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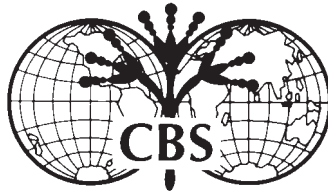
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# **Taxonomy and Pathology of *Togninia (Diaporthales)* and its *Phaeoacremonium* Anamorphs**

Lizel Mostert\*, Johannes Z. Groenewald, Richard C. Summerbell, Walter Gams  
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#### Abbreviations used:

ACT	Actin	LSU	Large subunit (28S) of ribosomal RNA gene
DIC	Differential interference contrast	MEA	2 % Malt extract agar
DM	Dissecting microscope	RFLP	Restriction fragment length polymorphism
dNTP	Deoxyribonucleotide triphosphate	rRNA	Ribosomal ribonucleic acid
GWA	Sterile grapevine cane in water agar	SEM	Scanning electron microscopy
L/W	Length/width ratio	SSU	Small subunit (18S) of ribosomal RNA gene
OA	Oatmeal agar	TUB	$\beta$ -tubulin
PDA	Potato-dextrose agar	WA	Water agar



## PREFACE

For many years plant pathologists in Europe encountered *Cephalosporium*- or *Acremonium*-like species that were associated with a grapevine decline disease. These fungi were initially documented in Sicily by Petri in 1912, and later in California by Chiarappa in 1959. In the U.S.A., researchers found that diseased grapevines examined in cross section oozed a dark, sticky substance; this material was compellingly nicknamed “black goo.” The unidentified cephalosporioid fungi seemed to be associated with its production. Coincidentally, in the medical field, Ajello *et al.* 1974 described a fungus similar to those isolated from grapevines as *Phialophora parasitica*. It was a regularly occurring causal agent of subcutaneous phaeohyphomycosis in humans.

Hawksworth *et al.* (1976) made the initial link between the medical and agricultural isolates, but also commented on the morphological diversity observed among isolates initially identified as *P. parasitica*. As the disease in grapevines spread and became more serious, researchers were forced to resolve the issues surrounding the taxonomy of the causal organisms. Research on this topic really gained momentum when Walter Gams sent all strains available at the time in the Centraalbureau voor Schimmelcultures (CBS) to Pedro Crous in South Africa. This led to a chain of events, namely the description of the genera *Phaeoacremonium* (with *P. parasitica* redispersed as

the type species *Phaeoacremonium parasiticum*) and *Phaeomoniella*, the initiation of the *International Council on Grapevine Trunk Diseases*, and a strongly focused international research programme on grapevine trunk diseases. Special emphasis was given to fungi associated with black goo or brown wood streaking, particularly *Phaeomoniella chlamydospora* and certain *Phaeoacremonium* species. In subsequent years, several students worked on this topic. The bulk of one such study forms the basis of the current issue of the *Studies in Mycology*. The relocation of Lizel Mostert and Pedro Crous from Stellenbosch University, South Africa, to CBS in the Netherlands, and the subsequent involvement of Richard Summerbell, led to the inclusion of several previously unrecognised medically important species into this study. The discovery of *Togninia* sexual states for many of the new plant- and human-derived species, and the elucidation of the differing mating strategies employed by different species, brought a further level of complexity to the project, and also raised several tantalizing new research questions.

We hope that the present monograph, together with its dichotomous and novel polyphasic online identification keys, will provide a solid foundation for *Phaeoacremonium* biosystematics, as well as a springboard facilitating future research into this exciting group of fungi.

The Authors

March 2006





# Taxonomy and Pathology of *Togninia* (*Diaporthales*) and its *Phaeoacremonium* Anamorphs

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**Abstract:** The genus *Togninia* (*Diaporthales*, *Togniniaceae*) is here monographed along with its *Phaeoacremonium* (*Pm.*) anamorphs. Ten species of *Togninia* and 22 species of *Phaeoacremonium* are treated. Several new species of *Togninia* (*T.*) are described, namely *T. argentinensis* (anamorph *Pm. argentinense*), *T. austroafricana* (anamorph *Pm. austroafricanum*), *T. krajdenii*, *T. parasitica*, *T. rubrigena* and *T. viticola*. New species of *Phaeoacremonium* include *Pm. novae-zealandiae* (teleomorph *T. novae-zealandiae*), *Pm. iranianum*, *Pm. sphinctrophorum* and *Pm. theobromatis*. Species can be identified based on their cultural and morphological characters, supported by DNA data derived from partial sequences of the actin and  $\beta$ -tubulin genes. Phylogenies of the SSU and LSU rRNA genes were used to determine whether *Togninia* has more affinity with the *Calosphaerales* or the *Diaporthales*. The results confirmed that *Togninia* had a higher affinity to the *Diaporthales* than the *Calosphaerales*. Examination of type specimens revealed that *T. cornicola*, *T. vasculosa*, *T. rhododendri*, *T. minima* var. *timidula* and *T. villosa*, were not members of *Togninia*. The new combinations *Calosphaeria cornicola*, *Calosphaeria rhododendri*, *Calosphaeria transversa*, *Calosphaeria timidula*, *Calosphaeria vasculosa* and *Jattaea villosa* are proposed.

Species of *Phaeoacremonium* are known vascular plant pathogens causing wilting and dieback of woody plants. The most prominent diseases in which they are involved are Petri disease and esca, which occur on grapevines and are caused by a complex of fungi, often including multiple species of *Phaeoacremonium*. Various *Phaeoacremonium* species are opportunistic fungi on humans and cause phaeoohyphomycosis. The correct and rapid identification of *Phaeoacremonium* species is important to facilitate the understanding of their involvement in plant as well as human disease. A rapid identification method was developed for the 22 species of *Phaeoacremonium*. It involved the use of 23 species-specific primers, including 20 primers targeting the  $\beta$ -tubulin gene and three targeting the actin gene. These primers can be used in 14 multiplex reactions. Additionally, a multiple-entry electronic key based on morphological, cultural and  $\beta$ -tubulin sequence data was developed to facilitate phenotypic and sequence-based species identification of the different *Phaeoacremonium* species. Separate dichotomous keys are provided for the identification of the *Togninia* and *Phaeoacremonium* species. Keys for the identification of *Phaeoacremonium*-like fungi and the genera related to *Togninia* are also provided.

The mating strategy of several *Togninia* species was investigated with ascospores obtained from fertile perithecia produced *in vitro*. *Togninia argentinensis* and *T. novae-zealandiae* have homothallic mating systems, whereas *T. austroafricana*, *T. krajdenii*, *T. minima*, *T. parasitica*, *T. rubrigena* and *T. viticola* were heterothallic.

**Taxonomic novelties:** *Calosphaeria rhododendri* (Rehm) L. Mostert comb. nov., *C. transversa* (Sacc. & Farim.) L. Mostert comb. nov., *Jattaea villosa* (Nitschke) L. Mostert comb. nov., *Phaeoacremonium iranianum* L. Mostert, Gräf., W. Gams & Crous sp. nov., *Pm. sphinctrophorum* L. Mostert, Summerb. & Crous sp. nov., *Pm. theobromatis* L. Mostert, H.C. Evans, Summerb. & Crous sp. nov., *Togninia argentinensis* L. Mostert, W. Gams & Crous sp. nov. (anamorph *Pm. argentinense* L. Mostert, W. Gams & Crous sp. nov.), *T. austroafricana* L. Mostert, W. Gams & Crous sp. nov. (anamorph *Pm. austroafricanum* L. Mostert, W. Gams & Crous sp. nov.), *T. krajdenii* L. Mostert, W. Gams & Crous sp. nov., *Pm. novae-zealandiae* L. Mostert, W. Gams & Crous sp. nov., *T. parasitica* L. Mostert, W. Gams & Crous sp. nov., *T. rubrigena* L. Mostert, W. Gams & Crous sp. nov., *T. viticola* L. Mostert, W. Gams & Crous sp. nov.

**Key words:** Actin,  $\beta$ -tubulin, BioloMICS, *Calosphaerales*, LSU, morphology, *Phaeoacremonium*, phylogeny, SSU, systematics, *Togninia*, *Togniniaceae*.

## INTRODUCTION

The genus *Togninia* Berl. was introduced by Berlese (1900) with 12 species and one variety. Subsequently, an additional three species of *Togninia* were described (Eriksson & Yue 1990, Hausner *et al.* 1992). Hausner (1992) commented that the anamorph of *Togninia* had characters of *Phialophora* Medlar and *Acremonium*

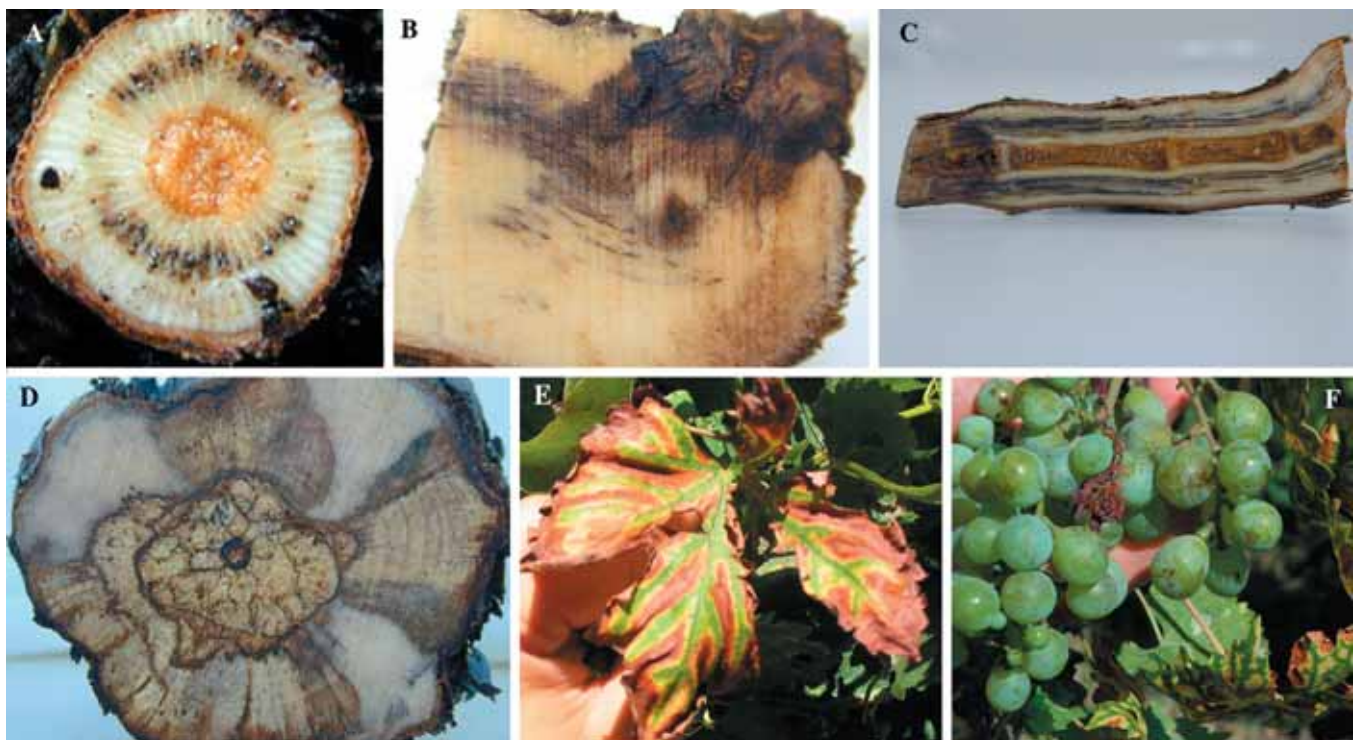
Link : Fr., but also did not fit the descriptions of these genera. The anamorph genus *Phaeoacremonium* W. Gams, Crous & M.J. Wingf. was introduced by Crous *et al.* (1996) with five new species; the type species was the only well known species in the group, *Phialophora parasitica* Ajello, Georg & C.J.K. Wang. Upon closer examination, *Phaeoacremonium chlamydosporum* W. Gams, Crous, M.J. Wingf. & L. Mugnai was found to

be morphologically and phylogenetically different from otherspeciesinthe genus, and a new genus was established for it, namely *Phaeomoniella* Crous & W. Gams (*Pa.*) (Crous & Gams 2000). *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams is distinct from *Phaeoacremonium* in that it has a *Phoma*-like synanamorph, a yeast-like growth in culture, conidiophores which are prominently darkened in their basal part, and conidia which are subhyaline and straight (Crous & Gams 2000). The significance of these differences was supported by DNA sequence data (Dupont *et al.* 1998, Groenewald *et al.* 2001). Since 1996, 11 new species of *Phaeoacremonium* have been described (Dupont *et al.* 2000, Groenewald *et al.* 2001, Mostert *et al.* 2005). The link between *Togninia* and its anamorph, *Phaeoacremonium* was only recently confirmed (Mostert *et al.* 2003, Pascoe *et al.* 2004, Rooney-Latham *et al.* 2005a).

The substrate range of *Phaeoacremonium* includes woody plants, humans and larvae of bark beetles. A few strains have also been isolated from soil (Crous & Gams 2000, Dupont *et al.* 2002). However, the majority of *Phaeoacremonium* species have been isolated from diseased woody host plants. The *Phaeoacremonium* strains from grapevines (*Vitis vinifera* L.) have been intensively studied because of the involvement of this genus in two complex fungal diseases, namely Petri disease and esca. *Phaeoacremonium aleophilum*

W. Gams, Crous, M.J. Wingf. & L. Mugnai appears to be the most widely distributed species, as well as the most common in grapevines (Larignon & Dubos 1997, Mugnai *et al.* 1999, Groenewald *et al.* 2001). The occurrence of the other *Phaeoacremonium* species differs among grape-growing countries. Other species that have also been isolated in relatively high frequencies from grapevines include *Pm. parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf in Argentina (Dupont *et al.* 2002) and *Pm. viticola* J. Dupont in France (Dupont *et al.* 2000). The relative importance of the different *Phaeoacremonium* species in Petri and esca diseases has been difficult to assess since strains are often not identified to species level, and since several new species have only recently been described.

Petri disease causes stunted growth and dieback of young and old grapevines. The disease can manifest as a sudden collapse of the foliage, but more often takes the form of a slow decline accompanied by weak growth, various leaf symptoms (interveinal chlorosis, leaf necrosis, wilting) and gradual death of grapevines. Petri disease often occurs on 1–5-yr-old grapevines and has caused significant losses in newly planted vineyards (Bertelli *et al.* 1998, Scheck *et al.* 1998, Ferreira *et al.* 1999, Mugnai *et al.* 1999, Morton 2000, Pascoe & Cottral 2000). Up to 50 % of newly planted vines have been lost (Pascoe & Cottral 2000).



**Fig. 1.** Symptoms associated with Petri disease (A–C) and esca (D–F). A. Black spots visible on rootstock ‘101–14 Mgt’ of a one-yr-old vine. B. Black streaking associated with natural pruning wound infection of a nine-yr-old ‘Shiraz’ vine. C. Spur showing typical brown to black streaking 14 months after inoculated on the pruning wound with a spore suspension of *Pa. chlamydospora*. D–E. Cross-section showing wood discoloration and “tiger stripes” on the leaves of an 18-yr-old ‘Chenin Blanc’ vine. F. Brown spots or “black measles” symptoms on berries of a ‘Chenin Blanc’ vine. Photographs B–D by F. Halleen and E–F by L. Morton.

Internal symptoms can normally be seen when transverse or longitudinal cuts are made in the trunk and shoots. These include black spots (Fig. 1A) and dark brown to black streaking (Fig. 1B–C) of the xylem tissues. The damaged xylem vessels often ooze black sap and therefore, the popular name “black goo” has emerged. The black discoloration of the xylem tissue is caused by the formation of tylosis, gums and phenolic compounds by the host as a reaction to the presence of the fungus in the xylem tissue (Mugnai *et al.* 1999, Del Rio *et al.* 2001). The blocking of the xylem tissue prohibits the normal uptake of water. An increase in Petri disease symptoms occurs during times of high water demand when the host is predisposed by water stress (Ferreira *et al.* 1999). Petri disease is caused by a combination of *Pa. chlamydospora* and several species of *Phaeoacremonium* (Scheck *et al.* 1998, Mugnai *et al.* 1999, Groenewald *et al.* 2001). *Phaeomoniella chlamydospora* has been more often associated with typical Petri disease symptoms than species of *Phaeoacremonium* (Mugnai *et al.* 1999, Chicau *et al.* 2000, Edwards & Pascoe 2004).

Esca can be typically identified by internal wood decay (Fig. 1D), symptoms on leaves (Fig. 1E) and berries (Fig. 1F). Various types of wood deterioration are observed when a transverse cut is made. Black spots appear as in the case of Petri disease, but also pink-brown or dark red-brown areas and a central pale-coloured necrosis of soft consistency (white rot) surrounded by a dark borderline (Larignon & Dubos 1997, Mugnai *et al.* 1999). Symptoms on the leaves consist of interveinal regions of chlorotic and yellowish tissue that turns yellow-brown or red-brown and have also been described as “tiger stripes.” Esca has been referred to as “black measles” because of the small, dark brown to purple spots that can develop on the berries. Foliar and fruit symptoms do not necessarily appear on the same diseased plant every year (Mugnai *et al.* 1999). In severe cases “apoplexy” can occur when vines or vine-parts suddenly wilt and die during hot, dry circumstances in the summer. Fungi that have been associated with esca symptoms include the wood-rotting basidiomycetes *Fomitiporia (F.) mediterranea* M. Fischer and *F. punctata* (P. Karst.) Murrill. To a lesser extent, *Stereum hirsutum* (Willd. : Fr) Pers. may also be involved. *Phaeomoniella chlamydospora* and *Pm. aleophilum* are among the principal hyphomycetes associated with esca symptoms (Larignon & Dubos 1997, Mugnai *et al.* 1999, Ari 2000, Cortesi *et al.* 2002, Fischer 2002). It is the combination of these fungi that causes “esca proper” (Surico 2001), affecting mostly vines older than 15 yr. However, over the past decade younger vines have also been observed with esca symptoms (Edwards *et al.* 2001b). Young vines infected with *Pa. chlamydospora* and/or *Phaeoacremonium* species revealing Petri disease



**Fig. 2.** A foot with white grain eumycetoma caused by *Phaeoacremonium kraidenii* (photograph by A.A. Padhye).

symptoms can later develop esca symptoms after the infection and colonisation of *F. mediterranea*, *F. punctata* or *S. hirsutum*. The degree of involvement of different *Phaeoacremonium* species in esca is uncertain because the *Phaeoacremonium* strains have often not been identified to species level (Serra *et al.* 2000, Gatica *et al.* 2001).

*Phaeoacremonium* species associated with human infections cause phaeohyphomycosis (defined as tissue invasion by fungi with melanised cell walls), usually specifically seen as phaeohyphomycotic cyst, a closed, painless, pus-filled cavity under the skin, seen in biopsy to have a border of fungal growth into the surrounding dermis (Fig. 2) (Ajello *et al.* 1974, Crous *et al.* 1996, Padhye *et al.* 1998, Guarro *et al.* 2003). The species of *Phaeoacremonium* most commonly causing human infections are *Pm. parasiticum* and *Pm. kraidenii* (Mostert *et al.* 2005). Observations over several years have shown that species of *Phaeoacremonium* are opportunistic pathogens needing a traumatic subcutaneous injection or a predisposed host to be able to infect and cause disease.

## OVERVIEW OF *TOGNINIA* AND *PHAEOACREMONIUM*

### Classification of *Togninia* and its relatives

*Togninia* has historically been classified in the *Calosphaeriales* (Barr 1983). The order *Calosphaeriales* was erected to accommodate members of *Ascomycetes* with broad and tapered paraphyses and hyaline, often allantoid ascospores (Barr 1983). Other features of this order included the presence of asci and paraphyses along the entire inner region of the centrum, and asci that are stipitate and clavate, forming small fascicles from short ascogenous hyphae, or sessile and oblong or subglobose, in a spicate cluster from proliferating

ascogenous hyphae. Barr (1985) outlined the history of the *Calosphaeriaceae* and the corresponding genera, and published the first modern concept of this family. Eight genera were included: *Calosphaeria* Tul. & C. Tul., *Enchnoa* Fr., *Graphostroma* Piroz., *Jattaea* Berl., *Pleurostoma* Tul. & C. Tul., *Romellia* Berl., *Scoptria* Nitschke and *Togninia* Berl. (Barr 1985). Barr (1985) found that differences in the morphology of ascospores were of insufficient value to employ at generic level, and instead chose features such as ascus shape, the arrangement of asci on ascogenous hyphae, the structure and presence or absence of papilla or necks, and the occurrence and arrangement of stromatic tissues or subiculum. In a later study, Barr (1993b) emended the concept of the *Calosphaeriales*, and five genera were recognised, namely *Calosphaeria*, *Enchnoa*, *Jattaea*, *Pachytrype* Berl. ex M.E. Barr and *Pleurostoma*. *Romellia*, *Togninia* and *Erostella* were synonymised under *Pleurostoma*, and *Wegelina* Berl. under *Calosphaeria* (Barr 1993b). The genus *Pachytrype* was thought to be diarthaceous because of the concentration of asci in its ascomatal centrum, but more detailed examination of the hymenial layer revealed the spicate arrangement of the asci indicating that it belongs in the *Calosphaeriales* (Barr 1993b). It was clear that the 8-spored *Togninia* was not a synonym of the multispored *Pleurostoma* (Mostert et al. 2003); this conclusion was soon confirmed with DNA sequence data (Vijaykrishna et al. 2004). There was uncertainty as to whether the name *Erostella* or *Togninia* should be taken up, since both had *Calosphaeria minima* Tul. & Tul. as lectotype. This issue was in fact resolved by Clements & Shear (1931), who designated *T. minima* as lectotype of *Togninia*. Arguments around the interpretation of the Latin used by Berlese (1900) support the lectotypification by Clements & Shear (1931) (Hausner et al. 1992, Holm 1992, Mostert et al. 2003).

*Graphostroma* has a *Nodulosporium*-like anamorph, a feature consistent with placement in the *Xylariales* (Pirozynski 1974). Barr (1993b) erected the family *Graphostromataceae* to accommodate this stromatic, formerly calosphaeriaceous genus. The *Calosphaeriales* until recently included six non-stromatic genera, namely *Calosphaeria*, *Jattaea*, *Pleurostoma*, *Romellia*, *Togninia*, *Wegelina*, and the stromatic *Pachytrype* (Réblová et al. 2004). Whether *Enchnoa* Fr. should remain within the *Calosphaeriales* is uncertain because its dark tomentum and subiculum appear to be inconsistent with placement in this family (Barr 1985). Fresh specimens of *Calosphaeria pulchella* (Réblová et al. 2004) and *Pleurostoma ootheca* (Vijaykrishna et al. 2004) were recently collected. Cultures of these fungi made DNA studies possible, and these studies in turn shed new light on the phylogenetic relationships of fungi in the *Calosphaeriales*. A collection was also

made of a new genus having morphological similarities with *Togninia* and was described as *Togniniella* Réblová, L. Mostert, W. Gams & Crous (Réblová et al. 2004). The phylogenetic analysis of the LSU and SSU rRNA genes showed that *Togninia* formed a unique cluster within the *Diaporthales* (Réblová et al. 2004). The *Diaporthales* and *Calosphaeriales* also appeared to be two of the more closely related groups among the perithecial ascomycetes, indicating that they share very recent common ancestry (Réblová et al. 2004). Two new families were also erected, namely the *Pleurostomataceae* Réblová, L. Mostert, W. Gams & Crous (*Calosphaeriales*), and the *Togniniaceae* Réblová, L. Mostert, W. Gams & Crous (*Diaporthales*). There is little similarity in morphology among the genera within the *Diaporthales* and the *Togniniaceae*. However, the *Togniniaceae* and the *Gnomoniaceae* (*Diaporthales*) have the following in common; dark, globose, long-beaked and non-stromatic perithecia, asci with a rounded base, floating freely within the centrum, a phialidic anamorph, and an ecology distinguished by phytopathogenic growth in woody plants (Réblová et al. 2004).

The anamorphs within the *Calosphaeriales* are reported as either phialidic or holoblastic. *Pachytrype* has a *Cytospora*-like anamorph (Barr 1993b); *Calosphaeria fagi* Samuels & Candoussau has *Ramichloridium*-like and *Sporothrix*-like synanamorphs (Samuels & Candoussau 1996); *Calosphaeria* (Pers. : Fr.) J. Schöt. has an *Acremonium*-like anamorph, namely *Calosphaeriophora* Réblová, L. Mostert, W. Gams & Crous; *Togniniella* Réblová, L. Mostert, W. Gams & Crous has a *Phaeoacremonium*-like anamorph named *Phaeocrella* Réblová, L. Mostert, W. Gams & Crous, and *Pleurostoma* has a *Phialophora*-like anamorph, *Pleurostomophora* D. Vijaykrishna, L. Mostert, R. Jeewon, W. Gams, K.D. Hyde & Crous. The coelomycetous anamorphs of the *Diaporthales* are different from the hyphomycete anamorphs of the *Togniniaceae* and *Calosphaeriales*, except that, as mentioned above, *Pachytrype*, though classified in the *Calosphaeriales*, has a *Cytospora*-like pycnidial anamorph. The collection of a fresh specimen of *Pachytrype* will make its phylogenetic placement possible and clarify the relevance of various morphological characters in the *Calosphaeriales*.

Most of the older names in *Togninia* belong to species initially described in *Calosphaeria* (Berlese 1900). Barr (1985) only treated *Togninia minima*, which she saw as a synonym of *T. alnicola*. The other species that Berlese (1900) illustrated, *T. ambigua*, *T. vasculosa* and *T. quarternarioides*, were excluded because they had clavate, stipitate asci not fitting the concept of *Togninia* (Barr 1985). Three additional species have since been accepted into *Togninia*, *T. inconspicua* (Rehm) J.Z. Yue & O.E. Eriksson (Eriksson & Yue 1990), *T.*

*fraxinopennsylvanica* (Hinds) Hausner, Eyjólfsdóttir & J. Reid, and *T. novae-zealandiae* Hausner, Eyjólfsdóttir & J. Reid (Hausner *et al.* 1992). The latter two species could be distinguished based on their ascospore morphology, ascus width, perithecial size and ornamentation, neck length and cultural growth rates. The *Togninia* teleomorphs described below for *Pm. argentinense*, *Pm. austroafricanum*, *Pm. parasiticum*, *Pm. viticola*, *Pm. krajdennii* and *Pm. rubrigenum* were found by means of *in vitro* mating studies (this study). The genus *Togninia* is distinguished by having ascomata with distinct necks (usually more prominent *in vitro* than *in vivo*), producing unitunicate asci that are oblong with clearly truncate bases and thickened apices. Asci are arranged in a spicate formation on the ascogenous hyphae, paraphyses are hyaline and septate, and ascospores are aseptate and hyaline with shapes ranging from allantoid to ellipsoidal to oblong-ellipsoidal (Barr 1985, Hausner *et al.* 1992).

The conditions under which perithecia are formed *in vitro* are variable for different species of *Phaeoacremonium*. Perithecia of *T. minima* formed *in vitro* after 2–3 wk of incubation on grapevine canes on water agar at 22 °C under continuous white light (Mostert *et al.* 2003). Rooney-Latham *et al.* (2005a) used a 12 h photoperiod with fluorescent light and grapevine shavings to induce perithecial formation after 4–5 wk. Perithecia formed after 11–22 wk when field-collected grapevine pieces showing vascular streaking were incubated under moist conditions at 18–22 °C (Pascoe *et al.* 2004). Hausner *et al.* (1992) found that perithecia of *T. fraxinopennsylvanica* formed sporadically and were not stimulated by light. However, temporarily flooding of cultures did promote the formation of perithecia. In the case of *T. novae-zealandiae*, protoperithecia formed after 5–7 wk, and were stimulated by exposure to light (Hausner *et al.* 1992). The mating strategy of *T. minima* was determined with *in vitro* mating tests to be bi-allelic heterothallic (Mostert *et al.* 2003, Rooney-Latham *et al.* 2005a).

#### **Classification of *Phaeoacremonium* and its relatives**

*Phialophora parasitica* was first described from a subcutaneous infection of a human patient who had undergone a kidney transplant (Ajello *et al.* 1974). Strains earlier identified as *Cephalosporium* Corda and associated with dieback symptoms of woody hosts (Petri 1912, Halliwell 1966) were confirmed by Hawksworth *et al.* (1976) as *Phialophora parasitica*. Petri (1912) identified fungi in the genera *Cephalosporium* and *Acremonium* Link from grapevines with esca symptoms. Chiarappa (1959) also reported a *Cephalosporium* species (represented by CBS 239.74) associated with grapevines affected by black measles, but Hawksworth *et al.* (1976) found this species to differ morphologically from the *Ph. parasitica* isolated from other woody hosts.

Upon later investigation, Chiarappa's *Cephalosporium* species was found to represent *Pm. chlamydosporum* (Crous *et al.* 1996) (later reclassified as *Phaeoconiella chlamydospora*). Subsequently, the *Cephalosporium* and *Acremonium* fungi isolated from grapevines were identified as *Pm. chlamydosporum* and *Pm. aleophilum* on the basis of Petri's description (Mugnai *et al.*, 1999). The genus *Phaeoacremonium*, with *Pm. parasiticum* as type, is morphologically intermediate between *Acremonium* and the traditional, pre-molecular concept of *Phialophora* Medlar. *Phaeoacremonium* can be distinguished from *Phialophora s. l.* by its aculeate phialides and inconspicuous, non-flaring collarettes, and from *Acremonium* by its pigmented vegetative hyphae (Crous *et al.* 1996). The genus *Phialophora* has proven to be polyphyletic, including various newly named genera (Gams & McGinnis 1983, Gams 2000, Harrington & McNew 2003).

Based on DNA phylogeny of the 28S rRNA gene, *Phaeoacremonium* has been stated to be close to the *Magnaporthaceae* (Dupont *et al.* 1998). This affinity now appears to have been an artifact of an analysis including an inadequate number of taxa. Later studies including more taxa have consistently shown that *Phaeoacremonium* is related to the *Diaporthales* (Mostert *et al.* 2003, Réblová *et al.* 2004). The anamorph genus characteristic of the *Magnaporthaceae*, *Harpophora* W. Gams, is morphologically different from *Phaeoacremonium* in that it has more broadly spreading colonies, conspicuous, divergent collarettes and prominently curved cylindrical conidia.

Six species of *Phaeoacremonium* were originally identified based on morphological and cultural characters (Crous *et al.* 1996). It soon became apparent that *Pm. chlamydosporum* represented a new genus, *Phaeoconiella* Crous & W. Gams, which resided within the *Chaetothyriales* (Crous & Gams 2000). Later, two additional species of *Phaeoacremonium*, *Pm. viticola* and *Pm. mortoniae*, were described based on phenotypic characters as well as DNA data (Dupont *et al.* 2000, Groenewald *et al.* 2001). A further nine species were identified based on morphological characters and combined  $\beta$ -tubulin, actin and calmodulin gene sequence data (Mostert *et al.* 2005). Micromorphological characters found to be useful in distinguishing species were conidiophore morphology, phialide type and shape, size of hyphal warts, and to a lesser extent, conidial size and shape (Mostert *et al.* 2005). Cultural characters that were useful included the colour of colonies on 2 % malt extract agar (MEA), the production of yellow pigment on potato-dextrose agar (PDA), the growth rate of colonies at 25 °C and the maximum temperature for growth *in vitro* (Mostert *et al.* 2005). Yellow pigment production on oatmeal agar (OA) was introduced as a definitive character by Dupont

et al. (2000), and our studies have confirmed that OA is an excellent medium to test pigment production.

The genus *Phaeoacremonium* is characterised by its mycelial bundles, conidiophores that can be branched or not, slender phialides occurring in three size types and bearing narrowly funnel-shaped collarettes, and conidia that aggregate in slimy heads, and that range from oblong-ellipsoidal to allantoid in shape. Generic descriptions of *Phaeoacremonium* have been published by Crous et al. (1996) and Mostert et al. (2005).

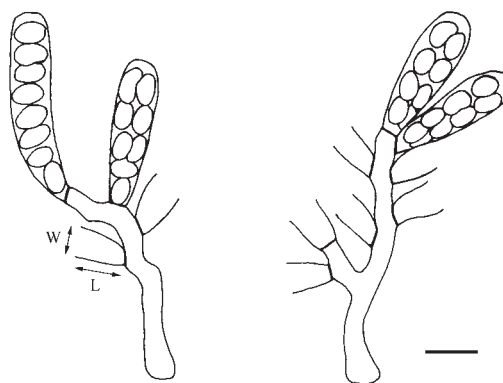
Within the *Calosphaeriales*, anamorphs are found that are morphologically more-or-less similar to *Phaeoacremonium*. These include *Pleurostomophora* (anamorph of *Pleurostoma*), *Calosphaeriophora* (anamorph of *Calosphaeria*) and *Phaeocrella* (anamorph of *Togniniella*). The conidiophores of *Pleurostomophora* resemble those of *Phialophora* s. str. in that they are single, separate and mostly monophialidic, and often have prominent, flaring collarettes (Vijaykrishna et al. 2004). The three species included in *Pleurostomophora* exhibit some morphological differences. *Pleurostomophora richardsiae* (Nannf. apud Melin & Nannf.) L. Mostert, W. Gams & Crous produces dimorphic conidia. One of the conidial types is a distinctive pigmented, subglobose type produced on phialides with flaring collarettes. The other *Pleurostomophora* species lack this feature. *Pleurostomophora repens* is distinct in that it has complex conidiophores with radiating phialides. *Calosphaeriophora*, represented by *Calosphaeriophora pulchella* Réblová, L. Mostert, W. Gams & Crous, is morphologically similar to *Acremonium*, but differs in producing subcylindrical, mostly unbranched conidiophores bearing hyaline phialides featuring a brown pigmented apical region below deep, flaring collarettes (Réblová et al. 2004). *Phaeocrella*, represented by a single species, *Phaeocrella acerosa* Réblová, L. Mostert, W. Gams & Crous, is morphologically similar to *Phaeoacremonium*, but differs in that the conidiophores are regularly branched and have prominent constrictions at the septa. Its collarettes are shallow and flaring, unlike those of *Phaeoacremonium*. Other hyphomycetous genera that are more or less morphologically similar to *Phaeoacremonium* include *Phialemonium* W. Gams & McGinnis, *Exophiala* J.W. Carmich., *Lecythophora* Nannf., *Margarinomyces* O. Laxa, *Catenulifera* Hosoya, *Monocillium* S.B. Saksena, *Chloridium* Link, *Exochalara* W. Gams & Hol.-Jech., *Monilochaetes* Ellis & Halsted, *Pseudogliomastix* W. Gams, and *Cadophora* Lagerberg & Melin. A key to allow morphological distinction of these genera is given in the 'Results' section.

### *Togninia*

*Perithecia*: Perithecia are aggregated or separate, occurring superficially or immersed in the periderm

without any stroma, globose to subglobose, with 1–3 dark brown to black, elongated necks, which are branched or not. In the generic circumscriptions of *Togninia*, Berlese (1900) and Barr (1985) described the perithecia as having short necks or beaks. Also, Tulasne & Tulasne (1863) described *Togninia minima* as having necks no longer than the diameter of the ostiole. However, Barr (1985) illustrated a perithecium with a relatively long, curved neck. Field collections made of *T. minima* revealed perithecia with long necks (275–880 µm), branched or not, with 1–3 necks per perithecium (Rooney-Latham et al. 2005b). The dimensions of necks given in the present study are mostly from perithecia induced in culture on grapevine canes, except in the case of *T. inconspicua*. *Togninia minima* produced necks of up to 1800 µm in culture. From these observations it is clear that cultural conditions favour the development of relatively long necks.

*Ascogenous hyphae*: Ascogenous hyphae are hyaline and branched, and elongate sympodially during ascus formation. The mature asci leave their basal part, seen as a remnant base, on the ascogenous hyphae after dehiscence (Fig. 3). These remnant bases do not always remain attached. Because of sympodial proliferation, the ascogenous hyphae appear to have a 'zig-zag' formation under the light microscope. This spicate arrangement of the asci on the ascogenous hyphae has been used by Barr (1985) as a feature distinguishing *Togninia* and *Pleurostoma* from other genera in the *Calosphaeriales*. Other genera that also have a spicate ascal arrangement include *Pachytrype* (Barr 1993b), *Romellia* and *Togniniella* (Réblová et al. 2004). The length of the ascogenous hyphae has proven to be a useful character for distinguishing the genera with spicately arranged asci. In the case of *Pleurostoma* (*Pleurostomataceae*), for instance, the ascogenous hyphae are shorter than those seen in *Togninia*, *Togniniella* and *Romellia*.



**Fig. 3.** Ascogenous hyphae of *T. viticola* with asci and remnant bases still attached [arrows indicate the direction of the length (L) and width (W) measurements as reported in the 'Taxonomy' section]. Scale bar = 10 µm.

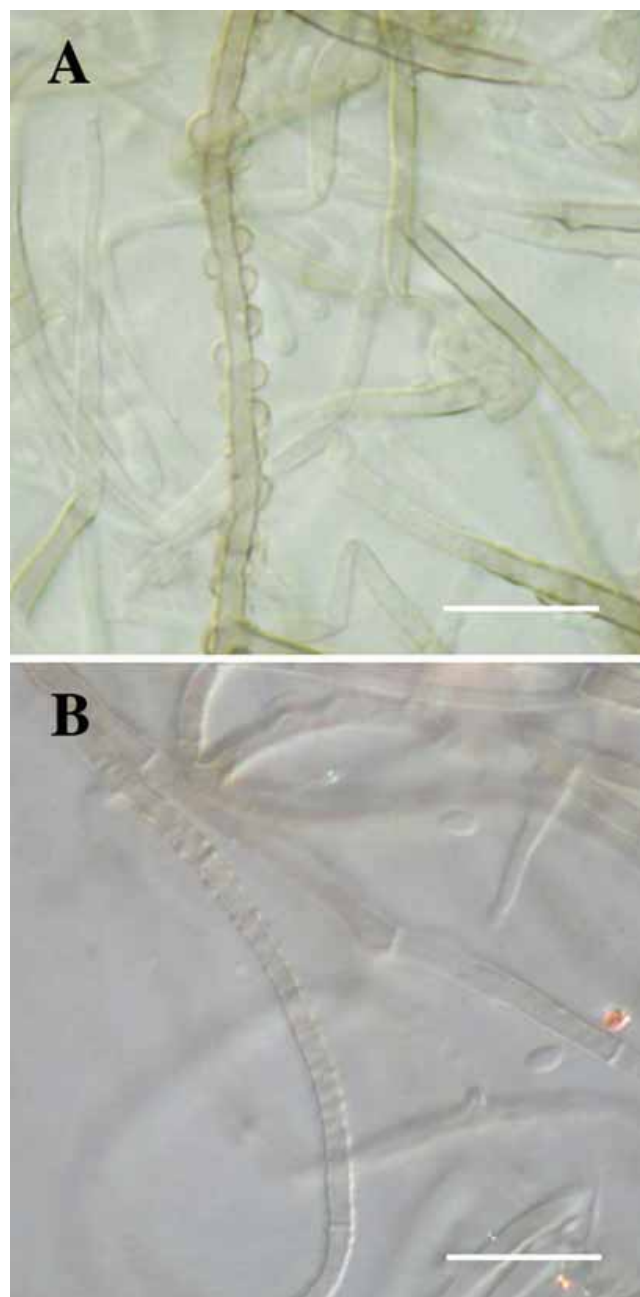
**Asci:** Asci arise from croziers, which although not easily seen; are arranged in acropetal succession on the ascogenous hyphae. The asci are unitunicate, 8-spored and mostly biseriata. The apical region is thickened with a nonamyloid apical ring (negative reaction in Melzer's reagent). Asci are clavate with bluntly rounded apices, and bluntly obtuse bases without a stalk. Ascus deliquescence occurs usually in the perithecium, as can be seen in the presence of ascospores in the spore droplets at the tip of perithecial necks in culture. However, when dried perithecia are remoistened and submerged in a film of water, whole asci are released from the necks, followed by forcible ascospore discharge (Rooney-Latham *et al.* 2004). Ascus size varies among *Togninia* species. *Togninia inconspicua* has longer and wider asci than any other species of the genus. In contrast, *T. parasitica* and *T. rubrigena* have asci that are shorter ( $< 20 \mu\text{m}$ ) than those of other *Togninia* species.

