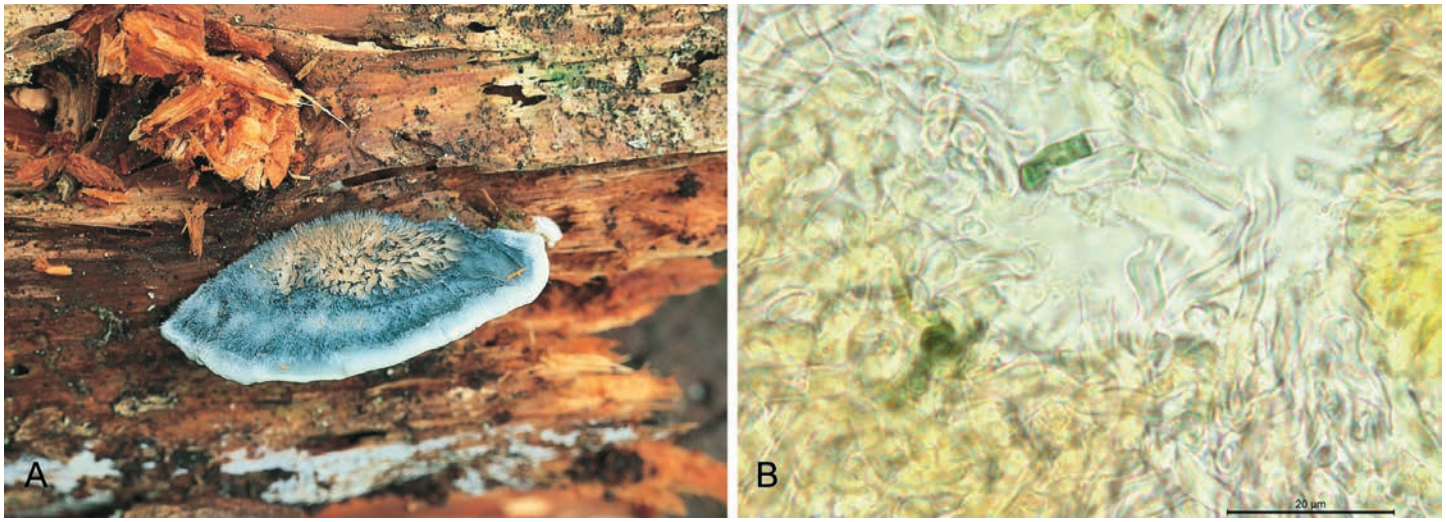

Fig. 11. *Postia caesia*. **A.** Basidiome *in situ* (Niemelä 7798). **B.** Microscopical slide of the neotype tube trama showing characteristic green hyphal segments in Melzer.

Specimens examined: **China**, Jilin, Antu, Changbaishan Nat. Res., *Pinus* sp., 5 Sep. 1993, *Dai 1059* (H). **Japan**, Hokkaido, Akan, 21 Sep. 1994, *Nuñez 602* (H, O). **Russia**, Khabarovsk Reg., Khabarovsk Dist., Bolshoi Khekhtsir Nat. Res., *Picea ajanensis*, 2 Sep. 2013, *Spirin 6532* (H); Malyi Niran, *P. ajanensis*, 5 Aug. 2012, *Spirin 4850** (H); 6 Aug. 2012, *Spirin 4987* (H), Malyi Kukachan, *Larix gmelinii*, 20 Aug. 2012, *Spirin 5461* (H); Primorie, Krasnoarmeiskii Dist., Valinku (holotype, see above).

Remarks: *Postia bifaria* is most similar to *P. glauca*. Both species inhabit coniferous hosts and sometimes occur side by side in the same habitats. Basidiocarps of *P. bifaria* discolor when bruised, as fresh specimens of *P. glauca* do. Smaller spore size (*P. bifaria*: 4.1 × 1.14 µm in average, *P. glauca*: 4.64 × 1.27 µm) is the best character for identification.

Postia caesia (Schrad.) P. Karst. *Revue mycol.*, Toulouse **3**(no. 9): 19. 1881. Figs 6–8, 11.
Basionym: *Boletus caesius* Schrad., *Spicilegium Florae Germaniae*: 167. 1794.

Neotype: **Germany**, Niedersachsen: Göttingen, Staufenberg, 51.58800° N 9.98217° E, alt. 390 m, *Picea abies*, 27 Sep. 2012, *Schuster** LY BR-6776, (selected here, MBT380822, duplicate H 7008647).
Synonyms: *Boletus coeruleus* A. Schumach., *Enumeratio Plantarum, in partibus Saellandiae septentrionalis et orientalis crescentium* **2**: 387. 1803.
Neotype: **Denmark**, Lolland, Krenkerup, Haveskov, 54.773° N 11.666° E, alt. 15 m, *Fagus sylvatica*, 4 Oct. 2007, *Schigel 5436* (H 7034978 selected here, MBT380823).
Polyporus caesiocoloratus Britzelmayr, *Bot. Centralblatt* **54**: 10. 1893.
Neotype: **Czech Republic**, Vysočina, Zbilidy, Panský les, alt. 650 m, *Picea abies*, 7 Aug. 1993 *Vampola**, Polyporales Exsiccati Čechoslovaciae 121, (H 7034977 selected here, MBT380824).

Basidiocarps conchate, often effused-reflexed, small or medium-sized, caps usually triquetrous (relatively thick at base). Upper surface first cream colored, matt, as a rule with bluish flecks, then plumbeous to bluish grey or greyish-brown, often distinctly pubescent. Tubes white to cream-colored, in older and dry specimens with light bluish-greyish tint, in vigorously growing specimens with bluish stains when bruised; *pores* 4–5(–6) per mm. *Section*: context 1–8 mm thick, tubes 2–6 mm thick. *Context hyphae* thin or only slightly thick-walled (walls 0.1–0.3 µm thick), (2.6–)3.7–5.2(–6.1) µm. *Tramal hyphae* thin- or only slightly thick-walled, clearly parallel, easily collapsing, (1.9–)2.8–3.6(–4.5) µm, hyphal segments with strongly amyloid (greenish-black in IKI) and cyanophilous/golden yellow content (in CB). *Basidia* (9.3–)10–15(–16.7) × 3.7–4.5 µm. *Basidiospores* (3.9–) 4.1–5.3(–6.0) × (1.2–)1.3–1.7(–1.9) µm, L=4.64 µm, W=1.48 µm, Q=3.13.

Distribution and ecology: Europe, common in temperate to hemiboreal, rare in south boreal zone; mostly *Picea* but also other coniferous (*Abies, Pinus*) and more rarely also deciduous trees (*Fagus, Salix, Syringa*).

Specimens examined: **Czech Republic**, Vysočina, Zbilidy (neotype of *P. caesiocoloratus*, see above). **Denmark**, Lolland, Krenkerup, *Fagus sylvatica*, 4 Oct. 2007, *Schigel 5436* (H, neotype of *Boletus coeruleus*). **Finland**, Uusimaa, Helsinki, Toukola, *Betula* sp., 25 Oct. 2008, *Miettinen 13610** (H); Uusimaa, Helsinki, Veräjämäki, *Pinus sylvestris*, 22 Sep. 2010, *Miettinen 14156.2** (H); Etelä-Häme, Hämeenlinna, Lammi, *Picea abies*, 17 Sep. 2015, *Miettinen 19424* (H); Satakunta, Ylöjärvi, Viljakkala, *P. abies*, 8 Sep. 2013, *Niemelä 9086** (H). **France**, Ardèche, St. Cirgues en Montagne, *P. abies*, 26 Sep. 2004, *Rivoire 2486* (LY, H); Aveyron, Salles-Curan, *P. abies*, 28 Oct. 2004, *Rivoire 2566* (LY, H); Haute Savoie, Sallanches, *Abies alba*, 13 Sep. 2010, *Rivoire 3859* (LY, H); Rhône, Courzieu, *P. abies*, 12 Sep. 2004, *Rivoire 2444* (LY, H); Rhône, Yzeron, Bois de Malval, *A. alba*, 20 Oct. 2006, *Rivoire 2985* (LY, H); Savoie, Doucy, *A. alba*, 19 Sep. 2005, *Rivoire 2756* (LY, H). **Germany**, Baden-Würtemberg, Freiburg, Triberg, 2 Sep. 1996, *Kytövuori 96–887* (H); Niedersachsen, Göttingen (neotype, see above). **Russia**, Leningrad Reg., Podporozhie Dist., Chogozero, *P. sylvestris*, 22 Sep. 2009, *Spirin 3307* (H); Vazhiny, *P. abies*, 29 Sep. 2010, *Spirin 3529* (H); Leningrad Reg., Volkhov Dist., Zagubie, *P. abies*, 18 Sep. 2011, *Spirin 4576* (H); Nizhny Novgorod Reg., Lukoyanov Dist., Razino, *P. abies*, 21 Aug. 2015, *Spirin 9787** (H); Panzelka, *P. sylvestris*, 3 Aug. 2004 *Spirin 2075* (H); Nizhny Novgorod Reg., Sharanga Dist., Kilemary Nat. Res., *Salix caprea*, 16 Aug. 2004, *Spirin 2102* (H), *P. abies*, 17 Aug. 2004, *Spirin 2143, 2157* (H); St. Petersburg, Volkovka, *Syringa vulgaris*, 16 Sep. 2003, *Spirin 1991* (H). **Slovakia**, Banská Bystrica, Dobroč, *A. alba*, Sep. 2009, *Vlasák 0909/23* (H, JV). **UK**, Scotland, South Lanarkshire, Cleghorn Glen, *P. sylvestris*, 6 Aug. 2010, *Miettinen 14133** (H).

Additional specimen examined: *Postia* aff. *caesia*. **Australia**, Tasmania, Mt Field NP, Russell Falls, 31 May 2003, *Gates* (H 7036111*, O 918359).

Remarks: Morphologically *P. caesia* is easy to recognize due to its hairy, often distinctly bluish basidiocarps and microscopically by its amyloid/cyanophilous hyphal segments in trama. It is most similar to *P. simulans*, which however does not produces pigmented, strongly amyloid hyphae in tube trama, and has longer basidiospores and smaller pores as a rule.

Boletus caesius was described based on material from Germany (Schrader 1794), and this name was subsequently sanctioned by Fries (1821). Fries's concept of the species and its current synonymy are unclear. No type material survives from Schrader or Fries. Fries (1821) referred directly to two illustrations and indirectly to a third one in his description:

1. Schaeffer's plate 124 (1763) depicting a pure white semistipitate polypore like *Postia floriformis* or *P. stiptica*;
2. Sowerby's plate 226 (1799), which is a pure white polypore like *Postia caesia* s.l., *P. lactea* or *P. stiptica*;
3. Fries cites Schrader (1794), who in turn cites Schaeffer's plate 314 (1774). The fungus in that figure is something strange, an unidentifiable, non-poroid species (possibly a young *Fomitopsis pinicola*). It is in direct conflict with Schrader's description stating that the species has a blue upper surface.

All of the above-mentioned illustrations are technically suitable as lectotypes, but are in conflict with Schrader's and Fries's description referring to a blue or bluish fungus and help in no way to anchor the name *Postia caesia* to modern species concepts. We therefore designate a sequenced neotype from *locus classicus* for *P. caesia*.

Fries (1821) considered *Boletus coeruleus* as a synonyms of *Polyporus caesius*, and this opinion persists in current literature and mycological databases. The protologue of *B. coeruleus* (Schumacher 1803) refers to a robust, bluish species occurring on oak in Denmark, and the two species that may come into question are *P. caesia* and *P. subcaesia*. In the name of nomenclatural stability we choose here a neotype for *B. coeruleus* among specimens of *P. caesia* - otherwise the name *B. coeruleus* might replace the much younger name *P. subcaesia*.

Identity of *Polyporus caesiocoloratus* described from Bavaria has been unclear. In the protologue, Britzelmayr (1893) stated that his species has basidiospores 5–6 × 0.75–1 µm. There are no polypore species known to us which possess spores of this size, and considering the quality of microscopes at the time these measurements are best viewed as an approximation. Otherwise the description by referring to bluish upper surface and steel blue pores fits only to the *Postia caesia* complex. Other hints in the description fit best *P. caesia sensu stricto* (brownish upper surface, pores turning blue, growth on spruce). No type material exists in M (Dagmar Triebel, pers. comm.), and Killermann (1922) did not report any specimens although he studied nearly all of Britzelmayr's material. To settle its identity we designate here a neotype from Czech Republic, in effect reducing *Polyporus caesiocoloratus* to a synonym of *Postia caesia*.

Postia caesiosimulans (G.F. Atk.) Spirin & Miettinen, ***comb. nov.*** MycoBank MB823899. Figs 6–8, 12.
Basionym: *Tyromyces caesiosimulans* G.F. Atk., *Ann. Mycol*. **6**: 61. 1908.

Lectotype: **USA**, New York, Tompkins Co., Ithaca, 'on rotten wood', 12 Oct. 1907, *Humphrey* (CUP A-022240 selected here, MBT380825).
Epitype: **USA**, New York, Essex Co., Catlin Lake, 44.06869° N 74.25795° W, alt. 560 m, *Fagus grandifolia* (?), 18 Sep. 2013, *Miettinen 16976.1** CUP (selected here, MBT380826, duplicate H 7008645).

Basidiocarps conchate, sometimes effused-reflexed, mostly thin, small or medium-sized. Upper surface first white to cream colored, matt, then greyish to pale ochraceous, very rarely with bluish flecks or faint zones, more or less glabrous. Tubes white to cream-colored, in older and dry specimens with light bluish-greyish tint; *pores* 5–7 per mm. *Section*: Context 1–3 mm thick, tubes 1–3 mm long. *Context hyphae* thin- to slightly thick-walled (rarely distinctly thick-walled), often sclerified, (2.2–)3.4–5.2(–6.4) µm. *Tramal hyphae* for the most part slightly to distinctly thick-walled (walls up to 1 µm thick), mainly interwoven, sometimes partly glued together, (2.0–)2.9–3.8(–4.8) µm. *Basidia* (9–)10.5–15.5(–19) × 3.2–5.2(–6.5) µm. *Basidiospores* (4.1–)4.2–5.5(–7.0) × (1.0–)1.1–1.4(–1.6) µm, L=4.80 µm, W=1.22 µm, Q=3.93.

Fig. 12. *Postia caesiosimulans*, Miettinen 15489.1, basidiomes from above (**A**) and below (**B**).

Distribution and ecology: Holarctic, temperate, common in North America; on fallen logs and branches of deciduous trees (in Europe preferably on *Corylus*, in North America on *Fagus*), in East Asia and North America also on *Abies* and *Picea* spp.

Specimens examined: **Finland**, Ahvenanmaa, Finström, Mangelbo Almskogen Nat. Res., *C. avellana*, 13 Oct. 2006, *Schigel 4798** (H). **Russia**, Khabarovsk Reg., Solnechnyi Dist., Suluk-Makit, *Abies nephrolepis*, 17 Aug. 2011, *Spirin 4199** (H); Gorin, *A. nephrolepis*, 13 Aug. 2011, *Spirin 4125** (H); Nizhny Novgorod Reg., Lukoyanov Dist., Sanki, *Corylus avellana*, 18 Aug. 2006, *Spirin 2610** (H). **USA**, Massachusetts, Worcester, Hadwen Arboretum, hardwood (branch), 20 Oct. 2013, *Miettinen 17340.1* (H); Minnesota, St. Louis Co., Independence, *Populus tremuloides*, 27 Sep. 2010, *Lindner 2010–062* (CFMR); 29 Sep. 2010, *Lindner 2010–081* (CFMR); Melrude, *P. tremuloides*, 29 Sep. 2010, *Lindner 2010–106, 2010–111* (CFMR); Minnesota, Orr, *P. tremuloides*, 28 Sep. 2010, *Lindner 2010–136* (CFMR); New York, Essex Co., Catlin Lake, *Acer* (?), 14 Aug. 2012, *Miettinen 15489.1* (H), *Fagus grandifolia* (?), 18 Sep. 2013, *Miettinen 16976.1** (CUP epitype, H isoepitype), New York, Essex Co., Wolf Lake, *F. grandifolia*, 20 Sep. 2013, *Miettinen 17075** (H); New York, Tompkins Co., Ithaca (lectotype, see above); North Carolina, Swain Co., Bryson City, *Picea* sp., Sep. 2005, *Vlasák 0509/24* (H, JV); Washington, Pend Oreille Co., Gypsy Meadows, *Abies lasiocarpa*, 17 Oct. 2014, *Spirin 8717** (H); *Picea/Abies*, 17 Oct. 2014, *Miettinen 18924* (H); *Picea* sp., 17 Oct. 2014, *Miettinen 18927* (H); Washington, Thurston Co., Nisqually Land Trust, *Abies procera*, 11 Oct. 2014, *Miettinen 18663*, 18665** (H).

Remarks: This species was introduced primarily on the basis of its globose basidiospores (Atkinson 1908). However, as Lowe (1974) stated, these spores belong to *Tremella polyporina*. We disagree with Lowe's opinion that alien spores in Atkinson's description are a reason to reject *T. caesiosimulans*. The protologue certainly refers to the polypore, not to a species of *Tremella*, and we select the lectotype here according to ICN Art. 9.14.

Mature basidiocarps with an ochraceous, glabrous upper surface, collected on deciduous hosts in Northeastern United States, are rather easy to recognize. However, in other cases morphological identification may be difficult, particularly without material of comparison at hand. Young, small-sized or half-resupinate specimens of *P. caesiosimulans* can be mistaken for *P. populi.* Both species have similar basidiospores, and hence attention should be paid to tramal hyphae: they are not so thick-walled and densely arranged in *P. caesiosimulans* as in *P. populi*. When small-sized, *P. livens* can be quite similar as well. Pubescent upper surface and in relative terms thick context should aid in correct identification. When well-developed, basidiocarps of *P. livens* are much larger in size than those of *P. caesiosimulans*.

In Europe, *P. caesiosimulans* can be mixed up with *P. alni* as well (see discussion under the latter name). *Postia simulans* is yet another potential look-alike, but wider basidiospores and collapsing, thin-walled tramal hyphae tell it apart.

Our picture of variation within and around *P. caesiosimulans* is still incomplete. Collections from Northwestern USA have wider basidiospores (W=1.46 µm) than rest of the material; and they have been excluded from the statistics. These basidiospores are similar to those of *P. simulans* (Suppl. 2). Minor intraspecific variation in ITS and *tef1* suggests that the Northwest population on conifers might be differentiated from the rest of *P. caesiosimulans*, but more extensive sampling and further markers would be needed to ascertain this. Also excluded from the description above are *P. caesiosimulans* material from Minnesota from the study by Brazee *et al.* (2012). These specimens are very similar to *Postia populi*, with narrower than average spores and thick-walled tramal hyphae; yet the ITS data places them in the *P. caesiosimulans* complex (no *tef1* data available).

Postia coeruleivirens (Corner) V. Papp, *Mycotaxon* **129**: 411. 2015. [2014]. Figs 7, 8.
Basionym: *Tyromyces coeruleivirens* Corner, Beih. *Nova Hedwigia* **96**: 163. 1989.

Basidiocarps conchate, small or medium-sized. Upper surface first cream-colored, then pale ochraceous, sometimes with bluish flecks or indistinct; pubescent. Pore surface white to cream-colored, developing a bluish-greyish tint when aging; *pores* 6–8 per mm. *Section*: Context 3–5 mm thick, tubes 3–4 mm long. *Context hyphae* thin-walled to slightly thick-walled, sometimes sclerified, (3.0–)3.6–6.0(–7.8) µm, with frequent finger-like outgrowths. *Tramal hyphae* thin- or moderately thick-walled (walls up to 0.8 µm thick), (1.8–)2.4–3.4(–4.0) µm. *Basidia* 8.8–13.5 × 3.3–4.3 µm. *Basidiospores* (3.6–)3.8–4.8(–5.2) × 1.0–1.3 µm, L=4.23 µm, W=1.16 µm, Q=3.64.

Distribution and ecology: East and Southeast Asia, warm temperate to tropical; fallen logs and branches of deciduous trees (*Populus, Tilia,* and *Ulmus* in East Asia).

Specimens examined: **China**, Jilin, Antu, Baoma, *Ulmus* sp., 7 Sep. 1993, *Dai 1134* (H); Wangqing, Lanjia, *Tilia* sp., 11 Sep. 1993, *Dai 1198* (H). **Indonesia**, Bali, Bedugul, Mt. Tapak, 24 Jul. 2007, *Miettinen 12214** (H). **Russia**, Khabarovsk Reg., Khabarovsk Dist., Ulika, *Tilia amurensis*, 15 Aug. 2012, *Spirin 5301** (H); Solnechnyi Dist., Elga, *Populus maximowiczii*, 22 Aug. 2011, *Spirin 4245** (H); Primorie, Ternei Dist., Maisa, *T. amurensis*, 15 Sep. 1990, *Parmasto* (H, O ex TAAM 151125).

Remarks: Corner (1989) described *Tyromyces coeruleivirens* and *T. amyloideus* from Borneo, and both are considered members of the *P. caesia* group (Hattori 2002). Appropriate combinations to *Postia* were made by Papp (2014). The three Asian collections we have studied possess mutually identical ITS sequences and are tentatively labelled as *P. coeruleivirens* sensu auct. Two of them, from Russian Far East, fit well with the protologue of *T. coeruleivirens*, and the description above is based on these Northeast Asian specimens. The third specimen collected in Bali (*Miettinen 12214*) is morphologically different, and is excluded from the description above. It has the smallest basidiospores in the whole group, L=3.69, W=1.00 (Suppl. 2). More material (especially from the type locality) and *tef*1 sequences are needed to settle proper identities in this group.

Postia coeruleivirens, as understood here, is morphologically most similar to *P. bifaria*, and these species can be separated by their preferred host (angiosperms versus gymnosperms). Tramal hyphae in *P. coeruleivirens* are predominately thick-walled (up to 0.8 µm) while in *P. bifaria* they are almost thin-walled (<0.5µm).

Postia comata Miettinen, ***sp. nov.*** MycoBank MB823900. Figs 7, 8.

Holotype: **USA**, Massachusetts, Petersham, Tom Swamp, 42.5121° N 72.2112° E, alt. 230 m, *Tsuga canadensis*, 19 Sep. 2011, *Miettinen 14755.1** (H 7005691).

Etymology: *Comatus* (Lat.), hairy.

Basidiocarps conchate or effused-reflexed, medium-sized. Upper surface cream colored to pale ochraceous, pubescent. Tubes cream-colored, in older specimens with bluish-greyish tint; *pores* 4–6 per mm, angular to sinuous. *Section*: Context 5–10 mm thick, tubes 3–6 mm long. *Context hyphae* slightly thick-walled, sclerified, (3.2–)4.2–5.3(–7.1) µm. *Tramal hyphae* predominantly thick-walled (walls up to 0.8–1 µm thick), (2.2–)2.8–3.8(–4.5) µm. *Basidia* 8.8–14.2(–15.2)×(3.4–)3.7–4.9 µm. *Basidiospores* (3.8–)4.1–4.9(–5.1) × 1.1–1.3 µm, L=4.36 µm, W=1.21 µm, Q=3.62.

Distribution and ecology: Northeastern United States, temperate, evidently uncommon; fallen logs and branches of conifers (*Tsuga canadensis*) and deciduous trees (*Acer*).

Specimens examined: **USA**, Massachusetts, Worcester Co., Petersham (holotype, see above); New York, Essex Co., Harris Lake, *Acer* sp., 23 Sep. 2013, *Miettinen 17180* (H); New York, Warren Co., Pack Demonstration Forest, *Tsuga canadensis*, 24 Sep. 2013, *Miettinen 17197* (H).

Remarks: *Postia comata* is morphologically similar to *P. livens* but it differs in having mostly thick-walled tramal hyphae and slightly smaller basidiospores. Its closest relative is East Asian *P. bifaria*, which has smaller pores and spores, and collapsing, thing-walled tramal hyphae (Table 3).

Postia cyanescens Miettinen, ***sp. nov.*** MycoBank MB823901. Figs 7, 8, 13A.

Holotype: **Finland**, Uusimaa, Helsinki, Veräjämäki, 60.222° N 24.973° E, alt. 40 m, on a cut stump of *Picea abies*, 19 Oct. 2008, *Miettinen 13602* (H 6014001, culture HAMBI/FBCC 2205*).

Etymology: *Cyanescens* (Lat.), turning bluish.

Basidiocarps conchate to flabelliform, rarely effused-reflexed, thin. Upper surface first white to cream colored, matt, then pale ochraceous, rarely with bluish-greyish hues. Tubes white to cream-colored, in older and dry specimens with light bluish-greyish tint, unchanged when bruised; *pores* (3–)5–6 per mm. *Section*: Context 0.5–3 mm thick, tubes 1–2 mm long. *Context hyphae* thin- to slightly thick-walled, sclerified, (2.6–)4.1–5.2(–6.8) µm. *Tramal hyphae* thin-walled or with distinctly thickened (0.2–0.8 µm thick) walls, more or less parallel, (2.0–)2.9–3.7(–4.4) µm. *Basidia* 11.4–19.8 × 3.7–5.4 µm. *Basidiospores* (4.2–)4.7–6.1(–6.8) × (1.0–)1.1–1.6(–1.9) µm, L=5.22 µm, W=1.33 µm, Q=3.92.

Distribution and ecology: Europe, temperate to boreal, rather common on fallen conifer logs. The Spanish records are from mountains and derive from *Abies alba* (?) and *A. pinsapo*. All other records derive from *Picea abies*, except one Finnish record on *Pinus sylvestris.*

Specimens examined: **Estonia**, Jõgevamaa, Umbusi, *Picea abies*, 19 Sep. 1993, *Kinnunen 35* (H); Tartumaa, Võnnu, Järvselja, *P. abies*, 6 Oct. 2001, *Niemelä 7224* (H). **Finland**, Uusimaa, Helsinki, Lammassaari, *P. abies*, 15 Nov. 2010, *Miettinen 14425** (H); Uusimaa, Helsinki, Veräjämäki (holotype, see above); Uusimaa, Kirkkonummi, *P. abies*, 24 Oct. 2012, *Miettinen 15815.1* (H); Satakunta, Ylöjärvi, Viljakkala, *P. abies*, 2 Oct. 2011, *Niemelä 8844** (H); Etelä-Häme, Padasjoki, Koivukannonsuo, *P. abies*, 6 Jul. 2003, *Miettinen 7511* (H); Pohjois-Karjala, Ilomantsi, Kaitavaara, *P. abies*, 15 Sep. 2003, *Penttilä 14560** (H); Pohjois-Karjala, Ilomantsi, Petkeljärvi, *Pinus sylvestris*, 6 Oct. 2012, *Niemelä 8998** (H); Etelä-Savo, Lappeenranta, Ihalinen, *P. abies* (?), 23 Sep. 2003, *Salo 9196** (H); Satakunta, Ylöjärvi, Viljakkala, *P. abies*, 2 Oct. 2011, *Niemelä 8844** (H); Kittilän Lappi, Kittilä, Linkukero, *P. abies*, 22 Sep. 2001, *Niemelä 7135* (H). **France**, Drôme, St. Agnan en Vercors, *P. abies*, 6 Oct. 2001, *Rivoire 2051* (LY, H), St. Martin en Vercors, *P. abies*, 6 Oct. 2001, *Rivoire 2052* (LY, H). **Poland**, Podlasie, Hajnówka, Białowieża, *P. abies*, 14 Oct. 2008, *Kinnunen 5087** (H). **Russia**, Leningrad Reg., Podporozhie Dist., Tokari, *P. abies*, 28 Sep. 2007, *Spirin 2752, 2756** (H); Nizhny Novgorod Reg., Sharanga Dist., Kilemary Nat. Res., *P. abies*, 26 Sep. 1999, *Spirin* (LE 211334). **Spain**, Huesca, Hecho, *Abies* sp., 10 Nov. 1977, *Ryvarden 15129* (O, H); Málaga, Estepona, Los Reales de Sierra Bermeja, *Abies pinsapo*, 21 Nov. 2012, *Miettinen 15919.2** (H); 23 Nov. 2012, *Miettinen 15988* (H); *15989** (H 7008640). **Sweden**, Halland, Halmstad, Biskopstorp Nat. Res., *P. abies*, 28 Sep. 2012, *Schigel 7436* (H).

Remarks: In most cases, *P. cyanescens* is easy to recognize. Thin, flabelliform basidiocarps on spruce and long, narrow basidiospores clearly separate it from *P. caesia*. *Postia simulans* has on average wider spores and its tramal hyphae are more loosely arranged and easily collapsing.

The Spanish specimens from Málaga have slightly larger spores than typical North European material and have more

Fig. 13. A. *Postia cyanescens* (Niemelä 8844). **B.** *Postia glauca* (Miettinen 10567).

interwoven hyphae. Their ITS and *tef1* sequences are identical with those of North European material, however.

Phylogenetic analysis utilizing ITS and *tef1* are not able to separate *P. cyanescens* specimens unequivocally to a separate clade from *P. caesiosimulans* (Fig. 3–5). European material of the two species differs ecologically (angiosperms vs. gymnosperms) as well as morphologically (basidiome shape, arrangement of tramal hyphae, basidial length, spore size). We have utilized a wide, pragmatic concept of *P. caesiosimulans* here, and American material from conifers is morphologically closer, yet still separable, from *P. cyanescens*. Our extensive *P. cyanescens* material has uniform and unique ITS- and *tef1*-sequences, so separation of the two is justified even in the face of only limited phylogenetic support. Adding further informative markers to phylogenetic analyses would no doubt increase the phylogenetic support for *P. cyanescens*.

Postia glauca Spirin & Miettinen, ***sp. nov.*** MycoBank MB823902. Figs 7, 8, 13B.

Holotype: **Russia**, Khabarovsk Reg.: Khabarovsk Dist., Hologu, 50.0677° N 134.4297° E, alt. 449 m, *Abies nephrolepis*, 17 Aug. 2012, *Spirin 5317** (H 7008648).

Etymology: *Glaucus* (Lat.), greyish bluish.

Basidiocarps conchate, small, thin to rather thick. Upper surface first greyish, matt to strigose, as a rule with bluish flecks, then plumbeous to bluish grey or greyish-brown. Tubes white to cream-colored, with light bluish-greyish tint in older and dry specimens, fresh specimens discoloring bluish when bruised; *pores* 5–8 per mm. *Section*: Context 1–3 mm thick, tubes 2–4 mm long. *Context hyphae* thin- to slightly thick-walled, some hyphae sclerified and appearing nearly solid, (2.5–)3.4–5.1(–6.3) µm. *Tramal hyphae* thin- or slightly thick-walled, (1.8–)2.6–3.3(–3.8) µm, amyloid (greenish in IKI) and cyanophilous hyphal segments common. *Basidia* 9.8–14.8(–17.0) × 3.1–4.3 µm. *Basidiospores* (4.0–)4.1–5.4(–6.2) × 1.1–1.5(–1.6) µm, L=4.64 µm, W=1.27 µm, Q=3.64.

Distribution and ecology: East Asia, cold temperate mountains, common; fallen conifer logs (*Abies, Picea*).

Specimens examined: **China**, Jilin, Antu Co., Changbaishan Nat. Res., *Picea* sp., 27 Aug. 2005, *Miettinen 10567** (H). **Russia**, Khabarovsk Reg., Khabarovsk Dist., Bolshoi Khekhtsir Nat. Res., *Picea ajanensis*, 2–3 Sep. 2013, *Spirin 6548, 6580** (H); Hologu (holotype, see above), Malyi Kukachan, *P. ajanensis*, 19 Aug. 2012, *Spirin 5424* (H); Levyi Ulun, *P. ajanensis*, 24 Aug. 2012, *Spirin 5577** (H); Solnechnyi Dist., Igdomi, *P. ajanensis*, 3 Sep. 2016, *Spirin 10884, 10892* (H); Verkhnebureinskii Dist., Dublikan, *P. ajanensis*, 20 Aug. 2014, *Spirin 7627* (H).

Remarks: *Postia glauca* is a common species, occurring mainly in spruce-dominated forests. It is most similar to *P. bifaria*, but their spore mean values differ a little (4.64 × 1.27 µm and 4.1 × 1.14 µm respectively), and only the specimens of *P. glauca* stain blue when bruised.

Postia gossypina (Moug. & Lév.) Spirin & Rivoire, ***comb. nov.*** MycoBank MB823903. Figs 7, 8.
Basionym: *Polyporus gossypinus* Moug. & Lév., *Ann. Sci. Nat. Bot*. **9**: 124. 1848.

Lectotype: **France**, "Vosges", fallen log, *Mougeot* (PC, isolectotype BPI US0209624, selected by Ryvarden 1981: 181, studied).

Basidiocarps conchate to flabelliform, small to medium-sized, thin. Upper surface first cream colored, then light grey, matt. Tubes cream-colored to bluish-greyish; *pores* 4–6 per mm. *Section*: Context 1–2 mm thick, tubes 2–3 mm long. *Context hyphae* thin- or slightly thick-walled, no sclerified hyphae seen, (2.9–)3.6–4.8(–5.4) µm. *Tramal hyphae* thin- to slightly thick-walled, (1.9–)2.3–3.0(–3.8) µm. *Basidia* 8.7–16.8 × 3.8–5.0 µm *Basidiospores* (4.0–)4.1–5.1(–5.2) × 1.2–1.7 µm, L=4.47 µm, W=1.44 µm, Q=3.11.

Distribution and ecology: Europe, temperate; logs of an unidentified fallen tree and *Cedrus atlantica*.

Specimens examined: **France**, "Vosges" (lectotype, see above); Vaucluse, Bonnieux, Cédraie du Luberon, *Cedrus atlantica*, 13 Oct. 2002, *Rivoire 6658** (LY, H).

Remarks: Our concept of *P. gossypina* derives from the lectotype and one recent collection from France. We did not find essential morphological differences between them, and therefore we consider these specimens conspecific. Only *tef1* sequence has been successfully produced from the newly collected specimen, and it places *P. gossypina* in the close vicinity of the East Asian *P. glauca*. The latter species possesses smaller pores (5–8 vs. 4–6 per mm) and on average narrower basidiospores (W=1.27 µm vs. W=1.44 µm), as well as partly amyloid tramal hyphae. Despite *tef1* similarity (3 bp) these morphological differences are significant enough to treat them as separate species.

Postia simulans is morphologically very similar, but has usually longer spores and wider tramal hyphae. More material is needed to confirm differences between these species, but after comparing them side by side, we think it is likely they are not conspecific and treat them as separate species. *Tef1* difference between our sequenced specimen and *P. simulans* material is very clear, over 50 bp.

Postia livens Miettinen & Vlasák, ***sp. nov.*** MycoBank MB823904. Figs 1, 6, 7, 14.

Holotype: **USA**, New York, Essex Co., Harris Lake, 43.9790° N 74.1472° W, alt. 500 m, *Larix laricina* (?), 23 Sep. 2013, *Miettinen 17177** (H 7008642, isotype BPI).

Etymology: *Livens* (Lat.), greyish bluish.

Basidiocarps conchate, small to medium-sized, rarely large, thin to thick. Upper surface first cream colored, matt to distinctly pubescent, then plumbeous to bluish grey to ochraceous, often with bluish tints. Tubes cream-colored, in older and dry specimens with light bluish-greyish tint; *pores* 4–6 per mm. *Section*: Context 1–15 mm thick, tubes 1–6 mm long. *Context hyphae* slightly thick-walled, often sclerified (2.5–)3.7–5.3(–7.2) µm. *Tramal hyphae* thin- to moderately thick-walled (walls up to 0.5–0.8 µm thick), (1.9–)2.9–4.0(–4.7) µm. *Basidia* 9.3–14.3(–15.7) × 4.0–5.3 µm. *Basidiospores* 4.1–5.7(–7.0) × 1.1–1.5(–1.7) µm, L=4.78 µm, W=1.28 µm, Q=3.74, variable within and between specimens.

Fig. 14. *Postia livens.* **A.** Miettinen 16714. **B.** Holotype.

Distribution and ecology: North America (from East coast to Rocky Mts.), temperate, common; fallen logs and branches of conifers (*Abies, Picea, Larix, Tsuga*) and deciduous trees (*Acer, Betula, Fagus*).

Specimens examined: **Canada**, Québec, Bas-Saint-Laurent, Notre-Dame-du-Portage, *Betula papyrifera*, 21 Aug. 2004, *Pieri* (LY-BR 2468); Québec, Estrie: Sherbrooke, *Acer rubrum*, 4 Sep. 2004, *Pieri* (LY-BR 2466, H). **USA**, Maine, Portland, Crescent Beech State Park, *Abies* sp., Sep. 2008, *Vlasák 0809/118* (H, JV); Massachusetts, Worcester Co., Worcester, deciduous tree (?), 20 Sep. 2011, *Miettinen 14775** (H); 8 Oct. 2011, *Miettinen 14878* (H); Holden, *Tsuga canadensis* (?), 26 Sep. 2011, *Miettinen 14828** (H); 6 Sep. 2013, *Miettinen 16816, 16819*, *16825* (H); *T. canadensis*, 14 Apr. 2013, *Miettinen 16056** (H); New York, Essex Co., Catlin Lake, rotten wood, 14 Aug. 2012, *Ortiz-Santana* (H); Harris Lake (holotype, see above); Arbutus Lake, *T. canadensis*, 16 Sep. 2013, *Miettinen 16899* (H); Minnesota, Waseca Co., Janesville, hardwood, 21 Aug. 2013, *Miettinen 16714** (H); North Carolina, Buncombe Co., Blue Ridge Assembly, *Tsuga* (?), 24 Sep. 2015, *Miettinen 19439** (H); *Betula* sp., 24 Sep. 2015, *Miettinen 19446* (H); North Carolina, Swain Co., Clingmans Dome, *Abies fraseri* (?), 1 Oct. 2015, *Miettinen 19644* (H); Pennsylvania, Montgomery Co., Schwenksville, Gossenhoppen Creek, hardwood, Sep. 2008, *Vlasák 0809/121* (H, JV); Pennsylvania, Wayne Co., Tobyhanna State Park, *Fagus grandifolia*, Sep. 2010, *Vlasák 1009/57** (H, JV); Tennessee, Cocke Co., Cosby Creek, *T. canadensis* (?), 2 Oct. 2015, *Miettinen 19666.2** (H); Washington, Pend Oreille Co., Gypsy Meadows, *Picea engelmannii*, 17 Oct. 2014, *Spirin 8728** (H).

Remarks: *Postia livens* is the most common representative of the *P. caesia* complex in North America. Its fully developed basidiocarps can be identified easily by their size and hairy upper surface. Identification of younger or dwarf-sized collections is more difficult, however. *Postia caesiosimulans* and *P. populi* have in general thicker-walled tramal hyphae, narrower context hyphae and spores, and their pores are slightly smaller. *Postia simulans* produces in average longer and wider basidiospores, and its tramal hyphae collapse commonly unlike those of *P. livens*.

One specimen from Washington (*Spirin 8728*) is excluded from the description above, having a slightly deviating ITS (3 bp) and larger spores than typical *P. livens*. Even so the intraspecific morphological variation (spore size, hyphal wall thickness) is among the highest in *Postia caesia* complex.

Postia luteocaesia (A. David) Jülich, *Persoonia* **11**(4): 423. 1982. Figs 7, 8.
Basionym: *Spongiporus luteocaesius* A. David, *Bull. Soc. Linn. Lyon* **49**: 29. 1980.

Holotype: **France**, Var, Massif des Maures, *Pinus* sp., 26 Dec. 1970, *David 929* (LY, studied).

Basidiocarps conchate, medium-sized. Upper surface first cream colored, then yellowish to bright yellow, pale to dark ochraceous on aging, often with brownish flecks, matt to pubescent. Pore surface first bright yellow, then with ochraceous tints; *pores* 3–5 per mm. *Section*: Context 2–5 mm thick, white to pale cream-colored, tubes 2–5 mm long. *Context hyphae* thin-walled, mostly sclerified, often with several, randomly oriented side branches, (3.3–)4.1–5.8(–6.7) µm. *Tramal hyphae* thin- to moderately thick-walled (walls up to 0.8 µm thick), partly glued together, (2.1–)2.7–3.2(–4.2) µm, partly with amyloid (greenish in IKI) and strongly cyanophilous content. *Basidia* 11.1–16.2(–18.2) × 4.0–5.2 µm. *Basidiospores* (4.2–)4.3–6.1(–7.1) × (1.4–)1.5–1.9(–2.0) µm, L=5.06 µm, W=1.68 µm, Q=3.02.

Distribution and ecology: Europe, Mediterranean France, rare; so far only on *Pinus halepensis* and *Pinus* sp.

Specimens examined: **France**, Var, Massif des Maures (holotype, see above); Port-Cros, *Pinus halepensis*, 12 Dec. 1992, *Rivoire 733* (LY, H); Porquerolles, *P. halepensis*, 13 Nov. 2004, *Rivoire 2605** (LY, H).

Remarks: Collections from *Pinus sylvestris* in Central and North Europe earlier addressed to *P. luteocaesia* belong to *P. auricoma*. The two species are very similar morphologically and grow on pine. The only constant morphological difference we can point to are the tramal hyphae, which are wider in *P. auricoma* (Table 1). According to current knowledge, their distribution areas do not overlap. Phylogenetically the closest relative of *P. luteocaesia* is *P. simulans*, a species without yellow coloration.

Postia magna Miettinen, ***sp. nov.*** MycoBank MB823905. Figs 7, 8, 15.

Holotype: **China**, Jilin, Antu Co., Changbaishan Nat. Res., Erdao Bai He, 42.3994° N 128.1015° E, alt. 730 m, *Populus koreana*, 28 Aug. 2005, *Miettinen 10634** (H 7008643, isotype BJFC).

Fig. 15. *Postia magna* holotype photographed in the field.

Etymology: *Magnus* (Lat.), big.

Basidiocarps conchate, medium-sized (7×4 cm), thick, margin sharp. Upper surface white, drying cream colored to light greyish and ochraceous, distinctly pubescent. Tubes white, drying ochraceous with bluish tint, *pores* (3–)4–5 per mm. *Section*: Context 3–10 mm thick, white, tubes 3–6 mm long. *Context hyphae* thin-walled (to slightly thick-walled), (3.4–)4.2–6.0(–6.6) µm. *Tramal hyphae* slightly thick-walled, regularly branched, (1.5–)2.2–3.3(–3.8) µm. *Basidia* 10–12.5×3.2–4 µm. *Basidiospores* 3.6–4.4(–4.5) × 1.0–1.2 µm, L=3.97 µm, W=1.13 µm, Q=3.51.

Distribution and ecology: Temperate China and South Korea, so far known from hardwoods only.

Specimen examined: *Postia magna*. **China**, Jilin, Antu (holotype, see above).

Remarks: This species reminds *P. subcaesia* and *P. livens* with its large, hairy basidiocarps. Microscopically *P. magna* seems to differ from them by its smaller spores. As far as we know their distribution areas do not overlap. Although we have examined only one collection from Northeast China, available ITS sequences indicate that the species is widespread in China and Korea (Shen *et al.* 2014, Kim *et al.* 2015, Jang *et al.* 2016).

Postia mediterraneocaesia M. Pieri & B. Rivoire, *Bull. Semestriel Féd. Assoc. Mycol. Méditerranéennes* **28**: 34. 2005. Figs 7, 8.

Holotype: **France**, Bouches du Rhône, St. Rémy de Provence, *Pinus halepensis*, 11 Nov. 2000, *Pieri & Rivoire 1946* (LY, studied).

Basidiocarps effused-reflexed or resupinate with detaching or adnate margin, thin. Upper surface white to cream colored or pale ochraceous, matt or almost glabrous. Tubes white to cream-colored, in older and dry specimens pale ochraceous, with light bluish-greyish tint; pores (4)5–6 per mm. *Section*: Context 0.2–1 mm thick, tubes 0.5–2 mm long. Context hyphae thin- to slightly thick-walled, often sclerified, (2.4–)3.1–4.0(–4.8) µm. Tramal hyphae thin to distinctly thick-walled (walls 0.2–1 µm thick), densely interwoven, collapsing easily, twisted, branching at random directions, (1.8–)2.3–3.2(–4.2) µm. *Basidia* (8.8–)12–18.5(–21.8) × 3.5–4.6(–5.1) µm. *Basidiospores* (3.9–)4.2–5.8(–6.3) × (1.2–)1.3–1.7(–1.9) µm, L=4.83 µm, W=1.48 µm, Q=3.26.

Distribution and ecology: Europe, warm temperate to Mediterranean (France, Spain), rather common; on fallen logs and dry branches of deciduous trees (*Buxus, Erica, Populus, Quercus*) and conifers (*Cedrus, Juniperus, Pinus*).

Specimens examined: **France**, Alpes-Maritimes, Lac de St. Cassien, *Juniperus oxycedrus*, 31 Oct. 1996 *Rivoire 1356* (LY, H); 19. Nov. 2000, *Rivoire 1903* (LY, H); Bouches du Rhône, St. Rémy de Provence (holotype, see above); Vaucluse, Goult, *Quercus pubescens*, 7 Nov. 2007, *Rivoire 3278* (LY, H); Vaucluse, Bédouin, *Pinus nigra*, 22 Oct. 2000, *Rivoire 1834* (LY, H). **Spain**, Navarra, Abaurea Alta, *Juniperus communis*, 28 Oct. 2001, *Rivoire 2083** (LY, H); *P. sylvestris*, 31 Oct. 2001, *Rivoire 2107* (LY, H); Garayoa, *Buxus sempervirens*, 30 Oct. 2001, *Rivoire 2100* (LY, H).

Remarks: *Postia mediterraneocaesia* produces small-sized, often resupinate or pendant basidiocarps, and it usually occurs on dry branches in xerophilic habitats. Its spores are similar to those of *P. caesia*, *P. cyanescens* and *P. simulans,* but tramal hyphae of *P. mediterraneocaesia* are strikingly different, unevenly thick-walled and irregularly branched. For a photo and illustration see Pieri & Rivoire (2005).

Postia populi Miettinen, ***sp. nov.*** MycoBank MB823906. Figs 6–8, 16.

Holotype: **USA**, New York, Essex Co., Wolf Lake, 44.0336° N 74.2262° W, alt. 560 m, *Populus tremuloides*, 20 Sep. 2013, *Miettinen 17043** (H 7008644, isotype BPI).

Etymology: After *Populus* spp., the most common host species.

Basidiocarps conchate to flabelliform, sometimes effused-reflexed, mostly thin, later fusing together, small to medium-sized; margin often undulating. Upper surface first white to cream colored, matt, then pale ochraceous to greyish, rarely with bluish flecks or indistinct zones, more or less glabrous. Tubes white to cream-colored, in older and dry specimens with light bluish-greyish tint; *pores* 5–7(–8) per mm, dissepiments first uneven, then strongly serrate. *Section*: Context 1–3 mm thick, tubes 1–5 mm long. *Context hyphae* slightly thick-walled, sclerified, tightly arranged, firm, (2.6–)3.2–4.8(–5.6) µm. *Tramal hyphae* predominantly thick- or very thick-walled (walls regularly over 1 µm thick), tightly arranged, (2.0–)2.7–3.3(–4.2) µm. *Basidia* (9–)10–16(–18.5) × (3.2–)3.5–4.2(–4.8) µm. *Basidiospores* (4.0–)4.2–5.6(–6.1) × 1.0–1.3(–1.6) µm, L=4.84 µm, W=1.17 µm, Q=4.14.

Distribution and ecology: Holarctic, boreal to temperate, common; on fallen logs and large branches of *Populus* spp., rarely on other deciduous trees (*Acer*, *Alnus*, *Betula, Salix*).

Specimens examined: **China**, Jilin, Antu Co., Changbaishan Nat. Res., *Populus* sp., 1 Sep. 1993, *Dai 960* (H); *Acer* sp., 2 Sep. 1993, *Dai 1001* (H). **Finland**, Uusimaa, Helsinki, Veräjämäki, *P. tremula*, 8 Nov. 2015, *Miettinen 19821* (H); Uusimaa, Kirkkonummi, *Betula* sp., 24 Oct. 2012, *Miettinen 15827.2** (H); Etelä-Häme, Hämeenlinna, Musta-Kotinen, *Populus/Salix*, 18 Sep. 2007, *Niemelä 8379** (H 6007874); Pohjois-Häme, Jyväskylä,

Fig. 16. *Postia populi*. **A.** Young, typically light-colored basidiome (Miettinen 14790.3). **B.** Old basidiomes with a characteristic wrinkled margin (Miettinen 15827.2).

Tourujoki, hardwood, 10 Sep. 2011, *Miettinen 14701** (H); Pohjois-Savo, Pieksämäki, Sorsasalo, *Populus tremula*, 16 Sep. 1996, *Haikonen 18147* (H); Kainuu, Hyrynsalmi, Paljakka, *P. tremula*, 27 Sep. 2010, *Miettinen 14211** (H); Kittilän Lappi, Kittilä, Aakenus, Vasalaki SW, 1 Sep. 2000, *Kinnunen 1155* (H). **Norway**, Østfold, Trøgstad, Håkås, *P. tremula*, 8 Sep. 2016, *Nordén* (H, NINA). **Poland**, Podlasie, Hajnówka, Białowieża, fallen deciduous log, 11 Oct. 2008, *Kinnunen 4938** (H). **Russia**, Chukchi Reg., Anadyr, *Alnus fruticosa*, 20 Aug. 2009, *Kotiranta 27132* (H 7033597); Kamtchatka, Bering Island, *Alnus* (?), 4 Sep. 2015, *Kotiranta 27600* (H 7033451); Khabarovsk Reg., Khabarovsk Dist., Bolshoi Khekhtsir Nat. Res., *Acer ukurunduense*, 3 Sep. 2013, *Spirin 6598** (H); Ulun, *P. maximowiczii*, 28 Aug. 2012, *Spirin 5771** (H); Solnechnyi Dist., Suluk-Makit, *Salix* sp., 19 Aug. 2011, *Spirin 4194** (H); Leningrad Reg., Boksitogorsk Dist., Vozhani, fallen log, 22 Sep. 2011, *Spirin 4587** (H); Shidrozero, *P. tremula*, 28 Sep. 2012, *Spirin 5869* (H); Podporozhie Dist., Nemzha, *P. tremula*, 20 Sep. 2009, *Spirin 3224* (H); Volkhov Dist., Chernetskoe, *P. tremula*, 17 Sep. 2009, *Spirin 3194* (H); Nizhny Novgorod Reg., Lukoyanov Dist., Razino, *P. tremula*, 4 Aug. 2004, *Spirin 2092* (H); 16 Aug. 2015, *Spirin 9353** (H). **USA**, Massachusetts, Worchester, *Acer* sp. (?), 25 Sep. 2011, *Miettinen 14790.3*, 14794* (H); hardwood, 8 Oct. 2011, *Miettinen 14876* (H); New York, Essex Co., Wolf Lake, *Populus tremuloides*, 20 Sep. 2013, *Miettinen 17043** (holotype, see above), *Miettinen 17048.3, 17052.1* (H).

Remarks: *Postia populi* is a narrow-spored species morphologically and phylogenetically very close to *P. alni* and *P. caesiosimulans*. In Eurasia narrow-spored specimens growing on *Populus tremula* belong almost always to *P. populi*, at least in cold temperate and boreal areas. However, records exist from other hosts, and in this case thick-walled, tightly arranged hyphae in trama and narrow context hyphae help in morphology-based identification. In North America growth on aspen is a less reliable character since *Populus tremuloides* is only one of many hosts of *P. populi*, and *P. caesiosimulans* is apparently common on aspen in the Midwest. In problematic cases, ITS or *tef1* sequences are required for identification.

Postia simulans (P. Karst.) Spirin & Rivoire, ***comb. nov.*** MycoBank MB823907. Figs 7, 8, 17.
Basionym: *Bjerkandera simulans* P. Karst., *Rev. Mycol*. **10**: 73. 1888.
Synonym: *Polyporus karstenii* Sacc., *Sylloge Fungorum* (Abellini) **9**: 170. 1891. (*nomen novum*)

Lectotype: **Finland**, Etelä-Pohjanmaa, Vaasa, *Picea abies*, 19 May 1864, *Karsten,* BPI 871870 (selected here, MBT380827, duplicate H 6060173). Both specimens were studied.
Epitype: **Finland**, Satakunta, Ylöjärvi, Viljakkala, Inkula, 61.72° N 23.25° E, *P. abies*, 2 Oct. 2011, *Niemelä 8846** (H 6034704 selected here, MBT380828).
Basidiocarps conchate, effused-reflexed or resupinate with detaching or adnate margin, thin to rather thick. Upper surface first white to cream colored, matt or almost glabrous, more rarely pubescent, occasionally turning blue, greyish or pale ochraceous. Tubes white to cream-colored, in older and dry specimens with light bluish-greyish tint and rarely with bluish dots; *pores* (4–)5–7 per mm. *Section*: Context 0.5–3 mm thick, tubes 0.5–4 mm long. *Context hyphae* thin to slightly thick-walled (walls 0.1–0.5 µm thick), rarely sclerified, easily collapsing, (2.9–)3.9–5.0(–6.4) µm. *Tramal hyphae* thin-walled to slightly thick-walled (walls up to 0.6 µm thick) and easily collapsing, often parallel, (1.7–)2.8–3.6(–4.8) µm, very rarely with strongly amyloid (greenish-black in IKI) and cyanophilous content; in East Asian material tramal hyphae sometimes thick-walled (up to 1 µm) and then not so easily collapsing. *Basidia* 10–14.8 × 3.7–5.2 µm. *Basidiospores* (4.1–)4.4–6.3(–8.1) × (1.2–)1.3–1.8(–1.9) µm, L=5.24 µm, W=1.46 µm, Q=3.6.

Distribution and ecology: Holarctic, warm temperate to boreal, common; mostly on conifers (*Abies, Cedrus, Juniperus, Picea, Pinus, Thuja, Tsuga*) but regularly also on deciduous trees (*Corylus, Fagus, Populus, Sorbus, Ulmus*).

Specimens examined: **Canada**, Québec, Saguenay – Lac-Saint-Jean, Alma, conifer, 31 Aug. 2004, *Pieri* (LY-BR 2467, H). **China**, Jilin, Antu Co., Changbaishan Nat. Res., *Pinus* sp., 12 Nov. 1995, *Dai 2054b* (H). **Estonia**, Hiiumaa, Käina, Nasva, *Juniperus communis*, 27 Sep. 2007, *Kotiranta 22018* (H); Valgamaa, Palupera, Käpa, *P. abies*, 13 Sep. 2012, *Spirin 5803* (H). **Finland**, Varsinais-Suomi, Kustavi, Kaurissalo, *Picea abies*, 2 Oct. 1979, *Alava 19067* (H), Varsinais-Suomi, Karjalohja, Karkali Nat. Res., *Corylus avellana*, 5.X.2006 *Niemi 183b** (H); Satakunta, Ylöjärvi (epitype, see above); Etelä-Pohjanmaa, Vaasa (lectotype, see above); Etelä-Häme, Padasjoki, Vesijako, *Pinus sylvestris*, 23 Sep. 2016, *Miettinen 20422** (H); Kainuu, Hyrynsalmi, Paljakka, *P. abies*, 24 Sep. 2010, *Miettinen 14167.2** (H); *Betula*, 26 Sep. 2010, *Miettinen 14190* (H). **France**, Loire, Bessat, *P. abies*, 5 Oct. 2002, *Rivoire 2201* (LY, H); Loire,

Fig. 17. *Postia simulans*. **A**. A half-resupinate, young basidiome with no blue color (Spirin 4689). **B.** Pileate basidiome with a characteristic blue upper surface (Miettinen 20422).

Chalmazel, *Fagus sylvatica*, 1 Sep. 2006, *Rivoire 2936* (LY, H); Rhône, Larajasse, *P. sylvestris*, 30 Sep. 2002, *Rivoire 2195* (LY, H); Rhône, Sérézin, *Populus nigra*, 30 Sep. 1995, *Rivoire 1166* (LY, H); Vaucluse, Bonnieux, *Cedrus atlantica*, 12 Nov. 1994, *Rivoire 1046* (LY, H); Vosges, La Bresse, *A. alba*, 22 Sep. 2002, *Rivoire 2177* (LY, H). **Germany**, Hesse, Alsfeld, Kestrich, *Fagus sylvatica*, 3 Sep. 1972, *Hupke* (H). **Norway**, Akershus, Ski, Svartoren, 12 Sep. 2014, *Ursin** (O 75832). **Russia**, Leningrad Reg., Boksitogorsk Dist., Perelesok, *P. abies*, 27 Sep. 2011, *Spirin 4689* (H); Podporozhie Dist., Chogozero, *P. abies*, 22 Sep. 2009, *Spirin 3407* (H); Kurba, *P. sylvestris*, 19 Sep. 2009, *Spirin 3239* (H); Ostrechiny, *P. abies*, 28 Sep. 2008, *Spirin 2834, 2841* (H); Tokari, *P. abies*, 28 Sep. 2007, *Spirin 2746* (H); Tikhvin Dist., Urya, *Ulmus glabra*, 25 Sep. 2011, *Spirin 4629* (H); Khabarovsk Reg., Khabarovsk, Voronezhskoe, *Quercus mongolica*, 19 Aug. 2013, *Spirin 6177* (H); Khabarovsk Dist., Malyi Niran, *Picea ajanensis*, 6–8 Aug. 2012, *Spirin 4983, 5062* (H); Malyi Kukachan, *P. ajanensis*, 19 Aug. 2012, *Spirin 5418* (H); Solnechnyi Dist., Igdomi, *Pinus pumila*, 2 Sep. 2016, *Spirin 10810* (H); Suluk-Makit, *P. ajanensis*, 19 Aug. 2011, *Spirin 4217* (H); Razlivnoi, *P. ajanensis*, 22–24 Aug. 2011, *Spirin 4271*, 4273, 4386** (H); Evoron, *P. ajanensis*, 26–27 Aug. 2011, *Spirin 4397, 4424* (H); Nizhny Novgorod Reg., Lukoyanov Dist., *P. sylvestris*, 15 Aug. 2006, *Spirin 2546* (H); Primorie, Krasnoarmeiskii Dist., Valinku, *Betula ermanii*, 25 Aug. 2013, *Spirin 6306* (H); *P. ajanensis*, 25 Aug. 2013, *Spirin 6310* (H); *Spirin 6328** (H 7008649); Sverdlovsk Reg., Sysert' Dist., Dvurechensk, *P. sylvestris*, 25 Aug. 2002, *Kotiranta 19754* (H). **USA**, Idaho: Bonner Co., Trapper Creek, *Tsuga heterophylla*, 14 Oct. 2014, *Miettinen 18735** (H); Idaho, Boundary Co., Upper Priest River, *Thuja/Tsuga*, 16 Oct. 2014, *Miettinen 18882** (H); New York, Essex Co., Catlin Lake, *Fagus grandifolia*, 19 Sep. 2013, *Miettinen 17015* (H); New York, Warren Co., Pack Demonstration Forest, *Acer*, 24 Sep. 2013, *Miettinen 17197* (H); North Carolina, Swain Co., Clingmans Dome, *Sorbus americana*, 1 Oct. 2015, *Miettinen 19612** (H); *Abies fraseri/ Picea rubens*, 1 Oct. 2015, *Miettinen 19632.3* (H); Washington, Pend Oreille Co., Slate Creek, *Pinus ponderosa*, 15 Oct. 2014, *Spirin 8542** (H); *8587** (H 7008650); *Thuja plicata*, 15 Oct. 2014. *Miettinen 18817* (H).

Remarks: This species was described by Karsten (1888) as *Bjerkandera simulans* and later tentatively placed in the synonyms of *Postia lactea* (as *Polyporus tephroleucus*) (Lowe 1956, Ryvarden 1991). However, the presence of characteristic amorphous aggregates developing in CB as well as spore dimensions in the type material preclude this synonymy. The most similar species is *P. yanae*. For an overview of differences, see remarks under that species and Tables 1–3.

In herbaria, *Postia simulans* has been mixed up mainly with *P. caesia* in the strict sense, but also mislabeled as other *Postia* species. This is not surprising due to its wide morphological variation. Young basidiocarps of *P. simulans* are white and lack bluish tints, and only microscopical study can reveal their identity. Senescent, distinctly bluish basidiocarps can be mistaken for other species of the complex, in Europe *P. caesia* in particular. In this case, rather long basidiospores (regularly reaching 6 µm) and almost total absence of colored hyphae in tube trama are the best features to recognize *P. simulans*. Most of the European, distinctly pubescent, blue specimens on conifers belong to *P. caesia*.

The East Asian material differs from other Eurasian material we have studied in having often more thick-walled and slightly narrower tramal hyphae (Suppl. 3). This population seems to be genetically slightly different as well - the ITS sequences differ only by 1 bp between European and North American *P. simulans* while *tef1* differs by 5–7 bp, albeit with only one East Asian specimen sequenced for *tef1*. However, the ITS sequences of this East Asian material are identical with the Mediterranean *P. luteocaesia*. Although otherwise morphologically very similar, *P. luteocaesia* sensu typi differs from *P. simulans* in having yellow basidiocarps and wider basidiospores. In the light of these differences and the absence of *tef1* sequences from *P. luteocaesia* we have kept *P. simulans* and *P. luteocaesia* separate. We are also refraining from split the *P. simulans* complex further. Extensive sampling of material and sequencing *tef1,* and possibly other markers, are needed to clarify whether these taxa and populations represent more than one species.

Postia subcaesia (A. David) Jülich, *Persoonia* **11**(4): 424. 1982. Figs 6–8, 18.
Basionym: *Tyromyces subcaesius* A. David, *Bull. Soc. Linn. Lyon* **15**: 120. 1974.

Holotype: **France**, Isère, Crémieu, *Malus domestica*, Oct.1968, *David 652* (LY, isotype H 7034976* studied).

Fig. 18. *Postia subcaesia*, Rivoire 1370.

Basidiocarps conchate to triquetrous, small to medium-sized. Upper surface first cream colored, then pale to dark ochraceous, first matt to later distinctly pubescent, rarely with bluish flecks or faint zones. Pore surface white to cream-colored, bluish-greyish on aging; *pores* 4–6(–7) per mm. *Section*: Context 3–17 mm thick, tubes 2–10 mm long. *Context hyphae* thin- to slightly thick-walled, (2.3–)4.2–6.6(–7.8). *Tramal hyphae* thin- or only slightly thick-walled, (2.2–)3.1–4.1(–5.8) µm. *Basidia* 10.3–17.8 × 3.3–4.6 µm. *Basidiospores* (3.8–)4.0–5.3(–6.1) × 1.0–1.4(–1.5) µm, L=4.60 µm, W=1.21 µm, Q=3.80.

Distribution and ecology: Europe, temperate, rather rare; fallen logs of deciduous trees (*Alnus, Carpinus, Crataegus, Corylus, Fagus, Fraxinus, Malus, Populus, Quercus, Salix, Ulmus*).

Specimens examined: **Czech Republic**, Jihomoravský Reg., Břeclav, Lanžhot, *Carpinus betulus*, 31 Aug. 1989, *Vampola* (H, MJ 1563); *Crataegus* sp., Oct. 2001, *Vlasák 0110/24** (H, JV). **France**, Allier, Tronçais, *Quercus* sp., 19 Oct. 1969, *David 785* (LY, H); Alpes Maritimes: Montauroux, *Salix* sp., 2 Nov. 1996, *Rivoire 1370* (LY, H); Isère (holotype, see above); Loire, St. Foy St. Sulpice, *Populus tremula*, 31 Oct. 2000, *Rivoire 1868* (LY, H); Saône-et-Loire: Tramayes, *Quercus* sp., 29 Aug. 1965, *David 150* (LY, H). **Russia**, Nizhny Novgorod Reg., Lukoyanov Dist., Sanki, *Corylus avellana*, 12 Aug. 2005, *Spirin 2390* (H), 10 Aug. 2013, *Spirin 6083** (H). **UK**, England, Hampshire, New Forest, *Alnus glutinosa*, 14 Oct. 1995. *Legon** (K(M) 31967, O, H).

Remarks: In most cases, *P. subcaesia* is easily recognized by its large, soft basidiocarps with a matt or pubescent upper surface. The distinct bluish color only appears in senescent specimens. Microscopically, it differs from other European narrow-spored species (*P. alni*, *P. caesiosimulans*, *P. populi*) in having thin-walled, wide and loosely arranged hyphae both in context and tubes. Papp (2014) provides a detailed comparison with *P. alni*.

Postia subviridis (Ryvarden & Guzmán) Spirin, ***comb. nov.*** MycoBank MB823908. Figs 7, 8.
Basionym: *Tyromyces subviridis* Ryvarden & Guzmán, *Mycotaxon* **78**: 252. 2001.

Holotype: **Mexico**, Veracruz, Cofre de Perote, fallen log, 8 Jan. 1991, *Tapia 468* (XAL, isotypes O, H studied).

Basidiocarps conchate, small to medium-sized, thin. Upper surface first pale ochraceous, then ochraceous to greyish, glabrous to matt. Tubes white to cream-colored, with light bluish-greyish ting upon aging and drying; *pores* 6–8 per mm, angular to sinuous. *Section*: Context 1–2 mm thick, tubes 1–4 mm long. *Context hyphae* slightly thick-walled, often sclerified, (2.8)4.8–5.8(7.8) µm. *Tramal hyphae* predominantly thick-walled (walls up to 1 µm thick) to slightly thick-walled, (2.2–)2.5–3.2(–3.7) µm. *Basidia* (9–)10–13(–14) × 3.2–4.4 µm. *Basidiospores* (3.5–)3.8–4.5(–4.6) × 1.0–1.3 µm, L=4.11 µm, W=1.15 µm, Q=3.58.

Distribution and ecology: North America and Europe, temperate to boreal. Only three records so far (mountains in Mexico, western US, Finland); conifer logs (*Picea, Pinus*; the type probably from *Abies* or *Picea*).

Specimens examined: **Finland**, Pohjois-Karjala, Ilomantsi, Lahnavaara, *Pinus sylvestris*, 5 Sep. 2003, *Penttilä 14376** (H). **Mexico**, Veracruz, Cofre de Perote (holotype, see above). **USA**, Washington, Jefferson Co., Hoh River, *Picea sitchensis*, 20 Oct. 2014, *Spirin 8774a** (H).

Remarks: Our concept of the species may still include several species. *Tyromyces subviridis* was described from a highland conifer forest in Mexico (Guzmán & Ryvarden 2001). We studied the type specimen, as well as another collection from Washington, USA. The morphological description above is based on these two specimens. A third collection from Finland has a *tef1* sequence identical to the Washington specimen, but morphologically it is deviating, being pure white and having nearly thin-walled tramal hyphae and longer spores (L=4.92 µm, Tables 1, Suppl. 2).

Both morphological and DNA data show that *P. subviridis* is a member of the difficult narrow-spored complex which also includes *P. alni*, *P. populi*, and *P. caesiosimulans*. Of them, *P. subviridis* has the smallest pores and the shortest basidiospores, and it might be restricted to conifers.

Postia yanae Miettinen & Kotiranta, ***sp. nov.*** MycoBank MB823909. Figs 7, 8.

Holotype: **Russia**, Sakha, Verkhoyansk Reg., Verkhoyansk-Batagai road, 67.5° N 133.67° E, alt. 370 m, old larch dominated forest, decorticated *Larix gmelinii* branch (3 cm diam, decay stage 2/5), 10 Aug. 2016, *Kotiranta 27454** (H 7034942).

Etymology: After river Yana, the type locality.

Basidiocarps small to medium-sized, effused-reflexed or resupinate with detaching or adnate margin. Upper surface white to cream colored, pale ochraceous or bluish, deep brown in large specimens, matt to glabrous. Pore surface white, with light to strong bluish-greyish tint; *pores* 5–7 per mm, angular to sinuous. *Section*: Context 0.5–2 mm thick, white, tubes 1–3 mm long, concolorous with pore surface. *Context hyphae* thin- to slightly thick-walled, (2.0–)3.0–4.0(–5.2) µm. *Tramal hyphae* thin- to slightly thick-walled (walls 0.2–0.5 µm thick), winding, collapsing easily, (1.5–)2.2–2.9(–4.3) µm. *Basidia* 9–14 × 3.5–4.2 µm. *Basidiospores* (4.0–)4.3–5.8(–7.5) × 1.2–1.6(1.7) µm, L=5.02 µm, W=1.41 µm, Q=3.56.

Distribution and ecology: Known from four localities in Eastern Siberia, on branches, cut wood and small diameter trunks of *Pinus* and *Larix* in dry environments.

Specimens examined: **Russia**, Sakha, Churapacha Dist., Telei, *Larix gmelinii*, 22 Aug. 2016, *Kotiranta 27772** (H 7036326); Verkhoyansk Dist. (holotype, see above); Sakha, Xañalas Dist., Buotama, *L. gmelinii*, 17 Aug. 2016, *Kotiranta 27606** (H); *P. sylvestris*, 18 Aug. 2016, *Kotiranta 27677** (H); Nizhny Bestjah, *Pinus sylvestris*, 23 Aug. 2016, *Kotiranta 27879** (H 7036282).

Remarks: *Postia yanae* belongs to the wide-spored group of conifer-dwelling species within the *P. caesia* complex. It is morphologically very similar to *P. simulans. Postia simulans* shares with *P. yanae* collapsing, thin-walled tramal hyphae, but its tramal and context hyphae are usually wider (Suppl. 3, context: 3.9–5.0 vs. 3.0–4.0 µm, trama: 2.8–3.6 vs. 2.2–2.9 µm). Spores of *P. simulans* are often longer in average (L=5.24 µm vs. 5.02 µm), but some specimens deviate from this trend (Suppl. 2). Possibly of importance is autecology: *P. simulans* inhabits various tree species (rarely *Pinus*) in mesic forests, whereas *P. yanae* collections derive from dry continental forests from *Larix* and *Pinus* branches. *Postia yanae* basidiomes are mostly very small while *P. simulans* usually makes sturdier basidiomes. In borderline cases sequencing is needed for definite identification. Also the basidiocarps and spores of *P. mediterraneocaesia* are similar, but beside the widely different distribution area, the richly branched tramal hyphae tell this species apart.

Further species associated with *Postia caesia* complex

Bjerkandera ciliatula Karst., *Medd. Soc. Fauna Flora Fennica* **14**: 80. 1887.

Remark: We have studied the type and it represents *Tyromyces chioneus*.

Boletus candidus Roth, *Catalecta botanica quibus plantae novae et minus cognitae describuntur atque illustrantur* **1**: 244. 1797.

Remarks: Fries (1821) treated this species as a synonym of *Postia caesia*, but he later (Fries 1836–1838) considered it a form of *Bjerkandera adusta*. No type is known to exist and the description is vague: all we can say is that it is a light-colored wood-inhabiting polypore. The name should be treated as *nomen dubium*.

Postia africana (Ryvarden) V. Papp, *Mycotaxon* **129**: 411. 2015. [2014]
Basionym: *Oligoporus africanus* Ryvarden, *Mycotaxon* **31**: 407. 1988.

Remarks: The species has been described based on the single collection from highlands of Burundi (Ryvarden 1988a). Large and thick basidiocarps, wide tramal hyphae and short and narrow basidiospores of *P. africana* point towards *P. subcaesia* and related species. The identity of this species should be reestablished after sequencing newly collected specimens from Central Africa.

Postia amyloidea (Corner) V. Papp, *Mycotaxon* **129**: 411. 2015. [2014]
Basionym: *Tyromyces amyloideus* Corner, *Beih. Nova Hedwigia* **96**: 160. 1989.

Remarks: Corner (1989) described this species alongside with *P. coeruleivirens*, and the only reliable characters in the protologue to distinguish these species are amyloid tramal hyphae and slightly smaller basidiospores of *P. amyloidea*. However, the taxonomic value of these features, as well as identity of both aforementioned species, must be revised after collecting new material in the type locality, Mount Kinabalu.

Postia atrostrigosa (Cooke) Rajchenb., *N.Z. Jl Bot.* **33**: 104. 1995.
Basionym: *Polyporus atrostrigosus* Cooke, *Grevillea* **19** (89): 2. 1890.

Remarks: Rajchenberg (1995) re-introduced this species after morphological and cultural studies of specimens from New Zealand. We sequenced a Tasmanian specimen, which differs by just 1 bp from two Argentinian ITS sequences available in GenBank (JX090109 and JX090110); they represent a unique species that may well be *P. atrostrigosa*.

Postia caesioflava (Pat.) V. Papp, *Mycotaxon* **129**: 411. 2015. [2014]
Basionym: *Polyporus caesioflavus* Pat., *Bull. Soc. Mycol. France* **8**: 114. 1892.

Remarks: Patouillard described this species from Ecuador. It is the only yellow-colored representative of the *P. caesia* complex known so far from the tropics. It differs from similarly colored European species, *P. auricoma* and *P. luteocaesia*, in having larger basidiocarps, not turning green when bruised, and smaller pores, 8–10 per mm (Ryvarden 2016).

Postia wakefieldiae (Kotl. & Pouzar) Pegler & E.M. Saunders, *Mycologist* **8**: 28. 1994.
Basionym: *Tyromyces wakefieldiae* Kotl. & Pouzar, *Česká Mykol*. **43**: 39. 1989.

Specimens examined: **Germany**, Schleswig-Hollstein, Travemünde, Hermannshöhe, dicot, 24 Jun. 2007, *Miettinen 11703* (H). **UK**, England, Hampshire, New Forest, *Quercus* sp., 4 Nov. 1995, *Legon* (O, H). Suffolk, Brettenham, Nov. 1935, Pearson (PRM 611292, holotype).

Remarks: Pegler & Saunders (1994) transferred this species to *Postia* due to "close affinity of this species to the *Postia caesia* complex". They emphasize a context "bruising blue" as a key character. When Kotlaba & Pouzar (1989) described *Tyromyces wakefieldiae*, they cited a letter from D.A. Reid stating that the species sometimes shows blue coloration. Also Wakefield stated, according to Kotlaba and Pouzar, that "the flesh" stained blue when fresh. Ryvarden & Melo (2014) note that the blue discoloration sometimes persists even in dry specimens.

Despite mentioning the blue color, neither Kotlaba & Pouzar (1989) nor Ryvarden & Melo (2014) suggest that the species would belong in the vicinity of *P. caesia*. Pieri & Rivoire (1998) rejected affinity to *P. caesia* due to the lack of amyloid reaction and blue spore color. We have seen the type and other material from England without any blue coloration and conclude that *P. wakefieldiae* does not belong to the *P. caesia* complex, but is a good member of the genus *Postia*.

Tyromyces setiger (Cooke) G. Cunn., *Bull. N.Z. Dept. Sci. Industr. Res., Pl. Dis. Div.*: 763. 1963.
Basionym: *Polyporus setiger* Cooke, *Grevillea* **19**(89): 1. 1890.

Remarks: Cunningham (1965) mentions that he reported this species previously under *Postia caesia*, so there is some resemblance. Another Austro-American species, *P. atrostrigosa*, differs in having wider basidiospores. Ryvarden (1988b) also treated this species under *Tyromyces*. New collections are needed to settle its proper taxonomic position.

DISCUSSION

Our study has raised the species number in the *Postia caesia* complex from 10 to 24. The most important contribution of this paper are the rigorous type studies that allowed us to combine existing temperate names to phylogenetic species concepts and previously unaccounted diversity.

Considering that we did not revise tropical or Southern Hemisphere material, the number of species known from Northern Hemisphere more than tripled, from 6 to 20 species. Why this diversity has gone unnoticed or at least undescribed for so long is due to the combination of minute morphological differences and small interspecific differences in the most commonly used genetic marker ITS (in some cases below 1% between species). Obviously, the insight provided by DNA sequences proved fundamental in revising species concepts accurately.

ITS sequences were commonly polymorphic, exhibiting double bases and even length variation. Considering that ITS sequence differences between some species are very low (< 1 %) they may fall within error margins of current mass sequencing methods used in environmental studies. *Tef1* sequences are in general more variable between species and in this sense more reliable for molecular identification, but reference sequences are available for much fewer species.

Our sampling and analyses allowed distinguishing 20 species in the temperate Northern Hemisphere. However, even in the best sampled area, Europe, we have indications that still more species are present: *Postia* cf. *subviridis* from Finland does not agree well the North American material though their *tef1* sequence are identical; two English specimens collected on *Acer* and *Fraxinus* from the study of Yao *et al.* (2005) have distinct ITS sequences. Variation within the wide-spread *P. caesiosimulans* leaves open the possibility that our concept of the species encompasses more than one taxon (species or subspecies). We can also expect a good number of new species from the Southern Hemisphere, the Himalayas as well as subtropical and tropical areas.

While species differences at the extremes of the morphological variations within the complex are very clear (e.g. *P. alni* vs. *P. auricoma*), some species are very difficult to separate, even to the extent that DNA sequences are required for a reliable identification of some individuals. Such examples include *P. alni* vs. *P. caesiosimulans* in Europe, *P. arbuti* vs. *P. populi* in North America and *P. simulans* vs. *P. yanae* in Asia. Young or senescent specimens may also have to be left unnamed even after a careful microscopical examination. However, the great majority of specimens from the *Postia caesia* complex can be identified without DNA sequences, often in the field.

Most species of the *P. caesia* complex are restricted geographically to either western or eastern part of Eurasia or to North America, indicating that geographic isolation has probably played a role in speciation. Only three species (*P. caesiosimulans, P. populi, P. simulans*) and possibly a fourth (*P. subviridis*) are distributed in both Eurasia and North America. In addition, one further species (*P. auricoma*) is found in Europe and East Asia. Within North America some species have been recorded only from the east (*P. comata*) or from the west coast (*P. arbuti*), but collection intensity is too low to judge whether this is just accidental or a true pattern of distribution. Continental divide and possibly ice ages - we expect that some of the species divisions such as *P. alni – P. cyanescens – P. caesiosimulans – P. populi* are relatively young - have thus shaped the species diversity.

Host tree, particularly division between angiosperms and gymnosperms, is an important character for identification but also when contemplating speciation patterns in this complex. Six out of 20 north temperate species grow only on angiosperms (*P. arbuti, P. alni, P. coeruleivirens, P. magna, P. populi, P. subcaesia*), and eight only on gymnosperms (*P. auricoma, P. bifaria, P. cyanescens, P. glauca, P. gossypina, P. luteocaesia, P. subviridis, P. yanae*). Six species (*P. caesia, P. caesiosimulans, P. comata, P. livens, P. mediterraneocaesia, P. simulans*) grow both on gymno- and angiosperms, although in the case of *P. caesiosimulans* the conifer records from North America may represent distinct, possibly differentiating populations.

In a few cases *Postia* species strongly prefer a particular tree species, at least regionally. Thus *Postia cyanescens* is almost always found on *Picea abies* in the North, while *Postia populi* prefers *Populus tremula* in Europe. *P. luteocaesia* have been recorded only from *Pinus* spp., and *P. auricoma* only from *Pinus sylvestris* in Europe.

Although host specialization clearly plays a role in the evolution of the *P. caesia* complex, it is an open question whether sympatric speciation has significance. We can point to one case where sympatric speciation seems likely: the spruce-inhabiting species *P. cyanescens* is distributed in Europe, where its closest relatives are *P. alni, P. caesiosimulans* and *P. populi*, all angiosperm-inhabiting species. *Tef1* sequence places *P. cyanescens* in the vicinity of *P. caesiosimulans*, which occurs on conifers in East Asia and North America. In this case host jump as a primary speciation process would appear to be a plausible option.

Despite small ITS differences, overlapping distribution areas and similar ecology we did not observe clear cases of hybridization between species with the exception of a single individual: Collection *Spirin 9353* from Nizhny Novgorod produced polymorphic ITS sequence that can be interpreted as having ITS copies from two parental species, *P. alni* and *P. populi*. Since the *tef1* sequence is a typical *P. populi* sequence this specimen is not a first generation hybrid. It nevertheless indicates that limited gene flow may be taking place between closely related species.

A number of *Postia* species have been red-listed or are considered indicators of old-growth forests in Northern Europe. Most species of the *P. caesia* complex do not seem to be associated with endangered habitats at the continental level. It is worth mentioning that *P. auricoma* records derive from forests with a continuum of big pine logs, so this species may well be old-growth-forest dependent. Also all the records of *P. bifaria* derive from virgin or near virgin conifer forests. The number of records is so low for many species that it is premature to assess how common and ecologically specialized they are.

We hope to see other researchers continue where we have left in studying this fascinating, easily recognizable species group. While doing a decent job at delineating species, our

study did not fare as well in establishing a robust phylogeny for the *Postia caesia* complex. For this, we need further genetic markers, whose sequencing will require cultures or recently collected specimens. Fresh collections are really needed since even ITS sequence production in this group is difficult from specimens older than a few years, and other markers are likely to be more difficult to sequence.

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Supplement 1 - INSDC accession numbers

INSDC accession numbers for DNA sequences used in this study. Specimens provided with collector and collection number information have been sequenced for this study, the rest retrieved from the INSDC database.

Supplement 2 - Spore measurements in the *Postia caesia* complex

Bold-face values represent composite statistics for species. L = average of spore length, W = average of spore width, Q = L/W, and n = number of spores measured. The whole range is given in parentheses; 90% range excluding 5% extreme values from both ends of variation is given without parentheses; in case the values are identical, parentheses are omitted. Specimens marked with asterisk (*) have been excluded from the combined statistics for that species.

Supplement 3 - Hyphal measurements in the *Postia caesia* complex

Bold-face values represent composite statistics for species, n = number of spores measured. The whole range is given in parentheses; 60% range excluding 20% extreme values from both ends of variation is given without parentheses; in case the values are identical, parentheses are omitted.

Supplement 4 – Distribution maps of north temperate *Postia caesia* species

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Neocosmospora perseae sp. nov., causing trunk cankers on avocado in Italy

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Abstract: Trunk and branch cankers are among the most important diseases compromising avocado production worldwide. A novel species, *Neocosmospora perseae* sp. nov. is described isolated from trunk lesions on *Persea americana* in the main avocado producing area of Sicily, Italy. The new species is characterised using a polyphasic approach including morphological characters and a multilocus molecular phylogenetic analysis based on partial sequences of the translation elongation factor-1α, the internal transcribed spacer regions plus the large subunit of the rDNA cistron, and the RNA polymerase II second largest subunit. Pathogenicity tests and the fulfilment of Koch's postulates confirm *N. perseae* as a novel canker pathogen of *Persea americana*.

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INTRODUCTION

Fusaria are omnipresent fungi belonging to *Nectriaceae*, commonly found in soil, water, air, dead or living plant material, food, and many other substrates, where they are acting mainly as saprobes (Lombard *et al.* 2015). Nevertheless, some species are of great importance as mycotoxin producers which can affect human and animal health. The genus *Fusarium sensu lato* has recently been segregated into several fusarium-like genera, i.e. *Albonectria*, *Bisifusarium*, *Cyanonectria, Geejayessia, Neocosmospora* and *Rectifusarium* (Gräfenhan *et al.* 2011, Lombard *et al.* 2015). These taxa are among the most impactful human, animal and plant pathogens, affecting an extensive variety of hosts (O'Donnell *et al.* 2008, 2010, Lombard *et al.* 2015).