**Ascospores:** Ascospores are hyaline and aseptate, and may be allantoid, reniform, cylindrical or oblong-ellipsoidal. They are sometimes biguttulate. Their size range is  $3\text{--}5\text{--}(6.5) \times 1\text{--}2\text{--}(2.5) \mu\text{m}$ , except in the large-spored *T. inconspicua*, where the range is  $7\text{--}10 \times 1.5\text{--}2 \mu\text{m}$ . Ascospore shape is a useful character in that species consistently have either allantoid or non-allantoid ascospores. Species with allantoid ascospores include *T. inconspicua*, *T. minima*, *T. krajdennii*, *T. parasitica* and *T. rubrigena*. *Togninia argentinensis*, *T. fraxinopennsylvanica*, *T. novae-zealandiae* mostly have oblong-ellipsoidal ascospores, and the species *T. austroafricana* and *T. viticola* mostly reniform ascospores.

**Paraphyses:** Paraphyses are abundant. They are long, hyaline structures, sometimes branched in the basal region, broadly cellular, slightly constricted at the septa, and tapered towards the apex. In a few species, namely *T. krajdennii*, *T. parasitica* and *T. rubrigena*, the paraphyses become distinctly thread-like towards the apex. According to Hausner *et al.* (1992), paraphyses were abundant in young perithecia of *T. fraxinopennsylvanica* and *T. novae-zealandiae*, but collapsed and became inconspicuous in more mature perithecia.

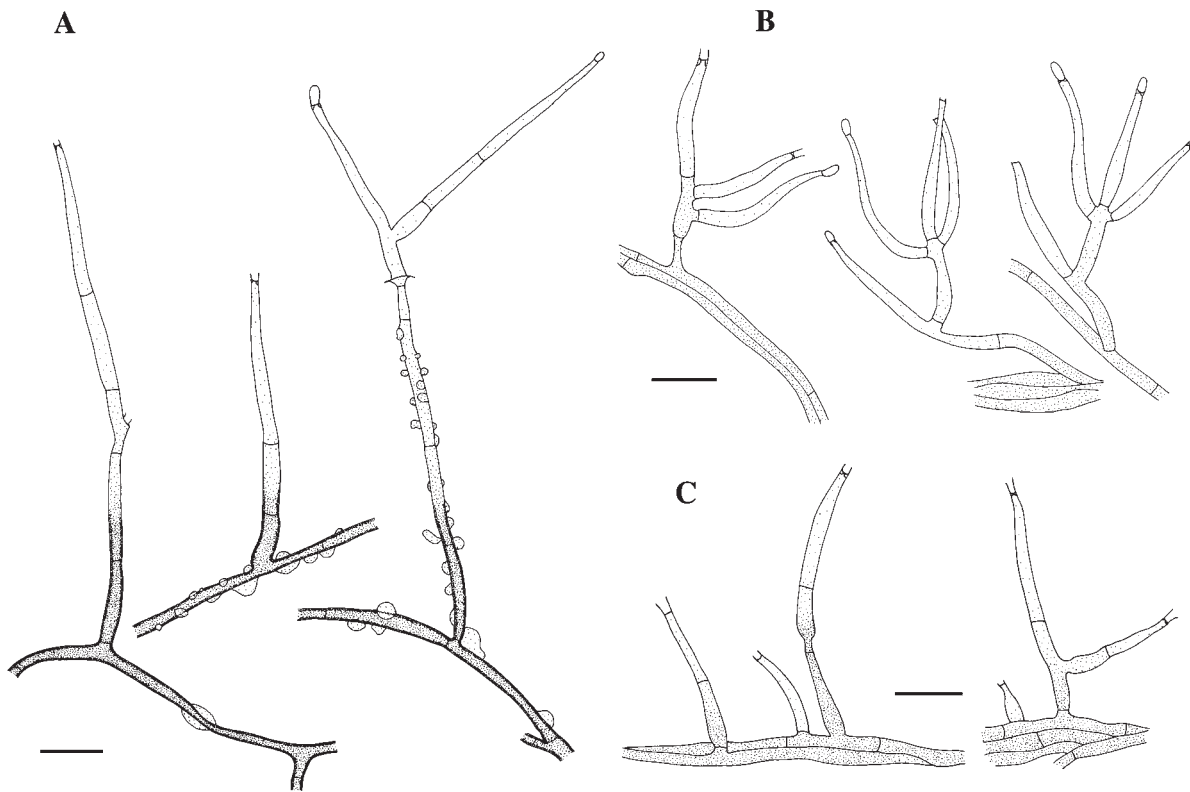
### ***Phaeoacremonium***

**Mycelium:** Mycelium consists of branched, septate hyphae that occur singly or in bundles of 4–27. The colour of the mycelium varies so that there are relatively pale and relatively deeply brown-coloured species, but in general the mycelium is medium brown, becoming paler towards the area where the conidia are formed. Exudate droplets, perceived as wart-like structures under the light microscope, differ in density and size among the different species. *Phaeoacremonium parasiticum*, for instance, can easily be identified

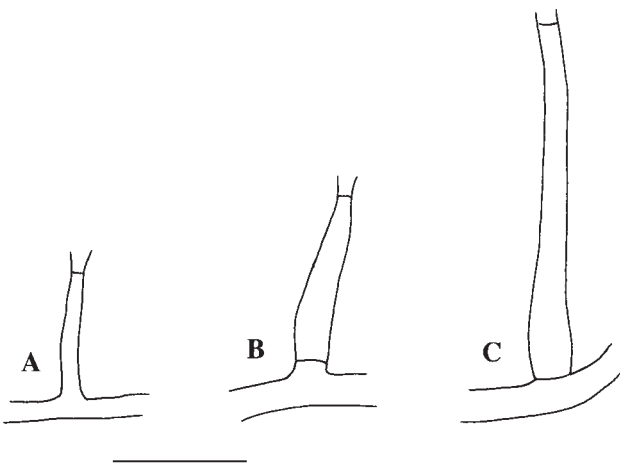


**Fig. 4.** A. Dense, prominent exudate droplets (perceived as warts under the light microscope) on mycelium of *Pm. parasiticum* (CBS 860.73). B. Smaller, less dense exudate droplets on mycelium of *Pm. alvesii* (CBS 110034) grown on MEA. Scale bars =  $10 \mu\text{m}$ .

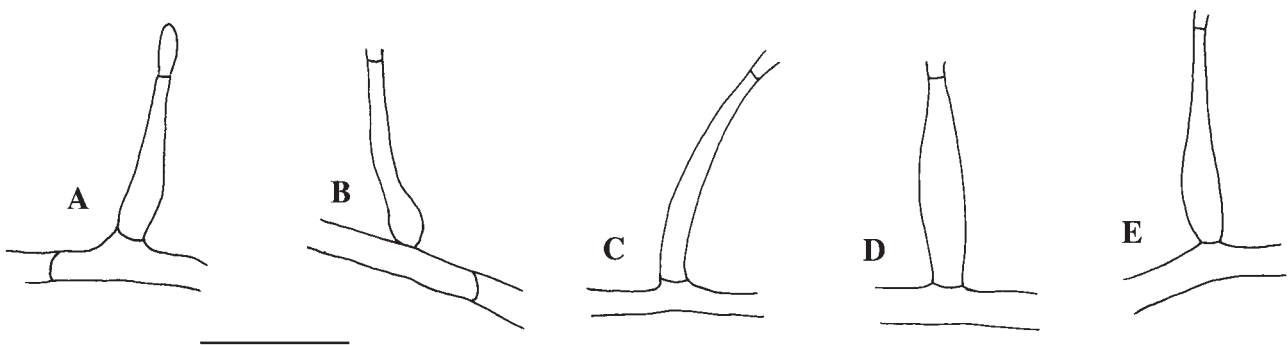
based on the frequent occurrence of very prominent hyphal warts up to  $3 \mu\text{m}$  diam (Fig. 4A). By contrast, the hyphae of other taxa have smaller warts that form in moderate densities (Fig. 4B). The occurrence of warts is influenced by the age of the culture and the medium used. Warts are not common on mycelium at the margin of a colony or on mycelium from colonies younger than 9 d (Mostert *et al.* 2005). They are also less frequently observed on the low-nutrient media potato-carrot agar (PCA) and water agar (WA) than on 2 % MEA (Mostert *et al.* 2005). Additional mycelial ornamentation was due to irregularities in the cell wall surface, seen as verruculose (lightly textured) to



**Fig. 5.** Conidiophore morphology pattern of *Phaeoacremonium*. A. Long, branched conidiophores of *Pm. parasiticum* (CBS 860.73). B. Branched conidiophores of *Pm. inflatipes* (CBS 391.71). C. Short, usually unbranched conidiophores of *Pm. rubrigenum* (CBS 498.94). Scale bars = 10  $\mu$ m.



**Fig. 6.** Three types of phialides. A. Type I phialide (adelophialide). B. Type II phialide. C. Type III phialide. Scale bar = 10  $\mu$ m.



**Fig. 7.** Different shapes of Type II and III phialides. A. elongate-ampulliform and attenuated at the base. B. elongate-ampulliform and constricted at the base. C. subcylindrical. D. navicular; E. subulate. Scale bar = 10  $\mu$ m.



verrucose (roughly textured) roughening. The presence of verrucose mycelium is a useful character for distinguishing taxa that possess this feature from those with smooth hyphae.

**Conidiophores:** Conidiophore structure is an important taxonomic feature. It distinguishes species having predominantly long (*Pm. parasiticum*) or frequently branched (*Pm. inflatipes*, *Pm. sphinctrophorum*) conidiophores from species with short and infrequently branched or unbranched conidiophores (Fig. 5A–C).

**Conidiogenous cells:** Phialides are either discrete (arising directly from the mycelium) or integrated in conidiophores. Three types of phialides were identified in *Phaeoacremonium* occurring on aerial mycelium (Hausner *et al.* 1992), i.e. types I–III (Fig. 6A–C). Phialides differ in size and shape as shown in Fig. 7A–E. Phialide sizes mostly overlap among the different species of *Phaeoacremonium*. However, the predominance of one or more of these types proved to be distinctive for certain species. Type I phialides, for which the specialised term “adelophialides” (Gams 1971) may also be used, are the shortest, (2–)3–11(–17)  $\mu\text{m}$ , and have no basal septum; type II phialides are medium-sized, (5–)9–14(–16)  $\mu\text{m}$ , and are elongate-ampulliform or navicular in shape; type III phialides are the longest, (10–)15–23(–34)  $\mu\text{m}$ , and are subcylindrical, navicular or subulate. Phialides are mostly monophialidic, that is, they produce conidia from a single apical aperture. However, some type II and a few type I phialides proliferate to become polyphialidic (see descriptions of *Pm. scolyti* and *Pm. krajdenii*), ultimately terminating in a forked apex consisting of two conidiogenous apertures. Phialide rejuvenation (the growing of a new phialide through the tip of an existing phialide) is observed in several species. In this process, each newly formed phialide often becomes strongly swollen at the base, just above the point where it has grown out of the narrow apex of the preceding phialide. Phialides produced on and in the agar surface are described separately; they are predominantly hyaline and adelophialidic.

**Conidia:** Conidia occur in slimy heads at the phialide apices, and are hyaline and aseptate. They vary in shape from oblong-ellipsoidal to obovate to cylindrical to allantoid to reniform. In contrast, the conidia that are produced on and in the agar are relatively long and are mostly allantoid or oblong-ellipsoidal. The size difference between the aerial and agar conidia can easily be seen under the light microscope, but is also apparent in calculated averages of length / width (L/W) ratios for aerial and submerged conidia. Conidia have homogenous cellular contents when young, but can become two-guttulate after 7–14 d. Guttulation is relatively frequently observed in conidia produced on and in the agar. Aerial conidial

shape and size are relatively uninformative characters for species discrimination. However, *Pm. angustius*, *Pm. austroafricanum* and *Pm. tardicrescens* produce relatively long, allantoid or oblong-ellipsoidal aerial conidia (av. length = 5  $\mu\text{m}$ ), in comparison with the mostly shorter, obovoid or oblong-ellipsoidal conidia of other species (av. length of 3–4  $\mu\text{m}$ ).

### Distribution and host range

*Phaeoacremonium* species have a world-wide distribution (Table 1). *Phaeoacremonium parasiticum* has the widest known distribution, with *Pm. aleophilum* and *Pm. krajdenii* also being known to have broad distributions. The countries known for the *Phaeoacremonium* species are listed in Table 1 along with their hosts and substrata.

*Phaeoacremonium* species have been isolated from a range of woody hosts (Table 1), either as endophytes or as suspected pathogens associated with wilting or dieback or death of these hosts. The first unpublished identification was in 1970, when *Pm. parasiticum*, then named *Phialophora parasitica*, was isolated from stems and leaf sheaths of date palm (*Phoenix dactylifera*) trees in Iraq that showed wilting symptoms (Hawksworth *et al.* 1976). Halliwell (1966) isolated a fungus identified as “*Cephalosporium* sp.” that was associated with decline of oak trees (*Quercus virginiana*) in Texas. “*Phialophora parasitica*” together with *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. caused dark brown cambial staining, dieback and death of 7-yr-old *Nectandra* trees from Costa Rica (Hawksworth *et al.* 1976). Several isolates were reidentified by Hawksworth *et al.* (1976) as *Ph. parasitica*, but Halliwell’s and the *Nectandra* fungus were again found to be representative of *Pm. inflatipes* (Crous *et al.* 1996). Wilting apricot trees (*Prunus armeniaca*) had *Pm. parasiticum* and *Graphium penicillioides* Corda associated with the disease (Hawksworth *et al.* 1976). *Phaeoacremonium aleophilum* has recently been reported from *Prunus* species showing dieback symptoms in the Western Cape, South Africa (Damm *et al.* 2005). Serious dieback symptoms, caused by *Pm. parasiticum*, were observed in cherry trees [*Prunus avium* (L.) L.] in Greece (Rumbos 1986). In Italy, Greece and France, kiwifruit vines (*Actinidia deliciosa* var. *deliciosa*) showing dieback and wood decay symptoms (Di Marco *et al.* 2004b) have yielded a complex of species including *Fomitiporia mediterranea*, *Pm. aleophilum* and *Pm. parasiticum* (Di Marco *et al.* 2004b). *Phaeoacremonium mortoniae* was shown to cause brown wood staining of *Fraxinus pennsylvanica* in North Dakota, U.S.A. (Hausner *et al.* 1992).

Three *Phaeoacremonium* species have been isolated from soil, including *Pm. aleophilum* in California (Rooney *et al.* 2001), *Pm. argentinense* in Argentina (Crous & Gams 2000) and *Pm. parasiticum* in Tahiti

(Dupont *et al.* 2002). Strains originally identified as *Pm. rubrigenum* have been isolated from the galleries and larvae of an oak bark beetle, *Scolytus intricatus* Ratzeburg (*Coleoptera: Scolytidae*), on *Quercus robur*, as well as from adults of the bark beetle *Leperisinus fraxini* Panzer (*Coleoptera: Scolytidae*) on *Fraxinus excelsior* in the Czech Republic (Kubátová *et al.* 2004). These strains proved to be representative of a new species, *Pm. scolyti* (Mostert *et al.* 2005).

*Phaeoacremonium* infections from humans have been reported from various countries (Table 1). Nine

*Phaeoacremonium* species, including the newly described *Pm. sphinctrophorum* of the present study, have been confirmed as causal agents of human infections (Crous *et al.* 1996, Mostert *et al.* 2005). The occurrence of *Phaeoacremonium* infections in humans appears to have increased over the past two decades. The first reported medical case known to have involved a *Phaeoacremonium* species was a Venezuelan case in which *Pm. venezuelense*, then identified as *Cephalosporium serrae* Maffei, was identified as the etiologic agent of a mycetoma (de Albornoz 1974).

**Table 1.** List of known *Phaeoacremonium* species, their host/ substrate range and world-wide distribution<sup>a</sup>.

<i>Phaeoacremonium</i> species	Host/ Substrate	Countries
<i>Pm. aleophilum</i>	<i>Actinidia chinensis</i> , <i>Vitis vinifera</i> , <i>Olea europaea</i> , <i>Prunus pennsylvanica</i> , <i>Prunus</i> sp., <i>Salix</i> sp., Soil	Argentina <sup>b</sup> , Australia <sup>b</sup> , Austria <sup>b</sup> , Canada, Chile <sup>b</sup> , Iran <sup>b</sup> , Italy <sup>b</sup> , France <sup>b</sup> , South Africa <sup>b</sup> , Spain <sup>b</sup> , Turkey <sup>b</sup> , U.S.A. <sup>b</sup> , Yugoslavia <sup>b</sup>
<i>Pm. alvesii</i>	<i>Dodonaea viscosa</i> , Human	Australia, Brazil <sup>c</sup> , U.S.A. <sup>c</sup>
<i>Pm. amstelodamense</i>	Human	Netherlands <sup>c</sup>
<i>Pm. angustius</i>	<i>Vitis vinifera</i>	Portugal <sup>b</sup> , U.S.A. <sup>b</sup>
<i>Pm. argentinense</i>	Soil	Argentina
<i>Pm. australiense</i>	<i>Vitis vinifera</i>	Australia <sup>b</sup>
<i>Pm. austroafricanum</i> sp. nov.	<i>Vitis vinifera</i>	South Africa <sup>b</sup>
<i>Pm. griseorubrum</i>	Human	Japan <sup>c</sup> , U.S.A. <sup>c</sup>
<i>Pm. inflatipes</i>	<i>Hypoxyton truncatum</i> , <i>Nectandra</i> sp., <i>Quercus virginiana</i> , <i>Vitis vinifera</i>	Chile <sup>b</sup> , Costa Rica, U.S.A.
<i>Pm. iranianum</i> sp. nov.	<i>Actinidia chinensis</i> , <i>Vitis vinifera</i>	Iran <sup>b</sup> , Italy
<i>Pm. krajdjenii</i>	Human, <i>Vitis vinifera</i>	Canada <sup>c</sup> , India <sup>c</sup> , Japan <sup>c</sup> , Norway <sup>c</sup> , South Africa <sup>b</sup> , U.S.A. <sup>c</sup> , Dem. Rep. Congo <sup>c</sup>
<i>Pm. mortoniae</i>	<i>Fraxinus excelsior</i> , <i>Fraxinus latifolia</i> , <i>Fraxinus pennsylvanica</i> , <i>Vitis vinifera</i>	Sweden, U.S.A. <sup>b</sup>
<i>Pm. parasiticum</i>	<i>Actinidia chinensis</i> , <i>Aquilaria agallocha</i> , <i>Cupressus</i> sp., Dog, Human, <i>Nectandra</i> sp., <i>Phoenix dactylifera</i> , <i>Prunus armeniaca</i> , <i>Prunus avium</i> , <i>Quercus virginiana</i> , Soil, <i>Vitis vinifera</i>	Argentina <sup>b</sup> , Australia <sup>b</sup> , Brazil <sup>c</sup> , Canada <sup>c</sup> , Chile <sup>b</sup> , Costa Rica, Finland <sup>c</sup> , Greece, Iran <sup>b</sup> , Iraq, Italy, South Africa <sup>b</sup> , Tunisia, U.S.A. <sup>bcd</sup>
<i>Pm. rubrigenum</i>	Human	U.S.A. <sup>c</sup>
<i>Pm. scolyti</i>	<i>Vitis vinifera</i> , larvae of <i>Scolytus intricatus</i>	Czech Republic, France <sup>b</sup> , South Africa <sup>b</sup>
<i>Pm. sphinctrophorum</i> sp. nov.	Human	Laos <sup>c</sup> , U.S.A. <sup>c</sup>
<i>Pm. subulatum</i>	<i>Vitis vinifera</i>	South Africa <sup>b</sup>
<i>Pm. tardicrescens</i>	Human	U.S.A. <sup>c</sup>
<i>Pm. theobromatis</i> sp. nov.	<i>Theobroma gileri</i>	Ecuador
<i>Pm. viticola</i>	<i>Sorbus intermedia</i> , <i>Vitis vinifera</i>	Iran <sup>b</sup> , France <sup>b</sup> , Germany, South Africa <sup>b</sup> , U.S.A. <sup>b</sup>
<i>Pm. venezuelense</i>	Human, <i>Vitis vinifera</i>	Canada <sup>c</sup> , South Africa <sup>b</sup> , Venezuela <sup>c</sup>

<sup>a</sup>(Hawksworth & Gibson 1976a, Hausner *et al.* 1992, Crous *et al.* 1996 Dupont *et al.* 1998, Larignon & Dubos 1997, Ari 2000, Chicau *et al.* 2000, Crous & Gams 2000, Dupont *et al.* 2000, Pascoe & Cottral 2000, Péros *et al.* 2000, Reizenzein *et al.* 2000, Armengol *et al.* 2001, Groenewald *et al.* 2001, Rooney *et al.* 2001, Rumbos & Rumbo 2001, Dupont *et al.* 2002, Auger *et al.* 2005, Damm *et al.* 2005a, Eskalen *et al.* 2005, Mostert *et al.* 2005b, Overton *et al.* 2005b, T. Gräfenhan, pers comm.).

<sup>b</sup>Countries where *Phaeoacremonium* strains were isolated from *Vitis vinifera*.

<sup>c</sup>Countries where *Phaeoacremonium* strains were isolated from human infections.

<sup>d</sup>Country where *Pm. parasiticum* was isolated from an infected dog.

Shortly thereafter, a subcutaneous infection in a kidney transplant patient was reported involving *Pm. parasiticum*, newly described in the same report as *Phialophora parasitica* (Ajello *et al.* 1974). Since then, various other species of *Phaeoacremonium* have been reported from humans (Hironaga *et al.* 1989, Crous *et al.* 1996, Padhye *et al.* 1998, Matsui *et al.* 1999, Guarro *et al.* 2003, Mostert *et al.* 2005). Several strains originally identified as *Pm. inflatipes* proved to be misidentified or to be representative of new species, which were described as *Pm. alvesii*, *Pm. amstelodamense*, *Pm. krajdenii*, *Pm. tardicresens* and *Pm. venezuelense* (Mostert *et al.* 2005). Strains originally identified as *Phialophora repens* (Meyer *et al.* 1975) were revealed to represent *Pm. krajdenii* (Mostert *et al.* 2005).

There has been one case where *Phaeoacremonium* was isolated from an animal in circumstances that suggested infection. *Phaeoacremonium parasiticum* and *Arthrographis kalrae* (Tewari & Macpherson) Sigler & Carmichael were isolated from the blood and urine of a dog in California that had renal failure, and had been treated with Prednisone and antibacterials in connection with peripheral lymphadenopathy (A. Wang, pers comm.).

### Epidemiology

*Plants*: The life cycle of *Togninia/Phaeoacremonium* has mostly been investigated with the primary aim of understanding the spread of the *Phaeoacremonium* species and *Pa. chlamydospora* in Petri disease and esca on grapevines. The sources of inoculum and the portals of entry for these diseases will be discussed.

The main sources of inoculum in vineyards include infected propagation material, infected soils and aerial spores. Infected mother vines have proven to be a source of infected propagation material (Mugnai *et al.* 1999, Pascoe & Cottral 2000, Rego *et al.* 2000, Fourie & Halleen 2002, Halleen *et al.* 2003, Ridgway *et al.* 2003, Edwards *et al.* 2004, Retief *et al.* 2005a). Propagation material can also become infected during the grafting process. In the studies of Halleen *et al.* (2003), species of *Phaeoacremonium* were frequently isolated from rootstock-and-graft unions of vine cuttings before and after planting was done in nurseries, indicating that these infections derived from infected mother material or from nursery operations. Zanzotto *et al.* (2001) also found *Phaeoacremonium* species in certified, grafted plants and 1-yr-old plants, although very little infection was found in rootstock and scion cuttings made from the corresponding mother plants.

The infection of field grapevines can be through the roots or through pruning wounds. *Phaeoacremonium aleophilum* has recently been detected in soil (Rooney *et al.* 2001). Pathogenicity studies have shown that *Pm. aleophilum* can infect and colonise grapevine roots

(Adalat *et al.* 2000). However, root symptoms are not always present in diseased vines (Morton 2000).

Conidia of *Phaeoacremonium* species can be aerially dispersed. The presence of aerial inoculum of *Pm. aleophilum* and *Pm. mortoniae* has been detected in the field with petroleum jelly-covered glass slides (Larignon & Dubos 2000, Eskalen & Gubler 2001, Eskalen *et al.* 2005a). Pruning wounds are the most obvious port of entry for aerial inoculum. Several studies have shown that *Pm. aleophilum* can readily infect pruning wounds inoculated with conidia (Adalat *et al.* 2000, Larignon & Dubos 2000). Adalat *et al.* (2000) found that pruning wounds were particularly vulnerable to colonisation when pruning was done early in the season, and that they remained vulnerable for 9–12 wk after pruning. The extent to which the aerial inoculum is a source of pruning wound infections was assessed by Larignon & Dubos (2000), who found that *Pm. aleophilum* occurred with the same frequency on pruned and unpruned canes, unlike the co-occurring *Pa. chlamydospora*, which occurred in increased numbers of pruned canes. Conidia of *Pm. aleophilum* were not obtained in traps in the winter, but were found throughout the vegetative period, indicating that this fungus might enter the plant via some other route than pruning wounds (Larignon & Dubos 1997). Despite the ability of *Pm. aleophilum* to penetrate pruning wounds, Larignon & Dubos (2000) suggested that this might not be the way it invades grapevines in France, mainly because of the absence of airborne spores during winter pruning. Eskalen & Gubler (2001) found that airborne inoculum of *Pm. aleophilum* was present during winter and spring, but also found that conidia of *Pm. aleophilum* occurred more frequently in early to mid-summer than in the colder periods of the year. *Phaeoacremonium aleophilum* was also found in symptomatic berries (Eskalen & Gubler 2001), indicating that berries can become infected during the time when aerial conidia are present in the summer. Van Niekerk *et al.* (2005) correlated the occurrence of *Phaeoacremonium* spp. in cordons of mature grapevines with rainfall patterns and found that *Phaeoacremonium* spp. predominantly occurred in winter rainfall regions. Inoculation of grapevine spurs with *Pm. aleophilum* and the ecologically co-occurring *Pa. chlamydospora* revealed that the latter is much more aggressive than the former as a pruning wound invader (Adalat *et al.* 2000).

Aerial spores tend to come from the production of anamorphic or teleomorphic structures on the grapevine surface. Recently, perithecia of *T. minima* (Rooney-Latham *et al.* 2005b), *T. fraxinopennsylvanica* (Eskalen *et al.* 2005a) and *T. viticola* (Eskalen *et al.* 2005b) were observed on grapevines in the field. The presence of both mating types on the same grapevine (Mostert *et al.* 2003) and the formation of *T. minima* perithecia on incubated grapevine wood (Pascoe *et al.* 2004)

have indicated that the teleomorph could easily form in the field under the right environmental conditions. Perithecia of *T. minima* were indeed found in the field on dead vascular tissue in deep cracks along the trunks and cordons (Rooney-Latham *et al.* 2005b). They were also seen on the surface of decayed pruning wounds. The presence of perithecia in the field indicates that, under sufficiently moist environmental conditions, ascospore dispersal could also be a source of inoculum. *In vitro* studies showed that forcible discharge of ascospores can take place from rehydrated perithecia, and led Rooney-Latham *et al.* (2004) to conclude that ascospores of *T. minima* are an important inoculum source in the field. Aerial spore catch data confirmed that propagules of *Pm. aleophilum* / *T. minima* were indeed present in the air after rainfall (Rooney-Latham *et al.* 2004). Asexual and sexual spores could conceivably occur simultaneously under field conditions, since conidia may form on mycelium on and around perithecia, as is seen on colonized wood in moist chambers (Pascoe *et al.* 2004; also seen in our own observations). Perithecia of *T. fraxinopennsylvanica* (Eskalen *et al.* 2005a) and *T. viticola* (Eskalen *et al.* 2005b) were found not just on grapevines but also on ash trees (*Fraxinus latifolia*) in California. This shows that neighbouring trees can harbour the vine pathogens. Presumably, all woody hosts from which grape-infecting *Phaeoacremonium* species have been isolated can serve as sources of infective inoculum.

Insects probably contribute more to the dispersal of the slimy inoculum than aerial translocation. Ascospore droplets on the tips of long perithecial necks are ideal for smearing ascospores onto passing insects (Cain & Weresub 1957, Cassar & Blackwell 1996). The evidence for this type of dispersal has been found in the isolation of *Pm. scolyti* from insect larvae as well as in the isolation of *Pm. parasiticum* and *Pm. mortoniae* from larval galleries inside tree bark. Boring beetles were present in *Nectandra* sp. trees in Costa Rica from which *Pm. parasiticum* was isolated from discoloured vascular tissue (Hawksworth *et al.* 1976). Cherry trees that showed serious dieback symptoms caused by *Pm. parasiticum* also yielded bark beetles (*Coleoptera: Scolytidae*) and metallic wood-boring beetles (*Coleoptera: Buprestidae*) from which the fungus was isolated (Rumbos 1986). *Togninia fraxinopennsylvanica* (teleomorph of *Pm. mortoniae*) was isolated from a brown stain on wood of living *Fraxinus pennsylvanica* in North Dakota. The affected tree also had larval galleries of *Leperisimus californicus* Swaine (Hausner *et al.* 1992).

The role of insects in the spread of inoculum on grapevines remains uncertain. There have been few reports of bark beetles on grapevines. Exotic Lyctid beetles, *Trogoxylon impressum* Comolli (*Coleoptera: Lyctidae*), have been found on grapevines in Israel

(Halperin & Geis 1999) and a small wood-borer, *Xyloperthodes* cf. *incertus*, was identified from grapevines in South Africa (Allsopp 2004).

**Humans:** The environmental sources of *Phaeoacremonium* causing human infections could be contaminated wood splinters (Guarro *et al.* 2003), soil (Dupont *et al.* 1998) or the air (Mostert *et al.* 2005). Of the nine species of *Phaeoacremonium* that can infect humans, only *Pm. alvesii*, *Pm. krajdenii*, *Pm. parasiticum* and *Pm. venezuelense* have been isolated from woody hosts. Though splinters are widely suspected to be a major source of human infections, given that forceful introduction of inoculum under the skin is generally necessary to initiate a phaeohyphomycotic cyst or mycetoma, no case has been directly linked to ingress of a splinter. Aerial inoculum has not been detected in connection with human infections, but given what is known about the ecology of the fungi involved, aerial inoculum could be released by fruiting structures on infected woody hosts in residential or farming areas.

### Pathogenesis

**Plant hosts:** Dieback and related disease symptoms have been demonstrated experimentally with the inoculation of *Phaeoacremonium* species onto various woody hosts including apricot, cherry, grapevines, kiwifruit, oak, olive and peach (Halliwell 1966, Rumbos 1986, Sparapano *et al.* 2001a, Di Marco *et al.* 2004a).

The expression of symptoms caused by the different fungi involved in Petri disease and esca on grapevines has been extensively investigated with artificial inoculations. Various pathogenicity studies have investigated symptom expression of *Pm. aleophilum*. Inoculation studies have shown that *Pm. aleophilum* can cause brown wood streaking (Adalat *et al.* 2000, Sparapano *et al.* 2000b, Feliciano *et al.* 2004, Halleen *et al.* 2005), reduced shoot growth (Gubler *et al.* 2001) and esca symptoms on leaves and berries (Sparapano *et al.* 2001a, Feliciano *et al.* 2004). Inoculation studies have also shown that *Pm. krajdenii*, *Pm. parasiticum*, *Pm. subulatum*, *Pm. venezuelense* and *Pm. viticola* can reproduce brown wood streaking (Halleen *et al.* 2005). Laignon & Dubos (1997) concluded that in esca, *Pm. aleophilum* was similar to *Pa. chlamydospora* in being a pioneering fungus colonising living wood but leaving it in relatively sound condition, whereas the secondarily appearing basidiomycete fungi were responsible for the typical decay associated with esca. Mugnai (1999) found that *Pa. chlamydospora* and *Pm. aleophilum* were both able to break down polyphenolic compounds *in vitro*, suggesting that they might counteract substances produced in plant disease-resistance responses. This observation, together with the finding that inoculations with *F. punctata* alone very seldomly reproduced esca symptoms, suggested to Mugnai that the

*Phaeocremonium* and *Phaeomoniella* components of esca play a pioneering function in disease development. However, inoculation of *F. punctata* on grape cultivars Italia and Matilde caused wood deterioration and spongy wood decay after 6 mo, showing that this fungus has an ability to act as a primary pathogen and to act alone in causing symptoms compatible with esca when efficiently inoculated (Sparapano *et al.* 2000a).

The interactions among the fungi isolated from esca have been tested under controlled conditions as well as in the field. A marked antagonistic effect of *Pm. aleophilum* against *F. punctata* was found in paired fungal cultures (Sparapano *et al.* 2000b) and also in inoculated plants (Sparapano *et al.* 2001a). Bruno & Sparapano (2005b) also showed that colonies of *Pm. angustius*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola* had antagonistic effects on the colonies of *F. mediterranea* in assays done *in vitro* on malt extract agar.

Mugnai *et al.* (1999) theorised that foliar and berry symptoms were mainly caused by substances produced by melanogenic fungi in the discoloured woody tissues of the trunk and branches and translocated in the transpiration stream. Evidence to support this theory was given by Sparapano *et al.* (2001a) when they observed black measles (spotting on berries) on cv. 'Matilde' after inoculation of branches and spurs with *Pm. aleophilum*. Esca-like lesions developed on most berries that had been wounded and inoculated with *Pm. aleophilum* (Gubler *et al.* 2004). This shows that this fungus alone is potentially pathogenic to grape berries.

Variability in symptom expression has been found and has been ascribed to differences in cultivar susceptibility (Sparapano *et al.* 2001a, Feliciano *et al.* 2004). In a 3-yr trial where *Pm. aleophilum* was inoculated on spurs of cv. 'Italia' and 'Matilda', the latter cultivar was more resistant (Sparapano *et al.* 2001a). Also, inoculation of pruning wounds with *Pm. aleophilum* caused esca symptoms on leaves and berries of cv. 'Thompson Seedless', and on one of the 'Grenache' vines, while no symptoms developed on cv. 'Cabernet Sauvignon' (Feliciano *et al.* 2004).

Root inoculations have shown that *Pm. aleophilum* can successfully infect via the roots. When it does so, it may cause wood streaking as well as reductions in various growth parameters such as number of roots, plant height, number of internodes, extent of root elongation and accumulation of dry weight in above-ground parts (Scheck *et al.* 1998, Adalat *et al.* 2000). In grapevine nurseries the grafting of the scion and rootstock canes becomes successful when adequate callus tissue is formed at the grafting wound. The influence of *Pm. aleophilum* on callus formation was tested. This species was shown to inhibit callus formation in the cultivar 'Chardonnay' (Adalat *et al.* 2000). However, when inoculated into the base of seven other rootstock and

five scion varieties, it caused no inhibition of callus, whereas its ecological partner *Pa. chlamydospora* did so (Wallace *et al.* 2004).

*Phaeocremonium parasiticum*, identified as *P. parasitica*, was inoculated onto shoots of young apricot (*Prunus armeniaca*), cherry (*Prunus avium*), peach (*Prunus persica*) and olive (*Olea europaea*) trees. It caused significant vascular discoloration in all of the trees measured after 7 mo (Rumbos 1986). Strains of *Pm. inflatipes* (then identified as *Cephalosporium* sp.) from declining oak trees, upon being inoculated into stems and roots of *Quercus virginiana*, *Q. falcata* and *Q. palustris*, caused vascular discoloration within the stems after 8 wk. Roots showed intermitted symptoms after 2–4 mo (Halliwell 1966). When two-year-old potted kiwifruit vines were artificially inoculated with either *Pm. parasiticum* or *Pm. aleophilum*, wood discoloration was observed after 6 mo (Di Marco *et al.* 2004a). The wood discoloration observed was similar to naturally diseased kiwifruit vines.

Several substances involved in pathogenesis have been identified from fungi causing Petri and esca disease. These include phytotoxic compounds, pectic enzymes and enzymes involved in lignin degradation. Phytotoxic metabolites extracted from culture filtrates of *Pm. aleophilum* were identified as pullulans, scytalone and isosclerone (Sparapano *et al.* 2000c). When these substances were allowed to be absorbed by detached leaves, they caused foliar symptoms similar to those shown by esca-affected vines (Sparapano *et al.* 2000c). Similar symptoms were seen when the materials were injected into the shoot or branch xylem of standing grapevines. Evidente (2000) also isolated scytalone and isosclerone from culture filtrates of *Pm. aleophilum* and showed that these substances cause leaf symptoms on detached leaves. Tabacchi *et al.* (2000) isolated *p*-hydroxybenzaldehyde from culture filtrates of *Pm. aleophilum*, *Pa. chlamydospora* and *F. punctata*. This metabolite showed marked toxicity towards grapevine callus growth. Abou-Mansour *et al.* (2004) isolated seven compounds from liquid cultures of *Pm. aleophilum*, namely scytalone, isosclerone, 4-hydroxy scytalone, 2,4,8-trihydroxytetralone, 3,4,8-trihydroxytetralone, 1,3,8-trihydroxynaphthalene and flaviolin. These investigators found that scytalone and isosclerone hardly inhibited grapevine callus tissue growth. They divided the metabolites into two classes, tetralones, including scytalone, isosclerone, 2,4,8-trihydroxytetralone and 3,4,8-trihydroxytetralone, and naphthoquinones, including 2-hydroxyjuglone and flaviolin. The tetralones promoted callus growth while the naphthoquinones inhibited it. The ability of culture filtrates of *Pm. angustius*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola* to cause phytotoxic reactions on detached leaves of 'Italia' or 'Sangiovese' grapevines was linked to the production

**Table 2.** Body sites and types of lesions associated with *Phaeoacremonium* infections of humans.

<i>Phaeoacremonium</i> species	Symptom or place isolated from	Reference
<i>Pm. alvesii</i>	Subcutaneous infection	(Guarro <i>et al.</i> 2003)
	Synovial fluid, keratitis patient	(Present study)
	Subcutaneous lesion of foot	(Padhye <i>et al.</i> 1998)
<i>Pm. amstelodamense</i>	Elbow joint interior	(Mostert <i>et al.</i> 2005)
<i>Pm. griseorubrum</i>	Blood	(Mostert <i>et al.</i> 2005)
	Subcutaneous phaeohyphomycosis	(Matsui <i>et al.</i> 1999)
<i>Pm. krajdennii</i>	Skin lesion	(Meyer <i>et al.</i> 1975)
	Mycetoma on foot	(Mostert <i>et al.</i> 2005)
	Granuloma on hand	(Hironga <i>et al.</i> 1989)
	Mass on foot	(Mostert <i>et al.</i> 2005)
	Foot lesion	(Mostert <i>et al.</i> 2005)
<i>Pm. parasiticum</i>	White grain eumycetoma in foot	(Mostert <i>et al.</i> 2005)
	Abscess on arm	(Ajello <i>et al.</i> 1974)
	Subcutaneous infection	(Guarro <i>et al.</i> 2003)
	Left lower lobe of lung	(Mostert <i>et al.</i> 2005)
	Toenail	(Mostert <i>et al.</i> 2005)
<i>Pm. rubrigenum</i>	Synovial fluid	(Mostert <i>et al.</i> 2005)
	Pneumonia patient	(Crous <i>et al.</i> 1996)
	Infected eye	(Mostert <i>et al.</i> 2005)
<i>Pm. sphinctrophorum</i>	Phaeohyphomycotic cyst	(Present study)
	Subcutaneous cyst	(Present study)
<i>Pm. tardicrescens</i>	Unknown	(Mostert <i>et al.</i> 2005)
<i>Pm. venezuelense</i>	Mycetoma on foot	(De Albornoz 1974)
	Tissue from ankle	(Mostert <i>et al.</i> 2005)

of isosclerone, scytalone and pullulan by these fungi (Bruno & Sparapano 2005a). Pullulan is toxic to plants in general, and in particular causes severe symptoms on grapevine leaves (Sparapano *et al.* 2000c). On the other hand, isosclerone and scytalone tend to promote plant growth, suggesting that pullulan is the principal phytotoxic element associated with *Phaeoacremonium* (Sparapano *et al.* 2000c, Bruno & Sparapano 2005a).