The agri-food production sector has been undergoing major changes over the last few decades in Italy. These changes especially concern the introduction of alternative crops such as avocado. In the 20th century, avocado (*Persea americana*) was introduced to Italy and cultivated for ornamental purposes. However, due to a decline in demand for lemon, and a global increasing demand for avocado, it took the place of lemon orchards in eastern Sicily, where it represents an important fruit industry and a viable alternative crop to citrus (Guarnaccia *et al*. 2016). Unfortunately, avocado production is compromised by several pathogens causing branch cankers (Menge & Ploetz 2003, Guarnaccia *et al*. 2016). Frost or mechanical injuries such as pruning wounds may represent the initial access wounds for these canker-causing pathogens. Moreover, species belonging to *Nectriaceae* are well-known as responsible for diseases on avocado plants (Vitale *et al*. 2012, Parkinson *et al*. 2017), including several members of *Fusarium* and fusarium-like genera, such as *Albonectria* and *Neocosmospora* (Farr & Rossman 2018).

In one of the most renowned cases, damage was inflicted to avocado trees in Israel in 2009, caused by the ambrosia beetle *Euwallacea fornicatus,* and a vectored symbiotic fungal species belonging to *Neocosmospora* (formerly the *Fusarium solani* species complex, FSSC; O'Donnell *et al*. 2008, Lombard *et al*. 2015, Aoki *et al*. 2018). The affected plants showed dieback, wilt, including sugar or gum exudates, and ultimately host tree mortality (Mendel *et al*. 2012). In 2012, the beetle was recorded on several tree species in southern California and Israel, playing a major role as serious threat to avocado production (Mendel *et al*. 2012, Freeman *et al*. 2013, Kasson *et al*. 2013). "*Fusarium" euwallaceae*, found associated with the beetle is closely related to *Neocosmospora ambrosia*, another obligate symbiont occurring in Sri Lanka and India causing damage to tea plantations (Lombard *et al*. 2015). Both fungal pathogens are nested in an exclusive lineage (the Ambrosia clade) within Clade 3 of *Neocosmospora,* together with at least another eight unnamed phylogenetic species, all symbionts of the fungus-farming *Euwallacea ambrosia* beetles and one of the best examples of host-fungus co-evolution (Freeman *et al*. 2013, O'Donnell *et al*. 2016, Aoki *et al*. 2018). The fulfilment of Koch's postulates (Mendel *et al*. 2012) demonstrated the ability of "*Fusarium" euwallaceae* to cause wilt and dieback on avocado in Israel and California with no beetle-association (Freeman *et al*. 2013).

After the observation of prominent trunk cankers on avocado trees in an orchard located in the Catania province (eastern Sicily) during 2015, efforts were made to identify the causal agent.

In this study, a new fungal pathogen of avocado belonging to the genus *Neocosmospora* is proposed. The fungus is described on the basis of morphological and cultural characteristics as well as phylogenetic analyses of combined DNA sequences. Moreover, the pathogenicity on the host from which the fungus was isolated, is evaluated.

MATERIALS AND METHODS

Field sampling and isolation

During 2015, trunk canker symptoms were observed in a 14-yr-old avocado (Hass cultivar) orchard, located in the avocado plant-production region in eastern Sicily. The disease incidence (DI) was

recorded based on the number of symptomatic plants compared to the total number present. Branch canker samples were taken from 10 plants. Fragments (5 × 5 mm) of symptomatic tissues were cut from the lesion margins, surface-sterilised in a sodium hypochlorite solution (10 %) for 20 s, followed by 70 % ethanol for 30 s, and rinsed three times in sterilised water. Tissue fragments were dried between sterilised filter papers, placed on 2 % potato dextrose agar (PDA; Difco, Leeuwarden, The Netherlands) amended with 100 µg/mL penicillin and 100 µg/mL streptomycin (PDA-PS) and incubated at 25 °C until characteristic fungal colonies were observed. Pure cultures were obtained by transferring germinating single conidia to fresh PDA plates with the aid of a Nikon SMZ1000 dissecting microscope.

Fungal isolates and morphological characterization

The cultural and micromorphological features of all the isolates included in this study were evaluated following the procedures of Aoki *et al*. (2003) with some modification as described previously (Sandoval-Denis *et al*. 2018). Colour notation followed the mycological colour charts of Rayner (1970). Micromorphological characteristics were examined and photographed using a Nikon Eclipse 80i microscope with Differential Interference Contrast (DIC) optics and a Nikon AZ100 stereomicroscope, both equipped with a Nikon DS-Ri2 high definition colour digital camera. Photographs and measurements were taken using the Nikon software NIS-elements D software v. 4.50.

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA for 4–7 d at room temperature, under a natural day/night photoperiod. Total genomic DNA was extracted from fresh mycelium scraped from the colony surface using the Wizard® Genomic DNA purification Kit (Promega Corporation, Madison, WI, USA). Fragments of four nuclear loci including the translation elongation factor 1-alpha (*EF-1α*), the internal transcribed spacer region of the rDNA (ITS), the large subunit of the rDNA (LSU) and the RNA polymerase second largest subunit (*RPB2*) were PCR amplified as described previously (O'Donnell *et al*. 2009, 2010, Sandoval-Denis *et al.* 2018) and sequenced using the following primer pairs: EF-1/EF-2 for *EF-1α* (O'Donnell *et al*. 2008), ITS4/ITS5 for ITS (White *et al*. 1990), LR0R/LR5 for LSU (Vilgalys & Hester 1990, Vilgalys & Sun 1994) and 5f2/7cr and 7cf/11ar for *RPB2* (Liu *et al*. 1999, Sung *et al*. 2007). Sequences generated in this study were uploaded to GenBank and the European Nucleotide Archive (ENA) databases (Table 1).

Phylogenetic analyses and molecular identification

Sequence alignments were performed individually for each locus using MAFFT on the European Bioinformatics Institute (EMBL-EBI) portal (http://www.ebi.ac.uk/Tools/msa/mafft/). BLASTn searches on GenBank and pairwise sequence alignments on the *Fusarium* MLST database of the Westerdijk Fungal Biodiversity Institute (http://www.westerdijkinstitute.nl/fusarium/) were performed using *EF-1α* and *RPB2* sequences in order to preliminary identify the fungal isolates to generic level. Following this initial identification, a combination of DNA sequences from four loci (*EF-1α*, ITS, LSU and *RPB2*) was used for the final molecular identification and phylogenetic analyses (O'Donnell *et al*. 2008).

The different gene datasets were analysed independently and combined using RAxML (ML) and Bayesian methods (BI) as described previously (Sandoval-Denis *et al*. 2018). Evolutionary models for the four loci (GTR+I+G for ITS, LSU and *RPB2*; GTR+G for *EF-1*α) were calculated using MrModelTest v. 2.3 (Nylander 2004) selecting the best-fit model for each data partition according to the Akaike criterion.

Pathogenicity tests

Pathogenicity tests were performed on potted, healthy avocado seedlings (6-mo-old) with a subset of two representative isolates. Each experiment was conducted twice. For each experiment three replicates per isolate were used with 10 plants per replicate. Twigs were superficially wounded between two nodes forming a slit using a sterile blade. Inoculations were conducted by placing a 1-wk-old, 6-mm-diam colonised agar plug from each fungal isolate on a wound. Wounds were then wrapped with Parafilm® (American National Can, Chicago, IL, USA). Ten twigs were inoculated as described above with 6-mm-diam non-colonised MEA plugs as negative controls. The same number of wounds/plants were inoculated with sterile MEA plugs and served as controls. After inoculation, plants were covered with a plastic bag for 48 h and maintained at 25 ± 1 °C and 95 % relative humidity (RH) under a 12-h fluorescent light/dark regime. All plants were irrigated 2–3 times per week and examined weekly for disease symptom development. Disease incidence (DI) was recorded as described above.

RESULTS

Field sampling and fungal isolation

Symptoms referable to fusaria species were detected in an avocado orchard in the main avocado-producing region of Eastern Sicily, Italy (GPS coordinates: 37.687247, 15.175479). The disease was observed on established plants (14-yr-old) in an open field. Disease incidence was ascertained at 10 %. The symptoms observed on avocado plants consisted of trunk cankers. Bark appeared cracked, darkly discoloured and/or slightly sunken. Occasionally, a sugar exudate was present on the surface. Cankers were internally reddish brown in colour and variable in shape. Transverse cuts revealed a characteristic wedge-shaped canker extending deep into the xylem (Fig. 1). Only fusarium-like isolates growing in pure culture were obtained from the symptomatic avocado trees, from which five monosporic strains were retained.

Phylogenetic analyses and species identification

Pairwise sequence alignments on the *Fusarium* MLST database and GenBank BLASTn searches demonstrated that the five fungal isolates belonged to the genus *Neocosmospora*.

Subsequently, more inclusive multilocus phylogenetic analyses were performed based on *EF-1α*, ITS, LSU, and *RPB2* sequences. A first analysis spanned the currently known phylogenetic diversity of the genus *Neocosmospora*, and included sequences from a total of 365 strains, based on the alignments published by O'Donnell *et al*. (2008). According to this analysis, the five strains from avocado formed an exclusive new linage in the genus *Neocosmospora* (data not shown, alignments, trees and statistics all available at TreeBASE). A second analysis was run based on a selected subset of DNA data representing most of the species of *Neocosmospora* currently assigned with Latin binomials, plus several yet unnamed phylogenetic clades phylogenetically related to the new lineage (Fig. 2). This final analysis included sequences from 80 strains, representing 48 taxa and a total of 2 917 character sites, of which 2 203 were conserved (*EF-1*α 212, ITS 372, LSU 441 and *RPB2* 1178), and 555 were variable and phylogenetically informative (*EF-1*α 69, ITS 101, LSU 35 and *RPB2* 350). The BI analyses identified a total of 774 unique sites (*EF-1*α 134, ITS 179, LSU 43 and *RPB2* 418) and sampled a total 315 000 trees, from which 236 250 were used to calculate the 50 % consensus tree and posterior probability (PP) values, after discarding 25 % of trees as burn-in fraction. Results from ML and BI methods showed that the

Table 1. Collection details and GenBank accession numbers of isolates included in this study.

Species	Clade number[a]	Strain number[b]	Country and substrate	GenBank/EBI accession number[c]			
				EF-1α	ITS	LSU	*RPB2*
Fusarium brasiliense		NRRL 22743	Brazil, *Glycine max*	EF408407	FJ919502	FJ919502	EU329525
Fusarium cuneirostrum		NRRL 31104	Japan, *Phaseolus vulgaris*	EF408413	FJ919509	FJ919509	EU329558
Fusarium ensiforme	FSSC 15	NRRL 28009	USA, human eye	DQ246869	DQ094351	DQ236393	EF470136
	FSSC 15	NRRL 32792	Japan, human	DQ247101	DQ094561	DQ236603	EU329621
Fusarium euwallaceae		CBS 135855 = NRRL 54723	Israel, Beetle from Avocado Tree	JQ038008	JQ038015	JQ038015	JQ038029
		CBS 135856 = NRRL 54724	Israel, Beetle from Avocado Tree	JQ038009	JQ038016	JQ038016	JQ038030
Fusarium keratoplasticum	FSSC 2	CBS 490.63^{T} = NRRL 22661	Japan, human eye	DQ246846	DQ094331	DQ236373	EU329524
	FSSC 2	NRRL 28561	USA, human	DQ246902	DQ094375	DQ236417	EU329552
Fusarium lichenicola	FSSC 16	NRRL 34123	India, human eye	DQ247192	DQ094645	DQ236687	EU329635
Fusarium paranaense		CML 1830^{T}	Brazil, Soybean root	KF597797			KF680011
		CML 1833	Brazil, Soybean root	KF597798			KF680012
Fusarium petroliphilum	FSSC 1	NRRL 22141	New Zealand, cucurbit	AF178329	DQ094307	DQ236349	EU329491
	FSSC 1	NRRL 43812	USA, contact lens solution	EF453054	EF453205	EF453205	EF470093
Fusarium solani f. sp. *pisi*	FSSC 11	NRRL 22820	USA, *Glycine max*	AF178355	DQ094310	DQ236352	EU329532
	FSSC 11	NRRL 45880	USA, Lab cross T10 (pea) and T219 (soil)	FJ240352	EU329689	EU329689	EU329640
Fusarium solani f. sp. *batatas*	FSSC 23	NRRL 22400	USA, *Ipomoea batatas*	AF178343	AF178407	DQ236345	EU329509
Fusarium solani f. sp. *xanthoxyli*	FSSC 22	NRRL 22163	Japan, *Xanthoxylum* sp.	AF178336	AF178401	AF178370	FJ240380
Fusarium striatum	FSSC 21	NRRL 22101	Panama, cotton cloth	AF178333	AF178398	AF178367	EU329490
Neocosmospora ambrosia	FSSC 19	NRRL 20438	India, *Camellia sinensis*	AF178332	AF178397	DQ236357	JX171584
	FSSC 19	NRRL 22346	India, *Camellia sinensis*	FJ240350	EU329669	EU329669	EU329503
Neocosmospora croci		CBS 142423^{T} = CPC 27186	Italy, *Citrus sinensis*	LT746216	LT746264	LT746264	LT746329
Neocosmospora croci		CPC 27187	Italy, *Citrus sinensis*	LT746217	LT746265	LT746265	LT746330
Neocosmospora cyanescens	FSSC 27	CBS 518.82^{T} = NRRL 37625	Netherlands, human foot	FJ240353	EU329684	EU329684	EU329637
Neocosmospora falciformis	FSSC 3+4	NRRL 32757	USA, sand	DQ247075	DQ094536	DQ236578	EU329614
	FSSC 3+4	NRRL 32828	USA, human	DQ247135	DQ094594	DQ236636	EU329626
Neocosmospora illudens		NRRL 22090	New Zealand, *Beilschmiedia tawa*	AF178326	AF178393	AF178362	JX171601
Neocosmospora macrospora		CBS 142424^{T} = CPC 28191	Italy, *Citrus sinensis*	LT746218	LT746266	LT746281	LT746331
		CPC 28192	Italy, *Citrus sinensis*	LT746219	LT746267	LT746282	LT746332

Table 1. (Continued).

Species	Clade number[a]	Strain number[b]	Country and substrate	GenBank/EBI accession number[c]			
				EF-1α	ITS	LSU	*RPB2*
		CPC 28193	Italy, *Citrus sinensis*	LT746220	LT746268	LT746283	LT746333
Neocosmospora perseae		CBS 144142T[#] = CPC 26829	Italy, *Persea americana*	**LT991902**	**LT991940**	**LT991947**	**LT991909**
		CBS 144143[#] = CPC 26830	Italy, *Persea americana*	**LT991903**	**LT991941**	**LT991948**	**LT991910**
		CBS 144144 = CPC 26831	Italy, *Persea americana*	**LT991904**	**LT991942**	**LT991949**	**LT991911**
		CBS 144145 = CPC 26832	Italy, *Persea americana*	**LT991905**	**LT991943**	**LT991950**	**LT991912**
		CBS 144146 = CPC 26833	Italy, *Persea americana*	**LT991906**	**LT991944**	**LT991951**	**LT991913**
Neocosmospora plagianthi		NRRL 22632	New Zealand, *Hoheria glabrata*	AF178354	AF178417	AF178386	JX171614
Neocosmospora pseudensiformis	FSSC 33	NRRL 22354	French Guiana, bark	AF178338	AF178402	DQ236358	EU329504
Neocosmospora solani	FSSC 5	CBS 140079[ET] = NRRL 66304	Slovenia, *Solanum tuberosum*	KT313611	KT313633	KT313633	KT313623
	FSSC 5	CPC 27736	Italy, *Ficus carica*	**LT991907**	**LT991945**	**LT991952**	**LT991914**
	FSSC 5	CPC 27737	Italy, *Ficus carica*	**LT991908**	**LT991946**	**LT991953**	**LT991915**
	FSSC 5	NRRL 32741	USA, human eye	DQ247061	DQ094522	DQ236564	EU329608
Neocosmospora sp.	FSSC 6	CBS 143194 = NRRL 22782	Spain, human corneal ulcer	DQ246850	EU329670	EU329670	EU329528
	FSSC 6	CBS 143210 = NRRL 32785	USA, human toenail cancer	DQ247094	*	FJ240371	EU329618
	FSSC 7	CBS 130181 = NRRL 43502	USA, human eye	DQ790488	DQ790532	DQ790532	DQ790576
	FSSC 7	CBS 143209 = NRRL 32770	USA, human eye	DQ247083	DQ094544	DQ236586	EU329615
	FSSC 9	CBS 143208 = NRRL 32755	USA, turtle head lesion	DQ247073	DQ094534	DQ236576	EU329613
	FSSC 10	NRRL 22098	USA, cucurbit	DQ247073	DQ094534	DQ236576	EU329613
	FSSC 10	NRRL 22153	Panama, cucurbit	AF178346	DQ094302	DQ236344	EU329492
	FSSC 12	CBS 143212 = NRRL 32821	USA, turtle eggs	DQ247128	DQ094587	DQ236629	EU329625
	FSSC 12	NRRL 22642	Japan, *Penaceous japonicus*	DQ246844	DQ094329	DQ236371	EU329522
	FSSC 13	NRRL 22161	Japan, *Robinia pseudoacacia*	AF178330	DQ094311	DQ236353	EU329494
	FSSC 13	NRRL 22586	Japan, *Robinia pseudoacacia*	AF178353	AF178416	AF178385	EU329516
	FSSC 14	NRRL 32705	USA, human skin	DQ247025	DQ094488	DQ236530	EU329594
	FSSC 14	NRRL 32736	USA, human eye	DQ247056	DQ094517	DQ236559	EU329605
	FSSC 17	NRRL 22157	Japan, *Morus alba*	AF178359	DQ094306	DQ236348	EU329493
	FSSC 17	NRRL 22230	Japan, *Morus alba*	AF178358	DQ094305	DQ236347	EU329499
	FSSC 18	NRRL 31158	USA, human	DQ246916	DQ094389	DQ236431	EU329559
	FSSC 18	NRRL 32301	USA, human eye	DQ246929	EU329677	EU329677	EU329567

Table 1. (Continued).

Species	Clade number[a]	Strain number[b]	Country and substrate	GenBank/EBI accession number[c]			
				EF-1α	ITS	LSU	*RPB2*
	FSSC 20	CBS 143214 = NRRL 32858	USA, human wound	DQ247163	DQ094617	DQ236659	EU329630
	FSSC 20	NRRL 28001	USA, human skin	DQ246866	DQ094348	DQ236390	EF470129
	FSSC 24	CBS 117481 = NRRL 22389	USA, *Liriodendron tulipifera*	AF178340	AF178404	DQ236356	EU329506
	FSSC 25	CBS 130328 = NRRL 31169	USA, human oral wound	DQ246923	DQ094396	DQ236438	KR673999
	FSSC 26	NRRL 28541	USA, human synovial fluid	DQ246882	EU329674	EU329674	EU329542
	FSSC 28	CBS 109028 = NRRL 32437	Switzerland, human subcutaneous nodule	DQ246979	DQ094446	DQ236488	EU329581
	FSSC 29	NRRL 28008	USA, human	DQ246868	DQ094350	DQ236392	EF470135
	FSSC 30	NRRL 22579	Indonesia, tree bark	AF178352	AF178415	AF178384	EU329515
	FSSC 31	NRRL 22570	Brazil, *Piper nigrum*	AF178360	AF178422	AF178391	EU329513
	FSSC 32	NRRL 22178	Venezuela, dicot tree	AF178334	AF178399	AF178368	EU329498
	FSSC 34	NRRL 46703	Spain, nematode	HM347126	EU329712	EU329712	EU329661
	FSSC 35	NRRL 46707	Brazil, human	HM347127	EU329716	EU329716	EU329665
	FSSC 37	NRRL 25137	New Guinea, diseased cocoa pods	JF740757	JF740899	JF740899	JF741084
	FSSC 37	NRRL 25138	New Guinea, diseased cocoa pods	DQ247537	JF740900	JF740900	JF741085
	FSSC 38	NRRL 52781	Benin, *Hypothenemus hampei* adult	JF740849	*	*	JF741175
	FSSC 38	NRRL 52782	Benin, *Hypothenemus hampei* adult	*	JF740850	JF740850	JF741176
	FSSC 38	NRRL 52783	Benin, *Hypothenemus hampei* adult	JF740851	*	*	JF741177
	FSSC 39	FRC S-2432	USA, building	JN235756	JN235326	JN235326	JN235941
	FSSC 43	NRRL 54992	USA, Zebra shark multiple tissues	KC808213	KC808255		KC808354
	FSSC 43	NRRL 54993	USA, Zebra shark multiple tissues	KC808214	KC808256		KC808355
	FSSC 45	NRRL 62797	USA, *Xylosandrus compactus*	KF906129	KF906130	KF906130	KF906132
Neocosmospora vasinfecta	FSSC 8	CBS 130182 = NRRL 43467	USA, human eye	EF452940	EF453092	EF453092	EF469979
	FSSC 8	NRRL 22436	South Africa, soil	AF178348	AF178412	DQ236359	JX171610

[a] Clade nomenclature follows O'Donnell *et al.* (2008, 2016).

[b] CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CPC: Culture collection of P.W. Crous, housed at Westerdijk Fungal Biodiversity Institute; CML: Coleção Micológica de Lavras, Universidade Federal de Lavras, Minas Gerais, Brazil; F: College of Forestry, Northwest A&F University, Taicheng Road, Yangling, Shaanxi China; FRC: Fusarium Research Center, University Park, PA, USA; NRRL: Agricultural Research Service, Peoria, IL, USA. Ex- and ex-epitype strains are indicated with [T], and [ET], respectively. [#] Strains used in the pathogenicity tests.

[c] *EF-1α*: Translation elongation factor 1-alpha; ITS: Internal transcribed spacer regions of the rDNA and 5.8S region; LSU: Partial large subunit of the rDNA; *RPB2*: RNA polymerase II largest subunit. * Sequences not publicly available, provided as DNA datasets by Kerry O'Donnell.

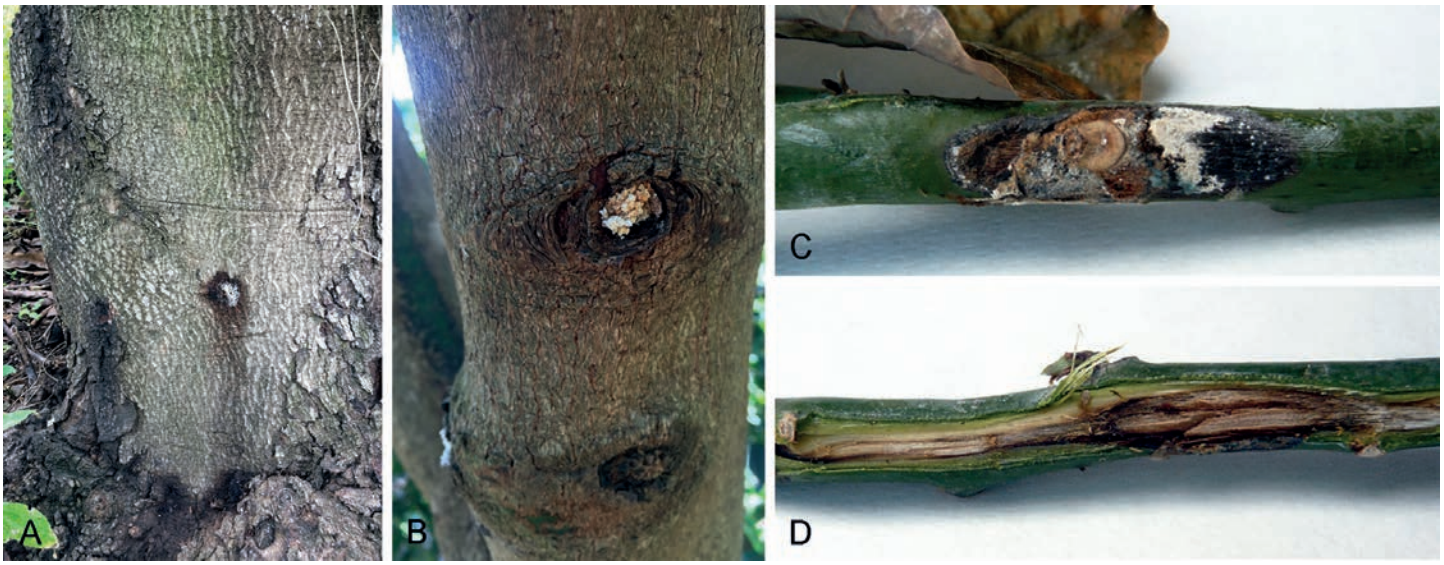


Fig. 1. Natural and artificial symptoms referable to *Neocosmospora perseae.* **A, B**. Sugar exudation from avocado trunk cankers. **C, D**. External and internal canker caused by *N. perseae* inoculation.

clade encompassing the five strains from cankers on *P. americana* (CPC 29829 to 26833) correspond to a new linage in *Neocosmospora* (BS 96 / PP 1), closely related to the unnamed phylogenetic species FSSC 37 and 38, and clearly unrelated with the common *Persea* pathogens in the Ambrosia clade of *Neocosmospora* (clade nomenclature according to O'Donnell *et al*. 2008, 2016). The new lineage is proposed here as the new species *Neocosmospora perseae*.

Pathogenicity tests

Two *Neocosmospora* isolates tested were pathogenic to the *Persea americana* seedlings inoculated, and produced symptoms similar to those observed on diseased plants in the avocado orchard. Canker and internal discolouration symptoms were observed corresponding to inoculation points on avocado plants. Initial symptoms were observed after 1 mo. High DI (100 %) was observed after 3 mo with serious symptoms leading to plant death (Fig. 1). Similar results were obtained in both tests performed.

The pathogen was re-isolated from the artificially inoculated plants and identified as previously described, completing Koch's postulates. No symptoms were observed on control plants.

TAXONOMY

Neocosmospora perseae Sandoval-Denis & Guarnaccia, ***sp. nov.*** MycoBank MB824587. Fig. 3.

Etymology: Named after the host genus *Persea*.

Sporulation abundant from conidiophores formed directly on the substrate and aerial mycelium, and from sporodochia. *Conidiophores* straight to slightly flexuous, up to 350 µm tall, solitary and simple or branched one to several times irregularly and laterally, verticillately or sympodially, each branch bearing a single terminal monophialide; *phialides* subulate to subcylindrical, smooth- and thin-walled, (40.5–)45–66.5(–90.5) µm long, (2–)2.5–3(–3.5) µm wide at the base, tapering to (1–)1.5–2(–2.5) µm wide at the apex, often with conspicuous periclinal thickening and a minute, discrete collarette; *conidia* formed on aerial conidiophores, hyaline, obovoid, ellipsoidal, short clavate to cylindrical, symmetrical or gently bent dorsoventrally, smooth- and thin-walled, 0(–1)-septate, (4.5–)6–10.5(–13.5) × (1.5–)2.5–4(–6) µm, clustering in false heads at the tip of monophialides. *Sporodochia* at first white to cream-coloured, becoming pale luteous, green to dark blue-green when mature, formed abundantly on the surface of carnation leaves and lately on and under the agar surface. *Conidiophores* in sporodochia 26–54 µm tall, densely packed in a cushion-like structure, irregularly or verticillately branched, with terminal branches bearing verticills of 1–3 monophialides; *sporodochial phialides* doliiform, subulate to subcylindrical, (13.5–)14.5–18.5(–20.5) × 2.5–3.5(–4.5) µm, smooth- and thin-walled, with periclinal thickening and an inconspicuous apical collarette. *Sporodochial conidia* falcate, wedge-shaped, tapering toward the basal part, robust; smaller sized conidia often conspicuously curved; large sized conidia somewhat straight on its ventral line with a moderate dorsal curvature; apical cell blunt, more or less equally sized than the adjacent cell; basal cell distinctly notched, (3–)4–5(–6)-septate, hyaline, thick- and smooth-walled. Three-septate conidia: 30.5–32.5 × 5–5.5 µm; four-septate conidia: (39–)40.5–47(–49) × 5–5.5(–6.5) µm; five-septate conidia: (39.5–)45.5–51.5(–56) × (4.5–)5.5–6(–6.5) µm; six-septate conidia: 49–53.5(–55) × (5–)6–7 µm; overall (30.5–)43.5–52(–55.5) × (4.5–)5.5–6(–7) µm. *Chlamydospores* abundant and rapidly formed on agar media (approx. 7 d), hyaline to pale brown, spherical to subspherical (4.5–)6–8(–9) µm diam, solitary or in chains, terminal, intercalary or borne on short lateral pegs, smooth- and thick-walled.

Cardinal temperatures for growth: Minimum 9 °C, maximum 36 °C, optimum 27–30 °C.

Culture characteristics: *Colonies* on PDA showing radial growth rates of 4.4–7.2 mm/d at 27 °C and 4.1–6.8 mm/d at 30 °C in the dark, reaching a diameter of 72–74 mm after 7 d at 24 °C. Colony surface straw to pale luteous, flat, felty to floccose, aerial mycelium and sporulation abundant, white, becoming pale luteous to sulphur yellow; colony margins regular and filiform. Reverse amber to sulphur yellow, becoming bright red to scarlet with the production of abundant diffusible pigment. Colonies on OA showing a diameter of 62–66 mm after 7 d at 24 °C. Colony colour white with sienna to umber patches, flat to slightly umbonate and radiate, felty to floccose, aerial mycelium and sporulation abundant; margins filiform and slightly undulate. Reverse pale luteous with slight production of a scarlet to sienna coloured diffusible pigment.

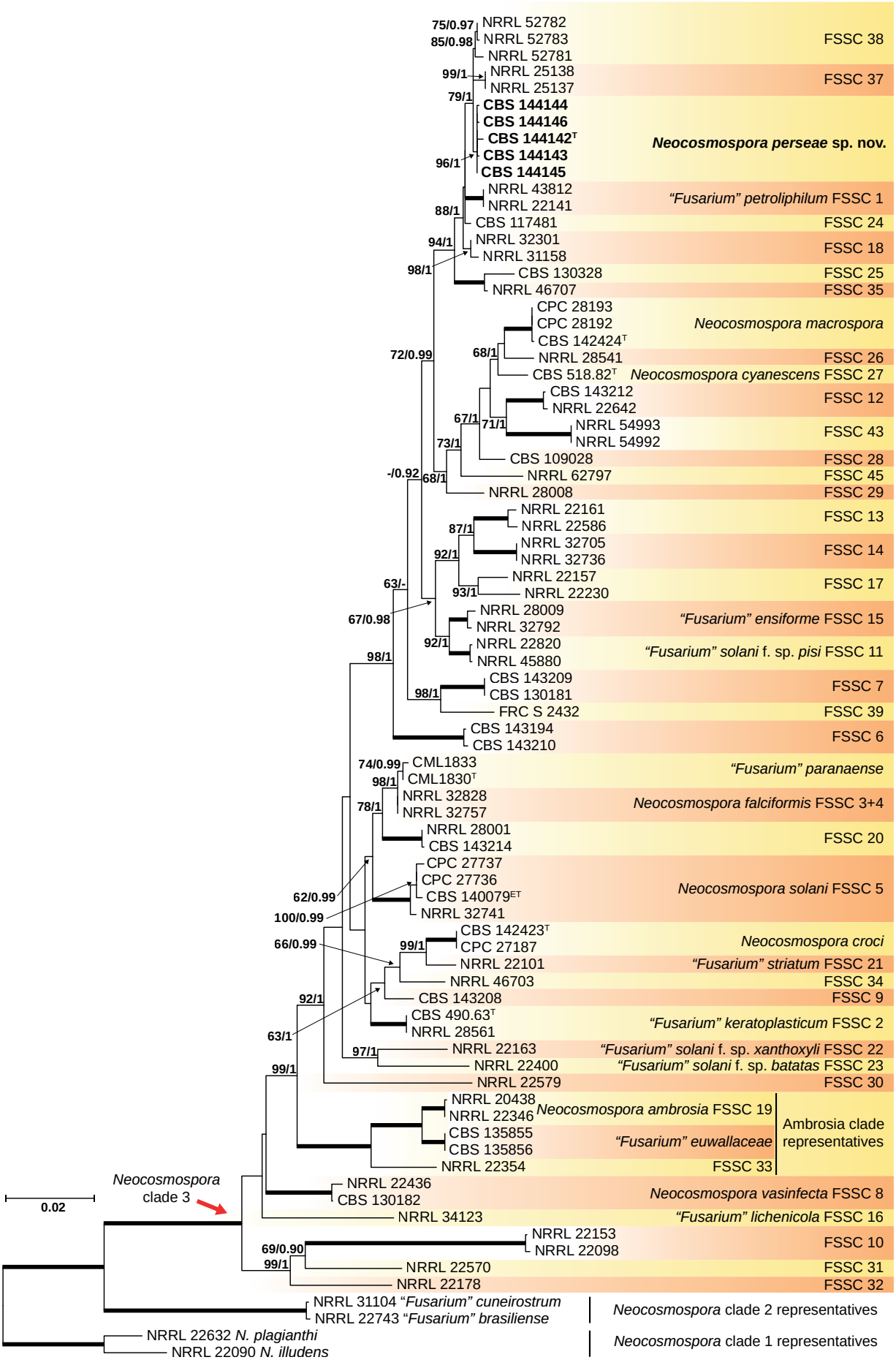
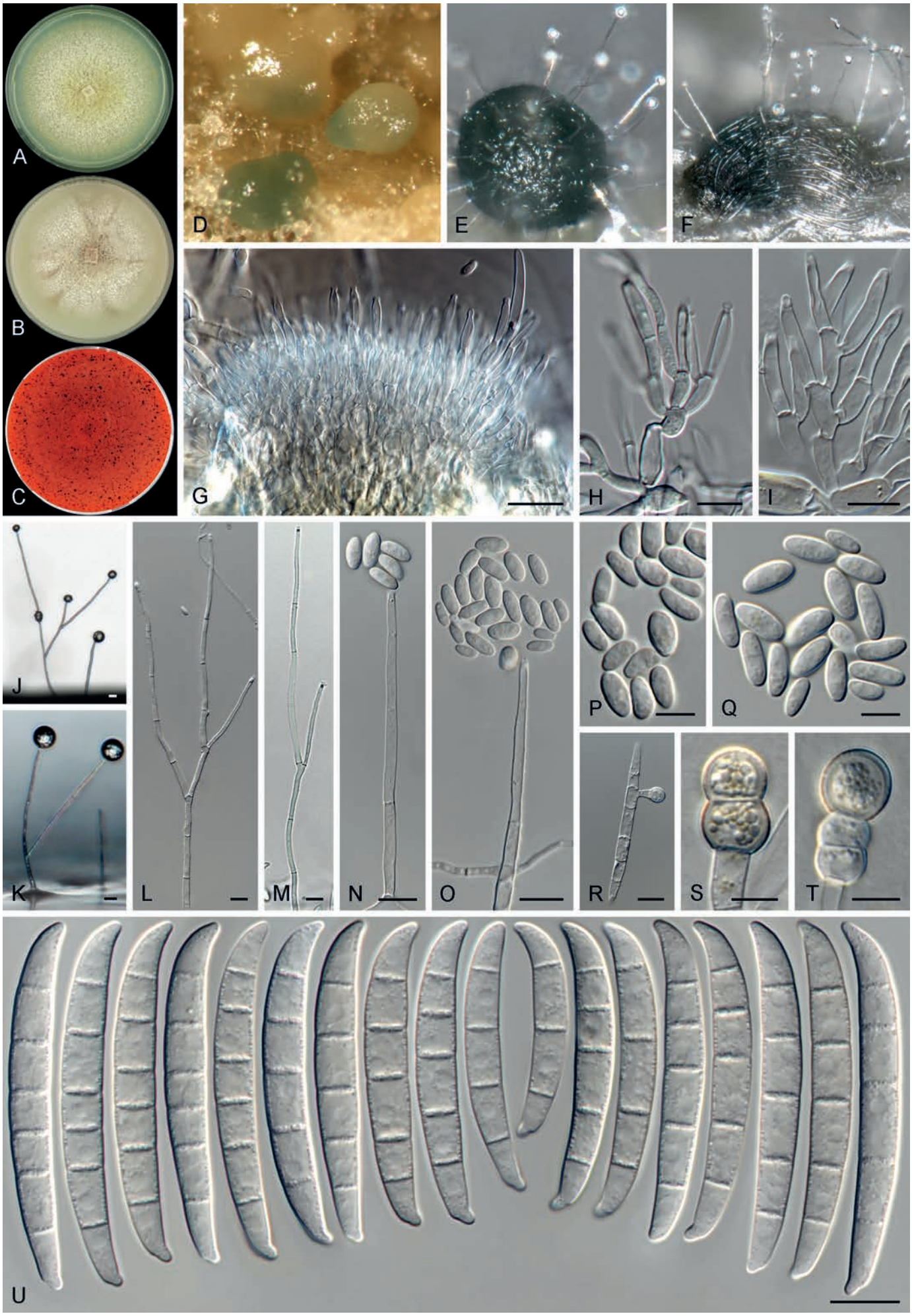


Fig. 2. Maximum-likelihood (ML) phylogram of the genus *Neocosmospora* obtained from combined *EF-1α*, ITS, LSU and *RPB2* sequences. Branch lengths are proportional to distance. Numbers on the nodes are ML bootstrap values (BS) above 55 %; and Bayesian posterior probability values (PP) above 0.95. Full supported branches (BS = 100 and PP = 1) and isolates obtained from *Persea americana* are indicated in bold. Ex-type and ex-epitype strains are indicated with ^{T}, and ^{ET}, respectively.



Typification: **Italy**, Catania, San Leonardello, from trunk canker lesions on *Persea americana*, 25 Mar. 2015, *G. Polizzi* (holotype CBS H-23433, culture ex-type CBS 144142 = CPC 26829).

Additional isolates examined: **Italy**, Catania, San Leonardello, from trunk canker lesions on *Persea americana*, 25 Mar. 2015, *G. Polizzi* (CBS 144143 = CPC 26830; CBS 144144 = CPC 26831; CBS 144145 = CPC 26832; CBS 144146 = CPC 26833).

DISCUSSION

In this study, five *Neocosmospora* isolates were recovered from *Persea americana* trees showing trunk canker symptoms in Sicily (Southern Italy) during 2015, and identified based on single and multilocus phylogenetic analyses of four loci (*EF-1α*, ITS, LSU and *RPB2*), as well as morphological characters. These analyses revealed that the five isolates belonged to a novel species, described here *N. perseae*.

The robust four-loci based analysis allowed to distinguish *N. perseae* from "*Fusarium*" *euwallaceae* and *N. ambrosia*, already known as canker-causing species associated with symbiotic *Euwallacea* beetles. In spite of the recent detection of similar cankers caused by other fungal species in the same area (Guarnaccia *et al*. 2016), *N. perseae* was found as the only fungus associated with the disease. Because cankers developed in the absence of *Euwallacea* beetles, the fungus is clearly able to cause wood cankers independently. Furthermore, pathogenicity tests confirmed that *N. perseae* causes a high disease incidence on *Persea americana*, thereby fulfilling Koch's postulates.

Neocosmospora perseae was clearly not related phylogenetically or morphologically with the most significant *Neocosmospora* canker pathogens affecting *Persea*, known to belong to the Ambrosia clade (Aoki *et al*. 2018). Moreover, while the new species exhibits the typical hyaline, falcate and multiseptate macroconidia and short clavate to cylindrical microconidia commonly attributed to this genus, the *Persea* pathogens in the Ambrosia clade of *Neocosmospora* are characterised by their irregularly clavate, somewhat swollen conidia, a putative evolutionary adaptation to its host (Freeman *et al.* 2013). Additionally, all currently known members of the Ambrosia clade exhibit a symbiotic lifestyle, associated with species of the shot hole borer beetle genus *Euwallacea* (Coleoptera, Xyleborini) (Mendel *et al.* 2012, Freeman *et al.* 2013, Kasson *et al.* 2013). In contrast, *N. perseae* showed no evidence of association with any vector, as demonstrated by the absence of wood galleries or any other sign of insect infestation in the trees. Its transmission is therefore more likely to respond to soil contamination and plant-associated reservoirs. Furthermore, the new species proved to be genetically closely related to two undescribed lineages (FSSC 37 and FSSC 38), yet, being phylogenetically and ecologically distinct. So far, phylogenetic species FSSC 37 is only known from diseased cocoa pods in New Guinea. However, FSSC 38, known from Benin & Uganda, has been isolated from the coffee borer beetle *Hypothenemus hampei* (Coleoptera, Scotylini) (O'Donnell *et al.* 2012), a relative to *Euwallacea* beetles. Similarly, the unrelated phylogenetic species FSSC 45 is known to inhabit the abdomen and external surfaces of *Xylosandrus compactus* (Coleoptera, Xyleborini) and its galleries (Bateman *et al*. 2016), which could suggest either that a similar insect-fungus mutualism or opportunism could also exist in other *Neocosmospora* lineages. However, no clear indication exists of FSSC 38 or FSSC 45 having either a pathogenic or symbiotic lifestyle with their insect hosts.

This study has revealed and characterised a new pathogenic fungal species, *N. perseae,* associated with trunk cankers on avocado in Italy, and includes information on its pathogenicity. As no epidemiological data are yet available it is not possible to suggest any control strategies to avoid *N. perseae* infections. Previous studies in the same geographical area have revealed a diversity of soil-borne fungal species (Polizzi *et al*. 2012, Vitale *et al*. 2012), including species pathogenic to avocado trees (Dann *et al*. 2012). Thus, these and other diseases might threaten avocado production, and could become a major limiting factor for future production.

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Fig. 3. *Neocosmospora perseae* (from ex-type CBS 144142). **A, B.** Colonies on PDA and OA, respectively, after 7 d at 24 °C in the dark. **C.** Colony on PDA after 20 d at 24 °C under continuous white light. **D–F.** Sporodochia formed on the surface of carnation leaves. **G–I.** Sporodochial conidiophores. **J–O.** Aerial conidiophores and phialides. **P, Q.** Aerial conidia (microconidia). **R–T.** Chlamydospores. **U.** Sporodochial conidia (macroconidia). Scale bars: P, Q, S, T = 5 µm, G = 20 µm, all others = 10 µm.

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The culturable mycobiota associated with three Atlantic sponges, including two new species: *Thelebolus balaustiformis* and *T. spongiae*

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two new taxa

Abstract: Covering 70 % of Earth, oceans are at the same time the most common and the environment least studied by microbiologists. Considering the large gaps in our knowledge on the presence of marine fungi in the oceans, the aim of this research was to isolate and identify the culturable fungal community within three species of sponges, namely *Dysidea fragilis*, *Pachymatisma johnstonia* and *Sycon ciliatum*, collected in the Atlantic Ocean and never studied for their associated mycobiota. Applying different isolation methods, incubation temperatures and media, and attempting to mimic the marine and sponge environments, were fundamental to increase the number of cultivable taxa. Fungi were identified using a polyphasic approach, by means of morpho-physiological, molecular and phylogenetic techniques. The sponges revealed an astonishing fungal diversity represented by 87 fungal taxa. Each sponge hosted a specific fungal community with more than half of the associated fungi being exclusive of each invertebrate. Several species isolated and identified in this work, already known in terrestrial environment, were first reported in marine ecosystems (21 species) and in association with sponges (49 species), including the two new species *Thelebolus balaustiformis* and *Thelebolus spongiae*, demonstrating that oceans are an untapped source of biodiversity.

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INTRODUCTION

Water covers almost 70 % of our planet. Nonetheless, its biodiversity and habitats remain largely unexplored. For the last 580 M years, oceans have been hosting the most ancient metazoans on Earth: sponges. *Porifera* contains more than 8 600 described species and about 15 000 species waiting to be discovered by scientists (Webster & Thomas 2016). Sponges are key components of marine ecosystems, because of their incredible ability to filter seawater: according to recent estimates, they are able to process 24 000 L/kg of seawater per day and to detain over 80 % of its particles (Taylor *et al.* 2007, Rozas *et al.* 2011).

Over the millions of years of their evolution, species of *Porifera* have formed a close association with a wide variety of microorganisms including bacteria, archaea, fungi, and algae (Taylor *et al.* 2007). This close association was described for the first time by Vacelet & Donadey (1977), who observed bacteria within sponges' tissues. Today, it is well recognised that microorganisms can represent up to 40–60 % of the sponge biomass (Yarden 2014). The term "sponge holobiont", is used when sponges and the associated microbial communities are considered as a whole (He *et al.* 2014). Different degrees of complexity characterise the interactions among sponge holobiont components, including mutualism, commensalism and parasitism (Rodríguez-Marconi 2005). Non-pathogenic microorganisms can positively contribute to sponge metabolism, by increasing the uptake of carbon, nitrogen and sulphur. Furthermore, by the production of secondary metabolites, they could be involved in host defence systems and in the regulation of the microbial community associated with sponges (Taylor *et al*. 2007). Interestingly, metabolites previously ascribed to sponges were recognised to be structurally similar to those produced by the associated bacteria (Imhoff & Stöhr 2003). As a consequence, the use of microorganisms for the bio-discovery of new molecules would avoid several problems related to the use of sponges. The isolation of new molecules and their production in the required amount from sponges is always very problematical for reasons such as their rare occurrence, difficulties with sponge collections, or irreproducible production of metabolites due to specimen variability (Imhoff & Stöhr 2003). Nowadays studies on microorganisms associated with sponges are primarily focused on prokaryotic organisms while the fungal community remain less studied, despite recent results emphasizing its great biodiversity and biotechnological potential (Raghukumar 2012). Fungi represent suitable biotechnological resources but require specific expertise for the isolation and the correct identification. Many taxa already known for their bioactivity lack a precise identification and correct preservation in culture collections hampering their possible future exploitation as recently highlighted by the 2nd International Conference of Marine Fungal Natural Products (MaFNaP 2017).

In this study we present for the first time the mycobiota associated with *Dysidea fragilis*, *Pachymatisma johnstonia* (*Demospongiae*), and *Sycon ciliatum* (*Calcarea*). The two *Demospongiae* have been extensively examined for their production of secondary metabolites. The metabolome of *D. fragilis* was characterised by Yu *et al.* (2006), although its biological activity included only a single compound, which acted as fish feeding deterrent (Marin *et al.* 1998). *Pachymatisma johnstonia* is also well known for its production of secondary metabolites, whose anticancer (Zidane *et al.* 1996, Ferreira *et al.* 2011) and antibacterial (Warabi *et al.* 2004) activity has been demonstrated. On the contrary, the metabolome of *S. ciliatum* has never been studied.

In light of the above-mentioned considerations, it is likely that microorganisms, including fungi, could be the true producers of the bioactive molecules isolated so far but could also be a source of additional novel compounds of interest. Aiming at the biotechnological exploitation of new molecules, the scope of this study was to isolate and identify the fungal community associated with three Atlantic sponges, with particular emphasis on the proper systematic identification of taxa. Moreover, in this paper two novel *Thelebolus* species are described.

MATERIAL AND METHODS

Sampling sites and axenic isolation

The sponges *Dysidea fragilis* and *Pachymatisma johnstonia* (three specimens each) were collected by scuba divers in Gurraig Sound (Co. Galway, Ireland; N 53°, 18.944; W 09°, 40.140). The sampling site was at 15 m depth, characterised by fairly strong current and suspended sediments. Three specimens of *Sycon ciliatum* exposed to a fast water flow due to the tide going out were collected in Coranroo rapids (Co. Clare, Ireland; N 053°09.100, W 009°,00. 550).

Specimens were surface sterilised with ethanol 70 % (for 30 s) to prevent contaminants and serially washed (three times) in artificial sterile Sea Water (SW) to get rid of unrefined sediments and to wash out propagules, in order to leave only fungi actively growing on the surface or into the sponge tissues.

Working in sterile conditions, the sponge samples were divided into three parts to be used for two different fungal isolation techniques and for a taxonomic voucher of the sponge. For the first isolation method, one third of the sponge was further cut in 20 pieces of about 0.5 cm^3 by means of sterile tools and directly plated in Petri dishes (six cm Ø) containing two different media: Sea Water Agar – SWA (Sea Salts 30 g, Agar 15 g, up to 1 L dH_2O) and Corn Meal Agar Sea Water - CMASW (Corn Meal 2 g, Agar 15 g, Sea Salts 30 g, up to 1 L dH_2O). Five replicates for each medium and incubation temperatures (15 °C and 25 °C) were performed.

Approximately 5 g of each sample were also homogenised (homogenizer blade Sterilmixer II - PBI International) and diluted 1:10 w/v in SW. One mL of suspension was included in Petri dishes (nine cm Ø) containing CMASW or Gelatin Agar Sea Water–GASW (Gelatin 20 g, Sea Salts 30 g, Agar 15 g, up to 1 L dH_2O), rich in collagen and mimicking sponge tissue composition. Five replicates for each medium and incubation temperatures (15 °C and 25 °C) were performed. All media were supplemented with an antibiotic mix (Gentamicin Sulfate 40 mg/L, Piperacillin plus Tazobactam 11 mg/L) to prevent bacterial growth. Plates were incubated in the dark and periodically checked for 30 d to isolate slow growing fungi.

Fungal identification

Fungal morphotypes were isolated in pure culture and identified by means of a polyphasic approach combining morpho-physiological and molecular features. After determination of genera via macroscopic and microscopic features (Domsch *et al.* 1980, Von Arx 1981, Kiffer & Morelet 1997), fungi were transferred to genus-specific media (Klich 2002, Braun *et al.* 2003, Samson & Frisvad 2004).

In parallel for molecular analyses, fungi were pre-grown on Malt Extract Agar - MEA (Malt Extract 20 g, Glucose 20 g, Peptone 2 g, Agar 20 g, up to 1 L dH_2O) at 25 °C for 1 wk, for fast growing fungi, and from 2–4 wk for slow growing fungi. DNA was extracted using the NucleoSpin kit (Macherey Nagel GmbH, Duren, DE, USA), according to the manufacturer instructions. Based on the taxonomic assignment attributed to each fungus by morphological observations, specific primers were used for PCR as detailed in Table 1. Briefly, PCR reactions were performed in 50 µL final volumes and consisted of 0.5 µL Taq DNA Polymerase (Qiagen 5 U/µL), 10 µL PCR Buffer (10 ×), 2.5 µL dNTP Mixture (dATP, dCTP, dGTP, dTTP; 10 mM), 2 µL $MgCl_2$ (25 mM), 2.5 µL of each primer (10 µM), 1 µL genomic DNA extract (80 ng/mL) and 34 µL distilled-deionised water. PCR products were visualised under UV light (BIO-RAD Universal Hood II) on 1.5 % agarose electrophoresis gels stained with ethidium bromide. Macrogen, Inc. (Seoul, South Korea) Europe Lab carried out the purification and sequencing of PCR products.

Taxonomic assignments were based both on high percentage homologies with sequences available in public databases (GenBank - NCBI database and CBS-KNAW Collection, Westerdijk Fungal Biodiversity Institute) and the consistency of morphological features with available literature descriptions. The taxonomic position of doubtful strains (low homologies with sequences available in public databases) or sterile mycelia (i.e. not showing morphological features useful to confirm taxonomical assignments) were inferred via molecular phylogenetic analyses based on DNA sequences from the large ribosomal subunit LSU, (Vilgalys & Hester 1990). Separate alignments were created for the orders *Pleosporales*, *Capnodiales* and *Chaetothyriales* and two for the classes *Leotiomycetes* and *Sordariomycetes*. Alignments were generated using MEGA v. 7.0 and manually refined. Phylogenetic analyses were performed using a Bayesian Inference (BI; MrBayes v. 3.2.2 four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC), run over 10 M generations, (under GTR + Γ + I evolutionary model approach). BPP values are reported in the resulting trees. A full alignment of the dataset was submitted to TreeBASE (submission number 21746).

Representative strains of each species isolated in pure culture during this work are preserved at *Mycotheca Universitatis Taurinensis* (MUT- www.mut.unito.it) of the Department of Life Sciences and Systems Biology, University of Turin (Italy). The Accession numbers of the sequences deposited in GenBank are available in the supplementary material 1.

Table 1. Gene loci sequenced, primers for molecular analysis and PCR programs.

Fungi	Gene loci and DNA regions sequenced[a]	Primers (Forward and Reverse)	PCR amplification Conditions	References for primers[b]
Alternaria	*GAPDH*	GPD1 and GPD2	96 °C: 2 min, (96 °C: 1 min, 50 °C: 1 min, 72 °C: 50 sec) × 35 cycles; 72 °C: 5 min	(1)
Aspergillus	*CAL*	CL1 and CL2a	95 °C: 10 min, (95 °C: 50 sec, 55 °C: 50 sec, 72 °C: 1 min) × 35 cycles; 72 °C: 7 min	(2)
Aspergillus, Penicillium, Thelebolus	*TUB*	BT–2a and BT–2b	94 °C: 4 min, (94 °C: 35 sec, 58 °C: 35 sec, 72 °C: 50 sec) × 35 cycles; 72 °C: 5 min	(3)
Cladosporium	*ACT*	ACT–512F and ACT–783R	94 °C: 8 min, (94 °C: 15 sec, 61 °C: 20 sec, 72 °C: 40 sec) × 35 cycles; 72 °C: 10 min	(4)
Yeast like fungi (*Holtermanniella, Metschnikowia, Pseudozyma*, Sporidiobolales)	D1-D2	NL1-NL2	94 °C: 4 min, (94 °C: 1 min, 52 °C: 35 sec, 72 °C: 1.5 min) × 35 cycles; 72 °C: 5 min	(5)
Alternaria, Thelebolus, sterile mycelia and taxa for whom no specific primers are required	ITS	ITS1 and ITS4	95 °C: 5 min, (95 °C:40 s, 55 °C: 50 s, 72 °C: 50 sec) × 35 cycles; 72 °C: 8 min	(6)
Sterile mycelia	LSU	LROR and LR7	95 °C: 5 min, (95 °C: 1 min, 50 °C: 1 min, 72 °C: 2 min) × 35 cycles; 72 °C: 10 min	(7)

[a] *GAPDH*: partial glyceraldehyde-3-phosphate dehydrogenase gene; *CAL*: partial calmodulin gene; *TUB*: partial beta-tubulin gene; *ACT*: partial actin gene; D1-D2: D1-D2 region of the nuclear ribosomal DNA large subunit; ITS: internal transcribed spacer regions and intervening 5.8S nrRNA gene; LSU: partial nuclear ribosomal DNA large subunit.

[b](1) Berbee *et al*. 1999, (2) O'Donnell *et al*. 2000, (3) Glass & Donaldson 1995, (4) Carbone & Kohn 1999, (5) De Barros Lopes *et al*. 1998, (6) White *et al*. 1990, (7) Vilgalys & Hester 1990.

Table 2. Fungal taxa isolated from *D. fragilis* (DF), *P. johnstonia* (PJ) and *S. ciliatum* (SY) and their relative abundance (RA) in percentage. The species already found in marine environment (MA) and associated with sponges (SP) are reported, as well as the first record (FR).

	RA %				
	DF	PJ	SY	MA	SP
Ascomycota					
Acremonium breve	0.6			(20)	FR
Acremonium implicatum	0.6			(1), (2), (3), (4), (5), (19)	(3)
Acremonium persicinum	1.3			(7)	(33)
Acremonium potronii[a]	5.8		1.0	(7), (34)	FR
Acremonium tubakii	5.8			(1), (34)	FR
Acremonium zonatum			2.9	FR	FR
Alternaria molesta[a]		1.4		(30)	FR
Alternaria sp.[a]			1.0	-	-
Aspergillus creber	4.5	2.7		(23)	FR
Aspergillus flavipes		5.4		(22)	(35)
Aspergillus fumigatus	2.6		1.0	(1), (4), (5), (7), (19)	(6), (18), (27)
Aspergillus jensenii	0.6	2.7		FR	FR
Aspergillus puulaauensis	3.8			FR	FR
Aureobasidium pullulans	1.3	2.7		(2), (7), (19), (22), (34)	(9), (36)
Beauveria bassiana	1.9		2.9	(7), (34)	(17), (37)
Bimuria novae-zelandiae[a]	0.6			(31)	FR
Boeremia exigua[a]	0.6			(32), (34)	FR
Botrytis cinerea	0.6			(5), (24), (34)	FR
Cadophora luteo-olivacea	0.6		4.9	(11)	FR
Cladosporium aggregatocicatricatum	0.6			FR	FR
Cladosporium allicinum	2.6	1.4	3.9	(2)	FR
Cladosporium cladosporioides	2.6	6.8	1.0	(1), (2), (5), (7), (19), (21), (22), (34)	(9), (12), (14), (15), (18), (27)
Cladosporium halotolerans		5.4	10.8	(5), (11), (22)	FR

Table 2. (Continued).

	RA %				
	DF	**PJ**	**SY**	**MA**	**SP**
Cladosporium perangustum	0.6			(25)	FR
Cladosporium pseudocladosporioides	2.6	4.1		(5)	FR
Cladosporium psychrotolerans	1.3			(22)	FR
Cladosporium subtilissimum	0.6			(22)	FR
Cladosporium subuliforme		2.7		FR	FR
Cladosporium xylophilum	0.6			FR	FR
Coniothyrium obiones[a]			1.0	(7)	FR
Cyphellophora sp.[a]			1.0	-	-
Emericellopsis alkalina (asexual morph)	1.3			FR	FR
Emericellopsis maritima (asexual morph)		1.4		(7)	FR
Emericellopsis pallida (asexual morph)	1.3			(7)	FR
Epicoccum nigrum			4.9	(5), (34)	(40)
Fusarium pseudograminearum	0.6			FR	FR
Fusarium solani			1.0	(26)	(3), (41)
Gremmenia infestans[a]			1.0	FR	FR
Hypocreaceae sp.[a]	0.6			-	-
Metschnikowia bicuspidata			5.9	(7), (22)	(38)
Microascaceae sp.[a]	0.6			-	-
Mollisia sp.[a]			1.0	-	-
Myrothecium cinctum[a]	1.3			(39)	(39)
Neocamarosporium betae			1.0	FR	FR
Neocamarosporium calvescens	0.6			FR	FR
Paraphaeosphaeria neglecta (asexual morph)	0.6		1.0	FR	FR
Penicillium antarcticum	10.9	37.8	18.6	(2), (5)	(16)
Penicillium brevicompactum	3.2			(1), (2), (3), (4), (5), (7), (19), (22), (34)	(3), (10), (27)
Penicillium canescens		1.4		(5), (21), (34)	(40)
Penicillium chrysogenum	12.2	13.5		(5), (7), (22), (34)	(3), (6), (10), (27), (40)
Penicillium citreonigrum			1.0	(5), (7), (21)	(40)
Penicillium inflatum	0.6			FR	FR
Penicillium janczewskii	5.8			(7), (34)	FR
Penicillium roqueforti			1.0	(40)	(40)
Penicillium spinulosum		2.7		(1), (7), (34)	FR
Penicillium thomii		1.4		(7), (34)	FR
Penicillium waksmanii	1.3			(7), (34)	(12)
Periconia minutissima			1.0	(7)	FR
Periconia sp.[a]		1.4		-	-
Phaeosphaeria olivacea[a]			1.0	(7)	FR
Phaeosphaeria oryzae[a]			1.0	FR	FR
Phaeosphaeriopsis sp.[a]	0.6		4.9	-	-
Pleosporales sp.[a]			1.0	-	-
Pleosporaceae sp.[a]	0.6			-	-
Pochonia suchlasporia	0.6			(34)	FR
Preussia sp.[a]	0.6			-	-
Pseudeurotium bakeri			1.0	FR	FR
Pseudocercosporella sp.[a]		1.4		-	-
Pyrenochaetopsis microspora[a]	1.9			FR	FR

Table 2. (Continued).

	RA %				
	DF	**PJ**	**SY**	**MA**	**SP**
Roussoellaceae sp.[a]	0.6			-	-
Sarocladium strictum			14.7	(2), (7), (34)	FR
Scopulariopsis brevicaulis			1.0	(5)	(6), (13)
Thelebolus balaustiformis	0.6			FR	FR
Thelebolus spongiae	0.6			FR	FR
Thyronectria sp.[a]			1.0	-	-
Tilachlidium brachiatum	0.6			(28)	FR
Tolypocladium album	1.9			FR	FR
Tolypocladium cylindrosporum	2.6	1.4	4.9	(29), (34)	FR
Volutella ciliata	0.6			(4)	(40)
Xanthothecium peruvianum	0.6			FR	FR
Basidiomycota					
Bjerkandera sp.[a]	0.6			-	-
Holtermanniella sp.[a]			1.0	-	-
Agarycomycetes sp.[a]	0.6			-	-
Pseudozyma sp.[a]		1.4		-	-
Sporidiobolales sp.[a]		1.4		-	-
Trametes gibbosa[a]	1.3			FR	FR
Mucoromycota					
Absidia glauca	0.6			(19)	FR
Total taxa	54	21	32		
Total exclusive taxa	39	11	21		

(1) Panno *et al*. 2013, (2) Gnavi *et al.* 2017, (3) Paz *et al.* 2010, (4) Costello *et al.* 2001, (5) Bovio *et al.* 2017, (6), Ding *et al.* 2011, (7) Jones *et al.* 2015, (8) Thirunavukkarasu *et al.* 2012, (9) Henríquez *et al.* 2014, (10) Passarini *et al.* 2013, (11) Garzoli *et al.* 2015, (12) Rozas *et al.* 2011, (13) Yu *et al.* 2008, (14) Manriquez *et al.* 2009, (15) San-Martin *et al.* 2005, (16) Park *et al.* 2014, (17) Yamazaki *et al.* 2012, (18) Sayed *et al.* 2016, (19) Oren & Gunde-Cimerman 2012, (20) Kis-Papo *et al.* 2001, (21) Raghukumar & Ravindran 2012, (22) Zajc *et al.* 2012, (23) Jurjevic *et al.* 2012, (24) Suryanarayanan 2012, (25) Liu *et al.* 2016, (26) Hatai 2012, (27) Pivkin *et al.* 2006, (28) Gomes *et al.* 2008, (29) Rämä *et al.* 2014, (30) Tóth *et al.* 2011, (31) Suetrong *et al.* 2009, (32) Di Piazza *et al.* 2017, (33) Fraser *et al.* 2013, (34) Rämä *et al.* 2017, (35) Ratnaweera *et al.* 2016, (36) Shigemori *et al.* 1998, (37) Zhang *et al.* 2017, (38) Baker *et al.* 2009, (39) Wang & Zhu 2008, (40) Wiese *et al.* 2011, (41) Bolaños *et al.* 2015.

[a]Sterile mycelia.

Thelebolus spp. growth conditions and molecular study

Thelebolus spp. MUT 2357 and MUT 2359 were pre-grown on Potato Dextrose Agar - PDA (Potato extract 4 g, dextrose 20 g, agar 15 g, up to 1 L dH_2O) at 25 °C and then inoculated in triplicate onto Petri dishes (9 cm Ø) containing MEA, PDA and Carrot Agar (grated carrot 20 g boiled and filtered, agar 20 g, up to 1 L dH_2O) alone and with different concentration of NaCl (2.5 %, 5 %, 10 %, 15 %) and incubated at 4 °C, 15 °C and 25 °C. The fungal growth, as well as macroscopic and microscopic features, were evaluated at three, seven, 10, 14, 17, 21 d after the inoculum. Mature reproductive structures were observed and photographed with an optical microscope (LEICA DM4500 B) equipped with a camera (LEICA DFC320). Morphological data (micro- and macroscopic) were compared with the available description of *Thelebolus* species. DNA was extracted as mentioned above and the ITS and beta-tubulin regions were amplified as recommended by previous studies (de Hoog *et al.* 2005, Crous *et al.* 2015). A two-marker dataset (supplementary material 2) was built for a phylogenetic analysis, which was performed as described in the previous section.

Statistical analyses

Statistical analyses on the fungal community associated with sponges were performed using PRIMER v. 7.0 (Plymouth Routines In Multivariate Ecological Research; Clarke and Warwick 2001). The Similarity Percentages (SIMPER) analysis mostly highlighted the dissimilarity within the fungal community of the three sponges. The Permutational Multivariate Analysis of Variance (PERMANOVA; pseudo-F index; $p<0.05$) allowed the differences between the sponge mycobiotas to be assessed. Principal Coordinate Analysis (PCO) visualised data.

RESULTS

The use of different isolation techniques or culture conditions resulted in an increase in the number of fungal isolates. As reported in Fig. 1A the majority of the taxa were isolated exclusively by homogenisation of sponge tissues, while the remaining by directly plating the sponge tissue; less than 18 % were recovered with both techniques. Overall, from 67 % (*P. johnstonia*) to 75 % (*S. ciliatum*)

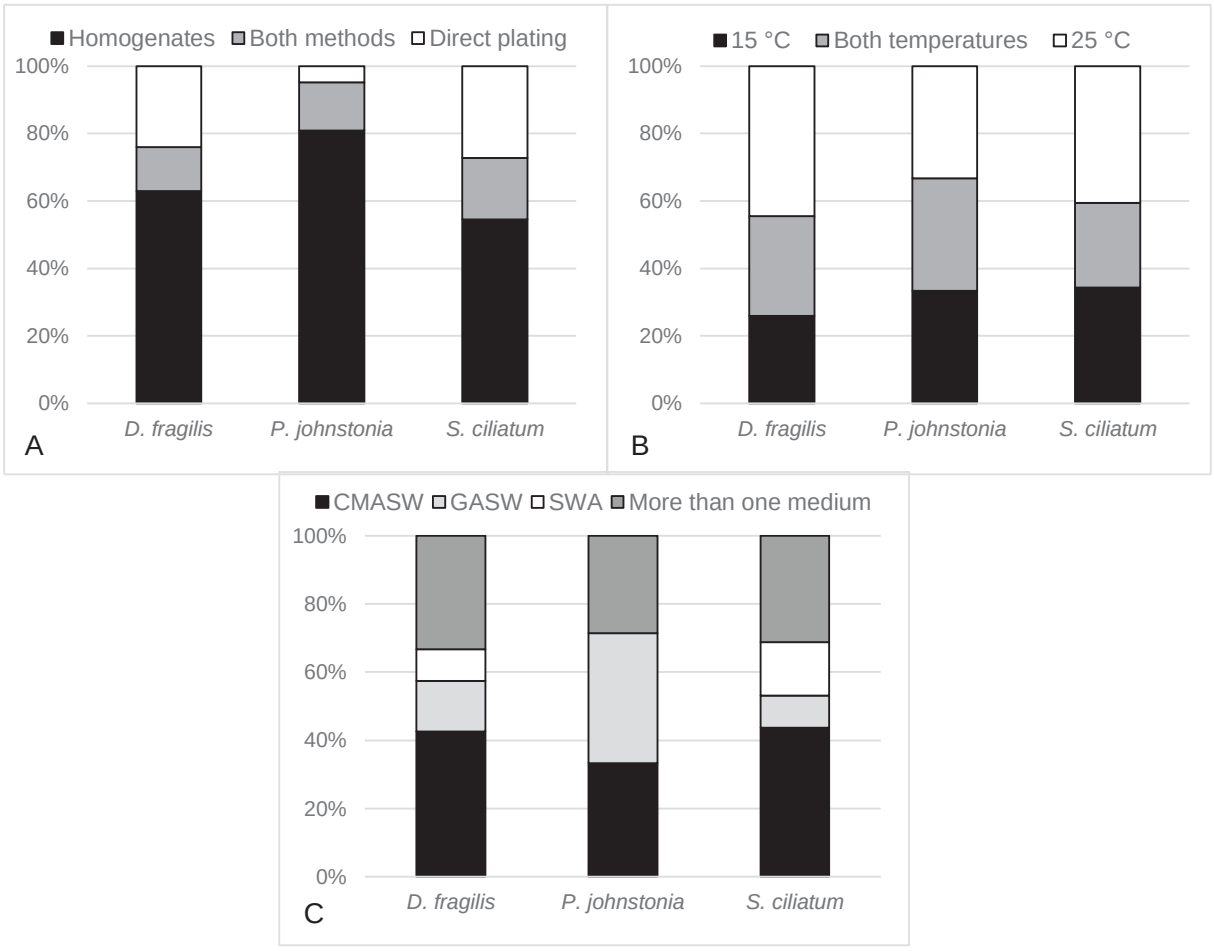


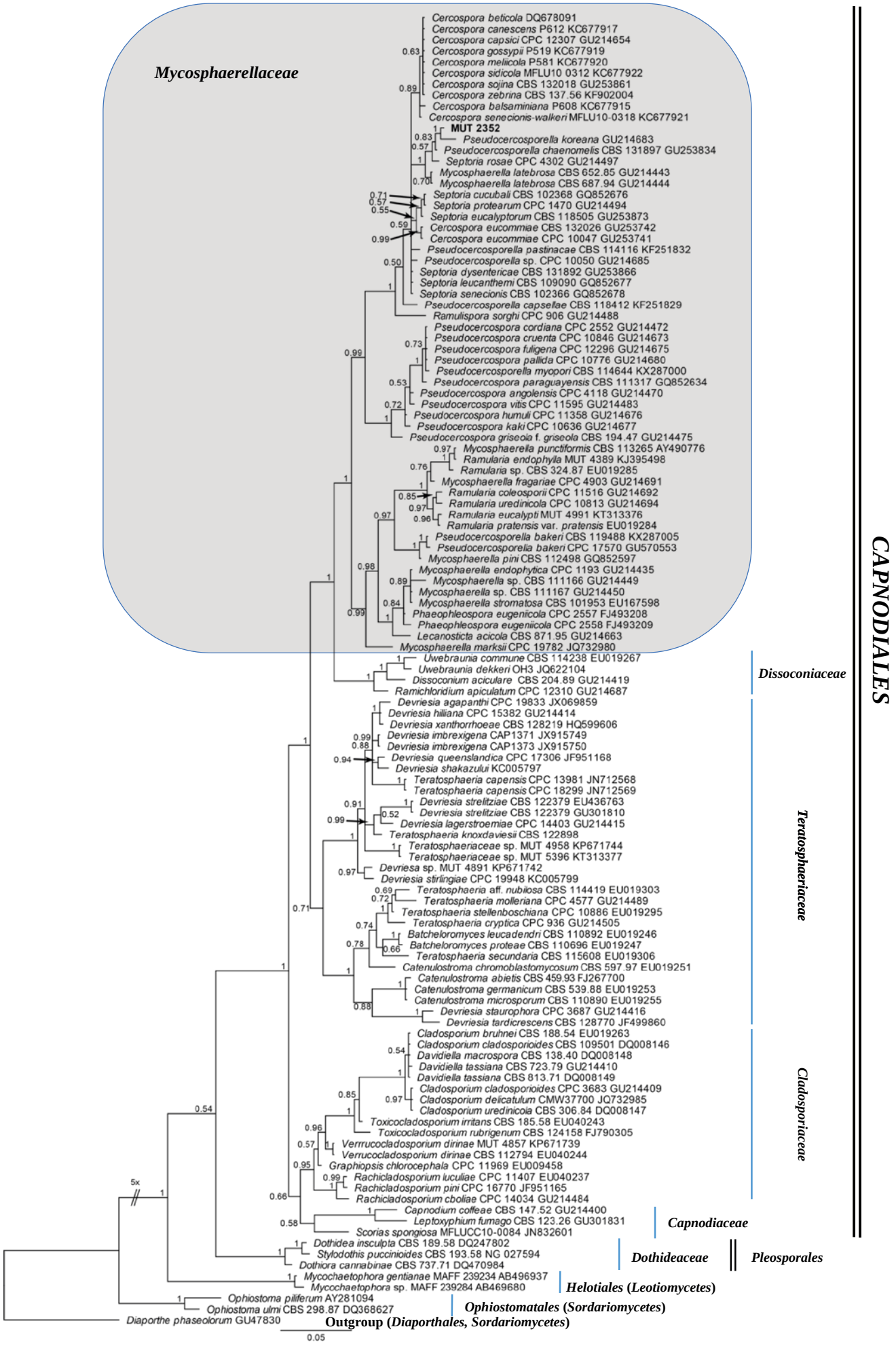
Fig. 1. Influence of **A.** Isolation methods. **B.** Incubation temperatures. **C.** Growth media, on the fungal community associated with *D. fragilis*, *P. johnstonia* and *S. ciliatum*.

of taxa were isolated only on one temperature condition (15 °C or 25 °C) as reported in Fig. 1B. Regarding the growth media (Fig. 1C), almost half of the taxa of *D. fragilis* (43 %) and of *S. ciliatum* (44 %) grew exclusively on CMASW; while 24 % and 25 % of taxa associated with *D. fragilis* and *S. ciliatum* were isolated only on oligotrophic media, mimicking sponges' composition (GASW) or marine water (SWA). The majority of the fungi associated with *P. johnstonia* were only isolated on GASW (38 %) or on CMASW (33 %); no exclusive taxa were reported on SWA. Interestingly, 66 %, 56 % and 38 % of taxa from *S. ciliatum, D. fragilis* and *P. johnstonia*, respectively, not only were recovered in one condition but were isolated only from one plate.

A total of 87 taxa were isolated: 54 taxa from *D. fragilis*, 32 from *S. ciliatum* and 21 from *P. johnstonia*; 79 % of the taxa were recognised at species level, 13 % at genus level, 5 % at family level, 2 % at order level, and 1 % at class level (Table 2). About one third of taxa were sterile despite the attempt to stimulate the production of reproductive structures using different culture media and incubation under near-UV light. Several sterile mycelia (Table 2) showed the same similarity percentages with different species and/or low homology with sequences deposited in public databases. In addition, some cryptic strains belonging to the *Pleosporales* order (MUT 2482, MUT 2870, MUT 2952, MUT 3080 and MUT 2425) presented only the asexual form in axenic culture (for many genera, only the description of the sexual morph is available). For these reasons, a phylogenetic analysis based on LSU region was necessary to achieve their best identification. In detail, *Capnodiales* (Fig. 2) and *Chaetothyriales* (Fig. 3) were represented by one isolate each; 17 strains belonged to *Pleosporales* order (Fig. 4); two and eight strains were grouped in *Leotiomycetes* (Fig. 5) and *Sordariomycetes* (Fig. 6), respectively.

The majority of taxa belonged to *Ascomycota* (92 %), with few representatives of *Basidiomycota* (7 %) and *Mucoromycota* (1 %). The genera *Cladosporium* and *Penicillium* (11 species), *Acremonium* (six species) and *Aspergillus* (five species) were the most represented in terms of species. In terms of first reports, 49 and 21 species were first recorded here as being associated with sponges and the marine environment, respectively (Table 2). Four species (*Cladosporium allicinum*, *Cladosporium cladosporioides*, *Penicillium antarcticum* and *Tolypocladium cylindrosporum)* were common among the three sponges (Fig. 7). *Dysidea fragilis* and *S. ciliatum* shared an additional six species (*Acremonium potronii, Aspergillus fumigatus, Beauveria bassiana, Cadophora luteo-olivacea, Paraphaeosphaeria neglecta, Phaeosphaeriopsis* sp.); while, *D. fragilis* and *P. johnstonia* had five additional species in common (*Aspergillus creber, Aspergillus jensenii, Aureobasidium pullulans, Cladosporium pseudocladosporioides*

Fig. 2. Bayesian phylogram of *Capnodiales* (*Dothideomycetes*) based on rDNA large subunit (LSU). One fungal isolate (MUT 2352) is included and identified as *Cercospora* sp. within the *Mycosphaerellaceae*. Branch numbers indicate BPP values.


Mycosphaerellaceae
Cercospora beticola DQ678091
Cercospora canescens P612 KC677917
Cercospora capsici CPC 12307 GU214654
Cercospora gossypii P519 KC677919
Cercospora meliicola P581 KC677920
Cercospora sidicola MFLU10 0312 KC677922
Cercospora sojina CBS 132018 GU253861
Cercospora zebrina CBS 137.56 KF902004
Cercospora balsaminiana P608 KC677915
Cercospora senecionis-walkeri MFLU10-0318 KC677921
MUT 2352
Pseudocercosporella koreana GU214683
Pseudocercosporella chaenomelis CBS 131897 GU253834
Septoria rosae CPC 4302 GU214497
Mycosphaerella latebrosa CBS 652.85 GU214443
Mycosphaerella latebrosa CBS 687.94 GU214444
Septoria cucubali CBS 102368 GQ852676
Septoria protearum CPC 1470 GU214494
Septoria eucalyptorum CBS 118505 GU253873
Cercospora eucommiae CBS 132026 GU253742
Cercospora eucommiae CPC 10047 GU253741
Pseudocercosporella pastinacae CBS 114116 KF251832
Pseudocercosporella sp. CPC 10050 GU214685
Septoria dysentericae CBS 131892 GU253866
Septoria leucanthemi CBS 109090 GQ852677
Septoria senecionis CBS 102366 GQ852678
Pseudocercosporella capsellae CBS 118412 KF251829
Ramulispora sorghi CPC 906 GU214488
Pseudocercospora cordiana CPC 2552 GU214472
Pseudocercospora cruenta CPC 10846 GU214673
Pseudocercospora fuligena CPC 12296 GU214675
Pseudocercospora pallida CPC 10776 GU214680
Pseudocercosporella myopori CBS 114644 KX287000
Pseudocercospora paraguayensis CBS 111317 GQ852634
Pseudocercospora angolensis CPC 4118 GU214470
Pseudocercospora vitis CPC 11595 GU214483
Pseudocercospora humuli CPC 11358 GU214676
Pseudocercospora kaki CPC 10636 GU214677
Pseudocercospora griseola f. griseola CBS 194.47 GU214475
Mycosphaerella punctiformis CBS 113265 AY490776
Ramularia endophylla MUT 4389 KJ395498
Ramularia sp. CBS 324.87 EU019285
Mycosphaerella fragariae CPC 4903 GU214691
Ramularia coleosporii CPC 11516 GU214692
Ramularia uredinicola CPC 10813 GU214694
Ramularia eucalypti MUT 4991 KT313376
Ramularia pratensis var. pratensis EU019284
Pseudocercosporella bakeri CBS 119488 KX287005
Pseudocercosporella bakeri CPC 17570 GU570553
Mycosphaerella pini CBS 112498 GQ852597
Mycosphaerella endophytica CPC 1193 GU214435
Mycosphaerella sp. CBS 111166 GU214449
Mycosphaerella sp. CBS 111167 GU214450
Mycosphaerella stromatosa CBS 101953 EU167598
Phaeophleospora eugeniicola CPC 2557 FJ493208
Phaeophleospora eugeniicola CPC 2558 FJ493209
Lecanosticta acicola CBS 871.95 GU214663
Mycosphaerella marksii CPC 19782 JQ732980
Uwebraunia commune CBS 114238 EU019267
Uwebraunia dekkeri OH3 JQ622104
Dissoconium aciculare CBS 204.89 GU214419
Ramichloridium apiculatum CPC 12310 GU214687
Devriesia agapanthi CPC 19833 JX069859
Devriesia hilliana CPC 15382 GU214414
Devriesia xanthorrhoeae CBS 128219 HQ599606
Devriesia imbrexigena CAP1371 JX915749
Devriesia imbrexigena CAP1373 JX915750
Devriesia queenslandica CPC 17306 JF951168
Devriesia shakazului KC005797
Teratosphaeria capensis CPC 13981 JN712568
Teratosphaeria capensis CPC 18299 JN712569
Devriesia strelitziae CBS 122379 EU436763
Devriesia strelitziae CBS 122379 GU301810
Devriesia lagerstroemiae CPC 14403 GU214415
Teratosphaeria knoxdaviesii CBS 122898
Teratosphaeriaceae sp. MUT 4958 KP671744
Teratosphaeriaceae sp. MUT 5396 KT313377
Devriesa sp. MUT 4891 KP671742
Devriesia stirlingiae CPC 19948 KC005799
Teratosphaeria aff. nubilosa CBS 114419 EU019303
Teratosphaeria molleriana CPC 4577 GU214489
Teratosphaeria stellenboschiana CPC 10886 EU019295
Teratosphaeria cryptica CPC 936 GU214505
Batcheloromyces leucadendri CBS 110892 EU019246
Batcheloromyces proteae CBS 110696 EU019247
Teratosphaeria secundaria CBS 115608 EU019306
Catenulostroma chromoblastomycosum CBS 597.97 EU019251
Catenulostroma abietis CBS 459.93 FJ267700
Catenulostroma germanicum CBS 539.88 EU019253
Catenulostroma microsporum CBS 110890 EU019255
Devriesia staurophora CPC 3687 GU214416
Devriesia tardicrescens CBS 128770 JF499860
Cladosporium bruhnei CBS 188.54 EU019263
Cladosporium cladosporioides CBS 109501 DQ008146
Davidiella macrospora CBS 138.40 DQ008148
Davidiella tassiana CBS 723.79 GU214410
Davidiella tassiana CBS 813.71 DQ008149
Cladosporium cladosporioides CPC 3683 GU214409
Cladosporium delicatulum CMW37700 JQ732985
Cladosporium uredinicola CBS 306.84 DQ008147
Toxicocladosporium irritans CBS 185.58 EU040243
Toxicocladosporium rubrigenum CBS 124158 FJ790305
Verrrucocladosporium dirinae MUT 4857 KP671739
Verrucocladosporium dirinae CBS 112794 EU040244
Graphiopsis chlorocephala CPC 11969 EU009458
Rachicladosporium luculiae CPC 11407 EU040237
Rachicladosporium pini CPC 16770 JF951165
Rachicladosporium cboliae CPC 14034 GU214484
Capnodium coffeae CBS 147.52 GU214400
Leptoxyphium fumago CBS 123.26 GU301831
Scorias spongiosa MFLUCC10-0084 JN832601
Dothidea insculpta CBS 189.58 DQ247802
Stylodothis puccinioides CBS 193.58 NG 027594
Dothiora cannabinae CBS 737.71 DQ470984
Mycochaetophora gentianae MAFF 239234 AB496937
Mycochaetophora sp. MAFF 239284 AB469680
Ophiostoma piliferum AY281094
Ophiostoma ulmi CBS 298.87 DQ368627
Diaporthe phaseolorum GU47830
5x
0.05
Dissoconiaceae
Teratosphaeriaceae
Cladosporiaceae
Capnodiaceae
Dothideaceae
Pleosporales
Helotiales (Leotiomycetes)
Ophiostomatales (Sordariomycetes)
Outgroup (Diaporthales, Sordariomycetes)
CAPNODIALES

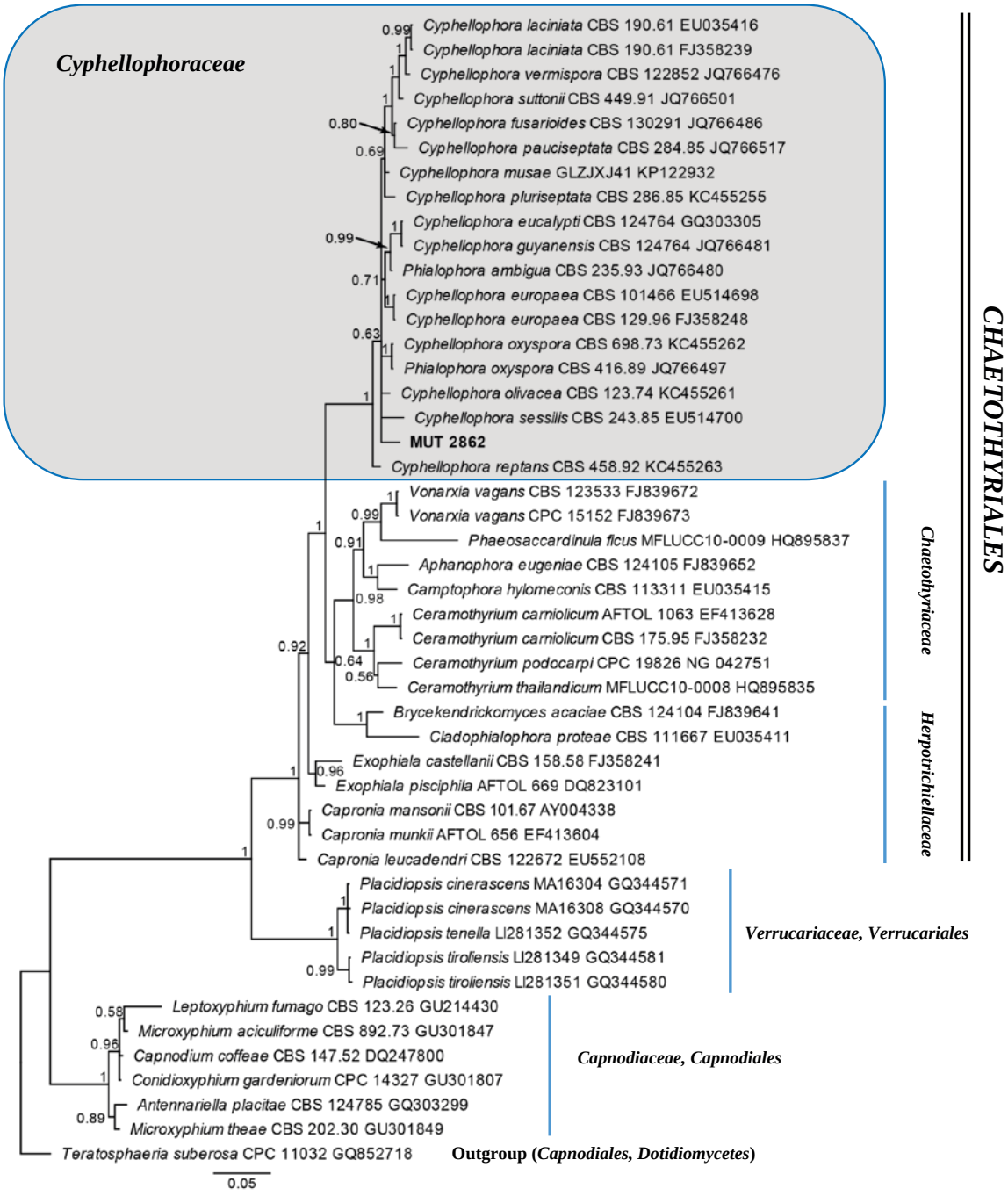


Fig. 3. Bayesian phylogram of *Chaetothyriales* (*Eurotiomycetes*) based on rDNA large subunit (LSU). MUT 2862 is included and clusters within the genus *Cyphellophora*. Branch numbers indicate BPP values.

and Penicillium chrysogenum). *Sycon ciliatum* and *P. johnstonia* shared one additional species (*Cladosporium halotolerans*).