The production of the pectic enzymes polygalacturonase and polymethylgalacturonase was detected in *Pm. aleophilum* and *Pm. rubrigenum* (Marchi *et al.* 2001). Pectic enzymes greatly aid the spread of a fungus inside its host by killing plant cells and macerating tissue. Analyses of the enzymes involved in lignin degradation showed that *Pm. aleophilum* expressed low specific activity for manganese peroxidase and high specific activity for both lignin peroxidase and laccase. This finding indicated that *Pm. aleophilum* has a greater capacity for degrading xylem walls than its ecological associate *Pa. chlamydospora*, which showed no activity in tests for lignin-degrading enzymes (Del Rio *et al.* 2004).

*Human and animal hosts:* Table 2 lists the *Phaeoacremonium* species infecting humans along with the associated clinical expressions seen. *Phaeoacremonium* infections in humans generally come under the broad banner of phaeohyphomycosis. This is a histopathological term proposed by Ajello *et al.* (1974) that refers to any cutaneous, subcutaneous or systemic infections where tissue preparations show diffusely arranged, dark-walled (melanised) fungal mycelium (which may be mixed with melanised yeast cells in infections by some species). Cases of phaeohyphomycosis caused by *Phaeoacremonium* have mostly involved subcutaneous abscesses and cysts, but there have also been some cases of chronic or acute osteoarthritis (Padhye *et al.* 1998). Immunocompetent patients are in the great majority, but some immunocompromised patients are also affected. Cases often appear to have been initiated by traumatic inoculation, but a few infections that were not caused by injury have also been reported (Matsui *et al.* 1999, Guarro *et al.* 2003). In a few cases involving immunocompromised patients (often patients who had received immunosuppressive therapy in connection

with major organ transplants), disseminated infection, fungemia or endocarditis occurred (Padhye *et al.* 1998). The innate immunity is mostly involved in the control of such opportunistic fungal diseases.

True mycetoma where tightly structured fungal grains are the main tissue presentation is classified separately from phaeoohyphomycosis. Only a few fungi have the specialised ability to cause this type of infection. Causation of mycetoma has been attributed to *Phaeoacremonium* strains on several occasions (Padhye *et al.* 1998) beginning with the original report of de Albornoz (1974) for *Pm. venezuelense* (identified as *Cephalosporium serra*).

#### Genetic diversity among *Phaeoacremonium* species

Few population studies have been done on *Phaeoacremonium* species. The genetic variation in populations of *Pm. aleophilum* has been studied in more detail than that of other species (Péros *et al.* 2000, Tegli *et al.* 2000b, Cottral *et al.* 2001). Using Random Amplified Polymorphic DNA (RAPD) and Random Amplified Micro- or Mini-Satellites (RAMS), Tegli *et al.* (2000b) showed that considerable variation existed among strains of *Pm. aleophilum* collected from a single field in Italy, suggesting that sexual reproduction might occur. RAPD analysis of *Pm. aleophilum* strains showed several different haplotypes within individual vineyards in France which Péros *et al.* (2000) ascribed to outside sources of inoculum. Considerable genetic variation, suggestive of ongoing recombination, was also found in universally primed-PCR studies done with *Pm. aleophilum* strains from Australia (Cottral *et al.* 2001). The presence in the field of perithecia of the corresponding teleomorph, *T. minima*, appears to explain these results (Rooney-Latham *et al.* 2005b). The genetic diversity of *Pm. parasiticum* strains was measured with PCR-RFLP analysis of the ITS rDNA and  $\beta$ -tubulin genes (Dupont *et al.* 2002). Two groups were distinguished among strains originating from grapevines in Argentina based on the presence or absence of one restriction enzyme site. The teleomorph of this species has recently been induced *in vitro* (present study), but its occurrence in the field is yet to be confirmed.

#### Molecular identification and detection

Molecular identification of *Phaeoacremonium* species has been done using RFLP patterns (Restriction Fragment Length Polymorphisms), phylogenetic analysis and direct PCR based on use of specific primers. RFLP patterns of the ITS region were used to distinguish *Pm. aleophilum*, *Pm. inflatipes* and *Pm. rubrigenum* (Tegli *et al.* 2000a). Dupont *et al.* (2002) distinguished five species of *Phaeoacremonium*, namely *Pm. aleophilum*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola*, using PCR-RFLP markers from the ITS regions and the partial  $\beta$ -tubulin gene.

DNA phylogenies based on the internal transcribed spacers (ITS 1 and 2) and 5.8 S rRNA gene, and  $\beta$ -tubulin, actin and calmodulin gene regions have been used in various studies to aid in the determination of new species of *Phaeoacremonium* (Dupont *et al.* 2000, Groenewald *et al.* 2001, Mostert *et al.* 2003, 2005). Recently Mostert *et al.* (2005) developed a polyphasic identification tool including morphological and cultural characters as well as  $\beta$ -tubulin sequences generated with primers T1 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). This *Phaeoacremonium* database including all of the known *Phaeoacremonium* species can be accessed from the website of the Centraalbureau voor Schimmelcultures ([www.cbs.knaw.nl/phaeoacremonium.htm](http://www.cbs.knaw.nl/phaeoacremonium.htm)).

Several PCR primer sets have been developed to facilitate rapid species identification and detection. Species-specific primers have been developed from the internal transcribed spacers ITS1 and ITS2 of the rRNA gene and used for the detection of *Pm. aleophilum* (Pal1N + Pal2) (Tegli *et al.* 2000a). Genus-specific primers for *Phaeoacremonium* (Pac1f + Pac2r) were developed from the ITS1 and ITS2 regions for use in real-time PCR detection with SYBR<sup>®</sup> Green (Overton *et al.* 2005a). These primers have been used to detect *Pm. aleophilum* in vines (Overton *et al.* 2005).

PCR detection is more reliable, sensitive and rapid than traditional plating methods. As little as 1 pg of DNA could be detected from spiked wood material (Retief *et al.* 2005b, Ridgway *et al.* 2002) and 50 fg of DNA with a nested PCR approach in artificially inoculated soils with *Pa. chlamydospora* (Whiteman *et al.* 2002). When traditional plating methods were compared with PCR detection, Retief *et al.* (2005b) found on average four times fewer positive detections with traditional plating methods than with PCR detection in naturally infected grapevine material. A disadvantage of PCR-based detection is that it does not distinguish dead material from viable cells. Retief *et al.* (2005b) demonstrated this shortcoming of PCR detection by comparing molecular detection and traditional plating from hot water treated and untreated, dormant nursery vines. They suggested that further work is necessary on the detection of pathogen ribonucleic acids (RNA), that would have a relatively short life span following pathogen death, and would be more reliable to indicate the presence of viable fungal material only.

#### Disease management

*Grapevines*: Several studies have been conducted to test for host resistance in scion and rootstock cultivars (Eskalen *et al.* 2001, Marchi 2001, Sparapano *et al.* 2001b, Feliciano *et al.* 2004, Santos *et al.* 2005). None of these studies has shown complete or high levels of resistance in any rootstock or scion cultivar tested. However, these studies did show that different cultivars had a wide range of susceptibility.

In several studies a range of fungicides was screened for their effectiveness against *Pa. chlamydospora* in Petri disease (Groenewald *et al.* 2000b, Jaspers 2001). These fungicides still need to be tested on the *Phaeoacremonium* species occurring on woody hosts. Studies also need to be conducted to verify the efficacy of these fungicides under field conditions.

Various control measures can be applied to ensure clean grapevine planting material. The presence of *Pa. chlamydospora*, *Pm. aleophilum*, *Botryosphaeria* and *Phomopsis* spp. in pruning wounds of rootstock mother blocks led Fourie & Halleen (2004a) to recommend that sanitation and pruning wound protection be practiced. Rootstock cuttings can be treated with hot water before grafting for 30 min at 50 °C, a measure that proved to be the most effective of several means tested for reducing the levels of these infections (Crous *et al.* 2001, Fourie & Halleen 2004b). Wounds made during the grafting processes can be protected by the addition to hydration and drench water of quaternary ammonium disinfectants (Sporekill®), fungicides (benomyl) or biological control agents (*Trichoderma harzianum*) (Fourie & Halleen 2004b, 2005). *Trichoderma* treatments carried out during grafting (Messina 1999, Di Marco *et al.* 2004a) resulted in nursery grapevines with stronger graft unions and root systems than those found in controls, as well as lower levels of infection. *Trichoderma* soil amendments in field nurseries had a similar effect (Fourie *et al.* 2001). Hot water treatment can also be applied on dormant nursery grapevines before planting (Fourie & Halleen 2004b). This treatment has shown to give similar reduction in *Phaeoacremonium* incidence than hot water treatment prior to grafting.

Sodium arsenite has been successfully applied to the trunk and cordons of diseased grapevines (Mugnai *et al.* 1999). Its toxicity, however, has caused it to be banned from various countries. Restricted use is still allowed in France, Portugal and Spain (Di Marco *et al.* 2000). Other chemical control measures have succeeded only in partially limiting symptom expression and disease development of Petri disease and esca. The chemical fosetyl-Al, the principal active ingredient of which is phosphorous acid, has shown promising results. Phosphorous acid does not target the fungus directly but rather stimulates induced resistance responses in the host (Jaspers 2001). A mixture of phosphorous acid and the phytoalexin resveratrol inhibited *in vitro* mycelial growth of *Pm. aleophilum*, whereas the two compounds individually demonstrated poor efficacy (Di Marco *et al.* 1999). Fosetyl-Al applied as trunk injections in mature grapevines moderated the incidence of disease and preserved vine productivity (Di Marco *et al.* 2000). Foliar sprays of Fosetyl-Al on potted grapevines significantly reduced the extent of necrotic areas resulting from inoculation with *Pm. aleophilum* or *Pa. chlamydospora* (Di Marco & Osti

2005). Foliar sprays of fosetyl-Al on naturally infected field grapevines produced a reduction in esca disease (Di Marco & Osti 2005). Root zone application with triazoles and trunk injections with triazoles or fosetyl-Al in esca-affected vineyards resulted in significant reductions in foliar symptom development, but only when the treatments were made in vineyards where disease incidence was low and where plants were at an early stage of infection (Di Marco *et al.* 2000). Applications of composts, nutrient fertilizers, extra water, phosphonates and Brotomax (which increases the production of phenolic compounds) over periods of two to five years were ineffective in reducing disease occurrence (Edwards & Pascoe 2005).

Disease prevention seems to be the most effective means of managing Petri disease and esca. Propagation material should be of good quality and disease-free. Vascular discoloration of young vines can be attributed not only to Petri disease fungi, but also to various mechanical and biological stress factors (Stamp 2001). Therefore, to ensure healthy propagation, material without wood streaking should be selected (Mugnai *et al.* 1999). Preventive tactics should be used to avoid stress factors such as nutrient deficiencies, water stress, bad root development due to poor soil preparation, and heavy crop loads during the first three years of establishment (Ferreira *et al.* 1999, Gubler *et al.* 2004, Surico *et al.* 2004, Edwards & Pascoe 2005). The removal of infected plants, plant parts and pruning debris will help to reduce inoculum levels in vineyards. Pruning shears should be disinfected after the pruning of a vine. In addition, healthy-looking vines should be pruned before diseased vines (Mugnai *et al.* 1999). The protection of pruning wounds will limit infections. Pruning wound protection with benomyl and flusilazole reduced natural *Pa. chlamydospora* infections of pruning wounds by ca 80 % (Fourie & Halleen 2005). Pruning wound protection by *T. harzianum* and *T. longibrachiatum* against artificial infection by *Pa. chlamydospora* was demonstrated by Di Marco *et al.* (2004b). Fourie & Halleen (2005) also showed that *Trichoderma* species successfully colonised pruning wounds and reduced natural *Pa. chlamydospora* infections. These trials should include *Phaeoacremonium* species to verify the efficacy of the treatments on them.

*Human and animal hosts:* Various antifungal drugs have been tested against *Phaeoacremonium in vitro*. When strains of *Pm. parasiticum* were tested, amphotericin B and miconazole had minimum inhibitory concentrations (MIC) in the low sensitivity range (2.0–8.0 and 2.5–20 µg/mL, respectively), whereas most of the isolates were resistant to 5-fluorocytosine and ketoconazole (Weitzman *et al.* 1984). Voriconazole (0.03–0.6 µg/mL) gave lower MIC's than amphotericin B (1–16 µg/



mL) and itraconazole (0.25–32 µg/mL) in tests done on a single strain of *Pm. parasiticum* (McGinnis & Pasarell 1998). In tests on *Pm. rubrigenum* and *Pm. parasiticum*, voriconazole and ravuconazole gave the lowest MIC's (1 and 0.5–1 µg/mL, respectively) in comparison with amphotericin (2 µg/mL), fluconazole (8 µg/mL), itraconazole (8 and 16 µg/mL, respectively), ketoconazole (2–4 µg/mL) and terbinafine (2 µg/mL) (Guarro *et al.* 2003).

Localised *Phaeoacremonium* infections are usually readily treatable. As is common with fungal cysts and mycetomas, surgical removal of affected tissue has often been successfully used in combination with administration of antifungal drugs (Padhye *et al.* 1998). In immunocompromised patients, this combined approach has not always been effective. In one case surgical removal and the administration of fluconazole did not prevent the recurrence of subcutaneous *Pm. griseorubrum* infection (diagnosed at the time as *Pm. rubrigenum* infection) in an immunocompromised haemodialysis patient (Matsui *et al.* 1999). In another renal transplant case, *Pm. parasiticum* infection (diagnosed at the time as *Pm. rubrigenum* infection) returned after foot nodules were surgically removed twice in conjunction with serially administered itraconazole, terbinafine and fluconazole therapy (Guarro *et al.* 2003).

### Scope of this monograph

The aim of this monograph has been to compile a comprehensive taxonomic overview of *Togninia* and *Phaeoacremonium*. All known species have been described and illustrated here. New species have been identified on the basis of their morphological and cultural characters, phylogenies of actin and  $\beta$ -tubulin genes and mating studies. Known species of *Togninia* and *Phaeoacremonium* were reassessed by re-examining ex-type cultures and specimens.

The systematic placement of *Togninia* was investigated with phylogenies of the LSU and SSU rRNA genes. Descriptions of taxa related to *Togninia* were also included to compare the resemblance that these taxa have in teleomorph, and where known, in anamorph morphology.

Different identification systems have been developed. Dichotomous keys were written to identify the genera *Togninia* and *Phaeoacremonium* as well as keys for their respective species. With the aid of BioloMICS a multiple-entry electronic key has been developed that also incorporates  $\beta$ -tubulin sequence data that can be used for *Phaeoacremonium* species identification. Additionally, species-specific primers were developed to aid in the rapid identification of different *Phaeoacremonium* species.

## MATERIALS AND METHODS

**Strains:** The *Phaeoacremonium* strains included in this study are listed in Table 3. Living material of *Vitis vinifera* showing Petri disease and esca symptoms were primarily collected in South Africa for isolations. Other strains, isolated from human cases and various woody hosts were obtained from the Centraalbureau voor Schimmelcultures (CBS) culture collection in Utrecht, the Netherlands.

**Isolations:** Trunks and shoots of diseased grapevines were cut into disks and surface-sterilised using the following protocol: 30 s in 70 % ethanol, 2 min in NaOCl (1 %) and 15 s in 70 % ethanol. Small pieces of tissue were cut from just below the surface, around and in the darkened vascular tissues, and were placed onto potato-dextrose agar (PDA, 3.9 % Biolab, South Africa) amended with streptomycin (1 ml/L). PDA plates were incubated at 25 °C. Single-spore isolations were made from outgrowing colonies identified as species of *Phaeoacremonium*.

**Herbarium specimens:** All known or likely *Togninia* specimens were obtained from herbaria B, FH, M, NY, PAD, S and WIN [Herbarium abbreviations according to Holmgren *et al.* (1990)].

**Microscopic examination:** Strains were plated onto malt extract agar (MEA, 2 % malt extract, Oxoid Ltd., England; 1.5 % agar, Difco, U.S.A.) and placed at 25 °C in the dark for 2–3 wk until sporulation. Some plates were placed under near-UV light to enhance sporulation. Microscopic mounts were made with a fine needle from aerial mycelium 2–3 cm from the colony margin avoiding contact with the agar. Structures that formed on aerial mycelium were mounted in lactic acid on glass slides. To investigate the structures formed in and on the agar surface, an agar block 3–5 mm from the margin of the colony with very little to no aerial mycelium was cut and mounted in lactic acid on glass slides. Thirty measurements were made using a light microscope (Axioskop 2 plus, Carl Zeiss B.V., Germany) of each type of structure, except where otherwise stated. Standard morphological terms were used (Kirk *et al.* 2001). The 5<sup>th</sup> and 95<sup>th</sup> percentiles were determined for all measurements with the extreme values given in parentheses. Where the extreme values corresponded with the 5<sup>th</sup> and 95<sup>th</sup> percentiles, they were omitted. The average was calculated and given in parentheses, and the length/width ratios (L/W) of conidia were determined.

Perithecia of *Togninia* were induced on twice-autoclaved pieces of 3–4 cm of grapevine cane placed on 2 % water agar (Technical grade water agar, Oxoid) (grapevine water agar, GWA).

**Table 3.** Names, accession numbers and isolation details of *Phaeoacremonium* strains examined (original wrong identifications are given in parentheses in the first column).

<i>Phaeoacremonium</i> species	Accession numbers <sup>a</sup>	Origin	Host	Collector	Date of collection/ CBS accession	ACT	TUB
<i>Pm. aleophilum</i>	CBS 246.91 <sup>b</sup>	Yugoslavia	<i>Vitis vinifera</i>	M. Muntaniola-Cvetkovic	1991	AY735497	AF246811
	CBS 100397, C.P.C. 4029	Italy	<i>V. vinifera</i>	S. Serra	1998	AY735498	AF246806
	CBS 110701	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	CBS 110702	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	CBS 110703	South Africa	<i>V. vinifera</i>	L. Mostert	2001	DQ173115	DQ173094
	CBS 110705	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	CBS 110711	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	CBS 111015	South Africa	<i>V. vinifera</i>	F. Halleen	2001		
	CBS 110827	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	CBS 110753	South Africa	<i>V. vinifera</i>	M. Groenewald	1998		
	CBS 110831	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	CBS 111014	South Africa	<i>V. vinifera</i>	F. Halleen	2002		
	L.M. 44	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	L.M. 45	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	L.M. 46	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	L.M. 47	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	L.M. 48	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	L.M. 51	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	L.M. 58	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
	L.M. 61	South Africa	<i>V. vinifera</i>	L. Mostert	2001		
L.M. 77	South Africa	<i>V. vinifera</i>	L. Mostert	2001			
L.M. 78	South Africa	<i>V. vinifera</i>	L. Mostert	2001			
L.M. 467	South Africa	<i>V. vinifera</i>	M. Groenewald	1998			
L.M. 483	South Africa	<i>Prunus armeniaca</i>	F. Halleen	2002	DQ173116	DQ173095	
<i>Pm. abhesii</i> ( <i>Pm. inflatipes</i> )	CBS 408.78, CDC 78-042877	U.S.A.	Human	A.A. Padhye	1978	AY579236	AY579303
	CBS 729.97, CDC B-5747	U.S.A.	Human	A.A. Padhye	1997	AY579235	AY579302
	CBS 110034, FMR 7682 <sup>b</sup>	Brazil	Human	S.H. Alves	2002	AY579234	AY579301
<i>Pm. abhesii</i>	CBS 113590, VPRI 22409	Australia	<i>Dodonaea viscosa</i>	I. Pascoe	2000	AY579237	AY579304
	CBS 110627 <sup>b</sup>	Netherlands	Human	J. Bruins	2002	AY579228	AY579295

Table 3. (Continued).

<i>Phaeoacremonium</i> species	Accession numbers <sup>a</sup>	Origin	Host	Collector	Date of collection/ CBS accession	GenBank Accession numbers <sup>a</sup>	TUB
<i>Pm. angustius</i>	CBS 114991, LCP 93 3551	U.S.A.	<i>V. vinifera</i>	P. Larignon	1992	DQ173126	DQ173103
	CBS 114992, LCP 96 3897 <sup>b</sup>	U.S.A.	<i>V. vinifera</i>	P. Larignon	1992	DQ173127	DQ173104
<i>Pm. argentinense</i> ( <i>Pm. angustius</i> )	CBS 777.83	Argentina	Soil	A. Martinez	1983	DQ173135	DQ173108
<i>Pm. australiense</i>	CBS 113589, VPRI 22016a <sup>b</sup>	Australia	<i>V. vinifera</i>	T. Knaggs	1999	AY579229	AY579296
	CBS 113592, VPRI 22892	Australia	<i>V. vinifera</i>	J. Edwards	2000	AY579230	AY579297
<i>Pm. austroafricicum</i>	CBS 112949, C.P.C. 4656 <sup>b</sup>	South Africa	<i>V. vinifera</i>	L. Mostert	2001	DQ173122	DQ173099
	CBS 114993	South Africa	<i>V. vinifera</i>	F. Halleen	2002	DQ173124	DQ173101
	CBS 114994	South Africa	<i>V. vinifera</i>	F. Halleen	2002	DQ173125	DQ173102
	CBS 118482	South Africa	<i>V. vinifera</i>	F. Halleen	2002	DQ173123	DQ173100
<i>Pm. griseorubrum</i> ( <i>Pm. rubrigenum</i> )	CBS 566.97	Japan	Human	K. Nishimoto	1996	AY579226	AF246801
<i>Pm. griseorubrum</i>	CBS 111657, UTHSC 02-949 <sup>b</sup>	U.S.A.	Human	D. Sutton	2002	AY579227	AY579294
<i>Pm. inflatipes</i>	CBS 166.75	Costa Rica	<i>Nectandra</i> sp.	I.A.S. Gibson	1974	AY579258	AY579322
	CBS 391.71 <sup>b</sup>	U.S.A.	<i>Quercus virginiana</i>	R.S. Halliwell	1971	AY579259	AF246805
	CBS 113273, NRRRL 32148	U.S.A.	<i>Hypoxylon truncatum</i>	B. Horn	2000	AY579260	AY579323
<i>Pm. iranianum</i> ( <i>Pm. inflatipes</i> )	CBS 100400	Italy	<i>V. vinifera</i>	S. Serra	1998	DQ173119	DQ173096
<i>Pm. iranianum</i> ( <i>Pm. aleophilum</i> )	CBS 101357 <sup>b</sup>	Italy	<i>Actinidia chinensis</i>	F. Calzarano & S. Di Marco	1998	DQ173120	DQ173096
<i>Pm. iranianum</i>	CBS 117112	Iran	<i>V. vinifera</i>	T. Gräfenhan	2004		
	CBS 117113	Iran	<i>V. vinifera</i>	T. Gräfenhan	2004		
	CBS 117114	Iran	<i>V. vinifera</i>	T. Gräfenhan	2004	DQ173121	DQ173098
<i>Pm. krajidenii</i> ( <i>Phialophora repens</i> )	CBS 423.73	Democratic Republic of Congo	Human	K.J. Kwon-Chung	1973	AY579263	AY579326
<i>Pm. krajidenii</i>	CBS 633.93	Norway	Human	P. Sandven	1993	AY579264	AY579327
<i>Pm. krajidenii</i> ( <i>Pm. inflatipes</i> )	CBS 109479 <sup>b</sup>	Canada	Human	S. Krajden	2001	AY579267	AY579330
<i>Pm. krajidenii</i>	CBS 110118	South Africa	<i>V. vinifera</i>	G. van Coller	2001	AY579261	AY579324
<i>Pm. krajidenii</i> ( <i>Pm. inflatipes</i> )	CBS 110361, CDC B6093	India	Human	A.A. Padhye	2001	AY579270	AY579333
<i>Pm. krajidenii</i>	CBS 110365, UAMH 5723	U.S.A.	Human	A. Espinel	Unknown	AY579266	AY579329
<i>Pm. krajidenii</i> ( <i>Phialophora repens</i> )	CBS 110366, ATCC 58115, SM 3531	Japan	Human	M. Hironaga	Unknown	AY579265	AY579328
<i>Pm. krajidenii</i> ( <i>Pm. inflatipes</i> )	CBS 110367, CDC B6091	U.S.A.	Human	S. Weber	2001	AY579268	AY579331
	CBS 110368, CDC B6092	U.S.A.	Human	A.A. Padhye	2001	AY579269	AY579332
<i>Pm. krajidenii</i>	CBS 113588	South Africa	<i>V. vinifera</i>	F. Halleen	2002	AY579262	AY579325

Table 3. (Continued).

<i>Phaeoacremonium</i> species	Accession numbers <sup>a</sup>	Origin	Host	Collector	Date of collection/ CBS accession	GenBank Accession numbers <sup>b</sup>
						ACT      TUB
<i>Pm. mortoniae</i> ( <i>Pm. rubrigenum</i> )	CBS 211.97, C.P.C. 4027	Sweden	<i>Fraxinus excelsior</i>	J. Stenlid	1996	DQ173138      AF246810
<i>Pm. mortoniae</i> ( <i>Pm. inflatipes</i> )	CBS 101585 <sup>b</sup>	U.S.A.	<i>V. vinifera</i>	L. Morton & L. van de Water	1998	DQ173137      AF246809
<i>Pm. mortoniae</i>	CBS 110212, ATCC 26664	U.S.A.	<i>Fraxinus pennsylvanica</i>	T.E. Hinds	1970	DQ173136      DQ173109
<i>Pm. novae-zealandiae</i>	CBS 110156, UAMH 9589 <sup>b</sup>	New Zealand	<i>Cupressus macrocarpa</i>	J. Reid & S. Reid	1982	DQ173139      DQ173110
	CBS 110157, UAMH 9590	New Zealand	<i>Pinus radiata</i>	J. Reid & S. Reid	1982	DQ173140      DQ173111
	CBS 114512, C.P.C. 3394	New Zealand	<i>Desmoschoenus spiralis</i>	J. Rees-George	1999	DQ173141      DQ173112
<i>Pm. parasiticum</i>	CBS 184.75	Iraq	<i>Phoenix dactylifera</i>	H.Y. Al-Ani	1975	AY579251      AY579317
	CBS 514.82, UAMH 5054	U.S.A.	Human	I. Weitzman	1982	AY579240      AY579306
<i>Pm. parasiticum</i> ( <i>Pm. inflatipes</i> )	CBS 736.94	Finland	Human	University of Helsinki	1995	AY579250      AY579316
<i>Pm. parasiticum</i>	CBS 860.73, ATCC 26366, IMI 341971, IMI 181115, LCP 88.5537, C.P.C. 772, UAMH 36292	U.S.A.	Human	R.T. Steigbigel	1973	AY579253      AF246803
	CBS 984.73, IMI 192879	Tunisia	<i>Prunus armeniaca</i>	B. Jamoussi	1973	AY579249      AY579315
	CBS 101007	Italy	<i>Actinidia chinensis</i>	F. Calzarano & S. di Marco	1998	AY579252      AF246804
	CBS 109665	U.S.A.	Human	S. Moser	2001	AY579246      AY579312
	CBS 109666, FG 00 04652	U.S.A.	Human	S. Moser	2001	AY579245      AY579311
<i>Pm. parasiticum</i> ( <i>Pm. rubrigenum</i> )	CBS 110033, FMR 7681	Brazil	Human	S.H. Alves	1999	AY579247      AY579313
<i>Pm. parasiticum</i>	CBS 113594	South Africa	<i>V. vinifera</i>	F. Halleen	2000	AY579244      AY579310
	CBS 113585	South Africa	<i>V. vinifera</i>	L. Mostert	2001	AY579241      AY579307
	CBS 113586	South Africa	<i>V. vinifera</i>	L. Mostert	2001	AY579242      AY579308
	CBS 113591, VPRI 22542b	Australia	<i>V. vinifera</i>	I. Pascoe	2000	AY579243      AY579309
	CBS 113596, NOMH 568	Canada	Human	Sunnybrook Medical Centre	1987	AY579248      AY579314
	L.M. 8	South Africa	<i>V. vinifera</i>	J. van Niekerk	2001	
	L.M. 17	South Africa	<i>V. vinifera</i>	L. Mostert	2001	
	L.M. 18	South Africa	<i>V. vinifera</i>	L. Mostert	2001	
	L.M. 461	South Africa	<i>V. vinifera</i>	F. Halleen	2002	
	L.M. 462	South Africa	<i>V. vinifera</i>	F. Halleen	2002	
	L.M. 464	South Africa	<i>V. vinifera</i>	F. Halleen	2002	

Table 3. (Continued).

<i>Phaeoacremonium</i> species	Accession numbers <sup>a</sup>	Origin	Host	Collector	Date of collection/ CBS accession	GenBank Accession numbers <sup>c</sup>	TUB
<i>Pm. rubrigenum</i>	CBS 498.94 <sup>b</sup>	U.S.A.	Human	K.J. Kwon-Chung	1994	AY579238	AF246802
	CBS 112046, UTHSC 00-2395	U.S.A.	Human	C. Conover	2002	AY579239	AY579305
<i>Pm. scolyti</i>	CBS 112585, CCF 3266	Czech Republic	Larva of <i>Scolytus intricatus</i>	A. Kubátová	1998	AY579223	AY579292
	CBS 113593, LCP 97.4002	France	<i>V. vinifera</i>	P. Larignon	1997	AY579225	AY579293
	CBS 113597, C.P.C. 3092 <sup>b</sup>	South Africa	<i>V. vinifera</i>	S. Ferreira	1998	AY579224	AF246800
<i>Pm. sphinctrothorum</i> ( <i>Phialophora repens</i> )	CBS 337.90 <sup>b</sup>	Laos	Human	S. Kraiden & R.C. Summerbell	1988	DQ173142	DQ173113
	CBS 694.88	U.S.A.	Human	A.A. Padhye	1988	DQ173143	DQ173114
<i>Pm. subulatum</i>	CBS 113584, C.P.C. 4655 <sup>b</sup>	South Africa	<i>V. vinifera</i>	L. Mostert	2001	AY579231	AY579298
	CBS 113587	South Africa	<i>V. vinifera</i>	L. Mostert	2002	AY579232	AY579299
<i>Pm. tardicrescens</i> ( <i>Pm. inflatipes</i> )	CBS 110573, UTHSC 00-14 <sup>b</sup>	U.S.A.	Human	Levi	2000	AY579233	AY579300
<i>Pm. theobromatis</i> ( <i>Acremonium</i> sp.)	CBS 111586	Ecuador	<i>Theobroma gileri</i>	H.C. Evans	2000	DQ173132	DQ173106
<i>Pm. venezuelense</i> ( <i>Cephalosporium serrae</i> , later <i>Pm. inflatipes</i> )	CBS 651.85, ATCC 32628, UAMH 40342	Venezuela	Human	M.B. de Albornoz	1985	AY579256	AY579320
<i>Pm. venezuelense</i>	CBS 110119, C.P.C. 4648	South Africa	<i>V. vinifera</i>	L. Mostert	2001	AY579254	AY579318
	CBS 113595, SF 9587 (02)	Canada	Human	S. Kraiden	2002	AY579255	AY579319
<i>Pm. viticola</i> ( <i>Pm. inflatipes</i> )	CBS 113598, C.P.C. 3697	Unknown	Unknown	Unknown	Unknown	AY579257	AY579321
	CBS 428.95	Germany	<i>Sorbus intermedia</i>	K. Weise	1995	DQ173133	DQ173107
<i>Pm. viticola</i>	CBS 100947	Italy	<i>Olea europaea</i>	S. Frisullo	1998	DQ173134	AF246815
	CBS 101737, LCP 97.4014	France	<i>V. vinifera</i>	P. Larignon	1996	DQ173129	AF246817
	CBS 101738, LCP 96.3886 <sup>b</sup>	France	<i>V. vinifera</i>	P. Larignon	1993	DQ173131	AF192391
	CBS 101739, LCP 97.4004	France	<i>V. vinifera</i>	P. Larignon	1997	DQ173130	AF246816
	CBS 113065, C.P.C. 4653	South Africa	<i>V. vinifera</i>	L. Mostert	2001	DQ173128	DQ173105
	CBS 118235, LCP 97.4009	France	<i>V. vinifera</i>	P. Larignon	1999		
	LCP 97.4016	France	<i>V. vinifera</i>	P. Larignon	1999		

<sup>a</sup>Culture collections listed: ATCC: American Type Culture Collection, Manassas, VA, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CCF: Culture Collection of Fungi, Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic; CDC, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.; C.P.C.: Working collection of Pedro W. Crous, housed at CBS; FMR: Facultat Medicina de Reus, Reus, Spain; IMI: CABI Bioscience, Egham, U.K.; LCP: Laboratory of Cryptogamy, National Museum of Natural History, Paris, France; L.M.: Private collection L. Mostert; NCPF: National Collection of Pathogenic Fungi, Bristol, U.K.; NOMH: Ontario Ministry of Health, Toronto, ON, Canada; NRRL: USDA Agricultural Research Service Collection, Peoria, IL, USA; SM: Shiga University of Medical Science, Otsu, Japan; UAMH: University of Alberta Microfungus Collection, Devonian Botanic Garden, Edmonton, AB, Canada; UTHSC: University of Texas Health Sciences Center, San Antonio, TX, USA and VPRI, Knoxville Herbarium, Department of Primary Industries, Knoxfield, VIC, Australia.

<sup>b</sup>Ex-type strains of species.

<sup>c</sup>GenBank numbers starting with DQ were newly generated.

Perithecia were removed from the grapevines or water agar and squash mounts were made in lactic acid. Perithecia from herbarium specimens were removed and rehydrated in sterile water and 3 % KOH. One perithecium was crushed and mounted in Melzer's reagent (3.75 g KI, 1.25 g I<sub>2</sub>, in 50 mL H<sub>2</sub>O and 50 mL chloralhydrate) to observe the reaction of the ascus tips with iodine. Vertical sections (10 µm) of ascomata were made with a Leica CM3050 (Leica Microsystems, Rijswijk, the Netherlands) freezing microtome and were mounted in lactic acid. Perithecial and neck dimensions were determined for 10 ascomata from each specimen. As herbarium specimens often had few perithecia available, the number of perithecia examined is indicated in each description.

Low-temperature scanning electron microscopy (SEM) was used to visualise morphological features of the phialides and conidia. Strains were plated onto small agar blocks (< 25 mm<sup>2</sup>) of MEA (2 % malt extract and 4 % water agar) and incubated at 25 °C in the dark. These blocks were mounted in a specimen holder with a mixture of Cryoblock (Klinipath, Duiven, the Netherlands) and colloidal graphite (Emscope Laboratories, Ashford, U.K.). The specimens were flash-frozen (-212 °C) in nitrogen slush and transferred to the cryo-stage where a thin gold layer was splattered over the sample (75 s, 1.2 kV). The sample was then transferred to a cooled SEM stage chamber. Specimens were viewed with an acceleration voltage of 5 KV at -188 °C with a Jeol JSM 840A (JEOL Ltd., Tokyo, Japan) scanning electron microscope. Images were acquired as bitmaps using the Semafore 3.02 software package (JEOL Ltd., Tokyo, Japan).

*Culture descriptions:* Strains were plated onto MEA and incubated at 25 °C in the dark for 14 d. Mycelial plugs of 2 mm diam were cut from the outer growth of the colonies and plated on to MEA, PDA and oatmeal agar (OA, Gams *et al.* 1998). Plates were incubated at 25 °C and the colony characters and pigment production noted after 8 and 16 d. Colony colours were determined using Kornerup & Wanscher (1978). Cardinal temperatures for growth were determined by incubating MEA plates in the dark at temperatures ranging from 5 to 40 °C in 5 ° intervals, including 37 °C to simulate human body temperature. Radial growth was measured after 8 d at 25 °C.

### Molecular analyses

*DNA isolation and amplification:* Genomic DNA was extracted from 27 strains using approximately 200 mg mycelium with the FastDNA Kit (Bio101, Carlsbad, CA) according to the manufacturer's instructions. The partial β-tubulin (TUB) and actin (ACT) genes were amplified for 27 strains, as was the 18S rRNA (SSU) gene for seven strains and the 28S rRNA (LSU) gene

for two strains. A fragment of approximately 600 bp of the 5' end of the TUB gene was amplified using primers T1 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). Approximately 300 bp of the 5' end of the ACT gene were amplified using primers ACT-512F and ACT-783R (Carbone & Kohn 1999). A fragment of approximately 1700 base pairs at the 5' end of the SSU gene was amplified using the primers NS1, NS3, NS4, NS6 (White *et al.* 1990), and NS24 (Gargas *et al.* 1995). Approximately 1400 base pairs at the 5' end of the LSU gene were amplified using primers LR0R (Rehner & Samuels 1994), LR3R, LR5, and LR7 (Vilgalys & Hester 1990). Because Groenewald *et al.* (2001) had found that the internal transcribed spacers (ITS1 and ITS2) and 5.8S rRNA gene were not informative enough to distinguish *Phaeoacremonium* strains reliably at the species level, this region was excluded from consideration. The reaction mixture for PCR contained 5 µL of diluted sample, 1× PCR buffer (Bioline GmbH, Luckenwalde, Germany), 2.5 pmol of each primer, 200 µM of each of the dNTP's, 0.5 U of *Taq* DNA polymerase (Bioline GmbH), MgCl<sub>2</sub> at 1.5 mM, or 0.5 mM in the case of ACT, and each reaction was made up to a final volume of 25 µL with sterile water. The following PCR amplification cycles were run on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA): 96 °C for 5 min, followed by 36 cycles of denaturation (94 °C for 30 s), annealing for 30 s (at 52 °C for ACT, 58 °C for TUB and 50 °C for LSU and SSU) and elongation (72 °C for 90 s), and a final 7 min extension step at 72 °C. PCR products were analysed by electrophoresis at 85 V for 30 min in a 0.8 % (w/v) agarose gel in 0.5 × TAE buffer (0.4 M Tris, 0.05 M glacial acetic acid and 0.01 M ethylenediamine tetraacetic acid [EDTA], pH 7.85) and visualised under UV light following ethidium bromide staining. PCR products were purified according to the manufacturer's instructions using a commercial kit (GFX PCR DNA and Gel Band Purification, Amersham Biosciences, Roosendaal, the Netherlands). Sequencing reactions were carried out with the PCR primers using a DYEnamic ET Terminator Cycle Sequencing kit (Amersham Biosciences) according to the manufacturer's recommendations, and the resulting products were analyzed on an ABI Prism 3700 DNA Sequencer (Perkin-Elmer, Norwalk, Foster City, CA). A consensus sequence was computed from the forward and reverse sequences with SeqMan from the Lasergene package (DNASTar, Madison, WI). Sequences were deposited with GenBank (Table 3) and the alignments and trees in TreeBASE (TreeBASE accession number: S1479).

*Phylogenetic analysis:* Sequences were manually aligned in Sequence Alignment Editor v. 2.0a11 (Rambaut 2002) by inserting gaps.

The ACT and TUB sequences were added to the alignment generated by Mostert *et al.* (2005). A partition homogeneity test was conducted in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) to test the pairwise congruence between the ACT and TUB datasets. Phylogenetic analyses using parsimony were also conducted with PAUP. *Pleurostomophora richardsiae* (Nannf.) L. Mostert, W. Gams, & Crous (CBS 270.33; GenBank: ACT = AY579271, TUB = AY579334) and *Wuestneia molokaiensis* Crous & J.D. Rogers (CBS 114877; GenBank: ACT = AY579272, TUB = AY579335) were used as outgroups in the combined analysis of the ACT and TUB sequences.

Higher-order phylogenetic relationships were examined using 58 LSU sequences representing 11 orders and 2 families of perithecial ascomycetes, as well as 58 SSU sequences representing 9 orders and 2 families of perithecial ascomycete families. Members of the *Dothideomycetes* were used as outgroups in maximum parsimony analyses. LSU sequences were obtained for two strains of *Pm. sphinctrophorum*, CBS 337.90 and CBS 694.88. Also, SSU sequences were obtained for the following strains, CBS 337.90 and CBS 694.88 (*Pm. sphinctrophorum*), CBS 111586 (*Pm. theobromatis*), CBS 113065 (*Pm. viticola*), CBS 860.73 (*Pm. parasiticum*), CBS 111657 (*Pm. griseorubrum*) and CBS 110573 (*Pm. tardicrescens*). Homologous LSU and SSU sequences, of which the accession numbers are given in Figs 8–9, were retrieved from GenBank.

Maximum parsimony and distance analysis were conducted in PAUP. For distance analysis, neighbour-joining with the uncorrected “p,” the Jukes-Cantor and the Kimura 2-parameter substitution models were performed. Maximum parsimony analysis was performed using the heuristic search option with a 100 random taxon additions for the combined TUB and ACT dataset and 10 random taxon additions for the LSU and SSU datasets. Tree bisection and reconstruction (TBR) was used as the branch swapping algorithm. Gaps were treated as missing data for the LSU and SSU analysis and as a fifth character for the combined TUB and ACT dataset. All characters were unordered and of equal weight. Bootstrap support values were calculated from 1000 heuristic search replicates and 100 random taxon additions for the combined TUB and ACT dataset and from 10 random taxon additions for the LSU and SSU datasets. Other measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and the rescaled consistency index (RC) values.

Bayesian analyses were also performed on the three datasets using MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003). The combined TUB and ACT dataset was divided into two partitions representing

the two different genes. The program MrModeltest (J.J.A. Nylander, available from the internet: [www.ebc.uu.se/systzoo/staff/nylander.html](http://www.ebc.uu.se/systzoo/staff/nylander.html)) was used to select the optimal model of sequence evolution for each individual partition. The likelihood and prior settings were changed in MrBayes according to the models found with MrModeltest for each partition. Markov chains were initiated from a random tree and run for 1 million generations, keeping one out of every 100<sup>th</sup> generation. Convergence among chains was monitored by examining plots of log-likelihood values and observing when the values of the four chains have reached a plateau. The first 300 000 generations (burn-in) were discarded and the remaining samples were used to calculate the 50 % majority-rule tree and the posterior probability for the individual branches. The LSU and SSU datasets were not partitioned and analysed separately. MrModeltest was used to determine the optimal model of sequence evolution. The likelihood and prior settings were changed according to the model found. The LSU and SSU datasets were analysed in the same way as the combined dataset. Each run was performed twice.

*Molecular identification system with species-specific primers:* Primers were developed from the aligned 720-nucleotide TUB and 260-nucleotide ACT sequences generated in the section DNA isolation and amplification. The introns of the TUB gene were defined with reference to model strain *Pm. parasiticum* (CBS 860.73, AF246803), which has introns spanning nucleotide positions 1–129, 154–206, 233–306, 349–406 and 462–537. The introns of the ACT gene were defined using the introns at positions 51–144 and 176–227 of the same reference strain. Twenty-three primers (Table 4) were developed from the introns of the TUB and ACT genes. Reverse primers (names starting with the identifier “Pbr”) were developed in three of the introns of the TUB gene (Fig. 11). These reverse primers, used in combination with the universal T1 forward primer (O'Donnell & Cigelnik 1997), amplified products in three size ranges (Table 4). Forward primers were developed in the ACT gene and used in combination with the degenerate reverse primer, ACT-783R (Carbone & Kohn 1999), amplifying products of the same size (Table 4). To reduce the number of amplification reactions, the primers were multiplexed in combinations of two specific primers per reaction. A positive control reaction was included in the TUB amplifications by using the Bt2b (Glass & Donaldson 1995) primer in the multiplex reaction which, together with the T1 primer, produced a fragment of 720 base pairs. As the species-specific primers in the ACT gene were developed close to the annealing sites of the universal primers, a strategy that resulted in undetectable size differences among

amplicons, no positive control reaction was attempted for the ACT gene.

Initially, all species-specific primers were tested only in combination with the T1 primer, and the PCR reaction mixture contained 0.5  $\mu$ L of diluted sample, 1 $\times$  PCR buffer (Bioline), 2.5 pmol of each primer, 200  $\mu$ M of each of the dNTP's, 0.3 U of *Taq* DNA polymerase (Bioline), and 1.5 mM of  $MgCl_2$  and was made up to a final volume of 12.5  $\mu$ L with sterile water. Primer concentrations were optimised for each of the different primer combinations (Table 5) in the multiplex reactions and final reactions were made up to a volume of 25  $\mu$ L using 1  $\mu$ L of diluted genomic DNA. The DNA concentrations ranged from 10–100 ng/ $\mu$ L. A touch-down PCR was done with a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA), consisting of the following cycles: 94 °C for 5 min, followed by 5 cycles of denaturation at 94 °C for 30 s, annealing for 30 s at 66 °C and elongation at 72 °C for 60 s; 5 cycles of denaturation at 94 °C for 30 s, annealing for 30 s at 64 °C and elongation at 72 °C for 60 s and a final 25 cycles of denaturation at 94 °C for 30 s, annealing for 30 s at 62 °C and elongation at 72 °C for 60 s and a final 6 min extension step at 72 °C. PCR products were analysed as described in the “DNA isolation and amplification” section.

The specificity of the primers was tested on all the species of *Phaeoacremonium* listed in Table 3. Thereafter the primers were also tested on other fungi associated with vascular invasion of grapevines as well as other fungi associated with phaeohyphomycosis in humans (Table 6).

### Mating studies

Strains were grown on MEA plates for 2 wk. Conidia were dislodged from the agar surface by means of a glass rod and suspensions were prepared in 5 mL sterile distilled water. Two aliquots of 100  $\mu$ L each, representing two different strains, were pipetted onto the canes of GWA plates. Strains were mated in all possible combinations. Controls consisted of a 200  $\mu$ L aliquot of one isolate only. Plates were incubated at 22 °C under continuous white light. Matings of *T. krajdenii* and *T. rubrigena* were placed in darkness at 24 °C, which proved to promote perithecial formation in these species. Successful crosses were noted. For a mating to be considered successful, perithecia had to produce abundant ascospores that germinated readily in culture. One mating was chosen and up to 30 single-ascospore subcultures obtained. In the case of *Pm. rubrigenum*, ascospores were not exuded in droplets, and were therefore retrieved by cutting the perithecium and streaking out the asci releasing ascospores on MEA. Single ascospores were picked up with a micromanipulator (Axioplan, Carl Zeiss B.V., Germany). Further crosses were made with these

ascospore strains using the procedure described above. The ascospore strains used in the second crossing are given in parentheses for *T. argentinensis* (L.M. 562–L.M. 592), *T. austroafricana* (L.M. 797–L.M. 806), *T. minima* (L.M. 227–L.M. 240, L.M. 243–L.M. 247, L.M. 249), *T. parasitica* (L.M. 525–L.M. 526, L.M. 528–L.M. 539, L.M. 541, L.M. 543–L.M. 544, L.M. 546, L.M. 548, L.M. 550–L.M. 552, L.M. 554, L.M. 556), *T. krajdenii* (L.M. 807–L.M. 833), *T. rubrigena* (L.M. 893–L.M. 894, L.M. 896–L.M. 916) and *T. viticola* (L.M. 760–L.M. 779, L.M. 783–L.M. 788). Two strains found to be of opposite mating type in each species were arbitrarily designated as MAT1-1 and MAT1-2. Tester strains were designated for each species (CBS strain numbers are reported in the Taxonomy section). The null hypothesis of a 1:1 ratio of the two mating types of each mating was evaluated with a test for proportions i.e.  $H_0: p = 0.5$  where  $p = P(\text{Mat1-1})$  (Milton & Arnold 1990). Table 7 lists the number of conidial and ascospore strains used, the mating type distribution found and corresponding *P*-values. Inter-species crossings were done to investigate the biological species boundaries; however, *Pm. iranianum*, *Pm. sphinctrophorum* and *Pm. theobromatis* were not included.

### Numerical analysis of morphological and cultural characters for use in BioloMICS

An electronic identification key was developed using 23 micromorphological and cultural characters for 22 *Phaeoacremonium* species and TUB sequences generated with the primers T1 and Bt2b. Discrete data were scored for the main states as well as for the intermediate states. The minimum, 5<sup>th</sup> percentile, 95<sup>th</sup> percentile and maximum values from the size data for micromorphological structures were used. The micromorphological characters include: conidiophore structure and size, occurrence of three phialide types, type II phialide shape and size, type III phialide shape, percurrent occurrence of phialide rejuvenation, extent of wart formation, maximum wart diameter, mycelial texture, conidial shape and conidial size. Cultural characters used were colony colour on MEA at 25 °C after 8 d in the dark, yellow pigment production on PDA and OA, optimal and maximal growth temperatures, and colony radius at 25 °C and 30 °C after 8 d in the dark. A data matrix was compiled on a spreadsheet and imported into BioloMICS (Robert & Szoke 2003). Character weights were determined by excluding individual characters and comparing the distance matrices. Coherent coefficients of correlation were determined and used to indicate the extent to which a given character positively correlated with TUB sequences. The use of characters weighted according to a subjective perception of their relative usefulness was compared to the use of objective, unweighted characters (meaning that each character was automatically



**Table 4.** Primers developed for the identification of *Phaeoacremonium* species. The position of the primer binding sites was derived by comparison with GenBank accessions AF246803 ( $\beta$ -tubulin) and AY579253 (actin).

	Primer	Primer sequence (5'-3')	Primer binding site	Fragment size (bases)
<b><math>\beta</math>-tubulin (Reverse primers)</b>				
<i>Pm. angustius</i>	Pbr4_1	ACA ACA CAT GTA TAG GCT ATG AGT AA	531–556	556
<i>Pm. aleophilum</i>	Pbr6_1	TCG CGA TGG CCC ACT GCC TAC	521–541	548
<i>Pm. australiense</i>	Pbr1_1	CTA TCT CAA ATA TCG GGA GCC TC	561–583	583
<i>Pm. iranianum</i>	Pbr12	TCG CGC GAT GGG CTA TTG TCT G	524–545	545
<i>Pm. alvesii</i> and <i>Pm. rubrigenum</i>	Pbr5_1	ACG AGC TGA AGG TAA AAR GGA TC	544–548	548
<i>Pm. spinctophorum</i>	Pbr2_1	AGC RCC TGT AGC TTT GCA G	593–611	611
<i>Pm. subulatum</i>	Pbr7_1	AGA AAG GGT TGG AGT CTT CAC	533–553	553
<i>Pm. theobromatis</i>	Pbr10	TAC ATG GCT GGG CGA TGAA TAG	534–555	555
<i>Pm. venezuela</i>	Pbr3_1	ATC TCG AGA CAG AGC GGA TG	552–568	568
<i>Pm. viticola</i>	Pbr8	GGC TTT GAG TAG ATT TGG CA	523–542	542
<i>Pm. amstelodamense</i> and <i>Pm. griseorubrum</i>	Pbr9	CGG TGA ACA TCA CGG GGG AG	437–456	456
<i>Pm. parasiticum</i>	Pbr2_2	CGG TAG AGG TTT GGC GAC	430–446	446
<i>Pm. scolyti</i>	Pbr3_2	GCG GTG AGC ATC ATG GGA C	437–455	455
<i>Pm. tardicrescens</i>	Pbr1_2	TCC CGC TGA AGG AAA GGA AG	430–449	449
<i>Pm. argentinense</i>	Pbr5_2	TTC GGG ACA CTG AGA AAG GAC	228–248	248
<i>Pm. austroafricanum</i>	Pbr6_2	GTC AGT CGT GTC TAG AGG TAC TG	234–257	257
<i>Pm. inflatipes</i>	Pbr7_2	CAA ATC GTT AGA TAT ATT CCA GCG CG	235–260	262
<i>Pm. krajdenii</i>	Pbr13	AGA TCG TTA GAC GTG TCC CG	253–272	272
<i>Pm. mortoniae</i>	Pbr11	TGT CAG TTT CGT TCC AGG ATA C	236–257	257
<i>Pm. novae-zealandiae</i>	Pbr4_2	ACG TCG TCA GTC TTT TGC CGA ATC	239–262	262
<b>Actin (Forward primers)</b>				
<i>Pm. alvesii</i>	Paf2	GCC AAT CTG AGG CTA TGG AA	70–89	192
<i>Pm. griseorubrum</i>	Paf3	TCC GCC AAT TGA GGC TAC AA	66–85	194
<i>Pm. rubrigenum</i>	Paf1	GCC AAT CGA GGC TAT GGA G	70–88	191

assigned a weight equal to 1). Coherent coefficients of correlation were then calculated. Various algorithms were used to obtain the best fit for each of the data types.

## RESULTS

### Phylogenetic analyses based on DNA sequence data

*SSU rRNA sequence data:* The SSU sequence of *Pm. sphinctrophorum* (CBS 337.90) has a 440 nucleotide insertion (nucleotide positions 1118–1558) in the SSU, resembling a group I intron (Gargas *et al.* 1995). The intron was excluded from the analysis. A maximum parsimony analysis (Fig. 8) was performed using 296 parsimony-informative characters in an alignment containing 1769 nucleotides from 58 taxa, including the two outgroups. Five trees were obtained that differed in the arrangement of the *Togniniaceae* in the

*Diaporthales* and *Calosphaeriales* clades. In three of the five trees, the *Togniniaceae* grouped with the *Diaporthales*. In one tree, the *Togniniaceae* grouped with the *Calosphaeriales*, while in the remaining tree the *Togniniaceae* grouped basal to a clade embracing the *Diaporthales* and *Calosphaeriales*. Nine orders and five families of *Sordariomycetes* could be distinguished as different clades in the analysis, i.e. the *Diaporthales* clade containing the *Togniniaceae* clade (87 %), the *Calosphaeriales* clade containing the *Pleurostomataceae* clade (100 %) and the *Calosphaeriaceae* clade (100 %), the *Sordariales* clade (99 %), the *Chaetosphaeriales* clade (100 %), the *Boliniales* represented by one strain, the *Cephalothecaceae* clade (76 %), the *Magnaporthaceae* clade (96 %), the *Ophiostomatales* clade (100 %), the *Xylariales* clade (86 %), the *Hypocreales* clade (62 %), and the *Microascales* clade (100 %).

**Table 5.** The 14 primer combinations used for species identifications and end concentrations of primers present in the multiplex and single amplification reactions.

Species-specific primers		End concentrations (pmol/μL)					
First specific primer (SP I)	Second specific primer (SP II)	T1	SP I	SP II	Bt2b	Paf	ACT-783R
Pbr1_1	Pbr1_2	1.3	0.4	0.4	0.5		
Pbr2_1	Pbr2_2	1.3	0.5	0.4	0.4		
Pbr3_1	Pbr3_2	1.5	0.7	0.2	0.4		
Pbr4_1	Pbr4_2	1.5	0.8	0.2	0.4		
Pbr5_1	Pbr5_2	1.5	0.7	0.2	0.5		
Pbr6_1	Pbr6_2	1.5	0.7	0.2	0.4		
Pbr7_1	Pbr7_2	1.5	0.7	0.2	0.4		
Pbr8	Paf1	1.1	0.7		0.4	0.3	0.3
Pbr9	Paf2	0.8	0.4		0.4	0.4	0.4
Pbr10		0.9	0.4		0.5		
Pbr11		0.9	0.4		0.5		
Pbr12		0.9	0.4		0.5		
Pbr13		0.9	0.4		0.5		
Paf3						0.4	0.4

**Table 6.** Additional fungi on which the *Phaeoacremonium* species-specific primers were tested.

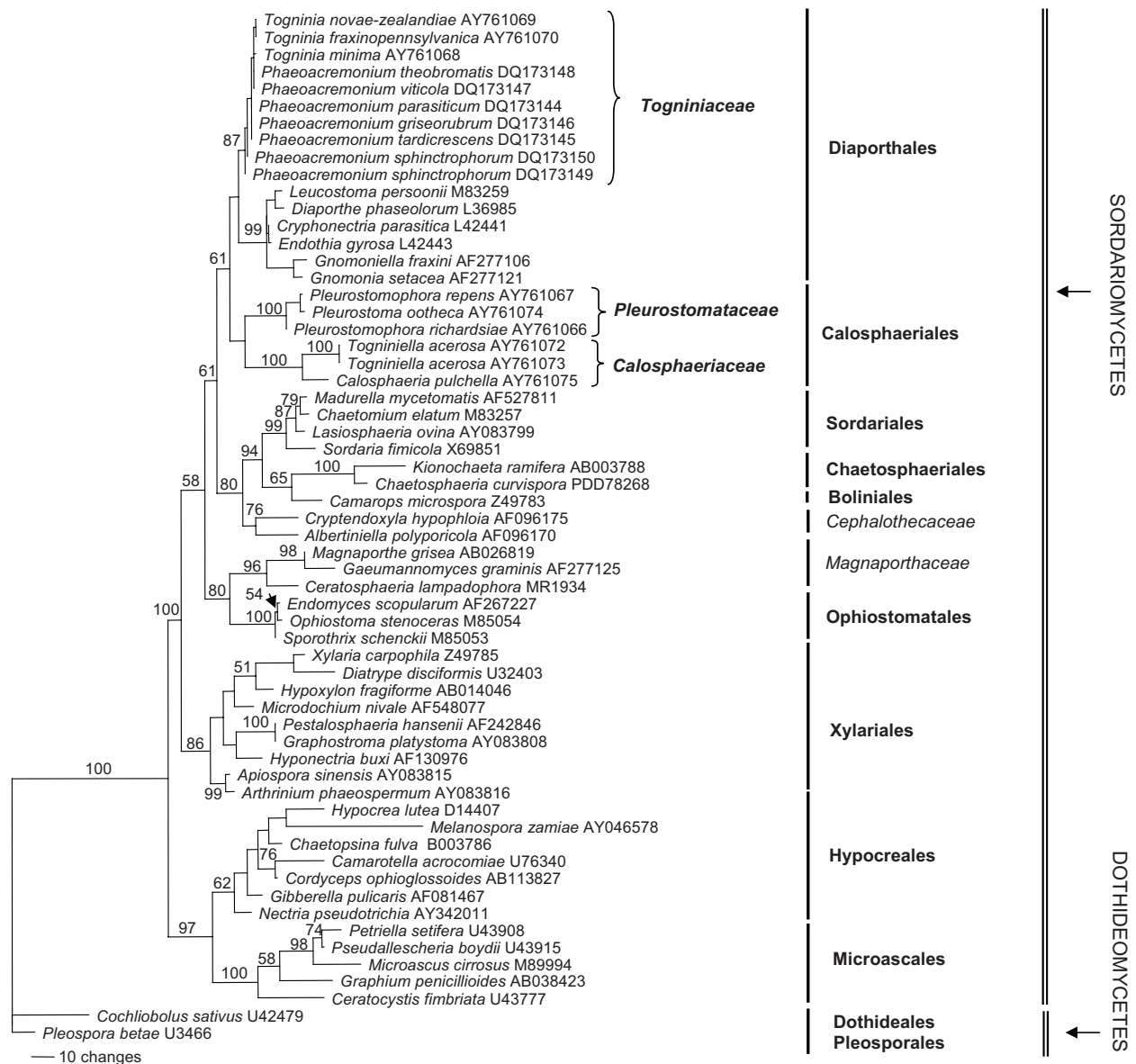
Fungus	Strain number	Host	Origin
<b>Grapevine vascular fungi</b>			
<i>Acremonium</i> cf. <i>charticola</i>	CBS 115996	<i>Vitis vinifera</i>	South Africa, Paarl
<i>Acremonium</i> cf. <i>ochraceum</i>	CBS 109930	<i>Vitis vinifera</i>	South Africa, Paarl
<i>Botryosphaeria australis</i>	CBS 113219	<i>Acacia</i> sp.	Australia, Bateman's Bay
<i>Botryosphaeria lutea</i>	CBS 110299	<i>Vitis vinifera</i>	Portugal, Oeiras, Quinta do Marquês
<i>Cadophora luteo-olivacea</i>	CBS 109928	<i>Vitis vinifera</i>	South Africa, Paarl
<i>Cylindrocarpon destructans</i>	CBS 112236	<i>Vitis vinifera</i>	South Africa
<i>Eutypa lata</i>	CBS 208.87	<i>Tilia</i> sp.	Switzerland, Vaud, Chênaies/Villeneuve
<i>Fomitiporia punctata</i>	CBS 100121	<i>Salix</i>	Germany, Bayern, Straubing
<i>Lophiostoma</i> sp.	CBS 109932	<i>Vitis vinifera</i>	South Africa, Paarl
<i>Phaeomoniella chlamydospora</i>	L.M. 40	<i>Vitis vinifera</i>	South Africa, Paarl
<i>Phialemonium</i> cf. <i>curvatum</i>	CBS 115998	<i>Vitis vinifera</i>	South Africa, Wellington
<i>Phialemonium viticola</i>	CBS 252.38	<i>Vitis vinifera</i>	Italy
<b>Fungi commonly associated, correctly or falsely (*), with subcutaneous phaeohyphomycotic infections</b>			
<i>Alternaria alternata</i>	CBS 109803	Human	Germany
<i>Cladophialophora bantiana</i>	CBS 101158	Human	Japan
<i>Dactylaria gallopava</i>	d.H.13020	Human	Netherlands, Rotterdam
<i>Exophiala dermatitidis</i>	CBS 207.35	Human	Japan
<i>Exophiala jeanselmei</i>	CBS 115833	Human	Kuwait
<i>Exophiala spinifera</i>	CBS 110628	Bark	Venezuela
<i>Phialemonium obovatum</i>	CBS 396.82	Human	U.S.A.
<i>Pleurostomophora repens</i> *	CBS 294.39	Pine lumber	U.S.A., Florida, Caryville
<i>Pleurostomophora richardsiae</i>	CBS 270.33	Unknown	Sweden
<i>Phialophora verrucosa</i>	d.H.12666	Unknown	Unknown
<i>Scytalidium hyalinum</i> *	CBS 145.78	Human	U.K.

The neighbour-joining analyses produced three trees with similar topology (not shown), one for each substitution model analysed. The nine orders and five families were represented in separate clades. The trees were different from the trees produced with maximum parsimony analysis, in that the *Diaporthales* clade containing the *Togniniaceae* had a bootstrap support value of 72 % (uncorrected “p” substitution model). Two of the taxa, *Camarotella acrocomiae* (mont.) K.D. Hyde & P.F. Cannon, representing the *Phyllachoraceae*, and *Melanospora zamiae* Corda, of the *Ceratostomaceae*, grouped basal to the *Hypocreales* and *Microascales* clades.

Bayesian analysis produced a 50 % majority-rule consensus tree (not shown) with the nine orders and

five families represented by the corresponding clades. The tree was different from the trees produced with maximum parsimony analysis in that the *Diaporthales* clade containing the *Togniniaceae* had a posterior probability value of 0.69 in the Bayesian analysis. The *Calosphaeriales* clade had an even lower posterior probability value of 0.55.

*LSU rRNA sequence data:* A maximum parsimony analysis (Fig. 9) was performed using 338 parsimony-informative characters from an alignment of 1240 nucleotides. The data were derived from 58 strains, including the two outgroups. Twelve trees were obtained and these trees differed in the internal arrangement of the taxa in the *Togniniaceae*,

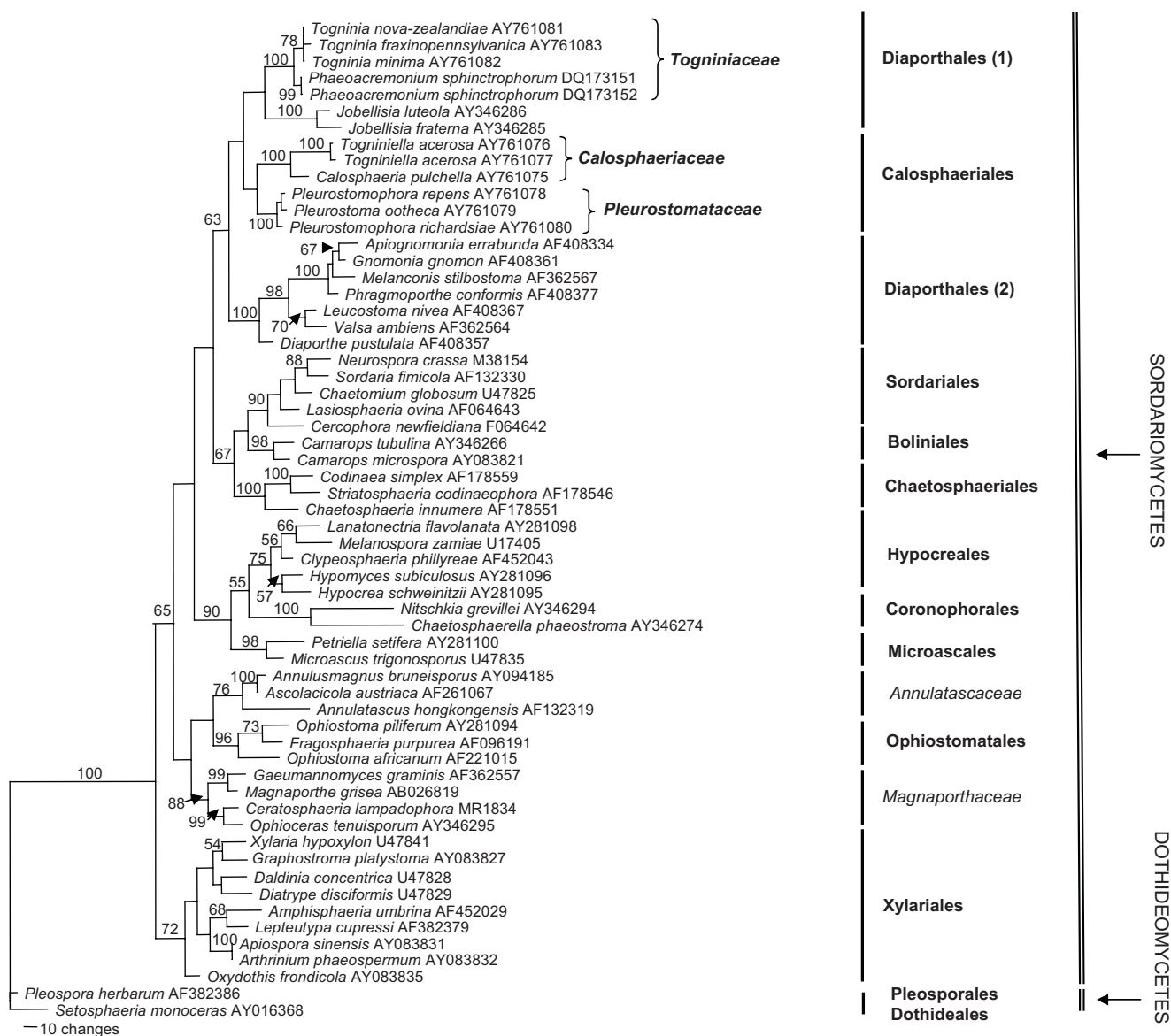


**Fig. 8.** One of five most parsimonious trees obtained from heuristic searches of an alignment of the SSU rRNA gene sequences (TL = 1046 steps, CI = 0.502, RI = 0.728 and RC = 0.365). Bootstrap support values (1000 replicates) above 50 % are shown at the nodes. *Cochliobolus sativus* and *Pleospora betae* were used as outgroups. GenBank numbers starting with DQ were newly generated.

*Diaporthales* (2) and *Xylariales* clades. Eleven orders and five families could be observed in different clades in the analysis, i.e. the *Diaporthales* (1) clade containing the *Togniniaceae* clade (100 %) and the *Jobellisia* clade (100 %), the *Calosphaeriales* clade containing the *Pleurostomataceae* clade (100 %) and the *Calosphaeriaceae* clade (100 %), the *Diaporthales* (2) clade (100 %), the *Sordariales* (90 %) clade, the *Boliniales* clade (98 %), the *Chaetosphaeriales* clade (100 %), the *Hypocreales* clade (75 %), the *Coronophorales* clade (100 %), the *Microascales* clade (98 %), the *Annulatascaceae* clade (76 %), the *Ophiostomatales* clade (96 %), the *Magnaporthaceae* clade (88 %) and the *Xylariales* clade (72 %). The *Diaporthales* (1) and *Calosphaeriales* clades clustered together, but with no bootstrap support. There was no direct association of the *Togniniaceae* and *Jobellisia*

clades with that of the *Diaporthales* (2) clade. The *Calosphaeriales*, *Diaporthales* (1) and *Diaporthales* (2) clades clustered together with a bootstrap support value of 63 %.

The neighbour-joining analyses produced three trees of similar topology, one for each substitution model analysed (not shown). The 11 orders and five families were represented in separate clades. The trees were different from the trees produced with maximum parsimony analysis, in that the *Togniniaceae* clade did not cluster with the *Jobellisia* clade, but formed a basal element within a broad cluster that also contained the *Calosphaeriales* and the *Diaporthales* (2) clades. The *Calosphaeriales* clade and the *Diaporthales* (2) clade clustered together with 64 % bootstrap support (uncorrected “p”). The *Calosphaeriales* and *Diaporthales* (1 and 2) clustered together with

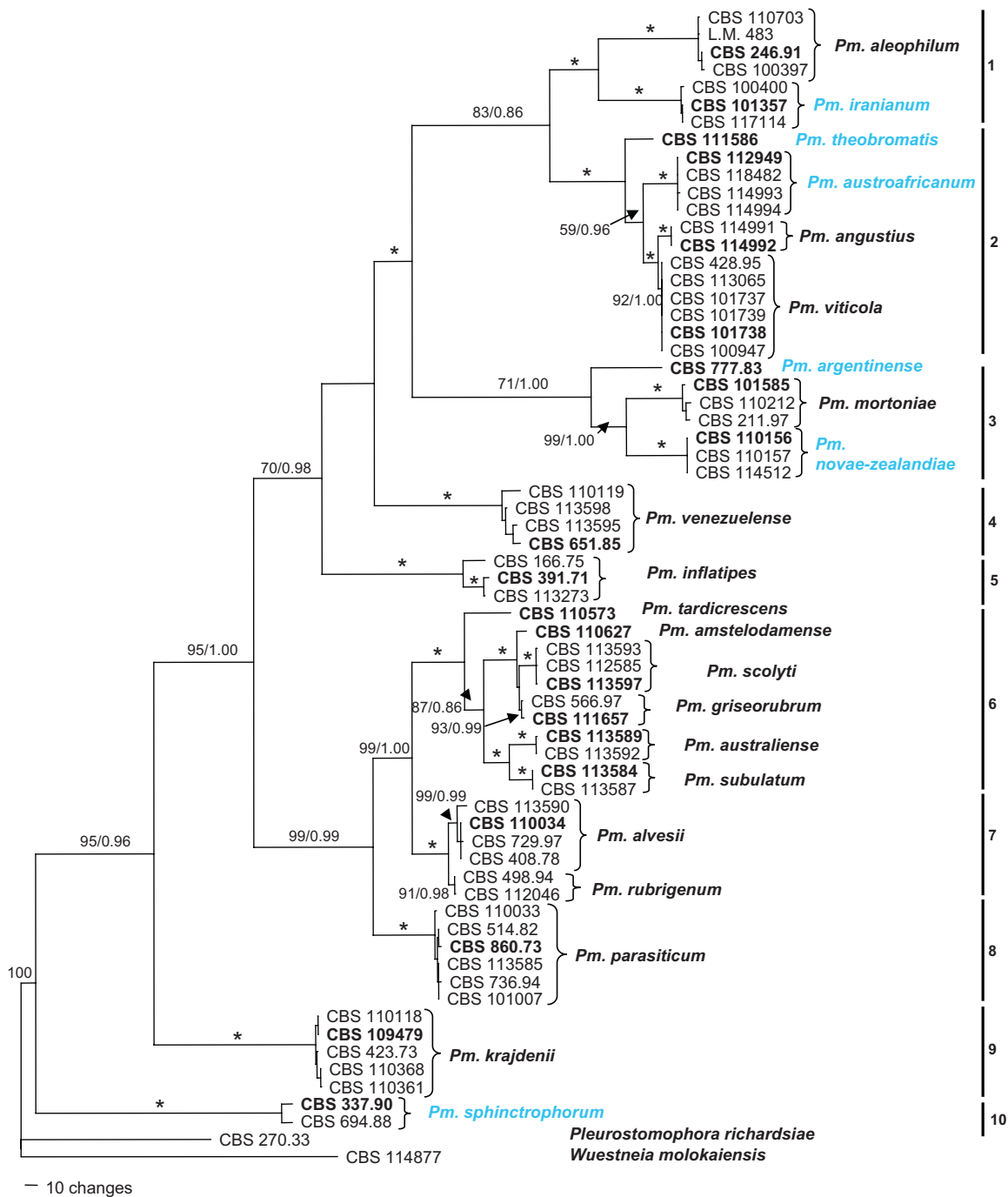


**Fig. 9.** One of 12 most parsimonious trees obtained from heuristic searches of an alignment of the LSU rRNA gene sequences (TL = 2121 steps, CI = 0.373, RI = 0.643 and RC = 0.240). Bootstrap support values (1000 replicates) above 50 % are shown at the nodes. *Pleospora herbarum* and *Setosphaeria monoceras* were used as outgroups. GenBank numbers starting with DQ were newly generated.

54 % bootstrap support (uncorrected “p”). The *Magnaporthaceae* clade appeared to cluster with the *Sordariales*, *Bolineales* and *Chaetosphaeriales*, but without any bootstrap support.

The Bayesian analysis produced a 50 % majority-rule consensus tree (not shown) with the 11 recognised orders and five families again represented as clades. The *Calosphaeriales* and *Diaporthales* (1 and 2) clades (each with a posterior probability value of

1.00) had a hierarchy similar to that obtained with the neighbour-joining analyses. The *Calosphaeriales* clade (1.00) grouped with the *Diaporthales* (2) clade with a posterior probability value of 0.87. The *Togniniaceae* clade did not cluster with the *Jobellisia* clade, but was basal in the clade combining the *Calosphaeriales* and *Diaporthales*. The *Magnaporthaceae* clade clustered with no posterior probability support with the *Sordariales*, *Boliniales* and *Chaetosphaeriales*.



**Fig. 10.** One of 10 most parsimonious trees obtained from heuristic searches of a combined alignment of the ACT and TUB gene sequences (length = 2280 steps, CI = 0.589, RI = 0.895 and RC = 0.527). Bootstrap support values (1000 replicates) above 50 % are shown at the nodes. An asterisk (\*) indicates bootstrap support values of 100 % and posterior probability values of 1.00 obtained for a node. *Pleurostomophora richardsiae* and *Wuestneia molokaiensis* were used as outgroups. Type strains are indicated in bold print. Species names in blue are new species.

*Combined  $\beta$ -tubulin and actin sequence data:* A maximum parsimony analysis (Fig. 10) was performed using 475 parsimony-informative characters in a combined alignment containing 1018 nucleotides. The data were derived from 66 strains including the two outgroups. The result of the partition homogeneity test showed that the ACT and TUB data sets were congruent ( $P = 0.395$ ) and could therefore be combined. Ten trees were obtained. They differed in the internal arrangement of taxa in the *Pm. aleophilum* and *Pm. parasiticum* clades. Neighbour-joining analyses gave similar tree topologies and bootstrap support values, but were different only in that *Pm. venezuelense* and *Pm. inflatipes* clustered together with relatively high bootstrap values, which were as high as 91 %. The 50 % majority-rule consensus tree obtained with the Bayesian analysis gave a topology similar to that seen in maximum parsimony analysis. The phylogenetic tree consisted of 10 major clades. The first clustered *Pm. aleophilum* and *Pm. iranianum* together with 100 % bootstrap support. The second, equally strongly supported, included *Pm. theobromatis*, *Pm. austroafricanum*, *Pm. angustius* and *Pm. viticola*. The third grouped *Pm. argentinense*, *Pm. mortoniae* and *Pm. novae-zealandiae* with 71 % support. *Phaeoacremonium venezuelense* and *Pm. inflatipes* made up clades 4 and 5, each with 100 % support. A sixth clade gave 100 % support to the clustering of *Pm. tardicrescens*, *Pm. amstelodamense*, *Pm. scolysi*, *Pm. griseorubrum*, *Pm. australiense* and *Pm. subulatum*. The final four clades consisted in turn of *Pm. alvesii* plus *Pm. rubrigenum* (99 % bootstrap support), *Pm. parasiticum* (100 %), *Pm. krajdenii* (100 %) and *Pm. sphinctrophorum* (100 %). From this phylogenetic analysis (Fig. 10), six new species could be identified. Four of these, *Phaeoacremonium austroafricanum*, *Pm. iranianum*, *Pm. novae-zealandiae* and *Pm. sphinctrophorum*, each had 100% bootstrap support. Two new species, *Pm. theobromatis* (CBS 111586) and *Pm. argentinense* (CBS 777.83), are known from only one isolate each. Their distant grouping and distinct morphological and cultural characteristics support their designation as new species.