Despite this species overlap, the three sponges host specific fungal communities (Fig. 7); *Dysidea fragilis* mycobiota was represented by 72.2 % exclusive taxa, followed by *S. ciliatum* (65.6 %) and *P. johnstonia* (52.4 %). The specificity of the sponge-mycobiota association was highlighted also by the Permanova analysis that reported a significant difference (p = 0.011) among sponges. Almost 45 % of the multivariate variability via two-dimensional Principal Coordinate Analysis (PCO) can be explained by the different fungal communities associated with the sponges (Fig. 8). The dissimilarity among the sponges, highlighted by the SIMPER analysis, was higher between *P. johnstonia* and *S. ciliatum* (89.9 %), with the major contribution given by *P. chrysogenum*, *C. pseudocladosporioides* and *C. luteo-olivacea*. The dissimilarity value of *S. ciliatum* and *D. fragilis* was still high (87.4 %) and *A. potronii*, *Sarocladium strictum* and *Cladosporium psychrotolerans* mostly contribute to the value. The lowest dissimilarity was between *D. fragilis* and *P. johnstonia* (82.6 %), with the major contribution given by *A. potronii*, *C. psychrotolerans* and *Tolypocladium album*.

Overall, *D. fragilis* presented the most diverse mycobiota, including two fungi MUT 2357 and MUT 2359, attributed to the genus *Thelebolus* both by molecular and morphological analyses. No matches in morphological features were observed among our strains and the 16 species and two varieties of *Thelebolus* known (CBS-KNAW Collection, Westerdijk Fungal Biodiversity Institute, MycoBank). The phylogenetic tree (Fig. 9) based on two markers (ITS and beta-tubulin) confirmed the uniqueness of *Thelebolus* MUT 2357 and *Thelebolus* MUT 2359.

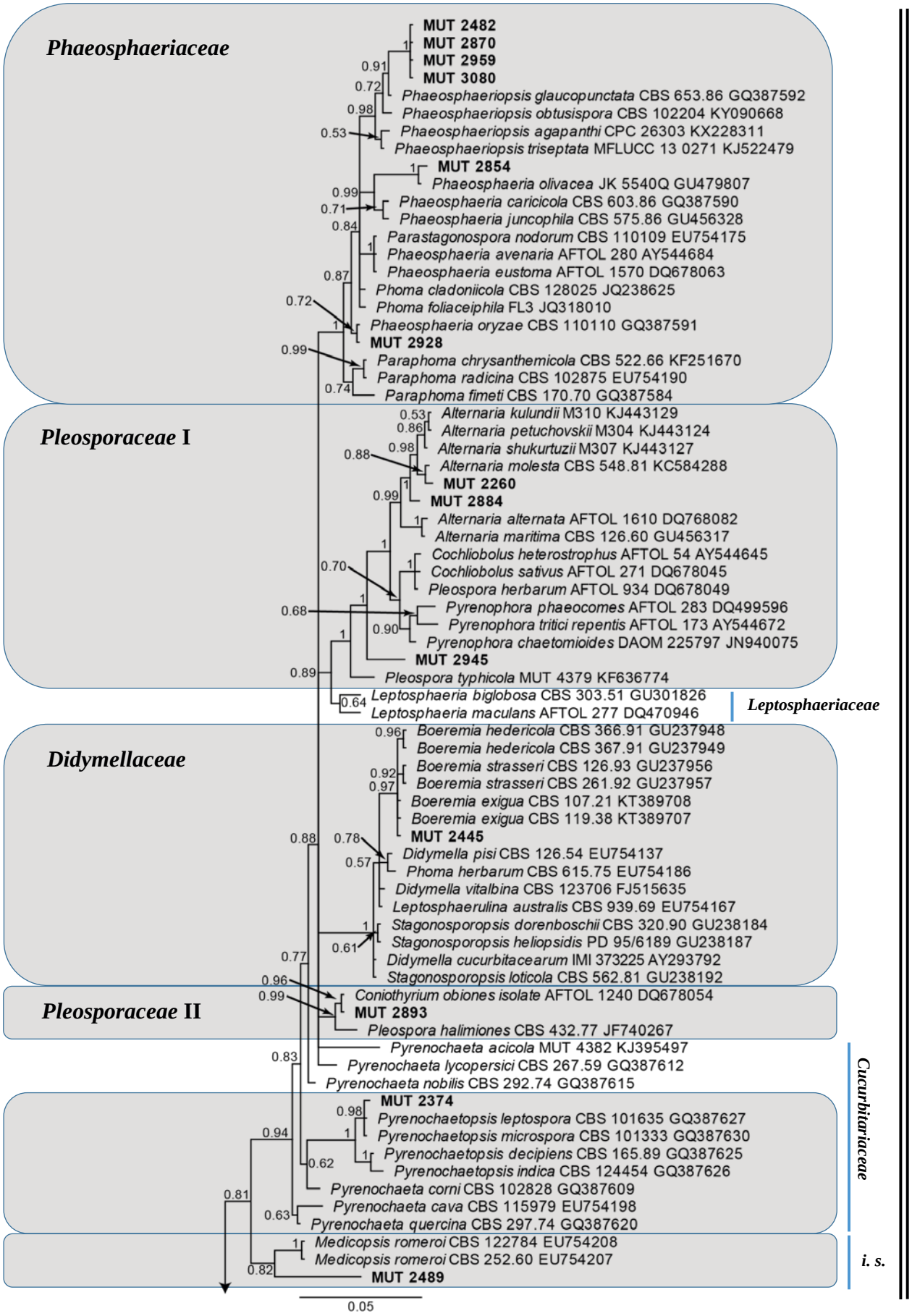


Fig. 4. Bayesian phylogram of *Pleosporales* (*Dothideomycetes*) based on rDNA large subunit (LSU). Six and four fungal isolates clustered within the *Phaeosphaeriaceae* and the *Pleosporaceae*, respectively. Six fungal taxa clustered individually within the *Didymellaceae*, *Cucurbitariaceae*, *Montagnulaceae*, *Periconiaceae*, *Sporormiaceae* and *Roussoellaceae*/*Thyridariaceae*. One fungus was included in the *Pleosporales* order. Branch numbers indicate BPP values. *i.s.* = *incertae sedis*.

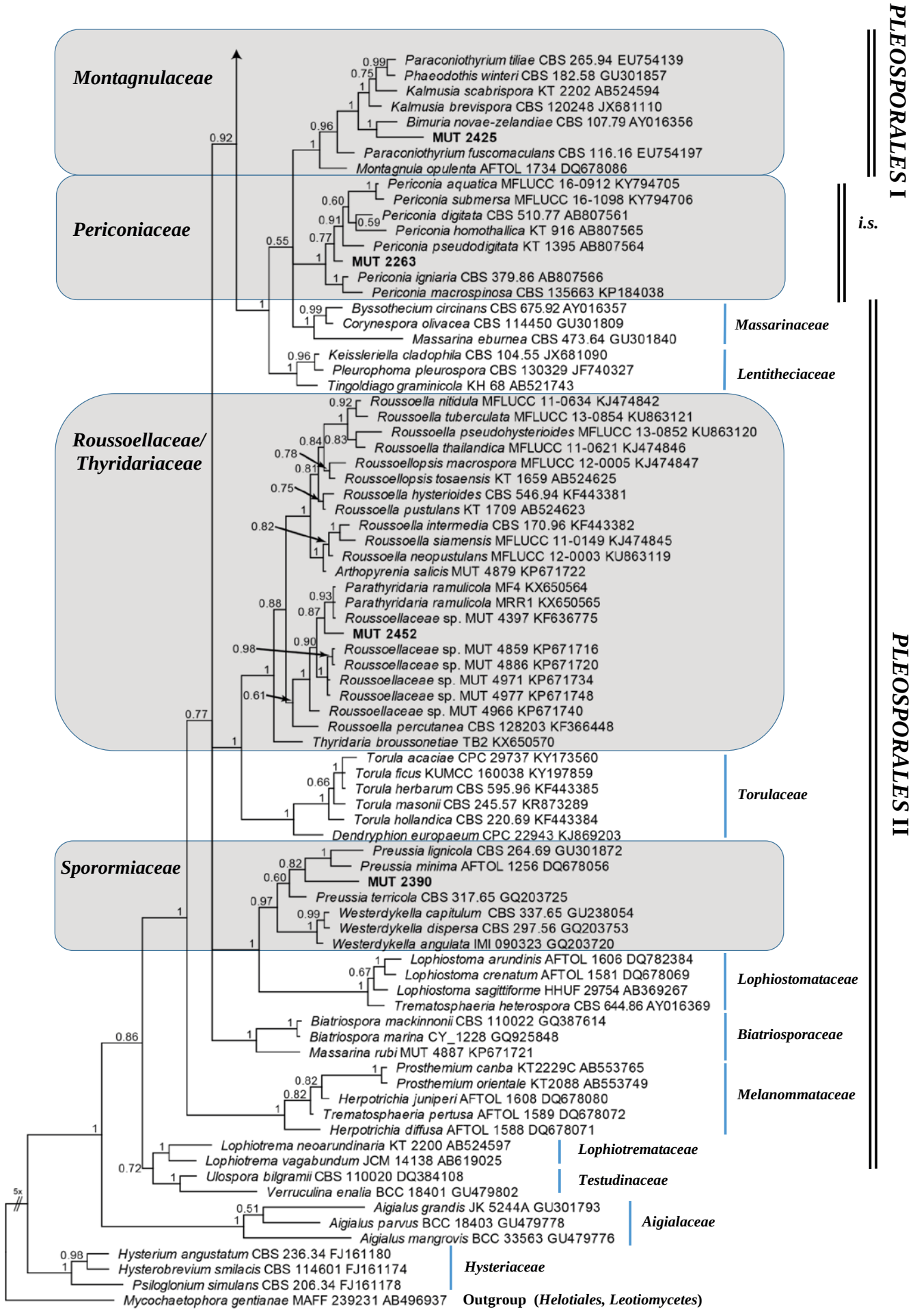


Fig. 4. (Continued).

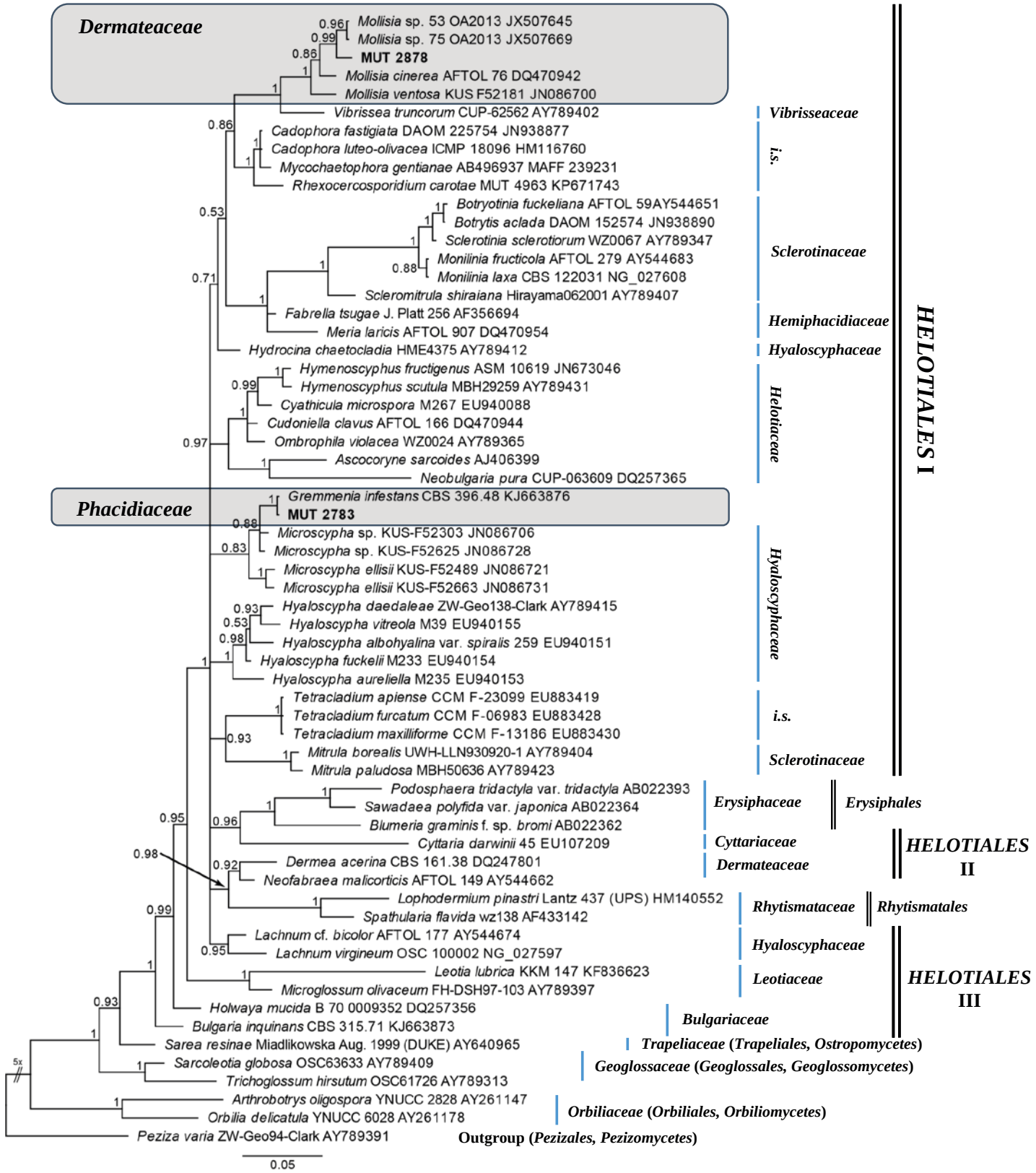


Fig. 5. Bayesian phylogram of *Leotiomycetes* based on rDNA large subunit (LSU). Two fungal isolates were identified as *Mollisia* sp. and *Gremmenia infestans* within the *Dermateaceae* and *Phacidiaceae*, respectively. Branch numbers indicate BPP values.

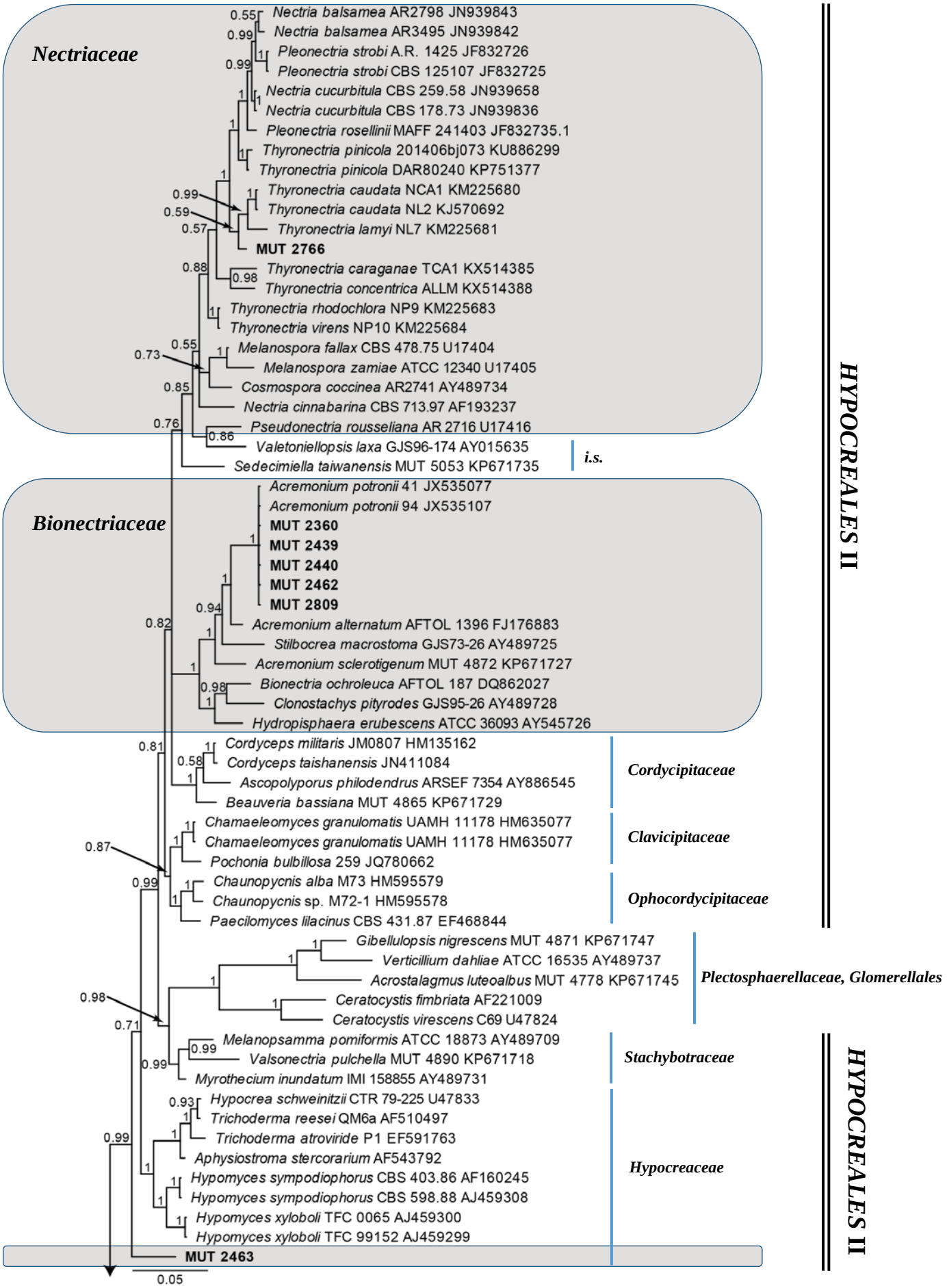


Fig. 6. Bayesian phylogram of *Sordariomycetes* based on rDNA large subunit (LSU). Three fungal taxa clustered individually within the *Nectriaceae*, *Hypocreaceae* and *Microascaceae*. Five fungal isolates clustered within the *Bionectriaceae*. Branch numbers indicate BPP values.

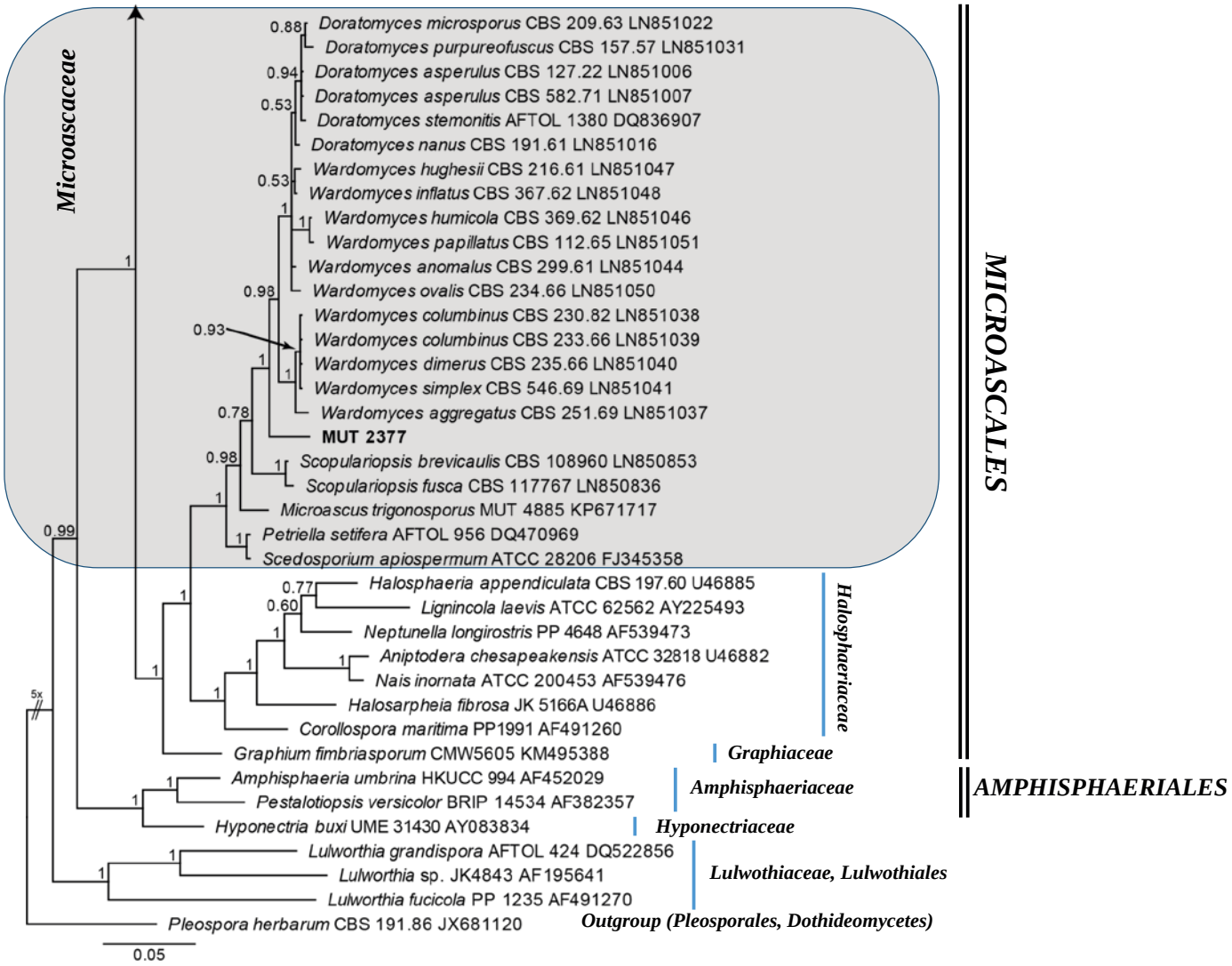


Fig. 6. (Continued).

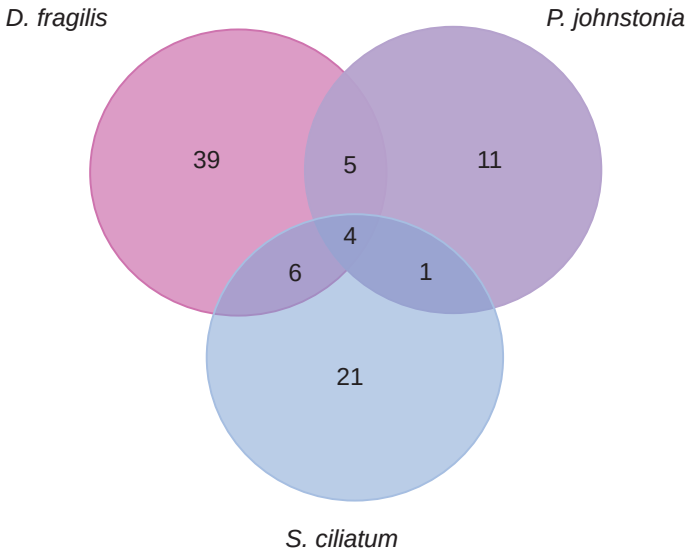


Fig. 7. Exclusive and common fungal taxa species occurring in *D. fragilis*, *S. ciliatum* and *P. johnstonia*

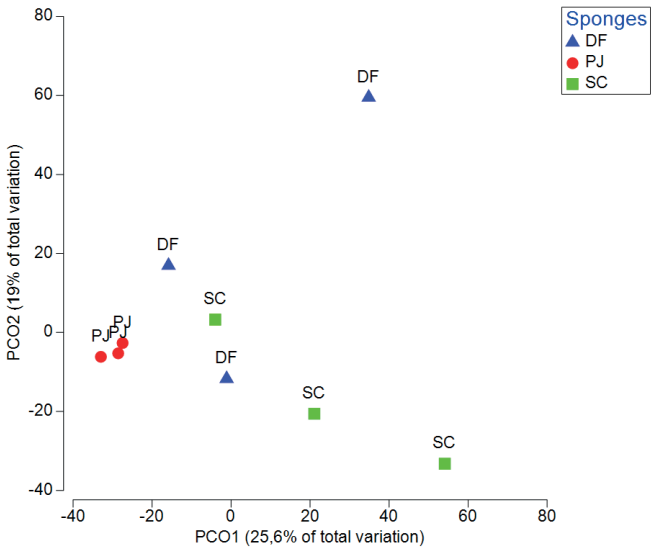


Fig. 8. PCO on the fungal communities of the three Atlantic sponges *D. fragilis* (DF), *P. johnstonia* (PJ) and *S. ciliatum* (SC).

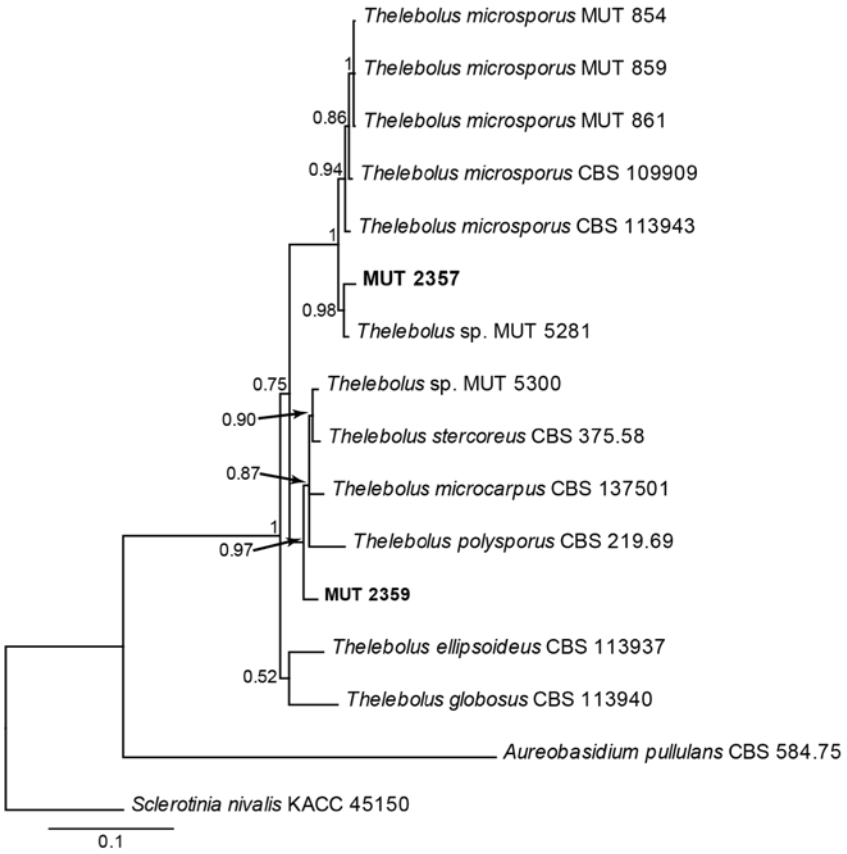


Fig 9. Bayesian phylogram of the genus *Thelebolus* based on a combined dataset of ITS and beta-tubulin partial sequences. MUT 2357 and MUT 2359 were identified as new species, *T. balaustiformis* and *T. spongiae*, respectively. Branch numbers indicate BPP values

Taxonomy

Classification: *Thelebolaceae*, *Thelebolales*, *Leotiomycetes.*

Thelebolus balaustiformis E. Bovio, L. Garzoli, A. Poli, V. Prigione, G.C. Varese, ***sp. nov.*** MycoBank MB824102. Figs 10–13.

Etymology: The specific epithet *balaustiformis* is derived from the similarity of ascomata, either whole or in section, with the pomegranate (*Punica granatum*) fruit, which, in botanical terms, is called *balaustium*.

Ascomata were produced only on MEA at 4 °C, after 3 wk of incubation (Fig. 10). *Mycelium* hyaline to pale yellow consisting of irregularly swollen, septate hyphae 1.5–5 µm wide. *Ascomata* hyaline or pale yellow, partially immersed in the colony, (87–)100–120 × 100 µm, at first subglobose cleistohymenial then opening by rupturing of the cortical excipulum in the upper part and becoming semiglobular, appearing 'apothecioid' at maturity. *Hymenium* with a palisade of asci. *Cortical excipulum ca*. 6–10 µm thick, consisting of several layers of flattened cells (*textura epidermoidea*). *Asci* 20–30 per ascoma, broadly clavate, rather thick-walled (1–1.5 µm), 48–64-spored, 11–20 × 43–57 µm. *Ascospores* irregularly disposed, ellipsoid with rounded ends (length/width ratio 1.4–1.6), 4–4.7 × 2.8–3 µm, hyaline with a homogenous content, smooth-walled, without mucilaginous substance. *Spores* are forcefully discharged as a single projectile through the subapical part of the ascus. *Paraphyses* absent. *Asexual morph* not observed.

Colony description and physiological features: Colonies on CA attaining 12–17 mm diam in 21 d at 25 °C, plane, thin, mycelium mainly submerged, margins irregular (also at 5 % NaCl), becoming regular in presence of 2.5 % NaCl; at 15 °C and 4 °C colonies very similar with regular margins, reaching 56–59 mm and 36–37 mm diam in 21 d, respectively. The sizes of the colonies (diam in mm) at different salt concentrations and temperature are shown in Fig. 14A–C; the morphologies in Fig. 11. Colonies on PDA attaining 8–9 mm diam in 21 d at 25 °C, developing in height, pink to orange, reverse of the same colour of the surface. At 15 °C colonies reaching 65–73 mm diam in 21 d, plane, pink-orange, margins regular (slightly irregular at 5 % NaCl), slimy; reverse of the same colour of the surface. At 4 °C colonies very similar, reaching 42–48 mm diam in 21 d. Colonies' sizes (diam in mm) and morphologies are shown in Fig. 14D–F and Fig. 12, respectively. Colonies on MEA not growing at 25 °C in 21 d; in presence of 2.5 % and 5 % NaCl, mycelium developing in height, pale orange, attaining 11–13 mm (2.5 % NaCl) and 8–11 mm (5 % NaCl) diam in 21 d. At 15 °C and 4 °C colonies plane, pink, reverse as the surface, reaching 46–48 mm and 25–27 mm diam in 21 d, respectively; Fig. 14 (G–I) report the growth curves (diam in mm); Fig. 13 show the colonies morphologies.

Thelebolus balaustiformis reached the optimal growth at 15 °C, regardless of media and/or salt concentrations utilised (Fig. 14); 25 °C was the most inhibiting temperature (Fig. 14). Regarding the salt concentration, the fungus grew up to 10 % NaCl only on CA at 4 °C (Fig. 14A) and 15 °C (Fig. 14B). On CA at 4 °C and 15 °C the faster growth was reached at 2.5 % NaCl, followed by 0 %, 5 % and 10 % NaCl (Fig. 14A, B). At 25 °C the media with NaCl (2.5 % and 5 %) better supported the fungal growth (Fig. 14C). On PDA at 4 °C the growth was faster with the decreasing of salt (until 0 % NaCl). At 15 °C the conditions with 0 % and 2.5

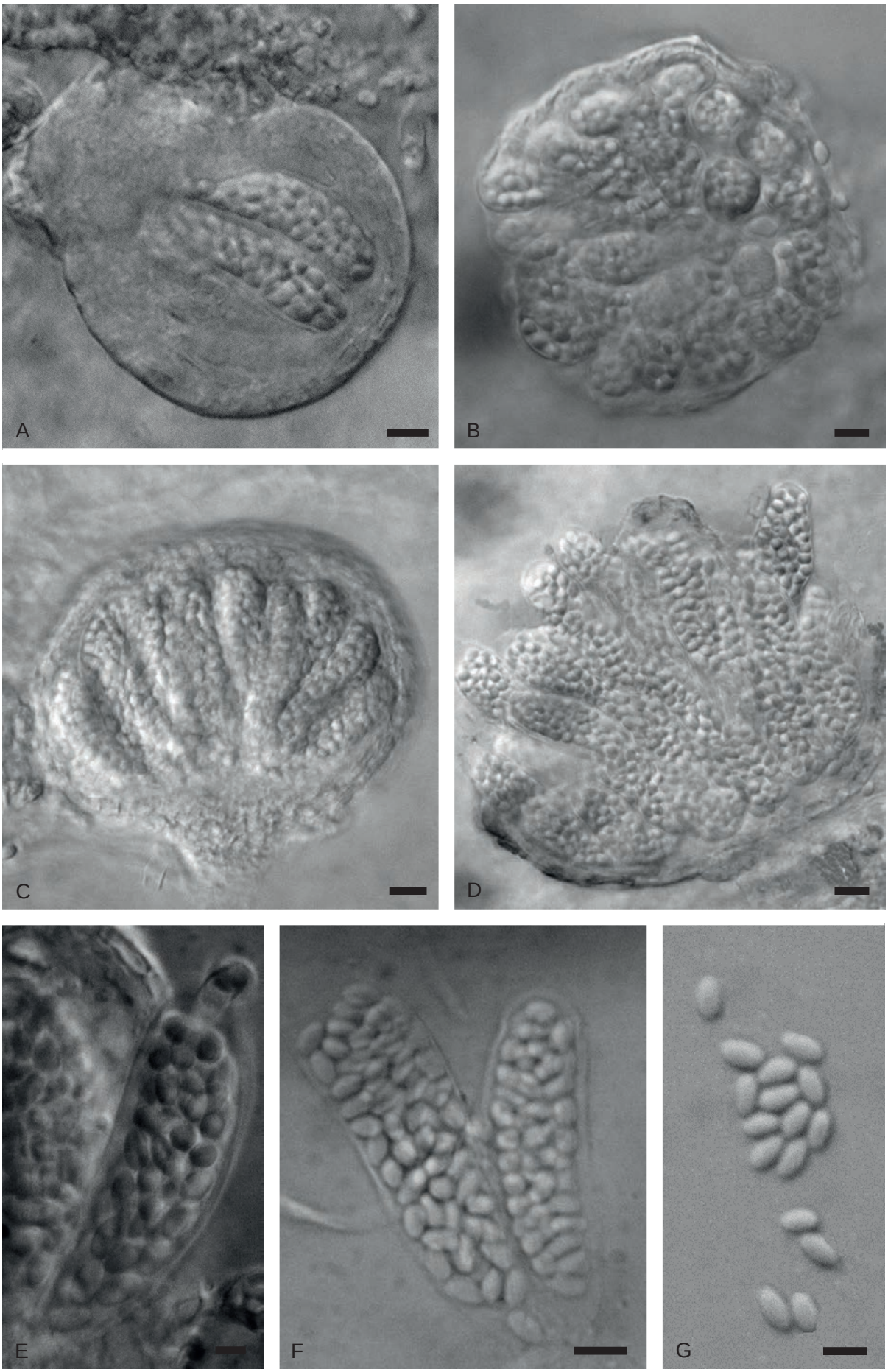


Fig. 10. *Thelebolus balaustiformis* MUT 2357. **A, B.** Closed subglobosus ascoma in the first stage of development. **C.** Ascoma becoming apothecial with mature asci. **D.** Apothecial ascoma with cortical excipulum dehiscent. **E, F.** Mature asci with 48–64 ascospores. **G.** Ascospores. Scale bars: A–D, F = 10 µm; E, G = 5 µm.

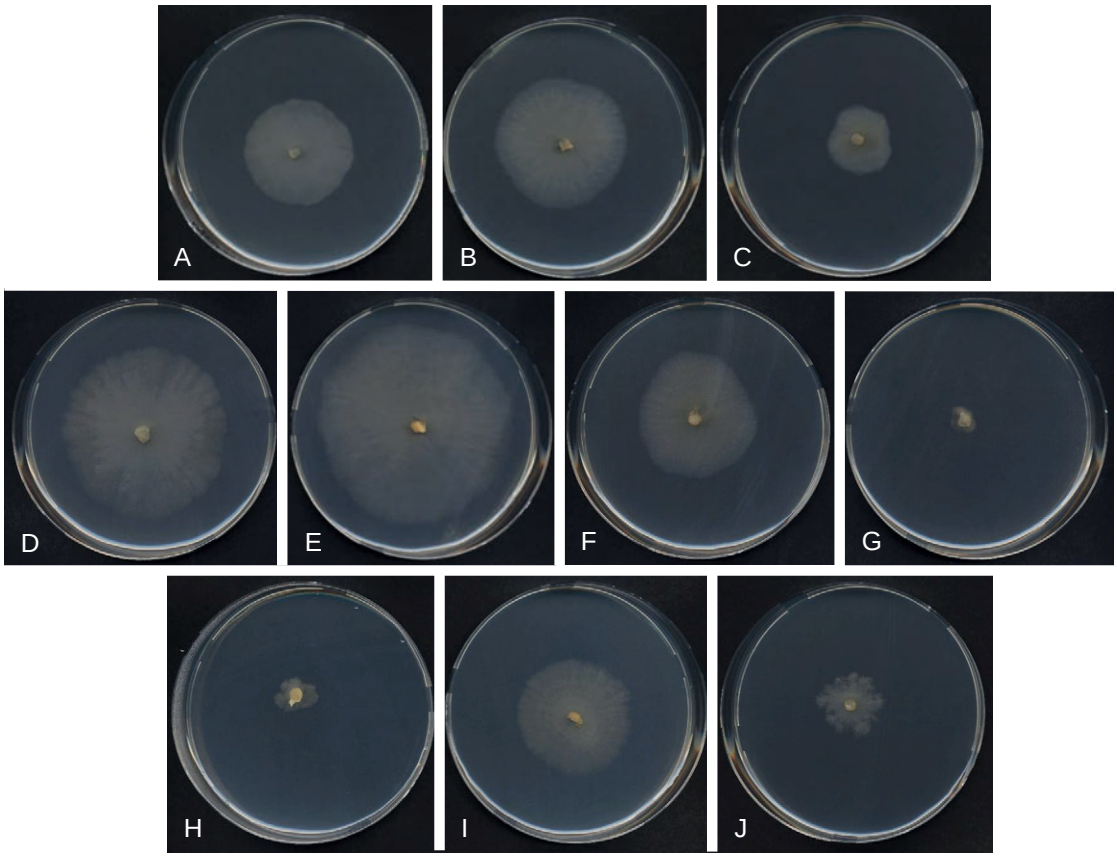


Fig. 11. *Thelebolus balaustiformis* MUT 2357: 21-d-old colonies on CA at 4 °C with **A.** 0 % NaCl, **B.** 2.5 % NaCl, **C.** 5 % NaCl; at 15 °C with **D.** 0 % NaCl, **E.** 2.5 % NaCl, **F.** 5 % NaCl, **G.** 10 % NaCl; at 25 °C with **H.** 0 % NaCl, **I.** 2.5 % NaCl, **J.** 5 % NaCl.

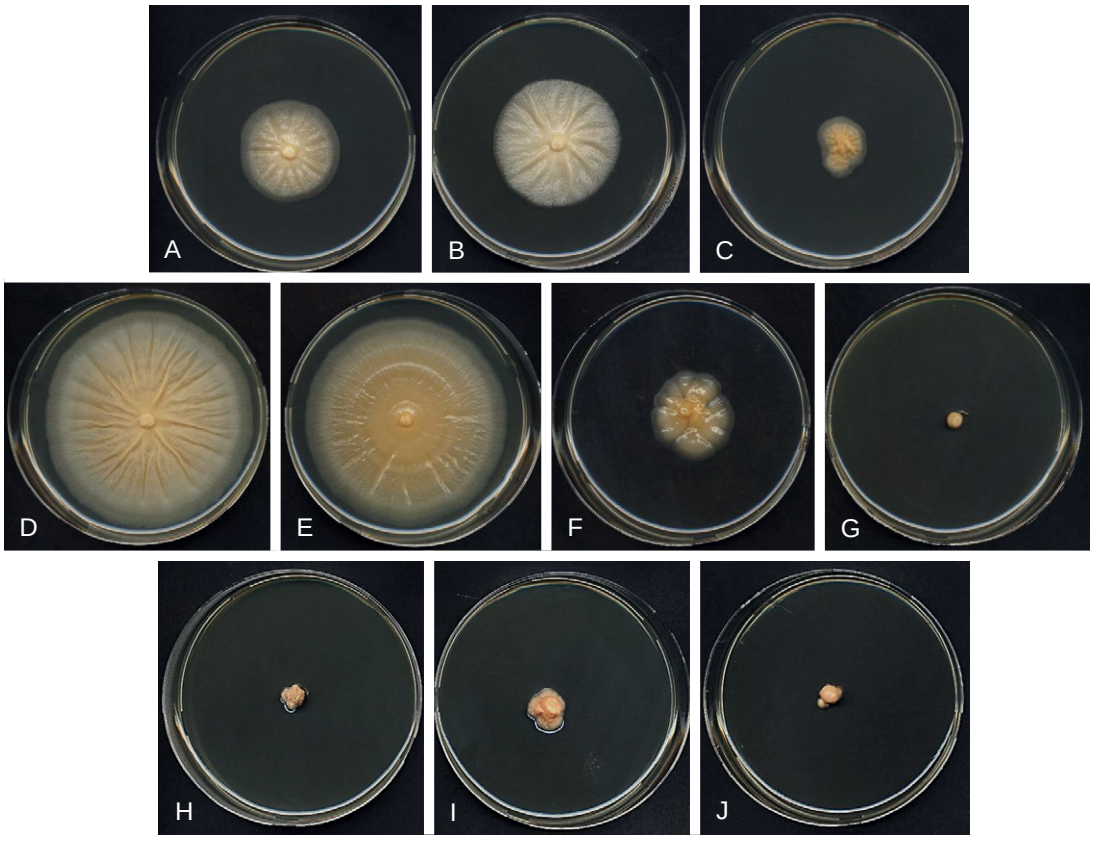


Fig. 12. *Thelebolus balaustiformis* MUT 2357: 21-d-old colonies on PDA at 4 °C with **A.** 0 % NaCl, **B.** 2.5 % NaCl, **C.** 5 % NaCl; at 15 °C with **D.** 0 % NaCl, **E.** 2.5 % NaCl, **F.** 5 % NaCl, **G.** 10 % NaCl; at 25 °C with **H.** 0 % NaCl, **I.** 2.5 % NaCl, **J.** 5 % NaCl.

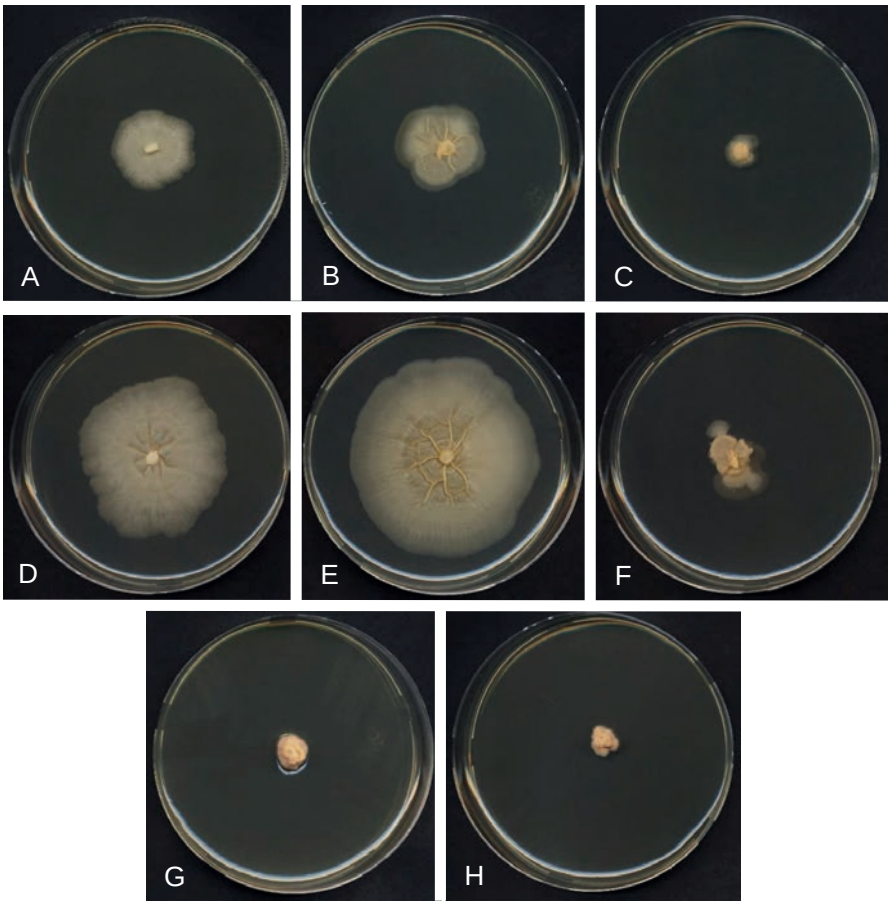


Fig. 13. *Thelebolus balaustiformis* MUT 2357: 21-d-old colonies on MEA at 4 °C with **A.** 0 % NaCl, **B.** 2.5 % NaCl, **C.** 5 % NaCl; at 15 °C with **D.** 0 % NaCl, **E.** 2.5 % NaCl, **F.** 5 % NaCl; at 25 °C with **G.** 2.5 % NaCl, **H.** 5 % NaCl.

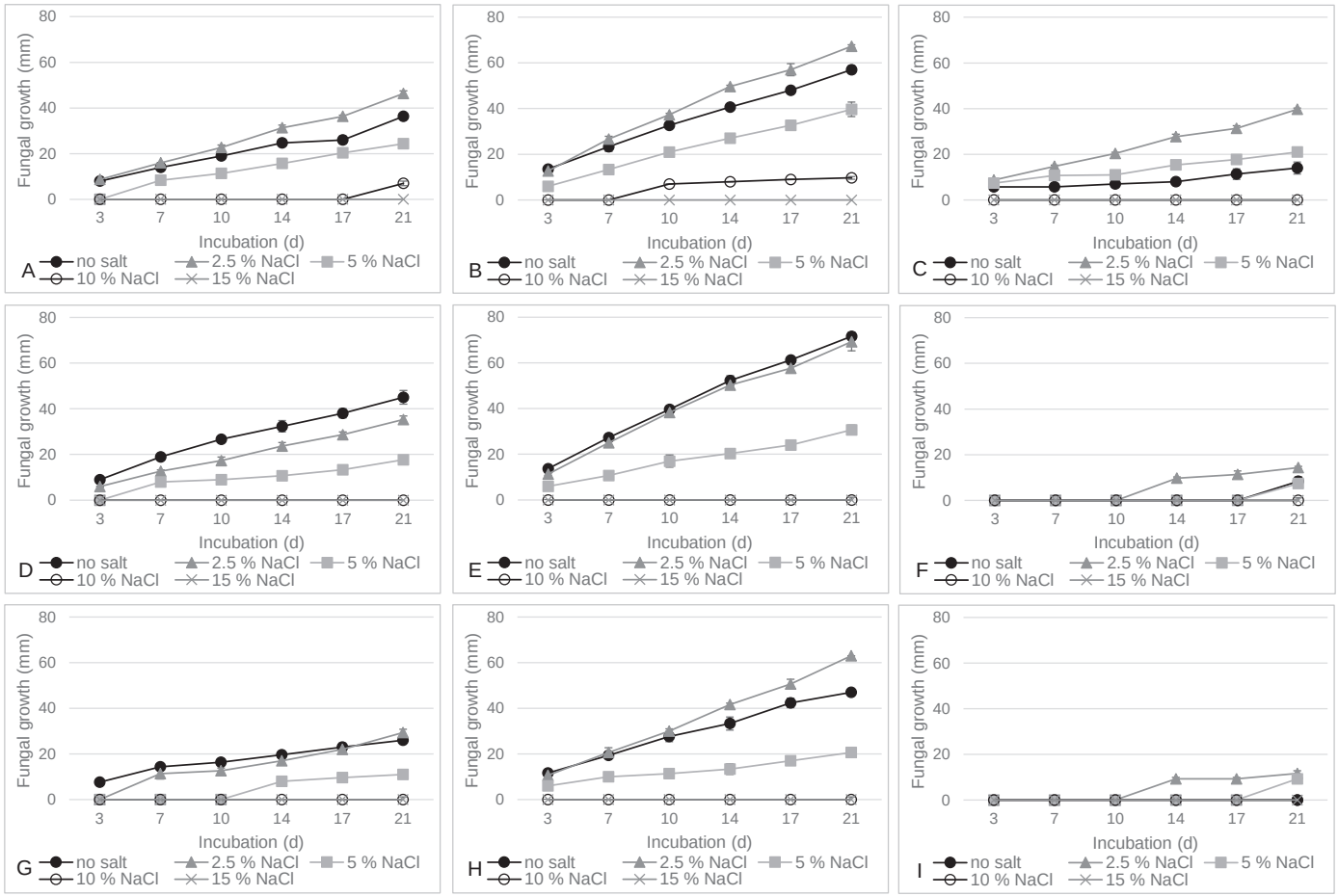


Fig. 14. *Thelebolus balaustiformis* MUT 2357 growth curve with no and different NaCl concentrations on CA at **A.** 4 °C, **B.** 15 °C, **C.** 25 °C; on PDA at **D.** 4 °C, **E.** 15 °C, **F.** 25 °C; on MEA at **G.** 4 °C, **H.** 15 °C, **I.** 25 °C.

% NaCl were comparable, while the slower growth was observed at 5 % NaCl (Fig. 14E). At 25 °C the fungus displayed a similar behaviour compared to CA at the same temperature, the growth was slow and better supported by salt (Fig. 14F). The growth of *T. balaustiformis* on MEA at 4 °C (Fig. 14G), 15 °C (Fig. 14H) and 25 °C (Fig. 14I) was similar to CA (in the same conditions), but there was a less evident difference between 0 % and 2.5 % NaCl at 4 °C, that became more evident at 15 °C, with a faster growth.

Specimen examined: **Ireland**, Galway, Gurraig Sound, Co. Galway, N 53°, 18.944; W 09°, 40.140, on the sponge *Dysidea fragilis*, 4 Jun. 2015, *G. McCormack & D. Firsova.* Holotype preserved as metabolically inactive culture MUT 2357.

Note: *Thelebolus balaustiformis* MUT 2357 was isolated by homogenisation of sponge tissues on CMASW, incubated at 15 °C.

Thelebolus spongiae E. Bovio, L. Garzoli, A. Poli, V. Prigione, G.C. Varese, ***sp. nov.*** MycoBank MB824103. Figs 15–18.

Etymology: The specific epithet *spongiae* is derived from the isolation of the fungus from a marine sponge and its strict association with it, due to the isolation by direct plating of the sponge.

Ascomata were produced only on PDA at 4 °C, after 3 wk of incubation (Fig. 15). *Mycelium* hyaline consisting of septate hyphae 3.2–4.7 µm wide, sometimes organised into bundles. *Ascomata* hyaline, superficial, scattered to grouped, from 50 × 40 µm for uni-ascal to 250 × 200 µm diam for multi-ascal, globose to subglobose cleistohymenial not becoming "apothecioid" with age. *Cortical excipulum* clearly differentiated, pale, 6–7 µm thick of 1–2 layers of flattened cells (*textura epidermoidea*). *Asci* 1–6 per ascoma, from globular to sacciform, rather thick-walled (1.5–3 µm), containing hundreds of spores, 37–57 × 50–70 µm. *Ascospores* irregularly disposed, ellipsoid with rounded ends (length/width ratio 2.2–2.4), 7–9.5 × 3.2–4 µm, hyaline with a homogenous content, smooth-walled, without mucilaginous substance. *Paraphyses* absent. *Asexual morph* not observed.

Colony description and physiological features: Colonies on CA attaining 47–51 mm diam in 21 d at 15 °C, smooth, mycelium sparse, pale pink, margins irregular also in presence of NaCl, reverse of the same colour of the surface. At 15 °C colonies similar but with more regular margins, reaching 49–50 mm diam in 21 d. Colonies with regular margins at 4 °C, 28–30 mm diam in 21 d. The sizes of the colonies (diameters in mm) and the morphologies at different salt concentrations and temperature are shown in Fig. 19A–C and Fig. 16, respectively. Colonies on PDA attaining 70–74 mm diam in 21 d at 25 °C, smooth, pale pink, radially sulcate (also in presence of 2.5 % NaCl, not with 5 % NaCl), margins mainly submerged; reverse of the same colour of the surface. At 15 °C and 4 °C colonies very similar, reaching 60–64 mm and 35–38 mm diam in 21 d, respectively. Colonies not radially sulcate, mucoid at 4 °C. Colonies' sizes (diam in mm) and morphologies are shown in Fig. 19D–F and Fig. 17, respectively. Colonies on MEA 30–32 mm diam in 21 d at 25 °C, smooth, mycelium partially submerged, pale pink, margins irregular (regular in presence of 2.5 % and 5 % NaCl); reverse of the same colour of the surface. At 15 °C and 4 °C colonies very similar with margins only slightly irregular, reaching 28–29 mm and 16 mm diam in 21 d, respectively. Fig. 19 (G–I) report the growth curves (diam in mm); Fig. 18 shows the colonies morphologies.

Thelebolus spongiae, as reported in Fig. 19, grew without NaCl and at 2.5 % and 5 % of NaCl; while at 10 % NaCl exhibited no growth, with the exception of PDA at 15 °C where the growth started 17 d after the inoculum and reached 7–9 mm diam in 21 d (Fig. 19E). The fungus grew better with the increasing of the incubation temperature, from 4°C to 25 °C. On PDA and CA at all temperatures, the growth of *T. spongiae* without and with 2.5 % NaCl was comparable (Fig. 19A–F); only at 25 °C on CA the difference was more pronounced: after 10 d the fungus started to grow faster with 2.5 % NaCl (Fig. 19C). The presence of 5 % NaCl made slower *T. spongiae* growth. *T. spongiae* grew faster on MEA in the presence of NaCl (2.5–5 %) compared to its absence; this difference was evident since the firsts stage of development at 15 °C and 25 °C (Fig. 19H, I), while at 4 °C it took 10 d to take shape (Fig. 19G).

Specimen examined: **Ireland**, Galway, Gurraig Sound, Co. Galway, N 53°, 18.944; W 09°, 40.140, on the sponge *Dysidea fragilis*, 4 Jun. 2015, *G. McCormack & D. Firsova.* Holotype preserved as metabolically inactive culture MUT 2359.

Note: *Thelebolus spongiae* MUT 2359 was isolated by direct plating of the sponge on SWA plate and incubated at 15 °C.

DISCUSSION

Isolation techniques

Our study clearly demonstrates the astonishing diversity of fungi inhabiting marine environments: 87 taxa were isolated from three sponges, many of them representing new records in marine ecosystems. This was chiefly due to the use of different isolation techniques and culture conditions. The homogenisation of sponge tissues yielded the highest number of taxa compared to the direct plating. This could be due to the specific requirements of marine fungi and the technique itself. Direct plating resulted in the isolation of only one fungus for each piece of sponge plated; on the contrary, the homogenization best suits the isolation of more marine fungi. These results are in agreement with other comparative studies (Paz *et al.* 2010, Sayed *et al.* 2016). Noteworthy, the direct plating, even if performing less well, allowed the isolation of fungi that otherwise would have not been recorded.

As for the isolation techniques, the use of three different media, also mimicking marine environment and sponge composition resulted in an increase of the number of cultivable fungi. The best performing condition for both *D. fragilis* and *S. ciliatum* was CMASW, a complete medium able to support fungal growth, not extremely rich in nutrients but containing sea salts to provide a condition as much as possible similar to marine environment. Interestingly, the medium that yielded the higher number of isolates in *P. johnstonia* was the gelatine-based medium, specially developed in this research to mimic the host organisms. Usually, media rich in nutrients allow for the isolation of a high number of fungi, but this not necessary means a high biodiversity (Caballero-George *et al.* 2013).

We considered the possible influence of temperature in the isolation of marine fungi from sponges. To mimic marine conditions as much as possible, two different temperatures were set: 25 °C commonly used to culture fungi and 15 °C closer to the environmental conditions of the sponge sampling sites.

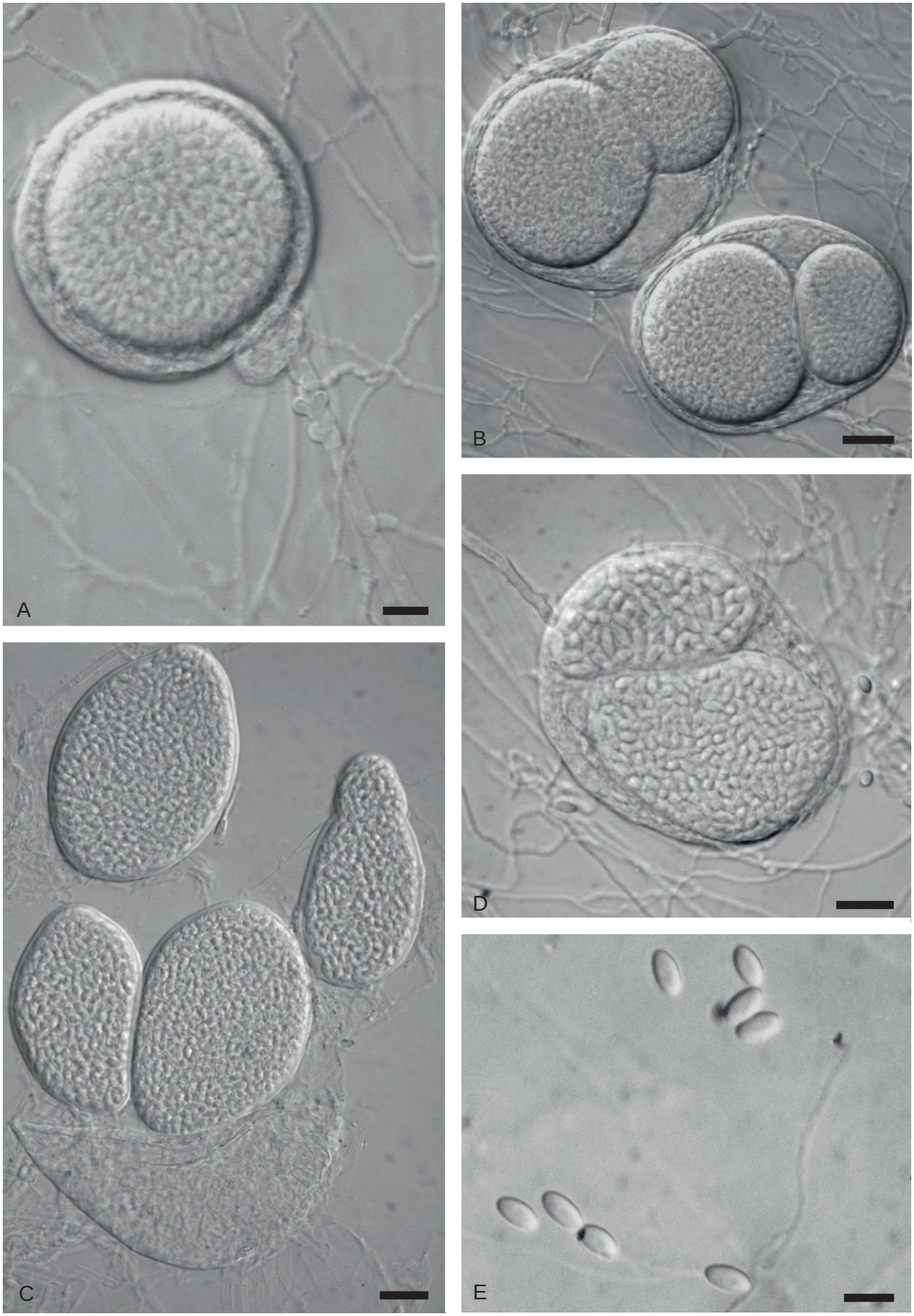


Fig. 15. *Thelebolus spongiae* MUT 2359: **A.** Initial ascoma. **B.** Ascomata with two globular asci. **C.** Mature ascoma opening with four asci. **D.** Ascoma with two sacciform asci. **E.** Ascospores. Scale bars: A, E = 10 µm; B–D = 30 µm.

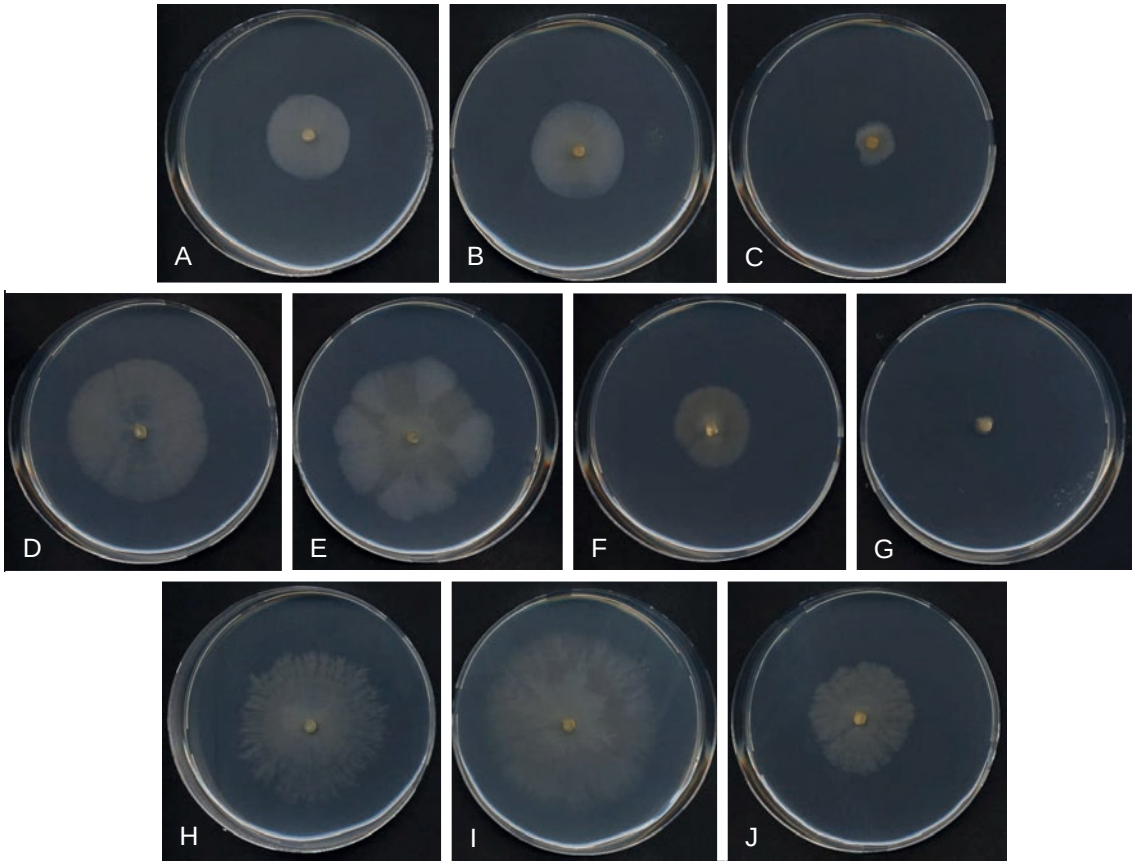


Fig. 16. *Thelebolus spongiae* MUT 2359: 21-d-old colonies on CA at 4 °C with **A.** 0 % NaCl, **B.** 2.5 % NaCl, **C.** 5 % NaCl; at 15 °C with **D.** 0 % NaCl, **E.** 2.5 % NaCl, **F.** 5 % NaCl, **G.** 10 % NaCl; at 25 °C with **H.** 0 % NaCl, **I.** 2.5 % NaCl, **J.** 5 % NaCl.

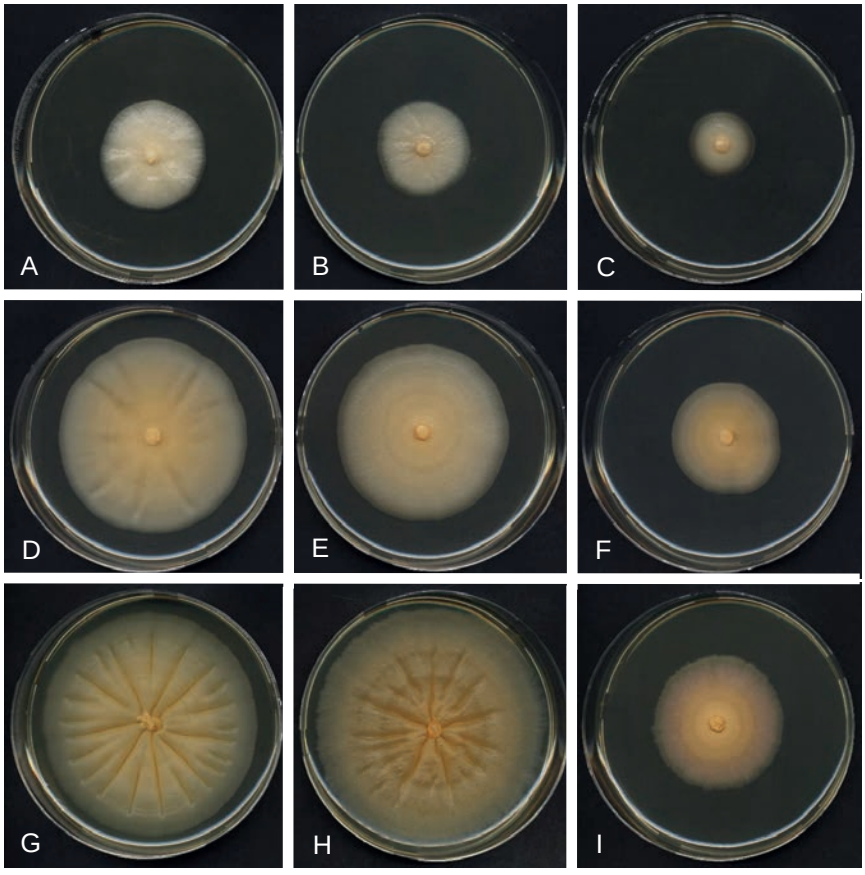


Fig. 17 *Thelebolus spongiae* MUT 2359: 21-d-old colonies on PDA at 4 °C with **A.** 0 % NaCl, **B.** 2.5 % NaCl, **C.** 5 % NaCl; at 15 °C with **D.** 0 % NaCl, **E.** 2.5 % NaCl, **F.** 5 % NaCl; at 25 °C with **G.** 0 % NaCl, **H.** 2.5 % NaCl, **I.** 5 % NaCl.

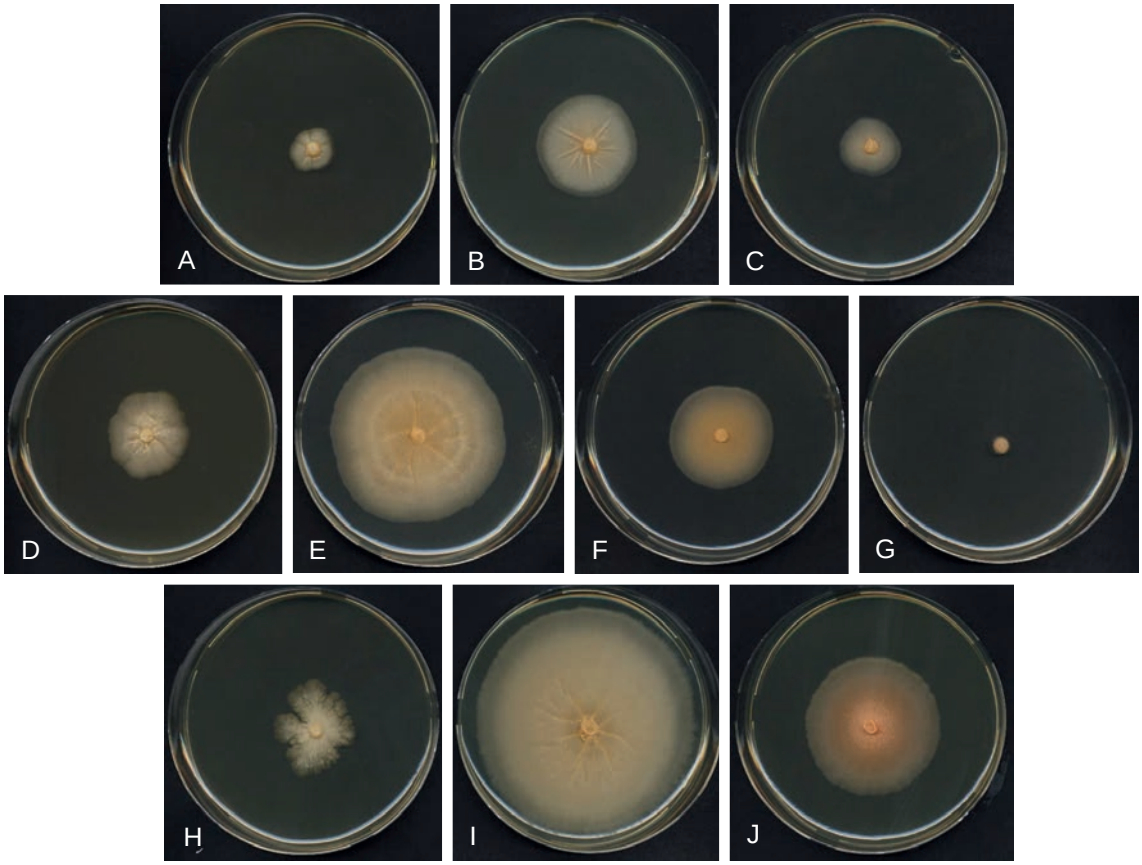


Fig. 18. *Thelebolus spongiae* MUT 2359: 21-d-old colonies on MEA at 4 °C with **A.** 0 % NaCl, **B.** 2.5 % NaCl, **C.** 5 % NaCl; at 15 °C with **D.** 0 % NaCl, **E.** 2.5 % NaCl, **F.** 5 % NaCl, **G.** 10 % NaCl; at 25 °C with **H.** 0 % NaCl, **I.** 2.5 % NaCl, **J.** 5 % NaCl.

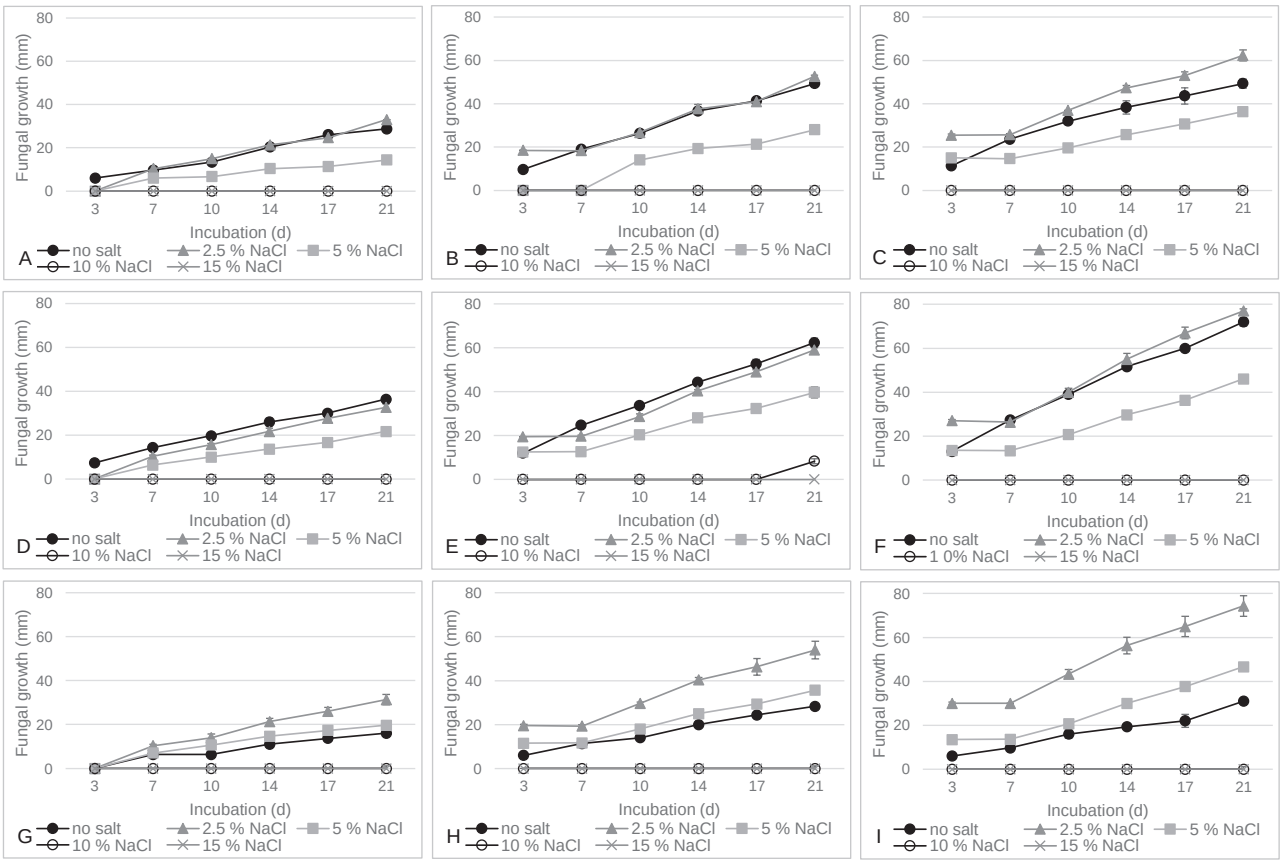


Fig. 19. *Thelebolus spongiae* MUT 2359 growth curve with no and different NaCl concentrations on CA at **A.** 4 °C, **B.** 15 °C, **C.** 25 °C; on PDA at **D.** 4 °C, **E.** 15 °C, **F.** 25 °C; on MEA at **G.** 4 °C, **H.** 15 °C, **I.** 25 °C.

Interestingly, the majority of the taxa grew exclusively at one temperature condition; this is particular evident for *P. johnstonia,* whose fungi were half isolated at 15 °C and half at 25°C.

Currently, several works on sponge-associated fungi employed different isolation techniques (Höller *et al*. 2000, Proksch *et al.* 2008, Wang *et al.* 2008, Li & Wang 2009, Ding *et al.* 2011, Wiese *et al*. 2011, Passarini *et al.* 2013, Henríquez *et al.* 2014, Diep *et al.* 2016). It would be extremely important to share these results to point out if some methods are more promising than other ones. In the attempt to increase the number of cultivable fungi, more efforts should be focused on development of innovative isolation techniques. For example, Rozas *et al.* (2011) succeeded in the isolation of fungi from single sponge cells. In parallel, the micro-Petri dishes as well as the iChip could be promising tools for the isolation of "uncultivable" (marine) microorganisms (Ingham *et al.* 2007, Nichols *et al.* 2010).

Mycobiota

Ascomycota (92 %) was the dominant phylum, as already reported for the marine environment (Jones *et al.* 2015) and for studies dealing with sponges' mycobiota (Suryanarayanan 2012). *Basidiomycota* represented a small percentage of isolates and were inferred phylogenetically by Poli *et al*. (in prep.). Their ecological role should not be underestimated: members of *Agaricales*, also detected in the present study, have already been acknowledged for their predominant role the mineralization of the organic matter in marine environment (Hyde *et al.* 1998).

Only one fungus (*Absidia glauca*) belonging to the phylum *Mucoromycota* was detected in association with *D. fragilis*. This species has already proven to withstand high salinities, as it was isolated also from Dead Sea waters. Members of *Mucoromycota* were also recorded in a small percentage in a few sponges (Höller *et al.* 2000, Thirunavukkarasu *et al*. 2012, Passarini *et al.* 2013).

According to Raghukumar (2017), sponges generally yield from zero to 21 genera of culturable fungi: while *P. johnstonia* (11 genera) is hosting an average biodiverse community, *S. ciliatum* (25) and *D. fragilis* (32) host a mycobiota communities above the mean values reported from other sponges worldwide. The most represented genera in terms of number of species were *Cladosporium* and *Penicillium* (11), followed by *Acremonium* (six) and *Aspergillus* (five). The presence of these genera and their abundance is not a surprise: they are among the most common within sponges and the most investigated for new secondary metabolites (Imhoff 2016).

Li & Wang (2009), worked on the mycobiota of three marine sponges and in the attempt to discriminate between fungi not strictly associated with sponges from those closely associated, proposed the following classification: "sponge specialist" for those genera exclusive of one sponge species, "sponge associates" for genera present in more than one species of sponge and "sponge generalist" for fungal genera present on all the species of sponges analysed. By applying this classification to the species of the present study, it was clear that the three sponges species host a specific mycobiota, as supported also by statistical analysis. For confirmation of how restricted the fungal strains are to specific sponges versus specific habitat, additional sponge species from each location should be assessed and compared to the diversity reported here.

In detail, "sponge specialist" fungi, represented more than half of the fungal community of each sponge species. Interestingly, in this group, the phylogenetic analysis highlighted several putative new species. Within the *Chaetothyriales*, MUT 2862 belongs to the genus *Cyphellophora,* which includes widespread species recorded on both animals and plants, but never described before in marine environment. This genus is in constant revision and three new species have been recently described by Gao *et al.* (2015). Within *Leotiomycetes*, MUT 2878 is well supported in the genus *Mollisia*, also known from the marine environment (Costello *et al.* 2001).

Pleosporales represent the largest group of *Dothideomycetes* and in this study, one of the most represented in terms of entities. Several fungi belong to genera already reported in the marine environment by Raghukumar (2017), this is the case of MUT 2884 (*Alternaria* sp.) MUT 2263 (*Periconia* sp.) and MUT 2390 (*Preussia* sp.). More cryptic are the position of the strains MUT 2945 (*Pleosporaceae* sp.), MUT 2489 (*Pleosporales* sp.) and MUT 2452 (*Roussoellaceae* sp.) for which further studies, are necessary. Within the *Sordariomycetes*, MUT 2766 belong to the genus *Thyronectria*, whose presence was described in the Antarctic environment by Seeler *et al*. (1940). More doubtful is the systematic classification of MUT 2463 and MUT 2377, that cluster within *Hypocreaceae* and *Microascaceae*, respectively; they both represent families already recorded in the marine environment (Jones *et al*. 2015).

Fourteen species were "sponge-associated" and common to two sponges; *D. fragilis* and *S. ciliatum* with six of these being the most similar sponges in terms of cultivable mycobiota. Among the "sponge-associated" strains, three species (*A. jensenii, A. puulaauensis* and *P. neglecta*) were reported for the first time from a marine environment. An additional seven species have never been retrieved in sponge samples but were present in the marine environment, from water samples to plants and algae samples (References details in Table 1). Two species were widespread, reported both in marine environment and associated with sponges: *B. bassiana* and *P. chrysogenum* (references in Table 2).

In the present study, the "sponge generalist" fungi were represented by *C. allicinum*, *C. cladosporioides, P. antarcticum* and *T. cylindrosporum;* all of them have been previously recorded in the marine environment and can be considered as widespread species (Bensch *et al*. 2012). *Penicillium antarcticum* is well-known both in marine (contaminated) water (Bovio *et al.* 2017) and on leaving organisms as sponges (Park *et al.* 2014) algae (Gnavi *et al*. 2017) and sea cucumbers (Marchese *et al.* 2016). *Cladosporium cladosporioides* has been reported in several marine environments, from the coral reef (Raghukumar & Ravindran 2012) to the extreme conditions of the salterns (Oren & Gunde-Cimerman 2012, Zajc *et al.* 2012) or from crude oil contaminated environments (Bovio *et al.* 2017). *Cladosporium cladosporioides* was also reported in association with marine algae (Gnavi *et al.* 2017), plants (Panno *et al.* 2013) and wood (Garzoli *et al.* 2014). Not least the presence on the sponges *Amphilectus digitata* (Pivkin *et al.* 2006), *Haliclona melana* (Rozas *et al.* 2011), *Cliona* sp. (San-Martin *et al.* 2005) and on four Red Sea sponges (Sayed *et al.* 2016). *Cladosporium allicinum* and *T. cylindrosporum* were recorded only once in the marine environment, on algae (Gnavi *et al.* 2017) and wood substrates (Rämä *et al.* 2014), respectively; while, here we documented the first report in association with marine sponges.

Considering the fact that some species, common to more than one sponge, have never been retrieved in the marine environment, it is hard to say if the classification proposed by Li & Wang (2009) is suitable to distinguish between transient

mycobiota, abundant in the water columns and true sponge-associated mycobiota. This is probably due to our still scant knowledge on fungi inhabiting sea sponges and the marine environment.

The specificity of the fungal community of each sponge could be related to several factors. Pivkin *et al.* (2006) highlighted that the number of fungi associated with sponges can be influenced by the sponge structure: the harder the structure, the lower the number of fungi. Interestingly, this hypothesis is well supported in our study. *Dysidea fragilis* which has a soft structure hosted the highest number of fungal taxa (54). Actually, the sponge name is due to its fragility outside water (Marine species identification portal http://species-identification.org/index.php). *Sycon ciliatum* was the second sponge in terms of number of taxa (32) and also in a scale of body rigidity since it presents calcareous spicules although the choanocyte chambers are free from each other, giving a "loose" consistency (Marine species identification portal http://species-identification.org/index.php). *Pachymatisma johnstonia*, which hosted the lowest number of taxa (21 taxa), is characterised by the hardest structure, given by the strong cortex of up to 1 mm thickness and the presence of both macro (megascleres) and micro (microscleres) spicules.

Several other factors could be involved in sponge recruitment of specific fungi, not least the sponge bioactivity; two sponges (*D. fragilis* and *P. johnstonia*) are known for the production of bioactive metabolites, although their antifungal activity has never been demonstrated. However, the strongest proof supporting the hypothesis of the ability of the sponge to recognise and select fungi is the discovery of sponge mitochondrial introns of fungal origin and of (1→3)-β-d-glucan-binding proteins on the sponge surface for fungus recognition (Suryanarayanan 2012).

Overall, the mycobiota examined in this study was one of the most diverse compared to other sponges even from the same environment. For instance, Baker *et al.* (2009) identified 19 fungal genotypes from the sponge *Haliclona simulans* isolated in the same study area (Gurraig Sound, Co. Galway) and interestingly 85 % of the identified fungal orders were also recorded in our research. In other environments too, the biodiversity recorded was lower: seven sponges collected in the Red Sea (Egypt) yielded 22 species (Sayed *et al.* 2016); 10 Antarctic sponges hosted 24 fungal genotypes (Henríquez *et al.* 2014) while 78 taxa were isolated from six sponges of Sakhalin Island, Russia (Pivkin *et al.* 2006). Contrast the Mediterranean sponge *Psammocinia* sp. with 85 fungal taxa (Paz *et al.* 2010) and an Atlantic sponge *Dragmacidon reticulatum* with 64 taxa (Passarini *et al*. 2013).

Finally, few species reported in the present study and isolated from healthy sponges, have been previously reported as pathogenic on marine plants and animals. *Alternaria molesta* was found on a skin lesion of *Phocaena phocaena*, a marine mammal (Tóth *et al.* 2011), and was first recorded in association with a sponge in the present study. *Fusarium solani* and *Metschnikowia bicuspidata* are a threat for shrimp and prawn aquaculture (Baker *et al.* 2009, Hatai *et al.* 2012); both species have already been reported in apparently healthy sponges (Baker *et al.* 2009, Paz *et al.* 2010, Bolaños *et al.* 2015). Concerning plants, *Cladosporium perangustum* (isolated from marine water) showed pathogenic activity against mangrove leaves under laboratory conditions (Liu *et al.* 2016). These fungi are probably opportunistic pathogens, not properly able to affect healthy organisms, like the sponges of the present studies; however, further studies will be necessary to better understand their ecological role.

Two novel species of *Thelebolus*

Proving the still untapped biodiversity of marine fungi in this study it was possible to describe two new *Thelebolus* species. *Thelebolus balaustiformis* and *T. spongiae* were isolated from the Atlantic sponge *Dysidea fragilis*.

The genus *Thelebolus* has been isolated from Tropical to Artic regions, often on animal dung and from freshwater and saline lakes (de Hoog *et al.* 2005). In the marine environment, members of *Thelebolus* were recorded also associated with *Padina pavonica*, a Mediterranean brown algae (Garzoli *et al.,* in prep) and from an Antarctic marine sponge (Henríquez *et al.* 2014). In both cases, isolates were reported as *Thelebolus* sp. and the identification was based on molecular data.

Morphological characters useful to classify this genus have been long debated and, since the '70s, the number of spores per ascus represents the main character for species definition (de Hoog *et al*. 2005). At present, the genus *Thelebolus* includes 16 species and two varieties, most of which described at the end of the 19th or in the first half of the 20th century. For this reason, many of the described species, are lacking of: i) original exhaustive descriptions (i.e. microscopic characters poorly described); ii) DNA barcode sequences available in public databases; iii) ex-type strains preserved in culture collections.

Since the two *Thelebolus* species isolated in this study presented unique morphological and molecular features, we performed a deep bibliographic search to define the main characters for each described *Thelebolus* species (Table 3) and for those available in culture collections, we obtained comparable sequences, which are now available to the scientific community. The two new marine species can be easily distinguished because they form well-defined lineages within the genus (Fig. 9). Interestingly, the isolates MUT 2357 clustered with a marine strain (*Thelebolus* sp. MUT 5281), already present in MUT culture collection and isolated from a Mediterranean brown alga; this indicates the strong affinity of this species with the marine environment. From a morphological point of view, all the dichotomous keys of the genus point out as first statement the presence of 8-spored or multisporic asci (Doveri 2004, de Hoog *et al.* 2005). Therefore, considering only the multi-spored species (not included in the tree because there were no available sequences) we can first exclude the similarity of *T. balaustiformis* with *T. monoascus* and *T. pilosus*, in fact, the last two mentioned species present only one ascus per ascoma and a higher number of spores compared to MUT 2357. *Thelebolus balaustiformis* differs also from the two varieties of *T. dubius,* by presenting a higher number of asci (20–30) and a lower number (48–64) of smaller spores.

Thelebolus spongiae MUT 2359, is characterised by a variable number of asci (from one to six), while in *T. monoascus* and *T. pilosus* is strictly limited to one; the latest mentioned species differs from *T. spongiae* MUT 2359 also for the shape and size of ascospores. *Thelebolus dubius* var. *lagopi* and *Thelebolus dubius* var. *dubius* present a variable number of asci, starting from three; the shape of asci, as well as those of ascospores differ from *T. spongiae*. In fact, MUT 2359 present globular to sacciform asci and peculiar ascospores with different ratio (2.2–2.4) from *T. dubius* var. *lagopi* (1.5) and *T. dubius* var. *dubius* (1.7).

Table 3. *Thelebolus* species and main morphological features (ascomata, asci and ascospores).

Species	Ascomata	Number of asci per ascoma	Asci	Number of ascospores	Ascospores	References
T. coemansii	-	numerous	85–110 × 20–25 µm, cylindrical-clavate	8	-	(13)
T. delicatus[a]	Subglobosus	-	-	-	-	(5)
T. dubius* var. *dubius	-	3–5	40–45 × 24.4 µm, broadly ovate or oblong-ovate	128 (?)	6 × 4 µm, ellipsoid, rather pointed at the ends	(4)
T. dubius* var. *lagopi	80–150 µm diam subglobosus	10–16	87–100 × 25–33 µm, cylindrical-clavate	more than 200	6.2–7.6 × 3.6–4.3 µm, ellipsoid to ovoid	(4)
T. ellipsoideus	17–46 µm diam, subglobosus or ovoid to ellipsoid	1–8 (rarely up to 25)	22 × 11–16 µm, shortly ellipsoid to subglobose	8	5–9.2 × 4–5.3 µm, shortly-ellipsoid	(6)
T. globosus	300–520 µm diam, subglobosus or ovoid to ellipsoid	1–4	12–15 × 9–12 µm, irregular shortly ellipsoid to subglobose	8	5–7.5 × 4.1–5.1 µm, broadly ellipsoid	(6)
T. hirsutus[a]	-	-	-	-	-	(2)
T. lignicola	-	-	-	60–100	3.4 x 4–4.5 µm	(8)
T. microcarpus	18–70 µm diam, globose to subglobose	1–5	12–17 × 10–15 µm, subglobose to broadly ellipsoid	8	5–9 × 3–4 µm, ellipsoid	(1)
T. microsporus	45–500 µm diam, subglobosus, hemispheric or subcylindric	5–100	80–125 × 20–26 µm, cylindrical to cylindrical-clavate	8	6–10 × 3–5	(4), (6), (7)
T. minutissimus[a]	-	-	-	-	-	(3)
T. monoascus	150–200 µm diam, hemispheric	1	150–170 µm	500	5–6.5 × 4– 4.5 µm, ovate	(9)
T. pilosus	-	1	300 × 250 µm	about 100	9–11 × 7– 8 µm	(11)
T. polysporus	60–200 µm diam	2–5	50–160 × 18–90 µm, subellipsoid to ovoid or sacciform	256	5–7.5 × 3–4 µm, ovoid to oblong-ellipsoidal	(4), (7), (13)
T. stercoreus	135–400 µm diam, ellipsoid to ovoid or subglobose	1, rarely 2–3	165–262 × 120–205 µm, ellipsoid to ovoid or subglobose	up to 3 000 spores	5 –7.7 × 2.3–4.5 µm, spores smooth, broadly elliptic, ellipsoid or oblong	(4), (7), (13)
T. striatus	-	-	124–162 × 9–10.8 µm, elongate cylindrical	8	11.3–13.5 × 6–6.7 µm, narrow ellipsoid	(12)
T. terrestris	-	-	-	-	18.4–25.6 × 8–9.6 µm	(10)

(1) Crous *et al.* 2015, (2) De Lamarck & De Candolle 1815, (3) De Schweinitz 1834, (4) Doveri 2004, (5) Fries 1823, (6) de Hoog *et al.* 2005, (7) Kimbrough 1981, (8) Lloyd 1918, (9) Mouton 1886, (10) Pfister 1993, (11) Schroeter 1908, (12) Thind *et al.* 1959, (13) Van Brummelen 1998.

[a]The original descriptions do not contain any information about the microscopic structures.

Interestingly, *T. balaustiformis* was isolated by homogenisation of sponges tissues on CMASW at 15 °C, while *T. spongiae* was isolated by direct plating of sponge tissue on SWA at 15 °C, highlighting once more the importance of using different isolation techniques and culture conditions.

In conclusion, with the present work, we highlighted the great and still unexplored fungal diversity that characterises the marine environment. The use of several isolation methods improved the yield of cultivable fungi that with few techniques and growth media, would have been impossible to isolate. The sponges proved to host a specific mycobiota and several fungi identified with the contribution of morphological, molecular and phylogenetic approach, were first reported from a marine environment, while *T balaustiformis* and *T.*

spongiae were here described as new. The present study again highlights the great mosaic of largely unknown marine microbial diversity.

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New and Interesting Fungi. 1

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Abstract: This study introduces two new families, one new genus, 22 new species, 10 new combinations, four epitypes, and 16 interesting new host and / or geographical records. *Cylindriaceae* (based on *Cylindrium elongatum*) is introduced as new family, with three new combinations. *Xyladictyochaetaceae* (based on *Xyladictyochaeta lusitanica*) is introduced to accommodate *Xyladictyochaeta. Pseudoanungitea* gen. nov. (based on *P. syzygii*) is described on stems of *Vaccinium myrtillus* (Germany). New species include: *Exophiala eucalypticola* on *Eucalyptus obliqua* leaf litter, *Phyllosticta hakeicola* on leaves of *Hakea* sp., *Setophaeosphaeria citricola* on leaves of *Citrus australasica,* and *Sirastachys cyperacearum* on leaves of *Cyperaceae* (Australia); *Polyscytalum chilense* on leaves of *Eucalyptus urophylla* (Chile); *Pseudoanungitea vaccinii* on *Vaccinium myrtillus* (Germany); *Teichospora quercus* on branch tissue of *Quercus* sp. (France); *Fusiconidium lycopodiellae* on stems of *Lycopodiella inundata, Monochaetia junipericola* on twig of *Juniperus communis, Myrmecridium sorbicola* on branch tissues of *Sorbus aucuparia, Parathyridaria philadelphi* on twigs of *Philadelphus coronarius,* and *Wettsteinina philadelphi* on twigs of *Philadelphus coronarius* (Germany); *Zygosporium pseudogibbum* on leaves of *Eucalyptus pellita* (Malaysia); *Pseudoanungitea variabilis* on dead wood (Spain); *Alfaria acaciae* on leaves of *Acacia propinqua, Dictyochaeta mimusopis* on leaves of *Mimusops caffra,* and *Pseudocercospora breonadiae* on leaves of *Breonadia microcephala* (South Africa); *Colletotrichum kniphofiae* on leaves of *Kniphofia uvaria, Subplenodomus iridicola* on *Iris* sp., and *Trochila viburnicola* on twig cankers on *Viburnum* sp. (UK); *Polyscytalum neofecundissimum* on *Quercus robur* leaf litter, and *Roussoella euonymi* on fallen branches of *Euonymus europaeus* (Ukraine). New combinations include: *Cylindrium algarvense* on leaves of *Eucalyptus* sp. (Portugal), *Cylindrium purgamentum* on leaf litter (USA), *Cylindrium syzygii* on leaves of *Syzygium* sp. (Australia), *Microdochium musae* on leaves of *Musa* sp. (Malaysia), *Polyscytalum eucalyptigenum* on *Eucalyptus grandis* × *pellita* (Malaysia), *P. eucalyptorum* on leaves of *Eucalyptus* (Australia), *P. grevilleae* on leaves of *Grevillea* (Australia), *P. nullicananum* on leaves of *Eucalyptus* (Australia), *Pseudoanungitea syzygii* on *Syzygium cordatum* leaf litter (South Africa), and *Setophaeosphaeria sidae* on leaves of *Sida* sp. (Brazil). New records include: *Sphaerellopsis paraphysata* on leaves of *Phragmites* sp., *Vermiculariopsiella dichapetali* on leaves of *Melaleuca* sp. and *Eucalyptus regnans*, and *Xyladictyochaeta lusitanica* on leaf litter of *Eucalyptus* sp. (Australia); *Camarosporidiella mackenziei* on twigs of *Caragana* sp. (Finland); *Cyclothyriella rubronotata* on twigs of *Ailanthus altissima*, *Rhinocladiella quercus* on *Sorbus aucuparia* branches (Germany); *Cytospora viticola* on stems of *Vitis vinifera* (Hungary); *Echinocatena arthrinioides* on leaves of *Acacia crassicarpa* (Malaysia); *Varicosporellopsis aquatilis* from garden soil (Netherlands); *Pestalotiopsis hollandica* on needles of *Cupressus sempervirens* (Spain), *Pseudocamarosporium africanum* on twigs of *Erica* sp. (South Africa), *Pseudocamarosporium brabeji* on branch of *Platanus* sp. (Switzerland); *Neocucurbitaria cava* on leaves of *Quercus ilex* (UK); *Chaetosphaeria myriocarpa* on decaying wood of *Carpinus betulus, Haplographium delicatum* on decaying *Carpinus betulus* wood (Ukraine). Epitypes are designated for: *Elsinoë mimosae* on leaves of *Mimosa diplotricha* (Brazil), *Neohendersonia kickxii* on *Fagus sylvatica* twig bark (Italy), *Caliciopsis maxima* on fronds of *Niphidium crassifolium* (Brazil), *Dictyochaeta septata* on leaves of *Eucalyptus grandis* × *urophylla* (Chile), and *Microdochium musae* on leaves of *Musa* sp. (Malaysia).

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Dedicated to Vadim Alexandrovich Mel'nik (*16 March 1937, †10 April 2017).

INTRODUCTION

New and Interesting Fungi (NIF) is introduced as a new series of papers that will supplement other series focussed on expanding existing knowledge of fungal biodiversity and fungal conservation. Another similar series such as the Fungal Planet (www.fungalplanet.org) aims to provide a rapid and simplified outlet for researchers to describe new fungal species as well as to highlight the environments where these fungi were isolated. The Fungal Planet series established in 2006 emphasises a holistic conservation of all life on the planet including not only plants and animals but also fungi (Crous *et al*. 2017a, b).

This new series of papers focusses not only on new fungal taxa but also on those that are generally interesting and that deserve notice. Like other series including the already mentioned Fungal Planet, the Genera of Fungi (GoF) series (Crous & Groenewald 2017, Giraldo *et al.* 2017), the Genera of Phytopathogenic Fungi (GOPHY) series (Marin-Felix *et al.* 2017), and the Fungal Systematics and Evolution series (Crous *et al.* 2015a, Hernandez-Restrepo *et al.* 2016, Krisai-Greilhuber *et al.* 2017) it has become evident that there are many undescribed species of fungi and new host or geographical records for which a scientific repository is lacking. Most of these could easily never be described or catalogued, and thus being lost to science. This justified the decision to launch the new series New and Interesting Fungi (NIF). It is hoped that this series will provide an attractive vehicle for mycologists to publish single new species or to highlight the relevance of important fungi.

Many known fungal species need to be recollected and epi- or neotypified in order to secure the application of old names already in use and resolve their DNA phylogeny. Subsequent to the end of the long-standing dual nomenclature for fungi (Hawksworth *et al*. 2011, Wingfield *et al*. 2012) and the connection of different morphs to a single name (Rossman *et al*. 2015, Réblová *et al.* 2016), it became clear that a vehicle was required to ensure that these data could be easily and effectively published. This would be comparable to "data release papers" published in other fields of science and biology (Miller et al. 2013, Vu *et al.* 2016). The New and Interesting Fungi series will link not only asexual and sexual morphs of species, but also provide opportunities to merge morphological observations with DNA sequence data, providing a means for rapid and accurate identification. New and Interesting Fungi will appear twice each year (June and December) in the journal Fungal Systematics and Evolution (www.FUSE-journal.org). Mycologists and other researchers wishing to contribute to future issues in this series are encouraged to contact Pedro Crous (p.crous@westerdijkinstitute.nl) before submission to ensure that potential conflicts with activities arising from other research groups can be avoided.

MATERIALS AND METHODS

Isolates

Leaves and twig samples were placed in damp chambers and incubated at room temperature for 1–3 d. Single conidial colonies were grown from sporulating conidiomata in Petri dishes containing 2 % malt extract agar (MEA) as described earlier by Crous *et al.* (1991). Leaf and stem tissues bearing ascomata were soaked in water for approximately 2 h, after which they were attached to the bottom side of the lids of Petri dishes containing MEA. After ascospores ejected onto the MEA, germination patterns were determined after 24 h, and single ascospore or conidial cultures were established following the method described by (Crous 1998). Colonies were sub-cultured on 2 % potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous *et al*. 2009b), autoclaved pine needles on 2 % tap water agar (PNA) (Smith *et al.* 1996), or autoclaved banana leaves (BLA), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains and specimens are maintained at the Westerdijk Fungal Biodiversity Institute in Utrecht, the Netherlands (CBS).

DNA extraction, amplification (PCR) and phylogeny

Fungal mycelium (Supplementary Table 1) was scraped from the agar surface of cultures with a sterile scalpel and the genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturers' protocols. The 28S nrRNA gene (LSU) and internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS) of the nrDNA operon were sequenced for all the isolates included in this study. Other loci were sequenced for various species or genera using primers and conditions specific for those groups of fungi (see references for details). The resulting fragments were sequenced in both directions using the respective PCR primers and the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA, USA); DNA sequencing amplicons were purified through Sephadex G-50 Superfine columns (Sigma-Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were analysed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA, USA). The DNA sequences were analysed and consensus sequences were computed using SeqMan Pro v. 13 (DNASTAR, Madison, WI, USA).

The sequences for each gene were subjected to megablast searches (Zhang *et al*. 2000) to identify closely related sequences in the NCBI's GenBank nucleotide database. The results are provided as part of the species notes or as selected phylogenetic trees. Phylogenetic trees were generated using Bayesian analyses performed with MrBayes v. 3.2.6 (Ronquist *et al*. 2012) for the overview trees and Maximum Parsimony analyses performed with PAUP v. 4.0b10 (Swofford 2003) as explained in Braun *et al*. (2018) for the genus and species trees. All resulting trees were printed with Geneious v. 11.0.3 (http://www.geneious.com, Kearse *et al.* 2012) and the layout of the trees was done in Adobe Illustrator v. CC 2017. Statistical measures calculated

included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC).

Morphology

Slide preparations were mounted in lactic acid, Shear's mounting fluid or water, from colonies sporulating on MEA, PDA, PNA, BLA or OA. Sections through conidiomata were made by hand. Observations were made with a Nikon SMZ25 dissection-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and images recorded on a Nikon DS-Ri2 camera with associated software. Colony characters and pigment production were noted after 2–4 wk of growth on MEA, PDA and OA (Crous *et al*. 2009b) incubated at 25 °C. Colony colours (surface and reverse) were scored using the colour charts of Rayner (1970). Sequences derived in this study were deposited in GenBank (Supplementary Table 1), the alignment in TreeBASE (www.treebase.org; study number S22442), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous *et al*. 2004).

RESULTS

Phylogeny

Dothideomycetes LSU phylogeny: The alignment contained 125 isolates and *Candida broadrunensis* (CBS 11838, GenBank KY106372.1) was used as outgroup. The final alignment contained a total of 808 characters used for the phylogenetic analyses, including alignment gaps. Based on the results of MrModelTest, dirichlet base frequencies and the GTR+I+G model was used for the Bayesian analysis. The Bayesian analyses generated 38 302 trees from which 28 728 were sampled after 25 % of the trees were discarded as burn-in. The posterior probability values (PP) were calculated from the 28 728 trees (Fig. 1 overview *Dothideomycetes*; PP >0.74 shown). The alignment contained a total of 345 unique site patterns.

Eurotiomycetes and *Leotiomycetes* LSU phylogeny: The alignment contained 44 isolates and *Orbilia vinosa* (GenBank DQ470952.1) was used as outgroup. The final alignment contained a total of 813 characters used for the phylogenetic analyses, including alignment gaps. Based on the results of MrModelTest, dirichlet base frequencies and the GTR+I+G model was used for the Bayesian analyses. The Bayesian analyses generated 9 702 trees from which 7 278 were sampled after 25 % of the trees were discarded as burn-in. The posterior probability values (PP) were calculated from the 7 278 trees (Fig. 2 overview *Eurotiomycetes*; PP >0.74 shown). The alignment contained a total of 253 unique site patterns.

Sordariomycetes LSU phylogeny: The alignment contained 148 isolates and *Candida broadrunensis* (CBS 11838, GenBank KY106372.1) was used as outgroup. The final alignment contained a total of 761 characters used for the phylogenetic analyses, including alignment gaps. Based on the results of MrModelTest, dirichlet base frequencies and the GTR+I+G model was used for the Bayesian analysis. The Bayesian analyses generated 34 702 trees from which 26 028 were sampled after 25 % of the trees were discarded as burn-in. The posterior probability values (PP) were calculated from the 26 028 trees (Fig. 3 overview *Sordariomycetes*; first value: PP >0.74 shown). The alignment contained a total of 361 unique site patterns.

Species phylogenies: Specific phylogenetic analyses were run for selected species and the resulting phylogenies are discussed in the species notes where applicable. Statistics associated with those phylogenies are provided in the figure legends.

Taxonomy

Alfaria acaciae Crous & M.J. Wingf., ***sp. nov.*** MycoBank MB824766. Fig. 4.

Etymology: Name refers to *Acacia,* the genus of the substrate from which this fungus was collected.

Conidiomata sporodochial, surrounded by setae, black with dark green to black slimy conidial masses, 80–250 µm diam. *Setae* flexuous, unbranched, thick-walled, apex obtuse, dark brown, verruculose, 3–6-septate, 100–150 × 5–7 µm. *Conidiophores* densely aggregated, arising from hyaline basal stroma, becoming pigmented and verruculose towards conidiogenous region, subcylindrical, 3–5-septate, branched, 30–55 × 2–2.5 µm. *Conidiogenous cells* integrated, terminal and intercalary, subcylindrical, becoming pigmented and verruculose at upper region, phialidic with periclinal thickening and flared collarette, 10–20 × 2–2.5 µm. *Conidia* solitary, fusoid-ellipsoid, straight, apex subobtuse, base truncate, 1.5–2 µm diam, aseptate, guttulate, granular, medium brown, smooth, (6–)8–10(–12) × (2.5–)3 µm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and smooth, lobate margins, reaching 50 mm diam after 2 wk at 25 °C. On MEA surface dirty white to pale luteous, reverse luteous. On PDA surface dirty white, reverse pale luteous. On OA surface pale luteous.

Specimens examined: **South Africa**, Western Cape Province, Stellenbosch, Helshoogte Pass, on leaves of *Acacia propinqua* (*Fabaceae*), Jul. 2012, *M.J. Wingfield* (holotype CBS H-23428, culture ex-type CPC 31882 = CBS 143504); *ibid.*, CPC 31940.

Notes: *Alfaria cyperi-esculenti* was originally described from *Cyperus esculentus* in Spain, where it causes a serious foliar disease (Crous *et al.* 2014). This species is currently known only from its sexual morph, which complicates a morphological comparison with the present, asexual isolate from South Africa. Although phylogenetically closely related (Fig. 5), we regard them as two distinct species (see bp differences below). Furthermore, culture characteristics also differ between the two species, with cultures of *A. acaciae* growing faster, and paler in colour than the ochreous / apricot cultures of *A. cyperi-esculenti* (Crous *et al.* 2014).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *A. cyperi-esculenti* (GenBank KJ869143; Identities 567 / 577 (98 %), 5 gaps (0 %)), *Myrothecium leucotrichum* (GenBank AJ301992; Identities 566 / 578 (98 %), 7 gaps (1 %)) and *A. thymi* (GenBank KU845990; Identities 559 / 572 (98 %), 8 gaps (1 %)). The ITS sequences of CPC 31882 and 31940 are identical. The highest similarities using the LSU sequence were *A. cyperi-esculenti* (GenBank KJ869200; Identities 803 / 804 (99 %), no gaps), *A. thymi* (GenBank KU845999; Identities 824 / 828 (99 %), 1 gap (0 %)) and *A. caricicola* (GenBank KU845992; Identities 822 / 828 (99 %), 1 gap (0 %)). The highest similarities using the *cmdA*

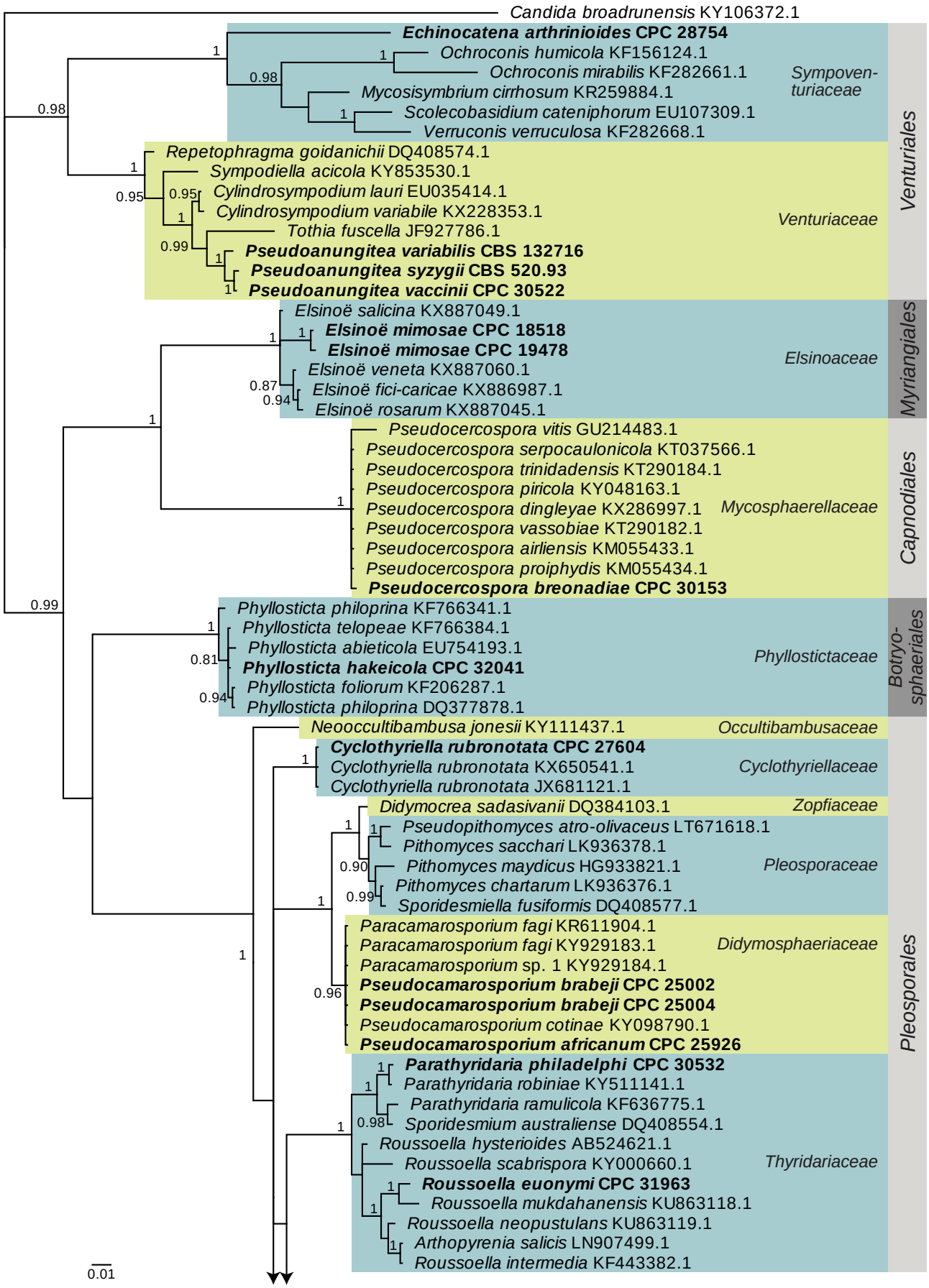

Fig. 1. Consensus phylogram (50 % majority rule) resulting from a Bayesian analysis of the *Dothideomycetes* LSU sequence alignment. Bayesian posterior probabilities (PP) > 0.74 are shown at the nodes and the scale bar represents the expected changes per site. Families and orders are indicated with coloured blocks to the right of the tree. GenBank accession or culture collection numbers are indicated behind the species names. The tree was rooted to *Candida broadrunensis* (GenBank KY106372.1) and the novelties treated in this study for which LSU sequence data were available are indicated in bold face.

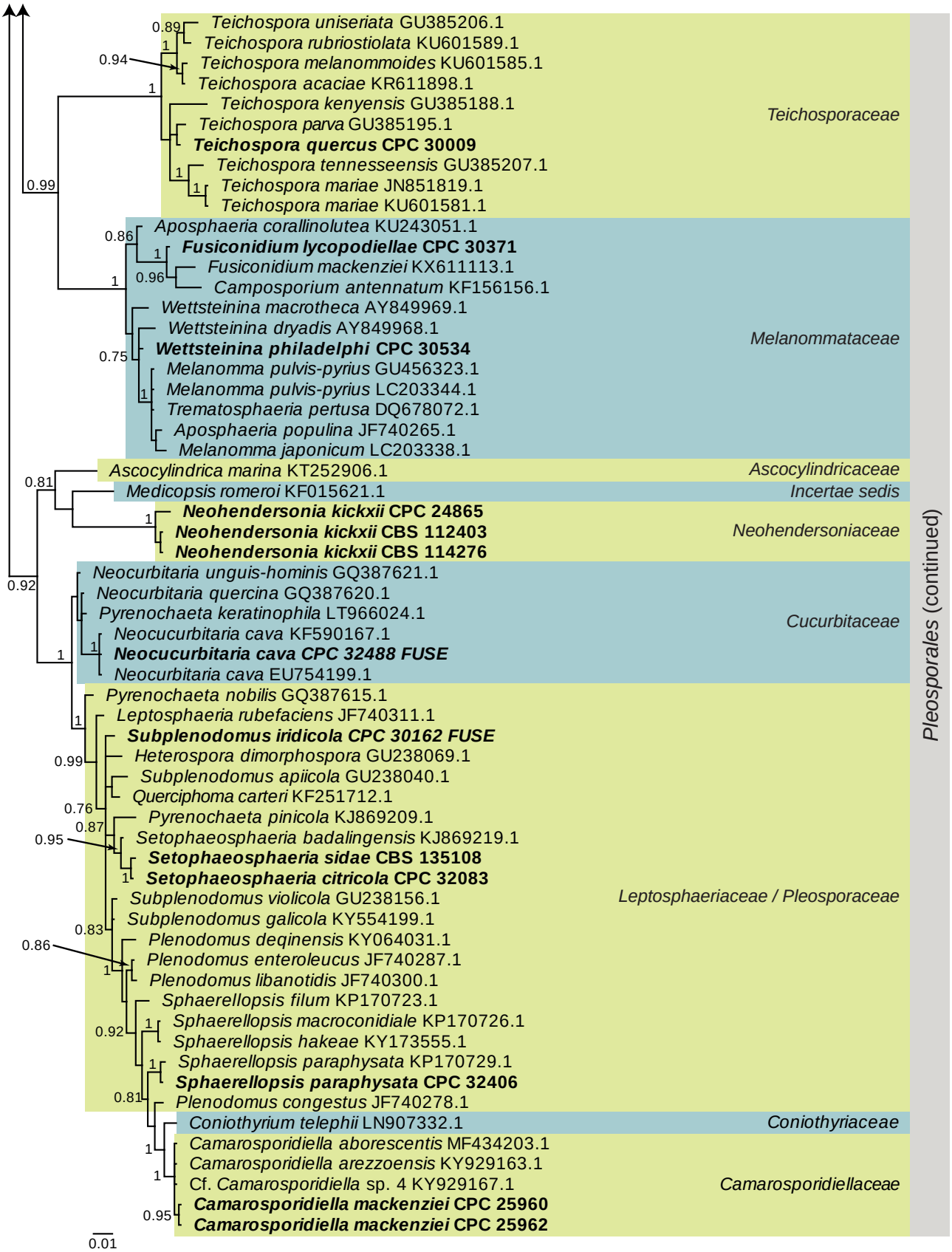

Fig. 1. (Continued).

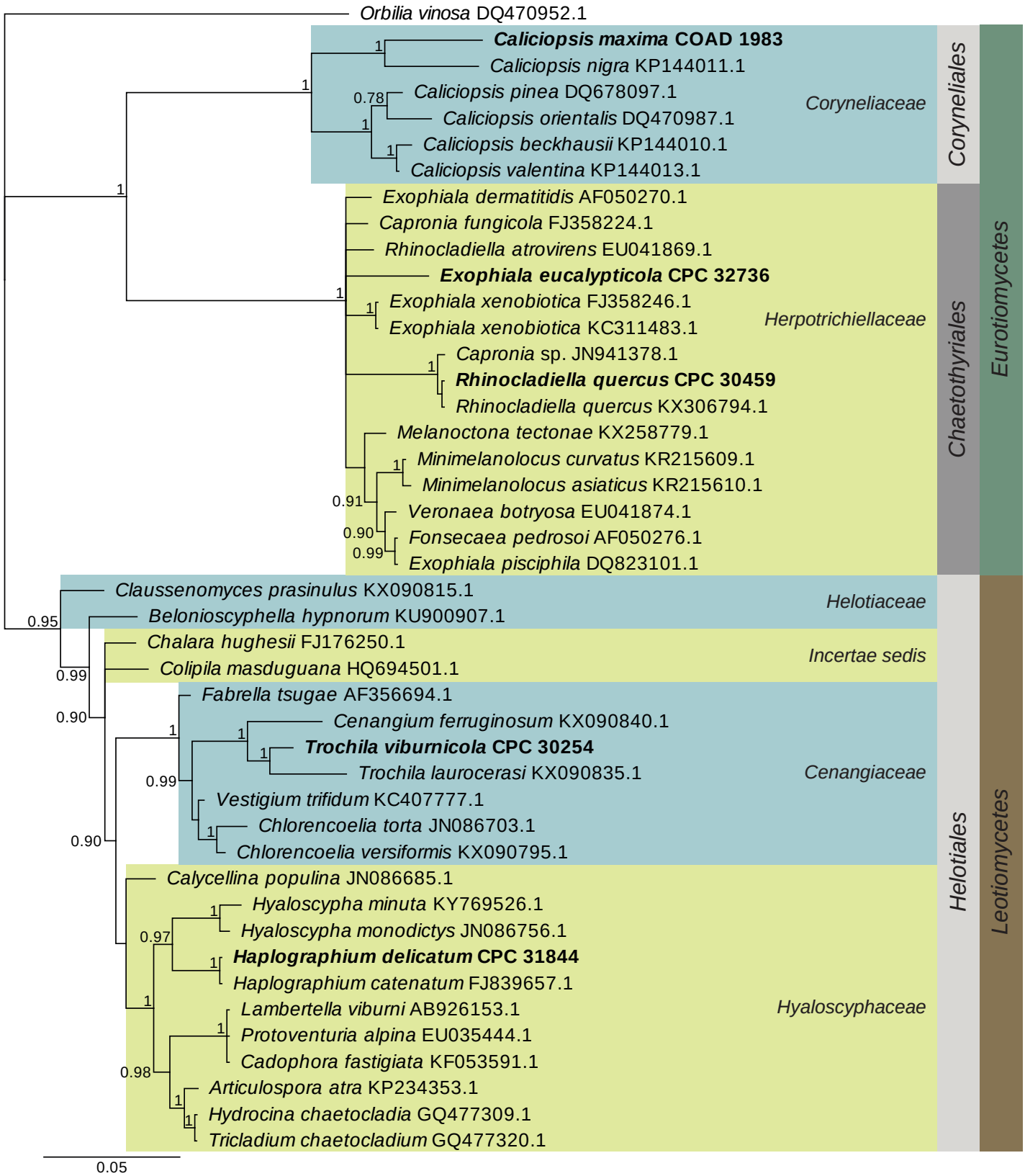

Fig. 2. Consensus phylogram (50 % majority rule) resulting from a Bayesian analysis of the *Eurotiomycetes* and *Leotiomycetes* LSU sequence alignment. Bayesian posterior probabilities (PP) > 0.74 are shown at the nodes and the scale bar represents the expected changes per site. Families, orders and classes are indicated with coloured blocks to the right of the tree. GenBank accession or culture collection numbers are indicated behind the species names. The tree was rooted to *Orbilia vinosa* (GenBank DQ470952.1) and the novelties treated in this study for which LSU sequence data were available are indicated in bold face.

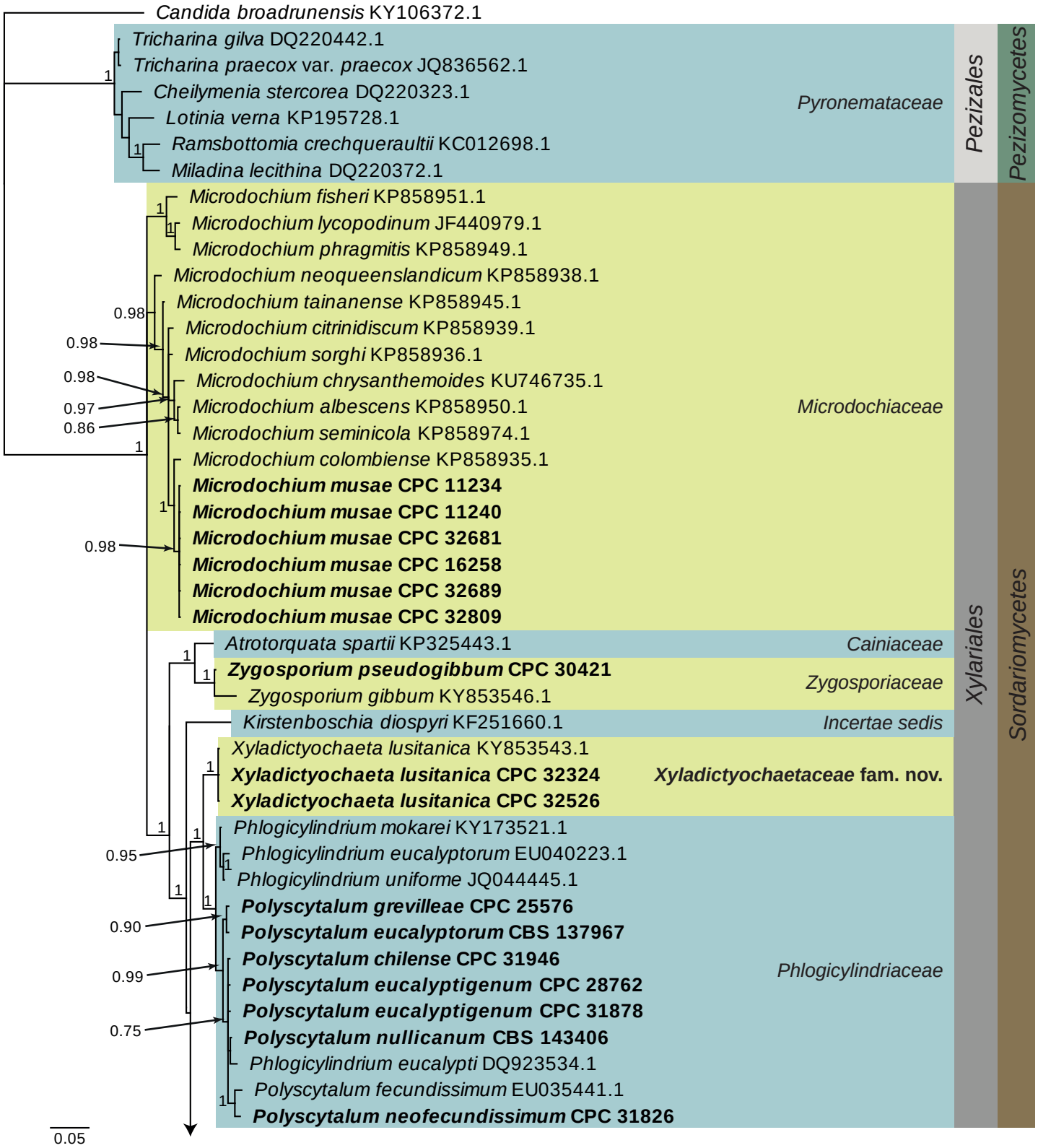

Fig. 3. Consensus phylogram (50 % majority rule) resulting from a Bayesian analysis of the *Pezizomycetes* and *Sordariomycetes* LSU sequence alignment. Bayesian posterior probabilities (PP) > 0.74 are shown at the nodes and the scale bar represents the expected changes per site. Families, orders and classes are indicated with coloured blocks to the right of the tree. GenBank accession or culture collection numbers are indicated behind the species names. The tree was rooted to *Candida broadrunensis* (GenBank KY106372.1) and the novelties treated in this study for which LSU sequence data were available are indicated in bold face.

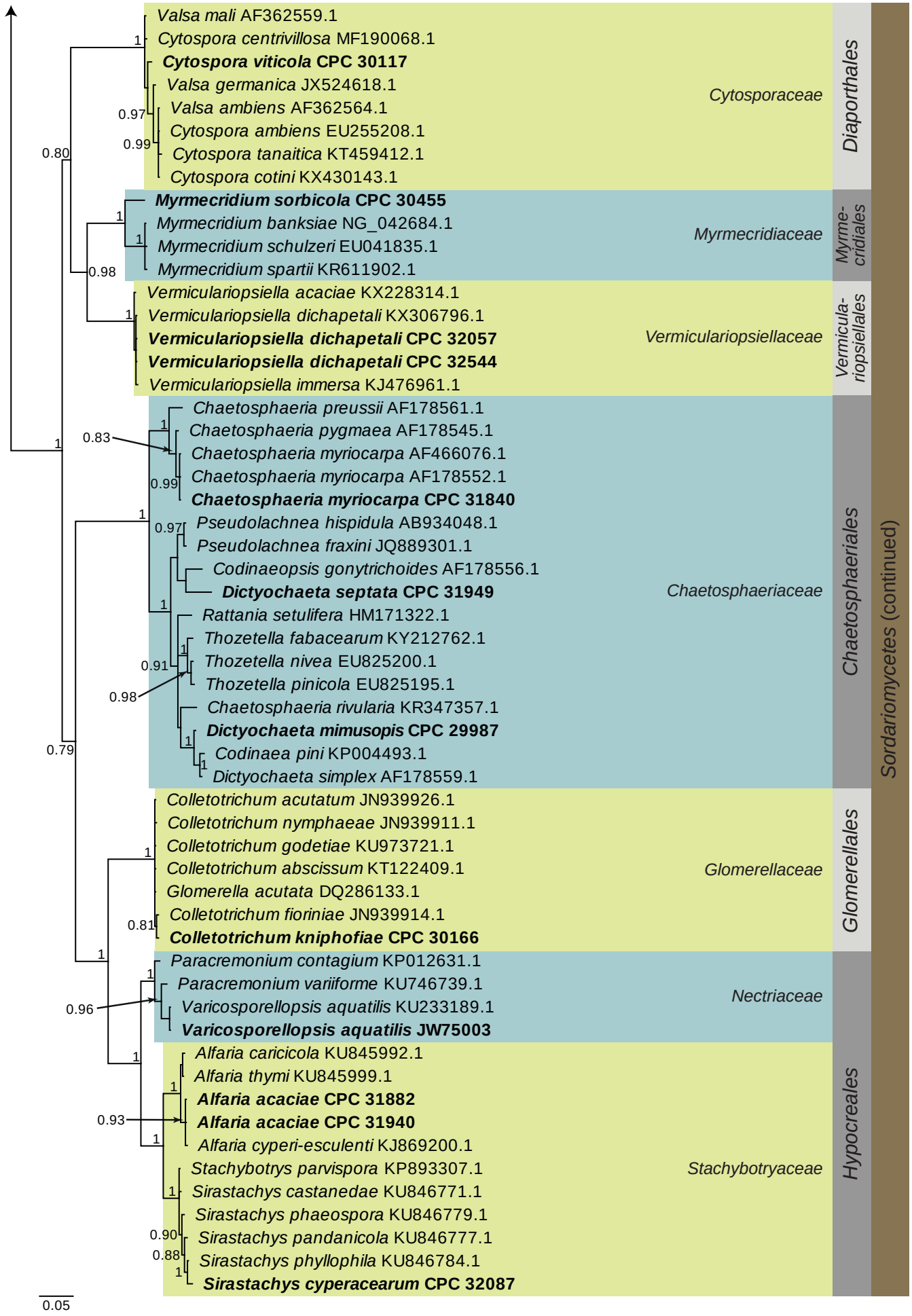

Fig. 3. (Continued).

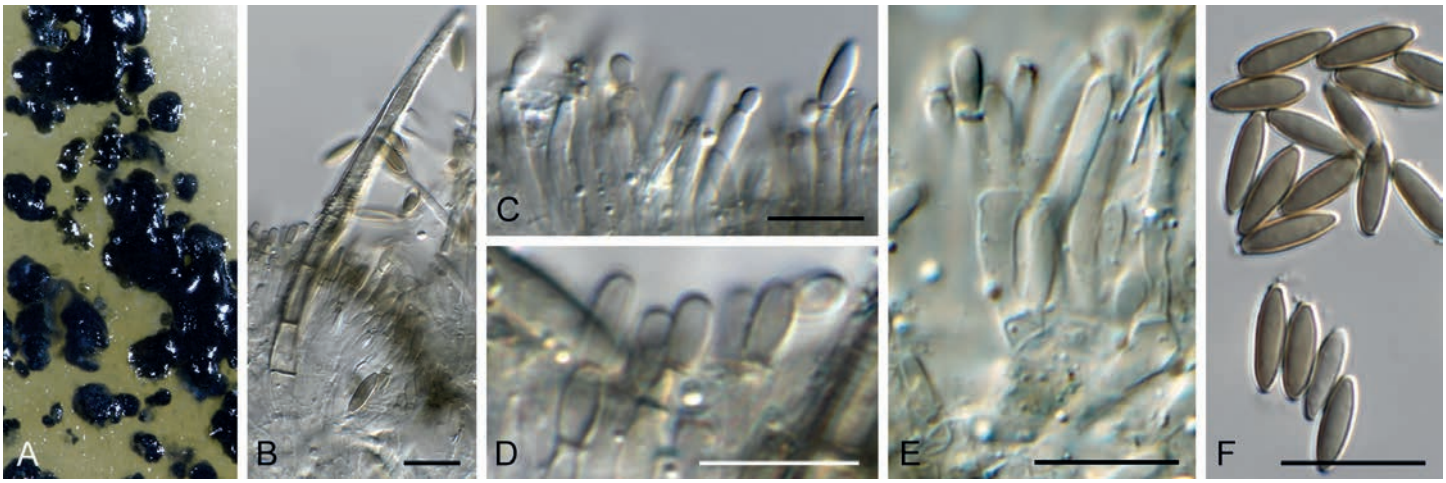

Fig. 4. *Alfaria acaciae* (CBS 143504). **A.** Colony on OA. **B.** Conidioma with seta. **C–E.** Conidiogenous cells. **F.** Conidia. Scale bars = 10 μm.

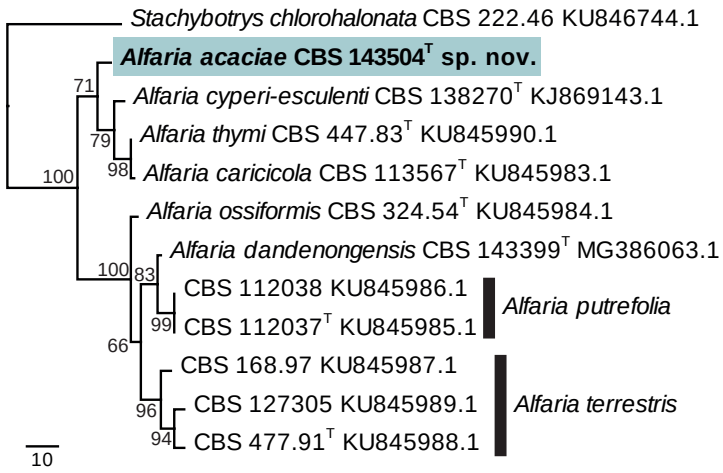

Fig. 5. Single most parsimonious tree obtained from a phylogenetic analysis of the *Alfaria* ITS alignment (12 strains including the outgroup; 538 characters analysed: 306 constant, 46 variable and parsimony-uninformative and 186 parsimony-informative). The tree was rooted to *Stachybotrys chlorohalonata* (GenBank KU846744.1) and the scale bar indicates the number of changes. Bootstrap support values higher than 49 % are shown at the nodes and novelties are highlighted with a coloured box and bold text. Species names are indicated to the right of the tree, or before the culture collection and GenBank accession numbers. A superscript T denotes strains with a type status. Tree statistics: TL = 130, CI = 0.900, RI = 0.892, RC = 0.803.

sequence of CPC 31882 were *A. terrestris* (GenBank KU845979; Identities 479 / 544 (88 %), 7 gaps (1 %)), *Gregatothecium humicola* (GenBank KU846285; Identities 475 / 544 (87 %), 7 gaps (1 %)) and *A. ossiformis* (GenBank KU845977; Identities 461 / 545 (85 %), 10 gaps (1 %)). The highest similarities using the *rpb2* sequence of CPC 31882 were *A. putrefolia* (GenBank KU846003; Identities 624 / 704 (89 %), 3 gaps (0 %)), *A. ossiformis* (GenBank KU846002; Identities 620 / 710 (87 %), 3 gaps (0 %)) and *A. caricicola* (GenBank KU846001; Identities 536 / 597 (90 %), no gaps). The *rpb2* sequences of CPC 31882 and 31940 were identical. The highest similarities using the *tef1* sequence of CPC 31882 were *A. terrestris* (GenBank KU846010; Identities 321 / 378 (85 %), 26 gaps (6 %)), *A. caricicola* (GenBank KU846008; Identities 370 / 441 (84 %), 20 gaps (4 %)) and *A. ossiformis* (GenBank KU846009; Identities 313 / 373 (84 %), 27 gaps (7 %)). The *tef1* sequences of CPC 31882 and 31940 were identical. The highest similarities using the *tub2* sequence of CPC 31882 were *A. terrestris* (GenBank KU846019; Identities 331 / 348 (95 %), 3 gaps (0 %)), *A. putrefolia* (GenBank KU846017; Identities 330 / 347 (95 %), 1 gap (0 %)) and *A. ossiformis* (GenBank KU846015; Identities 328 / 348 (94 %), 3 gaps (0 %)).

Caliciopsis maxima (Berk. & M.A. Curtis) Höhn., *Sitzungsber Akad. Wiss. Wien, Math.-Naturwiss. Kl., Abt. 1*, **128**: 84. 1919. Fig. 6.
Basionym: *Capnodium maximum* Berk. & M.A. Curtis, *J. Linn. Soc., Bot*. **10**(46): 391. 1868 (1869).

Stromata mostly abaxial, limited to sporangial sori of ferns, hidden from view beneath the host sporangia until black ascigerous, bristle-like stromatic columnar tubes push up and protrude from the sori, or otherwise are produced on wounded tissues, sometimes bordering entire pinnules; not associated with discoloration or necrosis of the opposite surface of the frond; rarely adaxial; starting as minute, erumpent cushions, increasing in diameter and thickness after emergence. *Ascigerous columns* long-stalked, prominently beaked, undergoing repeated apical proliferation; additional stromatic column material is formed in a renewed vegetative growth phase at the funnel-shaped apex of each stalk; the process is repeated as many as five times; primary column usually longer, reaching up to 1.7 mm long, all columns formed later, not exceeding 700 μm in length; stalk long, slender, flexuous,

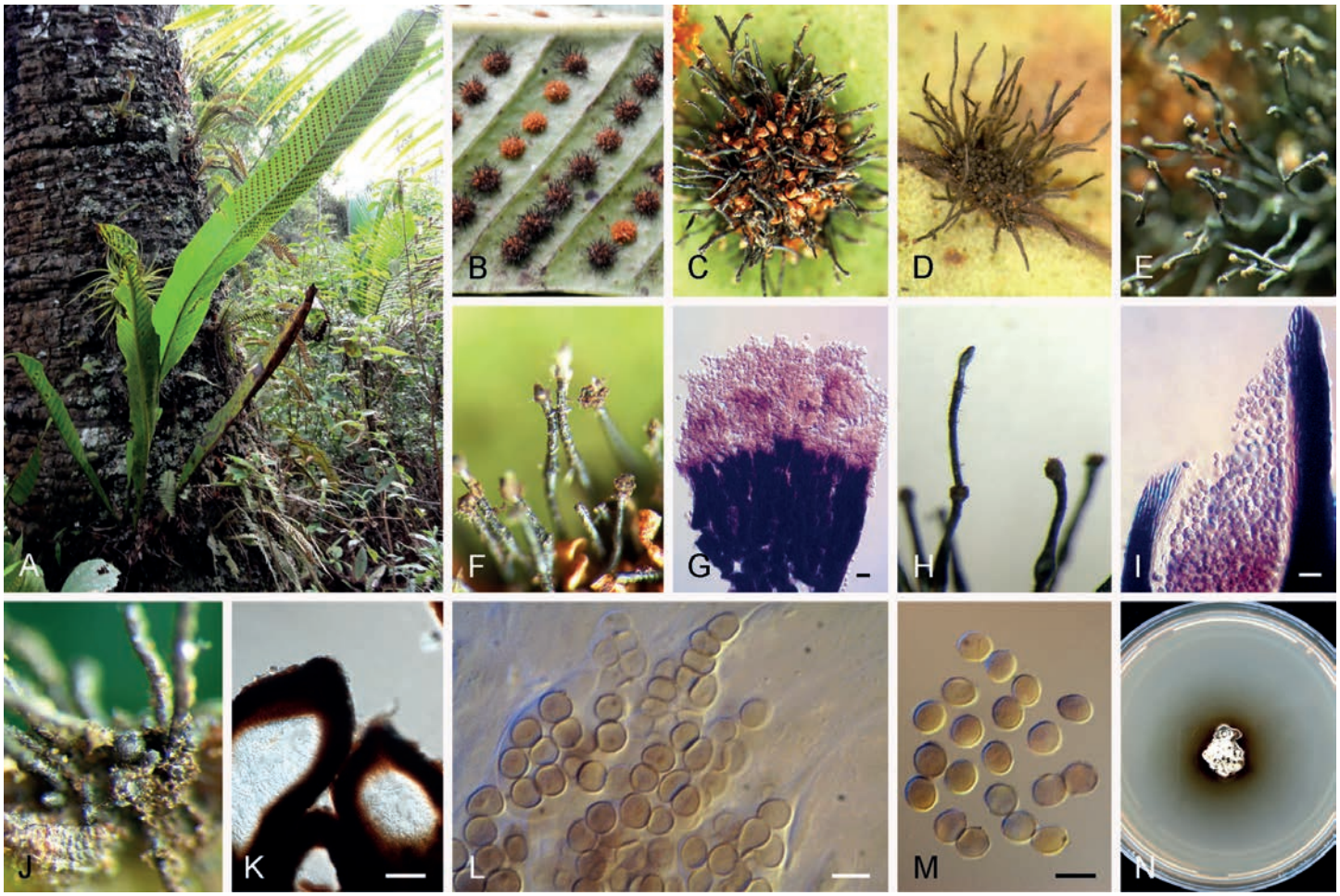

Fig. 6. *Caliciopsis maxima* (CPC 24674, VIC 42568). **A.** Habitat where the fungus and host (*Niphidium crassifolium*) was found – note growth of fern as an epiphyte on trunk of araucaria. **B–D.** Detail of fern sporangia colonized by the fungus. **E–F.** Detail of the ascospores aggregate formed by ascospores release. **G**. Squashed terminal portion of the ascomata, note the pulvurulent reddish-brown mass of ascospores. **H.** Detail of the percurrent proliferation of the ascoma. **I.** Vertical section of the upper portion of an ascoma. **J.** Spermogonium at the base of columnar ascomata. **K.** Vertical section of spermogonium, containing spermatia. **L.** Asci. **M.** Ascospores. **N.** Culture on PCA. Scale bars: G, I = 5 µm, K–M = 10 µm.

35–50 µm diam, covered with brown hyphae. *Ascigenous swelling* (locules) subterminal, ellipsoid, 125–150 µm diam, 200–350 µm in length, apical dehiscence, forming a reddish brown pulverulent of terminally aggregated ascospores. *Asci* bitunicate, evanescent, obclavate, pedicellate, straight or slightly curved, 15–17 × 8–10 µm, 8-spored, aparaphysate, hyaline, smooth. *Ascospores* inordinate, overlapping, globose or subglobose, 3–4 µm diam, aseptate, eguttulate, yellowish brown, thin-walled, smooth. *Spermogonium* subglobose, sessile or short stipitate, papillate, often covered in pale brown hyphae, aggregated below ascomatal tubes, black, smooth. *Spermatia* unicellular, narrowly fusiform, 11–24 × 3–4 µm, hyaline, smooth.

Culture characteristics: Colonies on PCA slow-growing, 15–20 mm diam after 1 mo; irregular, convex with papillate surface, edges entire, aerial mycelium sparse to absent, composed of black hyphal tufts, vinaceous buff towards periphery, pigmenting the medium with cinnamon taint; sepia in reverse; colonies sterile.

Specimens examined: **Cuba**, on fronds of *Niphidium* sp. (*Polypodiaceae*) (originally identified as *Polypodium* sp.), 1941, *Wright* (holotype CUP-029913). **Brazil**, Rio de Janeiro, Nova Friburgo, on fronds of *Niphidium crassifolium* (*Polypodiaceae*), 5 Nov. 2011, *R.W. Barreto* (epitype designated here VIC 42568, MBT373013, culture ex-epitype COAD 1983 = CPC 24674); *ibid,* on fronds of *Microgramma squamulosa* (*Polypodiaceae*), 10 Oct. 2013, *R.W. Barreto* (VIC 42602).

Notes: The epitype specimen of *Caliciopsis maxima* (*Coryneliaceae*) proposed here closely matches the morphology of the holotype and several additional collections studied by Fitzpatrick (1942), including one recorded on the same host and location in Brazil (NY-02928724). On all materials, stromata were produced on sori of sporangia, or on wounded tissues, not associated with discoloration or tissue necrosis of the opposite surface of the frond. Ascigerous columns had a tendency to undergo repeated apical proliferation, a feature that differs from all other known species; ascospores are typically globose or subglobose, yellowish brown, 3–4 µm diam (Fitzpatrick 1942). Based on phylogenetic evidence, the genus resides in the *Coryneliaceae*, within the *Eurotiomycetes*, as recently demonstrated through molecular analysis (Prieto *et al.* 2013, Garrido-Benavent & Pérez-Ortega 2015, Wood *et al.* 2016). In the combined ITS-LSU analysis (Fig. 7), *C. maxima* clustered in a basal position, suggesting that the fungal species associated with ferns are evolutionarily basal to the evolution of their relatives, as previously demonstrated for the cercosporoid and mycosphaerella-like species occurring on ferns (Guatimosim *et al.* 2016).

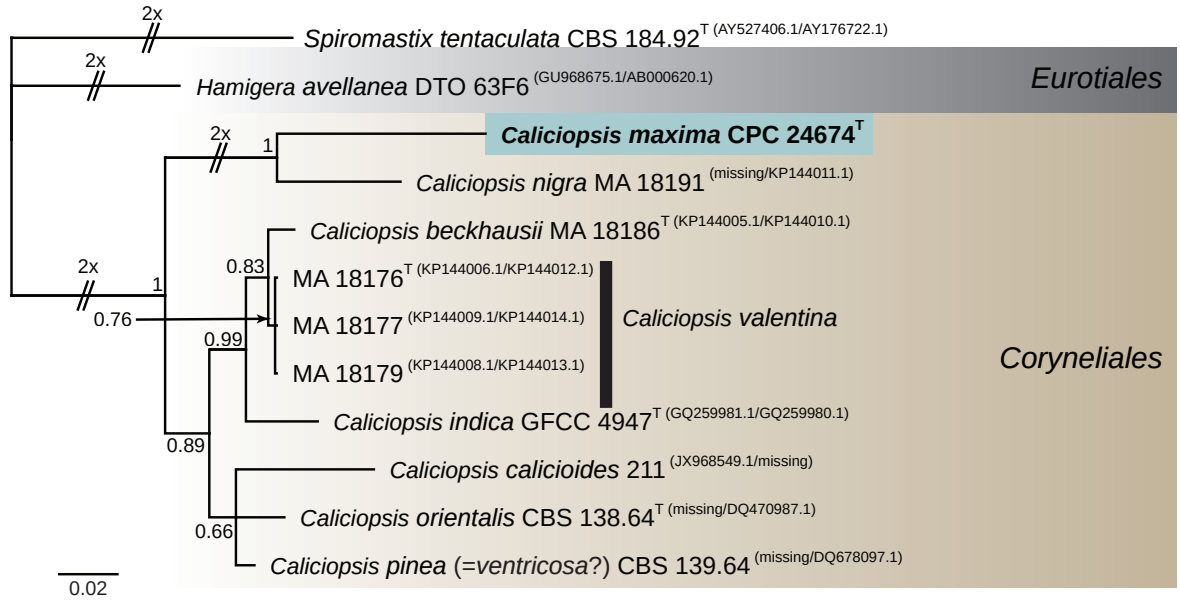

Fig. 7. Consensus phylogram (50 % majority rule) resulting from a Bayesian analysis of the combined ITS and LSU alignment of *Caliciopsis* species. Bayesian posterior probabilities are indicated at the nodes and the scale bar represents the expected changes per site. The tree was rooted to *Spiromastix tentaculata* (culture CBS 184.92) and the novelty treated in this study is highlighted with a coloured box and bold text. A superscript T denotes strains with a type status. GenBank accession and/or culture collection numbers are indicated behind the species names. Orders are indicated to the very right of the tree. The more basal branches were halved to facilitate easier layout. The ITS partition had 198 unique site patterns and the LSU partition had 139 unique site patterns out of the included 548 and 853 characters respectively.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Corynelia uberata* (GenBank KU204606; Identities 474 / 544 (87 %), 21 gaps (3 %)), *Caliciopsis pinea* (GenBank KY099604; Identities 323 / 361 (89 %), 6 gaps (1 %)) and *C. beckhausii* (GenBank NR_132090; Identities 330 / 370 (89 %), 11 gaps (2 %)). The highest similarities using the LSU sequence were *C. nigra* (GenBank KP144011; Identities 771 / 825 (93 %), 5 gaps (0 %)), *C. pinea* (GenBank DQ678097; Identities 779 / 839 (93 %), 6 gaps (0 %)) and *C. valentina* (GenBank KP144013; Identities 780 / 842 (93 %), 6 gaps (0 %)).

Camarosporidiella mackenziei Wanas*. et al*., *Stud. Mycol*. **87**: 236. 2017. Fig. 8.

Conidiomata separate, pycnidial, globose, 150–200 µm diam, with central ostiole; wall of 2–3 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* tightly aggregated, lining the inner cavity, hyaline, smooth, subcylindrical to ampulliform or doliiform, proliferating percurrently at apex, 5–8 × 3–4 µm. *Conidia* solitary, hyaline, smooth, guttulate to granular, subcylindrical to ellipsoid, apex obtusely rounded, base truncate, (3–)4–5(–6) × (2–)2.5(–3) µm. *Ascomata* pseudothecial, intra- to subcorticolous, singly to densely crowded, tufted if fully developed, erumpent, subglobose with flattened base, later somewhat fusing at the base, ostiole central and indistinct, black, finely rough, thick, soft, basally with a few red brown, thick-walled, smooth and gnarled hyphae, 0.5–0.75 mm diam. *Peridium* multi-layered, consisting of a *textura angularis* with red brown, thick-walled and smooth cells, inner layer hyaline, cells 10–17 µm diam. *Pseudoparaphyses* numerous, longer than the asci, basally moniliform otherwise cylindrical and filiform, short celled, multi-celled, branched, with a few anastomoses, hyaline, thin-walled, smooth, septa in the upper part smooth and thin-walled, eguttulate, 3–4 µm in diam. *Asci* 8-spored, cylindrical, bitunicate, fissitunicate, thick-walled, apically roundish, pedicel short and furcate, inamyloid (water plus Lugol), 131–210 × 15–16 µm, ascospores oblique uniseriate. *Ascospores* 8(–10)-celled, muriform, ellipsoid, mostly straight, both parts of the spore approx. equal in size, end cells conical or roundish, wall golden brownish, thick and always smooth, median septum constricted, otherwise smooth to faintly constricted, thick-walled and reddish, one longitudinal septum per cell, end cells aseptate, plasma eguttulate, without a gelatinous sheath and appendages, examined in water, living and mature, 30–35(–44) × 10–12.5 µm (av. 31.4 × 11.5).

Culture characteristics: Colonies spreading, with fluffy, moderate to abundant aerial mycelium. On MEA, PDA and OA surface and reverse dark mouse grey.

Specimens examined: **Finland**, Outokumpu, on twig of *Caragana* sp. (*Fabaceae*), 31 Dec. 2014, *M. Pennanen*, specimen CBS H-23430, culture CPC 25960 = CBS 144200; *ibid.*, CPC 25962.

Notes: Isolates CPC 25960 and CPC 25962 were treated as "*Camarosporium* sp. 2" in Crous & Groenewald (2017). The species was subsequently placed in the genus *Camarosporidiella* by Wanasinghe *et al.* (2017), clustering within the *C. mackenziei* clade. The latter taxon was described from twigs of *Caragana arborescens* collected in Russia. Although the present collection produced only the microconidial morph in culture, the sexual morph was observed on host tissue, which is a new observation for this species.

Based on a megablast search using the ITS sequence of CPC 25960, the closest matches in NCBI's GenBank nucleotide database were *C. mackenziei* (GenBank MF434159; Identities 542 / 543 (99 %), 1 gap (0 %)), *C. melnikii* (GenBank MF434162; Identities 540 / 544 (99 %), 1 gap (0 %)) and *C. caraganicola* (GenBank MF434124; Identities 540 / 544 (99 %), 1 gap (0 %)). The highest similarities using the LSU sequence of CPC 25960,

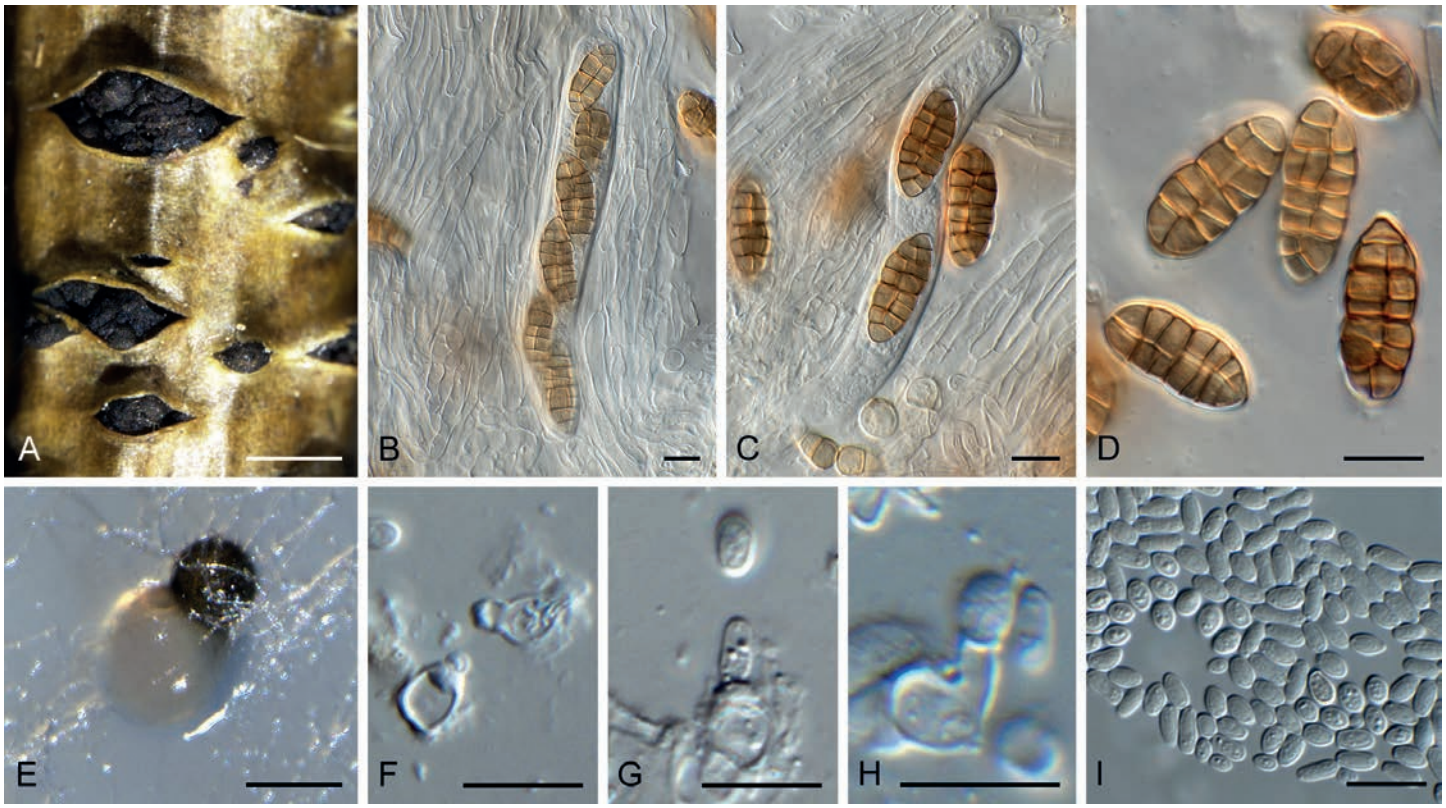

Fig. 8. *Camarosporidiella mackenziei* (CPC 25960). **A.** Ascomata on host tissue. **B, C.** Asci. **D.** Ascospores. **E.** Conidioma. **F–H.** Conidiogenous cells. **I.** Conidia. Scale bars: A = 0.75 mm, E = 200 μm, all others = 10 μm.

the closest matches in NCBI's GenBank nucleotide database were *C. aborescentis* (GenBank MF434203; Identities 821 / 822 (99 %), no gaps), "*Cf. Camarosporium* sp. 4" (GenBank KY929167; Identities 821 / 822 (99 %), no gaps) and *C. arezzoensis* (GenBank KY929163; Identities 821 / 822 (99 %), no gaps). The highest similarities using the *tef1* sequence of CPC 25960, the closest matches in NCBI's GenBank nucleotide database were *C. mackenziei* (GenBank MF434423; Identities 904 / 907 (99 %), no gaps), *C. italica* (GenBank MF434415; Identities 899 / 907 (99 %), no gaps) and *C. arborescentis* (GenBank MF434380; Identities 899 / 907 (99 %), no gaps).

Chaetosphaeria myriocarpa (Fr.) C. Booth, *Mycol. Pap*. **68**: 5. 1957. Fig. 9.
Basionym: *Sphaeria myriocarpa* Fr., *Kongl. Vetensk. Acad. Hand.* **267**. 1817.
Synonym: *Sphaeria myriocarpa* Fr., *Syst. mycol*. **2**(2): 459. 1823.

Mycelium consisting of medium brown, verruculose, branched, septate, 2–3 μm diam hyphae. *Conidiophores* solitary, erect, subcylindrical, flexuous, unbranched, at times rejuvenating percurrently in apical part, 1–5-septate, dark brown, thick-walled, roughened in lower region, 35–100 × 2.5–3 μm. *Conidiogenous cells* integrated, terminal, medium brown, smooth, subcylindrical to obovoid, 25–30 × 2.5–3 μm; apex with flared collarette, 2–3 μm diam. *Conidia* occurring in chains, aggregating in mucoid mass, hyaline, smooth, apex obtuse, abruptly tapering to a truncate base, creating triangular conidia, 2.5–3 × 2.5 μm.

Culture characteristics: Colonies flat, spreading, with sparse to moderate aerial mycelium and feathery, lobate margins, reaching 25 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface and reverse iron-grey.

Specimen examined: **Ukraine**, Ternopil region, Zalischyky district, Dniester Canyon, on decaying wood of *Carpinus betulus* (*Betulaceae*), 5 Oct. 2016, *A. Akulov*, specimen ex CWU (MYC) AS 6049 (dried culture CBS H-23426, culture CPC 31840 = CBS 143389).

Notes: *Chaetosphaeria myriocarpa* is commonly isolated from dead woody substrates in Europe, and represents a new record for Ukraine. Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Ch. myriocarpa* (GenBank JF340253; Identities 483 / 490 (99 %), 1 gap (0 %)), *Ch. pygmaea* (GenBank AF178545; Identities 473 / 496 (95 %), 3 gaps (0 %)) and *Phialophora phaeophora* (GenBank AF083191; Identities 503 / 533 (94 %), 2 gaps (0 %)). The highest similarities using the LSU sequence were *Ch. myriocarpa* (GenBank AF178552; Identities 841 / 841 (100 %), no gaps), *Ch. pygmaea* (GenBank AF178545; Identities 837 / 843 (99 %), 2 gaps (0 %)) and *Ch. preussii* (GenBank AF178561; Identities 816 / 835 (98 %), no gaps). Only distant matches were obtained with the *tub2* sequence, e.g. with *Chaetomium jodhpurense* (GenBank KP336854; Identities 292 / 361 (81 %), 19 gaps (5 %)).

Colletotrichum kniphofiae Crous & Denman, ***sp. nov.*** MycoBank MB824769. Fig. 10.

Etymology: Name refers to *Kniphofia*, the host genus from which it was isolated.

Asexual morph on OA (sterile on other media). *Conidiomata* acervular, conidiophores formed on a cushion of pale brown, angular cells, 6–15 μm diam. *Setae* rarely observed in culture, brown, flexuous, verruculose, tapering to subobtuse apices, 5–8-septate, up to 100 μm long. *Conidiophores* hyaline, septate, branched, smooth-walled, up to 60 μm long. *Conidiogenous*

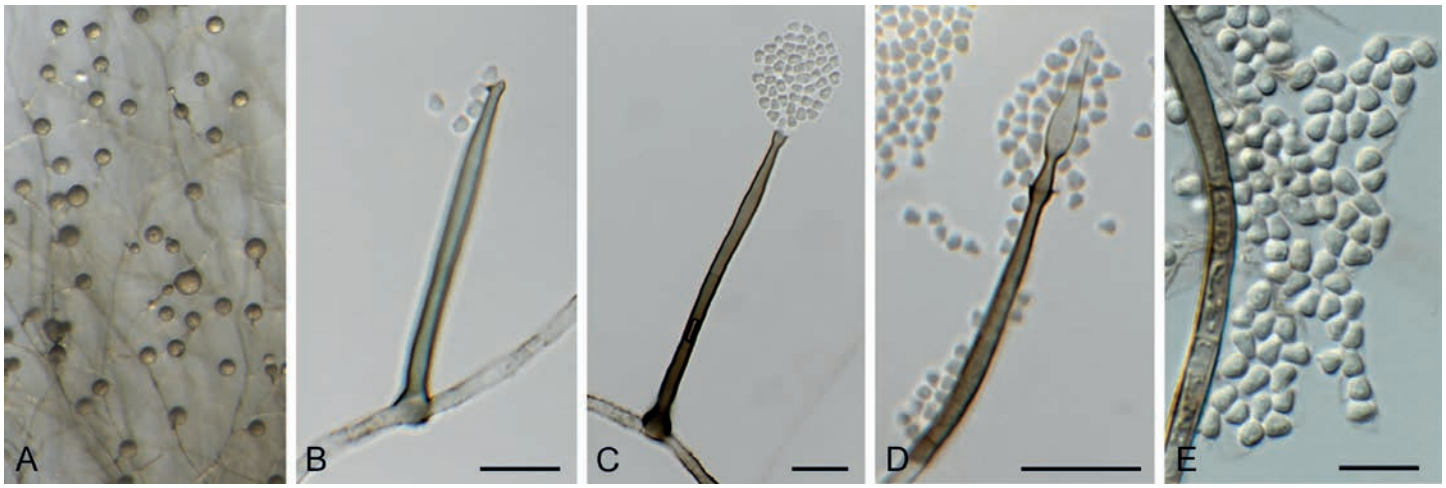

Fig. 9. *Chaetosphaeria myriocarpa* (CBS 143389). **A.** Conidiophores on SNA. **B–D.** Conidiophores. **E.** Conidia. Scale bars = 10 μm.

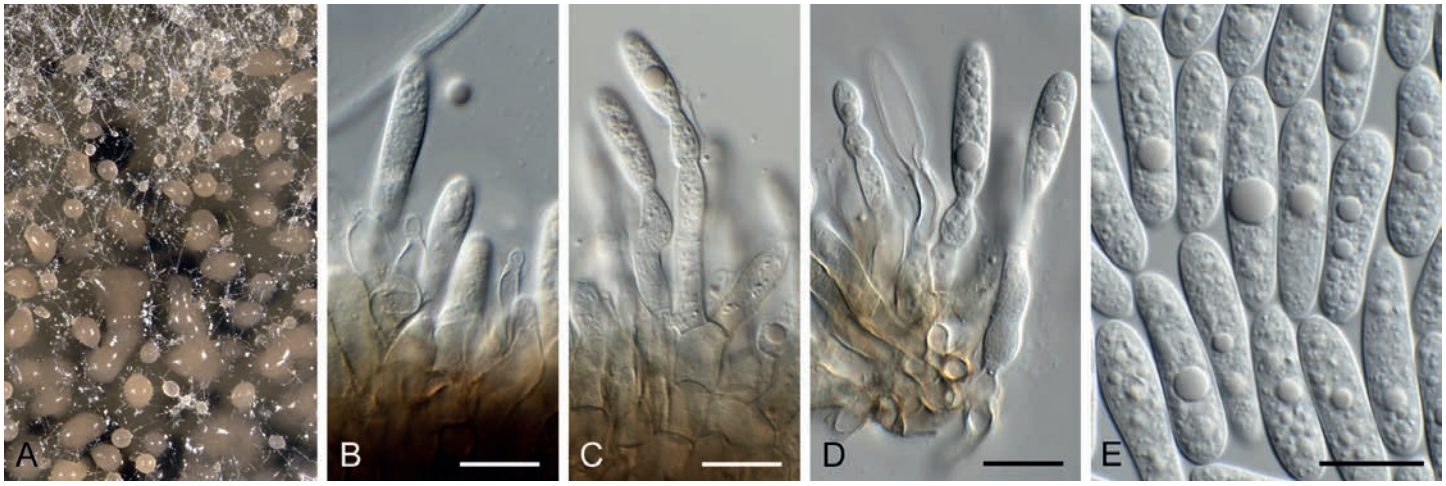

Fig. 10. *Colletotrichum kniphofiae* (CBS 143496). **A.** Colony on OA. **B–D.** Conidiogenous cells. **E.** Conidia. Scale bars = 10 μm.

cells hyaline, smooth, cylindrical, 12–20 × 4–7 μm, phialidic with periclinal thickening. *Conidia* hyaline, smooth-walled, aseptate, straight, rarely curved, prominently multi-guttulate, fusoid to subcylindrical, apex obtuse, tapering at base to truncate hilum, 2 μm diam, (17–)25–28(–37) × (5–)6(–7) μm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and even, lobate margins, covering dish after 2 wk at 25 °C. On MEA surface dirty white with patches of olivaceous grey, reverse amber with patches of olivaceous grey. On PDA surface and reverse olivaceous grey with patches of smoke grey. On OA surface olivaceous grey.

Specimen examined: **UK**, England, Upton Grey, on leaves of *Kniphofia uvaria* (*Xanthorrhoeaceae*), 28 Mar. 2016, *P.W. Crous* (holotype CBS H-23432, culture ex-type CPC 30166 = CBS 143496); *ibid*., CPC 30168.

Notes: Sexual morph not observed, but ascospores harvested from plant material, indicating that a sexual morph exists. *Colletotrichum kniphofiae* was isolated from dead leaves of *Kniphofia*, and nothing is known regarding its ecology, and no species of *Colletotrichum* have been described from this host. Supported by its distinct DNA phylogeny (Fig. 11), we believe this collection represents a distinct species.