*Placement of the Togniniaceae:* Réblová *et al.* (2004) placed the family *Togniniaceae* in the order *Diaporthales* since they grouped with this order (bootstrap support of 67 %) in a maximum parsimony analysis of SSU sequence data. In our LSU analysis that included the *Togniniaceae*, two taxa of *Jobellisia* also clustered with the *Togniniaceae*, and again clustered with taxa of the *Diaporthales*, supporting the placement of the *Togniniaceae* within the *Diaporthales* (Réblová *et al.* 2004). The genus *Jobellisia* M.E. Barr (Barr 1993a) was first placed in the *Xylariales* on the basis of morphology. Phylogenetic analysis of the LSU rRNA gene, however, revealed that *Jobellisia*

falls in the *Diaporthales* (Huhndorf *et al.* 2004). In the present study, the SSU sequence data showed that the *Togniniaceae* cluster within the *Diaporthales*. In the maximum parsimony analysis this association lacked bootstrap support. The Bayesian analysis gave a probability value of 0.69 and the neighbour-joining analysis a bootstrap support of 72 % for the *Togniniaceae* and *Diaporthales* cluster. In the analyses of LSU, *Jobellisia* grouped with the *Togniniaceae* (SSU not analysed). However, the other taxa of the *Diaporthales* do not have any specific association with the *Togniniaceae*. No bootstrap support was obtained with maximum parsimony analyses for the association of the *Togniniaceae*, including the *Jobellisia* species, with the *Calosphaerales* or *Diaporthales* clades. The *Calosphaerales* and *Diaporthales* clades (excluding the *Togniniaceae*) grouped together, but with low support. The LSU analyses did not give adequate resolution amongst the *Calosphaerales*, *Diaporthales* and *Togniniaceae* clades. The shortage of living cultures representing the *Calosphaerales* makes it difficult to infer the relation of this order to the *Diaporthales*. Réblová *et al.* (2004) stated that the *Togniniaceae* hold a unique position in the *Diaporthales* and do not fit the typical *Diaporthalean* morphology. Until the LSU and SSU sequences of more species in the *Calosphaerales* and *Diaporthales* are available and trees with adequate resolution can be generated, we follow Réblová *et al.* (2004) in retaining the *Togniniaceae* in the *Diaporthales*.

#### **Molecular identification of *Phaeoacremonium* with species-specific primers**

Twenty-three species-specific primers were developed. Twenty of these anneal within the TUB gene, while three bind within the ACT gene (Fig. 11). These primers can be used in 14 multiplex reactions to identify the 22 known species of *Phaeoacremonium*.

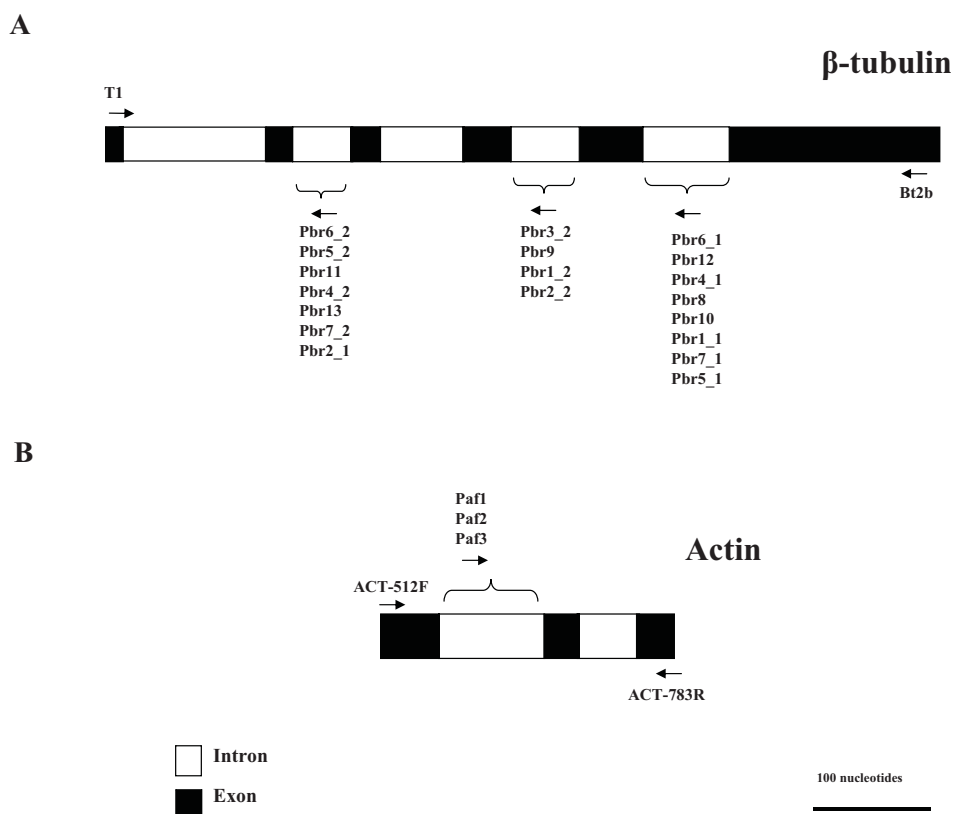
The primers were tested to confirm that they are species-specific. In two cases, pairs of species could not be distinguished using TUB primers. Primer Pbr5\_1 did not distinguish *Pm. rubrigenum* and *Pm. alvesii*, while primer Pbr9 did not distinguish *Pm. griseorubrum* and *Pm. amstelodamense*. Additional species-specific primers were therefore developed in an ACT gene intron. *Phaeoacremonium rubrigenum* and *Pm. alvesii* was distinguished by the ACT primers Paf1 and Paf2. A specific primer, Paf3, was developed for *Pm. griseorubrum* in the ACT region. *Phaeoacremonium amstelodamense* gives a positive reaction with the TUB primer Pbr9 and a negative one with Paf3.

In the multiplex PCR, the positive control band is not always present. In these cases, the presence of the product amplified by the specific primer confirms that the PCR was successful. Not all the primers could be successfully combined in a multiplex reaction, and

therefore nine multiplex reactions containing two specific primers and five reactions containing only a single specific primer were used (summarised in Table 5). The banding patterns produced when pairs of species or single species are present in test material can be seen in Fig. 12. When TUB primers were tested, products of different sizes were obtained. Those obtained when primers binding to intron 5 were used were 550–600 bp long. Intron 4 primers produced products 450 bp long, while intron 2 primers yielded products 250–270 bp long. Primer Pbr13 produced a single specific band of 270 bp, but when used in combination with the positive control primers a pale band of 550 bases is produced. Actin gene primers produced bands of approximately 200 bp. Concatemers sometimes formed from PCR products of different sizes, and could be recognised as relatively large pale bands on the gel (Fig. 12). To confirm that these products were indeed concatemers the bands were removed, melted and cleaned with a GFX column, after which the different-sized products could again be observed on a gel.

The species-specific primers did not amplify DNA of 23 heterogeneous fungal strains (Table 6) tested, showing that these primers are indeed specific. Under the conditions of the touchdown programme, six of the other fungal species gave a positive control band derived from the universal T1 and Bt2b. The other fungal species

only amplified a positive control band at annealing temperatures of 50–58 °C, lower than the temperatures used in our touchdown programme. The species that did produce a positive control band with the touchdown programme include *Acremonium ochraceum* (Onions & G.L. Barron) W. Gams, *Cylindrocarpon destructans* (Zinssm.) Scholten, *Exophiala dermatitidis* (Kano) de Hoog, *Exophiala jeanselmei* (Langeron) McGinnis & A.A. Padhye, *Phaeomoniella chlamydospora* and *Pleurostomophora repens* (R.W. Davidson) L. Mostert, W. Gams & Crous. In most cases the amplified product was between 650–800 bases long, but with *C. destructans* and *A. ochraceum*, the amplified PCR products were within the size range (550–600 bases) of the TUB intron 5 primers mentioned above. If these two fungi were tested with the multiplex reactions, they would give a false positive reaction. As this false positive would derive from amplification with the control primers, the product would be visible in all 14 tests done with specific primers, including tests not expected to yield a specific product of comparable size. The primers described here have been developed to facilitate identification of cultures, particularly in situations where the level of available mycological expertise is low. The use of these primers on grapevine material or clinical samples still needs to be validated.

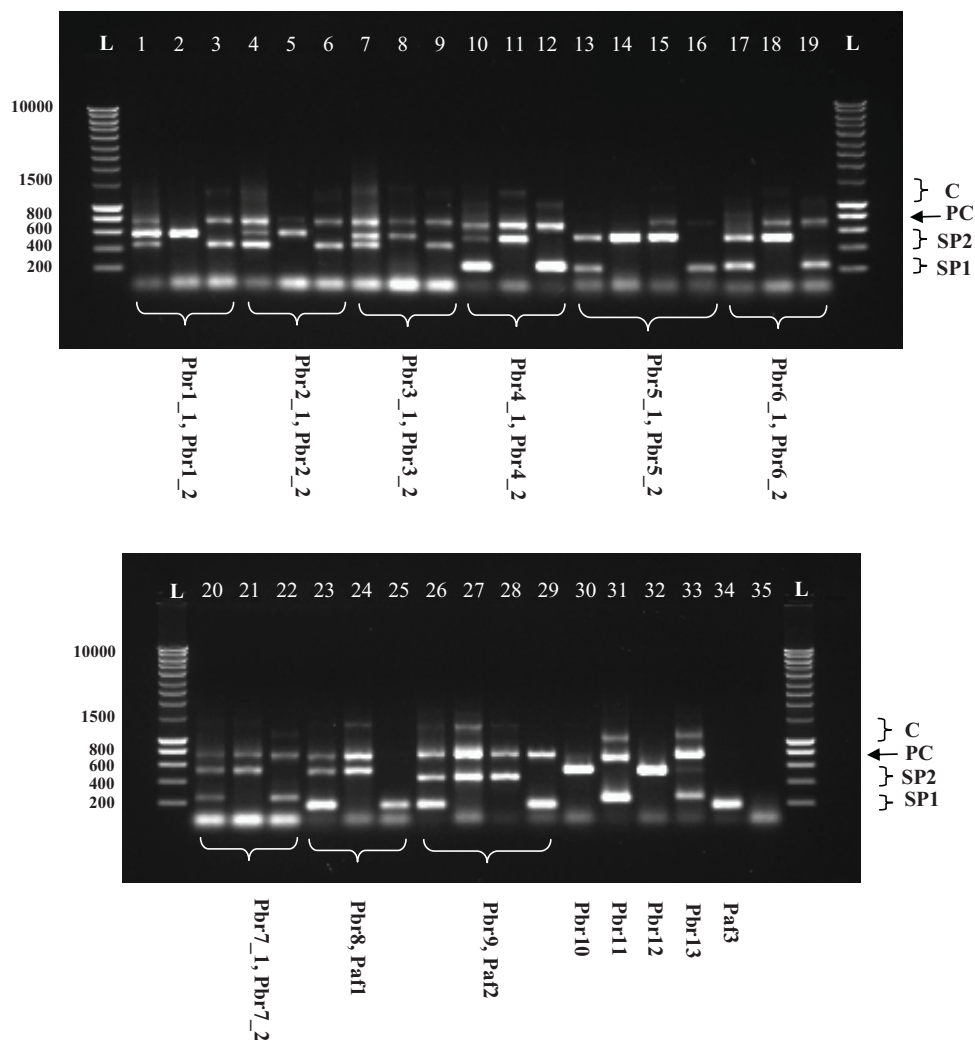


**Fig. 11.** Schematic representation indicating the introns and exons of the partial TUB (A) and ACT (B) genes. Braces indicate the approximate annealing positions and arrows the orientation of the primers.

### Mating systems

Eight *Togninia* teleomorphs formed in culture, including *T. argentinensis*, *T. austroafricana*, *T. krajdenii*, *T. minima*, *T. novae-zealandiae*, *T. parasitica*, *T. rubrigena* and *T. viticola*.

Single strains produced perithecia in two cases, namely CBS 777.83 (*T. argentinensis*) and CBS 114512 (*T. novae-zealandiae*). Single-ascospore strains of *T. argentinensis* (30 ascospores) and *T. novae-zealandiae* (29 ascospores) formed fertile perithecia, indicating that they have a homothallic mating system.



**Fig. 12.** PCR products amplified from genomic DNA of *Phaeoacremonium* species using the species-specific primer combinations indicated to the right of the lanes. L, DNA marker (SmartLadder, Eurogentec MW-1700-02, Seraing, Belgium) with sizes indicated in base pairs; Lane 1, *Pm. australiense* (CBS 113589) + *Pm. tardicrescens* (CBS 110573); Lane 2, *Pm. australiense* (CBS 113589); Lane 3, *Pm. tardicrescens* (CBS 110573); Lane 4, *Pm. sphinctrophorum* (CBS 337.90) + *Pm. parasiticum* (CBS 860.73); Lane 5, *Pm. sphinctrophorum* (CBS 337.90); Lane 6, *Pm. parasiticum* (CBS 860.73); Lane 7, *Pm. venezuelense* (CBS 651.85) + *Pm. scolyti* (CBS 113597); Lane 8, *Pm. venezuelense* (CBS 651.85); Lane 9, *Pm. scolyti* (CBS 113597); Lane 10, *Pm. angustius* (CBS 114992) + *Pm. novae-zealandiae* (CBS 110156); Lane 11, *Pm. angustius* (CBS 114992); Lane 12, *Pm. novae-zealandiae* (CBS 110156); Lane 13, *Pm. rubrigenum* (CBS 498.94) + *Pm. alvesii* (CBS 110034) + *Pm. argentinense* (CBS 777.83); Lane 14, *Pm. alvesii* (CBS 110034); Lane 15, *Pm. rubrigenum* (CBS 498.94); Lane 16, *Pm. argentinense* (CBS 777.83); Lane 17, *Pm. aleophilum* (CBS 246.91) + *Pm. austroafricanum* (CBS 112949); Lane 18, *Pm. aleophilum* (CBS 246.91); Lane 19, *Pm. austroafricanum* (CBS 112949); Lane 20, *Pm. subulatum* (CBS 113584) + *Pm. inflatipes* (CBS 391.71); Lane 21, *Pm. subulatum* (CBS 113584); Lane 22, *Pm. inflatipes* (CBS 391.71); Lane 23, *Pm. viticola* (CBS 101738) + *Pm. rubrigenum* (CBS 498.94); Lane 24, *Pm. viticola* (CBS 101738); Lane 25, *Pm. rubrigenum* (CBS 498.94); Lane 26, *Pm. griseorubrum* (CBS 111657) + *Pm. alvesii* (CBS 110034) + *Pm. amstelodamense* (CBS 110627); Lane 27, *Pm. griseorubrum* (CBS 111657); Lane 28, *Pm. amstelodamense* (CBS 110627); Lane 29, *Pm. alvesii* (CBS 110034); Lane 30, *Pm. theobromatis* (CBS 111586); Lane 31, *Pm. mortoniae* (CBS 101585); Lane 32, *Pm. iranianum* (CBS 101357); Lane 33, *Pm. krajdenii* (CBS 109479); Lane 34, *Pm. griseorubrum* (CBS 111657); Lane 35, negative control. — Legend: C, concatemers of amplification products; PC, positive control product of 720 bp; SP2, species-specific product within the size range 449–611 bp; SP1, species-specific product within the size range 191–262 bp.



**Table 7.** Distribution of mating types among conidial and ascospore strains of six *Togninia* species.

Species	First mating			Second mating		
	Number of conidial strains	Mating type distribution	P-value <sup>a</sup>	Number of ascospore strains	Mating type distribution	P-value <sup>a</sup>
<i>Togninia austroafricana</i>	3	1 : 1	0.750	10	1 : 8	0,016
<i>Togninia minima</i>	21	10 : 11	0.500	20	10 : 10	0,588
<i>Togninia parasitica</i>	10	3 : 4	0.500	23	10 : 13	0,339
<i>Togninia krajdenii</i>	9	1 : 4	0.188	27	1 : 24	P < 0.001
<i>Togninia rubrigena</i>	2	1 : 1	0.750	23	12 : 11	0,661
<i>Togninia viticola</i>	6	2 : 3	0.500	29	3 : 26	P < 0.001

<sup>a</sup>Probability value calculated with a proportion test under the null hypothesis of a 1:1 ratio.

Six of the *Togninia* species had a heterothallic mating system (Table 7). In most cases the null hypothesis of a 1 : 1 ratio between the mating types could not be rejected. The P-values obtained were greater than 0.05 (Table 7). This indicates that a 1 : 1 distribution underlies the mating type distribution for the species of *T. minima*, *T. parasitica*, *T. rubrigena* and *T. viticola*. For the species *T. austroafricana*, *T. krajdenii* and *T. viticola* the results from backcrosses clearly showed that these species did not have a 1 : 1 mating type ratio with P-values smaller than 0.05 (Table 7). The unequal distribution of mating types is mostly likely an artefact of too few strains used.

In the first mating of *T. krajdenii*, the four clinical strains (CBS 633.93, CBS 110366, CBS 109479, CBS 110367) all belonged to the same mating type. Only one strain from plants (strain CBS 110118) was tested and this strain proved to be of the opposite mating type. Only one clinical strain (strain CBS 860.73) of *T. parasitica* was used in the first crossing and did not provide insight into the mating type distribution among the clinical strains of this species. However, both mating types were present among the *Pm. parasiticum* strains from *Vitis vinifera*.

No fertile perithecia formed in any of the inter-species crossings. In just a few cases, sterile protoperithecia formed. These results confirm that these species behave according to the biological species concept (Taylor *et al.* 2000).

#### Numerical analysis of morphological and cultural characters for use in BioloMICS

In an evaluation of the utility of assigning different weights to the characters used in the analysis, the coefficient of correlation between phenotypic and phylogenetic analyses was found to be 0.40 when all characters were assigned an equal weight. Even though the correlation between phenotypic characters and phylogenetic relationships was not high, could the coefficient of correlation be used to see which

phenotypic characters do follow the same pattern of distribution as the phylogenetic species arrangement. Characters were tested individually for their effect on the overall correlation level by selectively removing them from the analysis. In cases where this removal caused the overall correlation coefficient to drop, the characters involved were recorded as making a positive contribution to the correlation. Characters with correlation coefficients below 0.39 were weighted. The coefficient of correlation between phenotypic and phylogenetic analyses was found to be 0.52 when *a priori* weightings were used. The morphological and cultural characters that were weighted included conidiophore structure, type III phialide shape, maximum wart diameter, mycelial texture, conidial length and maximum growth temperature. With the inclusion of nine species of *Phaeoacremonium* in addition to those studied by Mostert *et al.* (2005), the morphological and cultural characters changed in relation to the phylogeny reflected in the TUB data. Colony colour and colony radius at 25 °C and 30 °C no longer correlated positively with the TUB data as they had been found to do by Mostert *et al.* (2005). With the inclusion of the additional species, these various values for these characters did not follow the pattern of phylogenetic association, but were dispersed throughout the phylogenetic tree.

The *Phaeoacremonium* BioloMICS identification database is available on the CBS website at <http://www.cbs.knaw.nl/phaeoacremonium.htm> (Mostert *et al.* 2005). Any convenient number of characters can be entered (the more, the better) and, through pairwise comparison, the *Phaeoacremonium* species most similar to the query strain can be identified. The similarity of each character of the unknown species to those of the known *Phaeoacremonium* species can also be seen in the output file. This multiple-entry comparison key is similar to the yeast identification database available from CBS (Robert *et al.* 2003).

**Dichotomous keys****Key to genera related or similar to *Togninia***

1. Ascospores brown, ellipsoid, 0–1-septate, usually containing terminal germ pores ..... *Jobellisia*
1. Ascospores hyaline or pale pigmented, allantoid to suballantoid or oblong-ellipsoidal without germ pores ..... 2
2. Perithecia in valsoid, circinate groups with converging beaks ..... *Calosphaeria*
2. Perithecia gregarious, in rows or solitary ..... 3
3. Asci in spicate arrangement ..... 4
3. Asci not in spicate arrangement ..... 8
4. Asci subglobose or broadly oblong, short-stipitate and polysporous ..... *Pleurostoma*
4. Asci oblong or oblong-clavate, sessile, 8-spored ..... 5
5. Asci clavate to oblong-clavate, narrowly tapering towards the base ..... 6
5. Asci oblong, tapering gradually towards a truncate base ..... 7
6. Necks short, reaching to or extending slightly beyond the substrate surface ..... *Romellia*
6. Necks elongate, protruding beyond the substrate surface ..... *Togniniella*
7. Ascomata enclosed in a dull black erumpent, pulvinate stroma; with *Cytospora*-like anamorph ..... *Pachytrype*
7. Ascomata immersed beneath the periderm with necks erumpent or superficial; with *Phaeoacremonium* anamorph ..... *Togninia*
8. Perithecia with elongate necks ..... *Wegelia*
8. Perithecia with papilla or short, narrow beaks ..... 9
9. Perithecia 540–1500 µm diam, usually surrounded by dark-pigmented hyphal tomentum, gregarious to solitary; ascospores aseptate ..... *Enchnoa*
9. Perithecia 200–490 µm diam, without tomentum, gregarious, often in rows or small groups; ascospores aseptate or one- to several-septate ..... *Jattaea*

**Key to hyphomycete genera similar to *Phaeoacremonium***

1. Phialides often intercalary, integrated ..... 2
1. Phialides generally terminal, discrete ..... 5
2. Subtending hyphae and phialides brown, olive-grey or subhyaline ..... 3
2. Subtending hyphae and phialides hyaline; if sporodochia present, the exterior consists of golden-brown setose hairs ..... *Phialemonium*
3. Fertile necks short and narrow, conidiogenesis inconspicuously annellidic ..... *Exophiala*
3. Conidiogenesis phialidic ..... 4
4. Phialides generally intercalary besides 1 terminal, often swollen, with short lateral collarettes ..... *Lecythophora*
4. Phialides often longer, slender, only some distal ones intercalary, others discrete, lateral ..... *Margarinomyces*
5. Conidiophores and phialides hyaline ..... 6

5. Conidiophores or phialides pigmented ..... 8
6. Collarete well-developed, cylindrical to flared; conidia catenate,  
with truncate bases ..... *Catenulifera*
6. Collarete inconspicuous, small and not flaring; conidia in slimy heads or  
in some species catenate ..... 7
7. Conidiophores unbranched or sparingly branched in the lower part;  
phialides not thick-walled ..... *Acremonium*
7. Conidiophores unbranched; phialides thick-walled, at least in the lower part,  
and often swollen in the middle ..... *Monocillium*
8. Conidiophores unbranched, branched or diffusely branched, slender;  
phialides aculeate ..... 9
8. Conidiophores regularly branched or reduced to single and more or less  
flask-shaped phialides ..... 14
9. Conidiophores stiff, unbranched (or sympodially branched in *Exochalara*), with integrated  
terminal conidiogenous cells ..... 10
9. Conidiophores branched or unbranched, with discrete or sometimes also integrated  
conidiogenous cells ..... 12
10. Conidia in slimy heads or imbricate columns ..... *Chloridium*
10. Conidia in regular chains ..... 11
11. Conidia fusiform; conidiophores branched sympodially ..... *Exochalara*
11. Conidia oblong-ellipsoidal and truncate at the base;  
conidiophores simple ..... *Monilochaetes*
12. Conidiophores and conidia pigmented;  
collarettes short and inconspicuous ..... *Pseudogliomastix*
12. Conidiophores pigmented and conidia mostly hyaline;  
collarete funnel-shaped ..... 13
13. Conidiophores usually branched, gradually paler upwards; conidia hyaline, dimorphic,  
longer and allantoid or oblong-ellipsoidal, those in the aerial mycelium being shorter  
and oblong-ellipsoidal to obovoid ..... *Phaeoacremonium*
13. Conidiophores usually unbranched, only the basal stipe cell darkly pigmented;  
conidia (sub)hyaline, oblong-ellipsoidal to obovoid, straight ..... *Phaeomoniella*
14. Phialides constricted below the collarete; phialides and particularly collarettes  
pigmented ..... *Phialophora*
14. Collarete usually pale or hyaline ..... 15
15. Phialides hyaline, though pigmented in the apical region below the  
collarete ..... *Calosphaeriophora*
15. Phialides pigmented or hyaline, no localised pigmentation below the  
collarete ..... 16
16. Conidiophores usually unbranched, often reduced to a single phialide; conidia dimorphic,  
cylindrical or allantoid and hyaline, together with (sub)globose-brown  
or ellipsoidal-hyaline conidia ..... *Pleurostomophora ootheca* and *P. richardsiae*
16. Conidiophores branched; conidia generally monomorphic ..... 17
17. Collarettes distinct and flaring; branched conidiophores bearing few branches  
only ..... *Phaeocrella*

17. Collarettes inconspicuous, flaring or funnel-shaped; complex conidiophores more densely branched ..... 18
18. Complex conidiophores with phialides densely clustered; conidia oblong-ellipsoidal or obovoid; collarettes distinct, funnel-shaped ..... *Cadophora*
18. Complex conidiophores with radiating phialides; conidia cylindrical to allantoid; collarettes inconspicuous, cylindrical ..... *Pleurostomophora repens*

**Key to the species of *Togninia***

1. Ascospores mostly allantoid ..... 2
1. Ascospores mostly cylindrical, oblong-ellipsoidal or reniform ..... 6
2. Paraphyses thread-like at the apex ..... 3
2. Paraphyses tapering towards the apex, but not thread-like ..... 5
3. Perithecial necks 200–515 µm long ..... 4
3. Perithecial necks 515–1300 µm long ..... *T. rubrigena*
4. Ascospores allantoid to oblong-ellipsoidal, asci (16–)18–22(–23) × 4–5 µm; perithecial necks 220–440 µm long ..... *T. krajdieni*
4. Ascospores allantoid, asci (12–)14–18 × (3.5–)4–5 µm; perithecial necks 215–810 µm long ..... *T. parasitica*
5. Perithecial necks 83–113 µm long; asci 20–30(–32) × 6–8 µm; ascospores 7–10 × 1.5–2 µm ..... *T. inconspicua*
5. Perithecial necks 800–1800 µm long; asci (17–)19–20(–27) × 4–5 µm; ascospores (4–)5(–6.5) × 1–2 µm ..... *T. minima*
6. Maximum neck length up to 1250 µm ..... 7
6. Maximum neck length 1470 µm ..... 9
7. Ascus length up to 20 µm, av. 17 µm, long ..... *T. fraxinopennsylvanica*
7. Asci longer ..... 8
8. Perithecia up to 181 µm diam; asci up to 23 µm, av. 20 µm, long; ascospores ellipsoidal to oblong-ellipsoidal, 4–6 µm long ..... *T. novae-zealandiae*
8. Perithecia up to 377 µm diam; asci up to 26 µm, av. 21 µm, long; ascospores oblong-ellipsoidal to reniform, 3–5 µm long ..... *T. viticola*
9. Asci (16–)17–21(–22) × 4–5 µm; ascospores oblong-ellipsoidal or reniform, 1.5–2 µm wide ..... *T. austroafricana*
9. Asci (12–)13–18(–20) × (3–)3.5–4 µm; ascospores oblong-ellipsoidal or cylindrical, 1.0–1.5 µm wide ..... *T. argentinensis*

**Key to the species of *Phaeoacremonium***

1. Conidiophores mainly long or with extensive branching ..... 2
1. Conidiophores mainly short and unbranched or infrequently branched ..... 3
2. Mycelium medium to dark brown; hyphae verrucose, with prominent, coarse warts (up to 3 µm) diam; phialides generally cylindrical ..... *Pm. parasiticum*
2. Mycelium pale brown to hyaline; hyphae verruculose, sparsely and finely warted (warts up to 0.5 µm) diam; phialides often elongate-ampulliform ..... *Pm. inflatipes*

3.	Colony colour ranging from medium pink to greyish red on MEA .....	4
3.	Colony colour ranging from white to beige to brown on MEA.....	8
4.	Maximum growth temperature 30–35 °C.....	<i>Pm. viticola</i>
4.	Maximum growth temperature 37–40 °C.....	5
5.	Radial growth 6–7 mm after 8 d at 25 °C in the dark on MEA.....	<i>Pm. griseorubrum</i>
5.	Radial growth more than 9.5 mm after 8 d at 25 °C in the dark on MEA.....	6
6.	Type II phialides predominant.....	<i>Pm. scolyti</i>
6.	Type III phialides predominant.....	7
7.	Conidiophores (20–)23 × 51(–70) µm long, av. 34 µm; no yellow pigment on OA .....	<i>Pm. rubrigenum</i>
7.	Conidiophores (14–)17 × 43(–50) µm long, av. 27 µm; formation of yellow pigmentation on OA variable.....	<i>Pm. alvesii</i>
8.	Conidiophores often prominently constricted at the septa .....	<i>Pm. sphinctrophorum</i>
8.	Conidiphores seldom constricted at the septa.....	9
9.	Mycelium mostly verrucose .....	10
9.	Mycelium verruculose .....	11
10.	Colony surface medium brown to dark brown on MEA; colony radius 9–14 mm at 25 °C after 8 d in the dark; type II phialides mostly elongate-ampulliform and attenuated at the base, or subcylindrical; polyphialides often observed.....	<i>Pm. krajdienii</i>
10.	Colony surface medium brown to olivaceous-brown on MEA; colonies relatively slow growing with radius 8.5 mm at 25 °C after 8 d in the dark; type II phialides mostly subcylindrical to subulate; mostly monophialides.....	<i>Pm. tardicrescens</i>
11.	Colonies olive-brown becoming olive-coloured on OA; no production of yellow pigment on OA.....	<i>Pm. amstelodamense</i>
11.	Colonies ranging from white, beige to pale brown; in some cases yellow pigment is produced on OA.....	12
12.	Colony colour on OA reddish.....	13
12.	Colony colour on OA white, beige or brown.....	14
13.	Colonies slow-growing, reaching a radius of 5–8 mm after 8 d on MEA; type III phialides predominant.....	<i>Pm. austroafricanum</i>
13.	Colonies faster-growing, reaching a radius of 9–10 mm after 8 d on MEA; type I phialides is predominant.....	<i>Pm. angustius</i>
14.	Maximum temperature at which growth was observed 30 °C.....	15
14.	Maximum temperature at which growth was observed 35–40 °C .....	17
15.	No yellow pigment produced on OA; secondary proliferation of conidiophores often observed.....	<i>Pm. argentinense</i>
15.	Yellow pigment produced on OA .....	16
16.	Type I phialides common; conidia formed on and in the agar relatively slender (L/W = 5) .....	<i>Pm. theobromatis</i>
16.	Type III and type I phialides common; conidia formed on and in the agar relatively broad (L/W = 3).....	<i>Pm. novae-zealandiae</i>

17. Phialides tapering towards a narrow subulate neck; yellow pigment produced on MEA, PDA and OA ..... *Pm. subulatum*

17. Phialides also tapering towards the apex, but more gradually ..... **18**

18. Type II phialides mostly elongate-ampulliform, attenuated at the base ..... **19**

18. Type II phialides mostly subcylindrical or navicular ..... **21**

19. Conidiophores often branched; type III phialides short, av. length 17 µm ..... *Pm. australiense*

19. Conidiophores mostly unbranched; type III phialides longer, av. length 18–24 µm ..... **20**

20. Colonies honey-brown or beige on MEA; phialides mostly type II and III..... *Pm. aleophilum*

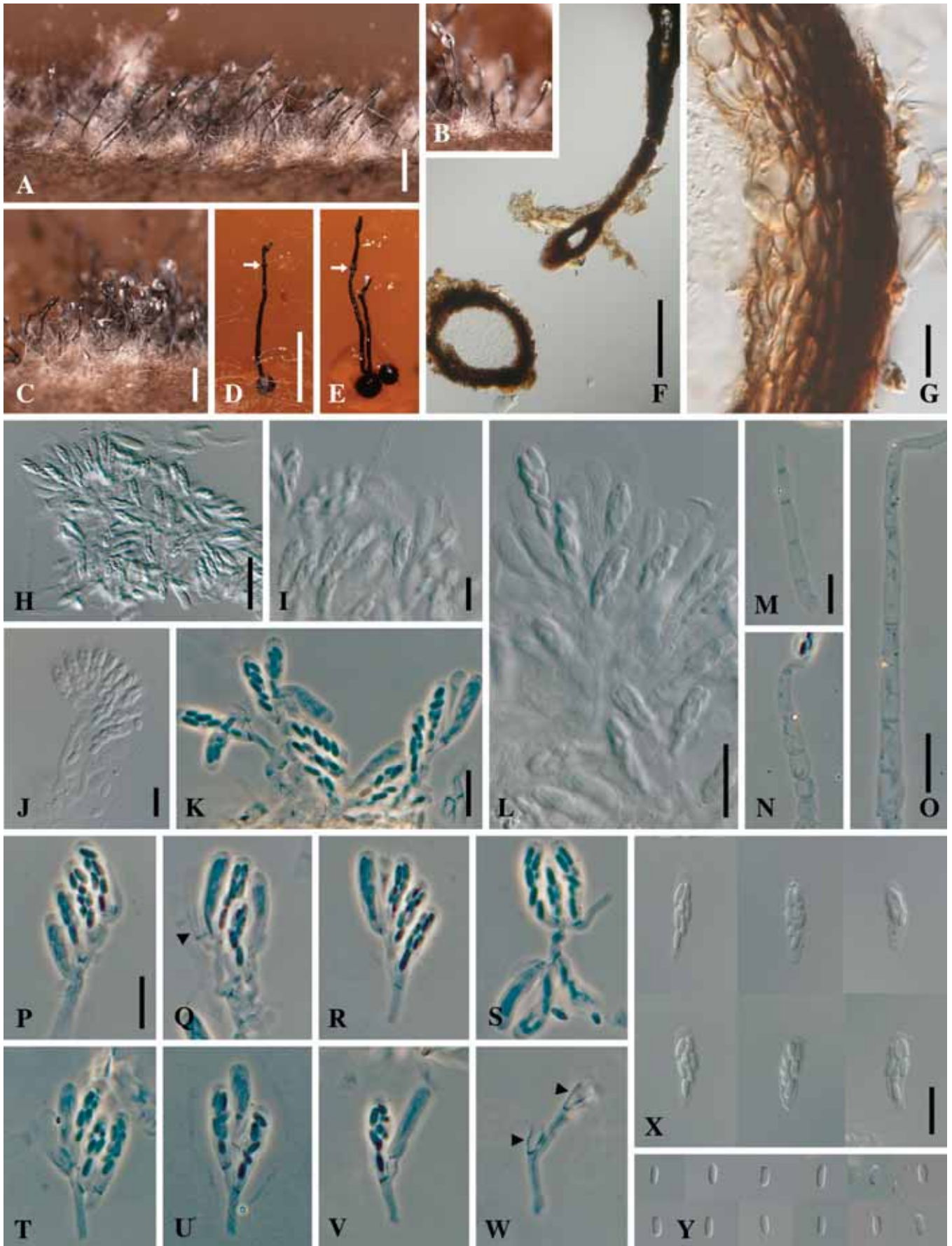
20. Colonies mostly white on MEA; phialides mostly type I and II ..... *Pm. mortoniae*

21. Colonies pale brown to grey-brown on MEA; maximum temperature at which growth was observed 37 °C ..... *Pm. iranianum*

21. Colonies beige to orange brown on MEA; maximum temperature at which growth was observed 40 °C ..... *Pm. venezuelense*

**Table 8.** Cardinal growth temperatures and radial growth (in mm after 8 d at 25 °C on MEA) of *Phaeoacremonium* species.

	Cardinal growth temperatures			Radial growth
	Minimum (°C)	Optimum (°C)	Maximum (°C)	
<i>Pm. aleophilum</i>	10	30	37–40	2.5–11
<i>Pm. alvesii</i>	15	30	37	9.5–11
<i>Pm. amstelodamense</i>	15	30	40	11.5–12.5
<i>Pm. angustius</i>	15	25	30	9–10
<i>Pm. argentinense</i>	15	25	30	8
<i>Pm. australiense</i>	15	30	35–37	9–10
<i>Pm. austroafricanum</i>	15	25	30	5–8
<i>Pm. griseorubrum</i>	10	30	40	6–7.5
<i>Pm. inflatipes</i>	10	25–30	35	12.5–13
<i>Pm. iranianum</i>	15	30	37	5–9
<i>Pm. krajdenii</i>	15	30	37	9–14
<i>Pm. mortoniae</i>	15	25–30	35	10–13
<i>Pm. novae-zealandiae</i>	15	25	30	10
<i>Pm. parasiticum</i>	15	30	40	10.5–11.5
<i>Pm. rubrigenum</i>	10	30	37	9.5–10
<i>Pm. scolyti</i>	15	25–30	37	10.5–12
<i>Pm. sphinctrophorum</i>	15	25–30	30–37	6–15
<i>Pm. subulatum</i>	15	25–30	37	8.5–11.5
<i>Pm. tardicrescens</i>	15	30	40	8–9
<i>Pm. theobromatis</i>	15	30	30	11
<i>Pm. venezuelense</i>	15	30	40	9–16
<i>Pm. viticola</i>	15	25–30	30–35	6–12



**Fig. 13.** *Togninia argentinensis*. A–C. Perithecia on canes of *Vitis vinifera*. D–E. Perithecia on WA with necks showing secondary proliferation. F–G. Longitudinal sections through perithecia; peridium (G). H–L. Asci intermingled with paraphyses. M–O. Paraphyses. P–V. Ascogenous hyphae with asci attached. W. Ascogenous hypha with remnant bases (arrow heads) showing positions where asci were attached. X. Asci. Y. Ascospores. A–P from CBS 17457 (holotype). A–E: DM; F–J, L, X, Y: DIC; K, P–W: PC. Scale bars: A–E = 500  $\mu$ m; F = 100  $\mu$ m; H = 20  $\mu$ m; G, I–Y = 10  $\mu$ m. Scale bar for P applies to Q–W; bar for X applies to Y.

**Table 9.** Summary of colony colour and morphological features useful for the identification of *Phaeoacremonium* species, sorted according to the colony colour (ranging from pink to brown to beige). Yellow pigment formation is defined as variable if the pigment is not formed by all the strains of a species.