Based on a megablast search using the ITS sequence of CPC 30166, the closest matches in NCBI's GenBank nucleotide database were *Co. godetiae* (GenBank KX756147; Identities 567 / 575 (99 %), 2 gaps (0 %)), *Co. pyricola* (GenBank KU963516; Identities 565 / 575 (95 %), 2 gaps (0 %)) and *Co. salicis* (GenBank KU498278; Identities 565 / 575 (95 %), 2 gaps (0 %)). The ITS sequences of CPC 30166 and CPC 30168 are identical. The highest similarities using the LSU sequence of CPC 30166 were *Co. godetiae* (GenBank KU973721; Identities 837 / 839 (99 %), no gaps), *Co. acutatum* (GenBank JN939926; Identities 837 / 839 (99 %), no gaps) and *Co. fioriniae* (GenBank JN939914; Identities 837 / 839 (99 %), no gaps). The highest similarities using the *actA* sequence were *Co. destructivum* (GenBank AY157843; Identities 596 / 657 (91 %), 16 gaps (2 %)), *Co. kahawae* (GenBank KU579251; Identities 595 / 659 (90 %), 26 gaps (3 %)) and *Co. orbiculare* (GenBank AB778553; Identities 584 / 654 (89 %), 22 gaps (3 %)). The highest similarities using the *chs1* sequence were *Co. salicis* (GenBank JQ949131; Identities 245 / 252 (97 %), no gaps), *Co. godetiae* (GenBank KY171916; Identities 244 / 252 (97 %), no gaps) and *Co. rhombiforme* (GenBank JQ949118; Identities 243 / 252 (97 %), no gaps). The highest similarities using the *gapdh* sequence were *Co. pyricola* (GenBank KU221341; Identities 560 / 604 (93 %), 4 gaps (0 %)), *Co. nymphaeae* (GenBank KP339289; Identities 556 / 602 (92 %), 2 gaps (0 %)) and *Co. fioriniae* (GenBank KF944354; Identities 542 / 589 (92 %), 9 gaps (1 %)). The highest similarities using the *tub2* sequence were with *Co. phormii* (GenBank KX069820.1; Identities 386 / 413 (93 %), 7 gaps (1 %)), *Co. australe* (GenBank JQ950106.1; Identities 385 /

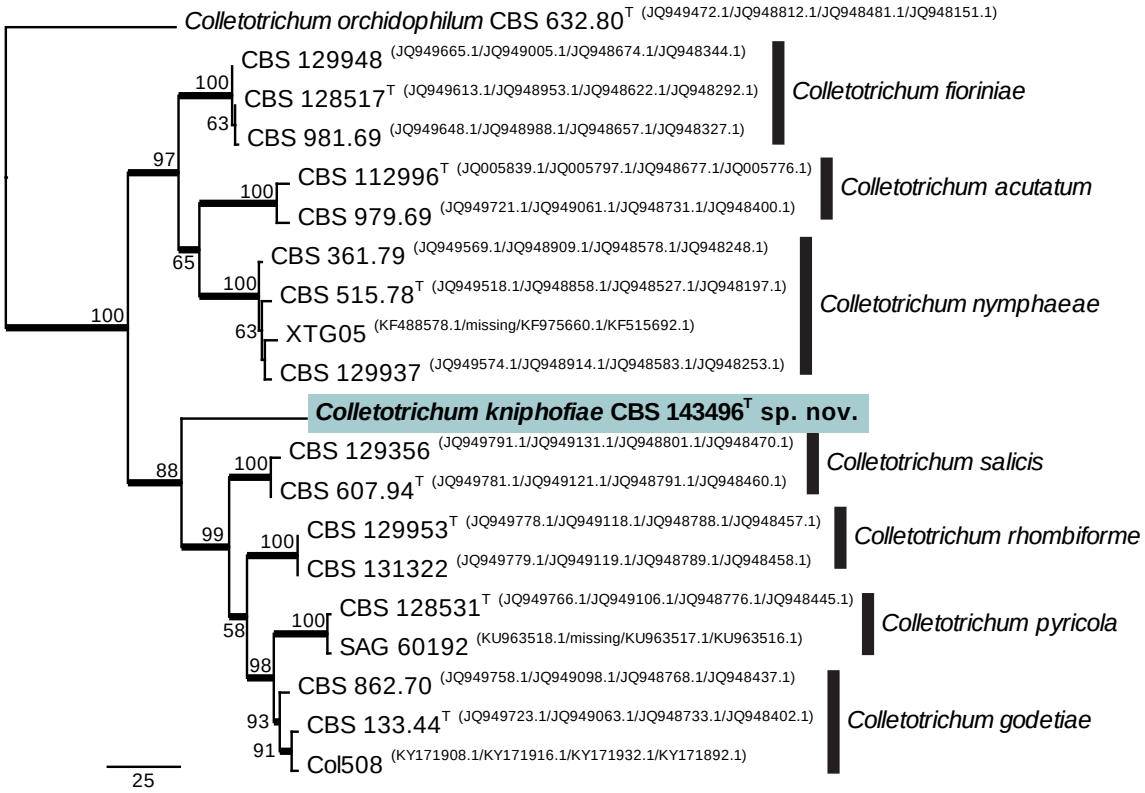

Fig. 11. The first of three equally most parsimonious trees obtained from a phylogenetic analysis of the combined *actA*, *chs-1*, *gapdh* and ITS alignment representing *Colletotrichum* species (20 strains including the outgroup; 1 301 characters analysed: 1 038 constant, 106 variable and parsimony-uninformative and 157 parsimony-informative). The tree was rooted to *Colletotrichum orchidophilum* (culture CBS 632.80) and the scale bar indicates the number of changes. Bootstrap support values higher than 49 % are shown at the nodes and novelties are highlighted with a coloured box and bold text. Species names are indicated to the right of the tree and GenBank accession numbers are indicated behind the culture collection numbers. A superscript T denotes strains with a type status and branches present in the strict consensus tree are thickened. Tree statistics: TL = 366, CI = 0.839, RI = 0.904, RC = 0.758.

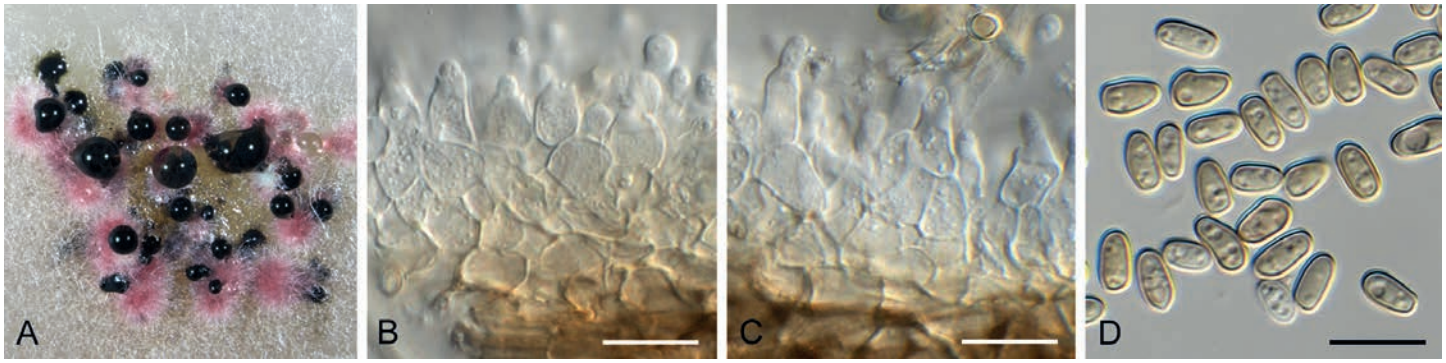

Fig. 12. *Cyclothyriella rubronotata* (CPC 27604). **A.** Colony on OA. **B, C.** Conidiogenous cells. **D.** Conidia. Scale bars = 10 µm.

413 (93 %), 7 gaps (1 %)) and *Co. scovillei* (GenBank KY475561.1; Identities 384 / 414 (93 %), 8 gaps (1 %)).

Cyclothyriella rubronotata (Berk. & Broome) Jaklitsch & Voglmayr, *Stud. Mycol*. **85**: 41. 2016. Fig. 12.
Basionym: *Melogramma rubronotatum* Berk. & Broome, *Ann. Mag. nat. Hist. Ser.* 3, **3**: 375. 1859.

Conidiomata erumpent, globose, 200–250 µm diam, with central ostiole; wall of 3–6 layers of brown *textura angularis*. *Conidiophores* lining the inner cavity, reduced to conidiogenous cells or with a single supporting cell. *Conidiogenous cells* hyaline, smooth, subcylindrical to ampulliform, 6–12 × 3–5 µm; apex with prominent periclinal thickening, rarely with percurrent proliferation. *Conidia* solitary, brown, smooth, thin-walled, guttulate, subcylindrical, mostly straight, apex obtuse, base truncate, (4.5–)5(–6) × (2.5–)3 µm.

Culture characteristics: Colonies spreading, flat on OA, erumpent on MEA and PDA, with moderate aerial mycelium and feathery margins, reaching 35 mm diam after 2 wk at 25 °C. On MEA surface dirty white, reverse apricot, on OA surface pale violet, on PDA surface coral with flesh in outer region, reverse sienna with patches of umber.

Specimen examined: **Germany**, near Berlin, on twig of *Ailanthus altissima* (*Simaroubaceae*), 4 Jun. 2015, *R.K. Schumacher* (specimen CBS H-23423, culture CPC 27604 = CBS 144201).

Notes: The genus *Cyclothyriella* was recently introduced by Jaklitsch & Voglmayr (2016), who also treated the taxonomic history of this genus in detail. The asexual morph isolated in this study closely resembles that described and illustrated by Jaklitsch & Voglmayr (2016), who reported conidia as (2–)4.5–6(–6.5) × (2–) 2.7–3.5(–4) µm, first hyaline, becoming medium brown with age. This fungus is common in Europe, and here we present a culture from Germany to supplement the Austrian material studied by Jaklitsch & Voglmayr (2016).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Cyclothyriella rubronotata* (GenBank NR_147651; Identities 564 / 564 (100 %), no gaps), *Melanomma pulvis-pyrius* (GenBank KY189979; Identities 400 / 476 (84 %), 20 gaps (4 %)) and *Ascochyta medicaginicola* (GenBank KX381183; Identities 359 / 419 (86 %), 10 gaps (2 %)). The highest similarities using the LSU sequence were *Cy. rubronotata* (GenBank KX650541; Identities 833 / 833 (100 %), no gaps), *Thyridaria rubronotata* (GenBank JX681121; Identities 833 / 833 (100 %), no gaps) and *Neooccultibambusa jonesii* (GenBank KY111437; Identities 781 / 812 (96 %), 2 gaps (0 %)).

Cylindriaceae Crous & L. Lombard, ***fam. nov.*** MycoBank MB824770.

Mycelium consisting of hyaline, smooth, septate, branched, hyphae. *Conidiophores* aggregated in sporodochia, or solitary, erect; hyphae and basal part of conidiophores becoming pale brown, smooth, subcylindrical, erect, septate, branched. *Conidiogenous cells* terminal and intercalary, subcylindrical, hyaline, smooth, with several sympodial flat-tipped loci, unthickened, not darkened. *Ramoconidia* hyaline, smooth, guttulate, subcylindrical. *Conidia* aseptate, hyaline, smooth, arranged in long, branched chains, scars unthickened, slightly refractive.

Type genus: Cylindrium Bonord.

Note: The genus *Cylindrium*, based on *C. elongatum*, was regarded by Lombard *et al*. (2015) as *incertae sedis*, and thus *Cylindriaceae* is herewith introduced to accommodate this genus.

Cylindrium algarvense (Cheew. & Crous) Crous, ***comb. nov.*** MycoBank MB824771.
Basionym: *Polyscytalum algarvense* Cheew. & Crous, *Persoonia* **23**: 73. 2009.

Description and illustration: Cheewangkoon *et al*. (2009).

Specimen examined: **Portugal**, Faro, Algarve, on *Eucalyptus* sp. (*Myrtaceae*), 24 Jan. 2007, *P.W. Crous* (holotype CBS H-20289, culture ex-type CPC 14936 = CBS 124770); *ibid*. (CPC 14937, CPC 14938).

Note: See discussion under *Polyscytalum* and Fig. 13.

Cylindrium purgamentum (Crous) Crous, ***comb. nov.*** MycoBank MB824772.
Basionym: *Polyscytalum purgamentum* Crous, *Persoonia* **37**: 363. 2016.

Description and illustration: Crous *et al*. (2016a).

Specimen examined: **USA**, Texas, Austin, on leaf litter, Aug. 2013, *P.W. Crous* (holotype CBS H-22899, culture ex-type CPC 29580 = CBS 142114).

Note: See discussion under *Polyscytalum* and Fig. 13.

Cylindrium syzygii (Crous & R.G. Shivas) Crous, ***comb. nov.*** MycoBank MB824773.
Basionym: *Pseudoidriella syzygii* Crous & R.G. Shivas, *Persoonia* **27**: 135. 2011.

Description and illustration: Crous *et al*. (2011).

Specimen examined: **Australia**, Queensland, Mackay, Eungella National Park, on leaves of *Syzygium* sp. (*Myrtaceae*), 14 Jul. 2009, *P.W. Crous & K.L. Crous*, (holotype CBS H-20758, cultures ex-type CPC 17233 = CBS 131307).

Note: See discussion under *Polyscytalum* and Fig. 13. By placing *Pseudoidriella syzygii* in *Cylindrium,* the genus *Pseudoidriella* is also reduced to synonymy with *Cylindrium*. This suggests that the conidiomata of *Cylindrium* could be reduced to solitary conidiophores, as well as sporodochia, as observed in this species. Interestingly enough, an LSU sequence attributed to *Tristratiperidium microsporum* also clustered in this clade (Fig. 3), which completely disagrees with the morphology of this fungus. This suggests that this sequence (MFLUCC) should be reconsidered. The ITS sequence from the same culture placed *Tristratiperidium microsporum* with *Kirstenboschia diospyri* (Fig. 13).

Cytospora viticola D.P. Lawr. *et al.*, *Pl. Pathol.* **66**: 718. 2017. Fig. 14.

Conidiomata (on PDA) with stromata up to 500 µm diam, rosette cytosporoid, subdivided by invaginations, up to four radially arranged. *Conidiophores* hyaline, smooth, branched, 1–3-septate, 15–20 × 2–3 µm, immersed in a mucilaginous layer. *Conidiogenous cells* phialidic with periclinal thickening and apical taper, 10–15 × 1.5–2 µm. *Conidia* hyaline, smooth, guttulate, allantoid, aseptate, (5–)6–7(–7.5) × 1(–1.5) µm.

Culture characteristics: Colonies spreading, with sparse aerial mycelium and smooth, lobate margins, covering dish after 1 mo at 25 °C. On MEA surface isabelline, reverse brown vinaceous. On PDA surface and reverse. On OA surface sepia.

Specimen examined: **Hungary**, Pécs wine region, on stems of *Vitis vinifera* (*Vitaceae*), 5 Nov. 2014, *K.Z. Váczy* (specimen CBS H-23278, culture T15 / 464 = CPC 30117 = CBS 143162).

Notes: Species of *Cytospora* are commonly known from woody plants and generally have wide host ranges. Two *Cytospora* species, *C. vinacea* and *C. viticola,* causing dieback and cankers of grapevines in the USA were recently described (Lawrence *et al*. 2017). Other species known from *Vitis* include *C. ceratosperma* (CBS 397.36), as well as the European taxa *C. vitis* and *Valsa vitis*, which are insufficiently known, and for which we could not trace type material. The isolate described here was associated with cankers on grapevines in Hungary, and is similar to *C. viticola*.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were

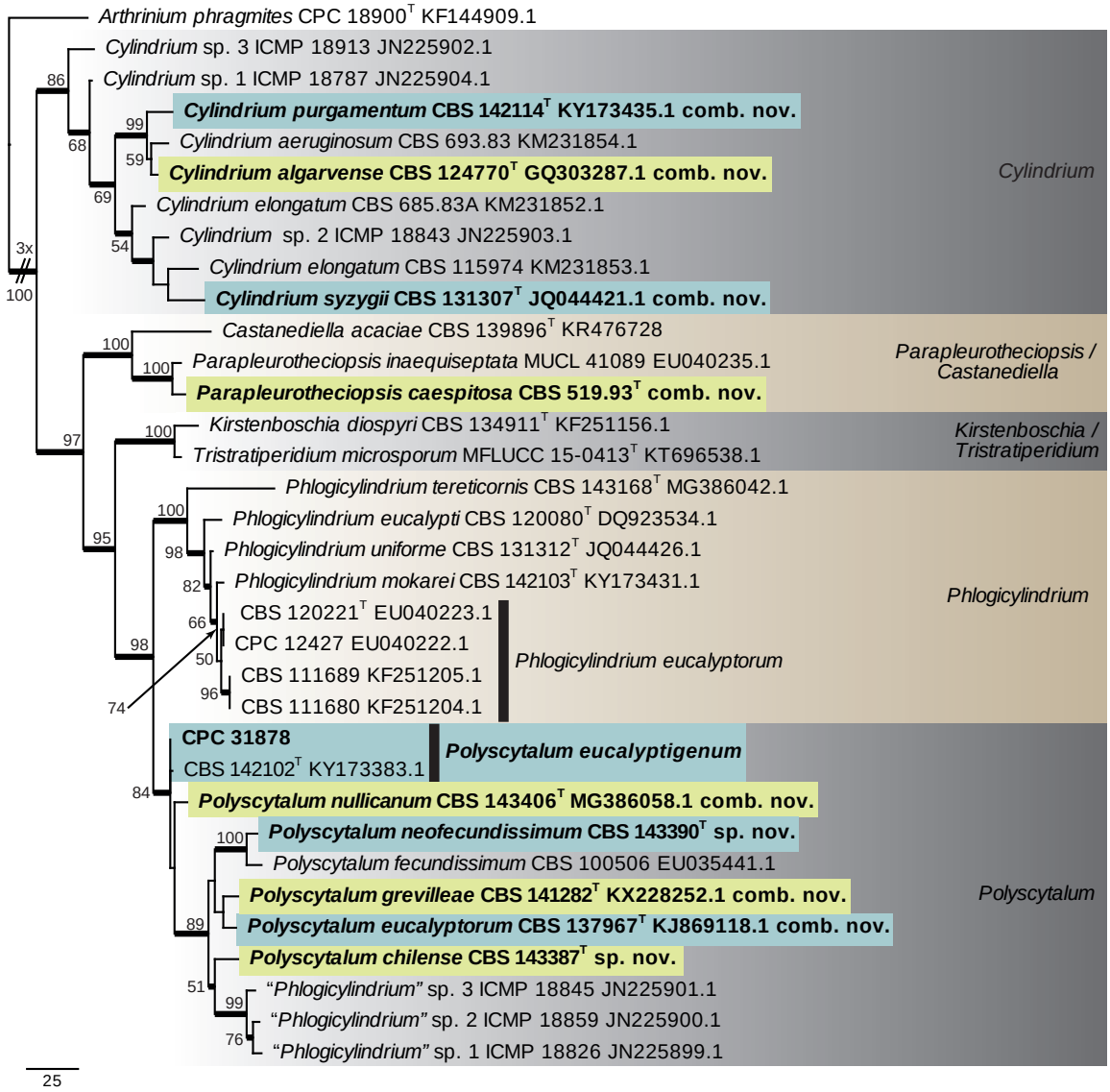

Fig. 13. The first of 72 equally most parsimonious trees obtained from a phylogenetic analysis of the ITS alignment representing the genera *Cylindrium*, *Parapleurotheciopsis*, *Phlogicylindrium* and *Polyscytalum* (34 strains including the outgroup; 538 characters analysed: 309 constant, 49 variable and parsimony-uninformative and 180 parsimony-informative). The tree was rooted to *Arthrinium phragmites* (GenBank KF144909.1) and the scale bar indicates the number of changes. Bootstrap support values higher than 49 % are shown at the nodes and novelties are highlighted with a coloured box and bold text. Species names are indicated to the right of the tree, or before the culture collection and GenBank accession numbers. Genera are indicated to the very right of the tree. A superscript T denotes strains with a type status and branches present in the strict consensus tree are thickened. The most basal branch was shortened three times to facilitate easier layout. Tree statistics: TL = 634, CI = 0.565, RI = 0.777, RC = 0.439.

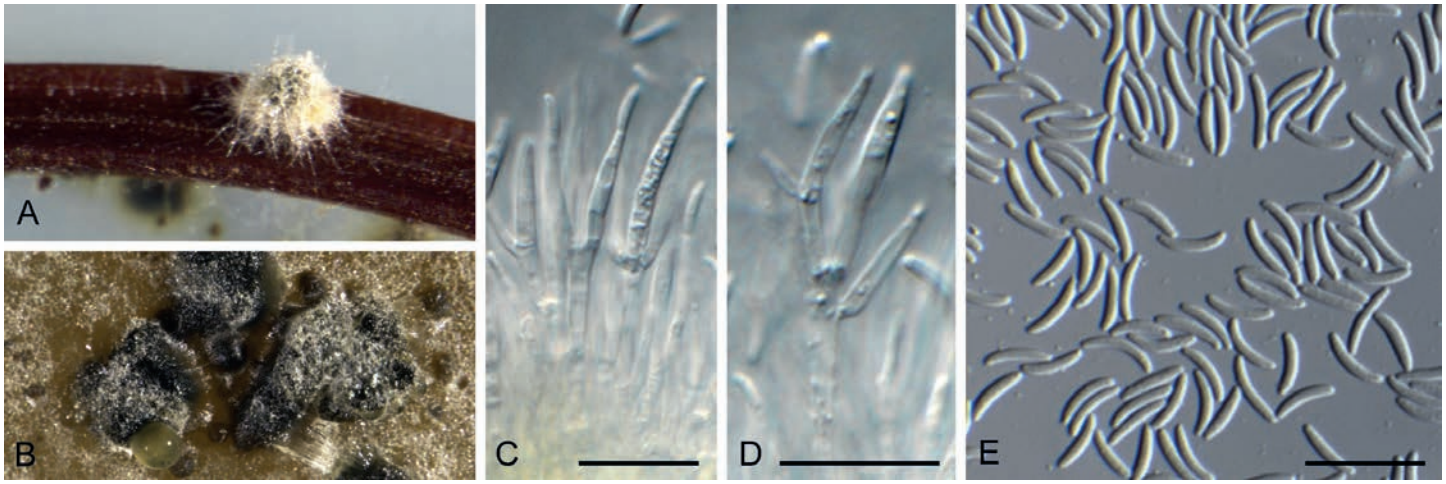

Fig. 14. *Cytospora viticola* (CBS 143162). **A.** Colony on PNA. **B.** Colony on OA. **C, D.** Conidiogenous cells. **E.** Conidia. Scale bars = 10 μm.

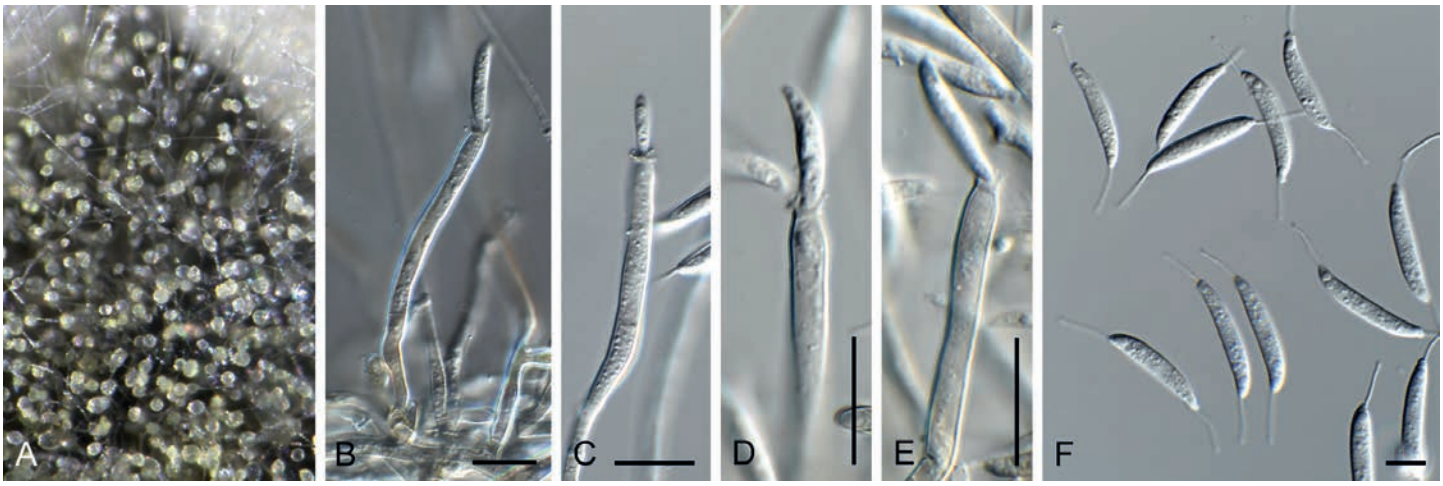

Fig. 15. *Dictyochaeta mimusopsis* (CBS 143435). **A.** Colony on OA. **B–E.** Conidiophores. **F.** Conidia. Scale bars = 10 µm.

Cy. sibiraeae (GenBank KR045651; Identities 566 / 591 (96 %), 8 gaps (1 %)), *Cy. chrysosperma* (GenBank KT692596; Identities 568 / 594 (96 %), 7 gaps (1 %)) and *Cy. germanica* (GenBank KX168596; Identities 563 / 590 (95 %), 7 gaps (1 %)). Our ITS sequence is identical to *Cy. viticola* (GenBank KX256239; Identities 423 / 423 (100 %), no gaps), but was not a result in the megablast search as roughly half of the first internal spacer region sequence is missing for the deposited sequences of that species. The highest similarities using the LSU sequence were *Valsa mali* (GenBank AF362559; Identities 837 / 842 (99 %), 1 gap (0 %)), *Cy. centrivillosa* (GenBank MF190068; Identities 830 / 837 (99 %), 1 gap (0 %)) and *Cy. ambiens* (GenBank EU255208; Identities 772 / 779 (99 %), no gaps). The highest similarities using the *actA* sequence were *Cy. salicicola* (GenBank KU982637; Identities 163 / 180 (91 %), 6 gaps (3 %)), *Cy. parasitica* (GenBank KT459410; Identities 190 / 212 (90 %), 10 gaps (4 %)) and *Cy. cincta* (GenBank KU710994; Identities 223 / 250 (89 %), 13 gaps (5 %)). The highest similarities using the *rpb2* sequence were *Cy. berberidis* (GenBank KU710948; Identities 658 / 727 (91 %), no gaps), *Cy. schulzeri* (GenBank KU710980; Identities 656 / 727 (90 %), no gaps) and *Cy. rostrata* (GenBank KU710974; Identities 656 / 727 (90 %), no gaps). The highest similarities using the *tef1* sequence were *Cy. viticola* (GenBank KX256274; Identities 253 / 253 (100 %), no gaps), *Cy. mali* (GenBank KU710928; Identities 359 / 422 (85 %), 24 gaps (5 %)) and *Cy. sophorae* (GenBank KU710941; Identities 422 / 517 (82 %), 26 gaps (5 %)). The highest similarities using the *tub2* sequence were *V. malicola* (GenBank KT934374; Identities 363 / 413 (88 %), 18 gaps (4 %)), *V. sordida* (GenBank KT428034; Identities 346 / 396 (87 %), 14 gaps (3 %)) and *Cy. carbonacea* (GenBank KP310825; Identities 343 / 395 (87 %), 16 gaps (4 %)).

Dictyochaeta mimusopis Crous & M.J. Wingf., ***sp. nov.*** MycoBank MB824774. Fig. 15.

Etymology: Name refers to *Mimusops,* the host genus from which this fungus was collected.

Mycelium consisting of branched, septate, hyaline, 3–4 µm diam hyphae. *Conidiophores* solitary, erect, pale brown, smooth, subcylindrical, unbranched, straight to flexuous, 1–6-septate, 40–150 × 3–4 µm. *Conidiogenous cells* monophialidic, integrated, terminal, pale brown, smooth, subcylindrical, 30–55 × 3(–3.5) µm; with flared apical collarette, 3–4 µm diam. *Conidia* solitary, aseptate, hyaline, smooth, guttulate to granular, inequilateral, fusoid, outer plane convex, apex subacute, base truncate, 1–1.5 µm diam, (11–)16–18(–20) × 2.5–3(–3.5) µm, with a single unbranched, flexuous appendage at each end, (6–)7(–8) µm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and smooth, lobate margins, reaching 50 mm diam after 2 wk at 25 °C. On MEA surface hazel with cinnamon pigment, reverse sepia. On PDA surface cinnamon, reverse amber. On OA surface sienna with patches of olivaceous grey.

Specimen examined: **South Africa**, Eastern Cape Province, Haga Haga, on leaves of *Mimusops caffra* (*Sapotaceae*), Dec. 2010, *M.J. Wingfield* (holotype CBS H-23412, culture ex-type CPC 29987 = CBS 143435).

Notes: *Dictyochaeta mimusopis* is closely allied to isolates in the *Di. simplex* complex (conidia 14–19 × 2.1–2.7 µm; Hughes & Kendrick 1968).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Di. simplex* (GenBank EF029193; Identities 482 / 503 (96 %), 1 gap (0 %)), *Di. fertilis* (GenBank AF178540; Identities 469 / 491 (96 %), 5 gaps (1 %)) and *Codinaea pini* (GenBank NR_137943; Identities 485 / 521 (93 %), 22 gaps (4 %)). The highest similarities using the LSU sequence were *Di. simplex* (GenBank AF178559; Identities 830 / 836 (99 %), no gaps), *Codinaea pini* (GenBank KP004493; Identities 829 / 838 (99 %), 1 gap (0 %)) and *Rattania setulifera* (GenBank HM171322; Identities 815 / 835 (98 %), no gaps).

Dictyochaeta septata (B. Sutton & Hodges) Whitton *et al.*, *Fungal Diversity* **4**: 148. 2000. Fig. 16.
Basionym: *Codinaea septata* B. Sutton & Hodges, *Nova Hedwigia* **26**(2–3): 520. 1975.
Synonym: *Dictyochaeta septata* (B. Sutton & Hodges) Aramb. & Cabello, *Mycotaxon* **34**(2): 682 (1989) (nom. inval. Art 41.5, Melbourne).

Mycelium consisting of hyaline, septate, branched, smooth, 2–3 µm diam hyphae. *Conidiophores* solitary, erect, brown, smooth, subcylindrical, straight to flexuous, 1–3-septate, 30–120 × 4–6 µm. *Conidiogenous cells* terminal, integrated, pale brown, smooth, mono-, rarely polyphialidic, 10–30 × 4–5 µm; collarette flared, 3–4 µm diam. *Conidia* solitary, hyaline, smooth, guttulate, granular, subcylindrical, falcate, ends subobtuse, (14–)15–19(–

Fig. 16. *Dictyochaeta septata* (CBS 143386). **A.** Colony on SNA. **B–D.** Conidiophores. **E.** Conidia. Scale bars = 10 μm.

20) × 2.5(–3) μm, medianly 1-septate, with a single unbranched, flexuous appendage at each end, 5–7 μm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and smooth, lobate margins, reaching 45 mm diam after 2 wk at 25 °C. On MEA surface hazel with cinnamon pigment, reverse sepia. On PDA surface cinnamon, reverse amber. On OA surface sienna with patches of olivaceous grey.

Specimens examined: **Brazil**, Espirito Santo, Vania, on *Eucalyptus* sp. (*Myrtaceae*), 11 Dec. 1973, *C.S. Hodges* (holotype K(M) IMI 181532f). **Chile**, on leaves of *Eucalyptus grandis* × *urophylla* (*Myrtaceae*), Jun. 2010, *M.J. Wingfield* (epitype of *Codinaea septata* designated here CBS H-23427, MBT381137, culture ex-epitype CPC 31949 = CBS 143386).

Notes: This collection closely resembles *Codinaea septata*, described from *Eucalyptus* leaves in Brazil with its conidia being 1(–2)-septate, (14.5–)17.5–23 × 2 μm, and conidiophores 30–105 × 4–6 μm (Sutton & Hodges 1975). Although we have in recent papers treated the genera *Codinaea* (setulate conidia) as distinct from *Dictyochaeta* (asetulate conidia) (Crous *et al*. 2015b), it appears that they could very well represent a single genus, with preference given to the older name, *Dictyochaeta*.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Pseudolachnella guaviyunis* (GenBank KJ834524; Identities 480 / 542 (89 %), 13 gaps (2 %)), *Di. simplex* (GenBank EF029193; Identities 462 / 519 (89 %), 9 gaps (1 %)) and *Thozetella fabacearum* (GenBank KY212754; Identities 478 / 544 (88 %), 27 gaps (4 %)). The highest similarities using the LSU sequence were *T. pinicola* (GenBank EU825195; Identities 810 / 837 (97 %), 2 gaps (0 %)), *T. nivea* (GenBank EU825200; Identities 807 / 837 (96 %), 2 gaps (0 %)) and *P. fraxini* (GenBank JQ889301; Identities 806 / 836 (96 %), 1 gap (0 %)). No close hits were obtained when the *tef1* sequence was used in a megablast search.

Echinocatena arthrinioides R. Campb. & B. Sutton, *Trans. Brit. Mycol. Soc*. **69**: 130. 1977. Fig. 17.

Mycelium consisting of branched, septate, pale brown, smooth, 1.5–2 μm diam hyphae. *Conidiophores* erect, solitary, 20–50 × 3–4 μm, unbranched, straight to flexuous, pale brown, smooth, 3–7-septate. *Conidiogenous cells* in simple or branched acropetal chains, 5–7 × 3–4 μm, separated by thick, dark brown, refractive septa, appearing like a separating cell, pale brown, echinulate, doliiform to cylindrical, constricted at septa, polyblastic,

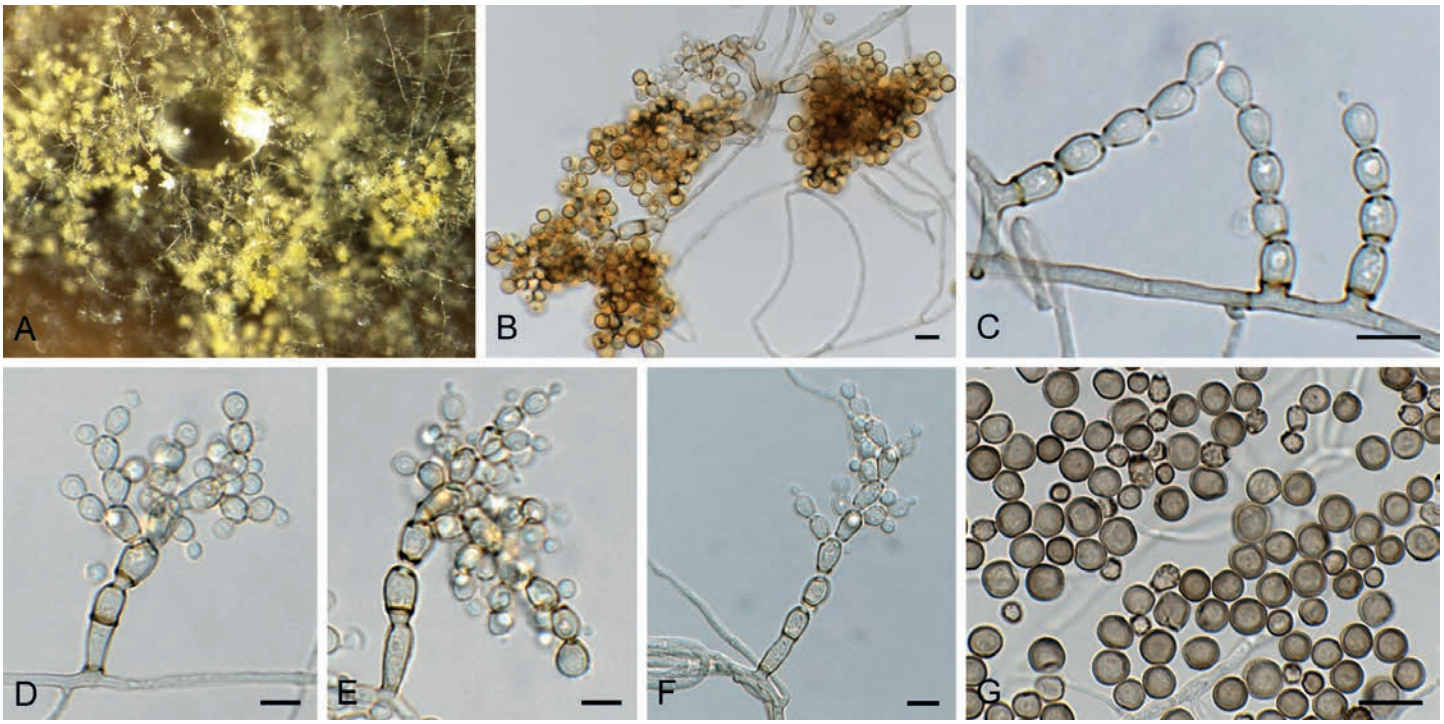

Fig. 17. *Echinocatena arthrinioides* (CPC 28754). **A.** Colony on MEA. **B–F.** Conidiophores. **G.** Conidia. Scale bars = 10 μm.

integrated with 5–7 conidiogenous loci. *Conidia* (4–)5–6(–7) µm diam, solitary, spherical, orange-brown, thick-walled, aseptate, echinulate.

Specimen examined: **Malaysia**, on leaves of *Acacia crassicarpa* (*Fabaceae*), 1 Jul. 2015, *M.J. Wingfield* (specimen CBS H-23424, culture CPC 28754 = CBS 144202).

Notes: *Echinocatena arthrinioides* was originally described from leaf litter collected in India. This collection has conidia that are slightly larger those observed for the type collection (3.5–4.5 µm; Campbell & Sutton 1977), and DNA data would be required to fully resolve if this strain is conspecific with the type (IMI 199279).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were "*Fusicladium* sp." from marine sponges in Panama (GenBank JN837045; Identities 524 / 530 (99 %), no gaps) and "*Sympoventuriaceae* sp." from a human nail in Hong Kong (GenBank LC158598; Identities 448 / 452 (99 %), no gaps); the remaining matches were with the 5.8S nrRNA genes of *Fusicladium* species. The highest similarities using the LSU sequence were *Mycosisymbrium cirrhosum* (GenBank KR259884; Identities 758 / 828 (92 %), 9 gaps (1 %)), *Scolecobasidium cateniphorum* (GenBank EU107309; Identities 758 / 829 (91 %), 11 gaps (1 %)) and *Verruconis verruculosa* (GenBank KF282668; Identities 757 / 829 (91 %), 11 gaps (1 %)).

Elsinoë mimosae Viégas, *Bragantia* **4**: 13. 1944.

Description and illustration: Fan *et al*. (2017).

Specimens examined: **Brazil**, São Paulo, Campinas, on *Mimosa* sp. (*Leguminosae*), 31 Mar. 1931, *H.P. Krug & O. Zagatto* (holotype IAC No. 2836); **Brazil**, Rio de Janeiro, Itaguaí, Mazomba, on *Mimosa diplotricha* (= *Mimosa invisa*), Mar. 1999, *R.W. Barreto* (epitype designated here MBT381423, preserved in metabolically inactive state, ex-epitype culture CBS 141878) = CPC 19478 = RWB 154. **Ecuador**, Coca, on *Mimosa diplotricha*, Nov. 2000, *R.W. Barreto*, specimen CBS H-22804, culture CPC 18518 = RWB 224 = CBS 141943.

Notes: The epitype was designated in Fan *et al*. (2017), but that epitypification was not effected since the holotype was not "explicitly" cited (Art 9.8, Melbourne Code). We correct this situation by citing the holotype as "IAC No. 2836".

Exophiala eucalypticola Crous & T.I. Burgess, ***sp. nov.*** MycoBank MB824775. Fig. 18.

Etymology: Name refers to *Eucalyptus,* the host genus from which it was collected.

Mycelium consisting of pale brown, smooth, septate, branched, 2–2.5 µm diam hyphae. *Conidiophores* arising as lateral ends of hyphae, or reduced to conidiogenous cells, integrated on hyphae, erect, medium brown, smooth, subcylindrical, 3–15 × 2–3 µm; scars thickened and darkened, 1 µm diam. *Conidia* occurring in branched chains, pale brown, smooth, 0–1-septate, fusoid-ellipsoidal, with hila that are thickened and darkened, 1 µm diam, (7–)10–13(–15) × (2.5–)3(–4) µm. Synasexual morph: *Conidiogenous cells* integrated as phialidic loci on creeping hyphae, 1–2 × 1 µm. *Conidia* dimorphic, with exophiala-like conidia pale brown, smooth, aseptate, ellipsoid, 4–7 × 2–3 µm.

Culture characteristics: Colonies flat, spreading, with sparse to moderate aerial mycelium and smooth, lobate margins, reaching 10 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface slimy, chestnut to black.

Specimen examined: **Australia**, Victoria, Melbourne, Dandenong Ranges, Silvan Reservoir Park, leaf litter of *Eucalyptus obliqua* (*Myrtaceae*), 1 Dec. 2016, *P.W. Crous* (holotype CBS H-23305, cultures ex-type CPC 32736 = CBS 143412).

Notes: The genera *Exophiala* and *Rhinocladiella* contain several clinically relevant species (de Hoog 1977). Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *R. aquaspersa* (GenBank AB091214; Identities 458 / 519 (88 %), 18 gaps (3 %)), *E. phaeomuriformis* (GenBank KP761151; Identities 485 / 555 (87 %), 27 gaps (4 %)) and *R. coryli* (GenBank KX306768; Identities 518 / 594 (87 %), 25 gaps (4 %)). The highest similarities using the LSU sequence were *E. xenobiotica* (GenBank KC311483; Identities 831 / 862 (96 %), 7 gaps (0 %)), *E. xenobiotica* (GenBank FJ358246; Identities 826 / 857 (96 %), 7 gaps (0 %)) and *Melanoctona tectonae* (GenBank KX258779; Identities

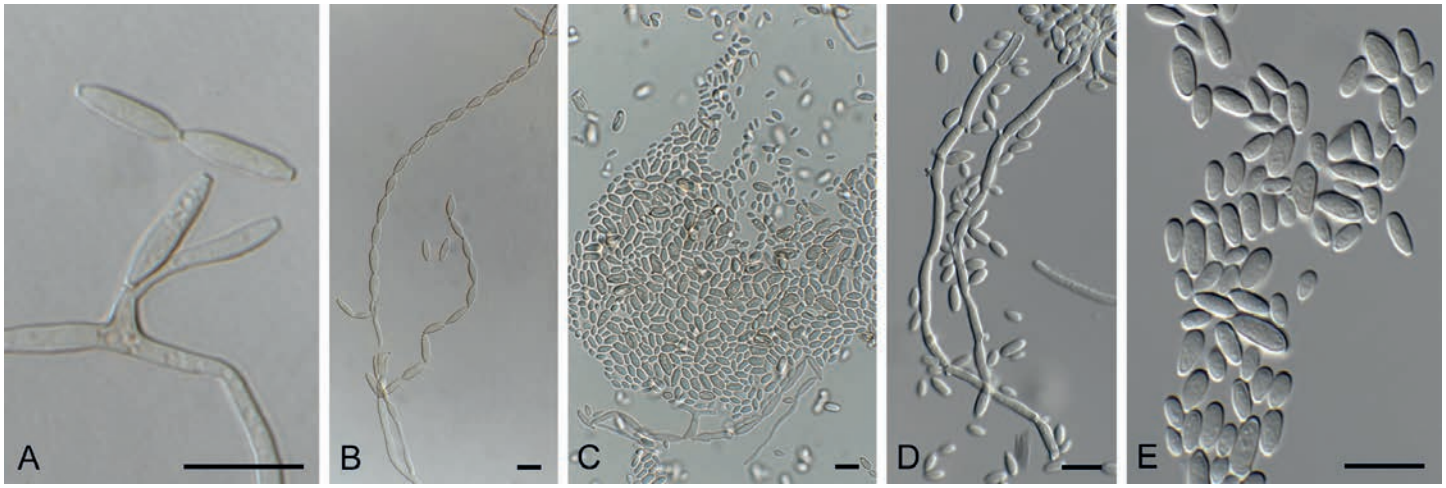

Fig. 18. *Exophiala eucalypticola* (CBS 143412). **A.** Conidiogenous cell. **B.** Conidial chain. **C, D.** Typical *Exophiala* morph with conidiogenous cells reduced to phialides. **E.** Conidia. Scale bars = 10 µm.

828 / 860 (96 %), 6 gaps (0 %)). The present collection is best allocated to this generic complex, and until better resolved it is best placed in *Exophiala*. No significant hits were obtained when the *tef1* and *tub2* sequences were used in a megablast search.

Fusiconidium lycopodiellae Crous & R.K. Schumach., ***sp. nov.*** MycoBank MB824776. Fig. 19.

Etymology: Name refers to *Lycopodiella,* the host genus from which this fungus was collected.

Mycelium consisting of hyaline, smooth, branched, septate, 2.5–4 µm diam hyphae. *Hyphopodia* absent. *Conidiophores* solitary, erect, subcylindrical, geniculate-sinuous, brown, smooth, 1–2-septate, 20–50 × 3–5 µm. *Conidiogenous cells* terminal, subcylindrical, brown, smooth, 10–17 × 3–4 µm, proliferating sympodially, holoblastically; scars unthickened, undarkened, 2–3 µm diam. *Conidia* solitary, brown, smooth, granular, subcylindrical, apex obtuse, tapering in lower cell to truncate hilum, 2–3 µm diam, (7–)8–9(–11)-septate, (65–)75–85(–100) × (7–)8(–9) µm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and smooth, lobate margins, covering dish after 1 mo at 25 °C. On MEA surface ochreous, reverse amber. On PDA surface and reverse amber. On OA surface luteous.

Specimen examined: **Germany**, near Berlin, on stems of *Lycopodiella inundata* (*Lycopodiaceae*), 25 Feb. 2016, *R.K. Schumacher* (holotype CBS H-23407, culture ex-type CPC 30371 = CBS 143437).

Notes: The present collection is reminiscent of *Clasterosporium*, except that it lacks hyphopodia (Ellis 1971). Based on LSU sequence data it is allied to *Fusiconidium* (Li *et al*. 2017), except that it lacks percurrent proliferation of the conidiogenous cells, and fusoid to ellipsoid conidia, and probably represents a new genus in this complex. However, due to the poor sporulation of the culture, we have tentatively named it in *Fusiconidium*.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Camposporium cambrense* (GenBank KY853428; Identities 481 / 502 (96 %), 3 gaps (0 %)), *Phragmocephala atra* (GenBank KP698721; Identities 495 / 523 (95 %), 4 gaps (0 %)) and *Phragmocephala garethjonesii* (GenBank NR_147636; Identities 493 / 523 (94 %), 3 gaps (0 %)). The highest similarities using the LSU sequence were *Fusiconidium mackenziei* (GenBank KX611113; Identities 806 / 815 (99 %), no gaps), *Paradendryphiella salina* (GenBank KF156156; Identities 780 / 791 (99 %), no gaps) and *Aposphaeria corallinolutea* (GenBank KU243051; Identities 804 / 817 (98 %), no gaps).

Haplographium delicatum Berk. & Broome, *Ann. Mag. Nat. Hist., Ser*. 3, **3**(17): 361. 1859. Fig. 20.

Conidiophores erect, subcylindrical, straight to flexuous, brown, thick-walled, verruculose, base with T-cell, lacking rhizoids, 70–160 × 5–6 µm, 5–10-septate. Conidiophores with swollen apical cell, pale brown, giving rise to 3–6 apical conidiogenous cells or primary branches; primary branches subcylindrical, straight to allantoid, hyaline, smooth, 5–10 × 2.5–3 µm. *Conidiogenous cells* hyaline, smooth, subcylindrical, straight to slightly curved, 6–12 × 2–2.5 µm, proliferating sympodially at apex. *Conidia* aggregating in mucoid mass, hyaline, smooth, guttulate, subcylindrical, straight, apex slightly swollen, obtuse, base truncate, (3–)5–6(–7.5) × 2(–2.5) µm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and smooth, lobate margins, reaching 13 mm diam after 2 wk at 25 °C. On MEA surface and reverse pale luteous. On PDA surface pale olivaceous grey, reverse ochreous. On OA surface umber, with diffuse sienna pigment.

Specimen examined: **Ukraine**, Ternopil region, Zalischyky district, Dniester Canyon, on decaying wood of *Carpinus betulus* (*Betulaceae*), 5 Oct. 2016, *A. Akulov*, specimen ex CWU (MYC) AS 6049 (dried culture CBS H-23417, culture CPC 31844 = CBS 143493).

Notes: The genus *Haplographium* is based on *H. delicatum* described from wood collected in Britain. The present collection is phylogenetically similar to strains identified as *H. catenatum* and *H. delicatum* (Crous *et al.* 2009a). Because the genus *Haplographium* lacks a type and the species concepts are still in flux, we have identified the present collection as *H. delicatum.* Species of *Haplographium* have been linked to *Dematioscypha* sexual morphs (Raitviir 2001), but this relationship also requires further study.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *H.*

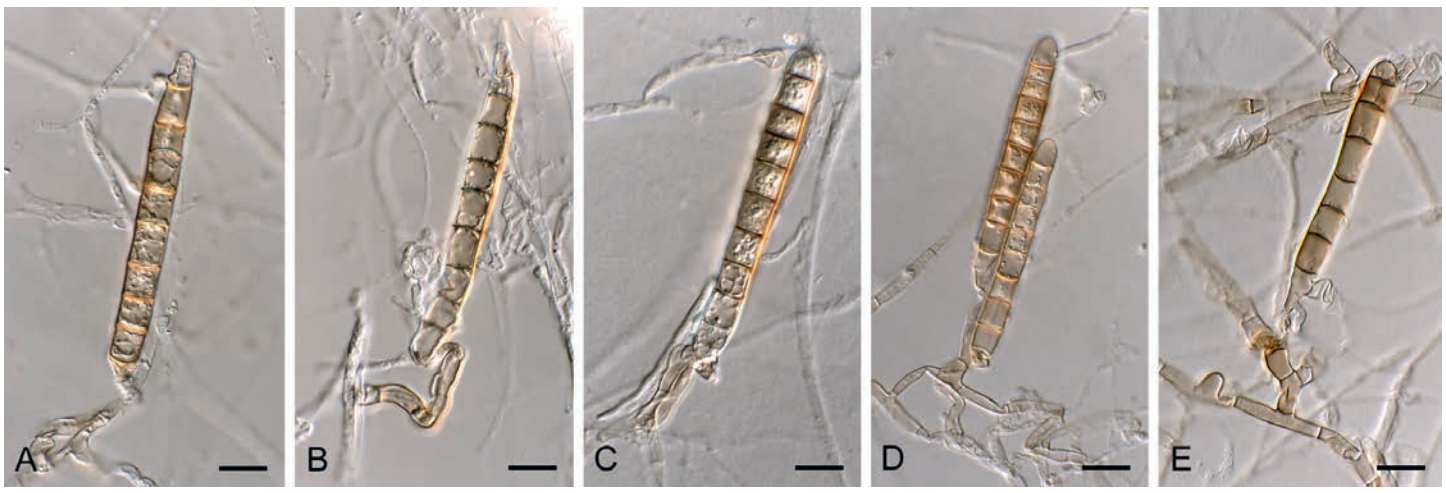

Fig. 19. *Fusiconidium lycopodiellae* (CBS 143437). **A–E.** Conidiophores giving rise to multiseptate conidia. Scale bars = 10 µm.

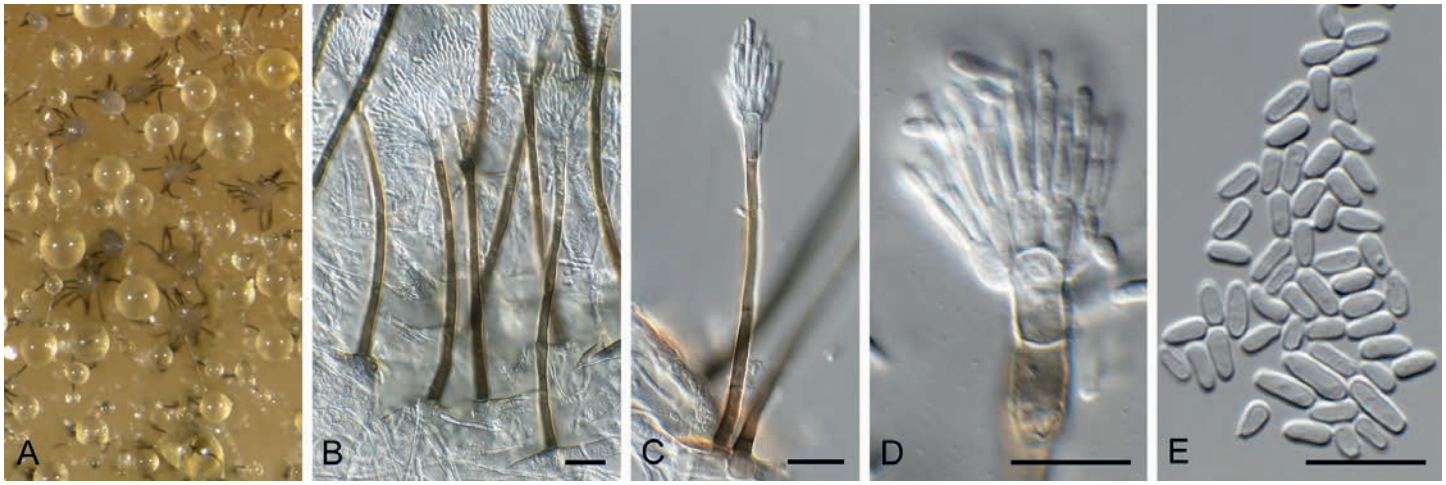

Fig. 20. *Haplographium delicatum* (CBS 143493). **A.** Colony on OA. **B–C.** Conidiophores. **D.** Conidiogenous cells. **E.** Conidia. Scale bars = 10 μm.

catenatum (GenBank FJ839620; Identities 528 / 533 (99 %), 1 gap (0 %)), *H. delicatum* (GenBank HF677177; Identities 495 / 500 (99 %), 1 gap (0 %)) and *Ciliciopodium brevipes* (GenBank KM231856; Identities 401 / 451 (89 %), 10 gaps (2 %)). The highest similarities using the LSU sequence were *H. catenatum* (GenBank FJ839657; Identities 855 / 855 (100 %), no gaps), *Hyaloscypha minuta* (GenBank KY769526; Identities 832 / 857 (97 %), 3 gaps (0 %)) and *Hy. monodictys* (GenBank JN086756; Identities 808 / 833 (97 %), 3 gaps (0 %)).

Microdochium musae (T.Y. Lin & J.M. Yen) Crous, ***comb. nov.*** MycoBank MB824777. Fig. 21.
Basionym: *Sphaerulina musae* T.Y. Lin & J.M. Yen, *Rev. Mycol.* (Paris) **35**: 326. 1971.

Ascomata (on OA) solitary, immersed on leaf tissue (superficial to immersed on banana leaf agar), globose, semi-papillate with central ostiole, pale brown, 200–250 μm diam; wall of 6–8 layers of pale brown *textura angularis*. *Paraphyses* intermingled among asci, hyaline smooth, septate, unbranched, constricted at septa, hyphae-like, 4–5 μm diam, with obtuse ends. *Asci* fasciculate, hyaline, unitunicate, apical mechanism staining blue in Meltzer's, broadly ellipsoid, straight to curved, 8-spored, stipitate, 80–100 × 17–22 μm. *Ascospores* bi- to triseriate, hyaline to faintly pinkish, smooth, guttulate, obovoid, apex obtuse, tapering from middle to base, straight to curved, 3–6-septate, at times with mucoid sheath, frequently constricted at median septum, (30–) 32–33(–35) × (6–)7(–8) μm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium, radially folded, and even margins, reaching 40 mm diam after 2 wk at 25 °C. On MEA surface and reverse saffron. On PDA surface and reverse salmon. On OA surface salmon.

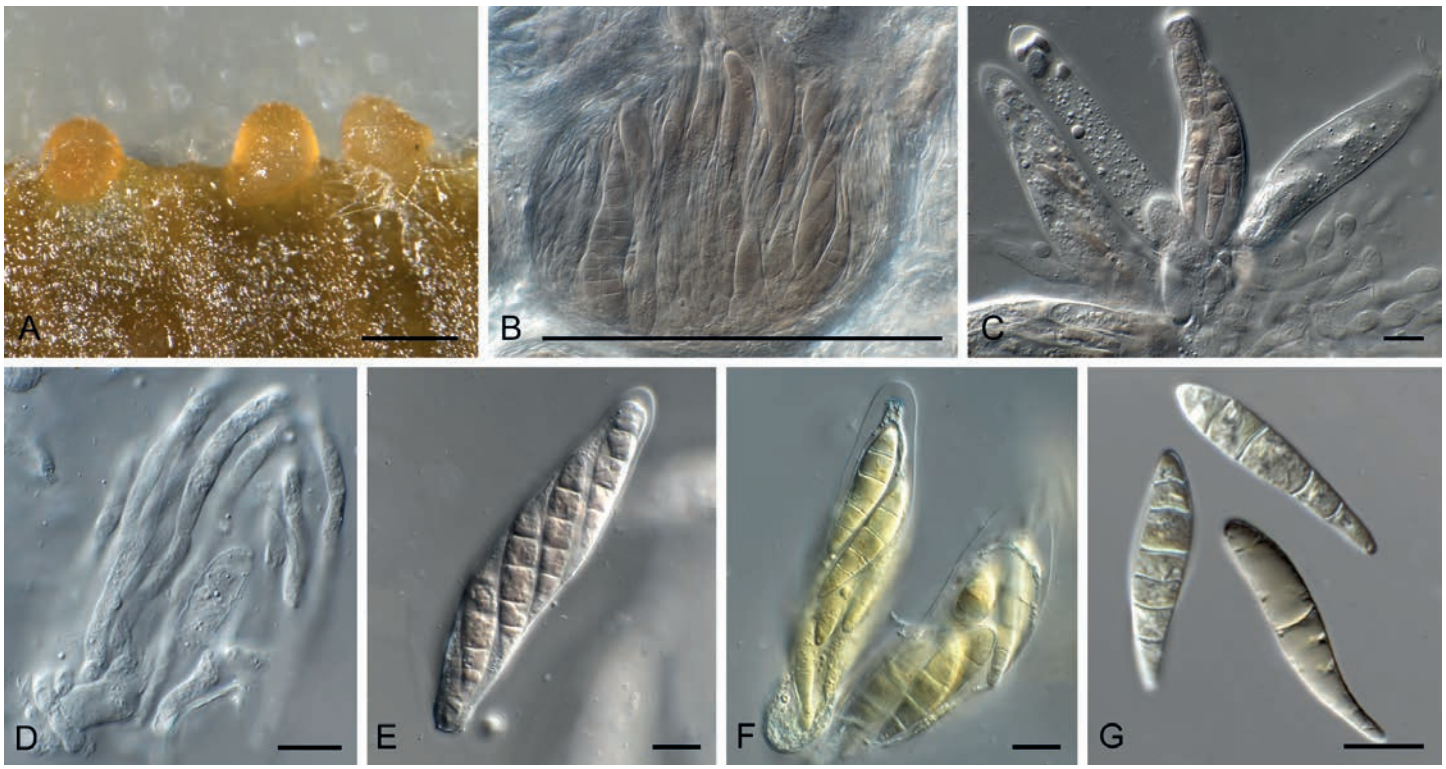

Fig. 21. *Microdochium musae* (CBS 143500). **A.** Ascomata on banana leaf. **B.** Vertical section through ascoma. **C.** Asci. **D.** Paraphyses. **E.** Subhyaline ascospores. **F.** Ascus in Meltzer's reagent. **G.** Ascospores. Scale bars: A, B = 250 μm, all others = 10 μm.

Specimens examined. **Republic of China** (Taiwan), on leaves of *Musa* sp. (*Musaceae*), 1970, *T. Wang*, holotype missing, lectotype designated here, MBT381229 (Lin & Yen 1971, fig. 2 D–F). **Malaysia**, Sabah, on leaves of *Musa* sp., 2016, *P.W. Crous* (epitype designated here, CBS H-23431, MBT381140, culture ex-epitype CPC 32689 = CBS 143500).

Additional cultures examined: **Costa Rica**, on *Musa* cv. Cavendish, May 2002, *P.W. Crous* (CBS 111018 = CPC 5380). **Malaysia**, on *Musa* leaves, 2010, *P.W. Crous* (CPC 32681), *ibid.* (CPC 32809). **Mauritius**, on *Musa* leaves, Jan. 2004, *Y. Jaufeerally-Fakim* (CPC 11234), *ibid.* (CPC 11240). **Mexico**, Chiapas, on *Musa* leaves, 16 Dec. 2008, *M. de J. Yanez Morales* (CPC 16258).

Notes: The genus *Sphaerulina* was treated by Quaedvlieg *et al.* (2013), and represents a genus in the *Mycosphaerellaceae*, to which *S. musae* (Lin & Yen 1971) is not related. "*Sphaerulina" musae* clusters among species of *Microdochium*, which have sexual morphs that are morphologically similar (Hernández-Restrepo *et al.* 2016), justifying this new combination.

Microdochium musae is commonly associated with brown necrotic areas on banana leaves, appears to be globally distributed along with its host, and is assumed to be weakly pathogenic (unpubl. data). Because the holotype could not be traced in Taiwan or Paris, the original illustration is proposed as lectotype and a neotype is designated. Colonies initially have a yeast-like appearance in culture, and single ascospores give rise to the sexual morph, suggesting that the species is homothallic.

Based on a megablast search using the ITS sequence of CPC 32689, the closest matches in NCBI's GenBank nucleotide database were *Sphaerulina musae* (GenBank AY293061; Identities 477 / 477 (100 %), no gaps), *Mi. stoveri* (GenBank FJ430601; Identities 537 / 540 (99 %), no gaps) and *Mi. colombiense* (GenBank KP858999; Identities 499 / 516 (97 %), 3 gaps (0 %)). The highest similarities using the LSU sequence of CPC 32689, the closest matches in NCBI's GenBank nucleotide database were *Mi. colombiense* (GenBank KP858935; Identities 834 / 842 (99 %), no gaps), *Mi. citrinidiscum* (GenBank KP858939; Identities 831 / 842 (99 %), no gaps) and *Mi. sorghi* (GenBank KP858936; Identities 831 / 842 (99 %), no gaps). The highest similarities using the *actA* sequence of CPC 16258 were *Chaetopsina fulva* (GenBank KM231165; Identities 404 / 422 (96 %), no gaps), *Fusarium phaseoli* (GenBank KM231203; Identities 400 / 419 (95 %), no gaps) and *Ch. acutispora* (GenBank KM231164; Identities 401 / 421 (95 %), no gaps). The *actA* sequences of CPC 11234, 11240, 16258, 32681 and 32809 are identical, but differ with two nucleotides from CPC 32689. No significant hits were obtained when the *cmdA* sequences were used in a megablast search. The *cmdA* sequences of CPC 11234, 11240, 16258, 32689 and 32809 are identical, but differ one nucleotide from CPC 32681. The highest similarities using the *rpb2* sequence of CPC 32689 were *Mi. colombiense* (GenBank KP859108; Identities 740 / 782 (95 %), no gaps), *Mi. majus* (GenBank KP859110; Identities 675 / 780 (87 %), 4 gaps (0 %)) and *M. nivale* (GenBank KP859117; Identities 679 / 785 (86 %), 6 gaps (0 %)). No significant hits were obtained when the *tef1* and *tub2* sequences of CPC 32681 were used in a megablast search. The *tef1* sequences of CPC 11234, 11240, 16258 and 32689 are identical. The *tub2* sequences of CPC 11234, 11240, 32689 and 32809 are identical, but differ one nucleotide from CPC 16258.

Monochaetia junipericola Crous & R.K. Schumach., ***sp. nov.*** MycoBank MB824778. Fig. 22.

Etymology: Name refers to *Juniperus,* the host genus from which this fungus was collected.

Conidiomata pycnidioid, separate to gregarious, erumpent, ovoid, 150–250 µm diam. *Conidiophores* arising from central stroma, hyaline, smooth, 3–6-septate, branched, subcylindrical, 40–100 × 3–4 µm. *Conidiogenous cells* terminal and intercalary, hyaline, smooth, subcylindrical, 10–30 × 2.5–3 µm, proliferating percurrently at apex. *Conidia* fusoid-ellipsoid, 4-septate, not constricted at septa, medium brown, finely verruculose, end cells hyaline, (22–)25–27(–28) × (5–)6(–7) µm, apical cell terminating in a single, unbranched, filiform, flexuous appendage, 10–20 µm long; basal cell with single, unbranched, flexuous, excentric appendage, 2–15 µm long. *Conidiomata* with *beta conidia* developing on OA, beta conidia hyaline, smooth, filiform, curved, apex obtuse, base truncate, 12–22 × 1.5–2 µm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and feathery, lobate margins, reaching 40 mm diam after 2 wk at 25 °C. On MEA surface pale luteous, reverse luteous. On PDA surface smoke grey with patches of isabelline, reverse pale luteous. On OA surface pale luteous with patches of amber.

Specimen examined: **Germany**, near Berlin, on twig of *Juniperus communis* (*Cypressaceae*), 20 Apr. 2016, *R.K. Schumacher* (holotype CBS H-23408, culture ex-type CPC 30561 = CBS 143391).

Fig. 22. *Monochaetia junipericola* (CBS 143391). **A.** Conidiomata on PDA. **B, C.** Conidiophores giving rise to conidia. **D.** Conidia. **E.** Beta conidia. Scale bars = 10 µm.

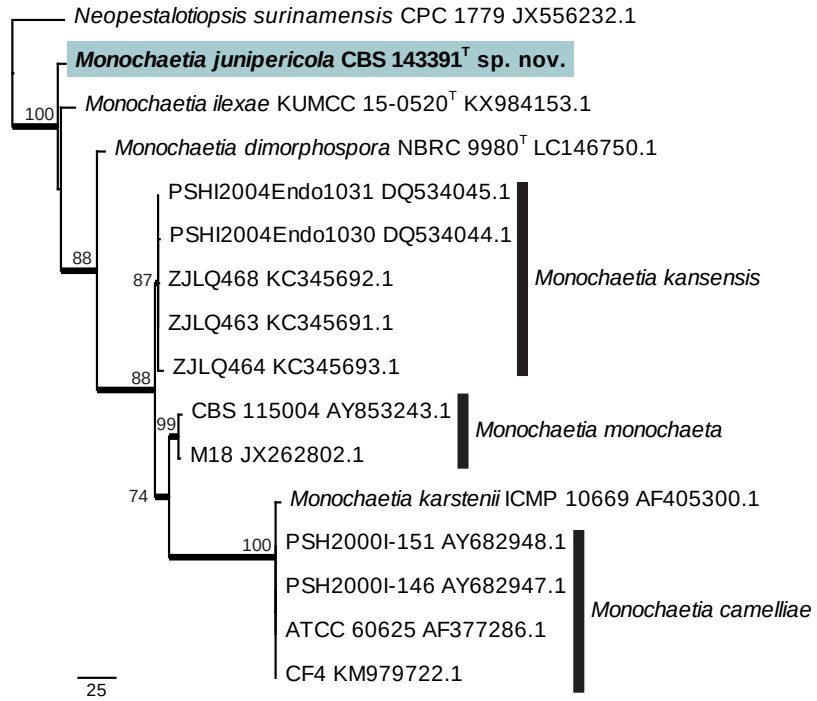

Fig. 23. The first of eight equally most parsimonious trees obtained from a phylogenetic analysis of the *Monochaetia* ITS alignment (16 strains including the outgroup; 524 characters analysed: 373 constant, 29 variable and parsimony-uninformative and 122 parsimony-informative). The tree was rooted to *Neopestalotiopsis surinamensis* (GenBank JX556232.1) and the scale bar indicates the number of changes. Bootstrap support values higher than 49 % are shown at the nodes and novelties are highlighted with a coloured box and bold text. Species names are indicated to the right of the tree, or before the culture collection and GenBank accession numbers. A superscript T denotes strains with a type status and branches present in the strict consensus tree are thickened. Tree statistics: TL = 238, CI = 0.782, RI = 0.899, RC = 0.702.

Notes: Nag Raj (1993) defined the genus *Monochaetia* to accommodate taxa with acervular conidiomata and fusiform, brown, transversely septate conidia with a single cellular apical, and single cellular basal appendage (when present). Nag Raj (1993) also regarded *Mo. juniperi* as synonym of *Sarcostroma foliicola*, occurring on needles of *Juniperus communis*. Morphologically *S. foliicola* has fusiform, 5-septate conidia, 18–22.5 × 7–8(–9) µm, apical appendage 3–8(–9) µm, and basal appendage excentric, 3–11 µm, thus smaller than those of *M. junipericola*. Phylogenetically, *M. junipericola* is basal to the other *Monochaetia* species known from ITS sequences (Fig. 23).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Mo. ilexae* (GenBank NR_148179; Identities 497 / 516 (96 %), 7 gaps (1 %)), *Mo. dimorphospora* (GenBank LC146750; Identities 498 / 527 (94 %), 20 gaps (3 %)) and *Synnemadiella eucalypti* (GenBank KY173467; Identities 497 / 538 (92 %), 10 gaps (1 %)). The highest similarities using the LSU sequence were *Mo. ilexae* (GenBank KX984152; Identities 846 / 847 (99 %), no gaps), *Mo. kansensis* (GenBank DQ534035; Identities 832 / 833 (99 %), no gaps) and *Mo. monochaeta* (GenBank KF590148; Identities 828 / 829 (99 %), no gaps). Only distant hits were obtained using the *rpb2* sequence; some of these were *Pestalotiopsis versicolor* (GenBank DQ368654; Identities 662 / 803 (82 %), 4 gaps (0 %)), *P. fici* (GenBank XM_007830789; Identities 657 / 800 (82 %), no gaps) and *Discosia brasiliensis* (GenBank KF827475; Identities 658 / 805 (82 %), 4 gaps (0 %)). No significant hits were obtained when the *tef1* sequence was used in a megablast search. The best hit using the *tub2* sequence was with *Mo. kansensis* (GenBank DQ534049; Identities 356 / 407 (87 %), 3 gaps (0 %)).

Myrmecridium sorbicola Crous & R.K. Schumach., ***sp. nov.*** MycoBank MB824779. Fig. 24.

Etymology: Name refers to *Sorbus,* the host genus from which this fungus was collected.

On OA (only medium with sporulation). *Mycelium* consisting of hyaline, smooth, branched, septate, 2–3 µm diam hyphae. *Conidiophores* solitary, erect, flexuous, unbranched, brown, subcylindrical, smooth, 1–18-septate, 50–200 × 4–7 µm. *Conidiogenous cells* integrated, terminal and intercalary, 20–65 × 3–4 µm, with a rachis of pimple-like denticles, 0.5–1 × 0.5 µm. *Conidia* solitary, obovoid, initially hyaline, but pale brown with age, apex obtuse, hilum 1 µm diam, (0–)1(–3) septate, with mucoid sheath surrounding conidium in median region, 1–2 µm diam, (7–)8–10(–15) × 4(–5) µm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and even, lobate margins, reaching 12 mm diam after 2 wk at 25 °C. On MEA surface and reverse luteous. On PDA surface and reverse pale luteous. On OA surface pale luteous.

Specimen examined: **Germany**, near Berlin, on branch of *Sorbus aucuparia* (*Rosaceae*), 17 Feb. 2016, *R.K. Schumacher* (holotype CBS H-23405, culture ex-type CPC 30455 = CBS 143433).

Notes: *Myrmecridium* was introduced by Arzanlou *et al.* (2007) for ramichloridium-like taxa having hyaline mycelium, and relatively unpigmented, pimple-like denticles, and obovoid to fusoid conidia with a wing-like gelatinous sheath. *Myrmecridium sorbicola* is distinct from known species based on its conidial morphology, with conidia being (0–)1(–3)-septate, (7–)8–10(–15) × 4(–5) µm.

Fig. 24. *Myrmecridium sorbicola* (CBS 143433). **A–E.** Conidiophores. **F.** Conidia with wing-like appendages. Scale bars = 10 µm.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *My. schulzeri* (GenBank KF986544; Identities 447 / 513 (87 %), 14 gaps (2 %)), *My. phragmitis* (GenBank NR_137782; Identities 494 / 567 (87 %), 18 gaps (3 %)) and *My. fluviae* (GenBank KX839679; Identities 419 / 481 (87 %), 15 gaps (3 %)). The highest similarities using the LSU sequence were *My. banksiae* (GenBank NG_042684; Identities 813 / 842 (97 %), 2 gaps (0 %)), *My. schulzeri* (GenBank EU041835; Identities 812 / 842 (96 %), 2 gaps (0 %)) and *My. spartii* (GenBank KR611902; Identities 812 / 843 (96 %), 3 gaps (0 %)).

Nematogonum ferrugineum (Pers.) S. Hughes, *Canad. J. Bot.* **36**: 789. 1958. Fig. 25.
Basionym: *Monilinia ferruginea* Pers., *Mycol. eur.* (Erlanga) **1**: 30. 1822.

Mycelium consisting of hyaline, smooth, branched, septate, 3–4 µm diam hyphae. *Conidiophores* dimorphic. *Microconidiophores* reduced to conidiogenous cells on hyphae, erect, golden-brown, smooth, cylindrical, 20–40 × 6–8 µm. *Macroconidiophores* erect, flexuous, subcylindrical, smooth, golden-brown, flexuous, up to 400 µm tall, 8–10 µm diam, 2–7-septate, unbranched, terminal conidiogenous cell clavate, but at times also intercalary (appears to be linked to rejuvenating conidiophore), 25–100 × 11–15 µm; loci sympodial, thickened, somewhat darkened, 1–2 µm diam. *Conidia* occurring in branched chains, obovoid to ellipsoid, thick-walled, golden-brown, smooth, granular, apex obtuse, tapering to a truncate hilum, thickened and somewhat darkened, 1–2 µm diam, attached via a narrow isthmus, aseptate; primary conidia 15–21 × 12–14 µm; secondary conidia 11–15 × 8–9 µm; tertiary conidia 7–10 × 6–7 µm.

Culture characteristics: Colonies not growing on MEA, PDA or SNA. Colonies on OA pale luteous, flat, spreading, with sparse aerial mycelium and feathery, lobate margins, reaching 40 mm diam after 2 wk at 25 °C.

Specimen examined: **Ukraine**, Ternopil region, Zalischyky district, Dniester Canyon, on ascomata of *Melogramma campylosporum* on trunk of fallen *Carpinus betulus* (*Betulaceae*), 7 Oct. 2016, *A. Akulov,* specimen ex CWU (MYC) AS 6079 (dried culture CBS H-23418, culture CPC 31872 = CBS 144203).

Notes: Matsushima (1975) firstly reported an aspergillus-like synasexual morph for *Nematogonum highlei* (a synonym of *N. ferrugineum*). His description and illustrations correspond well with the conidiophores we observed in this study. Walker & Minter (1981) studied the conidiogenesis of *N. ferrugineum* and cited conidia to be ellipsoid, 4–24 × 3–15 µm, which become progressively smaller towards the tips of the chains. However, no distinction was made between primary, secondary or tertiary

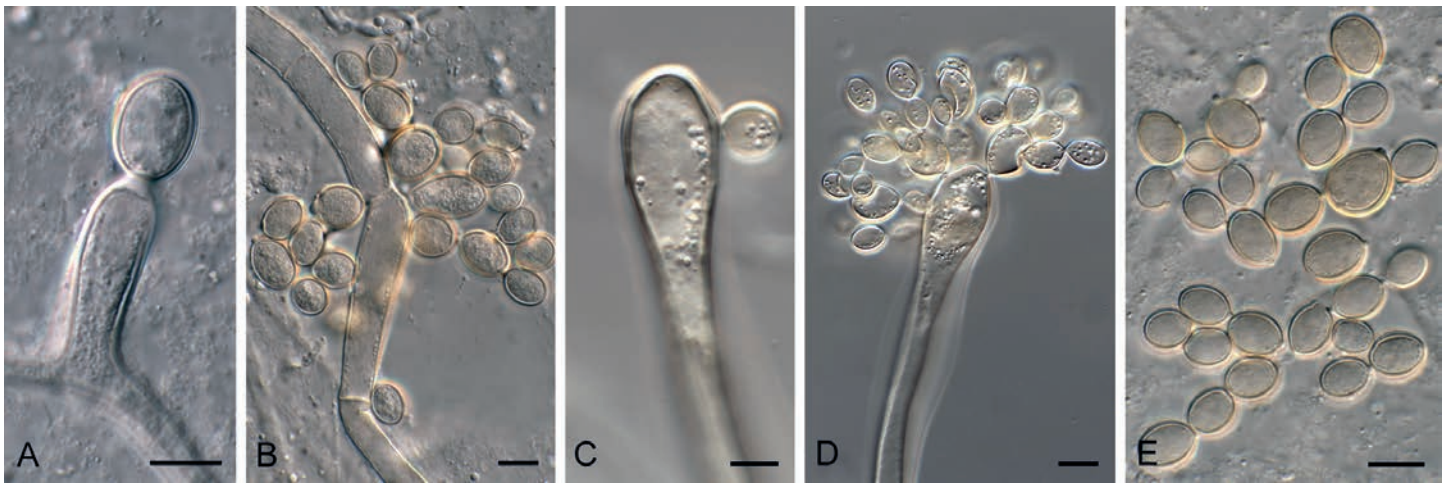

Fig. 25. *Nematogonum ferrugineum* (CPC 31872). **A.** Microconidiophore. **B–D.** Conidiophores with conidiogenous cells. **E.** Conidia. Scale bars = 10 µm.

conidia. The general conidium dimensions observed here, 7–21 × 6–15 µm, in the collection are different from the original species description, but correspond well with those provided by Matsushima (1975), 7–22 × 6.5–15 µm.

Nematogonum is not known from any sequence data that we were able to locate, and is listed as "*incertae sedis*" in MycoBank and Index Fungorum. In the present study, we were unable to generate an LSU sequence. However, based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Melanospora* spp. (*Melanosporales, Hypocreomycetidae, Sordariomycetes*), of which most members are also fungicolous. *Nematogonum ferrugineum* is known as an obligate fungicolous fungus on species of *Neonectria,* but has also been found on *Chaetomella, Cladosporium, Graphium, Melogramma, Tritirachium* and *Verticillium* representatives (Walker & Minter 1981, Gams *et al.* 2004, Akulov 2011).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Melanospora kurssanoviana* (GenBank KP981479; Identities 500 / 549 (91 %), 12 gaps (2 %)), *Me. singaporensis* (GenBank LC146748; Identities 519 / 582 (89 %), 20 gaps (3 %)) and *Papulaspora funabasensis* (GenBank LC228646; Identities 508 / 569 (89 %), 17 gaps (2 %)). The highest similarities using the *tef1* sequence were *My. banksiae* (GenBank NG_042684; Identities 813 / 842 (97 %), 2 gaps (0 %)), *My. schulzeri* (GenBank EU041835; Identities 812 / 842 (96 %), 2 gaps (0 %)) and *My. spartii* (GenBank KR611902; Identities 812 / 843 (96 %), 3 gaps (0 %)). No significant hits were obtained when the *tef1* sequence was used in a megablast search. All attempts to generate an LSU sequence for this culture failed, irrespective of using fresh DNA or different primer sets.

Neocucurbitaria cava (Schulzer) Valenzuela-Lopez *et al*., *Stud. Mycol*. **90**: 46. 2018. Fig. 26.
Basionym: *Phoma cava* Schulzer, *Verh. K. K. Zool.-Bot. Ges. Wien* **21**: 1248. 1871.

Conidiomata pycnidial, solitary, dark brown with 1–2 papillate ostioles, 150–250 µm diam; wall of 2–3 layers of brown *textura angularis. Conidiophores* lining the inner cavity, hyaline, smooth, subcylindrical, branched, 1–4-septate, 7–20 × 2–3 µm. *Conidiogenous cells* phialidic, with periclinal thickening, hyaline, smooth, subcylindrical, apical and intercalary, 4–7 × 2–3 µm. *Conidia* solitary, hyaline, smooth, aseptate, subcylindrical, guttulate, with bluntly rounded ends, (3–)3.5(–4) × 1.5 µm.
Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and smooth, lobate margins, reaching 40 mm diam after 2 wk at 25 °C. On MEA surface pale mouse grey, reverse mouse grey. On PDA surface olivaceous grey, reverse iron-grey. On OA surface iron-grey.

Specimen examined: **UK**, England, Bournmouth, on leaves of *Quercus ilex* (*Fagaceae*), 30 Dec. 2016, *P.W. Crous* (specimen CBS H-23414, culture CPC 32488 = CBS 143400).

Notes: The present collection is morphologically similar to that of the epitype, which was described from soil collected in Germany and has conidia that are aseptate, hyaline, smooth- and thin-walled, mostly cylindrical to slightly allantoid, 2.5–3.5 × 1–1.5 µm, guttulate (Valenzuela-Lopez *et al*. 2018). The present study adds a new culture of *N. cava* from the UK.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *N. cava* (GenBank JF440610; Identities 475 / 480 (99 %), 2 gaps (0 %)), *N. hakeae* (GenBank KY173436; Identities 512 / 533 (96 %), 3 gaps (0 %)) and *Ochrocladosporium frigidarii* (GenBank FJ755255; Identities 439 / 463 (95 %), no gaps). The highest similarities using the LSU sequence were *Ne. cava* (GenBank EU754199; Identities 855 / 855 (100 %), no gaps), *Ne. quercina* (GenBank GQ387620; Identities 848 / 855 (99 %), 1 gap (0 %)) and *Ne. unguis-hominis* (GenBank GQ387621; Identities 847 / 855 (99 %), 1 gap (0 %)). The highest similarities using the *actA* sequence were *Parastagonospora nodorum* (GenBank CP022855; Identities 469 / 508 (92 %), 2 gaps (0 %)), *Alternaria hordeicola* (GenBank JQ671637; Identities 478 / 520 (92 %), 2 gaps (0 %)) and *Al. triticimaculans* (GenBank JQ671631; Identities 478 / 520 (92 %), 2 gaps (0 %)). The highest similarities using the *rpb2* sequence were *Ne. populi* (GenBank MF795816; Identities 1035 / 1059 (98 %), no gaps), *Ne. juglandicola* (GenBank MF795815; Identities 1027 / 1059 (97 %), no gaps) and *Ne. cisticola* (GenBank MF795814; Identities 995 / 1058 (94 %), no gaps). The highest similarities using the *tub2* sequence were *Ne. populi* (GenBank MF795902; Identities 500 / 515 (97 %), 2 gaps (0 %)), *Ne. juglandicola* (GenBank MF795901; Identities 498 / 516 (97 %), 3 gaps (0 %)) and *Pyrenochaeta hakeae* (GenBank KY173613; Identities 500 / 534 (94 %), 10 gaps (1 %)).

Neohendersonia kickxii (Westend.) Sutton & Pollack, *Mycopathol. Mycol. Appl*. **52**: 334. 1974.
Basionym: *Stilbospora kickxii* Westend., *Bull. Séances Cl. Sci. Acad. Roy. Belgique* **18**: 409. 1851.

Description and illustration: Giraldo *et al*. (2017).

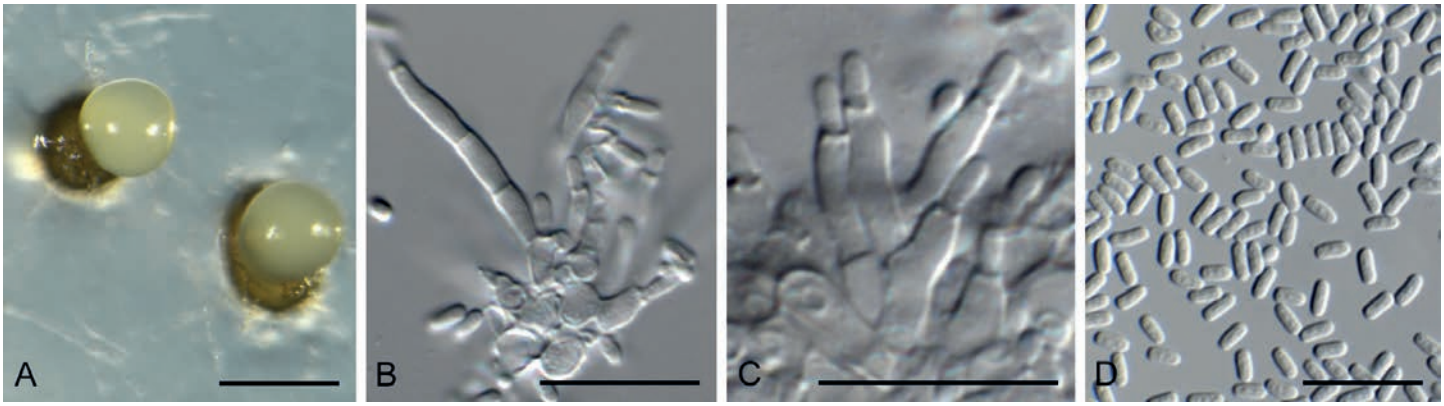

Fig. 26. *Neocucurbitaria cava* (CBS 143400). **A.** Conidiomata on SNA. **B, C.** Conidiogenous cells. **D.** Conidia. Scale bars: A = 200 µm, all others = 10 µm.

Specimens examined: **Belgium**, Courtrai, Parc Saint-George, on branch of *Fagus sylvatica* (*Fagaceae*) (substrate originally determined as *Betula pubescens* and later corrected with *Fagus sylvatica*), *G.D. Westendorp* (holotype BR5020162018281). **Italy**, Pian di Novello, on bark of twigs from *Fagus sylvatica*, 8 May 1996, *R. Danti* (epitype designated here of *Stilbospora kickxii* MycoBank MBT381143, preserved in metabolically inactive state, ex-epitype culture CBS 112403).

Notes: The epitype was originally designated in Giraldo *et al*. (2017), but a culture without any specimen was cited. This situation is herewith corrected, by stating that the culture is preserved as "metabolically inactive".

Parapleurotheciopsis caespitosa (Crous *et al*.) Crous, ***comb. nov.*** MycoBank MB824780.
Basionym: *Anungitea caespitosa* Crous *et al*., *Canad. J. Bot.* **73**(2): 225. 1995.

Description and illustration: Crous *et al*. (1995).

Specimen examined: **South Africa**, Mpumalanga, Sabie, on leaf litter of *Syzygium cordatum* (*Myrtaceae*), Nov. 1992, *M.J. Wingfield* (holotype PREM 51686, culture ex-type CPC 565 = CBS 519.93).

Note: See discussion under *Polyscytalum* and Fig. 13.

Parathyridaria philadelphi Crous & R.K. Schumach., ***sp. nov.*** MycoBank MB824781. Fig. 27.

Etymology: Name refers to *Philadelphus,* the host genus from which this fungus was collected.

Conidiomata (on OA) separate, pycnidial, brown, globose, erumpent, 250–300 µm diam, with central ostiole; wall of 6–8 layers of brown *textura angularis. Conidiophores* reduced to conidiogenous cells lining the inner cavity, hyaline, smooth, ampulliform, proliferating percurrently near phialidic apex, 4–7 × 3–4 µm. *Conidia* aseptate, solitary, subcylindrical, apex obtuse, base bluntly rounded, brown, smooth, at times slightly granular, (4–)5(–6) × 2 µm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and even, lobate margins, reaching 45 mm diam after 2 wk at 25 °C. On MEA surface pale olivaceous grey with patches of ochreous, reverse amber. On PDA surface amber, reverse chestnut. On OA surface amber with patches of sienna and vinaceous buff.

Specimen examined: **Germany**, near Berlin, on twigs of *Philadelphus coronarius* (*Hydrangeaceae*), 2 Apr. 2016, *R.K. Schumacher* (holotype CBS H-23409, culture ex-type CPC 30532 = CBS 143432).

Notes: The genus *Parathyridaria* was recently introduced by Jaklitsch & Voglmayr (2016). Phylogenetically (Fig. 28), *Pa. philadelphi* is allied to *Pa. robiniae*, a sexual species recently described from Italy on *Robinia pseudoacacia* (Tibpromma *et al*. 2017).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Pa. robiniae* (GenBank KY511142; Identities 709 / 715 (99 %), no gaps), *Roussoella mukdahanensis* (GenBank KU940129; Identities 602 / 718 (84 %), 20 gaps (2 %)) and *Pa. ramulicola* (GenBank NR_147657; Identities 406 / 429 (95 %), 3 gaps (0 %)). The highest similarities using the LSU sequence were *Pa. robiniae* (GenBank KY511141; Identities 854 / 855 (95 %), no gaps), *Sporidesmium australiense* (GenBank DQ408554; Identities 835 / 846 (99 %), 1 gap (0 %)) and *Pa. ramulicola* (GenBank KF636775; Identities 846 / 859 (98 %), no gaps). The highest similarity using the *tef1* sequence was with *Pa. ramulicola* (GenBank KX650536; Identities 314 / 352 (89 %), 7 gaps (1 %)).

Pestalotiopsis hollandica Maharachch. *et al*., *Stud. Mycol.* **79**: 164. 2014. Fig. 29.

Conidiomata pycnidial, globose, separate, immersed to erumpent on banana leaf agar, dark brown to black, 120–350 µm diam, exuding a globose, dark brown conidial mass. *Conidiophores* subcylindrical, branched, hyaline, smooth, 1–2-septate, 15–30 × 3–5 µm. *Conidiogenous cells* terminal and intercalary, subcylindrical, hyaline, smooth, 8–15 × 2.5–3.5 µm; proliferating percurrently at apex. *Conidia* solitary, fusoid-ellipsoid, 4-septate, versicoloured, central three cells brown, of which the median cell is dark brown, guttulate, verruculose, apical and basal cells hyaline, conidia (22–)24–26(–27) × (7–)8(–9) µm, apical cell 3–4 µm long, basal cell 3–5 µm long, apical cell with three flexuous appendages, unbranched, attachment apical, 17–25 µm long, basal cell with central unbranched appendage, 3–9 µm long.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and feathery, lobate margins, covering dish after 2 wk at 25 °C. On MEA surface dirty white, reverse luteous. On PDA surface pale luteous to luteous, reverse amber. On OA surface pale luteous.

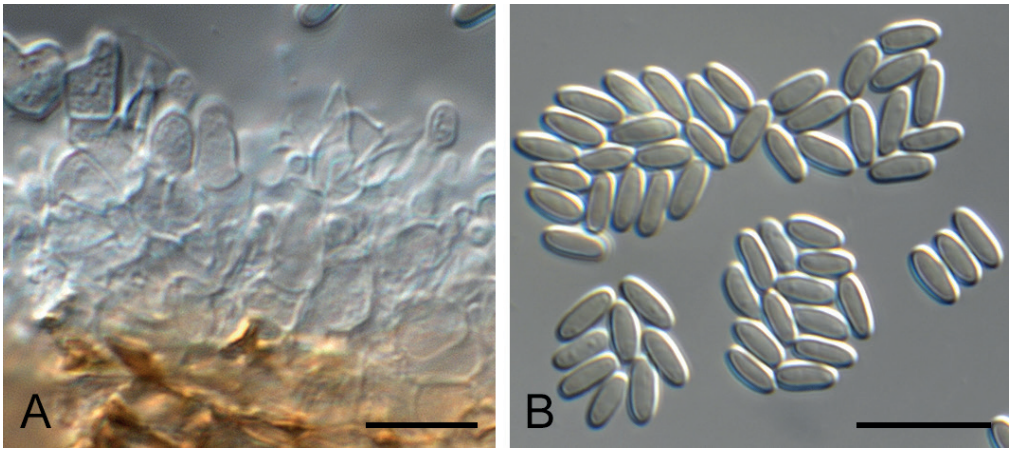

Fig. 27. *Parathyridaria philadelphi* (CBS 143432). **A.** Conidiogenous cells. **B.** Conidia. Scale bars = 10 µm.

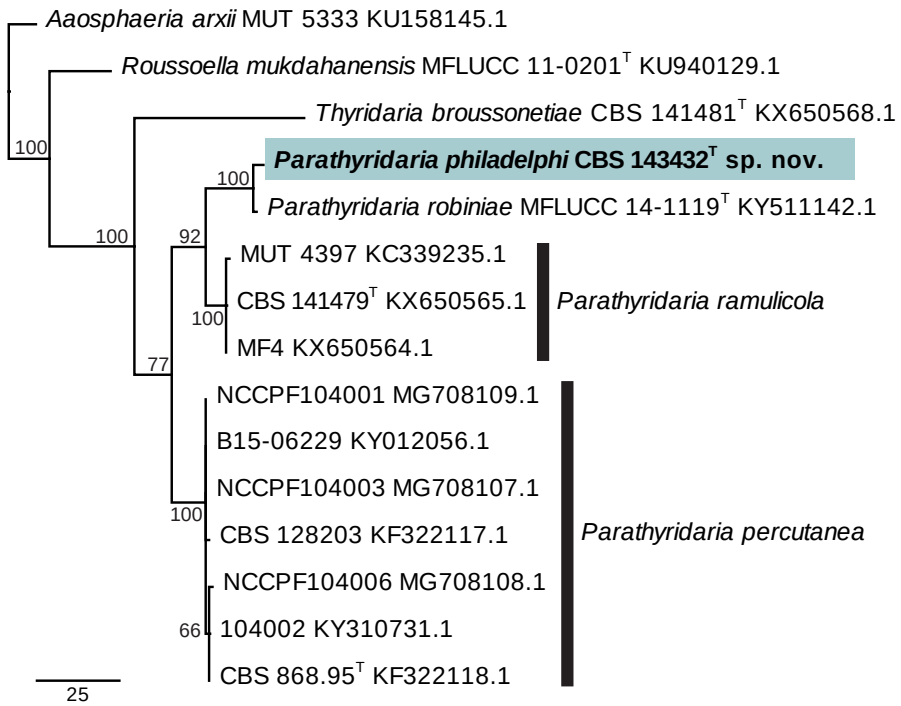

Fig. 28. Single most parsimonious tree obtained from a phylogenetic analysis of the *Parathyridaria* ITS alignment (15 strains including the outgroup; 412 characters analysed: 297 constant, 51 variable and parsimony-uninformative and 64 parsimony-informative). The tree was rooted to *Aaosphaeria arxii* (GenBank KU158145.1) and the scale bar indicates the number of changes. Bootstrap support values higher than 49 % are shown at the nodes and novelties are highlighted with a coloured box and bold text. Species names are indicated to the right of the tree, or before the culture collection and GenBank accession numbers. A superscript T denotes strains with a type status. Tree statistics: TL = 172, CI = 0.866, RI = 0.861, RC = 0.746.

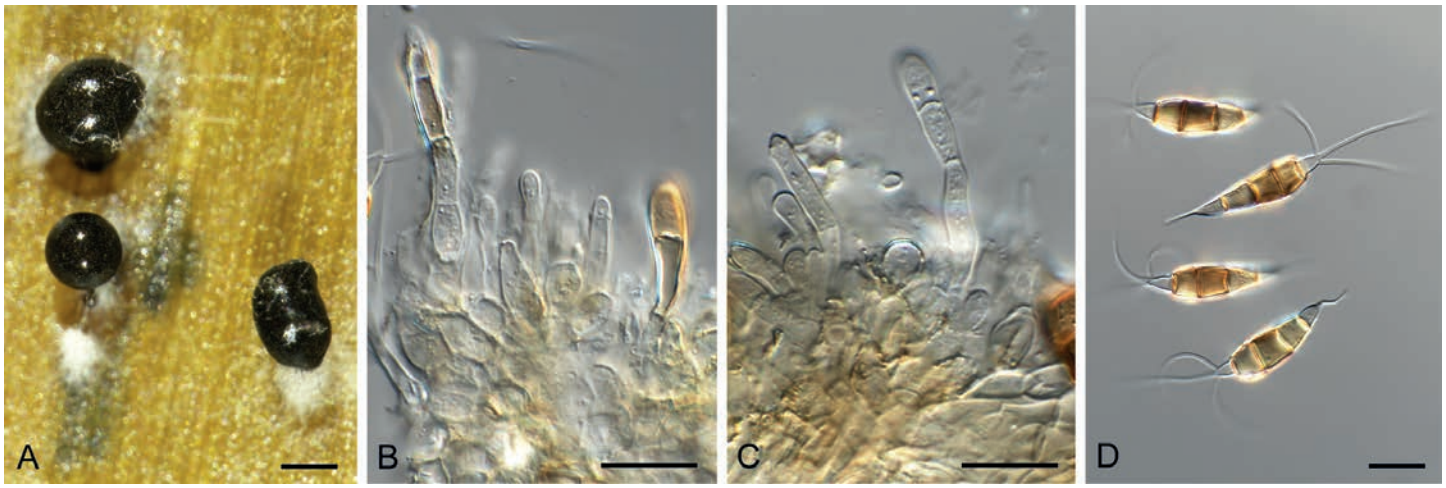

Fig. 29. *Pestalotiopsis hollandica* (CBS 143436). **A.** Conidiomata on BLA. **B, C.** Conidiogenous cells. **D.** Conidia. Scale bars: A = 300 μm, all others = 10 μm.

Specimen examined: **Spain**, Zaragoza, Carretera el Frago, on needles of *Cupressus sempervirens* (*Cupressaceae*), 7 Jan. 2016, *R. Blasco*, culture CPC 30399 = CBS 143436.

Notes: Phylogenetically the present collection is identical (based on ITS and LSU, and almost identical based on *tub2*) to *Pe. hollandica* (Maharachchikumbura *et al*. 2014). Morphologically, however, they are quite distinct, with *Pe. hollandica* having conidia that are larger, (25–)25.5–33(–34) × 8.5–10(–10.5) μm, with 1–4 tubular apical appendages, 20–40 μm long. Based on a megablast search using the ITS sequence in NCBI's GenBank nucleotide database, the ITS sequence is identical to *Pe. hollandica* (CBS 265.33; GenBank NR_147555), *Pe. monochaeta* (CBS 144.97; GenBank NR_147554) and *Pe. funerea* (ML4DY; GenBank EF055197). The highest similarities using the LSU sequence in NCBI's GenBank nucleotide database, the LSU sequence is identical to *Pe. monochaeta* (CBS 144.97; GenBank KM116229), *Pe. hollandica* (CBS 265.33; GenBank KM116228) and *Pe. hangzhouensis* (PSHI2002Endo390; GenBank DQ657865). The highest similarities using the *tef1* sequence were *Pe. verruculosa* (GenBank JX399061; Identities 298 / 299 (99 %), no gaps), *Pe. hollandica* (GenBank KM199481; Identities 281 / 286 (98 %), no gaps) and *Pe. brassicae* (GenBank KM199558; Identities 268 / 273 (98 %), no gaps). The highest similarities using the *tub2* sequence were *Pe. hollandica* (GenBank KM199388; Identities 446 / 447 (99 %), no gaps), *Pe. italiana* (GenBank KP781882; Identities 442 / 445 (99 %), 2 gaps (0 %)) and *Pe. monochaeta* (GenBank KX642435; Identities 448 / 452 (99 %), 2 gaps (0 %)).

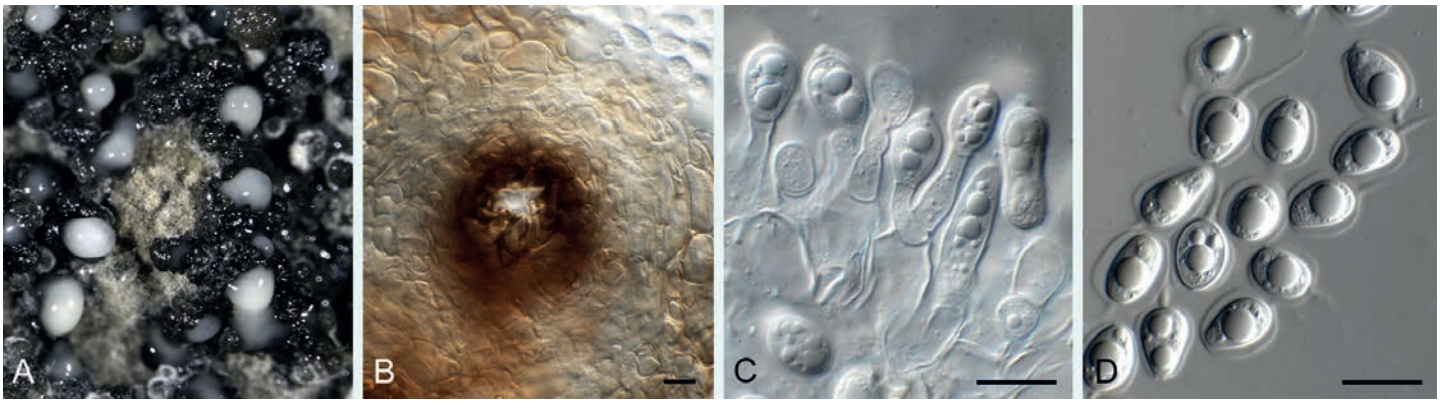

Fig. 30. *Phyllosticta hakeicola* (CBS 143492). **A.** Colony on PDA. **B.** Conidiomatal ostiole. **C.** Conidiogenous cells. **D.** Conidia. Scale bars = 10 μm.

Phyllosticta hakeicola Crous & T.I. Burgess, ***sp. nov.*** MycoBank MB824782. Fig. 30.

Etymology: Name refers to *Hakea*, the host genus from which it was collected.

Conidiomata pycnidial, solitary, globose, dark brown, 150–250 μm diam, with central ostiole, 25–40 μm diam; wall of 3–8 layers of brown *textura angularis. Conidiophores* lining the inner cavity, 1–2-septate, subcylindrical, hyaline, smooth, branched below, 20–30 × 6–10 μm. *Conidiogenous cells* terminal and intercalary, subcylindrical, hyaline, smooth, 8–15 × 3–5 μm, proliferating percurrently at apex. *Conidia* solitary, ellipsoid to obovoid, aseptate, smooth, hyaline, guttulate, granular, (9–)10–13(–15) × (6.5–)7 μm; conidia encased in a persistent mucoid sheath, 2–3 μm diam, but with a single apical mucoid appendage, 5–12 × 1.5–2 μm, tapering to subacutely rounded apex.

Culture characteristics: Colonies flat to erumpent, spreading, with sparse to moderate aerial mycelium and feathery, lobate margins, reaching 55 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface and reverse iron-grey.

Specimen examined: **Australia**, New South Wales, Fitzroy Falls, on leaves of *Hakea* sp. (*Proteaceae*), 26 Nov. 2016, *P.W. Crous* (holotype CBS H-23315, culture ex-type CPC 32041 = CBS 143492).

Notes: Van der Aa & Vanev (2002) placed *Phyllosticta hakeae* in the genus *Microsphaeropsis*, and presently no species of *Phyllosticta* are known from *Hakea*. Phylogenetically (Fig. 31),

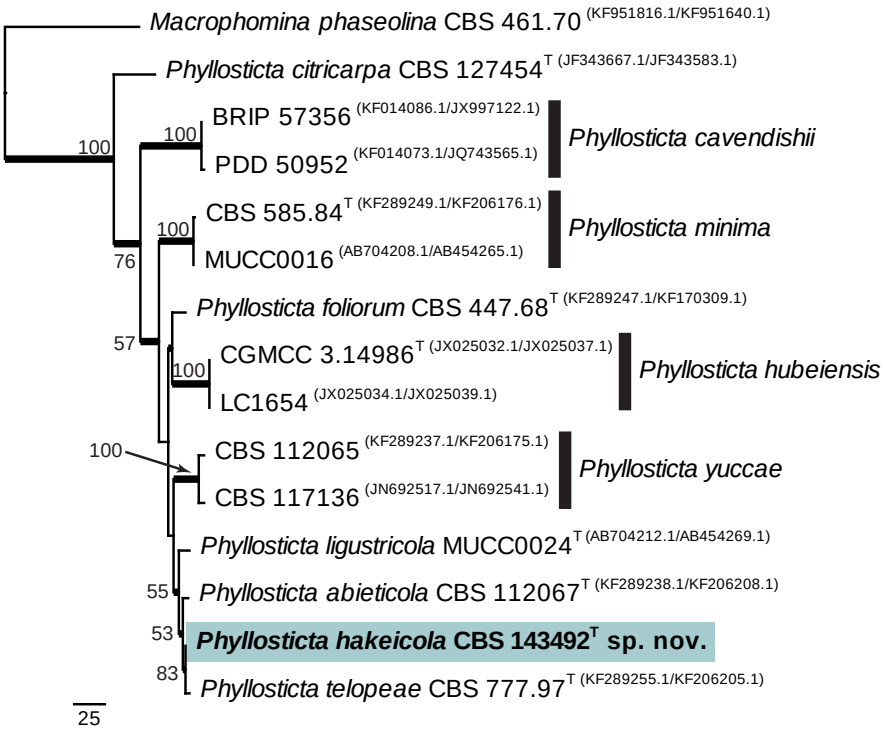

Fig. 31. The first of three equally most parsimonious trees obtained from a phylogenetic analysis of the combined *actA* and ITS alignment representing *Phyllosticta* species (15 strains including the outgroup; 659 characters analysed: 387 constant, 140 variable and parsimony-uninformative and 132 parsimony-informative). The tree was rooted to *Macrophomina phaseolina* (culture CBS 461.70) and the scale bar indicates the number of changes. Bootstrap support values higher than 49 % are shown at the nodes and novelties are highlighted with a coloured box and bold text. Species names are indicated to the right of the tree, or before the culture collection and GenBank accession numbers. A superscript T denotes strains with a type status and branches present in the strict consensus tree are thickened. Tree statistics: TL = 426, CI = 0.826, RI = 0.739, RC = 0.611.

Ph. hakeicola is closely related to *Ph. telopeae* (also *Proteaceae*; Crous *et al*. 2000), but can be distinguished by its larger conidia, (12–)13–16(–18) × (7–)8–9 μm (Swart *et al*. 1998).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Ph. telopeae* (GenBank KF206205; Identities 568 / 570 (99 %), no gaps), *Ph. abieticola* (GenBank NR_147344; Identities 562 / 570 (99 %), 2 gaps (0 %)) and *Ph. ligustricola* (GenBank NR_136951; Identities 609 / 626 (97 %), 4 gaps (0 %)). The highest similarities using the LSU sequence were *Ph. telopeae* (GenBank KF766384; Identities 841 / 841 (100 %), no gaps), *Ph. abieticola* (GenBank EU754193; Identities 852 / 854 (99 %), no gaps) and *Ph. philoprina* (GenBank DQ377878; Identities 852 / 854 (99 %), no gaps). The highest similarities using the *actA* sequence were *Ph. abieticola* (GenBank KF289238; Identities 225 / 225 (100 %), no gaps), *Ph. telopeae* (GenBank KF289255; Identities 222 / 225 (99 %), no gaps) and *Ph. foliorum* (GenBank KF289247; Identities 221 / 225 (98 %), no gaps). The highest similarities using the *gapdh* sequence were *Ph. hubeiensis* (GenBank JX025029; Identities 339 / 351 (97 %), 1 gap (0 %)), *Ph. cavendishii* (GenBank KU716083; Identities 324 / 337 (96 %), no gaps) and *Ph. citricarpa* (GenBank KX280614; Identities 323 / 336 (96 %), no gaps). The highest similarities using the *tef1* sequence were *Ph. telopeae* (GenBank KF766435; Identities 303 / 308 (98 %), no gaps), *Ph. yuccae* (GenBank JX227948; Identities 371 / 396 (94 %), 5 gaps (1 %)) and *Ph. minima* (GenBank KF766432; Identities 287 / 309 (93 %), 6 gaps (1 %)).

Polyscytalum chilense Crous & M.J. Wingf., ***sp. nov.*** MycoBank MB824783. Fig. 32.

Etymology: Name refers to Chile, the country where this fungus was collected.

Mycelium consisting of branched, septate, brown, smooth, 2–3 μm diam hyphae. *Conidiophores* solitary, erect, 1–3-septate, subcylindrical, brown, smooth, straight to geniculous-sinuous, 30–60 × 3–4 μm. *Conidiogenous cells* terminal and intercalary, subcylindrical to clavate, 7–12 × 3–4 μm; scars arranged in a rachis, prominent, thickened, darkened and refractive, 1–1.5 μm diam. *Conidia* cylindrical, pale brown, smooth, prominently guttulate, 1-septate, apex obtuse, base truncate, 1–1.5 μm diam, somewhat darkened and refractive, in very long, unbranched chains, (13–)15–18(–20) × (2–)2.5 μm.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery, lobate margins, reaching 15 mm diam after 2 wk at 25 °C. On MEA surface ochreous, reverse chestnut. On PDA surface and reverse amber. On OA surface iron-grey.

Specimen examined: **Chile**, on leaves of *Eucalyptus urophylla* (*Myrtaceae*), Jun. 2010, *M.J. Wingfield* (holotype CBS H-23403, culture ex-type CPC 31946 = CBS 143387).

Notes: Sutton (1973) established the genus *Anungitea* for a genus of hyphomycetes with dark, solitary conidiophores, bearing a head of denticles with flattened conidiogenous scars that are neither thickened nor darkened, and chains of cylindrical, 1-septate subhyaline conidia. Since its introduction, several taxa have been added to the genus, and because the type *A. fragilis* remains phylogenetically undefined, the generic concept has widened. As seen in the present study, several of these "*Anungitea*" species cluster with *Po. fecundissimum*, the type species of *Polyscytalum*. It has become clear that the generic concepts of these two genera overlap and that several species would be better accommodated in *Polyscytalum* than in *Anungitea*. *Polyscytalum* has cylindrical conidia that vary from being 0–1-septate, hyaline to pale brown, smooth, with truncate ends (those in *Anungitea* have obtuse ends), and the scars can be somewhat darkened and refractive, but unthickened in both genera (see *Pseudoanungitea* with thickened hila elsewhere in this manuscript).

Phylogenetically (Fig. 13), *Po. chilense* is distinct from all species known from *Eucalyptus* (Crous *et al.* 2017a) and is most similar to *Po. grevilleae*, which has setae, and smaller conidia, (10–)13–16(–22) × (2–)2.5–3 μm (Crous *et al.* 2016b).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Po. grevilleae* (GenBank KX228252; Identities 537 / 560 (96 %), no gaps), *Po. eucalyptorum* (GenBank NR_132904; Identities 534 / 560 (95 %), 1 gap (0 %)) and *Po. fecundissimum* (GenBank EU035441; Identities 371 / 391 (95 %), 2 gaps (0 %)). The highest similarities using the LSU sequence were *Po. eucalyptigena* (GenBank KY173477; Identities 818 / 821 (99 %), no gaps), *Phlogicylindrium eucalypti* (GenBank DQ923534; Identities 888 / 896 (99 %), 1 gap (0 %)) and *Po. eucalyptorum* (GenBank KJ869176; Identities 878 / 886 (99 %), no gaps).

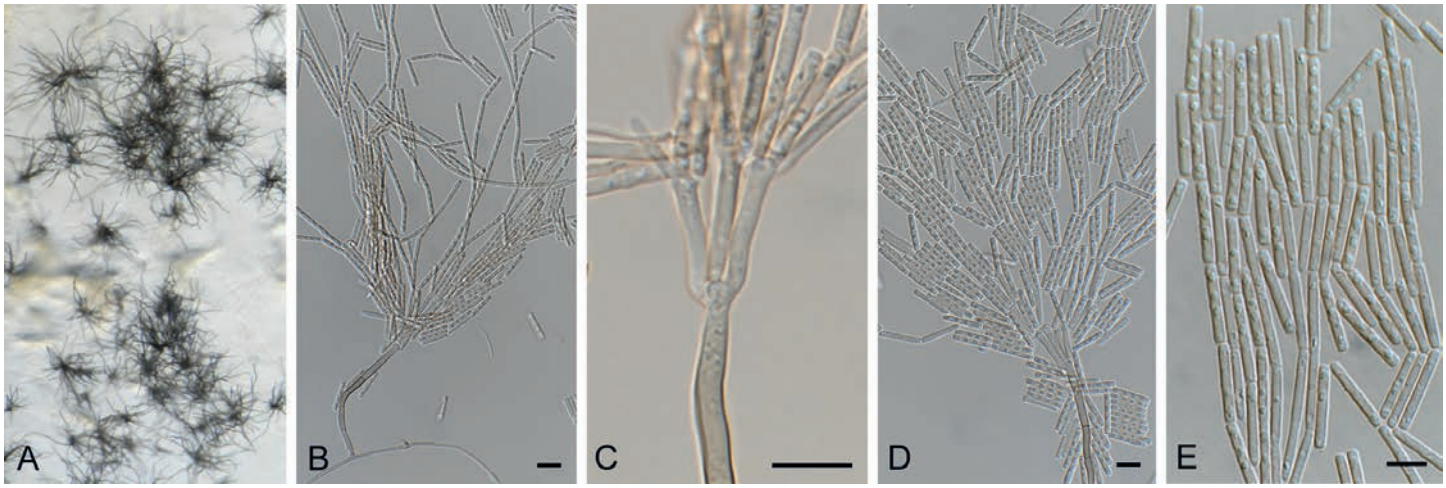

Fig. 32. *Polyscytalum chilense* (CBS 143387). **A.** Colony on SNA. **B–D.** Conidiophores giving rise to conidial chains. **E.** Conidia. Scale bars = 10 μm.

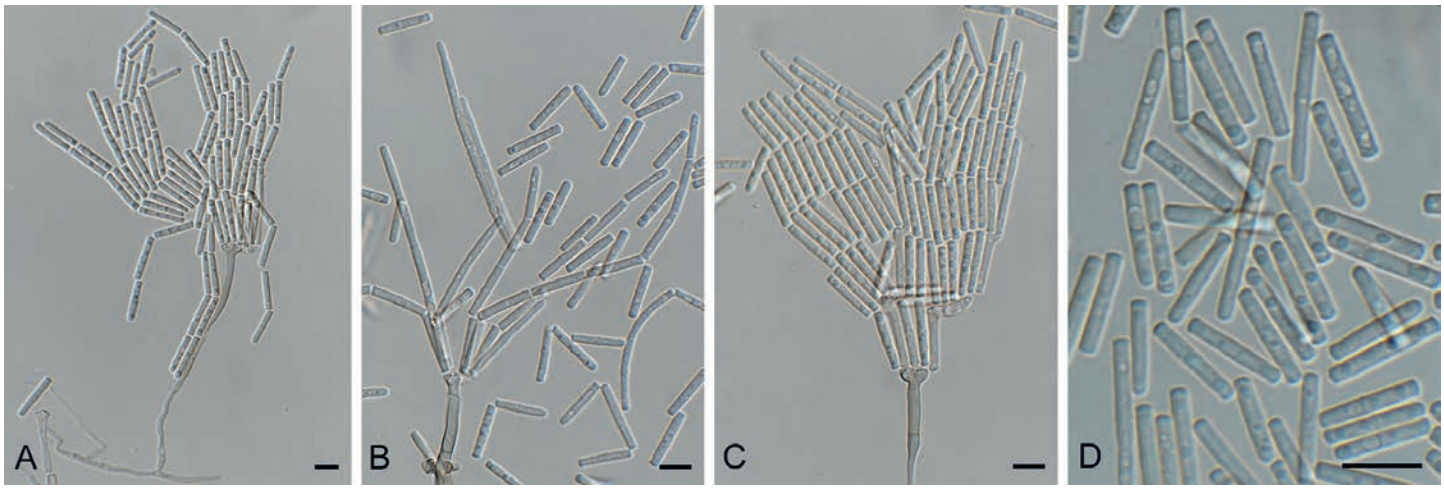

Fig. 33. *Polyscytalum eucalyptigenum* (CBS 143388). **A–C.** Conidiophores giving rise to conidial chains. **D.** Conidia. Scale bars = 10 µm.

Polyscytalum eucalyptigenum (Crous & M.J. Wingf.) Crous & M.J. Wingf., ***comb. nov.*** MycoBank MB824784. Fig. 33.
Basionym: *Anungitea eucalyptigena* Crous & M.J. Wingf., *Persoonia* **37**: 339. 2016.

Description and illustration: Crous *et al*. (2016a).

Mycelium consisting of brown, smooth, septate, 2–3 µm diam hyphae. *Conidiophores* erect, solitary, subcylindrical, unbranched, brown, smooth, flexuous, 1–3-septate, 20–100 × 3–4 µm. *Conidiogenous cells* integrated, terminal, 7–15 × 3–4 µm, apex swollen with several sympodial loci, denticulate, flat-tipped, 1–2 × 2–2.5 µm, not thickened nor darkened. *Ramoconidia* subcylindrical, pale brown, smooth, 0–1-septate, 12–20 × 2.5–3 µm. *Conidia* occurring in long, unbranched chains, cylindrical with truncate ends, hyaline, smooth, guttulate, medianly 1-septate, (11–)13–17(–20) × (2–)2.5 µm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and feathery, lobate margins, reaching 30 mm diam after 2 wk at 25 °C. On MEA surface ochreous, reverse iron-grey. On PDA surface amber, reverse brown vinaceous. On OA surface olivaceous grey.

Specimens examined: **Chile**, on leaves of *Eucalyptus grandis* × *uromycoides* (*Myrtaceae*), Jun. 2010, *M.J. Wingfield* (specimen CBS H-23421, culture CPC 31878 = CBS 143388). **Malaysia**, Kota Kinabalu, on leaf spots of *Eucalyptus grandis* × *pellita* (*Myrtaceae*), 30 May 2015, *M.J. Wingfield* (holotype CBS H-22888, culture ex-type CPC 28762 = CBS 142102).

Notes: The present collection from Chile is morphologically and phylogenetically (Fig. 13) similar to the ex-type strain of *Po. eucalyptigenum* from Malaysia (ramoconidia 16–20 × 2.5–3 µm, conidia (11–)14–16(–18) × (2–)2.5(–3) µm; Crous *et al*. 2016a). Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Po. eucalyptigena* (GenBank KY173383; Identities 570 / 571 (99 %), no gaps), *Po. grevilleae* (GenBank KX228252; Identities 548 / 571 (96 %), 11 gaps (1 %)) and *Po. eucalyptorum* (GenBank NR_132904; Identities 545 / 571 (95 %), 12 gaps (2 %)). The highest similarities using the LSU sequence were *Po. eucalyptorum* (GenBank KY173477; Identities 819 / 821 (99 %), no gaps), *Po. grevilleae* (GenBank KX228304; Identities 824 / 831 (99 %), no gaps) and *Phlogicylindrium eucalypti* (GenBank DQ923534; Identities 835 / 844 (99 %), 1 gaps (0 %)).

Polyscytalum eucalyptorum (Crous & R.G. Shivas) Crous, ***comb. nov.*** MycoBank MB824785.
Basionym: *Anungitea eucalyptorum* Crous & R.G. Shivas, *Persoonia* **32**: 199. 2014.

Description and illustration: Crous *et al*. (2014).

Specimen examined: **Australia**, Queensland, Dave's Creek, S28°12'13.7" E153°12'9.5", on *Eucalyptus* (*Myrtaceae*) leaf litter, 11 Jul. 2009, *P.W. Crous & R.G. Shivas,* (holotype CBS H-21678, culture ex-type CPC 17207 = CBS 137967).

Polyscytalum grevilleae (Crous & Jacq. Edwards) Crous, ***comb. nov.*** MycoBank MB824786.
Basionym: *Anungitea grevilleae* Crous & Jacq. Edwards, *Persoonia* **36**: 327. 2016.

Description and illustration: Crous *et al*. (2016b).

Specimen examined: **Australia**, Victoria, Royal Botanic Gardens Cranbourne, S38°7' 49.6" E145°16'9", on leaves of *Grevillea* sp. (*Proteaceae*), 7 Nov. 2014, *P.W. Crous & J. Edwards* (holotype CBS H-22591, culture ex-type CPC 25576 = CBS 141282).

Polyscytalum neofecundissimum Crous & Akulov, ***sp. nov.*** MycoBank MB824787. Fig. 34.

Etymology: Name refers to its morphological similarity to *Polyscytalum fecundissimum*.

Conidiophores reduced to conidiogenous cells or erect, flexuous, subcylindrical, branched, up to 100 µm tall, pale brown, smooth. *Conidiogenous cells* terminal and intercalary, subcylindrical, pale brown, smooth, 20–25 × 3–4 µm, proliferating sympodially at apex, scars unthickened, 2–2.5 µm diam. *Conidia* occurring in chains, cylindrical with obtuse ends, hyaline, smooth, medianly 1-septate, guttulate, (12–)14–17(–20) × 2(–3) µm.

Culture characteristics: Colonies erumpent, spreading, surface folded, with moderate aerial mycelium and smooth, lobate

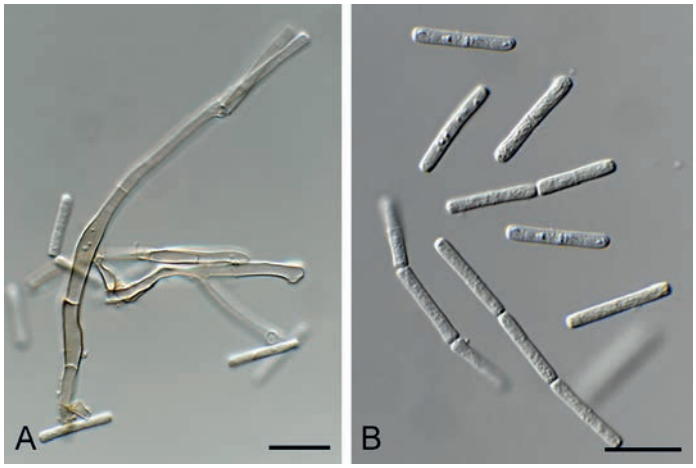

Fig. 34. *Polyscytalum neofecundissimum* (CBS 143390). **A.** Conidiophore. **B.** Conidial chains. Scale bars = 10 μm.

margins, reaching 20 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface olivaceous grey, reverse iron-grey.

Specimen examined: **Ukraine**, Ternopil region, Zalischyky district, Dniester Canyon, on leaf litter of *Quercus robur* (*Fagaceae*), associated with the mycelium of *Cladosporium* sp., 7 Oct. 2016, *A. Akulov,* specimen ex CWU (MYC) AS 6073 isotype (holotype CBS H-23419, culture ex-type CPC 31826 = CBS 143390).

Notes: *Polyscytalum neofecundissimum* is morphologically and phylogenetically (Fig. 13) similar to *Po. fecundissimum* (conidia 13–18 × 2 μm; Ellis 1971), except that it has larger conidia. Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Po. fecundissimum* (GenBank EU035441; Identities 562 / 578 (97 %), no gaps), *Subulispora britannica* (GenBank EF029198; Identities 535 / 571 (94 %), 2 gaps (0 %)) and *Pseudophloeospora eucalyptorum* (GenBank NR_145406; Identities 436 / 494 (88 %), 18 gaps (3 %)). The highest similarities using the LSU sequence were *Po. fecundissimum* (GenBank EU035441; Identities 800 / 809 (99 %), no gaps), *Po. eucalyptigena* (GenBank KY173477; Identities 812 / 823 (99 %), 2 gaps (0 %)) and *Po. eucalyptorum* (GenBank KJ869176; Identities 831 / 843 (99 %), 2 gaps (0 %)).

Polyscytalum nullicananum (Crous) Crous, ***comb. nov.*** MycoBank MB824788.
Basionym: *Anungitea nullicana* Crous, *Persoonia* **39**: 411. 2017.

Description and illustration: Crous *et al*. (2017b).

Specimen examined: **Australia**, New South Wales, Nullica State Forest, on leaf litter of *Eucalyptus* sp. (*Myrtaceae*), 29 Nov. 2016, *P.W. Crous* (holotype CBS H-23297, culture ex-type CPC 32528 = CBS 143406).

Pseudoanungitea Crous, ***gen. nov.*** MycoBank MB824789.

Etymology: Name refers to its morphological similarity to *Anungitea*.

Mycelium consisting of branched, septate, brown, smooth, 2–3 μm diam hyphae. *Conidiophores* solitary, erect, septate, subcylindrical, brown, smooth, straight to flexuous. *Conidiogenous cells* terminal and intercalary, subcylindrical to clavate; scars arranged in a rachis, prominent, thickened, darkened and refractive. *Conidia* fusoid-ellipsoid, pale brown, smooth, prominently guttulate, 0–1-septate, hila somewhat darkened and refractive, in short (1–2) unbranched chains.

Type species: *Pseudoanungitea syzygii* (Crous *et al.*) Crous.

Pseudoanungitea syzygii (Crous *et al*.) Crous, ***comb. nov.*** MycoBank MB824790.
Basionym: *Anungitea syzygii* Crous *et al*., *Canad. J. Bot*. **73**(2): 225. 1995.

Description and illustration: Crous *et al.* (1995).

Specimen examined: **South Africa**, Mpumalanga, Sabie, on leaf litter of *Syzygium chordatum* (*Myrtaceae*), Mar. 1993, *W.J. Swart* (holotype PREM 51687, culture ex-type CPC 578 = CBS 520.93).

Notes: *Anungitea* includes species with dark, solitary conidiophores, bearing a head of denticles with flattened conidiogenous scars that are unthickened and not darkened, and chains of cylindrical, 1-septate subhyaline conidia, with apical and basal scars (Sutton 1973). *Anungitopsis* is similar but includes taxa with indistinguishable scars arranged in a rachis. *Neoanungitea* is somewhat intermediate between these two genera, having a rachis, but with flat-tipped loci (Crous *et al.* 2017b).

Because the type species of *Anungitea* (*A. fragilis*) is not presently known from culture and needs to be recollected (leaves of *Abies balsamea*, Manitoba, Canada), the phylogeny of *Anungitea* remains unresolved, and several unrelated taxa have been described in the genus. The two species treated here cluster apart from the generic clade assumed to be *Anungitea s. str*. They differ from *Anungitea* in having terminal and intercalary conidiogenous cells, and refractive, thickened scars that give rise to short conidial chains with somewhat darkened and refractive hila.

Pseudoanungitea vaccinii Crous & R.K. Schumach., ***sp. nov.*** MycoBank MB824791. Fig. 35.

Etymology: Name refers to *Vaccinium,* the host genus from which this fungus was collected.

Mycelium consisting of branched, septate, brown, smooth, 2–3 μm diam hyphae. *Conidiophores* solitary, erect, 0–3-septate, subcylindrical, brown, smooth, straight to flexuous, 8–40 × 3–5 μm. *Conidiogenous cells* terminal and intercalary, subcylindrical to clavate, 5–20 × 3–5 μm; scars arranged in a rachis, prominent, thickened, darkened and refractive, 1–1.5 μm diam. *Conidia* fusoid-ellipsoid, pale brown, smooth, prominently guttulate, 0–1-septate, apex obtuse, base truncate, 1–1.5 μm diam, somewhat darkened and refractive, in short (1–2) unbranched chains, (8–)10–12(–13) × (2–)3(–4) μm.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and smooth, lobate margins, reaching 7 mm diam after 1 mo at 25 °C. On MEA, PDA and OA surface and reverse brown vinaceous.

Specimen examined: **Germany**, near Berlin, on stem of *Vaccinium myrtillus* (*Ericaceae*), 16 Jan. 2016, *R.K. Schumacher* (holotype CBS H-23422, culture ex-type CPC 30522 = CBS 143164).

Fig. 35. *Pseudoanungitea vaccinii* (CBS 143164). **A–E.** Conidiophores. **F.** Conidia. Scale bars = 10 μm.

Notes: Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Anungitea syzygii* (GenBank KY853424; Identities 499 / 526 (95 %), 4 gaps (0 %)), *Tothia fuscella* (GenBank JF927786; Identities 504 / 561 (90 %), 11 gaps (1 %)) and *T. spartii* (GenBank NR_132917; Identities 430 / 487 (88 %), 15 gaps (3 %)). The highest similarities using the LSU sequence were *Cylindrosympodium lauri* (GenBank EU035414; Identities 840 / 855 (98 %), no gaps), *Cy. variabile* (GenBank KX228353; Identities 836 / 852 (98 %), no gaps) and *An. syzygii* (GenBank KY853484; Identities 802 / 823 (98 %), 6 gaps (0 %)).

Pseudoanungitea variabilis Hern.-Restr., ***sp. nov.*** MycoBank MB824792. Fig. 36.

Etymology: Name refers to the variable conidial morphology.

Mycelium consisting of branched, septate, pale brown to brown, smooth, 1–2 μm diam hyphae. *Conidiophores* solitary, erect, simple, rarely branched, subcylindrical, straight to flexuous, 0–7-septate, brown paler to the apex, smooth, 18–100 × 2–3 μm. *Conidiogenous cells* terminal and intercalary, sympodial, denticulate, subcylindrical, 8.5–23.5 × 2.5–4 μm; denticles prominent, sometimes darkened, 1–1.5 μm diam. *Conidia* in short chains (1–2(–4)), two shapes *a*. fusoid-ellipsoid, hyaline, smooth, sometimes guttulate, 0–1-septate, apex obtuse or truncate, base truncate, 1–1.5 μm diam, somewhat darkened and refractive, 8–14 × 2–2.5(–3) μm; *b*. globose, subglobose to pyriform, hyaline, smooth, aseptate, apex obtuse, base truncated, 1–1.5 μm diam, 4–8.5 × 2–4 μm.

Culture characteristics: Colonies after 1 mo at 25 °C, on OA reaching 6 mm, velvety, brown vinaceous, entire to lobate margin; reverse brown vinaceous. On MEA and PDA reaching 6–12 mm, effuse becoming raised, aerial mycelium pale mouse grey, submerged mycelium black, lobate margin; reverse black.

Specimen examined: **Spain**, Castilla la Mancha, Hayedo de la Tejera Negra Natural Park, on dead wood, May 2011, *M. Hernández-Restrepo, J. Mena & J. Guarro* (holotype CBS H-23494, culture ex-type CBS 132716).

Notes: *Pseudoanungitea variabilis* is distinct from other species in the genus due to its having two conidial shapes. Some conidia are fusoid-ellipsoid resembling those of *Ps. syzygii* and *Ps. vaccini* (Crous *et al.* 1995, this study). However, *Ps. variabilis* can be distinguished by the presence of globose conidia.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Sympodiella acicola* (GenBank KY853468; Identities 370 / 412 (90 %), 17 gaps (4 %)), *Tothia fuscella* (GenBank JF927786; Identities 469 / 528 (89 %), 13 gaps (2 %)) and *T. spartii* (GenBank NR_132917; Identities 399 / 448 (89 %), 10 gaps (2 %)). The highest similarities using the LSU sequence were *Cylindrosympodium lauri* (GenBank EU035414; Identities 820 / 849 (97 %), 6 gaps (0%)), *Cyl. variabile* (GenBank KX228353; Identities 819 / 849 (96 %), 6 gaps (0%)) and *Repetophragma goidanichii* (GenBank DQ408574; Identities 802 / 836 (96 %), 9 gaps (1 %)).

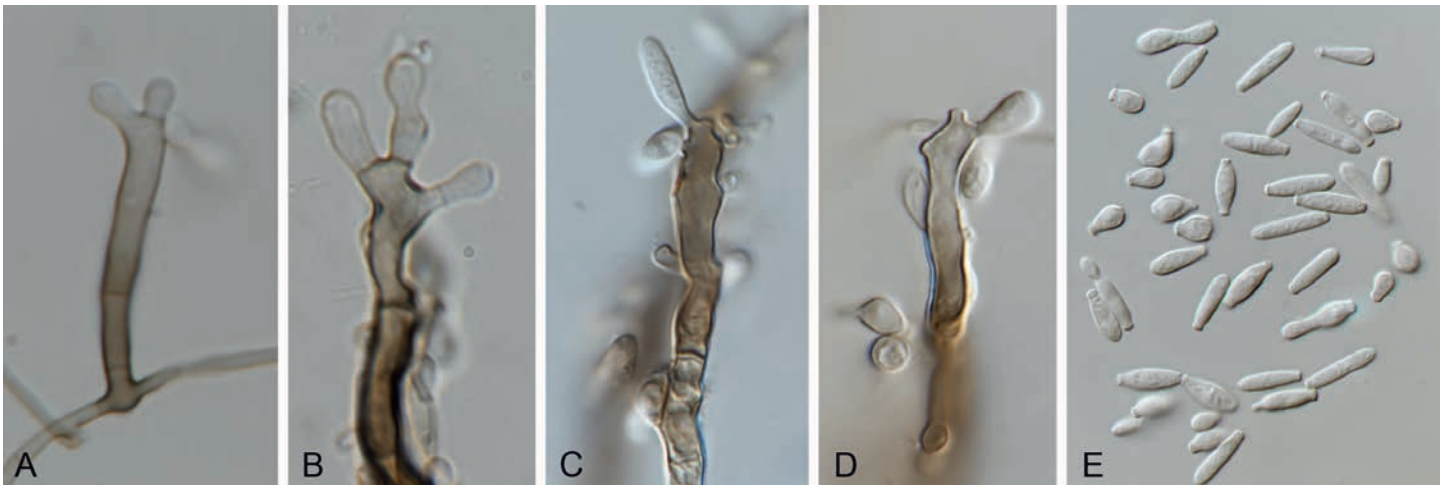

Fig. 36. *Pseudoanungitea variabilis* (CBS 132716). **A.** Conidiophores. **B–D.** Conidiogenous cells. **E.** Conidia. Scale bars = 10 μm.

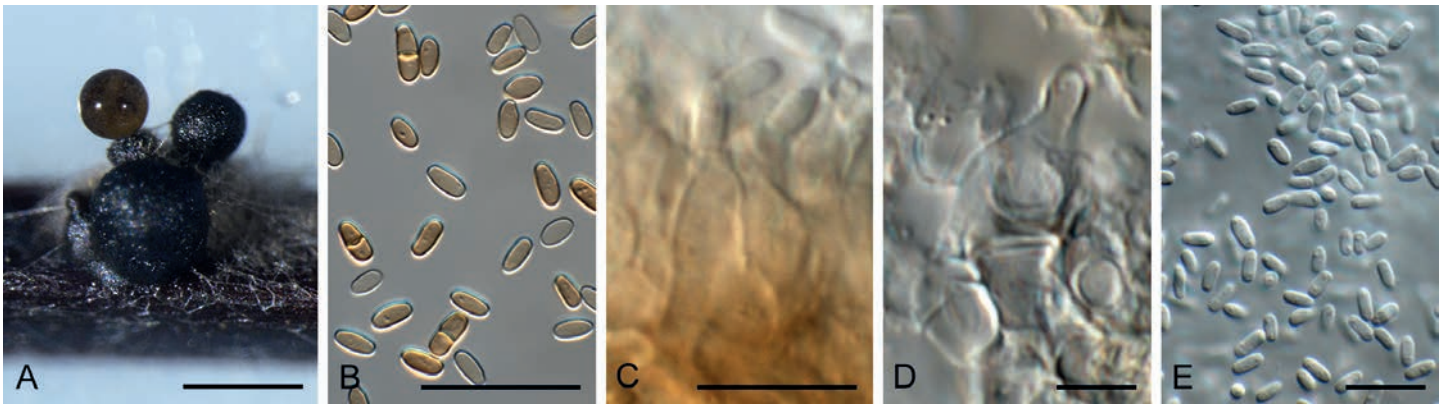

Fig. 37. *Pseudocamarosporium africanum* (CPC 25926). **A.** Conidioma on PNA. **B.** Pigmented macroconidia. **C, D.** Conidiogeous cells. **E.** Microconidia. Scale bars: A = 200 μm, all others = 10 μm.

Pseudocamarosporium africanum (Damm *et al.*) Crous, *Sydowia* **67**: 110. 2015. Fig. 37.
Basionym: *Paraconiothyrium africanum* Damm *et al.*, *Persoonia* **20**: 15. 2008.

Conidiomata separate, pycnidial, brown, erumpent, globose, 150–200 μm diam, with 1–2 ostioles, exuding a brown conidial mass; wall of 3–4 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity, hyaline, smooth, doliiform with periclinal thickening at apex, 4–9 × 3–6 μm. *Conidia* solitary, hyaline, smooth, becoming brown, finely roughened, subcylindrical, apex obtuse, at times slightly clavate, base truncate to bluntly rounded, 0–1-septate, (6–)7–8(–9) × (2.5–)3–3.5(–4) μm. *Spermatogonia* separate or in same conidioma as conidia, globose, brown, up to 150 μm diam, with central ostiole; wall of 3–4 layers of brown *textura angularis*. *Spermatophores* reduced to conidiogenous cells. *Spermatogenous cells* lining the inner cavity, ampulliform to doliiform, hyaline, smooth, 4–6 × 3–4 μm, apex with visible periclinal thickening and minute collarette. *Spermatia* solitary, smooth, hyaline, subcylindrical, straight to slightly curved, apex obtuse, base truncate, 3–5 × 1.5 μm.

Culture characteristics: Colonies spreading, with sparse to moderate aerial mycelium. On MEA surface pale mouse grey, reverse greyish sepia; on PDA surface and reverse fuscous black; on OA surface mouse grey.

Specimen examined: **South Africa**, Western Cape Province, Franschhoek pass, twigs of *Erica* sp. (*Ericaceae*), Nov. 2014, *M.J. Wingfield* (specimen CBS H-23425, culture CPC 25926 = CBS 144204).

Notes: The present collection is phylogenetically identical to *Pseudocamarosporium africanum*. The latter taxon was originally described from branches of *Prunus persica* in South Africa. Morphologically, the two collections are also similar in that conidia of the ex-type strain of *Ps. africana* are 1-septate, rarely 3- or 4-celled, brown and thick-walled, verruculose, (4–) 6.5–9.5(–12) × (2.5–)3–4(–5) μm (Damm *et al*. 2008).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Ps. africanum* (GenBank EU295650; Identities 457 / 457 (100 %), no gaps), *Ps. cotinae* (GenBank KY098789; Identities 475 / 477 (99 %), no gaps) and *Pseudocamarosporium* "sp. 2" (GenBank KY929162; Identities 475 / 477 (99 %), no gaps). The highest similarities using the LSU sequence were *Ps. cotinae* (GenBank KY098790; Identities 835 / 835 (100 %), no gaps), *Pseudocamarosporium* "sp. 2" (GenBank KY929187; Identities 835 / 835 (100 %), no gaps) and *Paracamarosporium* "sp. 1" (GenBank KY929184; Identities 835 / 835 (100 %), no gaps).

Pseudocamarosporium brabeji (Marinc. *et al.*) Crous, *Sydowia* **67**: 110. 2015. Fig. 38.
Basionym: *Camarosporium brabeji* Marinc. *et al*., in Marincowitz *et al.*, *CBS Diversity Ser.* (Utrecht) **7**: 90. 2008.

Conidiomata pycnidial, superficial on PNA, solitary, globose, brown, 200–250 μm diam, with central papillate ostiole up to 100 μm diam. *Peridium* of 3–6 layers of brown *textura angularis*, thick-walled, dark brown. *Conidiophores* reduced to

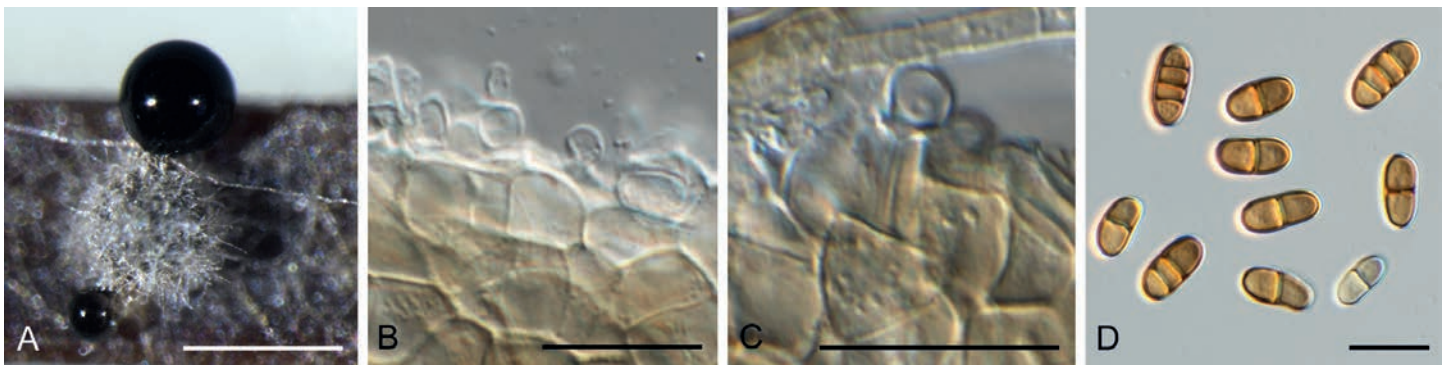

Fig. 38. *Pseudocamarosporium brabeji* (CPC 25002). **A.** Conidioma on PNA. **B, C.** Conidiogenous cells. **D.** Conidia. Scale bars: A = 250 μm, all others = 10 μm.

conidiogenous cells. *Conidiogenous cells* hyaline, smooth, 5–8 × 4–5 µm, ampulliform to doliiform with periclinal thickening at apex. *Conidia* brown, ellipsoid or subcylindrical, (9–)10–12(–13) × (4–)5(–6) µm, 1–3-transversely septate, straight or oblique, smooth to finely roughened, thick-walled.

Culture characteristics: Colonies flat, spreading with moderate aerial mycelium. On MEA surface pale mouse grey, reverse greyish sepia; on PDA surface and reverse fuscous black; on OA surface honey.

Specimens examined: **Switzerland**, on branch of *Platanus* sp. (*Platanaceae*), 24 Jun. 2014, *O. Holdenrieder* (specimen CBS H-23429, culture CPC 25002 = CBS 144205); *ibid*. (CPC 25004, 25843, 27400, 30973, 31482).

Notes: *Pseudocamarosporium* and *Paracamarosporium* were recently introduced to accommodate camarosporium-like taxa that reside in *Didymosphaeriaceae* (Wijayawardene *et al*. 2014). Both genera were also shown to include species with a coniothyrium-like morphology (Crous *et al.* 2015a). *Pseudocamarosporium brabeji* was treated as *Pseudocamarosporium* sp. 2. in Crous & Groenewald (2017).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Ps. tilicola* (GenBank KJ747050; Identities 555 / 555 (100 %), no gaps), *Ps. brabeji* (GenBank EU552105; Identities 578 / 579 (99 %), 1 gap (0 %)), and *Ps. lonicerae* (GenBank KJ747047; Identities 571 / 572 (99 %), 1 gap (0 %)). The highest similarities using the LSU sequence were *Ps. cotinae* (GenBank KY098790; Identities 882 / 882 (100 %), no gaps), *Paracamarosporium* "sp. 1" (GenBank KY929184; Identities 882 / 882 (100 %), no gaps) and *Pa. fagi* (GenBank KY929183; Identities 882 / 882 (100 %), no gaps).

Pseudocercospora breonadiae Crous & Jol. Roux, ***sp. nov.*** MycoBank MB824793. Fig. 39.

Etymology: Name refers to *Breonadia,* the host genus from which this fungus was collected.

Sporulation on the underside of leaves; lesions indistinct, pale to medium brown zones, containing several fungi, with *Pseudocercospora breonadiae* being intermixed with a *Zasmidium* sp., with superficial brown verruculose hyphae, and dark brown, verruculose, solitary, erect to flexuous conidiophores, giving rise to dark brown, verruculose obclavate conidia with thickened, darkened hila. *Mycelium* superficial on host surface, pale brown, smooth, branched, septate, 3–4 µm diam. *Conidiophores* solitary, arising from superficial hyphae, pale brown, smooth, erect, geniculate-sinuous, subcylindrical, 0–3-septate, 10–30 × 3–4 µm. *Conidiogenous cells* integrated, terminal, pale brown, smooth, subcylindrical with several terminal sympodial loci, flat-tipped, not thickened nor darkened, 1–1.5 µm diam, 7–12 × 3–4 µm. *Conidia* solitary, pale brown, smooth, guttulate, mostly gently curved, narrowly obclavate, apex subobtuse, base obconically truncate, 1–2 µm diam, 5–6(–8)-septate, (30–)50–80(–100) × (2.5–)3(–3.5) µm.

Culture characteristics: Colonies erumpent, spreading, surface folded with moderate aerial mycelium and even, lobate margins, reaching 20 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface grey olivaceous, reverse iron-grey.

Specimen examined: **South Africa**, Limpopo Province, Wolkberg, on leaves of *Breonadia microcephala* (*Rubiaceae*), Jan. 2010, *J. Roux* (holotype CBS H-23413, culture ex-type CPC 30153 = CBS 143489).

Notes: No species of *Pseudocercospora* have been described from *Breonadia microcephala*. The closest allied species to *Ps. breonadiae* was *Ps. planaltinensis*, which was described from leaves of a *Chamaecrista* sp. in Brazil (Fig. 40). However, it is morphologically distinct, having cylindrical to obclavate conidia, 1–8-septate, 49–129 × 3–5 µm (Silva *et al*. 2016).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Ps. planaltinensis* (GenBank KT290137; Identities 503 / 505 (99 %), no gaps), *Ps. fuligena* (GenBank GU214675; Identities 539 / 543 (99 %), 1 gap (0 %)) and *Ps. chengtuensis* (GenBank GU214672; Identities 539 / 543 (99 %), 1 gap (0 %)). The highest similarities using the LSU sequence were *Ps. dingleyae* (GenBank KX286997; Identities 840 / 841 (99 %), no gaps), *Ps. proiphydis* (GenBank KM055434; Identities 840 / 841 (99 %), no gaps) and *Ps. airliensis* (GenBank KM055433; Identities 840 / 841 (99 %), no gaps). The highest similarities using the *actA* sequence were *Ps. paraguayensis* (GenBank KF903444; Identities 507 / 521 (97 %), no gaps), *Ps. piricola* (GenBank KY048162; Identities 562 / 578 (97 %), no gaps) and *Ps. flavomarginata* (GenBank JX902134; Identities 522 / 537 (97 %), no gaps). The highest similarities using the *rpb2* sequence were *Ps. neriicola* (GenBank KX462647; Identities 681 / 686 (99 %), no gaps), *Ps. crispans* (GenBank KX462623; Identities 674 / 686 (98 %), no gaps) and *Ps. fukuokaensis* (GenBank KX462632; Identities 672 / 686 (98 %), no gaps). The highest similarities using the *tef1* sequence were *Ps. basiramifera* (GenBank DQ211677; Identities 464 / 518 (90 %), 13 gaps (2 %)), *Ps. parapseudarthriae* (GenBank

Fig. 39. *Pseudocercospora breonadiae* (CBS 143489). **A–C.** Conidiophores. **D.** Conidia. Scale bars = 10 µm.

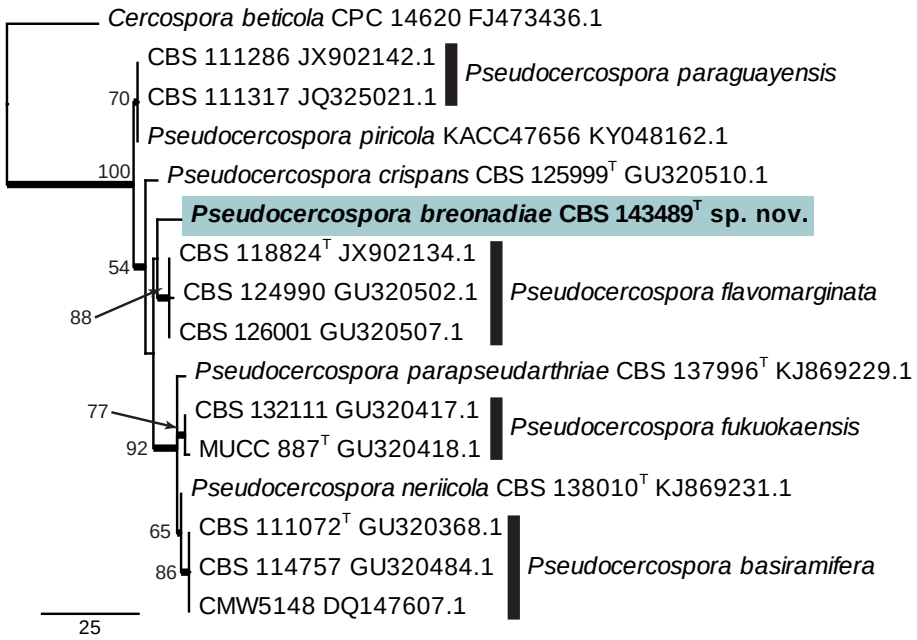

Fig. 40. The first of six equally most parsimonious trees obtained from a phylogenetic analysis of the combined *Pseudocercospora actA* alignment (16 strains including the outgroup; 191 characters analysed: 120 constant, 51 variable and parsimony-uninformative and 20 parsimony-informative). The tree was rooted to *Cercospora beticola* (GenBank FJ473436.1) and the scale bar indicates the number of changes. Bootstrap support values higher than 49 % are shown at the nodes and novelties are highlighted with a coloured box and bold text. Species names are indicated to the right of the tree, or before the culture collection and GenBank accession numbers. A superscript T denotes strains with a type status and branches present in the strict consensus tree are thickened. Tree statistics: TL = 89, CI = 0.899, RI = 0.870, RC = 0.782.

KJ869238; Identities 457 / 511 (89 %), 6 gaps (1 %)) and *Ps. jahnii* (GenBank KM393284; Identities 449 / 509 (88 %), 8 gaps (1 %)).

Rhinocladiella quercus Crous & R.K. Schumach., *Sydowia* **68**: 219. 2016. Fig. 41.

Mycelium consisting of pale brown, smooth, branched, septate, 2–3 μm diam hyphae. *Conidiophores* trimorphic. *Microconidiophores* exophiala-like, reduced to conidiogenous loci on hyphae, phialidic hyphal pegs solitary, 1–2 × 1 μm, giving rise to a mucoid conidial mass. *Macroconidiophores* ramichloridium-like, cylindrical, erect, medium brown, smooth, 1–2-septate, unbranched, straight, 20–30 × 2–3 μm. *Conidiogenous cells* terminal, medium brown, smooth, developing a rachis of pimple-like denticles, 0.5 μm diam, refractive, 11–25 × 2–3 μm. *Conidia* solitary, hyaline, smooth, ellipsoid to cylindrical, straight to slightly curved, (3–)4(–5) × 1.5–2 μm. Cladophialophora-like morph developing at hyphal ends, with cells becoming swollen, ellipsoid, aseptate, and prominently constricted at septa, in branched chains, 4–7 × 3–4 μm.

Culture characteristics: Colonies erumpent, spreading, with moderate aerial mycelium and even, lobate margins, reaching 20 mm diam after 2 wk at 25 °C. On MEA, PDA and OA, surface and reverse olivaceous grey.

Specimen examined: **Germany**, near Berlin, on branch of *Sorbus aucuparia* (*Rosaceae*), 17 Feb. 2016, *R.K. Schumacher* (specimen CBS H-23406, culture CPC 30459 = CBS 143495).

Notes: *Rhinocladiella quercus* was recently described from twigs of *Quercus robur* collected near Berlin in Germany (Hernández-

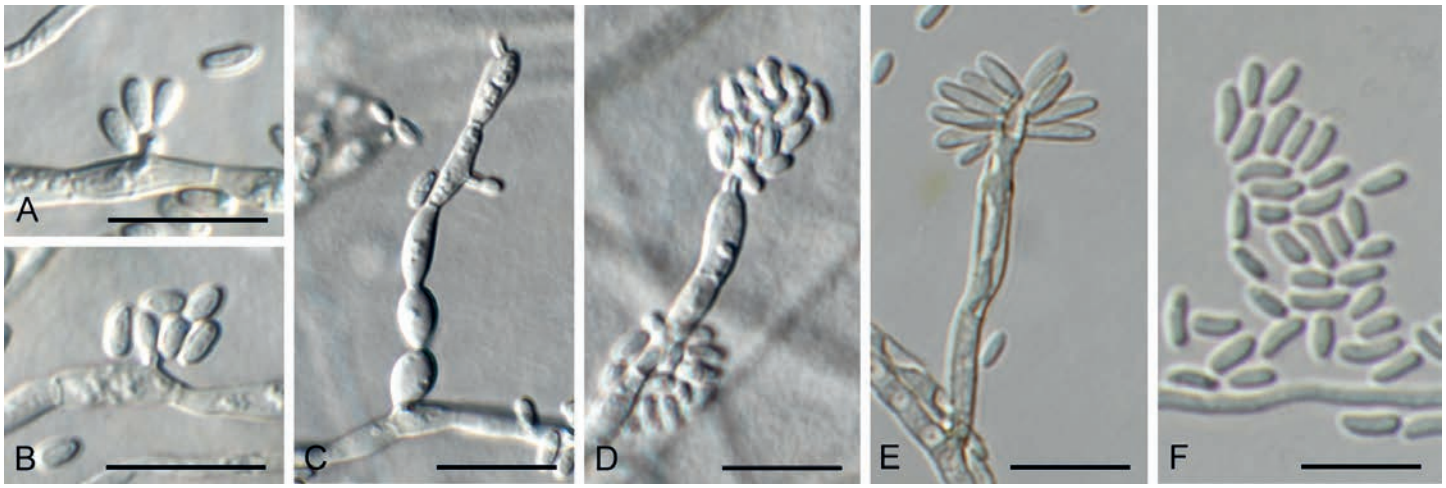

Fig. 41. *Rhinocladiella quercus* (CBS 143495). **A, B.** Conidiogenous loci. **C–E.** Conidiophores. **F.** Conidia. Scale bars = 10 μm.

Restrepo *et al.* 2016). The morphology of the present collection on *Sorbus aucuparia* closely matches that of the type.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *R. quercus* (GenBank KX306769; Identities 633 / 642 (99 %), 1 gap (0 %)), *Capronia* sp. (GenBank AF050240; Identities 613 / 617 (99 %), 1 gap (0 %)) and *Cladophialophora* sp. (GenBank JX494354; Identities 621 / 634 (98 %), 3 gaps (0 %)). The highest similarities using the LSU sequence were *R. quercus* (GenBank KX306794; Identities 792 / 792 (100 %), no gaps), *Capronia* sp. (GenBank JN941378; Identities 799 / 801 (99 %), no gaps) and *Ca. fungicola* (GenBank FJ358224; Identities 768 / 801 (96 %), 3 gaps (0 %)). No significant hits were obtained when the *tef1* and *tub2* sequences were used in a megablast search.

Roussoella euonymi Crous & Akulov, ***sp. nov.*** MycoBank MB824794. Fig. 42.

Etymology: Name refers to *Euonymus,* the host genus from which this fungus was collected.

Conidiomata erumpent, globose, brown, pycnidial, 150–300 µm diam, with central ostiole, exuding a black conidial mass. *Conidiophores* reduced to conidiogenous cells, lining the inner cavity, hyaline, smooth, ampulliform to doliiform, proliferating percurrently at apex, 5–12 × 5–7 µm. *Conidia* solitary, ellipsoid, guttulate, aseptate, apex obtuse, base 2 µm diam, bluntly rounded, thick-walled, becoming warty, golden-brown to red-brown, (6–)7(–8) × (4–)5–6 µm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and even, lobate margins, reaching 60 mm diam after 2 wk at 25 °C. On MEA surface olivaceous grey with patches of pale olivaceous grey, reverse pale olivaceous grey. On PDA surface olivaceous grey, reverse iron-grey. On OA surface iron-grey.

Specimen examined: **Ukraine**, Ternopil region, Zalischyky district, Dniester Canyon, on fallen branches of *Euonymus europaeus* (*Celastraceae*), 14 Oct. 2016, *A. Akulov*, specimen ex CWU (MYC) AS 6061 isotype (holotype CBS H-23420, culture ex-type CPC 31963 = CBS 143426).

Notes: Based on the LSU sequence, *Roussoella euonymi* is accommodated in the *Roussoellaceae*, being similar to other asexual species such as *Ro. solani* and *Ro. mexicana* (Crous *et al.* 2015b, c). Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were numerous unidentified "*Pleosporales* sp." sequences (e.g. GenBank HM116753; Identities 542 / 561 (97 %), 1 gap (0 %)), with the closest known species being *Ro. neopustulans* (GenBank KJ474833; Identities 441 / 474 (93 %), 5 gaps (1 %)) and *Ro. pustulans* (GenBank KJ474830; Identities 442 / 478 (92 %), 4 gaps (0 %)). The highest similarities using the LSU sequence were *Ro. mukdahanensis* (GenBank KU863118; Identities 837 / 847 (99 %), no gaps), *Arthopyrenia salicis* (GenBank LN907499; Identities 843 / 854 (99 %), no gaps) and *Ro. neopustulans* (GenBank KU863119; Identities 839 / 850 (99 %), no gaps). Only distant hits were obtained using the *actA* sequence; some of these were *Stagonosporopsis cucurbitacearum* (GenBank KX246908; Identities 459 / 509 (90 %), 10 gaps (1 %)), *S. citrulli* (GenBank KX246907; Identities 459 / 509 (90 %), 10 gaps (1 %)) and *S. caricae* (GenBank KX246909; Identities 458 / 509 (90 %), 10 gaps (1 %)). Only distant hits were obtained using the *rpb2* sequence; for example with *Torula herbarum* (GenBank KF443393; Identities 582 / 735 (79 %), 6 gaps (0 %)). No significant hits were obtained with the *tub2* sequence.

Setophaeosphaeria citricola Crous & M.J. Wingf., ***sp. nov.*** MycoBank MB824795. Fig. 43.

Etymology: Name refers to *Citrus,* the host genus from which this fungus was collected.

Ascomata on twigs immersed, black, 150–250 µm diam, globose, opening via a central ostiole that could with age become an irregular rupture in ascomatal wall; wall of 2–3 layers of brown *textura angularis. Asci* bitunicate, sessile, subcylindrical to narrowly ellipsoid, apical chamber 1–2 µm diam, stipitate, 50–70 × 11–15 µm. *Ascospores* multiseriate, hyaline, thin-walled, smooth, aseptate, fusoid-ellipsoidal, widest in upper third, apex subobtusely rounded, base obtuse, (16–)19–20(–22) × (4.5–)5(–6) µm. *Conidiomata* pycnidial, 150–250 µm diam, aggregated, globose, pale brown with dark brown central ostiole, 20–30 µm diam, ostiole surrounded by brown, thick-walled, verruculose, septate hyphae, up to 100 µm long, 4–5 µm diam at base, apex obtuse. *Conidiophores* reduced to conidiogenous cells lining the inner cavity, hyaline, smooth, ampulliform to doliiform, 5–7 × 5–6 µm, phialidic with prominent periclinal thickening. *Conidia* solitary, hyaline, smooth, aseptate, multiguttulate and granular, fusoid-ellipsoid, straight to irregularly twisted, apex obtuse, base truncate, 2 µm diam, (10–)12–14(–17) × 3–3.5(–4) µm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and even, lobate margins, reaching 45 mm diam after 2 wk at 25 °C. On MEA surface pale mouse grey to mouse grey, reverse mouse grey. On PDA surface olivaceous grey, reverse mouse grey. On OA surface olivaceous grey.

Specimen examined: **Australia**, New South Wales, Mount Annan Botanical Garden, on leaves of *Citrus australasica* (*Rutaceae*), 25 Nov. 2016, *P.W. Crous* (holotype CBS H-23271, culture ex-type CPC 32083 = CBS 143179).

Notes: *Coniothyrium sidae* was recently described from a *Sida* sp. collected in Brazil (Quaedvlieg *et al*. 2013). Although the ITS is identical (Fig. 44), the morphology is very different, with the sexual morph having hyaline, aseptate ascospores, those of *Con. sidae* being brown, (3–)5-septate, (18–)20–24(–26) × (4–)5(–5.5) µm, and conidia being smaller, fusoid-ellipsoidal, straight to slightly curved, (9–)10–12(–13) × (2.5–)3 µm. Conidia of *S. citri*, described from *Citrus* in Italy, are smaller than those of *S. citricola*, 3.5–5 × 2–3 µm (Crous *et al.* 2017b).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Con. sidae* (GenBank KF251149; Identities 518 / 520 (99 %), no gaps), *Phaeosphaeria setosa* (GenBank AF439500; Identities 472 / 476 (99 %), no gaps) and *S. hemerocallidis* (GenBank KJ869161; Identities 503 / 521 (97 %), 10 gaps (1 %)). The highest similarities using the LSU sequence were *Con. sidae* (GenBank KF251653; Identities 835 / 836 (99 %), no gaps), *S. badalingensis* (GenBank KJ869219; Identities 828 / 832 (99 %), no gaps) and *Leptosphaeria rubefaciens* (GenBank JF740311; Identities 843 / 854 (99 %), no gaps). The highest similarities

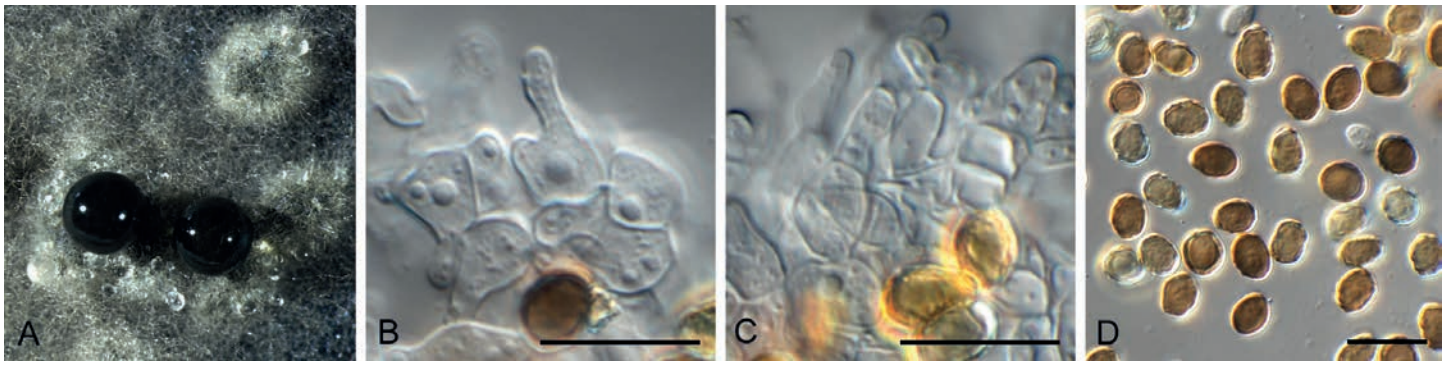

Fig. 42. *Roussoella euonymi* (CBS 143426). **A.** Conidiomata on PDA. **B, C.** Conidiogenous cells. **D.** Conidia. Scale bars = 10 μm.

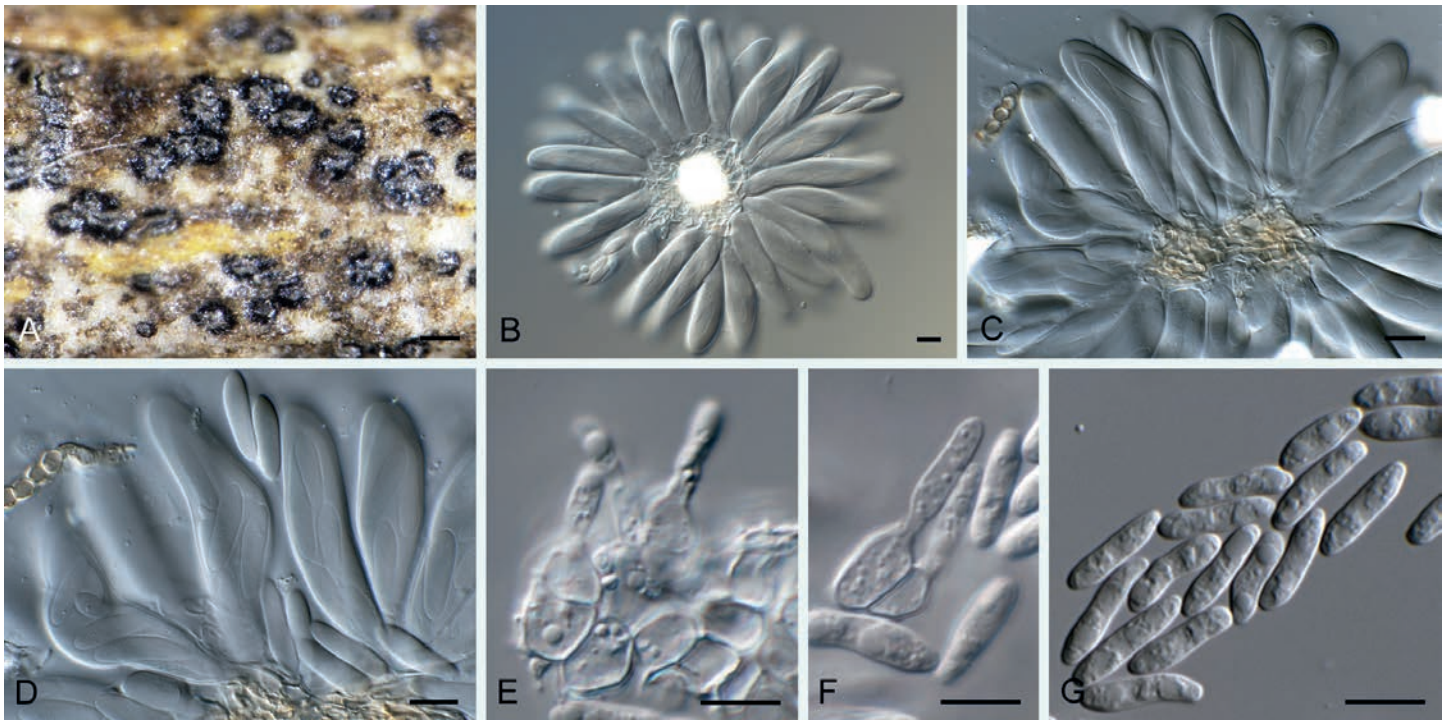

Fig. 43. *Setophaeosphaeria citricola* (CBS 143179). **A.** Ascomata submerged in host tissue. **B–D.** Asci and ascospores. **E, F.** Conidiogenous cells. **G.** Conidia. Scale bars: A = 250 μm, all others = 10 μm.