	Colony colour on MEA				Conidiophore			Conidiophore length		Mycelium		Maximum diam of warts (µm)		Predominant phialide type		Predominant Type II phialide shape		Yellow pigmentation on OA
	Colony colour on MEA	Conidiophore structure	Conidiophore length (µm)	Mycelium texture	Maximum diam of warts (µm)	Predominant phialide type	Predominant Type II phialide shape	Yellow pigmentation on OA										
<i>Pm. rubrigenum</i>	medium to purple pink	mostly short and unbranched	(20-)23-51(-70) av. 34	verruculose	1	Type III	elongate-ampulliform, attenuated at the base	No										
<i>Pm. viticola</i>	greyish red	mostly short and unbranched	(15-)18-49(-80) av. 31	verruculose	2	Type II and III	elongate-ampulliform attenuated at the base or subcylindrical	Variable										
<i>Pm. scolyti</i>	medium pink to translucent	mostly short and unbranched	(15-)17-35(-39) av. 26	mostly verruculose	1	Type II	elongate-ampulliform, attenuated or constricted at the base	No										
<i>Pm. griseorubrum</i>	dark pink	mostly short and occasionally branched	(21-)23-70(-85) av. 38	verruculose	1,5	Type II and III	elongate-ampulliform or navicular	No										
<i>Pm. alvesii</i>	medium pink or beige	mostly short and unbranched	(14-)17-43(-50) av. 27	verruculose	0,5	Type III	subcylindrical or navicular	Variable										
<i>Pm. inflatipes</i>	brown to grey-brown	mostly branched in the basal region	(14-)18-40(-43) av. 28	verruculose	0,5	Type III	elongate-ampulliform, attenuated at the base	No										
<i>Pm. parasiticum</i>	brown with medium brown center	mostly long and branched	(24-)27-80(-130) av. 47	verrucose	3	Type III	subcylindrical	No										
<i>Pm. krajdienii</i>	medium brown to dark brown	short and usually unbranched	(16-)20-45(-76) av. 28	verrucose	1	Type II	elongate-ampulliform, attenuated at the base	No										
<i>Pm. tardicrescens</i>	medium brown to olivaceous-brown	mostly short and unbranched	(13-)16-52(-67) av. 31	verrucose	0,5	Type I and III	subcylindrical to subulate	No										
<i>Pm. sphinctrophorum</i>	brown to orange grey	mostly short and often branched	(11-)13-39(-50) av. 23	verrucose	no warts	Type II	elongate-ampulliform attenuated or constricted at the base	No										
<i>Pm. australiense</i>	pale brown to medium brown	mostly short and unbranched	(14-)17-50(-64) av. 26	verruculose	1	Type I, II and III	elongate-ampulliform	Yes										
<i>Pm. iranianum</i>	pale brown to grey-brown	mostly short and unbranched	(17-)20-50 av. 30	verruculose	1	Type III	subcylindrical	Variable										
<i>Pm. aleophilum</i>	honey-brown or beige	mostly short and usually unbranched	(15-)17-42(-46) av. 29	mostly verruculose	1,5	Type II and III	elongate-ampulliform attenuated at the base	Yes										
<i>Pm. theobromatis</i>	brownish orange	mostly short and unbranched	18-40(-42) av. 24	verruculose	1	Type I	subcylindrical	Yes										
<i>Pm. subulatum</i>	pale yellow to pale brown	mostly short and unbranched	(17-)18-32(-45) av. 25	verruculose	0,8	Type I, II and III	subcylindrical to subulate	Yes										
<i>Pm. venezuelense</i>	beige to orange-brown	mostly short and occasionally branched	(20-)28-48(-52) av. 31	verruculose	1	Type III	subcylindrical or navicular	No										
<i>Pm. austroafricanum</i>	beige to brown-orange	mostly short and unbranched	(15-)16-42(-60) av. 25	verruculose	1	Type III	elongate-ampulliform attenuated at the base or subcylindrical	Yes										

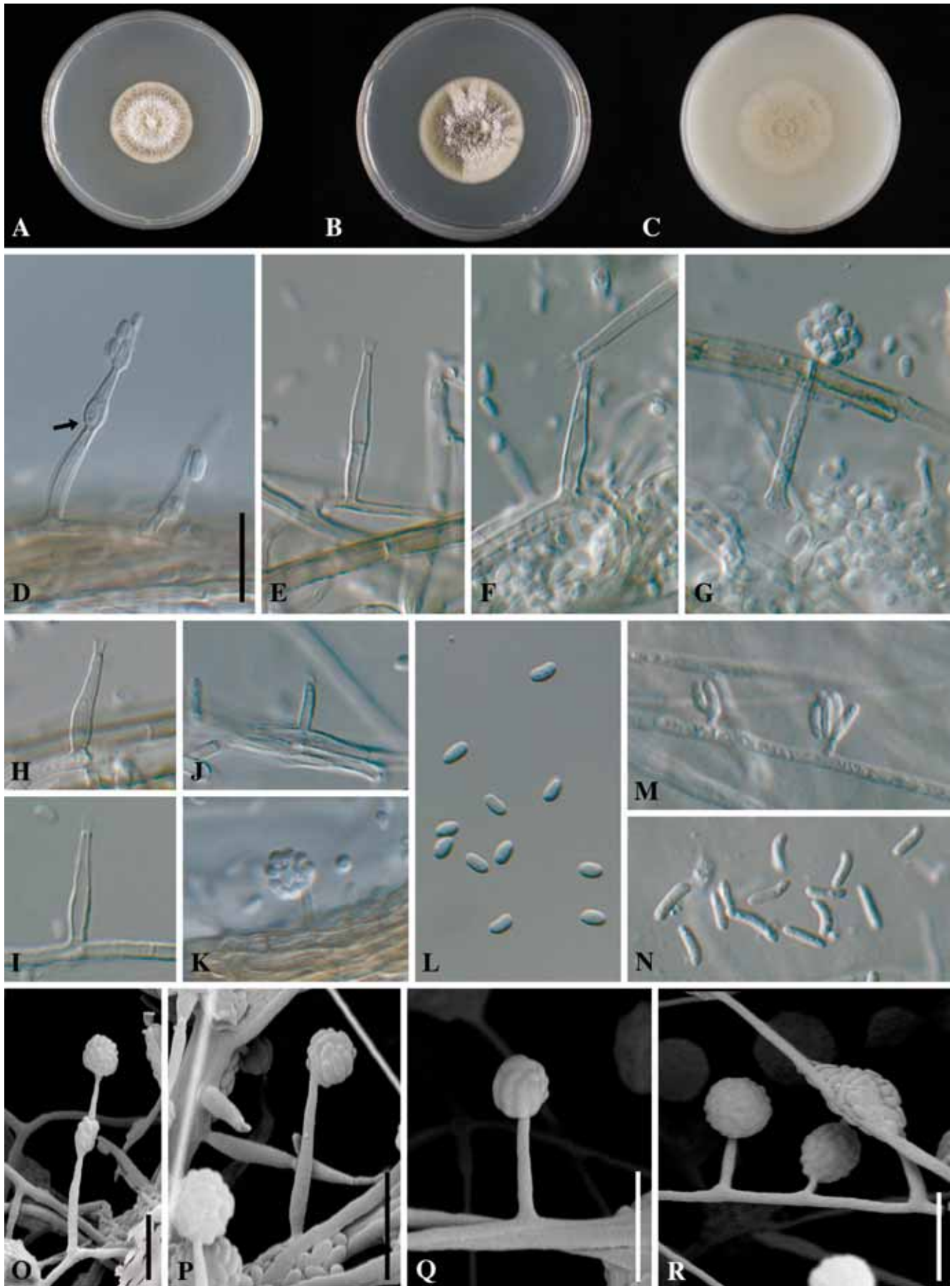


Table 9. (Continued).

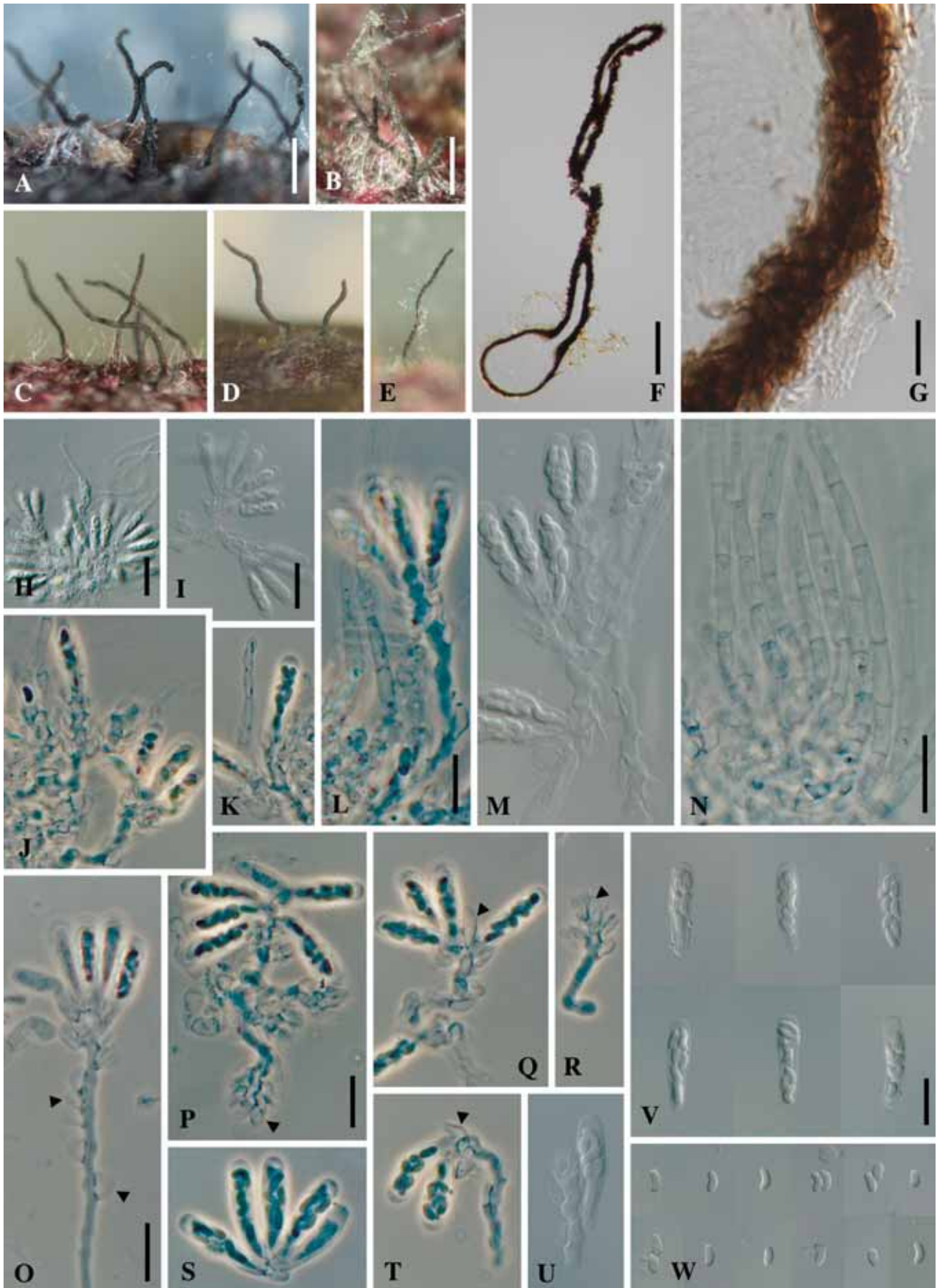
	Colony colour on MEA	Conidiophore structure	Conidiophore length (µm)	Mycelium texture	Maximum diam of warts (µm)	Predominant phialide type	Predominant Type II phialide shape	Yellow pigmentation on OA
<i>Pm. argentinense</i>	white to brownish grey	mostly short and unbranched	(15-)16-35(-44) av. 24	verruculose	1	Type II and III	elongate-ampulliform attenuated at the base	No
<i>Pm. amstelodamense</i>	beige to pale brown	mostly short and usually unbranched	(15-)16-61(-90) av. 36	verruculose	1	Type II	elongate-ampulliform, constricted at the base	No
<i>Pm. angustius</i>	pale yellow to grey-yellow	mostly short and unbranched	(15-)16-42(-60) av. 25	verruculose	1	Type I	subcylindrical or navicular	Yes
<i>Pm. novae-zealandiae</i>	white to olive-grey	medium length and often branched	(17-)19-55(-60) av. 35	verruculose	1	Type I and III	subcylindrical or navicular	Yes
<i>Pm. mortoniae</i>	white to yellow-grey	mostly short and unbranched	(16-)20-30(-40) av. 26	verruculose	0,5	Type I and II	elongate-ampulliform attenuated at the base or subcylindrical	Variable

Table 10. Micromorphology of the 10 *Togninia* species.

Species	Perithecial dimensions (µm)	Neck length (µm)	Ascus dimensions (µm)	Ascospore shape	Ascospore dimensions (µm)
<i>T. argentinensis</i>	(142-)144-245 tall (113-)115-171 diam	390-1470	(12-)13-18(-20) × (3-)3,5-4	oblong-ellipsoidal to cylindrical	3-4 × 1-1,5
<i>T. austroafricana</i>	(88-)92-193(-201) tall (64-)66-175(-181) diam	490-1470	(16-)17-21(-22) × 4-5	reniform to oblong-ellipsoidal	3-5 × 1,5-2
<i>T. fraxinopennsylvanica</i>	(181-)187-258(-270) tall (181-)185-252(-270) diam	390-1125	15-20 × 4(-5)	oblong-ellipsoidal to slightly curved	3,5-5 × 1
<i>T. inconspicua</i>	142-196 tall 74-167 diam	83-113	20-30(-32) × 6-8	allantoid or oblong-ellipsoidal	7-10 × 1,5-2
<i>T. krajdenii</i>	(202-)203-284(-287) tall (197-)203-275 diam	220-440	(16-)18-22(-23) × 4-5	allantoid to oblong-ellipsoidal	4-5(-6) × 1-1,5
<i>T. minima</i>	(200-)285-325(-400) tall (160-)250-285(-420) diam	800-1800	(17-)19-20(-27) × 4-5	oblong-ellipsoidal or allantoid	(4-)5(-6,5) × 1-2
<i>T. novae-zealandiae</i>	(147-)158-196 tall (142-)144-177(-181) diam	220-1250	(15-)17-23 × 4-5	oblong-ellipsoidal	3-4 × 1-2
<i>T. parasitica</i>	(215-)230-380(-410) tall (180-)200-345(-370) diam	215-810	(12-)14-18 × (3,5-)4-5	allantoid	4-5 × 1-1,5
<i>T. rubrigena</i>	(225-)234-354(-362) tall (172-)198-459(-470) diam	515-1300	(12-)16-19 × 4-4,5	allantoid or cylindrical	4-6 × 1-1,5
<i>T. viticola</i>	(211-)222-324(-328) tall 225-362(-377) diam	360-1030	(17-)18-24(-26) × (3-)3,5-4(-5)	oblong-ellipsoidal to reniform	3-5 × 1,5-2(-2,5)



**Fig. 14.** *Phaeoacremonium argentinense*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–L, O–R. Aerial structures on MEA. D–E. Conidiophores; conidiophore showing percurrent rejuvenation (D). F–G. Type III phialides. H–I. Type II phialides. J–K. Type I phialide. L. Conidia. M–N. Structures on the surface of and in MEA. M. Adelophialides with conidia. N. Conidia. O. Conidiophore. P. Type III phialide. Q–R. Type I phialides. A–R from CBS 777.83. D–N: DIC; O–R: SEM. Scale bars: D–R = 10  $\mu$ m. Scale bar for D applies to E–N.



**Fig. 15.** *Togninia austroafricana*. A–E. Perithecia on canes of *Vitis vinifera*. F–G. Longitudinal sections through perithecia; peridium (G). H–M. Asci intermingled with paraphyses. N. Paraphyses. O–Q, S–U. Ascogenous hyphae with asci attached; remnant bases indicated by arrow heads (O, P, Q, T, R). V. Asci. W. Ascospores. A–W from CBS 17458 (holotype). A–E: DM; F–I, U–W: DIC; J–L, N–T: PC. Scale bars: A–E = 500  $\mu$ m; F = 100  $\mu$ m; H = 20  $\mu$ m; G, I–W = 10  $\mu$ m. Scale bar for B applies to C–E; bar for I applies to J–K; bar for L applies to M; bar for P applies to Q–U; bar for V applies to W.

## Generic descriptions

*Togninia* Berl., Icon. Fung. (Abellini) 3: 9. 1900.

*Type species: T. minima* (Tul. & C. Tul.) Berl., lectotype designated by Clements & Shear (1931).

*Perithecia* aggregated or solitary, superficial to immersed, nonstromatic, globose to subglobose, dark, opaque, long-necked; neck straight or flexuous. *Perithecial wall* fragile to leathery, comprising two layers of *textura angularis*: outer layer brown to dark brown, with cells smaller and more rounded than those of the inner layer; inner layer, hyaline (centrum) to pale brown, cells more flattened. *Paraphyses* abundant, broadly cellular, slightly constricted at the septa, branching, hyaline, slightly tapering apically or thread-like towards the apex. *Ascogenous hyphae* hyaline, branched, elongating during ascus formation with remnant bases from which single asci arise. *Asci* arising in acropetal succession, appearing spicate when mature, unitunicate, 8-spored, ascus apex thickened without a discharge mechanism, basally bluntly obtuse, sessile. *Ascospores* hyaline, aseptate, allantoid, reniform, cylindrical or oblong-ellipsoidal, mostly biseriate or in a single row.

*Phaeocremonium* W. Gams, Crous & M.J. Wingf., Mycologia 88: 789. 1996.

*Type species: Pm. parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf.

*Colonies* on MEA flat with entire margins, mostly moderately dense, predominantly felty and sometimes woolly; ranging from different shades of brown, pale yellow to beige or pink to dark pink. *Mycelium* consisting of branched, septate hyphae, single or bundled; medium brown, becoming paler brown to hyaline near areas where conidia are formed, smooth, verruculose or verrucose; warts varying in density and size. *Conidiophores* branched in the basal region or unbranched, arising from aerial or submerged hyphae, erect, nearly cylindrical when unbranched, slightly tapering, straight or flexuous, variable in length, up to 7-septate, mostly pale brown, paler towards the tip; percurrent rejuvenation observed; small warts or verruculose ornamentation seen mostly at the base; usually with one integrated terminal phialide and one or two additional, discrete phialides at the uppermost septum. *Conidiogenous cells* phialidic, discrete or integrated, terminal or lateral, mostly monophialidic, sometimes polyphialidic, sparsely warted, verruculose or smooth, pale brown to hyaline, with an inconspicuous funnel-shaped collarette. Three distinct classes of phialides (Types I – III) can be observed (Fig. 6). *Conidia* aggregated into round, slimy heads at the apices of phialides, hyaline, aseptate, smooth-walled;

oblong-ellipsoidal to obovate, cylindrical, allantoid or reniform, uncommonly fusiform-ellipsoidal or globose, becoming biguttulate with age (after 7–14 d).

*Notes:* The growth temperatures and radial growth of the *Phaeocremonium* species are given in Table 8 and the key morphological features in Table 9. A summary of morphological characters of the *Togninia* species is given in Table 10.

## Treatment of species

1. *Togninia argentinensis* L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500689. Fig. 13A–Y.

*Anamorph: Phaeocremonium argentinense* L.

Mostert, W. Gams & Crous, **sp. nov.**

*Etymology:* Named after the country of origin where this species occurs.

Anamorphe *Phaeocremonium argentinense*. Perithecia plerumque aggregata et subepidermalia, subglobosa, (113–)115–171 µm diam; collis atris, singulis vel binis in quoque perithecio, rectis vel curvatis, verrucosis, 390–1470 µm longis. Paraphyses hyalinae, septatae, cylindricae, sursum angustatae, 30–70 (in medio 48) µm longae. Asci hyalini, clavati, (12–)13–18(–20) × (3–)3.5–4 (in medio 15 × 4) µm. Ascosporae unicellulares, hyalinae, oblongo-ellipsoideae vel cylindricae, utrinque rotundatae, 3–4 × 1–1.5 (in medio 4 × 1) µm.

Typus herb. CBS 17457.

*Perithecia* mostly aggregated, sometimes solitary, mostly subepidermal but less commonly superficial on the epidermis, subglobose, (113–)115–171 µm diam and (142–)144–245 µm tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 4–5 cells (individual cells not visible further outward) and 7–12 µm thick; inner region hyaline (centrum) to pale brown, 4–7 cells and 8–13 µm thick; surface covered with brown, septate hyphal appendages that become hyaline towards their tips (more abundant on older perithecia). *Perithecial necks* black, 1–2 per perithecium, straight to curved, verrucose, 390–1470 µm long, 15–50 µm wide at the base, and 29–74 µm wide at the apex, sometimes dividing into two near the apex; apex often proliferating secondarily upon aging and then appearing nodulose; nodules (65–70 µm diam) also appearing lower down on the neck. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 30–70 (av. 48) µm long, 3–4 (av. 4) µm wide at the base and 2 µm at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases (12–)13–18(–20) × (3–)3.5–4 (av. 15 × 4) µm; apical region (1–)1.5–2 µm thick, of indistinct structure, with a non-amyloid apical

ring. *Ascogenous hyphae* hyaline, branched, smooth-walled; remnant bases  $4-5 \times 2-2.5 \mu\text{m}$ . *Ascospores* aseptate, hyaline, oblong-ellipsoidal to cylindrical with rounded ends, sometimes containing small guttules at the ends, biseriate,  $3-4 \times 1-1.5$  (av.  $4 \times 1$ )  $\mu\text{m}$ .

***Phaeoacremonium argentinense*** L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500228. Fig. 14A–R.

In mycelio aërio hyphae singulae vel ad 14 fasciculatae, tuberculatae, verruculosae, medio brunneae vel hyalinae. Conidiophora plerumque brevia et simplicia, saepe in phialidem singulam exeuntia, (15–)16–35(–44) (in medio 24)  $\mu\text{m}$  longa. Phialides terminales vel laterales, praecipue typi II et III; phialides typi I cylindricae, (2–)3–9.5(–12) (in medio 6)  $\mu\text{m}$  longae; phialides typi II elongato-ampulliformes, ad basim attenuatae, (7–)8(–13) (in medio 11)  $\mu\text{m}$  longae; phialides typi III subcylindricae, (13–)15–17(–23) (in medio 16)  $\mu\text{m}$  longae. Conidia plerumque oblongo-ellipsoidea, nonnulla reniformia,  $3-5 \times 1-2$  (in medio  $4 \times 1.5$ )  $\mu\text{m}$ , long./lat. = 3. In superficie vel submersa in agar, phialides hyalinae, cylindricae, nonnullae subcylindricae, (1.5–)2–14(–43) (in medio 5)  $\mu\text{m}$ ; conidia hyalina, allantoidea vel oblongo-ellipsoidea,  $5-6(-7) \times 1-1.5$  (in medio  $6 \times 1.5$ )  $\mu\text{m}$ , long./lat. = 4.

Typus herb. CBS 17448.

**Aerial structures:** *Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 14, tuberculate with warts up to 1  $\mu\text{m}$  diam, verruculose, medium brown to hyaline and 1–2  $\mu\text{m}$  wide. *Conidiophores* mostly short and unbranched, arising from aerial or submerged hyphae, erect, simple, up to 3-septate, often ending in a single terminal phialide, pale brown becoming paler towards the tip, smooth to verruculose, often showing percurrent rejuvenation, (15–)16–35(–44) (av. 24)  $\mu\text{m}$  long and (1–)1.5–3 (av. 2)  $\mu\text{m}$  wide. *Phialides* terminal or lateral, mostly monophialidic, smooth to verruculose, subhyaline; collarettes 1.5–2.5  $\mu\text{m}$  long, 1–1.5  $\mu\text{m}$  wide; type II and type III phialides most common; type I phialides cylindrical, occasionally widened at the base, (2–)3–9.5(–12)  $\times$  1–1.5(–2) (av.  $6 \times 1$ )  $\mu\text{m}$ ; type II phialides elongate-ampulliform and attenuated at the base, tapering towards the apex, (7–)8(–13)  $\times$  1.5–2(–2.5) (av.  $11 \times 2$ )  $\mu\text{m}$ ; type III phialides subcylindrical, (13–)15–17(–23)  $\times$  1.5–2 (av.  $16 \times 2$ )  $\mu\text{m}$ , tapering gradually to a long neck. *Conidia* mostly oblong-ellipsoidal, some reniform,  $3-5 \times 1-2$  (av.  $4 \times 1.5$ )  $\mu\text{m}$ , L/W = 3.

**On surface or submerged in the agar:** *Phialides* hyaline, cylindrical, some subcylindrical, (1.5–)2–14(–43)  $\times$  1–2 (av.  $5 \times 1$ )  $\mu\text{m}$ . *Conidia* hyaline, allantoid to oblong-ellipsoidal,  $5-6(-7) \times 1-1.5$  (av.  $6 \times 1.5$ )  $\mu\text{m}$ , L/W = 4.

**Types:** **Argentina**, Buenos Aires, Nuñez, soil, 1983,

A. Martínez, herb. CBS 17448, **holotype** of anamorph, dried MEA colony; Herb. CBS 17457, **holotype** of teleomorph, perithecia formed on *Vitis vinifera* canes; ex-type culture CBS 777.83).

**Cultural characteristics:** Colonies reaching a radius of 8 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 25 °C, maximum 30 °C. Colonies on MEA flat, cottony, with entire edge; after 8 d white (3A1) above, in reverse yellowish white (3A2); after 16 d brownish grey (5C2) above, in reverse olive-grey (3E2). Colonies on PDA flat, hairy to woolly, with entire edge; after 8 d orange-grey (5B2) above and the same in reverse; after 16 d colonies brownish grey (5D2) above, in reverse grey to brownish grey towards the edge (5B1–5E2). Colonies on OA flat, felty with a few woollen tufts, with entire edge; after 8 d orange-white (5A1) above with some grey mycelial tufts (5A1–B1), after 16 d mostly orange-white (5A1) with a few sections olive-grey (3D2).

**Substrate:** Soil.

**Distribution:** Argentina.

**Notes:** A homothallic species. Formation of perithecia took 6 wk. *Phaeoacremonium argentinum* can be distinguished from the other brown-coloured species by its brownish grey colonies on MEA and PDA and pale orange-white colonies on OA, as well as by the absence of yellow pigment production on OA and the occurrence of percurrent rejuvenation.

**2. *Togninia austroafricana*** L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500690. Fig. 15A–W.

**Anamorph:** *Phaeoacremonium austroafricanum* L. Mostert, W. Gams & Crous, **sp. nov.**

**Etymology:** Named after South Africa, the country where this species is known to occur.

**Anamorphe *Phaeoacremonium austroafricanum*.** Perithecia plerumque aggregata, submerged et subglobosa, nonnulla obpyriformia, (64–)66–175(–181)  $\mu\text{m}$  diam; collis atris, singulis vel binis in quoque perithecio, rectis vel curvatis, verrucosis, 500–1500  $\mu\text{m}$  longis. Paraphyses hyalinae, septatae, cylindricae, sursum angustatae, 30–105 (in medio 68)  $\mu\text{m}$  longae. Asci clavati, (16–)17–21(–22)  $\times$  4–5 (in medio  $19 \times 4$ )  $\mu\text{m}$ . Ascospores unicellulares, hyalinae, reniformes vel oblongo-ellipsoideae, utrinque rotundatae,  $3-5 \times 1.5-2$  (in medio  $4 \times 2$ )  $\mu\text{m}$ .

Typus herb. CBS 17458

**Perithecia** mostly aggregated, sometimes solitary, mostly submerged but less commonly subepidermal; perithecia subglobose, sometimes obpyriform (64–)66–175(–181)  $\mu\text{m}$  diam and (88–)92–193(–201)  $\mu\text{m}$  tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 4–5

cells (individual cells not visible further outward) and 6–10 µm thick; inner region hyaline at the centrum, pale brown towards the periphery, 3–4 cells and 5–6 µm thick; surface covered with brown, septate hyphal appendages that become hyaline towards the tips (more abundant on older perithecia). *Perithecial necks* black, 1–2 per perithecium, straight to curved, verrucose, necks 490–1470 µm long, 34–54 µm wide at the base, and 25–44 µm wide at the apex, sometimes dividing into two near the apex. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 30–105 (av. 68) µm long, 2.5–4 (av. 3) µm wide at the base and 1.5–3 (av. 2) µm at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases, (16–)17–21(–22) × 4–5 (av. 19 × 4) µm; apical region 1–1.5 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascogenous hyphae* hyaline, branched, smooth-walled, remnant bases 4 × 2–2.5 µm. *Ascospores* aseptate, hyaline, reniform to oblong-ellipsoidal with rounded ends, often containing small guttules at the ends, biseriata, 3–5 × 1.5–2 (av. 4 × 2) µm.

*Phaeocremonium austroafricanum* L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500232. Fig. 16A–Q.

In mycelio aërio hyphae singulae vel ad 10 fasciculatae, tuberculatae, verrucosae, dilute brunneae vel hyalinae. Conidiophora plerumque brevia et simplicia, saepe in singulam phialidem, (15–)16–42(–60) (in medio 25) µm longam exeuntes. Phialides terminales vel laterales, praecipue typi III; phialides typi I cylindricae, 2–7 (in medio 5) µm longae; phialides typi II seu elongato-ampulliformes ad basim attenuatae, seu subcylindricae, 6–12(–13) (in medio 9) µm longae; phialides typi III subcylindricae vel naviculares, 13–19 (in medio 16) µm longae. Conidia hyalina, plerumque oblongo-ellipsoidea vel oblonga, 4–5(–6) × 1.5–2.5 (in medio 5 × 2) µm, long./lat. = 2.5. In superficie vel submersa in agaro, phialides hyalinae, cylindricae, nonnullae elongato-ampulliformes, 1–13(–17) (in medio 5) µm; conidia hyalina, oblongo-ellipsoidea vel allantoidea, 5–9 × 1.5–2(–2.5) (in medio 7 × 2) µm, long./lat. = 3.

Typus herb. CBS 17449.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 10, tuberculate with warts up to 1 µm diam, verrucose, pale brown to hyaline and 2–3 µm wide. *Conidiophores* mostly short and unbranched, arising from aerial or submerged hyphae, erect, simple, up to 4-septate, often ending in a single terminal phialide, pale brown, paler towards the tip, smooth to verrucose, (15–)16–42(–60) (av. 25) µm long and 1.5–2.5(–3) (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, smooth to verrucose, subhyaline to hyaline; collarettes 1–2 µm long, 1 µm wide; type III

phialides most common; type I phialides cylindrical, occasionally widened at the base, 2–7 × 1(–2) (av. 5 × 1) µm; type II phialides either elongate-ampulliform and attenuated at the base, or subcylindrical, tapering towards the apex, 6–12(–13) × 1.5–2(–2.5) (av. 9 × 1.5) µm; type III phialides subcylindrical or navicular, 13–19 × 1.5–2(–2.5) (av. 16 × 2) µm, tapering gradually to a long neck. *Conidia* mostly oblong-ellipsoidal or oblong, 4–5(–6) × 1.5–2.5 (av. 5 × 2) µm, L/W = 2.5.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, some elongate-ampulliform, 1–13(–17) × (0.5)1–2(–2.5) (av. 5 × 1) µm. *Conidia* hyaline, oblong-ellipsoidal to allantoid, 5–9 × 1.5–2(–2.5) (av. 7 × 2) µm, L/W = 3.

*Types:* **South Africa**, trunk of *Vitis vinifera*, 2001, L. Mostert, herb. CBS 17449 **holotype** of anamorph, dried MEA colony of CBS 112949; ex-type culture of anamorph CBS 112949 = C.P.C. 4656; Herb. CBS 17458, **holotype** of teleomorph, from *in vitro* crossing with tester strains CBS 114993 × CBS 114994 on *V. vinifera*.

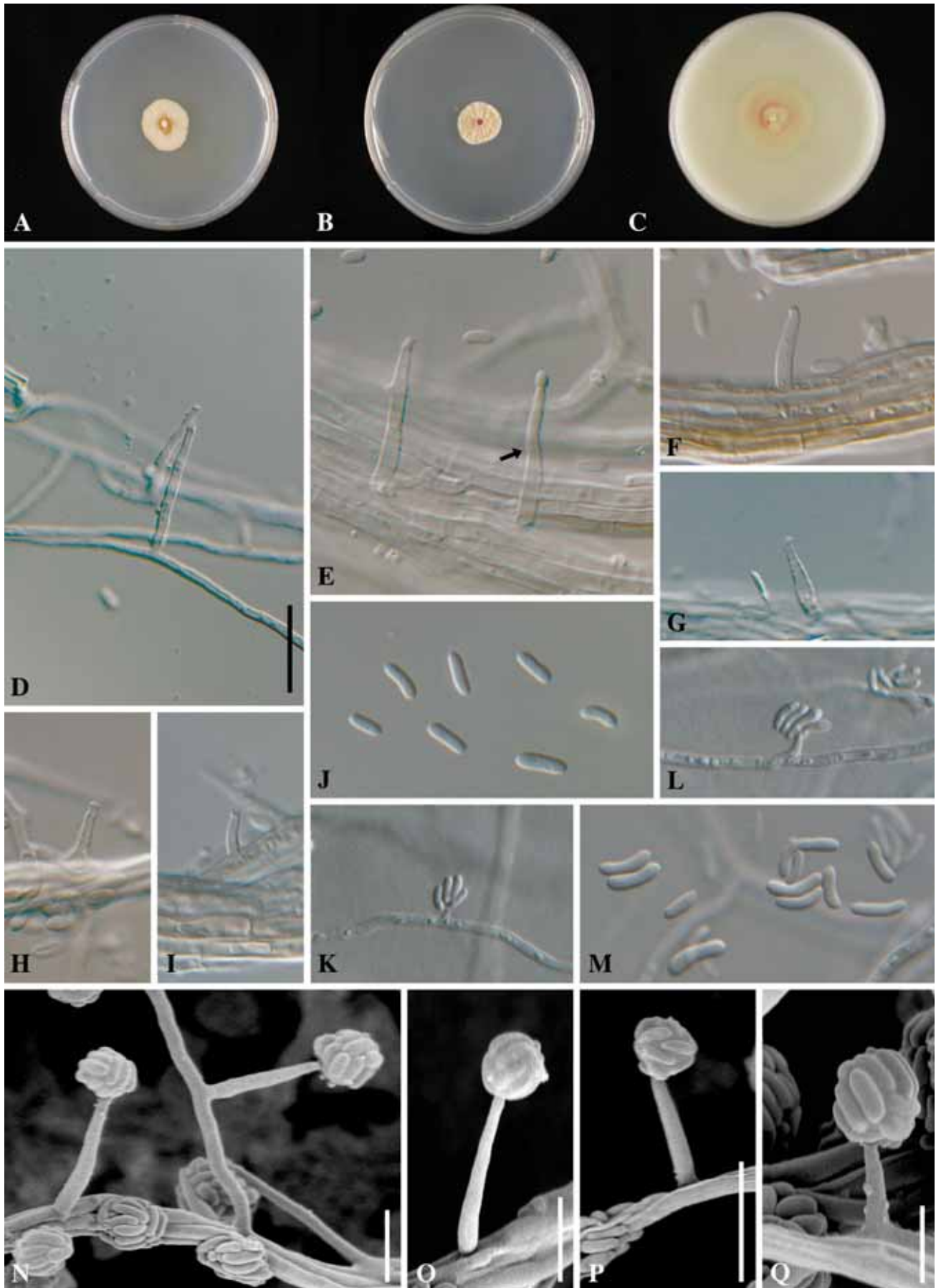
*Cultural characteristics:* Colonies reaching a radius of 5–8 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 25 °C, maximum 30 °C. Colonies on MEA flat, felty to cottony, yeast-like pustules sometimes forming on older colonies, with little aerial mycelium, with entire edge; after 8 d colonies yellowish white (3A2) above, in reverse pale yellow (3A3); after 16 d brownish orange to yellowish white towards the edge (5C4–3A2) above, in reverse greyish orange, to pale orange (5B3–A3). Colonies on PDA flat, felty, with entire edge; after 8 d orange-white (5A2) above and the same in reverse; after 16 d orange-white (5A2) above, in reverse pinkish white to orange-white towards the edge (10A2–5A2). Colonies on OA flat, felty, with entire edge; after 8 and 16 d red to yellowish white towards the edge (10B7–3A2). Yellow pigment produced on OA.

*Substrate:* *Vitis vinifera*.

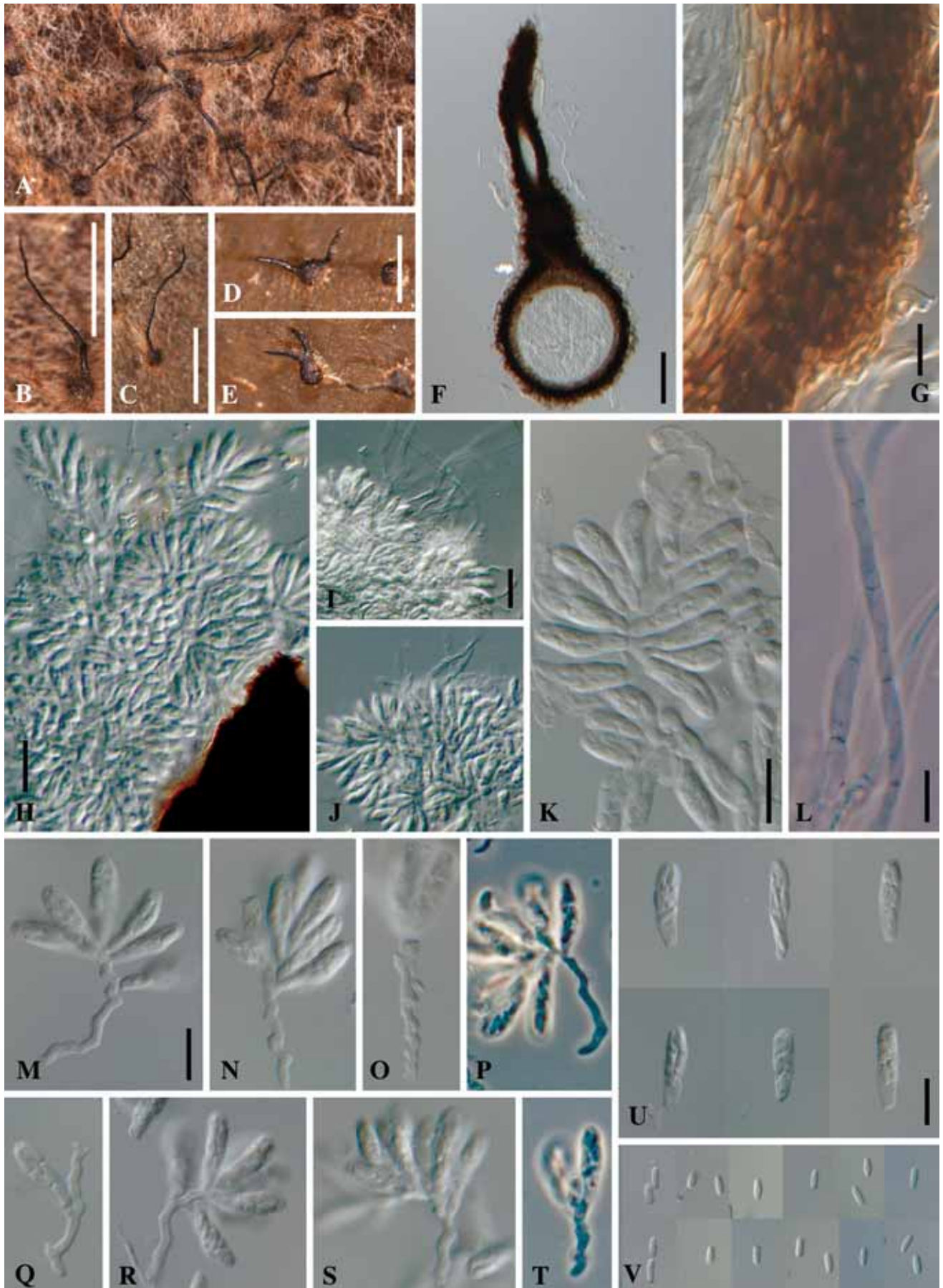
*Distribution:* South Africa.

*Additional cultures examined:* **South Africa**, *Vitis vinifera*, F. Halleen, 2002, pruning wound of *V. vinifera*, CBS 114993; graft union of *V. vinifera*, CBS 114994; pruning wound of *V. vinifera*, CBS 118482.

*Notes:* A heterothallic species. Formation of perithecia took 20–32 wk. *Phaeocremonium austroafricanum* and *Pm. angustius* can be distinguished from the other species by their reddish colony colour on OA. *Phaeocremonium austroafricanum* has a slower growth rate than *Pm. angustius*, with colonies reaching a radius of 5–8 mm after 8 d. Type III phialides were predominant in *Pm. austroafricanum*, while type I phialides predominated in *Pm. angustius*.