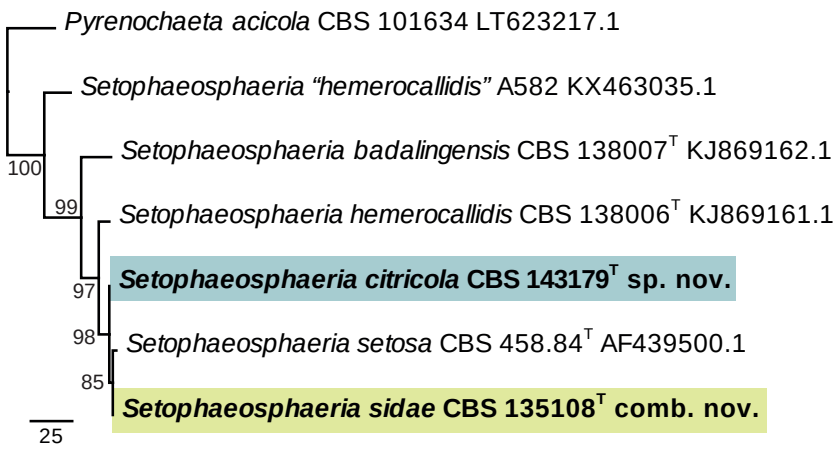

Fig. 44. Single most parsimonious tree obtained from a phylogenetic analysis of the *Setophaeosphaeria* ITS alignment (Seven strains including the outgroup; 487 characters analysed: 389 constant, 64 variable and parsimony-uninformative and 34 parsimony-informative). The tree was rooted to *Pyrenochaeta acicola* (GenBank LT623217.1) and the scale bar indicates the number of changes. Bootstrap support values higher than 49 % are shown at the nodes and novelties are highlighted with a coloured box and bold text. A superscript T denotes strains with a type status. GenBank accession and/or culture collection numbers are indicated behind the species names. Tree statistics: TL = 127, CI = 0.937, RI = 0.830, RC = 0.778.

using the *rpb2* sequence were *Pyrenochaeta unguis-hominis* (GenBank LT717682; Identities 715 / 847 (84 %), no gaps), *Py. cava* (GenBank LT717681; Identities 705 / 847 (83 %), no gaps) and *Py. hakeae* (GenBank KY173593; Identities 705 / 847 (83 %), no gaps). The best hit with the *tef1* sequence was with *Con. sidae* (GenBank KF253109; Identities 439 / 500 (88 %), 21 gaps (4 %)) while the *tub2* sequence was less than 87 % identical to species of *Pyrenochaeta*, *Neocucurbitaria* and *Cucurbitaria*.

Setophaeosphaeria sidae (Quaedvl. *et al.*) Crous, ***comb. nov.*** MycoBank MB824796.
Basionym: *Coniothyrium sidae* Quaedvl. *et al.*, *Stud. Mycol.* **75**: 374. 2013.

Specimen examined: **Brazil**, Rio de Janeiro, Nova Friburgo, Riograndina, along roadside on *Sida* sp. (*Malvaceae*), 24 Feb. 2008, *R.W. Barreto* (holotype CBS H-21315, culture ex-type CPC 19602 = RWB 866 = CBS 135108).

Sirastachys cyperacearum Crous & T.I. Burgess, ***sp. nov.*** MycoBank MB824797. Fig. 45.

Etymology: Name refers to *Cyperaceae*, the substrate from which this fungus was collected.

Conidiophores macro- and mononematous, single or in groups of 2–3, thin-walled, smooth, unbranched, erect, straight to flexuous, 3–4-septate, stipe 70–90 × 3–5 µm, bearing 5–10 conidiogenous cells. *Conidiogenous cells* phialidic, clavate to subclavate, hyaline (to faintly greenish), smooth, 10–12 × 3–5 µm, with collarettes. *Conidia* solitary, aseptate, ellipsoid, thick-walled, dark brown, guttulate, verrucose, (5–)6–7(–8) × (2.5–)3 µm, with rounded ends.

Culture characteristics: Colonies flat, spreading, with sparse to moderate aerial mycelium and even, lobate margins, reaching 25 mm diam after 2 wk at 25 °C. On MEA surface olivaceous grey, reverse olivaceous grey to smoke grey. On PDA surface olivaceous grey, reverse smoke grey. On OA surface iron-grey.

Specimen examined: **Australia**, New South Wales, Fitzroy Falls, on leaves of *Cyperaceae*, 26 Nov. 2016, *P.W. Crous* (holotype CBS H-23308, culture ex-type CPC 32087 = CBS 143444).

Notes: The genus *Sirastachys*, based on *Si. phaeospora*, was recently established by Lombard *et al*. (2016). Phylogenetically the present collection is closely related to *Si*. *phaeospora,* but distinct in that the latter has smaller conidia, 4–5 × 2–3 µm, and shorter conidiophores (40–65 µm long) (Lombard *et al*. 2016).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Si. phaeospora* (GenBank KU846667; Identities 581 / 588 (99 %), 2 gaps (0 %)), *Si. pandanicola* (GenBank KU846664; Identities 555 / 563 (99 %), 3 gaps (0 %)) and *Stachybotrys parvispora* (GenBank JN093263; Identities 543 / 552 (98 %), 2 gaps (0 %)). The highest similarities using the LSU sequence were *Si. phyllophila* (GenBank KU846784; Identities 822 / 827 (99 %), 1 gap (0 %)), *Si. pandanicola* (GenBank KU846777; Identities 820 / 827 (99 %), 1 gap (0 %)) and *Si. phaeospora* (GenBank KU846779; Identities 817 / 827 (99 %), 1 gap (0 %)).

Sphaerellopsis paraphysata Crous & Alfenas, *IMA Fungus* **5**: 411. 2014. Fig. 46.

Conidiomata eustromatic, pycnidioid, 200–300 µm diam, immersed to erumpent, dark brown, multilocular, ostiolate, ostioles 30–40 µm diam; wall of 4–6 layers of medium brown *textura angularis. Conidiophores* reduced to conidiogenous cells, or 1–2-septate, hyaline, smooth, ampulliform to subcylindrical, unbranched, 7–20 × 3–5 µm. *Conidiogenous cells* hyaline, smooth, subcylindrical to ampulliform with percurrent proliferation at apex, 7–13 × 3–5 µm. *Conidia* solitary, hyaline, smooth, guttulate, medianly 1-septate, constricted or not, ellipsoid with mucoid polar appendages, (12–)14–17(–18) × (4–)4.5–5.5(–6) µm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and feathery margin, reaching 15 mm diam after 2 wk. On MEA, PDA and OA surface and reverse olivaceous grey.

Specimen examined: **Australia**, New South Wales, Sussex Inlet, on leaves of *Phragmites* sp. (*Poaceae*), 27 Nov. 2016, *P.W. Crous*, CBS 143579 = CPC 32406.

Notes: *Sphaerellopsis paraphysata* was recently described on a rust on *Pennisetum* sp. collected in Brazil (Trakunyingcharoen *et al.* 2014), and this is the first record of this hyperparasite from Australia.

Fig. 45. *Sirastachys cyperacearum* (CBS 143444). **A.** Conidiophores on SNA. **B–D.** Conidiophores. **E.** Conidia. Scale bars = 10 µm.

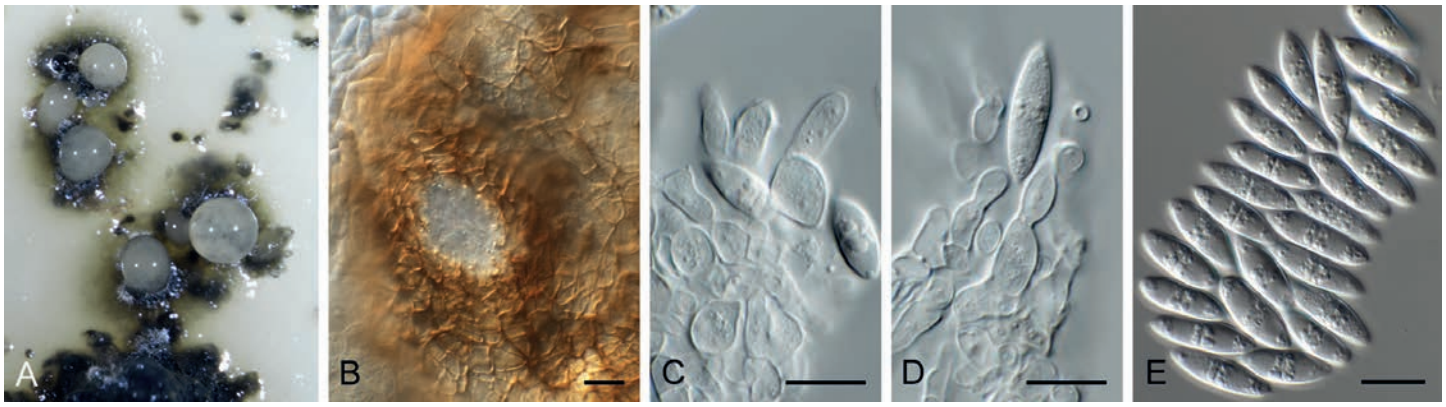

Fig. 46. *Sphaerellopsis paraphysata* (CPC 32406). **A.** Conidiomata on OA. **B.** Ostiolar region of conidioma. **C, D.** Conidiogenous cells. **E.** Conidia. Scale bars = 10 μm.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Sphaerellopsis paraphysata* (GenBank NR_137956; Identities 554 / 561 (99 %), no gaps), *Eudarluca caricis* (GenBank KP170655; Identities 426 / 475 (90 %), 7 gaps (1 %)) and *Subplenodomus drobnjacensis* (GenBank MG131867; Identities 426 / 481 (89 %), 14 gaps (2 %)). The highest similarities using the LSU sequence were *Sphaerellopsis paraphysata* (GenBank KP170729; Identities 840 / 841 (99 %), no gaps), *Plenodomus congestus* (GenBank JF740278; Identities 846 / 855 (99 %), 1 gap (0 %)) and *Con. telephii* (GenBank LN907332; Identities 847 / 857 (99 %), 1 gap (0 %)). The highest similarities using the *rpb2* sequence were *Leptosphaeria biglobosa* (GenBank FO905662; Identities 680 / 868 (78 %), 4 gaps (0 %)), *Curvularia affinis* (GenBank HG779159; Identities 674 / 871 (77 %), 15 gaps (1 %)) and *Plenodomus enteroleucus* (GenBank KY064042; Identities 603 / 770 (78 %), 8 gaps (1 %)). The highest similarity using the *tef1* sequence was *Sp. paraphysata* (GenBank KP170685; Identities 496 / 505 (98 %), 4 gaps (0 %)). The highest similarity using the *tub2* sequence was *Sp. paraphysata* (GenBank KP170710; Identities 300 / 304 (99 %), no gaps).

Subplenodomus iridicola Crous & Denman, ***sp. nov.*** MycoBank MB824798. Fig. 47.

Etymology: Name refers to the fact that the fungus is found on *Iris*.

Fig. 47. *Subplenodomus iridicola* (CBS 143395). **A.** Ascomata on BLA. **B.** Conidioma on SNA. **C–E.** Asci with ascospores. **F.** Paraphyses. **G, H.** Germinating ascospores. **I.** Conidia. Scale bars: A, B = 200 μm, all others = 10 μm.

Leaf spots pale brown with blackish margins, amphigenous, elongated, subcircular, 4–7 mm diam, up to 4 cm long. *Ascomata* immersed, globose, dark brown, 150–250 µm diam, with central ostiole, 20–30 µm diam; wall of 4–6 layers of brown *textura angularis*. *Pseudoparaphyses* intermingled among asci, subcylindrical, hyaline, smooth, hyphae-like, 2–3 µm diam. *Asci* 8-spored, fasciculate, stipitate, bitunicate, narrowly ellipsoid, ocular chamber 1.5–2 µm diam, 80–100 × 10–15 µm. *Ascospores* multiseriate, fusoid-ellipsoid, pale brown, guttulate, finely roughened, constricted at median septum, developing 1(–4) additional septa in both cells, at times first cell above median septum slightly swollen, (19–)21–25(–27) × (5–)6(–7) µm. Germinating ascospores become distorted, up to 8 µm diam, with germ tubes via terminal or intercalary cells. *Conidiomata* pycnidial, globose, pale brown, 100–200 µm diam, with central papillate ostiole, 20–30 µm diam; wall of 4–6 layers of brown *textura angularis. Conidiophores* reduced to conidiogenous cells lining inner cavity, doliiform, hyaline, smooth, phialidic with periclinal thickening, 4–7 × 4–6 µm. *Conidia* solitary, aseptate, hyaline, smooth, guttulate, subcylindrical to narrowly ellipsoid, apex obtuse, base truncate, (4–)5–6(–7) × (2.5–)3 µm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and smooth, lobate margins, reaching 50 mm diam after 2 wk at 25 °C. On MEA surface ochreous, reverse amber. On PDA surface and reverse isabelline. On OA surface rosy buff with patches of isabelline and cinnamon.

Specimen examined: **UK**, England, Upton Grey, on *Iris* sp. (*Iridaceae*), 28 Mar. 2016, *P.W. Crous* (holotype CBS H-23415, culture ex-type CPC 30162 = CBS 143395).

Notes: *Subplenodomus* was established by de Gruyter *et al.* (2013) for *Su. violicola*. Phylogenetically *Su. iridicola* is closely related to *Su. galicola*, but distinct in that the latter (described from a dead stem of *Galium* sp. collected in Italy) has larger ascospores [30–40 × 6–9 µm, (3–)4-septate] and asci (66–120 × 12–17 µm) (Tibpromma *et al*. 2017).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Alloleptosphaeria italica* (GenBank KT454722; Identities 428 / 458 (93 %), 6 gaps (1 %)), *Subplenodomus galicola* (GenBank KY554204; Identities 505 / 576 (88 %), 23 gaps (3 %)) and *Leptosphaeria rubefaciens* (GenBank KT804116; Identities 448 / 495 (91 %), 12 gaps (2 %)). The highest similarities using the LSU sequence were *Su. galicola* (GenBank KY554199; Identities 848 / 854 (99 %), no gaps), *Su. violicola* (GenBank GU238156; Identities XXX / 848 / 854 (99 %), no gaps) and *Plenodomus deqinensis* (GenBank KY064031; Identities 843 / 849 (99 %), no gaps).

Teichospora quercus Crous & R.K. Schumach., ***sp. nov.*** MycoBank MB824799. Fig. 48.

Etymology: Name refers to *Quercus,* the host genus from which this fungus was collected.

Ascomata solitary to gregarious, semi-immersed, becoming erumpent, dark brown, uniloculate, globose with papillate ostiole, 200–350 µm diam; peridium thick-walled, multi-layered, of *textura angularis*, brown, becoming hyaline inwards. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate, short-stipitate, rounded at apex, with ocular chamber, 60–110 × 10–15 µm. *Ascospores* tri- to multiseriate, hyaline, fusiform or ellipsoid-fusoid, straight, widest just above median septum, (1–)3-septate, but becoming golden brown, with mucoid sheath (up to 2.5 µm diam), (19–)20–22(–25) × (4–)5(–6) µm. *Pseudoparaphyses* longer than asci, filiform, cells cylindrical, branched, hyaline, thin-walled, smooth, 2–2.5 µm diam. *Conidiomata* globose to subglobose, 150–300 µm diam, with central ostiole, sessile on foot of brown stroma; wall of 6–8 layers of pale brown *textura angularis*, becoming hyaline towards inside. *Conidiophores*

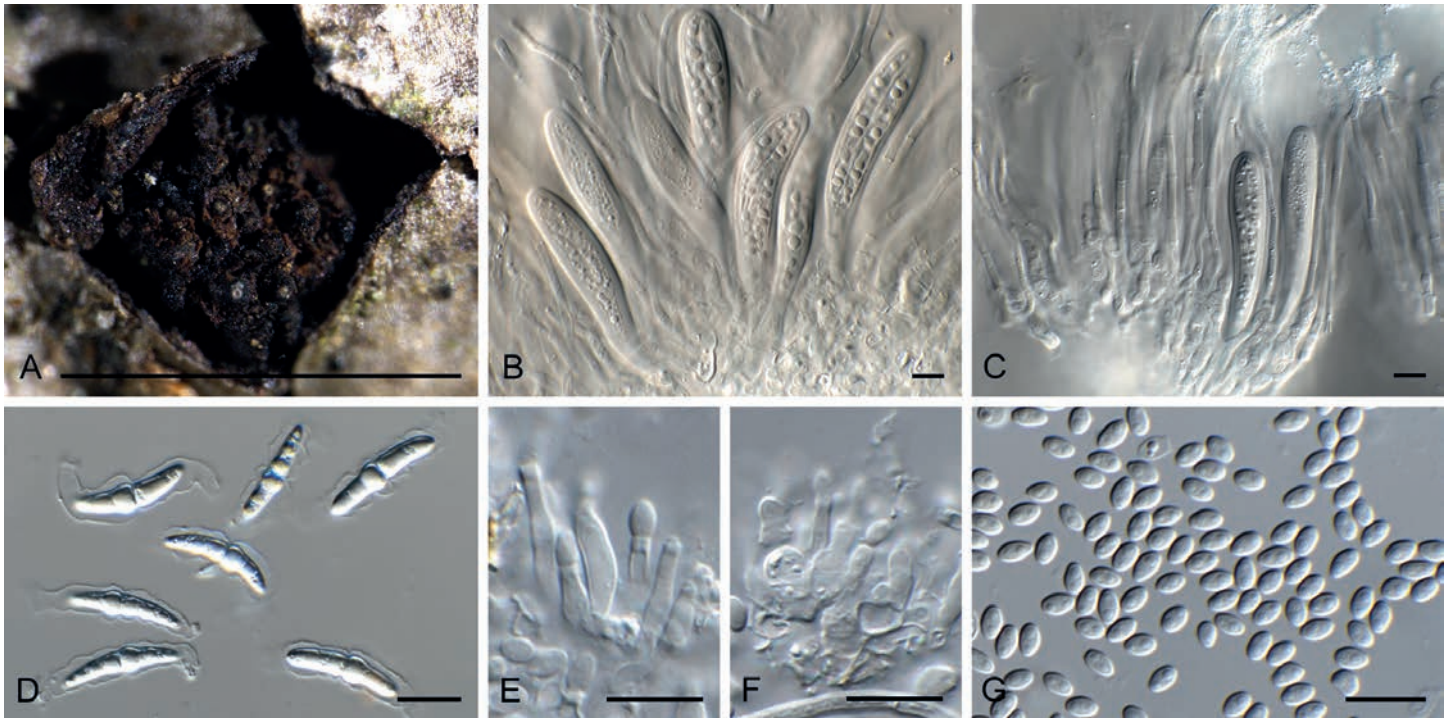

Fig. 48. *Teichospora quercus* (CBS 143396). **A.** Ascoma on host tissue. **B, C.** Asci and pseudoparaphyses. **D.** Ascospores. **E, F.** Conidiogenous cells. **G.** Conidia. Scale bars: A = 350 µm, all others = 10 µm.

subcylindrical, hyaline, smooth, branched at base, 10–20 × 3–5 µm. *Conidiogenous cells* terminal and intercalary, hyaline, smooth, subcylindrical, phialidic with prominent percurrent proliferation, 5–10 × 2–4 µm. *Conidia* solitary, ellipsoid, hyaline, smooth, guttulate, apex obtuse, base truncate, 1.5–2 µm diam, (4–)5(–6) × (2.5–)3 µm.

Culture characteristics: Colonies erumpent, spreading, with moderate aerial mycelium and smooth, lobate margins, reaching 30 mm diam after 2 wk at 25 °C. On MEA surface smoke grey in centre, olivaceous grey in outer zone, reverse olivaceous grey. On PDA surface pale olivaceous grey, reverse smoke grey with diffuse sienna pigment. On OA surface pale olivaceous grey.

Specimen examined: **France**, Cléron, on stroma of pyrenomycete, on branch of *Quercus* sp. (*Fagaceae*), 15 Nov. 2015, *G. Moyne* (holotype CBS H-23404, culture ex-type CPC 30009 = CBS 143396).

Notes: The genus *Teichospora* was treated in detail by Jaklitsch & Voglmayr (2016) and includes several generic synonyms. Although the present collection was initially assumed to represent a new genus, it clusters phylogenetically with other species of *Teichospora*. It is, however, morphologically distinct, in that the ascospores remain hyaline, and are surrounded by a mucoid sheath, and are only 1(–3) transversely septate. The asexual morph, however, is phoma-like, which again resembles those of *Teichospora*. Nevertheless, if additional gene loci eventually show this clade to represent more than one genus, *T. quercus* will most likely be placed in a separate genus.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *T. rubriostiolata* (GenBank KU601590; Identities 562 / 589 (95 %), 4 gaps (0 %)), *T. melanommoides* (GenBank KU601585; Identities 561 / 590 (95 %), 7 gaps (1 %)) and *T. acaciae* (GenBank NR_138410; Identities 559 / 591 (95 %), 8 gaps (1 %)). The highest similarities using the LSU sequence were *T. parva* (GenBank GU385195; Identities 851 / 854 (99 %), no gaps), *T. acaciae* (GenBank KR611898; Identities 800 / 810 (99 %), no gaps) and *T. melanommoides* (GenBank KU601585; Identities 841 / 852 (99 %), no gaps). The highest similarities using the *rpb2* sequence were *T. rubriostiolata* (GenBank KU601596; Identities 745 / 829 (90 %), no gaps), *T. trabicola* (GenBank KU601600; Identities 732 / 830 (88 %), no gaps) and *Melanomma radicans* (GenBank AY485625; Identities 701 / 826 (85 %), no gaps). The highest similarities using the *tef1* sequence were *T. trabicola* (GenBank KU601603; Identities 410 / 477 (86 %), 25 gaps (5 %)), *T. rubriostiolata* (GenBank KU601608; Identities 404 / 471 (86 %), 16 gaps (3 %)) and *T. melanommoides* (GenBank KU601610; Identities 399 / 466 (86 %), 9 gaps (1 %)).

Trochila viburnicola Crous & Denman, ***sp. nov.*** MycoBank MB824800. Fig. 49.

Etymology: Name refers to the fact that the fungus occurs (*icola* = dweller) on stems of *Viburnum*.

Conidiomata pale brown, globose, somewhat flattened, 120–250 µm diam, opening by irregular rupture, becoming acervular; wall of 3–6 layers of pale brown *textura angularis*. *Macroconidiophores* lining inner cavity, hyaline, smooth, subcylindrical, branched, 1–7-septate, 10–40 × 4–6 µm. *Macroconidiogenous cells* integrated, terminal and intercalary, hyaline, smooth, subcylindrical to doliiform, 5–13 × 4–5 µm, with semi-flared collarette, 1–3 µm tall, proliferating percurrently. *Macroconidia* solitary, hyaline, smooth, guttulate, aseptate, subcylindrical, straight, apex obtuse, base truncate, 3–4 µm diam with prominent marginal frill, (5–)6–7 × (3–)4 µm. *Microconidiophores* similar in morphology to *macroconidiophores*, 8–20 × 3–4 µm. *Microconidiogenous cells* terminal and intercalary, subcylindrical to ampulliform, 4–7 × 2.5–3 µm, proliferating percurrently. *Microconidia* similar to macroconidia but smaller, 3–4 × 2–2.5 µm, with minute marginal frill.

Culture characteristics: Colonies flat, spreading, surface folded, with sparse aerial mycelium and smooth, lobate margins, reaching 50 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface pale luteous to ochreous, reverse ochreous.

Specimen examined: **UK**, England, Upton Grey, on twig cankers of *Viburnum* sp. (*Adoxaceae*), 28 Mar. 2016, *P.W. Crous* (holotype CBS H-23416, culture ex-type CPC 30254 = CBS 144206).

Notes: The genus *Sirophoma* is known from *Viburnum*, but is distinct from the present collection in that it has pycnidial conidiomata with central ostioles, long flexuous conidiophores,

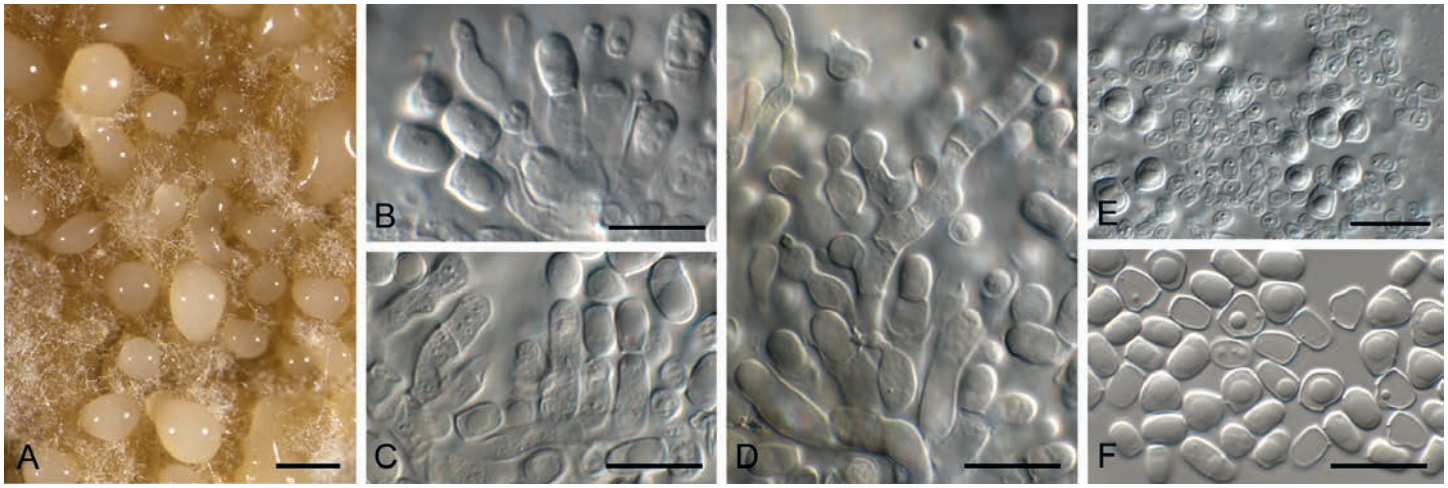

Fig. 49. *Trochila viburnicola* (CPC 30254). **A.** Conidiomata on OA. **B–D.** Conidiogenous cells. **E, F.** Micro- and macroconidia. Scale bars: A = 200 µm, all others = 10 µm.

and globose to pyriform conidia. Based on DNA sequence similarity, the present asexual collection is similar to sequences of the sexual morph *Trochila* (*Dermateaceae*). *Trochila* has been linked to cryptocline-like asexual morphs, and hence it is tentatively placed in this genus. A species of *Trochila* known from *Viburnum* is *T. tini*, but as this species is not known from culture and only the sexual morph is known, a comparison is impossible.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Crumenulopsis sororia* (GenBank KY941133; Identities 437 / 487 (90 %), 7 gaps (1 %)), *Cenangiopsis quercicola* (GenBank LT158425; Identities 506 / 552 (92 %), 4 gaps (0 %)) and *Vestigium trifidum* (GenBank NR_121556; Identities 502 / 551 (91 %), 3 gaps (0 %)). The highest similarities using the LSU sequence were *Vestigium trifidum* (GenBank KC407777; Identities 833 / 860 (97 %), 3 gaps (0 %)), *Fabrella tsugae* (GenBank AF356694; Identities 798 / 824 (97 %), 2 gaps (0 %)) and *Trochila laurocerasi* (GenBank KX090835; Identities 812 / 839 (97 %), no gaps). The highest similarities using the *rpb2* sequence were *Hyalopeziza nectrioides* (GenBank JN086836; Identities 551 / 689 (80 %), 6 gaps (0 %)), *Chlorencoelia torta* (GenBank JN086854; Identities 618 / 777 (80 %), 6 gaps (0 %)) and *Loramyces macrosporus* (GenBank JN086838; Identities 533 / 671 (79 %), 7 gaps (1 %)). Only distant hits to *Cucurbitaria* and *Trichoderma* were obtained when the *tef1* sequence was used in a megablast search.

Varicosporellopsis aquatilis Lechat & J. Fourn., *Ascomycete.org* **8**(3): 87. 2016. Fig. 50.

On SNA: *Mycelium* consisting of hyaline, branched, septate, smooth, 3–5 μm diam hyphae, lacking chlamydospores, and frequently forming hyphal coils. *Conidiophores* solitary, erect, branched at base, 0–2-septate, or reduced to conidiogenous cells; branched conidiophores consist of a basal stipe, 15–30 × 3–5 μm, giving rise to 1–3 lateral branches, 0–1-septate, or conidiogenous cells, 40–100 × 3–5 μm. *Conidiogenous cells* subcylindrical with slight apical taper, hyaline, smooth, 35–60 × 3–4 μm, apex phialidic with minute cylindrical collarette, 1–2 μm tall, giving rise to clusters of slimy conidia. *Conidia* solitary, hyaline, smooth, granular to guttulate, ellipsoid, aseptate, straight to curved, apex subobtuse, base tapered to a truncate hilum, 1–1.5 μm diam, (6–)11–13(–15) × (3–)4(–4.5) μm.

Culture characteristics: Colonies flat, spreading, aerial mycelium sparse, surface folded, with smooth, lobate margins, reaching 15–25 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface dirty white to pale luteous, reverse luteous to pale luteous.

Specimen examined: **The Netherlands**, Culemborg, from garden soil, Feb. 2017, *H. van Warenburg*, culture JW75003 = CBS 143509.

Notes: Morphologically *Varicosporellopsis aquatilis* resembles *Acremonium curvulum* in having curved, fusoid-ellipsoid conidia with truncate hila. However, it can be distinguished in that it lacks chlamydospores, and has much larger conidia than *A. curvulum* (4–6.7 × 1.4–2.1 μm; Gams 1971), from which it is also phylogenetically distinct. Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *V. aquatilis* (GenBank KU233187; Identities 524 / 530 (99 %), 2 gaps (0 %)), *Fusarium merismoides* var. *violaceum* (GenBank EU860060; Identities 842 / 906 (93 %), 27 gaps (2 %)) and *Thyronectria asturiensis* (GenBank KJ570690; Identities 854 / 919 (93 %), 25 gaps (2 %)). The highest similarities using the LSU sequence were *V. aquatilis* (GenBank KU233189; Identities 835 / 836 (99 %), no gaps), *Paracremonium variiforme* (GenBank KU746739; Identities 823 / 836 (98 %), no gaps) and *Pa. contagium* (GenBank KP012631; Identities 790 / 804 (98 %), no gaps). Only distant hits were obtained using the *actA* sequence; some of these were *Verticillium dahliae* (GenBank CP010981; Identities 874 / 973 (90 %), 15 gaps (1 %)), *Fusarium oxysporum* f. sp. *dianthi* (GenBank LT841228; Identities 870 / 977 (89 %), 17 gaps (1 %)) and *Fusarium graminearum* (GenBank HG970335; Identities 872 / 979 (89 %), 21 gaps (2 %)). The highest similarities using the *tub2* sequence were *Pa. inflatum* (GenBank KM232101; Identities 528 / 583 (91 %), 9 gaps (1 %)), *Pa. contagium* (GenBank KM232103; Identities 532 / 599 (89 %), 9 gaps (1 %)) and *Pa. pembeum* (GenBank KU053055; Identities 438 / 500 (88 %), 9 gaps (1 %)).

Varicosporellopsis aquatilis was recently described from submerged wood collected in freshwater in southwestern France. The acremonium-like asexual morph is morphologically similar, but its conidia are somewhat smaller, 6–11 × 2.8–3.2 μm (Lechat & Fournier 2016).

Vermiculariopsiella dichapetali Crous, *Persoonia* **32**: 213. 2014. Fig. 51.

Conidiomata sporodochial, on SNA erumpent, crystalline, up to 500 μm diam, with brown, erect setae distributed throughout conidioma, thick-walled, flexuous, finely roughened, 180–500 × 4–10 μm, 6–20-septate, with obtuse ends. *Conidiophores* aggregated in stroma, subcylindrical, 2–4-septate, branched below, 35–70 × 3–4 μm. *Conidiogenous cells* phialidic, terminal, cylindrical with curved apex, pale brown, smooth to finely roughened, 12–26 × 2.5–3 μm, apex 1.5–2 μm diam. *Conidia* solitary, hyaline, smooth, guttulate, aseptate, straight to slightly curved, inequilateral, outer plane convex, apex subobtusely rounded, with truncate hilum, excentric, 1 μm diam, (14–)16–19(–21) × 2.5(–3) μm.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and even, lobate margins, covering the dish after 2 wk at 25 °C. On MEA, PDA and OA surface and reverse pale luteous to ochreous.

Specimens examined: **Australia**, New South Wales, Barron Grounds Nature Reserve, on leaves of *Melaleuca* sp. (*Myrtaceae*), 26 Nov. 2016, *P.W. Crous* (culture CPC 32057 = CBS 143424); Victoria, La Trobe State Forest, on leaves of *Eucalyptus regnans* (*Myrtaceae*), 30 Nov. 2016, *P.W. Crous* (CBS H-23312, culture CPC 32544 = CBS 143440).

Notes: *Vermiculariopsiella dichapetali* was described from leaves of *Dichapetalum rhodesicum* collected in Botswana (Crous *et al*. 2014), and these are the first records from Australia. Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Ve. dichapetali* (GenBank KX306771; Identities 532 / 534 (99 %), no gaps), *Ve.immersa* (GenBank KY853476; Identities 532 / 534 (99 %), 2 gaps (0 %)) and *Ve. acaciae* (GenBank NR_145253; Identities 520 / 536 (97 %), 7 gaps (1 %)). The highest similarities using the LSU sequence were *Ve. dichapetali* (GenBank KX306796; Identities 716 / 716 (100 %), no gaps), *Ve. acaciae* (GenBank KX228314; Identities 837 / 839 (99 %), no gaps) and *Ve. immersa* (GenBank KJ476961; Identities 822 / 827 (99 %), 4 gaps (0 %)). Only distant

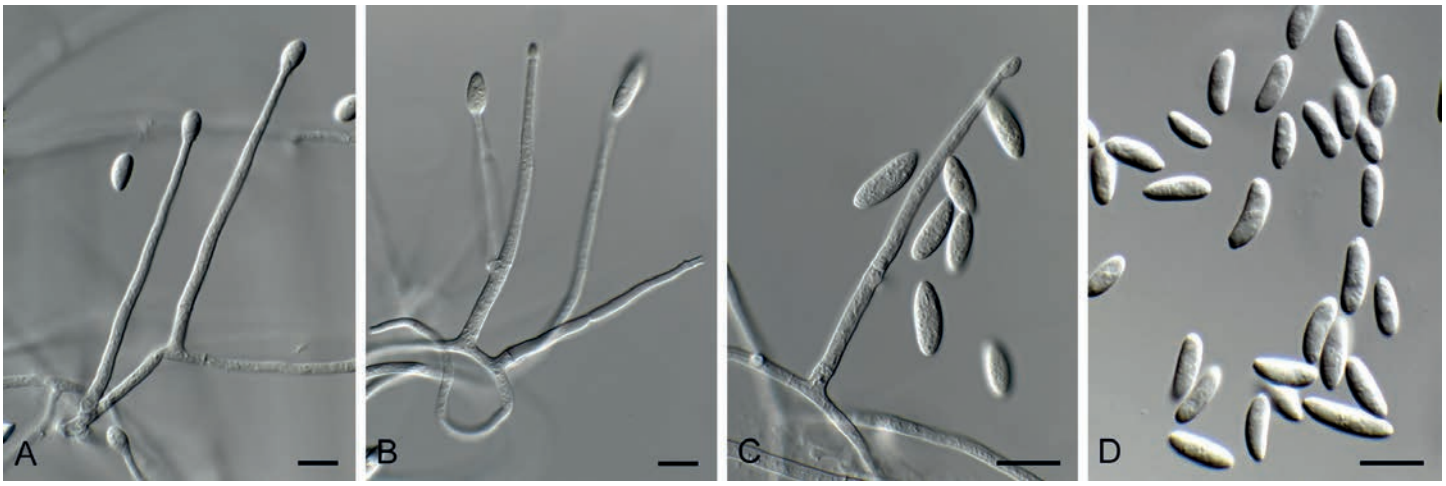

Fig. 50. *Varicosporellopsis aquatilis* (CBS 143509). **A–C.** Conidiophores. **D.** Conidia. Scale bars = 10 µm.

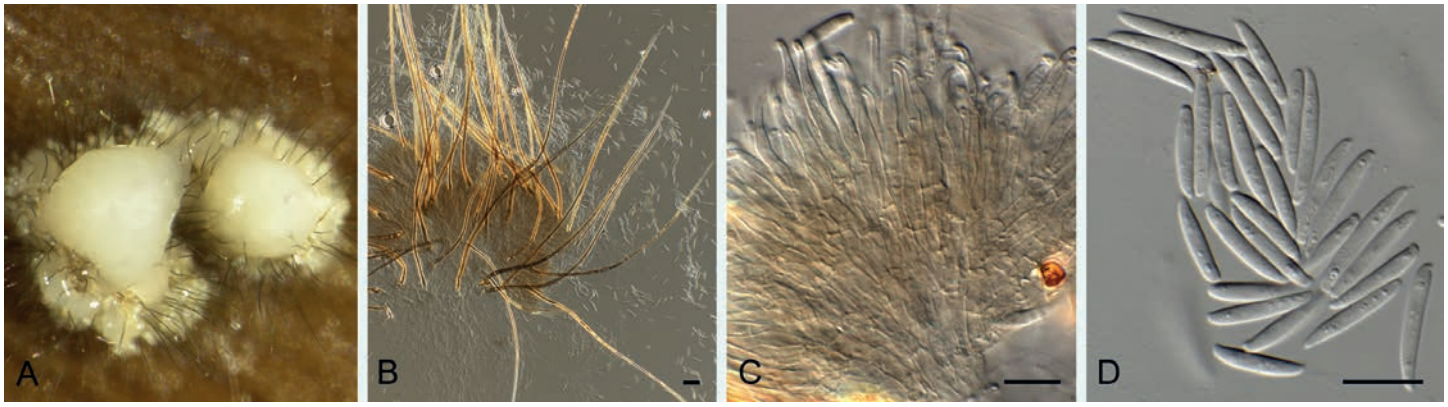

Fig. 51. *Vermiculariopsiella dichapetali* (CPC 32057). **A.** Conidiomata on BLA. **B.** Conidioma with setae on SNA. **C.** Conidiophores. **D.** Conidia. Scale bars = 10 µm.

hits were obtained using the *actA* sequence of CPC 32544; some of these were *Xenogliocladiopsis eucalyptorum* (GenBank KM231140; Identities 390 / 418 (93 %), no gaps), *Allantonectria miltina* (GenBank KM231247; Identities 388 / 418 (93 %), no gaps) and *X. cypellocarpa* (GenBank KM231141; Identities 388 / 418 (93 %), no gaps).

Wettsteinina philadelphi Crous & R.K. Schumach., ***sp. nov.*** MycoBank MB824801. Fig. 52.

Etymology: Name refers to *Philadelphus,* the host genus from which this fungus was collected.

Conidiomata pycnidial, solitary to aggregated, globose, 250–350 µm diam, with central ostiole; wall of 6–8 layers of brown *textura angularis. Conidiophores* reduced to conidiogenous cells lining inner cavity, hyaline, smooth, subcylindrical to ampulliform, 10–15 × 3–5 µm, with numerous, prominent percurrent proliferations in apical region. *Conidia* solitary, medium brown, finely roughened, guttulate, fusoid-ellipsoid, apex obtuse, tapering prominently in lower third to truncate hilum, 1–1.5 µm diam; with (2–)3(–5) transverse eusepta, and 1–3 muriform or vertical septa, (11–)15–20(–23) × (5–)6–7(–8) µm.

Culture characteristics: Colonies erumpent, spreading, with moderate to abundant aerial mycelium and smooth, lobate margins, covering the dish after 2 wk at 25 °C. On MEA surface and reverse smoke grey. On PDA surface and reverse olivaceous grey. On OA surface olivaceous grey.

Specimen examined: **Germany**, near Berlin, on twigs of *Philadelphus coronarius* (*Hydrangeaceae*), 2 Apr. 2016, *R.K. Schumacher* (holotype CBS H-23410, culture ex-type CPC 30534 = CBS 143392).

Notes: The present camarosporium-like collection is described in the genus *Wettsteinina*, although this genus is primarily known from its sexual morphs (Zhang *et al.* 2012), with one reference to a possible stagonospora-like asexual morph (Farr & Rossman 2018).

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Alpinaria rhododendri* (GenBank NR_147686; Identities 505 / 534 (95 %), 10 gaps (1 %)), *Herpotrichia juniperi* (GenBank JX981496; Identities 443 / 472 (94 %), 7 gaps (1 %)) and *He. pinetorum* (GenBank KP966102; Identities 442 / 471 (94 %), 8 gaps (1 %)). The highest similarities using the LSU sequence were *Melanomma pulvis-pyrius* (GenBank LC203344; Identities 847 / 853 (99 %), no gaps), *Trematosphaeria pertusa* (GenBank DQ678072; Identities 847 / 853 (99 %), no gaps) and *Wettsteinina macrotheca* (GenBank AY849969; Identities 838 / 844 (99 %), no gaps). Only distant similarity to *He. juniperi* sequences were obtained with the *tef1* sequence.

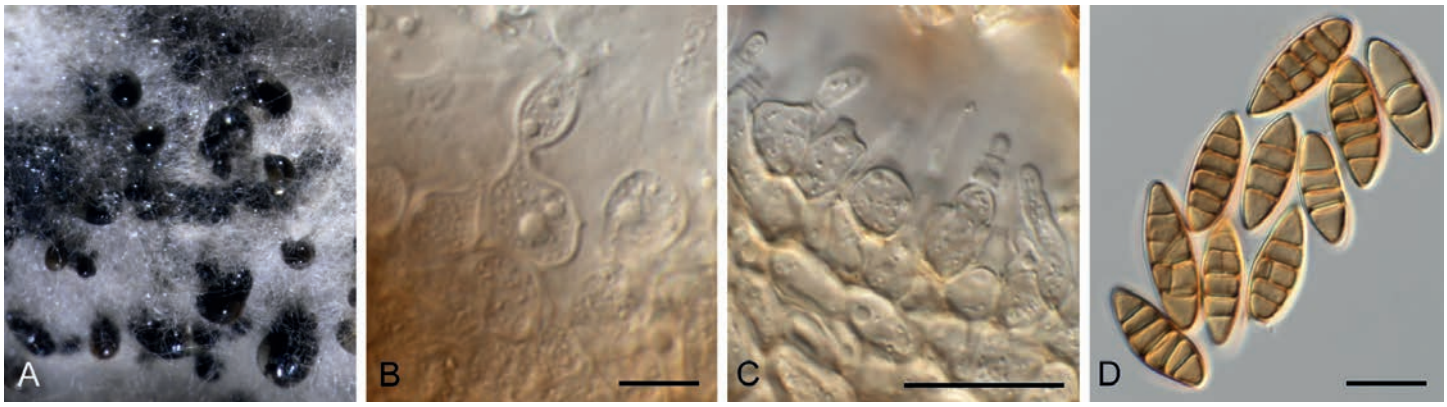

Fig. 52. *Wettsteinina philadelphi* (CPC 30534). **A.** Conidiomata on PDA. **B, C.** Conidiogenous cells. **D.** Conidia. Scale bars = 10 μm.

Xyladictyochaetaceae Crous & Hern.-Restr., ***fam. nov.*** MycoBank MB824802.

Mycelium consisting of medium brown, smooth, septate, branched, hyphae, forming globose, intercalary, brown, smooth, chlamydospore-like structures. *Conidiophores* erect, brown, smooth, subcylindrical, flexuous, multiseptate. *Conidiogenous cells* terminal and intercalary, polyphialidic; phialidic opening lacking flared collarettes. *Conidia* solitary, aggregating in slimy mass, hyaline, smooth, fusoid-ellipsoid, slightly curved, apex subacute, base truncate, medianly 1-septate; each end with flexuous, unbranched appendage.

Type genus: *Xyladictyochaeta* Hern.-Restr. *et al.*

Xyladictyochaeta lusitanica Hern.-Restr. *et al.*, *Stud. Mycol.* **86**: 94. 2017. Fig. 53.

Mycelium consisting of medium brown, smooth, septate, branched, 3–4 μm diam hyphae, that form globose, intercalary, brown, smooth, chlamydospore-like structures, 5–6 μm diam. *Conidiophores* erect, brown, smooth, subcylindrical, flexuous, multiseptate, 60–150 × 3–5 μm. *Conidiogenous cells* terminal and intercalary, polyphialidic, 2–6 × 2–2.5 μm; phialidic opening 1 μm diam, lacking flared collarettes. *Conidia* solitary, aggregating in slimy mass, hyaline, smooth, fusoid-ellipsoid, slightly curved, apex subacute, base truncate, 1 μm diam, medianly 1-septate, (10–) 11–12(–13) × (2.5–)3 μm; each end with flexuous, unbranched appendage, apex central, base excentric, 3–7 μm diam.

Culture characteristics: Colonies flat, spreading, surface folded, with sparse to moderate aerial mycelium and feathery, lobate margins, reaching 20 mm diam after 2 wk at 25 °C. On MEA and PDA surface and reverse amber. On OA surface olivaceous grey.

Specimens examined: **Australia**, New South Wales, Nullica State Forest, on *Eucalyptus* sp. (*Myrtaceae*) leaf litter, 29 Nov. 2016, *P.W. Crous*, (CBS H-23291, culture CPC 32324 = CBS 143502); *ibid*. (CPC 32526).

Notes: The genus *Xyladictyochaeta*, based on *Xy. lusitanica*, was recently described from *Eucalyptus* leaves collected in Portugal (Hernández-Restrepo *et al*. 2017), and this is the first record of this fungus from Australia. *Xyladictyochaeta* represents an undescribed family in *Xylariales*, and *Xyladictyochaetaceae* is introduced to accommodate it. Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Xy. lusitanica* (GenBank KY853479; Identities 571 / 573 (99 %), no gaps), *Anungitea eucalyptigena* (GenBank KY173383; Identities 517 / 578 (89 %), 16 gaps (2 %)) and *Beltraniopsis neolitseae* (GenBank NR_148072; Identities 521 / 583 (89 %), 19 gaps (3 %)). The ITS sequences of CPC 32324 and 32526 differs with three nucleotides (morphologically they are similar, except for prominent differences in conidiophore length). The highest similarities using the LSU sequence were *Xy. lusitanica* (GenBank KY853543; Identities 801 / 801 (100 %), no gaps), *Phlogicylindrium eucalypti* (GenBank DQ923534; Identities 822 / 844 (97 %), no gaps) and *Phl. mokarei* (GenBank KY173521; Identities 796 / 818 (97 %), no gaps). The LSU sequences of CPC 32324 and CPC 32526 are identical. No

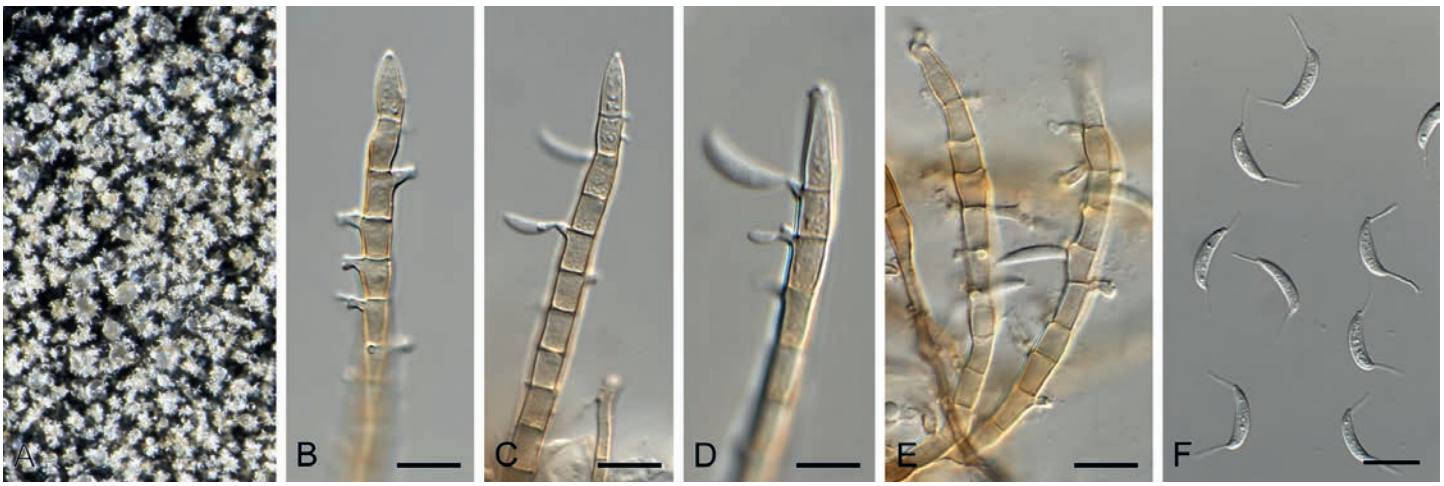

Fig. 53. *Xyladictyochaeta lusitanica* (CPC 32324). **A.** Conidiophores on PDA. **B–E.** Conidiophores on SNA. **F.** Conidia. Scale bars = 10 μm.

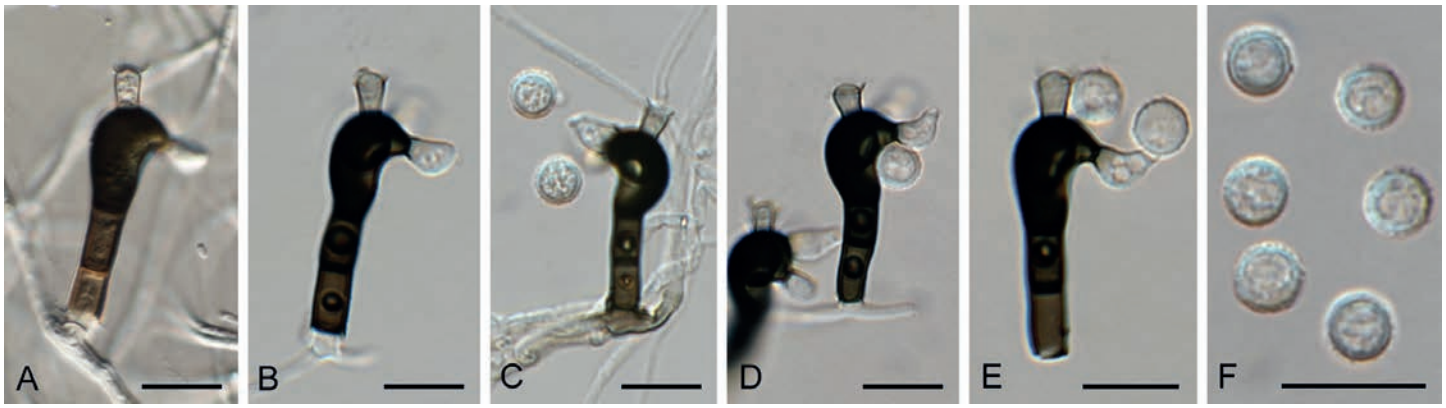

Fig. 54. *Zygosporium pseudogibbum* (CPC 30421). **A–E.** Conidiophores with conidiogenous cells. **F.** Conidia. Scale bars = 10 μm.

significant matches were obtained using the *tef1* sequences and the *tef1* sequences of CPC 32324 and CPC 32526 differ with one nucleotide and a single CA-repeat. No significant matches were obtained using the *tub2* sequence and the *tub2* sequences of CPC 32324 and CPC 32526 are 99 % identical (806 / 813, 1 gap).

Zygosporium pseudogibbum Crous, ***sp. nov.*** MycoBank MB824803. Fig. 54.

Etymology: Name refers to its morphological similarity to *Zygosporium gibbum.*

Conidiophores solitary, erect, consisting of 1–2 pale brown basal cells forming a stipe, 7–15 × 3–4 μm, giving rise to a curved, dark brown terminal vesicle, 11–12 × 6–8 μm. *Conidiogenous cells* arranged in a whorl of 3–4 on a terminal vesicle, hyaline, smooth, reniform, 4–6 × 3–4 μm. *Vesicle* with single apical cell, 4–5 × 3–4 μm, pale brown, cylindrical, with obtuse apex and prominent collarette. *Conidia* solitary, globose, verruculose, faintly olivaceous, 6(–7) μm diam.

Culture characteristics: Colonies flat, spreading, surface folded, with sparse to moderate aerial mycelium and smooth, lobate margins, reaching 50 mm diam after 2 wk at 25 °C. On MEA surface buff to dirty white, reverse luteous. On PDA surface buff to dirty white, reverse saffron. On OA surface buff to dirty white.

Specimen examined: **Malaysia**, Sabah, on leaves of *Eucalyptus pellita* (*Myrtaceae*), Mar. 2016, *M.J. Wingfield* (holotype CBS H-23411, culture ex-type CPC 30421 = CBS 143503).

Notes: Morphologically, the present collection matches the description of *Z. gibbum*, a European taxon (reference isolate, FMR 13130 = CBS 137306; leaf litter Canary Islands; Hernandez-Restrepo *et al*. 2017), which has a wide host range and wide geographical distribution (Ellis 1971). Phylogenetically, however, it clusters sister to this species, and thus a new taxon is introduced to accommodate it. The vesicle gives rise to a single apical cell that appears to be a conidiogenous cell, but could play a different role entirely (moisture droplet, insect dispersal). Active spore dispersal was observed on host tissue, and it could be that conidia on this cell can actively discharge.

Based on a megablast search using the ITS sequence, the closest matches in NCBI's GenBank nucleotide database were *Z. gibbum* (GenBank KY853482; Identities 481 / 504 (95 %), 1 gap (0 %)), *Podosordaria muli* (GenBank JX156377; Identities 462 / 499 (93 %), 14 gaps (2 %)) and *Poronia australiensis* (GenBank KP012826; Identities 384 / 434 (88 %), 21 gaps (4 %)). The highest similarities using the LSU sequence were *Z. gibbum* (GenBank KY853546; Identities 739 / 757 (98 %), 6 gaps (0 %)), *Atrotorquata spartii* (GenBank KP325443; Identities 818 / 846 (97 %), 2 gaps (0 %)) and *Circinotrichum cycadis* (GenBank KJ869178; Identities 804 / 849 (95 %), 6 gaps (0 %)). Only distant hits were obtained using the *actA* sequence; some of these were *Penicillifer pulcher* (GenBank KM231107; Identities 390 / 418 (93 %), no gaps), *Neonectria neomacrospora* (GenBank KM231143; Identities 389 / 418 (93 %), no gaps) and *Cylindrodendrum album* (GenBank KM231152; Identities 387 / 418 (93 %), no gaps). No significant hits were obtained when the *tub2* sequence was used.

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Position specificity in the genus *Coreomyces* (*Laboulbeniomycetes, Ascomycota*)

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Abstract: To study position specificity in the insect-parasitic fungal genus *Coreomyces* (*Laboulbeniaceae, Laboulbeniales*), we sampled corixid hosts (Corixidae, Heteroptera) in southern Scandinavia. We detected *Coreomyces* thalli in five different positions on the hosts. Thalli from the various positions grouped in four distinct clusters in the resulting gene trees, distinctly so in the ITS and LSU of the nuclear ribosomal DNA, less so in the SSU of the nuclear ribosomal DNA and the mitochondrial ribosomal DNA. Thalli from the left side of abdomen grouped in a single cluster, and so did thalli from the ventral right side. Thalli in the mid-ventral position turned out to be a mix of three clades, while thalli growing dorsally grouped with thalli from the left and right abdominal clades. The mid-ventral and dorsal positions were found in male hosts only. The position on the left hemelytron was shared by members from two sister clades. Statistical analyses demonstrate a significant positive correlation between clade and position on the host, but also a weak correlation between host sex and clade membership. These results indicate that sex-of-host specificity may be a non-existent extreme in a continuum, where instead weak preferences for one host sex may turn out to be frequent.

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INTRODUCTION

Members of the *Laboulbeniales* are minute ascomycete fungi that average 0.2 mm in length and seldom exceed 1 mm in length, although some species have been reported to grow beyond 2 mm (Giard 1892, Thaxter 1896, Santamaria 1998). They are obligatory ectoparasites on arthropods tied to their host throughout the entire life cycle, and many species appear to be specific to a single or a few closely related host species (Scheloske 1969, Huldén 1983, Majewski 1994). The order comprises four families with around 140 genera and in excess of 2000 described species (Kirk *et al.* 2008), but some estimates suggest that the true number of species worldwide is in the interval 15 000–75 000 (Weir & Hammond 1997).

Ascospores are assumed to be transmitted between hosts mainly through direct contact during various behavioural interactions, e.g., mating (Richards & Smith 1954, 1955, Whisler 1968, De Kesel 1993, 1995, 1996) or, to a more limited extent, through spore-contaminated host habitat (Arwidsson 1946, Lindroth 1948, Scheloske 1969, De Kesel 1995). Infection experiments in *Herpomyces* have shown that species infecting cockroaches are highly specific to one or a few species in the same genus (Richards & Smith 1954). Only occasionally did weak infections occur in host species distantly related to the main host. De Kesel (1996), on the other hand, demonstrated that *Laboulbenia slackensis*, seemingly strictly host specific in nature, was able to infect a broad range of carabid hosts under artificial conditions. This indicates that the physiological properties of the host do not explain host specificity in *L. slackensis*. The closely related *L. littoralis* is found in the same habitat (coastal marshland) but on a different host belonging to another coleopteran suborder, indicating that the species is restricted by habitat rather than host and that host shifts within the same habitat may result in speciation (De Kesel & Haelewaters 2014). Another example of habitat specificity is *Rickia wasmannii,* which infects several unrelated arthropod hosts sharing the same environment in *Myrmica* ant nests (Pfliegler *et al.* 2016). On the other hand, relatively few nominal species are known to exhibit a broad host range in nature, and some of these may turn out to consist of complexes of distinct species upon closer examination, e.g. with molecular tools (Weir & Blackwell 2005, Haelewaters & De Kesel 2017).

The *Laboulbeniales* are unique among the fungi in displaying "position specificity" (Peyritsch 1875, Thaxter 1896, Benjamin & Shanor 1952, Whisler 1968), a term coined to describe the phenomenon that a nominal parasite species only inhabits a specific, restricted part of its host species' anatomy. Position specificity is also known in a variety of parasites in the animal kingdom, for instance in the flatworm class Monogenea (Littlewood 1997) and among the water mites (Martin 2004). In many cases among the *Laboulbeniales*, however, there is no simple one-to-one relationship between the parasite and its position on the host. It has been observed, for example, that positions may become less unique as time after infection passes and that this could be explained by secondary infections mediated by the behaviour of the host (Whisler 1968). In some cases, the same nominal species has been observed to inhabit different positions in males and females that come into contact during mating (Peyritsch 1875). Thaxter (1896) countered that positions were not as strictly upheld as suggested by Peyritsch

and that mating alone could not fully explain this pattern. An even more extreme hypothesis, "sex-of-host specificity", was advanced by Benjamin & Shanor (1952) and Benjamin (1971), who suggested that each host sex is inhabited by one member of a pair of closely related parasite species, each member often with unique morphological traits and in a unique position on the host. Scheloske (1976) rejected sex-of-host specificity and argued that the different nominal species found on males and females are merely morphotypes of the same species (or exceptionally, a few species). Several examples of di- and polymorphic species have later been described where the authors follow Scheloske (e.g. Rossi & Kotrba 2004, Santamaria & Faille 2009). However, the parasite infects male and female hosts in the same position, which is difficult to explain by mating behaviour only (Majewski 1994, Rossi & Kotrba 2004, Santamaria & Faille 2009). Recent molecular investigations in the genera *Chitinomyces* and *Hesperomyces* suggest that nominal position-specific species more or less correspond to species as independent evolutionary units, that positions on the host may be different between the sexes, and that there may be intraspecific morphological differences correlated with either host sex and/or the position on the host (Goldmann & Weir 2012, Goldman *et al.* 2013).

This study is focused on the genus *Coreomyces* (Fig. 1), in which all members have been claimed to exhibit position specificity (identical between host sexes) but not sex-of-host specificity (Thaxter 1931, Majewski 1994). The genus is known from all continents except Australia (Tavares 1985) and includes 21 nominal and accepted species (MycoBank 2018), all of which parasitize members of the two closely related hemipteran families Corixidae and Micronectidae (Thaxter 1931, Nieser 2002). The host ranges of the *Coreomyces* species

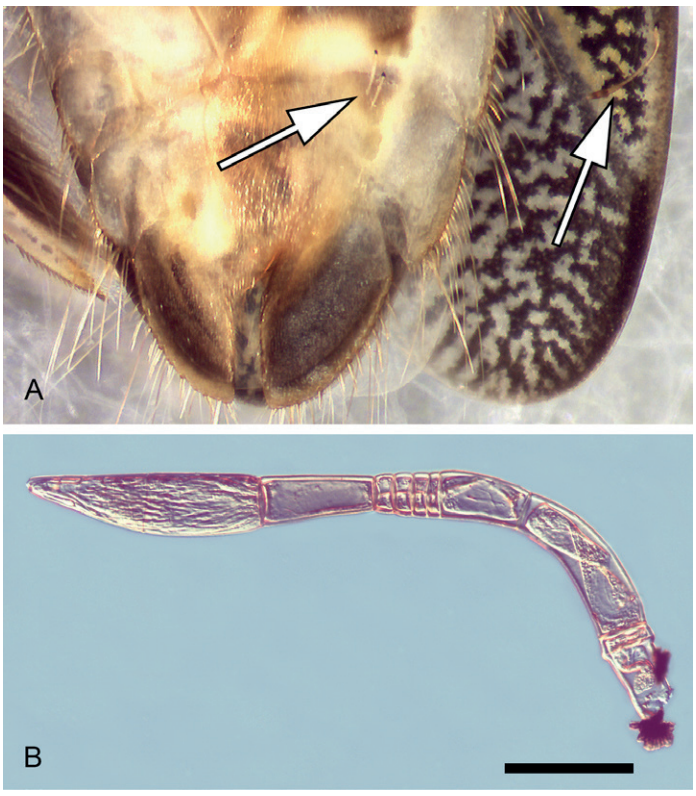

Fig. 1. Examples of *Coreomyces* sp. **A.** Thalli at the ventral side of the abdomen and at the inferior side of the left hemelytral margin of the corixid host. **B.** Detached thallus from the inferior margin of the left hemelytron. Scale bar = 100 µm.

are poorly understood, because claims of host and position specificity are often based on few observations. The most thorough morphological investigations of the genus, focus on the eastern European species (Majewski 1973, 1994, 2003, 2008). Distribution ranges are poorly known and many nominal species are known only from a few sites. Only a handful species have been reported from more than one country and a single one is considered more or less cosmopolitan (Thaxter 1931, Sugiyama & Hayama 1981, Majewski 1988, Santamaria *et al.* 1991, Majewski 1994, Shen *et al.* 2006).

The aims of this study were to test (1) to what extent thalli growing in different positions on their corixid hosts correspond to species as independent evolutionary units in the sense of de Queiroz (2007), and (2) the extent to which species (understood as independent evolutionary units) display position specificity, host specificity, or sex-of-host specificity. Our sampling included corixid populations in southern Sweden and Denmark, and we obtained DNA sequence data from several markers and numerous *Coremomyces* individuals from different host positions, sexes and species. This approach was made possible by recent advances in acquiring DNA sequence data from *Laboulbeniales* (Haelewaters *et al.* 2015, Sundberg *et al.* 2018).

MATERIALS AND METHODS

Sampling

During 2014–2015 we sampled corixids along a route from the province of Uppland in central Sweden to the province of Skåne in the southernmost part of the country, as well as in the Copenhagen area in Denmark. The geographical sampling range spans roughly 300 km in the longitudinal direction and 550 km in the latitudinal. Potential localities, i.e., small ponds, were identified from satellite images (see Table 1 for coordinates). The corixids were captured by sweeping a reinforced colander along the bottom of the pond. Captured animals were killed and preserved in 99.7 % ethanol, which was replaced after a few hours for the best preservation of the fungal DNA. Infected animals with the most developed thalli were sorted out under a dissecting microscope (Olympus SZ1145 TR), placed in 99.7 % ethanol, and then stored at -20 °C.

Thalli were detached from 76 corixid individuals and crushed according to the protocol by Sundberg *et al.* (2018). We used from one (or when poorly developed) up to six thalli from each position. The positions of the thalli were documented with a microscope camera (Moticam 5) connected to the dissecting microscope. After the study, host specimens were deposited at the Museum of Evolution (UPSZ).

Molecular procedures

For DNA amplification we used the Terra PCR Direct Polymerase Mix (Clontech) and KAPA3G Plant PCR Kit (Kapa Biosystems). The PCR settings and primers followed the protocol described by Sundberg *et al*. (2018). PCR products showing clear single bands on an agarose gel were enzymatically cleaned with FastAP Thermosensitive Alkaline Phosphatase combined with Exonuclease I (Thermo Fisher Scientific Inc.). The concentrations and quality of the PCR products were checked on a 2100 Bioanalyzer (Agilent Technologies). We used four molecular markers: the small subunit (nrSSU), internal transcribed spacer

Table 1. *Coreomyces* sp.: position on host, clade affiliation, isolate ID, host species and sex, site of collection with coordinates and GenBank accession numbers for the different molecular markers. CV = midventrally on the abdomen, in male hosts only, LW = inferior side of the exterior margin of the left hemelytron, LV = ventrally on the left side of the abdomen, RV = ventrally on the right side of the abdomen, RD= dorsally on the right side of the abdomen near the margin, in male hosts only. GenBank accession numbers beginning with KY were first published in Sundberg *et al.* (2017).

Position	Clade	Isolate	Host	Host sex	Collection site	WGS84 decimal (lat, lon)	ITS	nrLSU	nrSSU	mtSSU
LV	Green	Hl_1-1	*Sigara lateralis*	female	Sweden, Halland	57.136752, 12.271385	KY293257	KY350525	KY523236	KY523212
LW	Orange	Hl_2-1	*Sigara striata*	male	Sweden, Halland	57.131477, 12.265763	KY293277	KY350551	—	—
LW	Orange	Og_1-1	*Callicorixa praeusta*	male	Sweden, Östergötland	58.393054, 15.579268	—	—	—	MG602616
LV	?	Og_1-2	*Callicorixa praeusta*	male	Sweden, Östergötland	58.393054, 15.579268	—	—	—	MG602617
CV	Red	Sjae_1-1	*Sigara iactans*	male	Denmark, Sjælland	55.706659, 12.508814	MG602605	MG602627	—	MG602615
LW	Blue	Sjae_1-2	*Sigara iactans*	male	Denmark, Sjælland	55.706659, 12.508814	KY293265	KY350540	—	—
LW	Orange	Sjae_2-1	*Sigara striata*	male	Denmark, Sjælland	55.679423, 12.418692	KY293263	KY350533	KY523242	KY523217
LW	Orange	Sjae_3-1	*Sigara iactans*	male	Denmark, Sjælland	55.754983, 12.548575	KY293275	KY350549	—	—
LW	?	Sk_1-1	?	?	Sweden, Skåne	55.726704, 13.185147	—	—	—	MG602618
CV	Orange	Sk_1-10	*Sigara lateralis*	male	Sweden, Skåne	55.726704, 13.185147	KY293283	KY350557	—	—
LW	Orange	Sk_1-2	*Sigara iactans*	male	Sweden, Skåne	55.726704, 13.185147	KY293262	KY350532	KY523241	KY523216
LW	Orange	Sk_1-3	*Sigara lateralis*	female	Sweden, Skåne	55.726704, 13.185147	—	KY350534	KY523243	KY523218
LV	Green	Sk_1-4	*Sigara lateralis*	female	Sweden, Skåne	55.726704, 13.185147	KY293264	KY350535	—	—
LW	Blue	Sk_1-5	*Sigara lateralis*	female	Sweden, Skåne	55.726704, 13.185147	KY293267	KY350542	—	—
LW	Blue	Sk_1-6	*Callicorixa praeusta*	male	Sweden, Skåne	55.726704, 13.185147	KY293268	KY350543	—	—
LW	Orange	Sk_1-7	*Sigara striata*	male	Sweden, Skåne	55.726704, 13.185147	KY293269	KY350544	—	—
LW	Blue	Sk_1-8	*Sigara distincta*	male	Sweden, Skåne	55.726704, 13.185147	KY293270	KY350545	—	—
LW	Blue	Sk_1-9	*Sigara lateralis*	female	Sweden, Skåne	55.726704, 13.185147	KY293271	KY350546	—	—
LW	Blue	Sk_2-1	*Sigara striata* x *dorsalis*?	female	Sweden, Skåne	55.580009, 13.163021	KY293228	KY350499	—	—
LV	Green	Sk_2-2	*Sigara iactans*	female	Sweden, Skåne	55.580009, 13.163021	KY293229	KY350500	—	—
LW	Orange	Sk_2-3	*Sigara iactans*	female	Sweden, Skåne	55.580009, 13.163021	KY293230	—	—	—
LV	Green	Sk_2-4	*Sigara striata*	male	Sweden, Skåne	55.580009, 13.163021	—	—	KY523224	—
RV	Red	Sk_2-5	*Sigara iactans*	male	Sweden, Skåne	55.580009, 13.163021	KY293231	—	—	—
LW	Orange	Sk_2-6	*Sigara striata* x *dorsalis*?	male	Sweden, Skåne	55.580009, 13.163021	—	KY350501	KY523225	—
LV	Green	Sk_2-7	*Sigara striata* x *dorsalis*?	male	Sweden, Skåne	55.580009, 13.163021	KY293233	—	—	—
LW	Blue	Sk_3-1	*Sigara striata*	male	Sweden, Skåne	55.677399, 13.061255	KY293234	KY350503	KY523227	KY523205
LV	Green	Sk_3-2	*Sigara striata*	male	Sweden, Skåne	55.677399, 13.061255	—	KY350504	—	—

Table 1. (Continued).

Position	Clade	Isolate	Host	Host sex	Collection site	WGS84 decimal (lat, lon)	ITS	nrLSU	nrSSU	mtSSU
LV	Green	Sk_3-3	*Sigara striata* x *dorsalis*?	female	Sweden, Skåne	55.677399, 13.061255	KY293235	KY350505	KY523228	—
LV	Green	Sk_4-1	*Sigara lateralis*	female	Sweden, Skåne	55.557086, 14.337586	KY293238	KY350506	—	—
LV	Green	Sk_4-2	*Sigara lateralis*	female	Sweden, Skåne	55.557086, 14.337586	KY293239	KY350507	—	—
LV	Green	Sk_4-3	*Sigara lateralis*	male	Sweden, Skåne	55.557086, 14.337586	KY293240	KY350508	—	—
RD	Green	Sk_4-4	*Sigara lateralis*	male	Sweden, Skåne	55.557086, 14.337586	KY293241	KY350509	KY523229	—
LV	Green	Sk_4-5	*Sigara lateralis*	female	Sweden, Skåne	55.557086, 14.337586	KY293242	KY350510	—	KY523206
RV	Red	Sk_4-6	*Sigara striata* x *dorsalis*?	male	Sweden, Skåne	55.557086, 14.337586	KY293244	KY350511	—	—
LV	Green	Sk_4-7	*Sigara iactans*	female	Sweden, Skåne	55.557086, 14.337586	KY293243	KY350512	—	KY523207
RD	Green	Sk_4-8	*Sigara iactans*	male	Sweden, Skåne	55.557086, 14.337586	KY293245	KY350513	KY523230	KY523208
RD	Red	Sk_4-9	*Sigara iactans*	male	Sweden, Skåne	55.557086, 14.337586	KY293246	KY350514	—	—
LV	Green	Sk_5-1	*Hesperocorixa sahlbergi*	female	Sweden, Skåne	55.585991, 14.157691	—	KY350517	—	—
LW	Blue	Sk_5-2	*Sigara distincta*	male	Sweden, Skåne	55.585991, 14.157691	KY293250	KY350518	KY523232	—
LV	Green	Sk_5-3	*Sigara distincta*	male	Sweden, Skåne	55.585991, 14.157691	KY293251	KY350519	KY523233	KY523209
LW	Blue	Sk_5-4	*Callicorixa praeusta*	female	Sweden, Skåne	55.585991, 14.157691	KY293252	KY350520	KY523234	KY523210
LV	Green	Sk_5-5	*Callicorixa praeusta*	female	Sweden, Skåne	55.585991, 14.157691	KY293253	KY350521	—	—
LW	Blue	Sk_5-6	*Sigara lateralis*	female	Sweden, Skåne	55.585991, 14.157691	KY293254	KY350522	—	—
LW	Blue	Sk_5-7	*Callicorixa praeusta*	male	Sweden, Skåne	55.585991, 14.157691	KY293255	KY350523	KY523235	KY523211
LV	Green	Sk_5-8	*Callicorixa praeusta*	male	Sweden, Skåne	55.585991, 14.157691	KY293256	KY350524	—	—
LV	Green	Sk_6-1	*Sigara lateralis*	female	Sweden, Skåne	55.573821, 14.273054	KY293258	KY350527	KY523237	KY523213
CV	Blue	Sk_6-2	*Sigara lateralis*	male	Sweden, Skåne	55.573821, 14.273054	KY293272	KY350547	—	—
CV	Blue	Sk_6-3	*Sigara lateralis*	male	Sweden, Skåne	55.573821, 14.273054	KY293273	KY350548	—	—
RV	Red	Sk_7-1	*Sigara falleni*	male	Sweden, Skåne	55.57512, 13.208926	KY293259	KY350528	KY523238	KY523214
LW	Blue	Sk_7-2	*Sigara falleni*	male	Sweden, Skåne	55.57512, 13.208926	KY293276	KY350550	—	—
LW	Blue	Sk_7-3	*Sigara falleni*	female	Sweden, Skåne	55.57512, 13.208926	KY293278	KY350552	—	—
LW	Blue	Sk_7-4	*Sigara falleni*	male	Sweden, Skåne	55.57512, 13.208926	KY293282	KY350556	—	—
LW	Blue	Sk_8-1	*Sigara striata* x *dorsalis*?	male	Sweden, Skåne	55.771642, 12.955582	KY293266	KY350541	—	—
RV	Red	Sk_8-2	*Sigara striata*	male	Sweden, Skåne	55.771642, 12.955582	KY293274	—	—	—
LW	Blue	Sk_8-3	*Sigara striata*	male	Sweden, Skåne	55.771642, 12.955582	KY293281	KY350555	—	—

Table 1. (Continued).

Position	Clade	Isolate	Host	Host sex	Collection site	WGS84 decimal (lat, lon)	ITS	nrLSU	nrSSU	mtSSU
RV	Red	Sm_1-1	*Sigara fossarum*	male	Sweden, Småland	57.802609, 14.272442	KY293247	KY350515	_	_
LW	Blue	Sm_1-2	*Sigara fossarum*	female	Sweden, Småland	57.802609, 14.272442	_	_	KY523231	_
RV	Red	Sm_1-3	*Sigara falleni*	male	Sweden, Småland	57.802609, 14.272442	KY293249	KY350516	_	_
RV	Red	Sm_2-1	*Callicorixa praeusta*	female	Sweden, Småland	57.776057, 14.151442	_	KY350526	_	_
LW	Blue	Sm_3-1	*Callicorixa praeusta*	male	Sweden, Småland	57.778705, 14.228196	KY293261	KY350530	KY523240	_
LV	Green	Sm_3-2	*Callicorixa praeusta*	male	Sweden, Småland	57.778705, 14.228196	_	KY350531	_	_
LW	Orange	Upl_1-1	?	?	Sweden, Uppland	59.84386, 17.735076	_	_	_	MG602606
LV	Green	Upl_1-2	*Sigara dorsalis*	male	Sweden, Uppland	59.84386, 17.735076	MG602597	MG602619	MG640370	MG602607
LW	Orange	Upl_1-3	*Paracorixa concinna*	male	Sweden, Uppland	59.84386, 17.735076	MG602604	MG602626	MG640374	_
LW	Blue	Upl_1-4	*Sigara distincta*	male	Sweden, Uppland	59.84386, 17.735076	KY293279	KY350553	_	_
LW	Blue	Upl_1-5	*Sigara distincta*	female	Sweden, Uppland	59.84386, 17.735076	KY293280	KY350554	_	_
LW	Blue	Upl_2-1	*Callicorixa praeusta*	male	Sweden, Uppland	59.867169, 17.71259	MG602598	MG602620	MG640371	MG602608
LW	Blue	Upl_2-10	*Callicorixa praeusta*	male	Sweden, Uppland	59.867169, 17.71259	KY293237	KY350502	_	KY523204
LW	Blue	Upl_2-2	?	?	Sweden, Uppland	59.867169, 17.71259	MG602599	_	_	MG602609
LW	Blue	Upl_2-3	*Callicorixa praeusta*	male	Sweden, Uppland	59.867169, 17.71259	MG602600	MG602621	MG640372	MG602610
LV	Green	Upl_2-4	*Callicorixa praeusta*	male	Sweden, Uppland	59.867169, 17.71259	_	_	_	MG602611
LW	Blue	Upl_2-5	*Callicorixa praeusta*	male	Sweden, Uppland	59.867169, 17.71259	MG602601	MG602622	_	MG602612
LW	Blue	Upl_2-6	*Callicorixa praeusta*	female	Sweden, Uppland	59.867169, 17.71259	_	MG602623	MG640373	MG602613
LW	Blue	Upl_2-7	?	?	Sweden, Uppland	59.867169, 17.71259	MG602602	MG602624	_	MG602614
CV	Red	Upl_2-8	*Sigara iactans*	male	Sweden, Uppland	59.867169, 17.71259	MG602603	MG602625	_	_
LW	Blue	Upl_2-9	*Sigara distincta*	female	Sweden, Uppland	59.867169, 17.71259	KY293236	_	KY523226	_

(ITS) region (including ITS1, 5.8S, and ITS2), the large subunit (nrLSU) of the nuclear ribosomal RNA gene, and the small subunit of the mitochondrial ribosomal RNA gene (mrSSU). Sequencing was carried out by Wyzer Biosciences Inc., Cambridge (MA), and Macrogen Inc., Amsterdam, with the primers ITS4, ITS5, 5.8Shs2, 5.8Shs4, ctb6, LRhs1, LRhs3, LR7 NS4, NShs1, NShs4, NShs2, NShs3, mrSSU1, and mrSSU3R (Vilgalys & Hester 1990, White *et al.* 1990, Zoller *et al.* 1999, Sundberg *et al.* 2018).

Data analysis

Newly produced sequences were edited using Sequencher v. 5.4 and Geneious v. 7.1.9 and aligned together with previously published sequences (Sundberg *et al*. 2018) using MAFFT v. 7.312 (Katoh & Standley 2013). We used the E-INS-i algorithm with the PAM1 matrix for the nrSSU and mrSSU alignments, and the PAM20 matrix for the ITS and nrLSU alignments. The entire alignments were used in downstream analyses, i.e. no data were excluded.

Likelihood model selection as well as maximum likelihood analyses were performed using IQ-TREE v. 1.6 beta 4 (Nguyen *et al.* 2015, Kalyaanamoorthy *et al.* 2017). The best-fitting GTR family model was selected using the Bayesian Information Criterion (BIC) among candidates with one, two, or six substitution rates, with and without gamma-distributed rate heterogeneity among sites and a proportion of invariable sites. For the ITS data, we additionally selected the best-fitting time-irreversible RY Lie Markov model (Sumner *et al*. 2012, Fernández-Sánchez *et al.* 2015, Woodhams *et al*. 2015). Full likelihood optimization was carried out under each candidate model. For each marker, we performed searches for the best trees as well as non-parametric bootstrap analyses with 1 000 replicates under the selected model. The purpose of estimating a tree under a time-irreversible Lie Markov model was to infer the placement of the root. This is not a trivial task in Laboulbeniomycetes, because available DNA sequences are few and sequence divergence within the class is extreme compared to the amount of variation present in our data. The only marker displaying substantial variation in our data, the ITS, is essentially unalignable with any other available sequences. Therefore, we decided to instead use the information contained inside our data for rooting.

Bayesian inference was carried out using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003, Ronquist *et al.* 2012). We used the same best-fitting models as in the maximum likelihood analyses. Priors included a uniform distribution on topology, a uniform (0, 1) distribution on the proportion of invariable sites, a (1, 1) beta distribution on the transition/transversion rate ratio (when applicable), and a (1, 1, 1, 1) Dirichlet on state frequencies (when applicable). We assumed a compound Dirichlet prior on branch lengths (Rannala *et al*. 2012, Zhang *et al*. 2012). The gamma distribution component of this prior was set to $\alpha = 1$ and $\beta = \alpha/(\text{ML tree length})$, whereas the Dirichlet component was set to the default (1, 1). Three parallel Markov chain Monte Carlo (MCMC) runs were performed, each with four parallel chains and the temperature increment parameter set to 0.15 (Altekar *et al*. 2004). The appropriate degree of heating was determined by observing swap rates between the cold and hot chains in preliminary runs. Every 1000th tree was sampled. Runs were diagnosed every 10^6 generations, removing the first 50 % of the tree sample as burnin, and were set to halt automatically when converged before a maximum of 100×10^6 generations. Convergence was defined as an average standard deviation of splits (with frequency ≥ 0.1) across runs ≤ 0.01.

We used mPTP v. 0.2.3 in maximum likelihood mode to delineate species by locating the transition points between inter- and intraspecific processes in the accumulation of substitutions on the individual gene trees (Kapli *et al.* 2017). The original Poisson tree process (PTP) implementation assumes a single rate distribution across the tree for the coalescent process (Zhang *et al.* 2013), whereas the multi-rate Poisson tree process (mPTP) model allows multiple rate distributions, one for each inferred species. We applied both single-rate and multi-rate models to the already rooted Lie Markov model ITS tree and to a rooted version of the nrLSU tree. The nrSSU tree was not included in the analysis, because one potential species was represented by a singleton, whereas mrSSU data were excluded owing to lack of resolution. Likelihood ratio tests were performed to assess whether the multiple-rates model had better fit to the data than a single-rate model.

We performed multinomial logistic regression using the *multinom()* function of R package nnet v. 7.3.12 (Venables & Ripley 2002). Clade membership (four categories based on ITS, nrLSU, and nrSSU phylogenies) was treated as the response variable and position on host (five categories), host sex (male, female) and host genus (four categories) as predictor variables. We set green clade membership, host genus *Sigara*, host sex female, and left ventral position on the host as baseline categories. The *Anova()* function of R package car v. 2.1.6 (Fox & Weisberg 2011) was subsequently used to test the null hypotheses of no effect of each predictor term on the response using type-II chi-square likelihood ratio tests. We also carried out log-linear analysis on the same data using the *loglm()* function of R package MASS v. 7.3.47 (Venables & Ripley 2002). Starting from the saturated model, we carried out stepwise backward regression to find the best model according to the Akaike Information Criterion (AIC). All analyses, including 71 observations without missing data, were carried out with R v. 3.4.3 (R Core Team 2017).