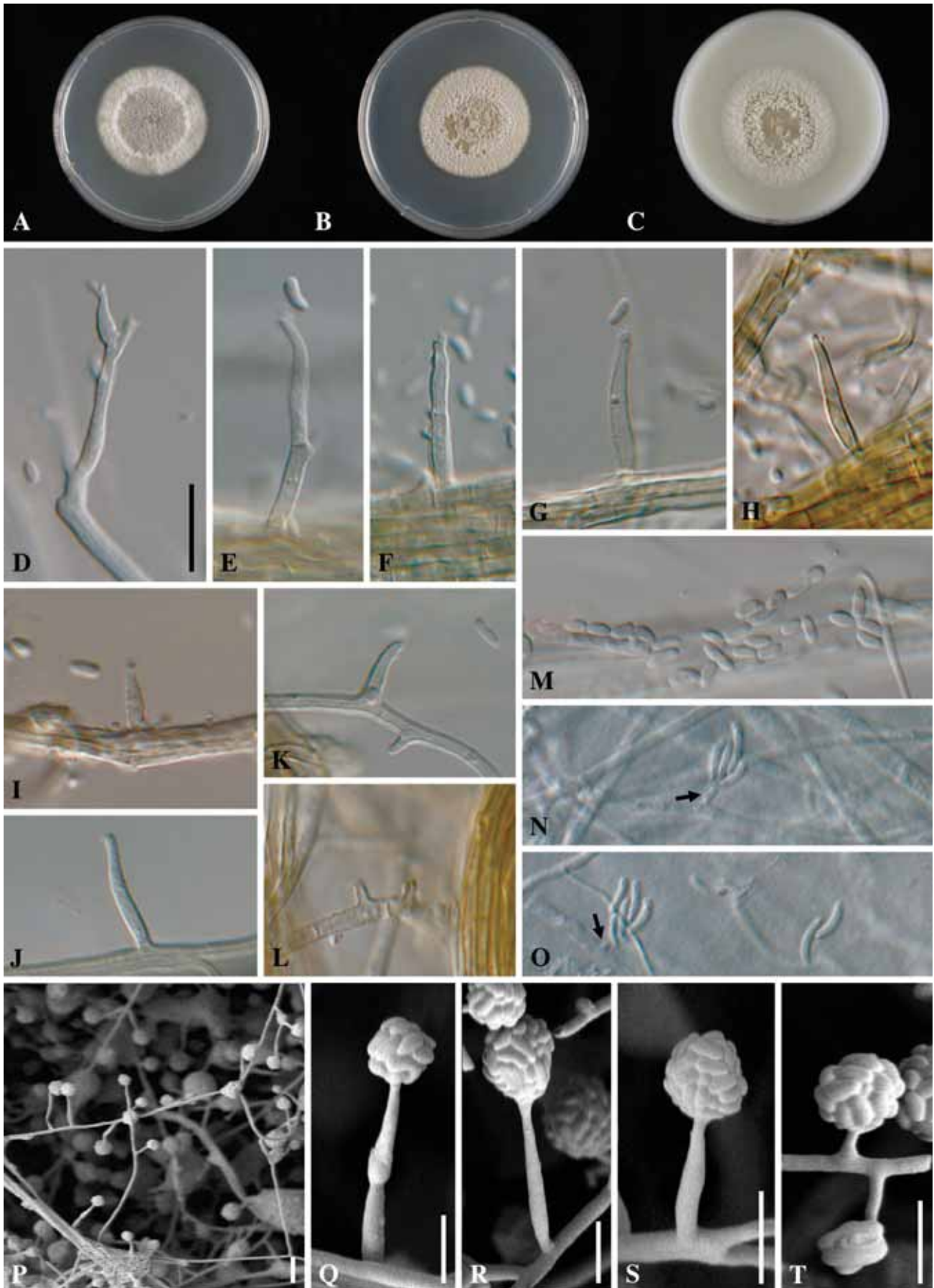


**Fig. 16.** *Phaeoacremonium austroafricanum*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–J, N–Q. Aerial structures on MEA. D. Conidiophore. E. Conidiophore and type III phialide (indicated by arrow). F–G. Type II phialides. H–I. Type I phialides. J. Conidia. K–M. Structures on the surface of and in MEA. K–L. Adelophialides with conidia. M. Conidia. N–O. Type III phialides. P. Type II phialide. Q. Type I phialide. A–Q from CBS 112949. D–M: DIC; N–Q: SEM. Scale bars: D–P = 10 µm; Q = 5 µm. Scale bar for D applies to E–M.



**Fig. 17.** *Togninia fraxinopennsylvanica*. A–E. Perithecia on dried MEA. F–G. Longitudinal sections through perithecia; peridium (G). H–K. Asci intermingled with paraphyses. L. Paraphyses. M–T. Ascogenous hyphae with asci attached. U. Asci. V. Ascospores. A–V from dried culture of CBS 110212 (holotype). A–E: DM; F–K, M–O, Q–S, U–V: DIC; L, P, T: PC. Scale bars: A–E = 500  $\mu$ m; F = 100  $\mu$ m; H–J = 20  $\mu$ m; G, K–V = 10  $\mu$ m. Scale bar for D applies to E; bar for I applies to J; bar for M applies to N–T; bar for U applies to V.





**Fig. 18.** *Phaeoacremonium mortoniae*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–L, P–T. Aerial structures on MEA. D–F. Conidiophores. G–H. Type III phialides. I–J. Type II phialides. K–L. Type I phialides. M. Conidia. N–O. Structures on the surface of and in MEA. N–O. Adelophialides (indicated with arrows) with conidia. P. Conidiophores and phialides. Q. Conidiophore. R. Type III. S. Type II phialide. T. Type I phialides. A–T from CBS 101585. D–O: DIC; P–T: SEM. Scale bars: D–Q = 10  $\mu$ m. Scale bar for D applies to E–O.

**3. *Togninia fraxinopennsylvanica*** (T.E. Hinds) Hausner, Eýjolfsdóttir & J. Reid, *Canad. J. Bot.* 70: 727. 1992. Fig. 17A–V.

*Basionym:* *Ceratocystis fraxinopennsylvanica* T.E. Hinds, *Mycologia* 67: 719. 1975.

= *Calosphaeria fraxinopennsylvanica* (T.E. Hinds) H.P. Upadhyay, In Upadhyay, *A monograph of Ceratocystis and Ceratocystiopsis*: 137. 1981.

*Anamorph:* *Phaeoacremonium mortoniae* Crous & W. Gams, *Mycol. Res.* 105: 655. 2001.

*Perithecia* single or in clusters, subglobose, sometimes obpyriform, (181–)185–252(–270)  $\mu\text{m}$  diam and (181–)187–258(–270)  $\mu\text{m}$  tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 4–8 cells (individual cells not visible further outward) and 10–13(–17)  $\mu\text{m}$  thick; inner region hyaline at the centrum, pale brown near the periphery, 3–8 cells and 8–15  $\mu\text{m}$  thick; surface covered with brown, septate hyphal appendages that become hyaline towards the tips (more abundant on older perithecia). *Perithecial necks* black, 1–2(–3) per perithecium, straight to curved, verrucose, (390–)410–1115(–1125)  $\mu\text{m}$  long, 40–60(–65)  $\mu\text{m}$  wide at the base, and 25–34  $\mu\text{m}$  wide at the apex, sometimes dividing into two near the apex; apex often proliferating secondarily upon aging, appearing nodulose; nodules (–70  $\mu\text{m}$  diam) also appearing lower down on the neck. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 45–100 (av. 66)  $\mu\text{m}$  long, 2–5 (av. 3)  $\mu\text{m}$  wide at the base and 1–2.5 (av. 2)  $\mu\text{m}$  at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases, 15–20  $\times$  4(–5) (av. 17  $\times$  4  $\mu\text{m}$ ); apical region 1–1.5  $\mu\text{m}$  thick, of indistinct structure, with a non-amyloid apical ring. *Ascogenous hyphae* hyaline, branched, smooth-walled, remnant bases 5–6  $\times$  3.5–4  $\mu\text{m}$  wide. *Ascospores* aseptate, hyaline, oblong-ellipsoidal to slightly curved with rounded ends, sometimes containing small guttules at the ends, biseriate, 3.5–5  $\times$  1 (av. 4  $\times$  1)  $\mu\text{m}$ .

*Phaeoacremonium mortoniae* Crous & W. Gams, *Mycol. Res.* 105: 655. 2001. Fig. 18A–T.

*Aerial structures:* *Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 19, tuberculate with warts up to 0.5  $\mu\text{m}$  diam, verruculose, medium to pale brown, 1.5–2.5  $\mu\text{m}$  wide. *Conidiophores* mostly short and usually unbranched, arising from aerial or submerged hyphae, erect, simple, up to 2-septate, often ending in a single terminal phialide, pale brown, paler towards the tip, smooth to verruculose, (16–)20–30(–40) (av. 26)  $\mu\text{m}$  long and (1.5–)2(–2.5) (av. 2)  $\mu\text{m}$  wide. *Phialides* terminal or lateral, mostly monophialidic, smooth to verruculose,

pale brown to hyaline; type I and type II phialides most common; collarettes 1  $\mu\text{m}$  long, 1  $\mu\text{m}$  wide; type I phialides cylindrical, occasionally widened at the base, 2–9(–10)  $\times$  1–1.5(–2) (av. 5  $\times$  1.5)  $\mu\text{m}$ ; type II phialides either elongate-ampulliform and attenuated at the base or subcylindrical, tapering towards the apex, 7–12  $\times$  1.5–2 (av. 10  $\times$  2)  $\mu\text{m}$ ; type III phialides subcylindrical or navicular, 13–29  $\times$  1.5–2 (av. 24  $\times$  2)  $\mu\text{m}$ , tapering gradually to a long neck. *Conidia* mostly oblong-ellipsoidal or reniform, 3–4(–5)  $\times$  1(–1.5) (av. 4  $\times$  1)  $\mu\text{m}$ , L/W = 4.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical 1–7(–9)  $\times$  1(–1.5) (av. 3  $\times$  1)  $\mu\text{m}$ . *Conidia* hyaline, allantoid, 5–6(–7)  $\times$  1(–1.5) (av. 5  $\times$  1)  $\mu\text{m}$ , L/W = 5.

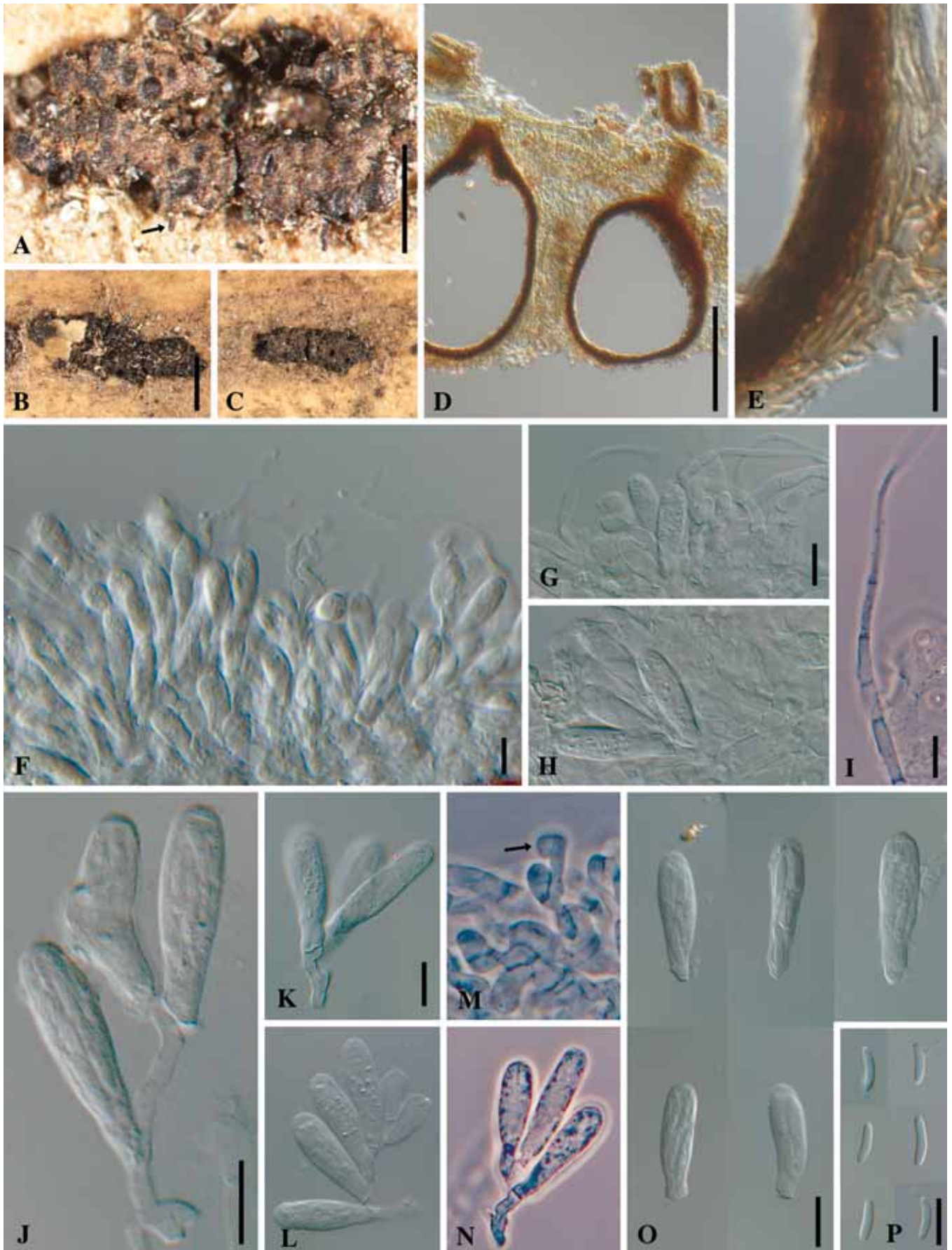
*Types:* **U.S.A.**, North Dakota, Bottineau county, isolated from brown stain of green ash, *Fraxinus pennsylvanica* Marsh., BPI 595570, **holotype** of teleomorph, dried colony of ATCC 26664, ex-type culture CBS 110212; California, Sonoma County, trunk of *Vitis vinifera*, 1998, L. Morton & L. van der Water, PREM 57084, **holotype** of anamorph, ex-type culture CBS 101585.

*Cultural characteristics:* Colonies reaching a radius of 10–13 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 25–30 °C, maximum 35 °C. Colonies on MEA flat, felty to cottony, with entire edge; after 8 d colonies white (3A1) above, with few grey (3B1) tufts in the centre, in reverse yellowish white (3A2); after 16 d mostly white (3A1) above, with yellowish grey (3B2) undertones, in reverse yellowish grey to yellowish white towards the edge (4B2–3A2). Colonies on PDA flat, felty to short woolly, with entire edge; after 8 d yellowish grey to white (3B2–A1) above, in reverse greyish yellow (2C5) or pale yellow (3A3); after 16 d yellowish white (3A2) or grey above, becoming yellowish grey (3B1–3B2), in reverse pale yellow (3A3) or greyish yellow to yellowish white toward the edge (4C4–3A2). Colonies on OA flat, felty, with entire edge; after 8 d colonies greyish yellow to yellowish white towards the edge (4B3–3A2) above; after 16 d yellowish white (3A2) or olive-brown to white (4D1–A1) towards the edge. Production of yellow pigment on OA variable.

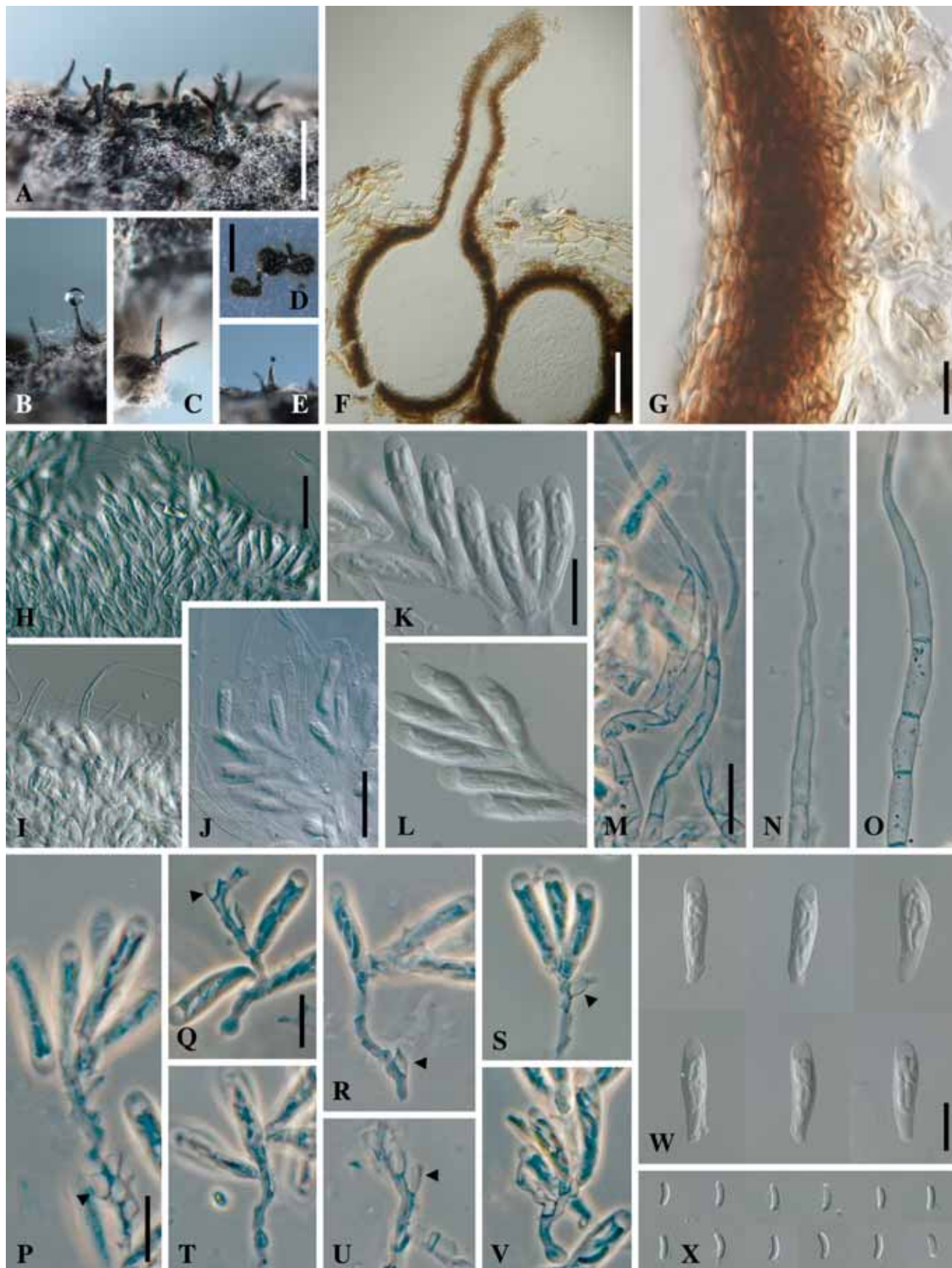
*Substrate:* *Fraxinus excelsior*, *F. pennsylvanica*, *Vitis vinifera*.

*Distribution:* Sweden, U.S.A. (California, North Dakota).

*Additional cultures examined:* **Sweden**, stem wound in *Fraxinus excelsior*, under stripped bark, 1996, J. Stenlid, CBS 211.97. **U.S.A.**, North Dakota, *Fraxinus pennsylvanica* associated with larval galleries of *Leperisinus californicus* Swaine, 1970, T.E. Hinds, CBS 110212 = ATCC 26664.



**Fig. 19.** *Togninia inconspicua*. A–C. Aggregated perithecia on *Bambusa vulgaris* (neck arrowed in A). D–E. Longitudinal sections through perithecia; peridium (E). F–H. Asci intermingled with paraphyses. I. Paraphysis. J–N. Ascogenous hyphae with asci attached; immature asci attached to ascogenous hyphae (K, L, N); crozier formation on apex of ascogenous hyphae (M). O. Asci. P. Ascospores. A–P from F6209 (holotype). A–C: DM; D–H, J–L, O, P: DIC; I, M, N: PC. Scale bars: A–C = 500  $\mu$ m; D = 100  $\mu$ m; E–P = 10  $\mu$ m. Scale bar for B applies to C; bar for G applies to H; bar for K applies to L–N.



**Fig. 20.** *Togninia krajdenii*. A–C, E. Perithecia on canes of *Vitis vinifera*. D. Perithecia on adjacent water agar. F–G. Longitudinal sections through perithecia; peridium (G). H–J. Asci intermingled with paraphyses. K–L. Asci on ascogenous hyphae. M–O. Paraphyses becoming thread-like towards the tips. P–T, V. Ascogenous hyphae with asci attached; remnant bases indicated by arrow heads (P, Q, R, U, S). U. Ascogenous hypha with remnant bases showing positions where asci were attached. W. Asci. X. Ascospores. A–X from CBS 17460 (holotype). A–E: DM; F–L, W–X: DIC; M–V: PC. Scale bars: A–E = 500 µm; F = 100 µm; H–J = 20 µm; G, K–X = 10 µm. Scale bar for A applies to B–C; bar for H applies to I; bar for K applies to L; bar for M applies to N–O; bar for Q applies to R–V; bar for W applies to X.

*Notes:* *Phaeoacremonium mortoniae* can be distinguished by its mostly white to pale grey colonies on MEA, and yellowish white colonies on PDA and OA. Type I and type II phialides are predominant, with type II phialides having a “short and stocky” appearance. The colony colours observed by Groenewald *et al.* (2001) were slightly darker than those described here, and these authors also saw a variable diffuse brown pigment in MEA. The same conditions were used by both authors indicating that these characters tend to be variable for this species.

**4. *Togninia inconspicua*** (Rehm) J.Z. Yue & O.E. Eriksson, Mycotaxon 38: 203. 1990. Fig. 19A–P.

*Basionym:* *Calosphaeria inconspicua* Rehm, Leafl. Philipp. Bot. 6: 2213. 1914.

≡ *Erostella inconspicua* (Rehm) Sacc., Syll. Fung. 24: 709. 1928.

= *Nitschkea bambusarum* Rehm, Leafl. Philipp. Bot. 8: 2956. 1916.

*Perithecia* densely aggregated, immersed, subepidermal, globose to subglobose, apex rounded, short-beaked, 74–167 µm diam and 142–196 µm tall. Peridium consisting of dark brown *textura angularis*, 8–10 µm thick. *Perithecial necks* black, straight to curved, 83–113 µm long and 15 µm wide at the base and 10 µm wide at the apex. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 65–120 µm long, 2–3.5 µm wide at the base and 1.5 µm at the apex, persistent, arising from the basal cells of each fascicle of asci. *Ascogenous hyphae* proliferating sympodially, hyaline, smooth-walled, no remnant bases observed. *Asci* appearing spicate when mature, 8-spored, unitunicate, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases, 20–30(–32) × 6–8 (av. 25 × 6 µm); apical region 2.5–4 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascospores* aseptate, hyaline, allantoid or oblong-ellipsoidal, with a smooth wall and small guttules, biserial, 7–10 × 1.5–2 (av. 9 × 2 µm).

*Anamorph:* Unknown.

*Substrate:* *Bambusa vulgaris*, *Gigantochloa schrebneriana*.

*Distribution:* Philippines.

*Type:* **Philippines**, Luzon, Laguna Prov., Los Baños, on *Gigantochloa schrebneriana*, 10 Sep. 1913, leg. M.B. Raimundo, no. 1698a, **holotype** herb. S F6209.

*Additional specimen examined:* **Philippines**, Luzon, Laguna Prov., Mt. Maquiling near Los Baños, on *Gigantochloa schrebneriana*, herb. C.F. Baker, herb. S F6215, part of Raimundo no. 1698.

*Notes:* This species is not available in fresh material

and could not be studied phylogenetically. It is a *Togninia* because of its nonstromatic perithecia that are globose to subglobose. The asci are also typically arranged in a spicate manner on the ascogenous hyphae. *Togninia inconspicua* have paraphyses that are broad at the base and taper towards the apex similar to those found in *Togninia*. Also the ascospores are aseptate and allantoid or oblong-ellipsoidal. It is a distinct species because of its small perithecia, short necks, absence of remnant bases on the ascogenous hyphae, large asci and ascospores.

**5. *Togninia krajdensis*** L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500691. Fig. 20A–X.

*Anamorph:* *Phaeoacremonium krajdensis* L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43:1761. 2005.

*Anamorphe Phaeoacremonium krajdensis.* Perithecia plerumque aggregata et subepidermalia, subglobosa, nonnulla obpyriformia, (197–)203–275 µm diam; collis atris, singulis vel binis in quoque perithecio, rectis vel curvatis, verrucosis, 220–440 µm longis. Paraphyses hyalinae, septatae, cylindricae, sursum angustatae, filiformes, 40–290 (in medio 95) µm longae. Asci clavati, (16–)18–22(–23) × 4–5 (in medio 21 × 4 µm) µm. Ascospores unicellulares, hyalinae, allantoidae vel oblongo-ellipsoideae, utrinque rotundatae, 4–5(–6) × 1–1.5 µm (in medio 5 × 1) µm.

Typus herb. CBS 17460.

*Perithecia* mostly aggregated, sometimes solitary, mostly subepidermal, but also on the surface of the epidermis; subglobose, sometimes obpyriform, (197–)203–275 µm diam and (202–)203–284(–287) µm tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 4–6 cells (individual cells not visible further outward) and 8–11 µm thick; inner region hyaline at the centrum, pale brown at the periphery, (4–)5–7 cells and 7–12 µm thick; surface covered with brown, septate hyphal appendages that become hyaline towards their tips (more abundant on older perithecia). *Perithecial necks* black, 1–2 per perithecium, straight to curved, verrucose, 220–440 µm long, 39–69 µm wide at the base, and 34–54 µm wide at the apex, necks sometimes dividing into two near the apex; sometimes proliferating secondarily upon aging, with nodules up to 17 µm diam. *Paraphyses* hyaline, septate, cylindrical, narrowing and becoming thread-like towards the tip, 40–290 (av. 95) µm long, 2.5–4.5 (av. 3) µm wide at the base and 1–3 (av. 2) µm at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases (16–)18–22(–23) × 4–5 (av. 21 × 4 µm); apical region 1.5–2.5 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascogenous hyphae* hyaline, branched, smooth-walled, remnant bases 5 × 1.5–4 µm. *Ascospores* aseptate, hyaline, allantoid

to oblong-ellipsoidal with rounded ends, sometimes containing small guttules at the ends, biseriate, 4–5(–6) × 1–1.5 µm (av. 5 × 1) µm.

***Phaeoacremonium krajdenui*** L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43: 1761. 2005. Fig. 21A–Q.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 8; hyphae strongly tuberculate with warts up to 1 µm diam, verrucose, dark to medium brown and 2–3 µm wide. *Conidiophores* short and usually unbranched, occasionally constricted at the basal septum, bases of older, percurrently rejuvenating cells often inflated, up to 5-septate, often bearing, besides the terminal phialide a second one at the apical septum, (16–)20–45(–76) (av. 28) µm long and 1.5–3 (av. 2) µm wide. *Phialides* terminal or lateral, often polyphialidic, sparsely tuberculate to verruculose, rarely smooth, pale brown to hyaline; collarettes, slightly flaring, 1–3 µm long and 1–2 µm wide; type I phialides cylindrical, occasionally widened at the base, tapering towards the apex, (2–)4–13(–17) × 1–1.5(–2) (av. 7 × 1) µm; type II phialides predominant, elongate-ampulliform and attenuated at the base, or subcylindrical, (8–)8.5–14 × 1.5–2(–2.5) (av. 12 × 2) µm; type III phialides navicular to subcylindrical, or sometimes elongate-ampulliform and attenuated at the base, 14–21(–25) × 1–2(–2.5) (av. 17 × 2) µm, gradually tapering towards the apex. *Conidia* subhyaline to hyaline, oblong-ellipsoidal or allantoid, 3–5 (–5.5) × 1–1.5(–2) (av. 4 × 1) µm, L/W = 3.5.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, (1.5–)2–15(–17) × 1–2 (av. 6 × 1) µm. *Conidia* hyaline, oblong-ellipsoidal to allantoid, some cylindrical, 4–8(–12) × 1–2 (av. 6 × 1) µm, L/W = 4.

*Types examined:* **Canada**, Ontario, Toronto, human, 2001, S. Krajden, herb. CBS 7959 **holotype** of anamorph, dried MEA colony of ex-type culture CBS 109479; **South Africa**, base of trunk of *V. vinifera*, 23 March 2001, G. van Coller, CBS 110118; CBS 110118 was crossed with CBS 109479, single-ascospore strains from fertile perithecia were crossed again: CBS 118230 × CBS 118239, Herb. CBS 17460, **holotype** of teleomorph.

*Cultural characteristics:* Colonies reaching a radius of 9–14 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 30 °C, maximum 37 °C. Colonies on MEA flat, mostly felty, with entire edge; after 8 d colonies hair-brown to dark blond (5E4–D4) above, in reverse the same; after 16 d greyish brown (7D3) above, in reverse dark brown (7F4). Colonies on PDA flat, felty, with entire edge; after 8 d brown (6E4) above, in reverse dark brown (6F4); after 16 d dark brown to brownish grey (6F4–D2) above, in reverse

greyish brown (6F3). Colonies on OA flat, felty with woolly tufts, with entire edge; after 8 d brown (5F4) above, after 16 d brown to greyish brown towards the edge (5F4–5E3).

*Substrate:* Human, *Vitis vinifera*.

*Distribution:* India, Japan, Norway, South Africa, U.S.A., Zaire.

*Additional cultures examined:* **India**, Karnataka State, Belgaum, white-grain eumycetoma in foot, 2001, A.A. Padhye, CBS 110361 = CDC B6093. **Japan**, granuloma on back of human hand, M. Hironaga, CBS 110366 = ATCC 58115. **Norway**, man, mycetoma of foot of 31-year-old male, 1993, P. Sandven, CBS 633.93. **Zaire**, human skin lesion, 1973, K.J. Kwon-Chung, CBS 423.73. **South Africa**, Western Cape Province, Wellington, crown of *Vitis vinifera*, 2001, G. van Coller, CBS 110118; Western Cape, rootstock of *V. vinifera*, 2002, F. Halleen, CBS 113588. **U.S.A.**, human clinical material, A. Espinel (depositor), CBS 110365 = UAMH 5723; Alabama, Mobile, mass on foot of female, 2001, S. Weber, CBS 110367 = CDC B6091; Maryland, Towson (greater Baltimore), foot lesion in female, 2001, A.A. Padhye, CBS 110368 = CDC B6092.

*Notes:* A heterothallic species. Formation of perithecia took 12–20 wk. Various brown-coloured *Phaeoacremonium* species have verrucose mycelium. *Phaeoacremonium krajdenui* can be distinguished from *Pm. parasiticum*, *Pm. tardicrescens* and *Pm. sphinctrophorum* by frequently producing polyphialides. These structures are rare or not seen in the other species. *Phaeoacremonium krajdenui* has short, mostly unbranched conidiophores, whereas *Pm. parasiticum* characteristically has very long conidiophores. *Phaeoacremonium krajdenui* has warts smaller (up to 1 µm diam) than those of *Pm. parasiticum* (up to 3 µm diam). *Phaeoacremonium krajdenui* can be distinguished from the slow-growing *Pm. tardicrescens* by its faster growth rate and darker colony colour on MEA. *Phaeoacremonium sphinctrophorum* can be distinguished by its conidiophores with prominent septal constrictions.

**6. *Togninia minima*** (Tul. & C. Tul.) Berl., Icon. Fung. 3: 11. 1900. Fig. 22A–U.

*Basionym:* *Calosphaeria minima* Tul. & C. Tul., Sel. Fung. Carpol. 2: 105. 1863.

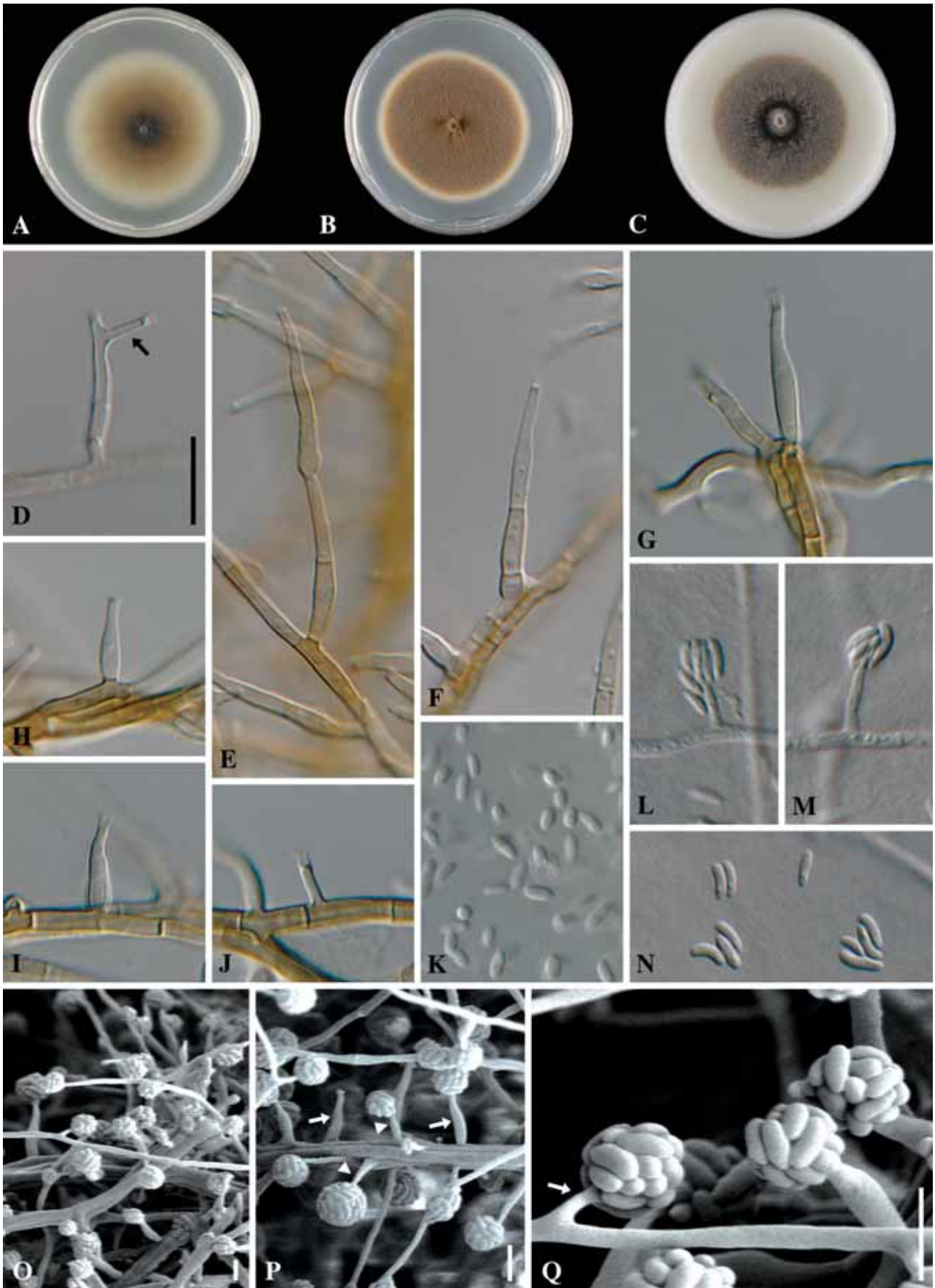
≡ *Calosphaeria (Erostella) minima* (Tul. & C. Tul.) Sacc., Syll. Fung. 1: 101. 1882.

≡ *Erostella minima* (Tul. & C. Tul.) Traverso, Fl. Ital. Crypt. 1: 156. 1905. 1906.

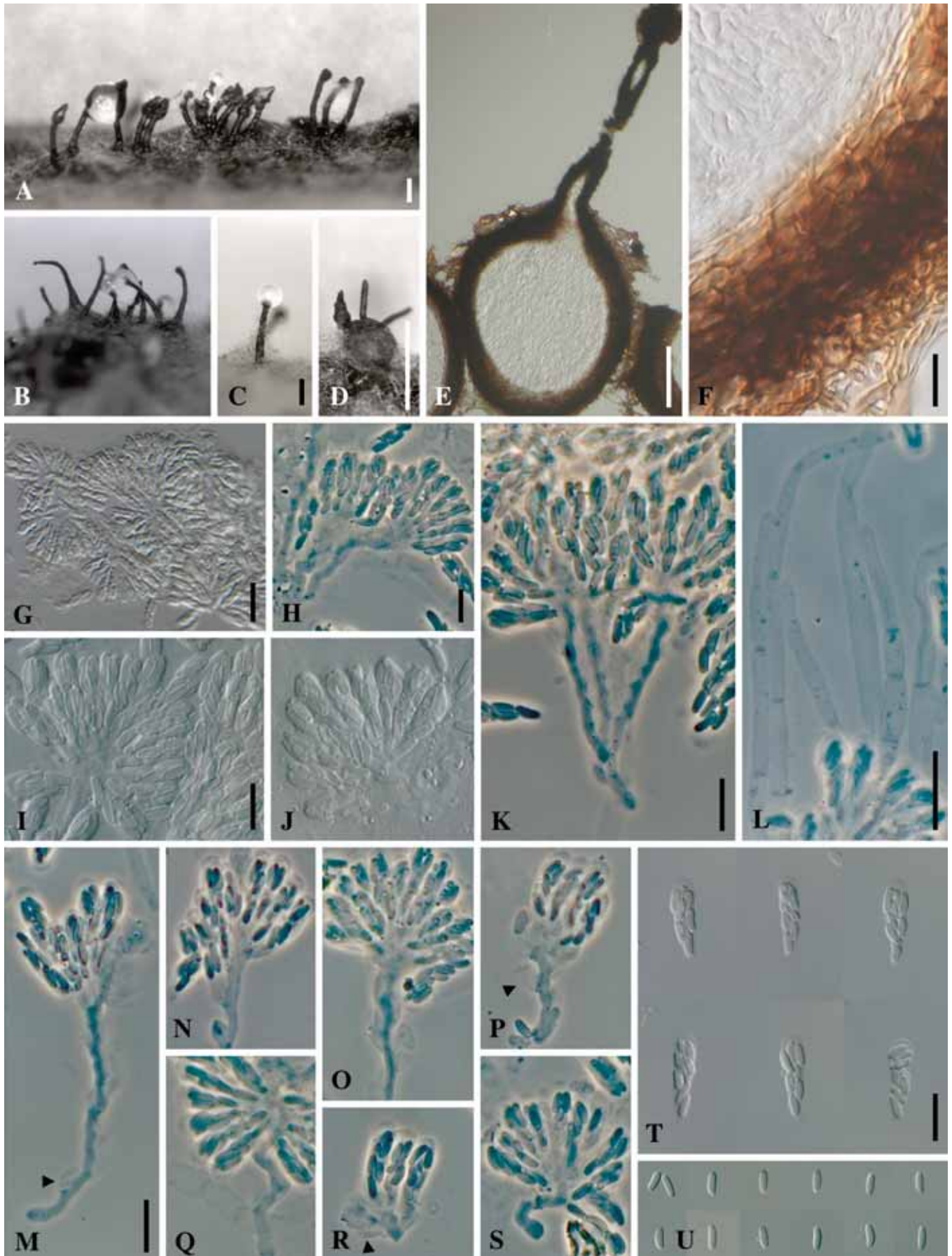
= *Calosphaeria alnicola* Ellis & Everh., Proc. Acad. Nat. Sci. Phila. 221. 1890. 1891.

≡ *Togninia alnicola* (Ellis & Everh.) Berl., Icon. Fung. 3: 10. 1900.

= *Longoa paniculata* Curzi, Atti Ist. Bot. R. Univ. Pavia, Ser. 3, 3: 204. 1927.