RESULTS

Sampling outcome

Members of the genus *Coreomyces* were found on 10 species of corixids belonging to four genera: *Callicorixa*, *Hesperocorixa*, *Paracorixa*, and *Sigara* (Table 1). Three new host species for *Coreomyces* were noted, *Sigara dorsalis*. *S. fossarum*, and *S. iactans*. Six individuals of *Sigara* showed intermediary traits and were determined as potential hybrids. Among the hosts, 48 were males and 24 females (four individuals were lost during handling and not included in downstream statistical analyses). *Coreomyces* thalli were encountered in five different positions on the corixids (Fig. 2): inferior side of the exterior margin of the hemelytron (LW), ventrally on the left side of the abdomen (LV), ventrally on the right side of the abdomen (RV), dorsally on the right side of the abdomen near the margin (RD), and mid-ventrally on abdomen (CV).

Molecular data

All sequences produced are listed in Table 1 with GenBank accession numbers. Those beginning with KY were published by Sundberg *et al.* (2018). Alignments and resulting gene trees are available from TreeBASE (https://treebase.org) under study number S21919.

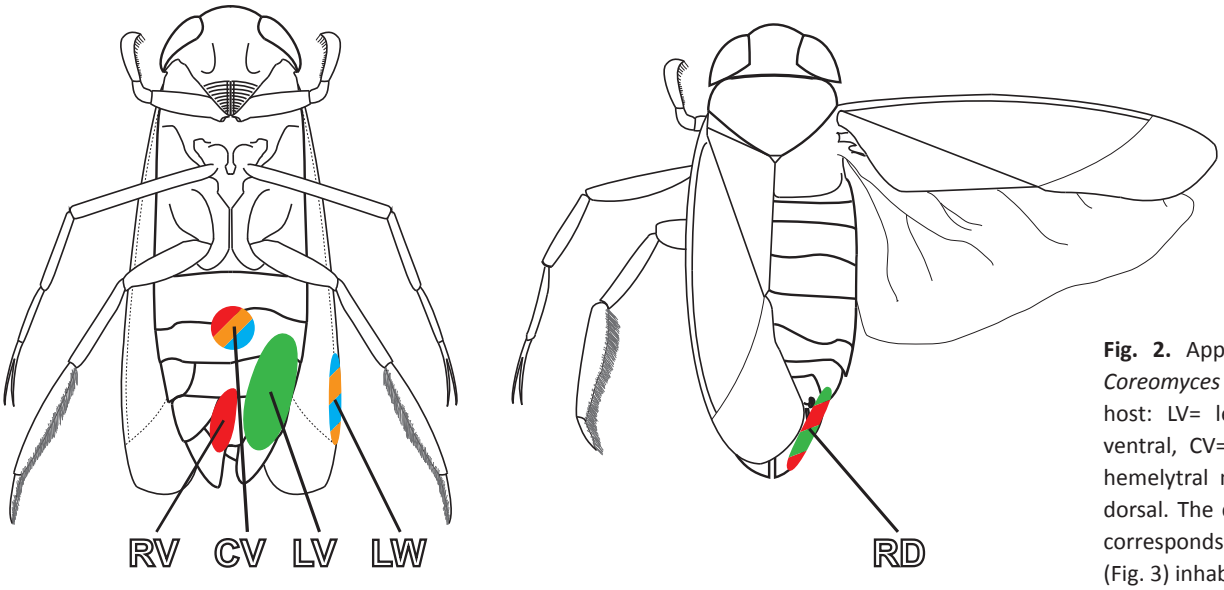

Fig. 2. Approximate positions of *Coreomyces* individuals on the host: LV= left ventral, RV= right ventral, CV= midventral, LW= left hemelytral margin, and RD= right dorsal. The colour of the positions corresponds to the colour of clades (Fig. 3) inhabiting these positions.

The ITS data is comprised of 62 sequences and 1 241 aligned positions, the nrLSU data 63 sequences and 1293 aligned positions, the nrSSU data 24 sequences and 1 059 aligned positions, and the mrSSU 27 sequences and 680 aligned positions. The ITS, nrLSU, nrSSU, and mrSSU data include 248, 21, 5, and 1 variable positions, respectively. The best among the 1-, 2-, and 6-rate GTR family models selected by the BIC were HKY+I for the ITS and nrLSU, and JC+I for the nrSSU. The best time-irreversible RY Lie Markov model selected for the ITS was RY6.7b. The ln likelihood scores of the best trees found by IQ-TREE under a GTR family model were -3363.666, -1953.347, and -1514.566 for the ITS, nrLSU, and nrSSU respectively, whereas the score was -3357.614 for the best ITS tree found under the RY6.7b model. The Bayesian inferences, depending on the marker, stopped after 2–4 × 10^6 generations under the convergence criterion in use. The ln harmonic mean estimations of marginal likelihoods in the Bayesian inference were -3496.416, -2009.554, and -1531.517 for the ITS, nrLSU, and nrSSU, respectively. The individual gene trees, with their corresponding bootstrap branch support and posterior probabilities, are summarized in Fig. 3A–D. Four major clades (green, red, orange, blue) are displayed in colour. The time-irreversible ITS tree in Fig. 3A indicates that the root of the tree is situated on the branch separating the green clade from the rest. This tree, contrary to all other, is rooted and has separate support for the two daughter branches attached to the root node. The relatively low bootstrap support values for these daughter branches compared to the corresponding branch in the unrooted and time-reversible ITS tree in Fig. 3B indicate that there is some uncertainty about the placement of the root. The four clades are monophyletic in the ITS and nrLSU trees (Fig. 3A–C). In the ITS tree, all four groups have strong support (≥ 80 % bootstrap proportions and ≥ 0.95 posterior probabilities), whereas in the nrLSU tree, three branches are strongly supported and the fourth slightly less so (71 %, 0.71). The nrSSU tree (Fig. 3D) provides strong support for two of the branches but weak support for the green clade (note that the red clade has no support as it is represented by a single sequence). Finally, the mrSSU data, consisting of only two haplotypes differing in a single mutation, is represented by a haplotype network in Fig. 3E. This network shows that the orange clade is resolved as a unique haplotype relative to the rest.

Poisson tree process modelling on the ITS and nrLSU trees indicates that the four major clades represent independent species. A model with multiple rates turned out to be worse than a model with a single rate for the ITS and a model with multiple rates was not significantly better than a model with a single rate for the nrLSU (likelihood ratio test, p=1.00). Therefore, we report here the results from the single-rate model.

Multinomial logistic regression rejects the null hypothesis that position on the host has no effect on clade membership ($p < 2 \times 10^{-16}$), whereas there is no indication that host genus or host sex affect clade membership (p = 0.87 and 0.34, respectively). Relative risk ratios (effect sizes), however, indicate that even among the non-significant predictors, some switches among host genera and host sex may confer an increased probability of clade membership: Relative risk ratios for *Sigara*→*Callicorixa* and *Sigara*→*Paracorixa* were 547 and 5 × 10^{13} for switching to the red and orange clade, respectively, whereas male→female was associated with relative risk ratios 1 and 8 × 10^8 for switching to the blue and orange clades, respectively. A model including all three predictors will, using the maximum predicted probability for each observation, classify 83 % of the observations correctly. Removing the only significant predictor, position on the host, results in 44 % correctly classified observations. For comparison, a random classification has a probability of 2×10^{-24} of being 44 % correct (four categories and 71 observations, 31 of which are correctly classified). The log-linear analysis with stepwise backward regression stopped at a model including the four variables plus the interactions between (1) clade and position and (2) host sex and position (chi-square likelihood ratio, p = 1). Taken together, the statistical analyses suggest what is obvious from looking at the phylogenetic trees in Fig. 3: There is a strong correlation between clade membership and the position of the fungus on the corixid host: Thalli in position LW came out as two sister groups (blue and orange), meaning that there seem to be two species occupying the same position (Fig. 2). All samples in position LV group in one clade (green). Position RV thalli group into the red clade. The thalli in position RD came out inside two different clades in the tree (the green and red). Finally, thalli from position CV belong to the red, orange and blue clades.

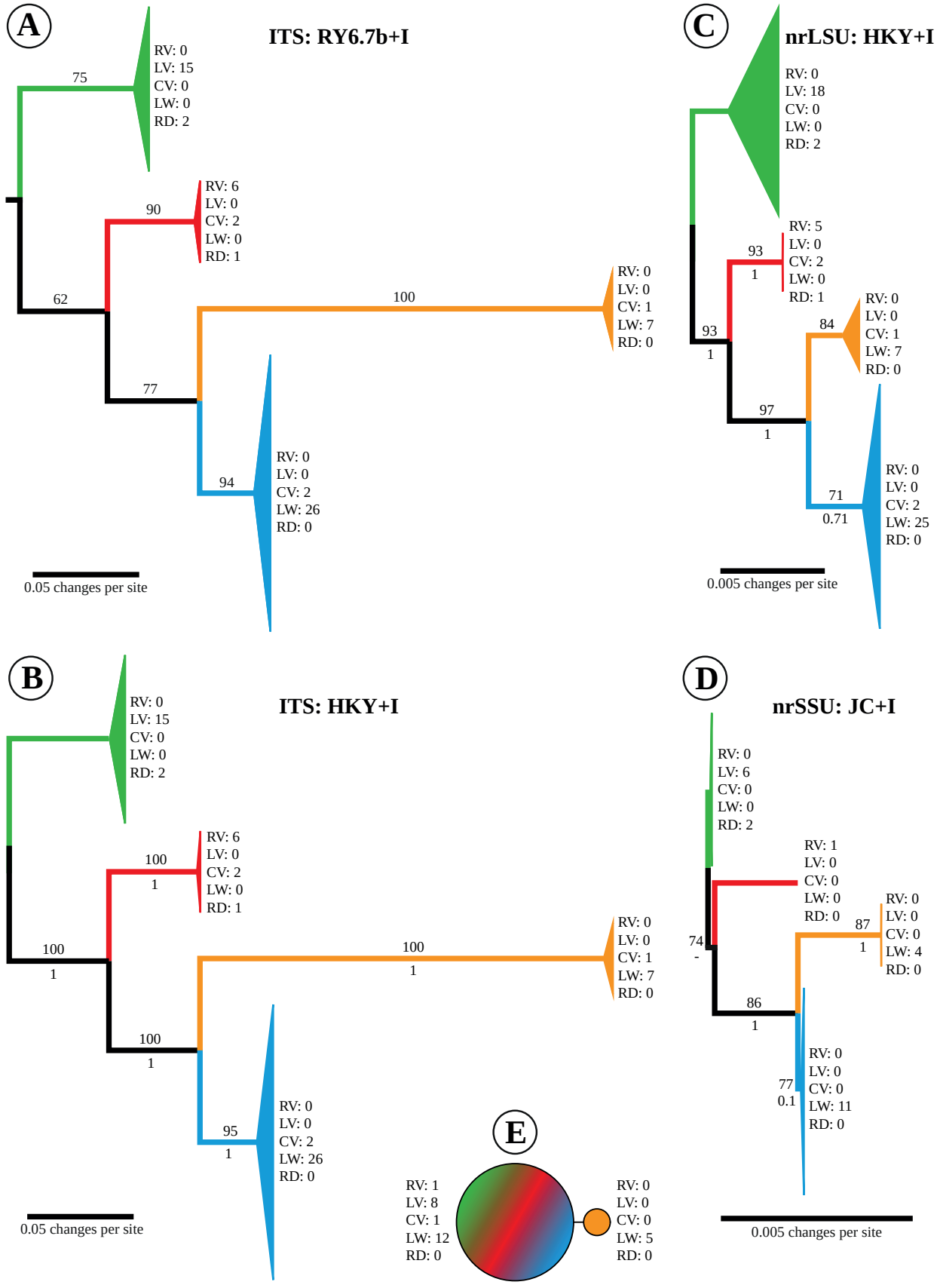

Fig. 3. Inferred phylogenies for each marker based on (A) ITS data under a RY6.7b time-irreversible model; (B) ITS data under a HKY+I model; (C) nrLSU data under a HKY+I model; (D) nrSSU data under a JC+I model. Branch support is indicated (upper: bootstrap proportion in ML analysis, lower: posterior probabilities in the Bayesian inference). Crown groups have been transformed into triangular cartoons, the width of which represents the branch length from the most recent common ancestor to the tip of the highest branch, and the height of which is proportional to the number of included individuals. The mrSSU data is represented by a haplotype network (E), the size of the filled circles being proportional to the number of individuals and a line representing one mutational step. The groups marked here in green, red, orange, and blue are topologically congruent at the level of individual. Clades and haplotypes have been annotated with the number of individuals in the five positions on the hosts (see Fig. 2 for an explanation to abbreviations).

DISCUSSION

Four species of *Coreomyces*

The four major clades (green, red, orange, blue) in Fig. 3 are identified and interpreted here as species, because (1) Poisson tree process modelling on the ITS and nrLSU trees indicates that the clades represent independent species, (2) they are topologically completely congruent across markers (although with different degrees of resolution), and (3) they correlate with ecological parameters, primarily the position on the host.

All species were present in the entire study area, from Uppland in the north to Skåne and Denmark in the south (Table 1). Species were also often mixed in the same host populations, and sometimes co-occurred on the same host individuals. The nomenclatural issues were not the focus of this study and assigning correct names, if available, will have to be the focus of a future research. However, thalli growing in three of the positions (CV, LV, and LW) are previously reported from Europe (Santamaria *et al.* 1991, Majewski 1994, De Kesel & Werbrouck 2008, Majewski 2008). The remaining RD and RV positions have not previously been explicitly reported in the literature. It remains unclear, however, whether they have been observed before, as early descriptions of *Coreomyces* tend to be vague about the exact positions on the host (Thaxter 1902, 1905, Spegazzini 1915, 1917, 1918, Thaxter 1931).

Specificity to host, position and sex

The *Coreomyces* taxa found in this study did not show any strict specificity to host species, as they occurred on five or seven corixid species belonging to two or three different but closely related genera within the subfamily Corixinae. This observation is in accord with available information on host preferences in the genus (e.g., Thaxter 1931, Majewski 1994). For instance, *Coreomyces corisae*, the species with the widest known host range, is known to parasitize a range of species belonging to at least four genera (Spegazzini 1918, Thaxter 1931, Santamaria *et al.* 1991, Majewski 1994, Santamaria 2003). Host ranges may, however, turn out to be narrower once species circumscriptions have been investigated by molecular means. Although we did not observe any strict host specificity, the effect sizes from the multinomial logistic regression suggest that some switches between host genera are associated with substantially increased odds for some of the clade memberships. The total effect of host genus is non-significant, however, and larger samples are needed to possibly detect subtle effects of this parameter.

Contrary to the strict position specificity reported for *Coreomyces* by Majewski (1973, 1994), our results indicate that none of the four species is restricted to only a single position on its host. Instead, each species inhabits two or three different positions, although one of them tends to be much preferred over the other(s). The latter observations explain why both the multinomial logistic regression and the log-linear analysis suggest a strong interaction between position on the host and clade membership. These findings are in agreement with Goldman & Weir (2012) and Goldmann *et al.* (2013). The former study demonstrated the presence of position specificity in *Chitinomyces*, with positions and morphology being different in male and female hosts. The latter investigation pointed to substantial morphological differences between conspecific thalli of *Hesperomyces* in different positions on the host, although it was not clear whether these phenotypes were also correlated with host sex.

All *Coreomyces* species in our study were found on both male and female corixid hosts (Table 1). The log-linear analysis indicates that there is an interaction between host sex and the position on the host, suggesting positions are not entirely identical in males and females. This result is probably explained by individuals in positions CV (orange, blue, and red clades) and RD (red and green clades) being known only from male hosts in our data. Other positions appear to be less unequally distributed between male and female hosts. The multinomial logistic regression did not reject the null hypothesis of no effect of host sex on clade membership, nor did the stepwise log-linear model selection indicate that the interaction between host sex and clade membership is needed to explain the observed data. However, the multinomial logistic regression does suggest that host sex confers increased odds for membership in two of the clades. Sex-of-host specificity may be a non-existent extreme in a continuum, where instead weak preferences for one host sex may turn out to be frequent.

Dispersal and positioning of thalli

In this study we encountered positions present in both host sexes (LW, LV, RV) and those that are seemingly male-specific in our data (CV and RD), which is in agreement with earlier studies of position specificity (Goldmann & Weir 2012, Goldmann *et al*. 2013). These studies demonstrated that occurrences of the same species in the same dorsal position of both females and males is explained by mixed hetero- and homosexual mounting by males. Additional occurrences on the ventral side or on the legs in males are more easily explained, as they represent contact surfaces during heterosexual mounting. In corixid mating, males position themselves on the back of the females, or males in case of homosexual mounting (Popham 1961, Aiken 1982). Peters (1962) provided a detailed description of corixid mating behaviour. After mounting, the male swings his abdomen to the left and forces it under the female abdomen and finally becomes curled around the body of the female. This manoeuvre could account for the LV and LW positions on the left side of the body and possibly also the RV position on the right. The remaining, possibly male-specific positions (CV and RD), are more difficult to explain. An intriguing clue to how this could come about may be found in a peculiarity of the male corixid morphology. They are not bilaterally symmetric, both dextral and sinistral forms existing in many species (Schilthuizen 2013), the former normally being much more common. In sinistral males, the abdomen and the copulatory apparatus are reversed compared to dextral males, and consequently they instead wrap their abdomen around the right side of the female (Peters 1962). It could be speculated that rare occurrence of sinistral males in combination with homosexual mounting may explain the CV and RD positions.

Other kinds of behaviour associated with mating may also affect the exact positioning of the thalli. Jansson (1979), for example, indicated that the copulating animals to some extent change positions when they need to ascend to the water surface to breathe and that this requires some struggling. Agonistic behaviour should also be considered. Candolin (2004) documented males of *Sigara falleni* trying to hinder each other's mounting attempts, while Jansson (1973, 1979) recorded a nudging behaviour among *Arctocorisa* males trying to

outcompete each other. Non-social behaviour like cleaning was suggested by Huldén (1983) to result in the fungus occupying precise positions similar in both sexes. We regard this theory as unlikely, however, as we would then expect the fungus to inhabit the host limbs. This does not rule out that they may be accounted for by some male behaviour that actively transmits thalli from positions that are not sex-specific, as suggested by Benjamin (1971)

Although behaviour may explain occurrence patterns, there is also the possibility of passive infection through the host substrate. This has been suggested as an important mode of dispersal under terrestrial conditions (Arwidsson 1946, Lindroth 1948). In the case of *Laboulbenia slackensis*, however, active transmission was found to be far more important, whereas passive transmissions were independent of soil characteristics and often unsuccessful owing to the short life-span of the spores (De Kesel 1995). In the case of *Coreomyces*, the aquatic environment possibly favours spore longevity and consequently increases the chance of infection. However, the distinctly non-random positions of the thalli on the host indicate that indirect transmission of spores may not be important. Indirect transmission through a contaminated host substrate is also likely to be unfavourable in small host populations and when spore production is modest, as is the case in many *Laboulbeniales* (Huldén 1983).

Concluding remarks

This study has demonstrated that there are four species of *Coreomyces* in our sample, that they prefer but are not strictly specific to certain positions on the host, and that there may or may not be weak preferences on the part of the fungus regarding host sex and genus. These findings lead to further questions of importance to understand the evolution of *Laboulbeniales*: How do species boundaries arise to start with, when host preferences are not discontinuous? How does position specificity arise and how is it related to substrate requirements? How does mating take place in the fungus, if at all? Genomic data as well as controlled observations of host behaviour under laboratory conditions may help solve these and other questions.

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AUTHORS' CONTRIBUTIONS

H.S. did the sampling and laboratory work. S.E. did the statistical and phylogenetic analysis. Figures were made by S.E. and H.S. Species determinations of the host material were made by J.B. The text was written jointly by H.S., Å.K. and S.E. with valuable input from J.B. Å.K. and S.E. supervised the project.

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A worldwide nomenclature revision of sequestrate *Russula* species

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57 new combinations
34 new names
Russulaceae

Abstract: Before the application of molecular techniques, evolutionary relationships between sequestrate genera and their epigeous counterparts in the *Russulaceae* were unclear. Based on overwhelming evidence now available, personal observations, and consideration of the International Code for Nomenclature of Algae, Fungi and Plants, we combine the overlapping sequestrate generic names *Bucholtzia, Cystangium*, *Elasmomyces*, *Gymnomyces*, *Macowanites*, and *Martellia* with the agaricoid genus *Russula*. This nomenclatural action follows precedents set by earlier mycologists and continues an effort to create clarity in our understanding of the evolutionary affiliations among sequestrate fungi - particularly the *Russulaceae*. We also provide the first comprehensive list of described sequestrate species of *Russula*.

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INTRODUCTION

There are many compelling arguments suggesting that sequestrate fruiting habits in fungi resulted from adaptations to abiotic environmental factors and symbiotic associations with vertebrate and invertebrate spore dispersers (Thiers 1984, Cázares & Trappe 1994, Trappe & Claridge 2005, Galante *et al.* 2011, Gehring *et al*. 2014). These evolutionary pressures led to the convergence of fruiting macromorphologies in many fungal lineages; however, early mycologists chose to classify fungi with "gasteroid" or "secotioid" fruiting habits in the obsolete class "*Gasteromycetes*". At the time, it was thought that this class was distantly related to non-sequestrate (agaricoid) fungi. The evolution of truffle-like fungi from non-sequestrate forms was recognized before the availability of molecular tools (Malençon 1931, Shaffer 1968, Heim 1971, Smith 1973, Trappe 1979, Thiers 1984) and later confirmed (Bruns *et al*. 1989, Hibbett *et al.* 1997, Krüger *et al.* 2001, Peintner *et al.* 2001, Binder & Bresinsky 2002, Geml 2004, Lebel *et al.* 2004, Vellinga 2004, Matheny *et al.* 2006, Albee-Scott 2007, Sheedy *et al.* 2016).

The spread of molecular approaches in mycology has taken this evolutionary perspective a step further by combining many sequestrate genera with closely affiliated non-sequestrate/ gymnocarpic genera. For example, in the *Agaricaceae,* sequestrate species of *Amogaster, Cryptolepiota*, *Endoptychum*, *Gyrophragmium*, and *Longula* have been variously described in or transferred to *Agaricus, Chlorophyllum*, *Lepiota*, and *Macrolepiota* (Vellinga 2002, Geml 2004, Geml *et al.* 2004, Lebel & Syme 2012, Ge & Smith 2013, Lebel 2013, Lebel & Vellinga 2013, Kerrigan 2016). In the *Amanitaceae*, *Torrendia* and *Amarrendia* were combined with *Amanita* (Justo *et al*. 2010). In the *Bolbitiaceae*, the sequestrate genus *Descomyces* and some members of the genera *Setchelliogaster* and *Timgrovea* have been combined with *Descolea* (Kuhar *et al.* 2017). In the *Boletaceae*, a new sequestrate species has been described in *Afroboletus*, and some *Gastroboletus* species have been combined with traditionally gymnocarpic genera (Nuhn *et al.* 2013, Wu *et al.* 2016, Han 2017). In the *Cortinariaceae*, *Thaxterogaster* and some species of *Protoglossum* have been combined with or described in *Cortinarius* (Peintner *et al.* 2002, Danks *et al.* 2010, Kuhar *et al.* 2017). In the *Entolomataceae*, the sequestrate genus *Richoniella* has been combined with *Entoloma* (Co-David 2009, Kinoshita *et al.* 2012). In the *Inocybaceae*, several sequestrate species have been placed in *Inocybe* (Braaten *et al.* 2013). In the *Physalacriaceae*, members of the sequestrate genus *Cribbea* have been combined with *Oudemansiella* (Lebel 2017). In the *Psathyrellaceae*, the sequestrate *Psathyrella secotioides* was described (Moreno *et al.* 2015). In the *Strophariaceae*, one species of the sequestrate genus *Nivatogastrium* has been placed in *Pholiota* and two species of the sequestrate genus *Weraroa* have been moved to *Leratiomyces* (Bridge *et al*. 2008). In the *Hymenogastraceae*, the sequestrate species *Weraroa novae-zelandiae* (previously in *Strophariaceae*) has been recombined and renamed *Psilocybe weraroa* (Borovička *et al.* 2011).

The wave of recent nomenclatural changes among sequestrate fungi is analogous to the "one fungus, one name" approach that is in use for revisions of pleomorphic fungus nomenclature (Hawksworth 2015). In the case of sequestrate fungi, it might be better termed "one fungus, several names." In the next section, we follow the precedents set by these aforementioned mycologists to bring the genus *Russula* and

its associated sequestrate genera up to date with current understandings of sequestrate fungus nomenclature.

SEQUESTRATE SPECIES OF RUSSULA

Since the early 19th century, new sequestrate *Russulae* have been placed in one or more of several genera created expressly for them. In early observations of amyloid spore reactions to iodine solutions, Bucholtz (1901, 1903) linked the sequestrate genus *Elasmomyces* to the *Russula* lineage and *Arcangeliella* to *Lactarius,* even though the phylogenetic affinities were not well understood at the time. The number of sequestrate genera regarded as russuloid in pre-molecular times reached its zenith at five in the seminal paper by Singer & Smith (1960): *Cystangium*, *Elasmomyces*, *Gymnomyces*, *Macowanites*, and *Martellia.* A sixth, *Bucholtzia*, had been validly described by Lohwag (1924) but never accepted by the taxonomist community. Species in two other genera of sequestrate *Russulaceae, Arcangeliella* and *Zelleromyces*, are mostly resolved within the epigeous genus *Lactarius*, although some individual species may eventually be resolved within *Russula* or other genera in the *Russulaceae* (Buyck *et al.* 2008). Each of these 10 genera was circumscribed by characters thought to separate them from the others, but all show various degrees of intergradation (Calonge & Martin 2000, Trappe *et al*. 2002). Moreover, some sequestrate russuloid species had initially been erroneously assigned to families unrelated to the *Russulaceae*, including the *Agaricaceae*, *Boletaceae*, and *Hydnangiaceae*. The champion in nomenclatural complexity is *Russula krjukowensis*, which has been treated in seven genera (see the following species list for the formal new combination).

By the late 20th century, the phylogenetic relationship of sequestrate russuloid taxa to *Russula* was firmly demonstrated (Lebel & Trappe 2000, Lebel 2007). Morphological distinctions between some of those genera soon became blurred as additional new species proved to be morphological intermediates. Calonge & Martin (2000), Lebel & Trappe (2000, 2002), and Lebel (2007) attempted to sharpen the generic morphological distinctions, but phylogenetic analyses by Miller *et al*. (2001, 2006) clearly showed that the *Russulaceae* are monophyletic, and members of the six named sequestrate russuloid genera are nested among non-sequestrate *Russula* species with no consistent genetic relationship to each other or to any clade within *Russula*. The generic names erected to describe sequestrate morphologies of species we now combine with *Russula* have shown to be nomenclatural artifacts that in hindsight have little bearing on the phylogenetic positions of these taxa. No available phylograms provide evidence that any sequestrate *Russula* spp. belong to distinct lineages within the genus. The para- or polyphyly within phylogenies that include both sequestrate and non-sequestrate *Russula* spp. are nomenclatural relics, not genetics.

Over the last twenty years, multiple papers have been published to either describe new sequestrate species in *Russula* or *Lactarius*, recombine species from sequestrate genera with *Russula* or *Lactarius*, or suggest close or monophyletic relationships between species with the different morphologies (Martín *et al.* 1999, Miller *et al.* 2001, Desjardin 2003, Nuytinck *et al.* 2003, Eberhardt & Verbeken 2004, Lebel & Tonkin 2007, Li *et al.* 2013, Verbeken *et al*. 2014, Beenken *et al.* 2016, Lebel 2017, Looney *et al.* 2018). It has been widely suggested through published phylogenies that all remaining members of *Bucholtzia, Cystangium*, *Elasmomyces*, *Gymnomyces*, *Macowanites*, and *Martellia* should be transferred to *Russula*. These combinations will reduce the confusion in the current "one fungus, several names" situation and will simplify how we refer to these fungi. Lebel (2007, 2017) did a meticulous job of combining all Australian sequestrate *Russulae* into *Russula*. Here we update the remaining described sequestrate *Russula* species. In an era of genetics, environmental sequencing, and analysis of ecological roles of fungi through sequencing of root tips, having multiple generic names representing fungi that share close genetic affinities leads to further confusion. As stated in the preamble to the 2012 International Code of Nomenclature for Algae, Fungi, and Plants: "The purpose of giving a name to a taxonomic group is not to indicate its characters or history, but to supply a means of referring to it and to indicate its taxonomic rank" (McNeill *et al.* 2012). Accordingly, we judge that the following nomenclatural revisions of the genus *Russula* provide the clearest and most accurate information to indicate their taxonomic rank. Some information is lost without the sequestrate generic names; however, this is easily remedied in phylograms by using an arbitrary symbol, colour, or font in association with sequestrate names in a list, caption, text, phylogram, etc., as done by Miller *et al.* (2001) and Hosaka *et al.* (2006).

METHODS

We considered genetics whenever possible, but it was impossible to source sequences of type specimens (or new enough specimens) collected from close to type localities to generate a full phylogeny of the species we cover. To resolve the nomenclatural issues in this study, we relied heavily on morphology and reviewed specimens and/or original descriptions of the species we combined to ensure that they belonged in *Russula*. With increased interest in the sequestrate *Russulaceae*, we hope that many of these taxa will be re-collected and sequenced to generate a world phylogeny of this group. Genetics would make it possible to resolve the placement of the sequestrate genera *Arcangeliella* and *Zelleromyces* within the closely affiliated gymnocarpic genera *Lactarius, Lactifluus*, and *Multifurca.*

All specific epithet headings are capitalized and arranged alphabetically in the list below. These species were originally published in the genera *Bucholtzia*, *Cystangium, Elasmomyces, Gymnomyces, Macowanites,* or *Martellia*, as listed in Index Fungorum (plus a few incorrectly placed in non-russuloid genera). In this way, readers can determine how and why an original sequestrate name has been converted to its corresponding *Russula* name. The protocols for these decisions follow Rossman (2014) and use an amended version of the format provided by Rossman *et al.* (2016). Sequestrate species with *Russula* names already or newly published herein are in boldface. The standard nomenclature abbreviation *comb. nov.* is used when the genus is being combined with *Russula* but the original specific epithet can still be retained*; nom. nov.* is used when a new name must be proposed to avoid creating a homonym with a previously described species of *Russula.* When a new name is needed to avoid producing *Russula* homonyms, its etymology and other relevant information are noted. MycoBank numbers are assigned and registered to each new combination or new name.

REVISED NOMENCLATURE OF SEQUESTRATE *RUSSULA* SPP.

Generic type species: *Russula emetica* (Schaeff.) Pers., *Observ. Mycol. (Lipsiae)* **1**: 100. 1796.

ABIETIS
Russula subabietis Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821258.
Replaced synonym: *Gymnomyces abietis* Trappe & Castellano, *Mycotaxon* **75**: 161. 2000, non *Russula abietum* (J. Blum) Bon, *Doc. Mycol*. **13** (no. 50): 27. 1983.

Note: *subabietis* ("almost of the fir") to avoid confusion between *abietis* ("of the fir") with the related epithet *abietum* ("of firs").

ACRIS
Russula acerba Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821259.
Replaced synonym: *Macowanites acris* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 86. 1960, non *Russula acris* Steinhaus, *Hedwigia* **27**: 51. 1888.

Etymology and note: *acerba* ("bitter") to avoid producing a homonym with the earlier *acris* ("sharp") of *Russula acris* Steinhaus (1888).

AGARICINUS
Russula agaricina (Kalchbr. ex Berk.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB 825252.
Basionym: *Macowania agaricina* Kalchbr. ex Berk., Gard. Chron. **5**: 785. 1876.
Synonyms: *Macowanites agaricinus* (Kalchbr. ex Berk.) Kalchbr., *Grevillea* **10**(55): 107. 1882. *Russula agaricina* (Kalchbr. ex Berk.) T. Lebel, [as '(Kalchbr.) T. Lebel.'], *Austral. Syst. Bot.* **20**: 360. 2007, nom. inval. (Art. 41.5).

Note: *Macowinia agaricina* is a legitimate name although published under Kalchbrenner's illegitimate genus name *Macowania* (Art. 55.1) and has to be considered in terms of priority issues (Art. 11.4). Lebel (in Lebel & Tonkin 2007) intended to reallocate this name to *Russula* but did not introduce a valid combination (basionym not cited). She only referred to *Macowanites agaricinus*, which is only an indirect reference to the true basionym and not sufficient to validate the combination (Art. 41.5).

ALBA
Russula stevemilleri Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821262.
Replaced synonym: *Hydnangium album* Harkn., *Proc. Calif. Acad. Sci., Ser. 3, Bot.* **1**: 251. 1899, non *Russula alba* Velen., *České Houby* **1**: 149. 1920.
Synonyms: *Martellia alba* (Harkn.) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 41. 1961.
Gymnomyces albus (Harkn.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 198. 2002.

Etymology and note: In honor of Dr. Steve Miller for his continuing research on taxonomy and phylogeny of sequestrate and other *Russula* species. This new name is erected to avoid producing a homonym with *Russula alba* Velen. (1920).

ALBIDIGLEBA
Russula albidigleba (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821263.
Basionym: *Macowanites albidiglebus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 85. 1960.

ALBIDOFLAVA
Russula albidoflava T. Lebel, *Austral. Syst. Bot.* **20**: 361. 2007. MycoBank MB532175.

ALBOBRUNNEA
Russula albobrunnea T. Lebel, *Austral. Syst. Bot.* **20**: 362. 2007. MycoBank MB532176.

ALPINUS
Russula alpicola Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821264.
Replaced synonym: *Macowanites alpinus* Zeller, *Mycologia* **39**: 291. 1947, non *Russula alpina* (A. Blytt & Rostr.) F.H. Møller & Jul. Schäff., *Ann. Mycol*. **38**(2/4): 333. 1940.
Synonym: *Elasmomyces alpinus* (Zeller) Zeller, *Mycologia* **40**: 643. 1948.

Etymology and note: *alpicola* ("dweller of alps"), to avoid producing a homonym with the earlier *Russula alpina* (A. Blytt & Rostr.) F.H. Møller & Jul. Schäff. (1940).

AMMOPHILUS
Russula ammophila (J.M. Vidal & Calonge) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821265.
Basionym: *Gymnomyces ammophilus* J.M. Vidal & Calonge, *Bol. Soc. Micol. Madrid* **24**: 66. 1999.
Synonym: *Macowanites ammophilus* (J.M. Vidal & Calonge) J.M. Vidal & Calonge, *Revista Catal. Micol.* **24**: 70. 2002.

ARENICOLA
Russula arenicola (S.L. Mill. & D. Mitch.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821266.
Basionym: *Macowanites arenicola* S.L. Mill. & D. Mitch., *Mycotaxon* **89**: 284. 2004.

BALPINEUM
Russula balpinea (Grgur.) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820015.
Basionym: *Cystangium balpineum* Grgur., Larger Fungi of South Australia (Adelaide): 53 (1997).

BISPORUM
Russula bispora (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820016.
Basionym: *Cystangium bisporum* T. Lebel, *Austral. Syst. Bot*. **16**: 373. 2003.

BORANUPENSIS
Russula boranupensis (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820018.
Basionym: *Gymnomyces boranupensis* T. Lebel, *Austral. Syst. Bot*. **16**: 403. 2003.

BRUNNEONIGRA
Russula brunneonigra T. Lebel, *Austral. Syst. Bot*. **20**: 363. 2007. MycoBank MB532177.

BRUNNESCENS
Russula shafferi Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821312.
Replaced synonym: *Martellia brunnescens* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 37. 1961, non *Russula brunnescens* Murrill, *Lloydia* **8**: 264. 1946 (1945).
Synonym: *Gymnomyces brunnescens* (Singer & A.H. Sm.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 199. 2002.

Etymology and note: To honor Dr. Robert Shaffer for his important contributions to knowledge of North American *Russulae.* This new name is erected to avoid producing a homonym with the earlier *Russula brunnescens* Murrill (1945).

CALIFORNICA
Russula californica (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821336.
Basionym: *Martellia californica* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 33. 1961.
Synonym: *Gymnomyces californicus* (Singer & A.H. Sm.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 199. 2002, non *Russula californiensis* Burl., *Mycologia* **28**: 262. 1936.

CANDIDUS

Note: Originally described as *Hydnangium candidum* Tul. & C. Tul. in 1843, this species was recombined by Zeller & Dodge (1935) as *Sclerogaster candidus* (Tul. & C. Tul.) Zeller & C.W. Dodge, and then by Vidal (2005) as *Macowanites candidus* (Tul. & C. Tul.) J.M. Vidal. According to Kirk (2010), *S. candidus* in the *Sclerogastraceae*, not the *Russulaceae*, is the correct name.

CAPITIS-ORAE
Russula capitis-orae (Dring) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820020.
Basionym: *Elasmomyces capitis-orae* Dring, in Dring & Pegler, *Kew Bull*. **32**(3): 564. 1978.
Synonym: *Cystangium capitis-orae* (Dring) T. Lebel, *Mycotaxon* **81**: 197. 2002.

CHLORINOSMUS
Russula chlorineolens Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821355.
Replaced synonym: *Macowanites chlorinosmus* A.H. Sm. & Trappe, *Mycologia* **55**: 423. 1963, non *Russula chlorinosma* Burl., *Mycologia* **16**: 22. 1924.

Etymology and note: "*chlorineolens*" (smelling like chlorine), to avoid producing a homonym with the earlier, similarly smelling *Russula chlorinosmus* Burl. (1924).

CINNAMOMEA
Russula unicalifornica Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821338.
Replaced synonym: *Gymnomyces cinnamomeus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 51. 1960, non *Russula cinnamomea* Banning, *Bot. Gaz*. **6**(1): 166. 1881.

Etymology and note: "*unicalifornica*" contraction of "University of California" in reference to the site of the type collection to avoid producing a homonym with the earlier *Russula cinnamomea* Banning (1881).

CITRINA
Russula harknessii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821339.
Replaced synonym: *Octaviania citrina* Harkn., *Proc. Calif. Acad. Sci., Ser. 3, Bot*. **1**: 252. 1899, non *Russula citrina* Gillet, *Revue mycol., Toulouse* **3**(no. 11): 5. 1881, non *Macowanites citrinus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 69. 1960.
Synonyms: *Hydnangium citrinum* (Harkn.) Zeller & C.W. Dodge, *Ann. Missouri. Bot. Gard.* **22**: 371. 1935.
Gymnomyces citrinus (Harkn.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 199. 2002.

Etymology and note: In honor of H. W. Harkness, pioneer in taxonomy of North American sequestrate fungi. This new name is erected to avoid producing a homonym with the earlier *Russula citrina* Gillet (1881).

CITRINUS
Russula alleinstanleyae T.F. Elliott & Trappe, ***nom. nov.*** MycoBank MB823193.
Replaced synonym: *Macowanites citrinus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 69. 1960, non *Russula citrina* Gillet, *Revue Mycol., (Toulouse)* **3**(no. 11): 5 (1881), non *Gymnomyces citrinus* (Harkn.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 199. 2002.

Etymology and note: In honor of mycologist and educator Allein Stanley for her more than 40 years of dedicated service to the North American Mycological Association. This new name is erected to avoid producing a homonym with the earlier *Russula citrina* Gillet (1881).

CLAVATUM
Russula clavata (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820021.
Basionym: *Cystangium clavatum* T. Lebel, *Austral. Syst. Bot*. **16**: 375. 2003.

CLELANDII
Russula aurantirosea T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820058.
Replaced synonym: *Gymnomyces clelandii* T. Lebel, *Austral. Syst. Bot.* **16**: 404. 2003, non *Russula clelandii* O.K. Mill. & R.N. Hilton, *Sydowia* **39**: 128. 1987.

COMPACTA
Russula mattsmithii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821354.
Replaced synonym: *Gymnomyces compactus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 53. 1960, non *Russula compacta* Frost, *Rep. (Annual) New York State Mus. Nat. Hist.* **32**: 32. 1879.

Etymology and note: In honor of Dr. Matt Smith for his extensive research on taxonomy and ecology of sequestrate fungi. This new name is erected to avoid producing a homonym with the earlier *Russula compacta* Frost (1879).

COSTATISPORUS
Russula costatispora (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820022.
Basionym: *Gymnomyces costatisporus* T. Lebel, *Austral. Syst. Bot.* **16**: 405. 2003.

CREMEA
Russula dodgei Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB824950.
Replaced synonym: *Arcangeliella cremea* Zeller & C.W. Dodge, *Ann. Missouri Bot. Gard*. **22**: 367. 1935, non *Russula cremea* (Murrill) Singer, *Lilloa* **22**: 711. 1951.
Synonyms: *Martellia cremea* (Zeller & C.W. Dodge) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 45. 1961 (1960).
Gymnomyces cremeus (Zeller & C.W. Dodge) Trappe *et al.*, *Mycotaxon* **81**: 199. 2002.

Etymology and note: In honor of C.W. Dodge, who collaborated with S. M. Zeller in describing numerous new species of sequestrate fungi, including this taxon and to avoid confusion with *Russula cremea* (Murrill) Singer.

CRISTATA
Russula korystospora T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB821530.
Replaced synonym: *Gymnomyces cristatus* T. Lebel, *New Zealand J. Bot.* **40**: 491. 2002, non *Russula cristata* Romagn., *Bull. Mens. Soc. Linn. Soc. Bot. Lyon* **31**(1): 177. 1962.

DEPAUPERATUM
Russula depauperata (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821344.
Basionym: *Cystangium depauperatum* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 69. 1960.

DOMINGUEZIAE
Russula laurae Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821357.
Replaced synonym: *Cystangium dominguezeiae* Nouhra & Trierv.-Per., *Mycologia* **107**: 94. 2014, non *Gymnomyces dominguezii* Mor.-Arr., J. Gómez & Calonge, *Mycol. Res.* **103**: 215. 1999.

Etymology and note: In honor of Argentine mycologist, Laura S. Dominguez after whom the species was originally named. This new name is erected to avoid producing a homonym with the earlier and similar name *Gymnomyces dominguezii* Mor.-Arr., J. Gómez & Calonge (see following entry).

DOMINGUEZII
Russula dominguezii (Mor.-Arrl, J. Gómez & Calonge) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821346.
Basionym: *Gymnomyces dominguezii* Mor.-Arr., J. Gómez & Calonge, *Mycol. Res.* **103**: 215. 1999.

DURANGENSIS
Russula durangensis (Guzmán) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821358.
Basionym: *Macowanites durangensis* Guzmán, *Revista Mex. Micol.* **4**: 118. 1988.

EBURNEA
Russula eburnea (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820023.
Basionym: *Gymnomyces eburneus* T. Lebel, *Austral. Syst. Bot.* **16**: 408. 2003.

ECHINOSPORUS
Russula tombrunsii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823196.
Replaced synonym: *Macowanites echinosporus* Zeller & C.W. Dodge, *Ann. Missouri Bot. Gard.* **6**: 57. 1919, non *Russula echinospora* Singer, *Bull. Soc. Mycol. France* **55**: 270. 1940, non *Russula echinospora* R. Heim, *Rev. Mycol. (Toulouse)* **32**: 207. 1967, non *Cystangium echinosporum* (Zeller & C.W. Dodge) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 197. 2002, non *Elasmomyces echinosporus* Zeller & C.W. Dodge, *Ann. Missouri Bot. Gard.* **22**: 370. 1935.

Etymology and note: In honor of Dr. Tom Bruns for his career-long research on taxonomy, phylogeny and ecology of ectomycorrhizal fungi. This new name is erected to avoid producing a homonym of *R. echinospora* Singer (1940).

EILDONENSIS
Russula eildonensis (G.W. Beaton *et al.*) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820024
Basionym: *Gymnomyces eildonensis* G.W. Beaton *et al.*, *Kew Bull*. **39**: 680. 1984.

ELLIPSOSPORUM
Russula ellipsospora (Zeller) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821361.
Basionym: *Hydnangium ellipsosporum* Zeller, *Mycologia* **31**: 13. 1939.
Synonyms: *Martellia ellipsospora* (Zeller) Singer & A.H. Sm., *Mem*. *Torrey Bot. Club* **21**: 30. 1960.
Gymnomyces ellipsosporus (Zeller) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 199. 2002.

FALLAX
Russula similaris Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821362.
Replaced synonym: *Martellia fallax* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 34. 1960, non *Russula fallax* (Schaeff.) Fr., *Hymenomyc. Eur.* (Upsaliae): 449. 1874.
Synonym: *Gymnomyces fallax* (Singer & A.H. Sm.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 199. 2002.

Etymology and note: "*similaris"* in reference to the similarity of the two epithets cited above, "*fallax"* (deceptive) deceiving their respective authors, and to avoid producing a homonym with the earlier *R. fallax* (Schaef.) Fr. (1874).

FERRUGINASCENS
Russula ferruginascens (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821363.
Basionym: *Gymnomyces ferruginascens* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 54. 1960.

FLAVOVIRENS
Russula olivaceoflava T. Lebel, Muelleria **36**: 12. 2017. MycoBank MB821532.
Replaced synonym: *Cystangium flavovirens* T. Lebel, *Austral. Syst. Bot.* **16**: 377. 2003, non *Russula flavovirens* J. Bommer & M. Rousseau, *Bull. Soc. Roy. Bot. Belgique* **23**(1): 310. 1884.

FOETENS
Russula taetra Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821365.
Replaced synonym: *Martellia foetens* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 36. 1960, non *Russula foetens* Pers., *Observ. Mycol.* (Lipsiae) **1**: 102. 1796.
Synonym: *Gymnomyces foetens* (Singer & A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 199. 2002.

Etymology and note: *taetra* ("foul, offensive"), a related term to express the original epithet *foetens* ("stinking") to avoid producing a homonym of the earlier *Russula foetens* Pers. (1796).

FRAGRANS
Russula aromatica Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821366.
Replaced synonym: *Martellia fragrans* A.H. Sm., *Mycologia* **55**: 437. 1963.
Synonym: *Gymnomyces fragrans* (A.H. Sm.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 199. 2002, non *Russula fragrans* Romagn., *Bull. Mens. Soc. Linn. Soc. Bot. Lyon* **23**: 112. 1954.

Etymology and note: "aromatica", a related term to express the original epithet *fragrans* "becoming fragrant" to avoid producing a homonym of the earlier *Russula fragrans* Romagn. (1954).

FULVESCENS
Russula vilgalysii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821367.
Replaced synonym: *Macowanites fulvescens* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 84. 1960, non *Russula fulvescens* Burl., *N. Amer. Fl.* (New York) **9**: 229. 1915.

Etymology and note: In honor of Dr. Rytas Vilgalys for his research and teaching of all aspects of mycology. This new name is erected to avoid producing a homonym of the earlier *Russula fulvescens* Burl. (1915).

FULVISPORA
Russula fulvispora (A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821368.
Basionym: *Martellia fulvispora* A.H. Sm., *Mycologia* **55**: 438. 1963.
Synonym: *Gymnomyces fulvisporus* (A.H. Sm.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 199. 2002.

FURCATISPINA
Russula furcatispina (T. Lebel) T. Lebel, Muelleria **36**: 12. 2017. MycoBank MB820025.
Basionym: *Gymnomyces furcatispinus* T. Lebel, *Austral. Syst. Bot.* **16**: 411. 2003.

FUSCOVIOLACEUS
Russula fuscoviolaceus (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821371.
Basionym: *Macowanites fuscoviolaceus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 87. 1960.

FUSCUS
Russula spinispora T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB821534.
Replaced synonym: *Gymnomyces fuscus* T. Lebel, *New Zealand J. Bot.* **40**: 495. 2002, non *Russula fusca* Quél., *Compt. Rend. Assoc. Franç. Avancem. Sci.* **15**: 486. 1887.

GALBANA
Russula galbana T. Lebel, Austral. Syst. Bot. **20**: 365. 2007. MycoBank MB532179.

GALILEENSIS
Russula galileensis (M.M. Moser, Binyam. & Aviz.-Hersh.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821373.
Basionym: *Macowanites galileensis* M.M. Moser, Binyam. & Aviz.-Hersh., *Trans. Brit. Mycol. Soc.* **68**: 371. 1977.

GAMUNDIAE
Russula gamundiae (Nouhra & Trierv.-Per.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821374.
Basionym: *Cystangium gamundiae* Nouhra & Trierv.-Per., *Mycologia* **107**: 95. 2014.

GILKEYAE
Russula gilkeyae (Zeller & C.W. Dodge) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821375.
Basionym: *Hydnangium gilkeyae* Zeller & C.W. Dodge, *Ann. Missouri Bot. Gard.* **22**: 371. 1935.
Synonyms: *Octaviania gilkeyae* (Zeller & C.W. Dodge) Svrček, Fl. ČSR, B-1, *Gasteromycetes*: 192. 1958.
Martellia gilkeyae (Zeller & C.W. Dodge) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 43. 1960.
Gymnomyces gilkeyae (Zeller & C.W. Dodge) Trappe *et al.*, *Mycotaxon* **81**: 199. 2002.

GLAREA
Russula glarea (T. Lebel) T. Lebel, Muelleria **36**: 12. 2017. MycoBank MB820026.
Basionym: *Gymnomyces glarea* T. Lebel, *Austral. Syst. Bot.* **16**: 412. 2003.

GRANDIHYPHATUM
Russula grandihyphata (Nouhra & Trierv.-Per.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821377.
Basionym: *Cystangium grandihyphatum* Nouhra & Trierv.-Per., *Mycologia* **107**: 96. 2014.

IDAHOENSIS
Russula idahoensis (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821378.
Basionym: *Martellia idahoensis* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 43. 1960.
Synonym: *Cystangium idahoense* (Singer & A.H. Sm.) Trappe *et al.* [as *'idahoensis'*], *Mycotaxon* **81**: 197. 2002.

ILICIS
Russula vidalii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821379.
Replaced synonym: *Gymnomyces ilicis* J.M. Vidal & Llistos., *Rivista Micol.* **38**: 160. 1995, non *Russula ilicis* Romagn. *et al.*, *Bull. Trimestriel Soc. Mycol. France* **88**: 33. 1972.

Etymology and note: In honor of J.D. Vidal, Spanish Catalonian mycologist who has specialized in taxonomy of sequestrate fungi. This new name is erected to avoid producing a homonym with the earlier *Russula ilicis* Romagn. (1972).

IODIOLENS
Russula iodiolens (A.H. Sm. & V.L. Wells) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821380.
Basionym: *Macowanites iodiolens*, A.H. Sm. & V.L. Wells, *Mycologia* **55**: 425. 1963.

JAVANICA
Russula boedijnii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823199.
Replaced synonym: *Hydnangium javanicum* Henn., *Hedwigia* **40**(Beibl.): 27. 1901, non *Russula javanica* Sacc. & P. Syd., *Syll. Fung. (Abellini)* **16**: 46. 1902.
Synonym: *Gymnomyces javanicus* (Henn.) M.E. Sm. & Schmull, *Mycol. Progr.* **10**: 253. 2011.

Etymology and note: In honor of Dutch mycologist K.B. Boedijn, who specialized in taxonomy of Indonesian fungi including hypogeous species. This new name is erected to avoid producing a homonym with the earlier *Russula javanica* Sacc. & P. Syd. (1902).

KERMESINA
Russula kermesina T. Lebel, *Austral. Syst. Bot.* **20**: 377. 2007. MycoBank MB531311.
Replaced synonym: *Macowanites carmineus* McNabb, *New Zealand J. Bot.* **9**: 359. 1971, non *Russula carminea* (Jul. Schäff.) Kühner & Romagn., in Romagnesi, *Russules d'Europe Afr. Nord,* Essai sur la Valeur Taxinomique et Specifique des Characteres des Spores et des Revetements: 447. 1967.

KRJUKOWENSIS
Russula krjukowensis (Bucholtz) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823514.
Basionym: *Secotium krjukowense* Bucholtz, *Hedwigia* **40**: 314. 1901.
Synonyms: *Secotium michailowskianum* Bucholtz [as *'michailowskjanum'*], *Hedwigia* **40**: 315. 1901.
Elasmomyces michailowskianus (Bucholtz) Sacc. & D. Sacc. [as *'michailowskjanus'*], *Syll. Fung. (Abellini)* **17**: 218. 1905.
Elasmomyces krjukowensis f. *pleurotopsis* Bucholtz, *Bull. Soc. Imp. Naturalistes Moscou, ser*. 2, **24**: 463. 1907.
Bucholtzia krjukowensis (Bucholtz) Lohwag, *Oesterr. Bot. Z.* **73(7-9)**: 173. 1924.
Arcangeliella krjukowensis var. *michailowskiana* (Bucholtz) Zeller & C.W. Dodge, *Ann. Missouri. Bot. Gard.* **22**: 368. 1935.
Arcangeliella krjukowensis (Bucholtz) Zeller & C.W. Dodge, *Ann. Mo. Bot. Gdn* **22**: 368. 1935. var. *krjukowensis.*
Hydnangium krjukowense (Bucholtz) Svrček, Fl. ČSR, B-1, *Gasteromycetes*: 206. 1958. var. *krjukowense.*
Macowanites krjukowensis (Bucholtz) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 77. 1960.

LEUCOCARPUS
Russula leucocarpa (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820035.
Basionym: *Gymnomyces leucocarpus* T. Lebel, *New Zealand J. Bot.* **40**: 497. 2002.

LONGISPORUS
Russula longispora (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820036.
Basionym: *Gymnomyces longisporus* T. Lebel, *Austral. Syst. Bot.* **16**: 415. 2003.

LONGISTERIGMATUM
Russula longisterigmata (Nouhra & Trierv.-Per.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821384.
Basionym: *Cystangium longisterigmatum* Nouhra & Trierv.-Per., *Mycologia* **107**: 97. 2014.

LUTEIROSEA
Russula luteirosea (Bougher) T. Lebel, *Muelleria* **36**: 13 (2017). MycoBank MB822048.
Basionym: *Macowanites luteiroseus* Bougher, *Mycotaxon* **63**: 38. 1997.
Synonym: *Russula luteirosea* (Bougher) T. Lebel, *Austral. Syst. Bot.* **20**(4): 378. 2007, *nom*. *inval*.

LUTEOBRUNNEUM
Russula luteobrunnea (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820037.
Basionym: *Cystangium luteobrunneum* T. Lebel, *Austral. Syst. Bot.* **16**: 380. 2003.

LUTEOLUS
Russula luteola (Harkn.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821386.
Basionym: *Hydnangium luteolum* Harkn., *Proc. Calif. Acad. Sci*., Ser. 3, Bot. **1**: 251. 1899.
Synonym: *Gymnomyces luteolus* (Harkn.) Trappe *et al.*, *Mycotaxon* **81**: 200. 2002, non *Macowanites luteolus* A.H. Sm. & Trappe, *Mycologia* **55**: 427. 1963.

LUTEOLA
Russula stricklandorum T.F. Elliott & Trappe, ***nom. nov.*** MycoBank MB821387.
Replaced synonym: *Macowanites luteolus* A.H. Sm. & Trappe, *Mycologia* **55**: 427. 1963, non *Russula luteola* (Harkn.) Trappe & T.F. Elliott (this paper, see preceding entry).

Etymology and note: *stricklandorum*, in honor of Bob and Babs Strickland for their support of the natural sciences and contributions to conservation. This new name is erected to avoid producing a homonym with the preceding *Russula luteola* (Harkn.) Trappe & T.F. Elliott (2017).

LYMANENSE
Russula lymanensis (Cázares & Trappe) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821388.
Basionym: *Macowanites lymanensis* Cázares & Trappe, *Mycotaxon* **42**: 335. 1991.
Synonym: *Cystangium lymanense* (Cázares & Trappe) Trappe *et al.* [as *'lymanensis'*], *Mycotaxon* **81**: 197. 2002.

MACROCYSTIDIUM
Russula macrocystidia (T. Lebel) T. Lebel, Muelleria **36**: 12. 2017. MycoBank MB820038.
Basionym: *Cystangium macrocystidium* T. Lebel, *Austral. Syst. Bot.* **16**: 380. 2003.

MACULATA
Russula extramaculata Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821390.
Replaced synonym: *Martellia maculata* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 28. [1960] 1961, non *Russula maculata* Quél., *Bull. Soc. Bot. France* **24**: 323. [1877] 1878.

Synonym: *Cystangium maculatum* (Singer & A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 197. 2002.

Etymology and note: *extramaculatum* ("outside *maculata*") to avoid producing a homonym with the earlier *Russula maculata* Quél. (1878).

MATTIROLOANUS
Russula mattiroloana (Cavara) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820039.
Basionym: *Elasmomyces mattiroloanus* Cavara [as *'mattirolianus'*], *Malpighia* **11**(9–10): 426. 1897.
Synonyms: *Secotium mattiroloanum* (Cavara) E. Fisch., in Engler & Prantl, *Nat. Pflanzenfam., Teil. I (Leipzig)* **1**: 301. 1900.
Macowanites mattiroloanus (Cavara) T. Lebel & Trappe (as *'mattirolianus'*), *Mycologia* **92**: 1194. 2000.

MEDITERRANEA
Russula mediterranea (G. Moreno *et al.*) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821392.
Basionym: *Martellia mediterranea* G. Moreno *et al.*, *Mycotaxon* **42**: 227. 1991.

MEDLOCKII
Russula medlockii (Trappe & Castellano) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821393.
Basionym: *Martellia medlockii* Trappe & Castellano, *Mycologia* **78**: 918. 1986.
Synonym: *Cystangium medlockii* (Trappe & Castellano) Trappe *et al.*, *Mycotaxon* **81**: 197. 2002.

MEGASPORUS
Russula megaspora (Rodway) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820040.
Basionym: *Gymnomyces megasporus* Rodway, *Pap. & Proc. Roy. Soc. Tasmania* '1925': 168. 1926 (1925).
Synonyms: *Octaviania megaspora* (Rodway) G. Cunn., *New Zealand J. Sci. Technol*. **22**: 300B. 1941.
Cystangium megasporum (Rodway) T. Lebel & Castellano, *Mycologia* **94**: 335. 2002.

MESSAPICOIDES
Russula messapicoides (Llistos. & J.M. Vidal) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821395.
Basionym: *Macowanites messapicoides* Llistos. & J.M. Vidal, *Rivista Micol*. **38**: 155. 1995.

MEXICANUS
Russula guzmanii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821396.
Replaced synonym: *Macowanites mexicanus* Guzmán, *Revista Mex. Micol*. **4**: 116. 1988, non *Russula mexicana* Burl., *Mycologia* **3**: 26. 1911.

Etymology and note: In honor of Prof. Gastón Guzmán, much accomplished Mexican mycologist and taxonomist. This new name is erected to avoid producing a homonym of the earlier *Russula mexicana* Burl. (1911).

MISTIFORMIS
Russula mistiformis (Mattir.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821397.
Basionym: *Martellia mistiformis* Mattir., *Malpighia* **14**: 78. 1900.
Synonyms: *Hydnangium mistiforme* (Mattir.) Zeller & C.W. Dodge, *Ann. Missouri Bot. Gard.* **22**: 372. 1935.
Octaviania mistiformis (Mattir.) Svrček, Fl. ČSR, B-1, *Gasteromycetes*: 192. 1958.
Gymnomyces mistiformis (Mattir.) T. Lebel & Trappe, *Mycologia* **92**: 1999. 2000.

MOLLIS
Russula castellanoi Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821398.
Replaced synonym: *Macowanites mollis* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 79. 1960, non *Russula mollis* Quél., *Compt. Rend. Assoc. Franç. Avancem. Sci.* **11**: 397. 1883 (1882).
Synonym: *Elasmomyces mollis* (Singer & A.H. Sm.) Pegler & T.W.K. Young, *Trans. Brit. Mycol. Soc.* **72**: 368. 1979.

Etymology and note: In honor of Dr. Michael (Caz) Castellano for his career-long fascination with and research on sequestrate fungi. This new name is erected to avoid producing a homonym with the earlier *Russula mollis* Quél. (1882).

MONOSPORA
Russula monospora (Boud. & Pat.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821399.
Basionym: *Hydnangium monosporum* Boud. & Pat., *J. Bot., Paris* **2**: 445. 1888.
Synonyms: *Octaviania monospora* (Boud. & Pat.) Lloyd, *Mycol. Writ.* **7**(Letter 67): 1141. 1922.
Martellia monospora (Boud. & Pat.) Astier & Pacioni, *Doc. Mycol*. **28**(nos 109–110): 9. 1998.

MONOSPORUS
Russula stewartii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB821400.
Replaced synonym: *Gymnomyces monosporus* E.L. Stewart & Trappe, *Mycotaxon* **2**: 209. 1975.

Etymology and note: In honor of Dr. Elwin L. Stewart, mycologist and plant pathologist, for his contributions to taxonomy of sequestrate fungi. This new name is erected to avoid producing a homonym of the earlier *Hydnangium monosporum* Boud. & Pat. (1888). See previous entry.

MONTICOLA
Russula monticola (Harkn.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB821401.
Basionym: *Octaviania monticola* Harkn., *Proc. Calif. Acad. Sci., Ser. 3, Bot*. **1**: 254. 1899.
Synonyms: *Hydnangium monticola* (Harkn.) Zeller & C.W. Dodge, *Ann. Missouri Bot. Gard.* **22**: 372. 1935.
Martellia monticola (Harkn.) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 40. 1961 (1960).
Gymnomyces monticola (Harkn.) Trappe *et al.*, *Mycotaxon* **81**: 200. 2002.

NANJINGENSIS
Russula nanjingensis (B. Liu & K. Tao) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823202.
Basionym: *Martellia nanjingensis* B. Liu & K. Tao, *Acta Mycol. Sin.* **12**: 103. 1993.
Synonyms: *Gymnomyces nanjingensis* (B. Liu & K. Tao) Trappe *et*

al., *Mycotaxon* **81**: 200. 2002.
Arcangeliella nanjingensis (B. Liu & K. Tao) J.M. Vidal, *Revista Catal. Micol*. **26**: 73. 2005 (2004).

NAUSEOSUS
Russula hunsuckeri T.F. Elliott & Trappe, ***nom. nov.*** MycoBank MB823515.
Replaced synonym: *Macowanites nauseosus* A.H. Sm., *Mycologia* **55**: 428. 1963, non *Russula nauseosa* (Pers.) Fr., *Epicr. Syst. Mycol. (Upsaliae)*: 363. [1836–1838] 1838.

Etymology and note: In honor of naturalist, mycologist, botanist, and philosopher Dr. Robert Hunsucker. This new name is erected to avoid producing a homonym with the earlier *Russula nauseosa* (Pers.) Fr. (1838).

NONDISTINCTA
Russula nondistincta (Trappe & Castellano) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823207.
Basionym: *Gymnomyces nondistincta* Trappe & Castellano, *Mycotaxon* **75**: 166. 2000.

NOTHOFAGI
Russula nothofagi (E. Horak) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823517.
Basionym: *Elasmomyces nothofagi* E. Horak, *Sydowia* **17**(1–6): 211. [1963] 1962.
Synonym: *Cystangium nothofagi* (E. Horak) Trappe *et al.*, *Mycotaxon* **81**: 198. 2002.

OCCIDENTALIS
Russula thiersii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823208.
Replaced synonym: *Martellia occidentalis* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 42. 1960, non *Russula occidentalis* (Singer) Singer, *Lilloa* **22**: 705. [1949] 1951, non *Russula occidentalis* Bon, *Doc. Mycol*. **12**(no. 46): 32. 1982, nom. illeg.

Etymology and note: In honor of Dr. Harry Thiers for his long interest in and taxonomic studies of fungi and the *Russulaceae*. This new name is erected to avoid producing a homonym with the earlier *Russula occidentalis* Singer (1951).

ODORATUS
Russula odorifera Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823209.
Replaced synonym: *Elasmomyces odoratus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 57. 1960, non *Russula odorata* Romagn., *Bull. Mens. Soc. Linn. Soc. Bot. Lyon* **19**: 76. 1950.
Synonym: *Macowanites odoratus* (Singer & A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 202. 2002.

Etymology and note: *odorifera* (bearing an odor), to avoid producing a homonym with the earlier *Russula odorata* Romagn. (1950).

OLIDUS
Russula olida (A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823210.
Basionym: *Macowanites olidus* A.H. Sm., *Mycologia* **55**: 428. 1963.

OREGONENSIS
Russula oregonensis (Zeller) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823211.
Basionym: *Hydnangium oregonense* Zeller, *Mycologia* **33**: 200. 1941
Synonyms: *Martellia oregonensis* (Zeller) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 27. 1961 (1960).
Cystangium oregonense (Zeller) Trappe *et al.*, *Mycotaxon* **81**: 198. 2002.

PALLIDUS
Russula paneeroides T. Lebel, Muelleria **36**: 12. 2017. MycoBank MB821533.
Replaced synonym: *Gymnomyces pallidus* Massee & Rodway, in Massee, *Bull. Misc. Inform. Kew*: 125. 1898, non *Russula pallida* P. Karst., *Hedwigia* **35**: 43. 1896.
Synonym: *Octaviania pallida* (Massee & Rodway) G. Cunn., *Proc. Linn. Soc. New South Wales* **60**: 119. 1935.

PARKSII
Russula parksii (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823213.
Basionym: *Gymnomyces parksii* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 55. 1960.

PARVISAXOIDES
Russula parvisaxoides (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB822047.
Basionym: *Gymnomyces parvisaxoides* T. Lebel, *New Zealand J. Bot.* **40**: 498. 2002.

PHYMATODISPORUM
Russula phymatodispora (G.W. Beaton *et al.*) T. Lebel *Muelleria* **36**: 12. 2017. MycoBank MB820041.
Basionym: *Cystangium phymatodisporum* G.W. Beaton *et al.*, *Kew Bull.* **39**: 672. 1984.

PILA
Russula pila (Pat.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823216.
Basionym: *Hydnangium pila* Pat., *Bull. Soc. Mycol. France* **26**: 202. 1910.
Synonyms: *Octaviania pila* (Pat.) Svrček, Fl. ČSR, B-1, *Gasteromycetes*: 199. 1958.
Martellia pila (Pat.) J.M. Vidal, *Bull. Soc. Catalana Micol.* **14–15**: 172. 1991.
Gymnomyces pila (Pat.) Trappe, T. Lebel & Castellano, *Mycotaxon* **81**: 200. 2002.

PILOSUS
Russula pilosa (Zeller & C.W. Dodge) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823217.
Basionym: *Arcangeliella pilosa* Zeller & C.W. Dodge, *Ann. Missouri Bot. Gard.* **22**: 368. 1935.
Synonyms: *Elasmomyces pilosus* (Zeller & C.W. Dodge) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 66. 1960.
Macowanites pilosus (Zeller & C.W. Dodge) Trappe *et al.*, *Mycotaxon* **81**: 202. 2002.

PILOSELLA
Russula pilosella T. Lebel, *Austral. Syst. Bot.* **20**: 379. 2007. MycoBank MB532181.

Replaced synonym: *Hydnangium tomentosum* J.W. Cribb, *Pap. Dept. Bot. Univ. Queensland* **3**: 251. 1958, non *Russula tomentosa* Buyck, *Bull. Jard. Bot. Natl. Belg.* **58**: 476. 1988.
Synonyms: *Martellia tomentosa* (J.W. Cribb) A.H. Sm., *Mycologia* **54**: 631. 1963.

PINETI
Russula pineti (Singer) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823218.
Basionym: *Cystangium pineti* Singer, *Mycol. Helv.* **1**: 417. 1985.

PINICOLA
Russula piniamans Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823225.
Replaced synonym: *Macowanites pinicola* A.H. Sm., *Mycologia* **55**: 430. 1963, non *Russula pinicola* Murrill, *Lloydia* **6**: 213. 1943.

Etymology and note: *piniamans* ("friend of pine"), to avoid producing a homonym of the earlier *Russula pinicola* Murrill (1943)

PISIGLAREA
Russula pisiglarea (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820042.
Basionym: *Cystangium pisiglarea* T. Lebel, *Austral. Syst. Bot.* **16**: 383. 2003.

POLYCHROMUM
Russula collubrina T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820059.
Replaced synonym: *Cystangium polychromum* Trappe & Claridge, *Australas. Mycol*. **22**: 33. 2003, non *Russula polychroma* Singer ex Hora, *Trans. Brit. Mycol. Soc.* **43**: 457. 1960.

PSEUDOEMETICUS
Russula nancyweberae Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823545.
Replaced synonym: *Macowanites pseudoemeticus* A.H. Sm., *Mycologia* **55**: 431. 1963, non *Russula pseudoemetica* Secr. ex Singer, *Hedwigia* **66**: 190. 1926.

Etymology and note: In honor of Dr. Nancy Weber, for her extensive research on taxonomy of epigeous Ascomycetes, especially the genus *Morchella*. This new name is erected to avoid producing a homonym with the earlier *Russula pseudoemetica* Secr. ex Singer (1926).

PTEROSPERMUS
Russula pterosperma (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820043.
Basionym: *Gymnomyces pterospermus* T. Lebel, *Austral. Syst. Bot*. **16**: 416. 2003.

PUMICOIDEA
Russula pumicoidea T. Lebel, *Austral. Syst. Bot.* **20**: 367. 2007. MycoBank MB532182.

REDDELLII
Russula reddellii T. Lebel, *Austral. Syst. Bot.* **20**: 369. 2007. MycoBank MB532183.

REDOLENS
Russula osphranticarpa T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820061.
Replaced synonym: *Octaviania redolens* G. Cunn., *New Zealand J. Sci. Technol.* **23B**: 172. 1942, non *Russula redolens* Burl., *Mycologia* **13**: 133. 1921.
Synonym: *Gymnomyces redolens* (G. Cunn.) Pfister, *Occas. Papers Farlow Herb. Cryptog. Bot.* **9**: 43. 1976.

RODWAYI
Russula leonardii T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820060.
Replaced synonym: *Gymnomyces rodwayi* T. Lebel, *Austral. Syst. Bot.* **16**: 418. 2003, non *Russula rodwayi* (Massee) T. Lebel, *Muelleria* **36**: 11. 2017.

Russula rodwayi (Massee) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820044.
Basionym: *Secotium rodwayi* Massee, *Bull. Misc. Inform. Kew*: 158. 1901.
Synonyms: *Elasmomyces rodwayi* (Massee) Zeller, *Mycologia* **40**: 642. 1948.
Cystangium rodwayi (Massee) A.H. Sm., *Mycologia* **54**: 633. 1963.

ROGERSII
Russula rogersii (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823546.
Basionym: *Octavianina rogersii* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 12. 1960.
Synonym: *Gymnomyces rogersii* (Singer & A.H. Sm.) Trappe *et al. Mycotaxon* **81**: 201. 2002.

ROLFALEXII
Russula rolfalexii (Trappe *et al.*) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823552.
Basionym: *Gymnomyces rolfalexii* Trappe *et al.*, *Mycotaxon* **81**: 201. 2002.
Synonym: *Martellia parksii* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 32. 1960, non *Gymnomyces parksii* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 55. 1960. See *Russula parksii* (Singer & A.H. Sm.) Trappe & T.F. Elliott this publication.

ROSEIPES
Russula spataforae Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823547.
Replaced synonym: *Elasmomyces roseipes* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 64. 1960, non *Russula roseipes* Secr. ex Bres., *Fung. Trident.* **1**: 37. 1881.
Synonym: *Macowanites roseipes* (Singer & A.H. Sm.) Pegler & T.W.K. Young, *Trans. Brit. Mycol. Soc.* **72**: 363. 1979.

Etymology and note: In honor of Dr. Joey Spatafora for his teaching and extensive research on phylogeny and taxonomy of macrofungi. This new name is established to avoid producing a homonym with *Russula roseipes* Secr. ex Bres. (1881).

ROSEOMACULATUS
Russula roseomaculata (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823548.
Basionym: *Gymnomyces roseomaculatus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 50. 1960.

ROSTRATICYSTIDIA
Russula rostraticystidia T. Lebel, *Austral. Syst. Bot*. **20**: 371. 2007. MycoBank MB532184.

RUBROLUTEA
Russula rubrolutea (T. Lebel) T. Lebel, *Muelleria* **36**: 14. 2017. MycoBank MB822049.
Basionym: *Macowanites rubroluteus* T. Lebel, *New Zealand J. Bot.* **40**: 505. 2002.
Synonym: *Russula rubrolutea* (T. Lebel) T. Lebel, *Austral. Syst. Bot.* **20**: 378. 2007, *nom. inval*.

RUSSULOIDES
Russula russuloides (Setch.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823549.
Basionym: *Elasmomyces russuloides* Setch., *J. Mycol.* **13**: 240. 1907.
Synonym: *Macowanites russuloides* (Setch.) Trappe *et al.*, *Mycotaxon* **81**: 202. 2002.

SICHUANENSIS
Russula sichuanensis G.J. Li & H.A. Wen, *Mycotaxon* **124**: 179. 2013 MycoBank MB804645.

SEMINUDUS
Russula seminuda (Massee & Rodway) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820045.
Basionym: *Gymnomyces seminudus* Massee & Rodway, *Bull. Misc. Inform. Kew*: 125. 1898.
Synonyms: *Arcangeliella seminuda* (Massee & Rodway) Zeller & C.W. Dodge, *Ann. Missouri Bot. Gard.* **23**: 617. 1936.
Octaviania seminuda (Massee & Rodway) G. Cunn., *Trans. & Proc. Roy. Soc. New Zealand* **67**: 408. 1938.
Cystangium seminudum (Massee & Rodway) T. Lebel & Castellano, *Mycologia* **94**: 337. 2002.

SESSILE
Russula sessilis (Massee & Rodway) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820046.
Basionym: *Secotium sessile* Massee & Rodway, *Pap. & Proc. Roy. Soc. Tasmania* '1911': 31. [1911] 1912 [var. *sessile*].
Synonyms: *Elasmomyces sessilis* (Massee & Rodway) Rodway, *Pap. & Proc. Roy. Soc. Tasmania* '1924': 8. [1924] 1925.
Cystangium sessile (Massee & Rodway) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 27. 1960.

SETCHELLIANUS
Russula setchelliana (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823550.
Basionym: *Macowanites setchellianus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 92. 1960.

SETIGER
Russula setigera (Zeller) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823551.
Basionym: *Hydnangium setigerum* Zeller, *Mycologia* **31**: 14. 1939.
Synonyms: *Martellia setigera* (Zeller) Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 39. 1960.
Gymnomyces setiger (Zeller) Trappe *et al*. [as ‚*setigerus*'], *Mycotaxon* **81**: 201. 2002.

SHULTZIAE
Russula shultziae (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820047.
Basionym: *Cystangium shultziae* T. Lebel, *Austral. Syst. Bot.* **16**: 385. 2003.

SICHUANENSIS
Russula sichuanensis G.J. Li & H.A. Wen, *Mycotaxon* **124**: 179. 2013. MycoBank MB804645.

SINUATA
Russula sinuata T. Lebel, *Austral. Syst. Bot.* **20**: 374. 2007. MycoBank MB532185.

SOLIDUS
Russula solida (Rodway) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823522.
Basionym: *Gymnomyces solidus* Rodway, *Pap. & Proc. Roy. Soc. Tasmania* '1921': 157. 1921.

SPARSUM
Russula sparsa (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820048.
Basionym: *Cystangium sparsum* T. Lebel, *Austral. Syst. Bot.* **16**: 388. 2003.

STIPITATUS
Russula stipitata (H.A. Peters) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823523.
Basionym: *Elasmomyces stipitatus* H.A. Peters, *Mycologia* **54**: 112. 1962.
Synonym: *Macowanites stipitatus* (H.A. Peters) Trappe *et al.*, *Mycotaxon* **81**: 202. 2002.

SUBALPINUS
Russula orsonmilleri Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823524.
Replaced synonym: *Martellia subalpina* A.H. Sm., *Mycologia* **55**: 439. 1963, non *Russula subalpina* O.K. Mill., Arctic and Alpine Mycology, *First International Symposium on Arcto-Alpine Mycology*, 1980 (Seattle): 136. 1982.
Synonym: *Gymnomyces subalpinus* (A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 201. 2002.

Etymology and note: In honor of Dr. Orson K. Miller Jr. for his wide ranging contributions to taxonomy of North American fungi, including sequestrate and subalpine species. This new name is erected to avoid producing a homonym with the earlier *Russula subalpina* O.K. Miller (1963*).*

SUBFULVUS
Russula subfulva (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823525.
Basionym: *Martellia subfulva* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 35. 1960.
Synonym: *Gymnomyces subfulvus* (Singer & A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 201. 2002.

SUBLEVISPORUS
Russula andaluciana T.F. Elliott & Trappe, ***nom. nov.*** MycoBank MB823526.

Replaced synonym: *Gymnomyces sublevisporus* Mor.-Arr. *et al.*, *Revista Catal. Micol.* **24**: 179. 2002, non *Russula sublevispora* (Romagn.) Kühner & Romagn., in Romagnesi, *Russules d'Europe Afr. Nord,* Essai sur la Valeur Taxinomique et Specifique des Characteres des Spores et des Revetements: 299. 1967.

Etymology and note: In reference to Andalucia, the region where it was originally collected, and to avoid producing a homonym with *Russula sublevispora* (Romagn.) Kühner & Romagn (1967).

SUBOCHRACEUS
Russula subochracea (A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823527.
Basionym: *Martellia subochracea* A.H. Sm., *Mycologia* **55**: 440. 1963.
Synonym: *Gymnomyces subochraceus* (A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 201. 2002.

SUBOLIVACEUS
Russula subolivacea (A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823528.
Basionym: *Macowanites subolivaceus* A.H. Sm., *Mycologia* **55**: 432. 1963.

SUBROSACEUS
Russula subrosacea (A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823529.
Basionym: *Macowanites subrosaceus* A.H. Sm., *Mycologia* **55**: 433. 1963.

TAPAWERA
Russula tapawera (T. Lebel) T. Lebel, *Austral. Syst. Bot.* **20**: 379. 2007. MycoBank MB531354.
Basionym: *Macowanites tapawera* T. Lebel, *New Zealand J. Bot.* **40**: 507. 2002.

THAXTERI
Russula thaxteri (Singer) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823530.
Basionym: *Martellia thaxteri* Singer, Beih. *Nova Hedwigia* **29**: 357. 1969.
Synonym: *Cystangium thaxteri* (Singer) Trappe *et al.*, *Mycotaxon* **81**: 198. 2002.

THEODOROUI
Russula theodoroui (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820049.
Basionym: *Cystangium theodoroui* T. Lebel, *Austral. Syst. Bot.* **16**: 390. 2003.

TRAPPEI
Russula trappei (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820050.
Basionym: *Cystangium trappei* T. Lebel, *Austral. Syst. Bot.* **16**: 392. 2003.

VARIABILISPORA
Russula variabilispora (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823531.
Basionym: *Martellia variabilispora* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 27. 1960.
Synonym: *Cystangium variabilisporum* (Singer & A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 198. 2002.

VARIISPORA
Russula variispora T. Lebel, *Austral. Syst. Bot.* **20**: 375. 2007. MycoBank MB532186.

VESICULOSA
Russula marshallorum T.F. Elliott & Trappe, ***nom. nov.*** MycoBank MB823553.
Replaced synonym: *Martellia vesiculosa* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**: 29. 1960, non *Russula vesciculosa* (Coker & Couch) Trappe & T.F. Elliott (this paper, see below).
Synonym: *Cystangium vesiculosum* (Singer & A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 198. 2002.

Etymology and note: In honor of Australian truffle-growing Marshall family for their strong support of Australasian truffle research and conservation. This new name is erected to avoid producing a homonym with *Russula vesciculosa* (see following entry).

VESICULOSUS
Russula vesiculosa (Coker & Couch) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823554.
Basionym: *Gymnomyces vesiculosus* Coker & Couch, *Gasteromycetes of the Eastern U.S. and Canada* (Chapel Hill): 23. 1928.
Synonym: *Hydnangium vesiculosum* (Coker & Couch) Zeller, *Mycologia* **40**: 641. 1948.

VINACEODORUS
Russula vinaceodora (Calonge & J.M. Vidal) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823532.
Basionym: *Macowanites vinaceodorus* Calonge & J.M. Vidal, *Mycotaxon* **79**: 2. 2001.

VINICOLOR
Russula vinicolor (A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823533.
Basionym: *Macowanites vinicolor* A.H. Sm., *Mycologia* **55**: 434. 1963.

WESTRESII
Russula westresii (T. Lebel) T. Lebel, Muelleria **36**: 12. 2017. MycoBank MB820051.
Basionym: *Gymnomyces westresii* T. Lebel, *Austral. Syst. Bot.* **16**: 420. 2003.

WIRRABARENSIS
Russula wirrabarensis (Grgur.) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820052.
Basionym: *Gymnomyces wirrabarensis* Grgur., *Larger Fungi of South Australia* (Adelaide): 86. 1997.

XANTHOCARPUM
Russula xanthocarpa (T. Lebel) T. Lebel, *Muelleria* **36**: 12. 2017. MycoBank MB820053.
Basionym: *Cystangium xanthocarpum* T. Lebel, *Austral. Syst. Bot.* **16**: 394. 2003.

XANTHOSPORUS
Russula xanthospora (Hawker) Trappe & T.F. Elliott. ***comb. nov.*** MycoBank MB823534.
Basionym: *Hydnangium carneum* var. *xanthosporum* Hawker, *Trans. Brit. Mycol. Soc*. **35**: 281. 1952.

Synonym: *Gymnomyces xanthosporus* (Hawker) A.H. Sm., *Mycologia* **54**: 635. 1963.

XEROPHILUS
Russula xerophila (M.E. Sm. & Trappe) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823535.
Basionym: *Gymnomyces xerophilus* M.E. Sm. & Trappe, *Mycol. Res.* **110**: 577. 2006.

YUNNANENSIS
Russula zangii Trappe & T.F. Elliott, ***nom. nov.*** MycoBank MB823536.
Replaced synonym: *Macowanites yunnanensis* M. Zang, *Acta Bot. Yunnan*. **21**: 37. 1999, non *Russula yunnanensis* (Singer) Singer, *Mycologia* **34**: 72. 1942.

Etymology and note: In honor of M. Zang, original describer of the species. This new name is erected to avoid producing a homonym with the earlier *Russula yunnanensis* (Singer) Singer (1942).

ZELLERIANUS
Russula zelleriana (Singer & A.H. Sm.) Trappe & T.F. Elliott, ***comb. nov.*** MycoBank MB823537.
Basionym: *Elasmomyces zellerianus* Singer & A.H. Sm., *Mem. Torrey Bot. Club* **21**(3): 61. 1960.
Synonym: *Macowanites zellerianus* (Singer & A.H. Sm.) Trappe *et al.*, *Mycotaxon* **81**: 203. 2002.

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