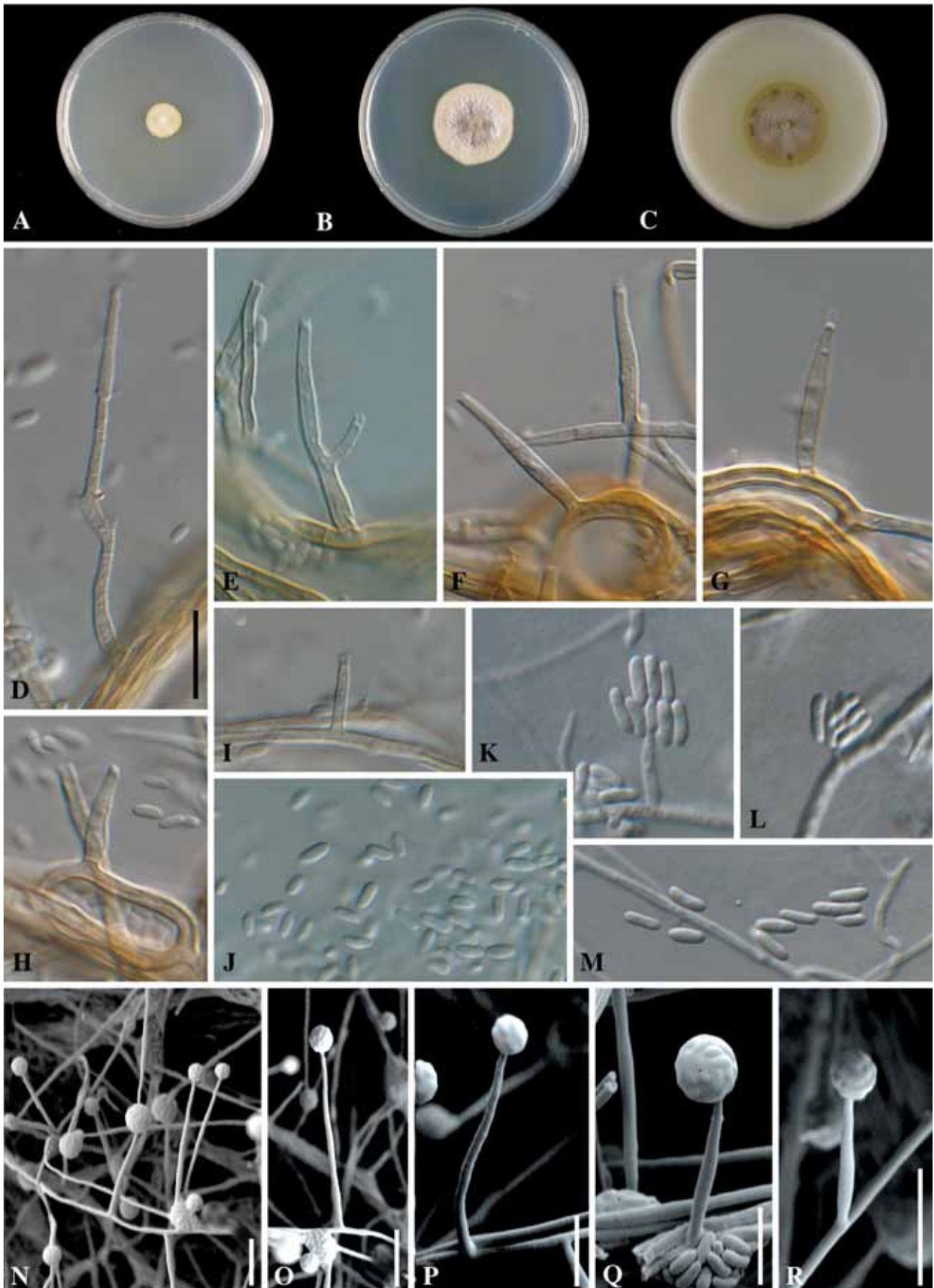


**Fig. 21.** *Phaeoacremonium krajdienii*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–K, O–Q. Aerial structures on MEA. D. Conidiophore with polyphialide (indicated by arrow). E–F. Conidiophores. G. Type III phialide. H–I. Type II phialides. J. Type I phialides. K. Conidia. L–N. Structures on the surface of and in MEA. L–M. Adelophialides with conidia. N. Conidia. O. Mycelium and phialides. P. Type II (indicated with arrows) and type I (indicated with arrow heads). Q. Type I phialide (indicated with arrow). A–Q from CBS 109479. D–N: DIC; O–Q: SEM. Scale bars: D–Q = 10  $\mu$ m. Scale bar for D applies to E–N.



**Fig. 22.** *Togninia minima*. A–D. Perithecia on canes of *Vitis vinifera*. E–F. Longitudinal section through perithecia; peridium (F). G–K. Asci attached to ascogenous hyphae. L. Paraphyses. M–S. Ascogenous hyphae with asci attached; remnant bases indicated by arrow heads (M, P, R). T. Asci. U. Ascospores. A–U from CBS 6580 (holotype). A–D: DM; E–G, I–J, T–U: DIC; H, K–S: PC. Scale bars: A–D = 500  $\mu$ m; E = 100  $\mu$ m; G = 20  $\mu$ m; F, H–V = 10  $\mu$ m. Scale bar for A applies to B; bar for I applies to J; bar for M applies to N–S; bar for T applies to U.





**Fig. 23.** *Phaeoacremonium aleophilum*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–J, N–R. Aerial structures on MEA. D–E. Conidiophores. F–G. Type III phialides. H. Type II phialide. I. Type I phialide. J. Conidia. K–M. Structures on the surface of and in MEA. K–L. Adelophialides with conidia. M. Conidia. N–P. Conidiophores. Q. Type III phialide. R. Type II phialide. A–R from CBS 246.91. D–M: DIC; N–R: SEM. Scale bars: D–R = 10 μm. Scale bar for D applies to E–M.

*Anamorph: Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & L. Mugnai, Mycologia 88: 791. 1996.

*Perithecia* mostly aggregated, sometimes solitary, mostly subepidermal also on the surface of the epidermis, subglobose, sometimes obpyriform, (160–)250–285(–420)  $\mu\text{m}$  diam and (200–)285–325(–400)  $\mu\text{m}$  tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 8–10 cells (individual cells not visible further outward) and 20–40  $\mu\text{m}$  thick; inner region hyaline at the centrum, paler brown towards the periphery, 5–7 cells and 12–28  $\mu\text{m}$  thick; surface covered with brown, septate hyphal appendages that become hyaline towards their tips (more abundant on older perithecia). *Perithecial necks* black, 1–3(–6) per perithecium, cylindrical, straight to curved, verrucose, 800–1800  $\mu\text{m}$  long, 35–130  $\mu\text{m}$  wide at the base, and 20–60  $\mu\text{m}$  wide at the apex, necks sometimes dividing into two near the apex; apex often proliferating secondarily upon aging and then appearing nodulose; nodules (–120  $\mu\text{m}$  diam) also appearing lower down on the neck; multi-necked perithecia often with a thin wall dividing the perithecial chamber. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 45–125 (av. 83)  $\mu\text{m}$  long, 2–4  $\mu\text{m}$  (av. 3) wide at the base and 1.5–2 (av. 2)  $\mu\text{m}$  at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases (17–)19–20(–27)  $\times$  4–5 (av. 19  $\times$  4)  $\mu\text{m}$ ; apical region 0.5–1  $\mu\text{m}$  thick, of indistinct structure, with a non-amyloid apical ring. *Ascogenous hyphae* hyaline, branched, smooth-walled, remnant bases 5  $\times$  2.5–4  $\mu\text{m}$ . *Ascospores* aseptate, hyaline, oblong-ellipsoidal to allantoid with rounded ends, sometimes containing small guttules at the ends, biserial, (4–)4.5–5(–6.5)  $\times$  1–2 (av. 5  $\times$  2)  $\mu\text{m}$ .

*Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & L. Mugnai, Mycologia 88: 791. 1996. Fig. 23A–R.

*Aerial structures: Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 25, tuberculate with warts up to 1.5  $\mu\text{m}$  diam, verruculose, medium to pale brown and 1–2.5  $\mu\text{m}$  wide. *Conidiophores* mostly short and usually unbranched, arising from aerial or submerged hyphae, erect, simple, up to 3-septate, often bearing a single phialide as the apical cell, pale brown, paler towards the tip, smooth to verruculose, (15–)17–42(–46) (av. 29)  $\mu\text{m}$  long and 1.5–2.5 (av. 2)  $\mu\text{m}$  wide. *Phialides* terminal or lateral, mostly monophialidic, smooth to verruculose, subhyaline; collarettes 1–1.5  $\mu\text{m}$  long 1.5–2  $\mu\text{m}$  wide; type II and type III phialides most common; type I

phialides cylindrical, occasionally widened at the base, (1.5–)2–9(–11)  $\times$  1–1.5 (av. 5  $\times$  1)  $\mu\text{m}$ ; type II phialides either elongate-ampulliform and attenuated at the base or navicular, tapering towards the apex, (6–)9–14(–15)  $\times$  1.5–2 (–2.5) (av. 11  $\times$  2)  $\mu\text{m}$ ; type III phialides subcylindrical or elongate-ampulliform and attenuated at the base, (14–)15–22  $\times$  1.5–2 (av. 18  $\times$  2)  $\mu\text{m}$ , tapering gradually to a long neck. *Conidia* mostly oblong-ellipsoidal or cylindrical, occasionally reniform, 3–5  $\times$  1–2 (av. 4  $\times$  1.5)  $\mu\text{m}$ , L/W = 3.

*On surface or submerged in the agar: Phialides* hyaline, cylindrical, some subcylindrical, (1.5–)2–13(–17)  $\times$  1–1.5(–2) (av. 4.5  $\times$  1)  $\mu\text{m}$ . *Conidia* hyaline, oblong-ellipsoidal to allantoid, 4–6(–7)  $\times$  (1–)1.5–2 (av. 5  $\times$  1.5)  $\mu\text{m}$ , L/W = 3.

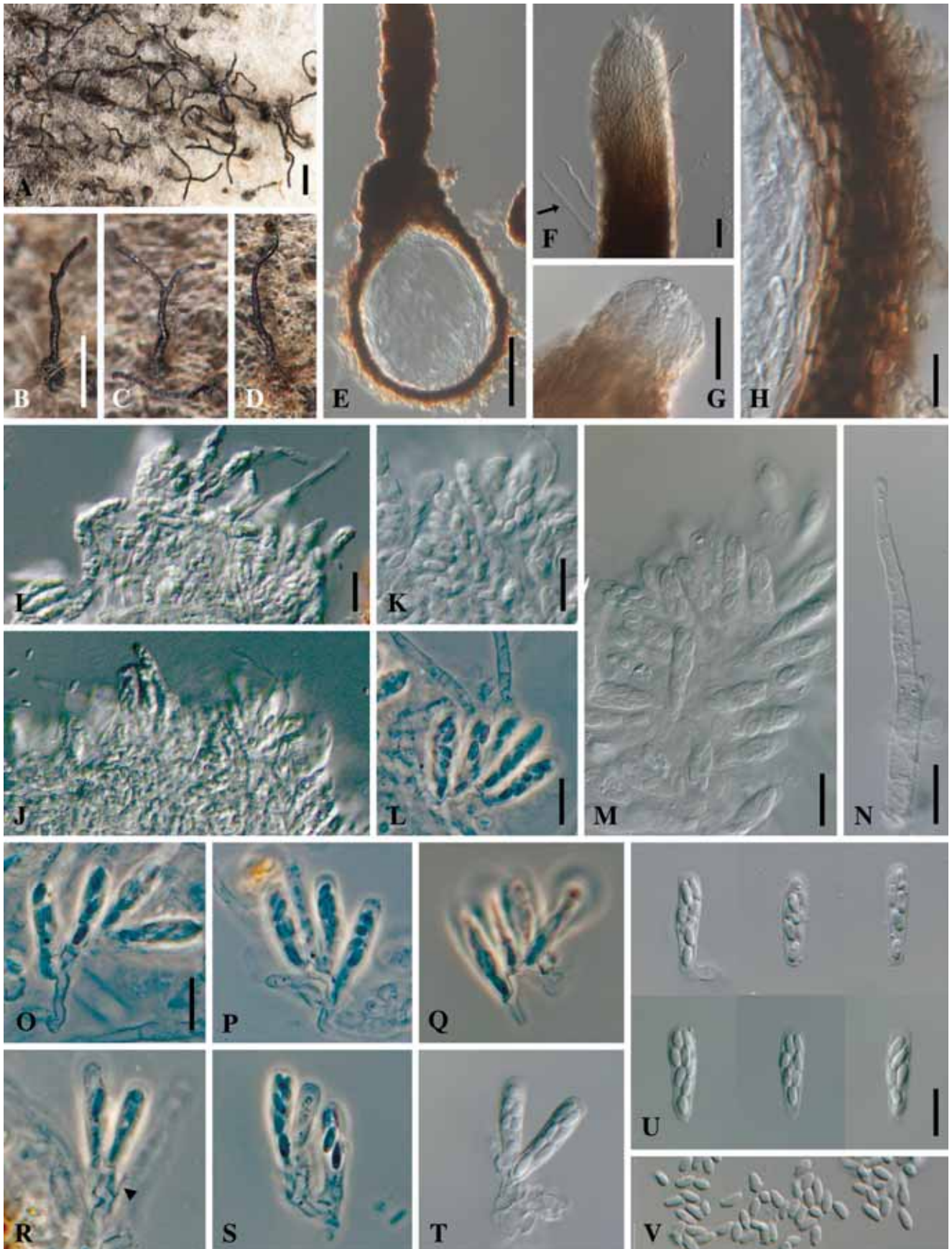
*Types: Yugoslavia*, on roots and stems of *Vitis vinifera*, 1990, M. Muntañola-Cvetković, herb. CBS 246.91 **holotype** of anamorph, dried colony; dried **isotype** lodged at PREM; cultures ex-type CBS 246.91 = C.P.C. 776. **South Africa**, Western Cape Province, Wellington and Paarl respectively, tester strains CBS 111015 and CBS 110703 on *Vitis vinifera*, 2001, L. Mostert; when crossed they yielded Herb. CBS 6580, the **epitype** of the teleomorph.

*Cultural characteristics:* Colonies reaching a radius of 2.5–11 mm in 8 d at 25 °C on MEA. Minimum temperature for growth 10 °C, optimum 30 °C, maximum 37–40 °C on MEA. Colonies on MEA flat, mostly felty textured, with entire edge; after 8 d yellowish white (3A2) or orange-grey (5B2) above, in reverse pale yellow (4A3) or brown-orange (5C3); after 16 d pale yellow (4A3) or grey (5C1) above, in reverse pale yellow (3A3) or brown (5F6). Colonies on PDA flat, felty or woolly textured, with entire edge; after 8 d pale brown (6D4) or grey-orange (5C4–B3) above, in reverse greyish orange (5B3) or brown (5F5); after 16 d dark blond to brownish grey towards the edge (5D4–7D2) above, in reverse pale brown to dark brown towards the edge (6D4–6F4). Colonies on OA flat, felty with a few woolly tufts, with entire edge; after 8 d yellowish white (4A2) above; after 16 d yellowish white to greyish yellow towards the edge (4A2–4B3). Colonies producing yellow pigment on PDA and OA.

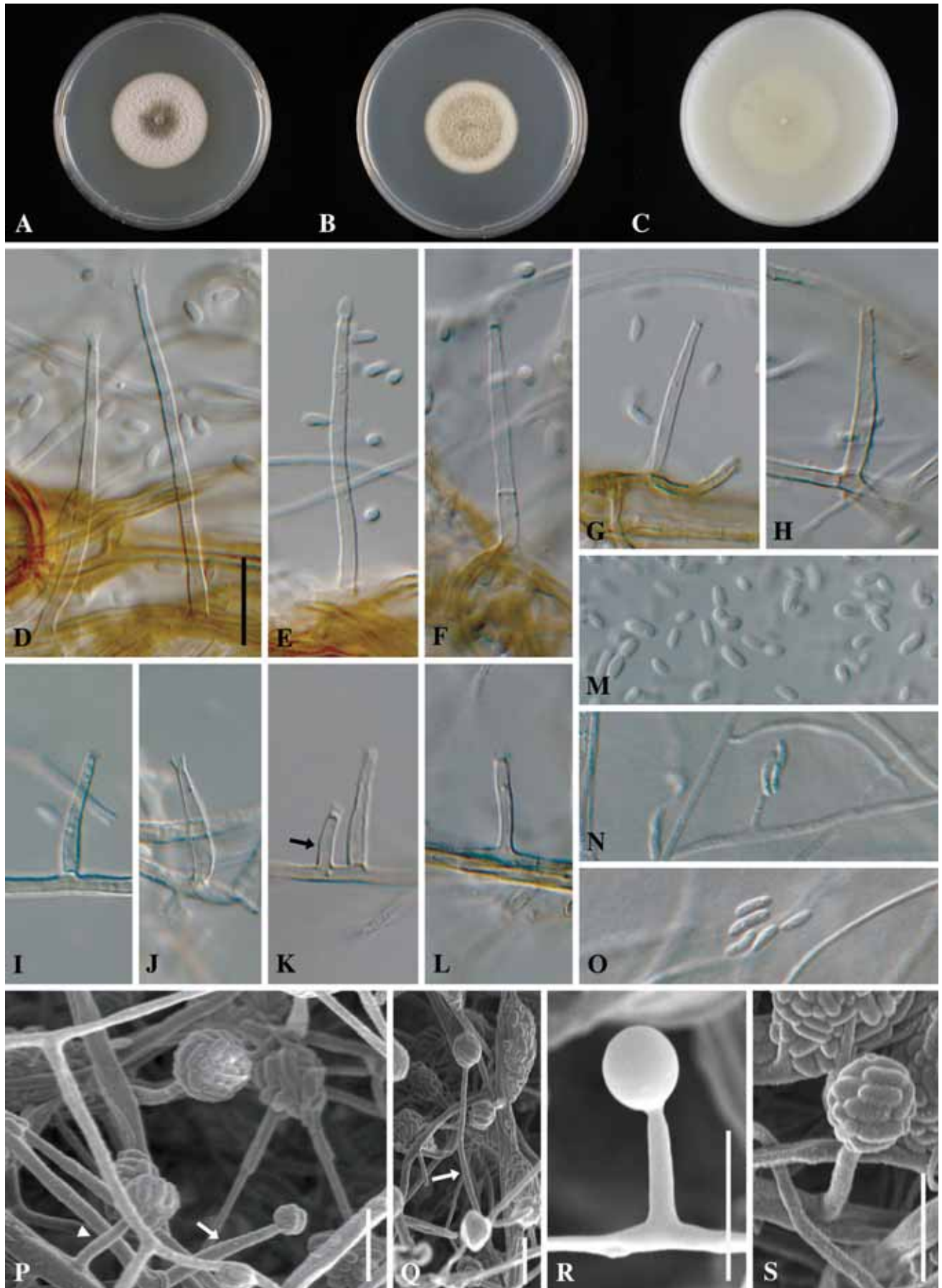
*Substrate: Actinidia chinensis, Olea europaea, Prunus pennsylvanica, Prunus* sp., *Salix* sp., *Vitis vinifera*.

*Distribution:* Argentina, Australia, Austria, Canada, Chile, France, Iran, Italy, South Africa, Spain, Turkey, U.S.A., Yugoslavia.

*Additional cultures examined: Italy, Vitis vinifera*, 1998, S. Serra, CBS 100397 = C.P.C. 4029. **South Africa**, base of trunk of *V. vinifera*, 2001, L. Mostert, CBS 110703; graft union of *V. vinifera*, 2001, L. Mostert, CBS 111015.



**Fig. 24.** *Togninia novae-zealandiae*. A–D. Perithecia on MEA. E. Longitudinal section through perithecium. F–G. Neck with pale brown to hyaline at the tip; conidiophores present on the surface of the neck (F); ascospores present in the channel at the tip of the neck (G). Peridium (H). I–L. Asci intermingled with paraphyses. M. Asci attached to ascogenous hyphae. N. Paraphysis. O–T. Ascogenous hyphae with asci attached; remnant base indicated by arrow head (R). U. Asci. V. Ascospores. A–V from dried culture of CBS 110156 (holotype). A–D: DM; E–K, M–N, T–V: DIC; L, O–S: PC. Scale bars: A–D = 500  $\mu$ m; E = 100  $\mu$ m; I–J = 20  $\mu$ m; F, G, H, K–V = 10  $\mu$ m. Scale bar for B applies to C–D; bar for I applies to J; bar for O applies to P–T; bar for U applies to V.



**Fig. 25.** *Phaeoacremonium novae-zealandiae*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–M, P–S. Aerial structures on MEA. D–F. Conidiophores. G–H. Type III phialides. I–J. Type II phialides. K–L. Type I phialides. M. Conidia. N–O. Structures on the surface of and in MEA. N. Adelophialide with conidia. O. Conidia. P. Type III (arrow) and type I (arrow head) phialides. Q. Conidiophore. R–S. Type I phialides. A–S from CBS 110156. D–O: DIC; P–S: SEM. Scale bars: D–Q = 10 µm. Scale bar for D applies to E–O.

*Notes:* A heterothallic species. Formation of perithecia took 3–4 wk. *Phaeoacremonium aleophilum* can be distinguished from other species with brown colonies, viz. *Pm. parasiticum* and *Pm. inflatipes*, by its short and usually unbranched conidiophores. *Phaeoacremonium parasiticum* has prominent warts not observed in the other brown-coloured species. *Phaeoacremonium parasiticum* and *Pm. inflatipes* both produce long or branched conidiophores. The equally brown-coloured *Pm. krajdenii* and *Pm. tardicrescens* do not produce a yellow pigment on OA. *Phaeoacremonium australiense* has more frequently branched conidiophores than *Pm. aleophilum*. *Phaeoacremonium iraniamum* is morphologically very similar to *Pm. aleophilum*, but can be distinguished by the absence of yellow pigmentation on PDA and its inability to grow above 37 °C.

**7. *Togninia novae-zealandiae*** Hausner, Eyjólfsdóttir & J. Reid, *Canad. J. Bot.* 70: 729. 1992. Fig. 24A–V.  
*Anamorph:* *Phaeoacremonium novae-zealandiae* Mostert, W. Gams & Crous, sp. nov.

*Perithecia* single or in clusters, subglobose, sometimes obpyriform, (142–)144–177(–181) µm diam and (147–)158–196 µm tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 4–5 cells (individual cells not visible further outward) and 7–11(–15) µm thick; inner region hyaline at the entrum, paler brown towards the periphery, 2–4 cells and (3–)5–8 µm thick; surface covered with brown, septate hyphal appendages that become hyaline towards the tips (more abundant on older perithecia). *Perithecial necks* black, 1–2 per perithecium, straight to curved, verrucose, (220–)298–1194(–1250) µm long, 29–39 µm diam at the base, and (15–)17–29 µm diam at the apex, necks sometimes dividing into two near the apex; apex often proliferating secondarily upon aging and then appearing nodulose; nodules (up to 147 µm diam) also appearing lower down on the neck. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 40–95 (av. 61) µm long, 3–5 (av. 4) µm wide at the base and 1.5–2 (av. 2) µm at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases (15–)17–23 × 4–5 (av. 20 × 4) µm; apical complex 1 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascogenous hyphae* hyaline, branched, smooth-walled, remnant bases 4–5 × 2.5–4 µm. *Ascospores* aseptate, hyaline, oblong-ellipsoidal with rounded ends, sometimes containing small guttules at the ends, biseriolate, 3–4 × 1–2 (av. 3 × 1) µm.

***Phaeoacremonium novae-zealandiae*** Mostert, W. Gams & Crous, sp. nov. MycoBank MB500230. Fig. 25A–S.

In mycelio aërio hyphae singulae vel ad 8 fasciculatae, tuberculatae, verruculosae, dilute brunneae vel hyalinae. Conidiophora longitudine media, saepe ramosa, vulgo in phialidem singulam exeuntia, (17–)19–55(–60) (in medio 35) µm longa. Phialides terminales vel laterales, praecipue typi I et III; phialides typi I cylindricae, nonnumquam deorsum dilatatae, (2–)3–11(–13) (in medio 7) µm longae; phialides typi II subcylindricae vel naviculares, 9–12 (in medio 10) µm longae; phialides typi III subcylindricae vel naviculares, 13–24 (in medio 18) µm longae. Conidia hyalina, plerumque oblongo-ellipsoidea vel reniformia, (3–)4–5(–5.5) × 1–1.5(–2) (in medio 4.5 × 1.5) µm, long./lat. = 3. In superficie vel submersa in agar, phialides hyalinae, cylindricae, (1–)2–18(–35) (in medio 6) µm; conidia hyalina, oblongo-ellipsoidea, 4–7(–7.5) × 1–1.5(–2) (in medio 5 × 1.5) µm, long./lat. = 3.

Typus herb. CBS 17451.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 8, tuberculate with warts up to 1 µm diam, verruculose, pale brown to hyaline and 1–2 µm wide. *Conidiophores* medium length, often branched, arising from aerial or submerged hyphae, erect, simple, up to 2-septate, often ending in a single terminal phialide, pale brown, paler towards the tip, smooth to verruculose, (17–)19–55(–60) (av. 35) µm long and 1.5–2 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, smooth to verruculose, subhyaline to hyaline; collarettes 1 µm long, 1–1.5 µm wide; type I and type III phialides most common; type I phialides cylindrical, occasionally widened at the base, (2–)3–11(–13) × 1–1.5 (av. 7 × 1) µm; type II phialides either subcylindrical or navicular, tapering towards the apex, 9–12 × 1.5–2 (av. 10 × 2) µm; type III phialides subcylindrical or navicular, 13–24 × (1–)1.5–2(–2.5) (av. 18 × 2) µm, tapering gradually to a long neck. *Conidia* mostly oblong-ellipsoidal or reniform, (3–)4–5(–5.5) × 1–1.5(–2) (av. 4.5 × 1.5) µm, L/W = 3.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, (1–)2–18(–35) × 1–1.5 (av. 6 × 1) µm. *Conidia* hyaline, oblong-ellipsoidal, 4–7(–7.5) × 1–1.5(–2) (av. 5 × 1.5) µm, L/W = 3.

*Types:* **New Zealand**, Auckland, Woodhill State Forest, Compartment 14, from *Cupressus macrocarpa* with inner bark & outer sapwood showing bark beetle activity, May 1982, J. Reid, WIN, dried colony of isolate 113 bi, **holotype** of teleomorph; herb. CBS 17451, **holotype** of anamorph, dried MEA colony; ex-type culture CBS 11015 = UAMH 9589.

*Cultural characteristics:* Colonies reaching a radius of 10 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 25 °C, maximum 30 °C.

Colonies on MEA flat, felty to cottony, with entire edge; after 8 d colonies white (3A1) above, with a few grey (3B1) tufts in the centre, in reverse yellowish grey to yellowish white towards the edge (3B2–3A2); after 16 d colonies olive-grey to white towards the edge (3E2–A1) above, in reverse olive to yellowish white (3F4–E2). Colonies on PDA flat, felty, becoming short woolly, with entire edge; after 8 d yellowish white (3A2) above, in reverse pale yellow (4A2); after 16 d brownish grey to white towards the edge (5D2–4A1) above, in reverse yellowish grey (3B2). Colonies on OA flat, felty to woolly towards the edge, with entire edge; after 8 d yellowish white (3A2) or greyish brown to yellowish white towards the edge (5D3–3A2) above; after 16 d yellowish white (3A2) or olive-brown to yellowish white towards the edge (4D4–2A2) above. Colonies producing yellow pigment on OA.

*Substrate:* *Cupressus macrocarpa*, *Desmoschoenus spiralis*, *Pinus radiata*.

*Distribution:* New Zealand.

*Additional cultures and specimens examined:* **New Zealand**, North Island, Auckland, Woodhill State Forest, Compartment 24, isolated from *Pinus radiata* with inner bark & outer sapwood showing bark beetle activity, May 1982, J. Reid, WIN, dried colony with perithecia 116c<sup>+</sup>; CBS 110157 = UAMH 9590; Coromandel, Whangapoua State Forest, off Road 41, *Pinus radiata*, WIN, dried colonies with perithecia of 89 bi and 105 aiii; Omaha beach, isolated from *Desmoschoenus spiralis*, 1999, J. Rees-George, herb. CBS 17462, dried colony with perithecia of CBS 114512 = C.P.C. 3394.

*Notes:* A homothallic species. Formation of perithecia took 4 wk. *Phaeoacremonium novae-zealandiae* can be distinguished from other grey-coloured species by its white to olive-grey colonies on MEA, yellow pigment production on OA and relatively long conidiophores (av. 35 µm).

**8. *Togninia parasitica* L. Mostert, W. Gams & Crous, sp. nov.** MycoBank MB500692. Fig. 26A–X.

*Anamorph:* *Phaeoacremonium parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf., *Mycologia* 88: 794. 1996.

*Anamorphe Phaeoacremonium parasiticum.* Perithecia plerumque aggregata et subepidermalia, subglobosa, nonnumquam obpyriformia, (180–)200–345(–370) µm diam in parte basilari, (215–)230–380(–410) µm alta. Colla peritheciorum atra, singula vel bina in quoque perithecio, recta vel curvata, verrucosa, 215–810 µm longa. Paraphyses hyalinae, septatae, cylindricae, sursum angustatae et filiformes, 35–150 (av. 85) µm longae. Asci hyalini, clavati, (12–)14–18 × (–3.5) 4–5 (in medio 16 × 4 µm). Ascospores unicellulares, hyalinae, allantoidea utrinque rotundatae, 4–5 × 1–1.5 (in medio 4.5 × 1 µm).

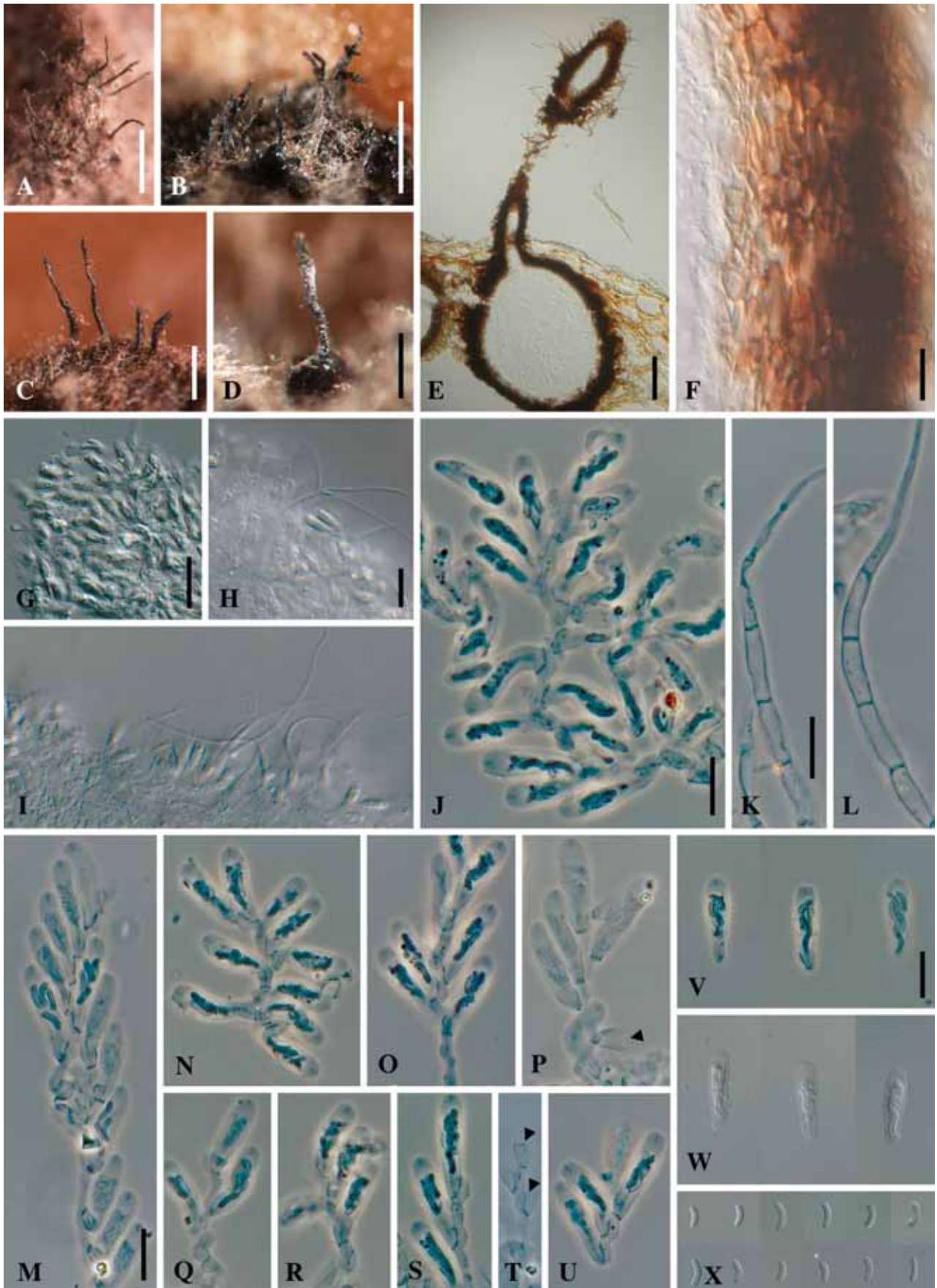
Typus herb. CBS 17463.

*Perithecia* mostly aggregated sometimes solitary, mostly subepidermal but less commonly on the surface of the epidermis, subglobose, sometimes obpyriform, (180–)200–345(–370) µm diam and basal part (215–)230–380(–410) µm tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 7–9 cells (individual cells not visible further outward) and 15–24 µm thick; inner region hyaline at the centrum, pale brown at the periphery, 5–9 cells and (10–)14–20 µm thick; surface covered with brown, septate hyphal appendages that become hyaline towards their tips (more abundant on older perithecia). *Perithecial necks* black, 1–2 per perithecium, straight to curved, verrucose, 215–810 µm long, 29–44 µm wide at the base, and 20–44 µm wide at the apex, necks sometimes dividing into two near the apex; apex often proliferating secondarily upon aging and then appearing nodulose; nodules (–59 µm diam) also appearing lower down on the neck. *Paraphyses* hyaline, septate, cylindrical, narrowing and thread-like towards the tip, 35–150 (av. 85) µm long, 2–4.5 (av. 3) µm wide at the base and 1–3 (av. 1) µm at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases, (12–)14–18 × (–3.5) 4–5 (av. 16 × 4 µm); apical region 2–3 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascogenous hyphae* hyaline, branched, smooth-walled; remnant bases 5–6 × 2–2.5 µm. *Ascospores* aseptate, hyaline, allantoid with rounded ends, sometimes containing small guttules at the ends, biseriate, 4–5 × 1–1.5 (av. 4.5 × 1 µm).

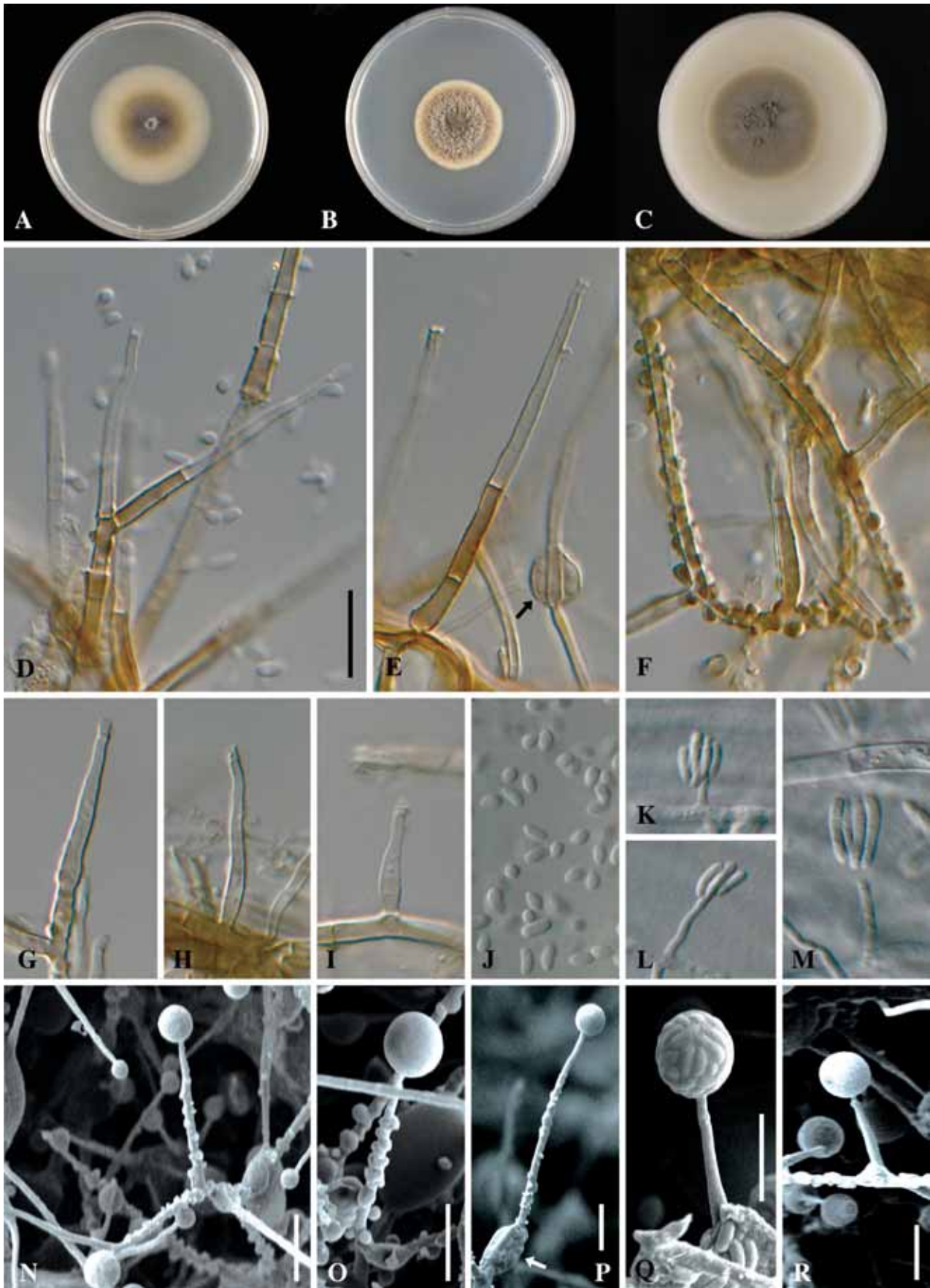
***Phaeoacremonium parasiticum*** (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf., *Mycologia* 88: 794. 1996. Fig. 27A–R.

≡ *Phialophora parasitica* Ajello, Georg & C.J.K. Wang, *Mycologia* 66: 493. 1974.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 20; hyphae densely tuberculate with warts up to 3 µm diam, verrucose, darker to medium brown and 1.5–3.5 µm wide. *Conidiophores* mostly long and branched, medium brown, becoming paler towards the tip, 1–7-septate, unbranched conidiophores sometimes slightly swollen at the base, often bearing a single phialide as the apical cell, (24–)27–80(–130) (av. 47) µm long and 1.5–2.5 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, frequently with percurrent rejuvenation, finely tuberculate to verrucose, smoother towards the apex, pale brown to hyaline; collarettes 0.5–2 µm long and 1–2 µm wide; type I and II phialides rare; type I phialides cylindrical, occasionally widened at the base, tapering towards the apex,

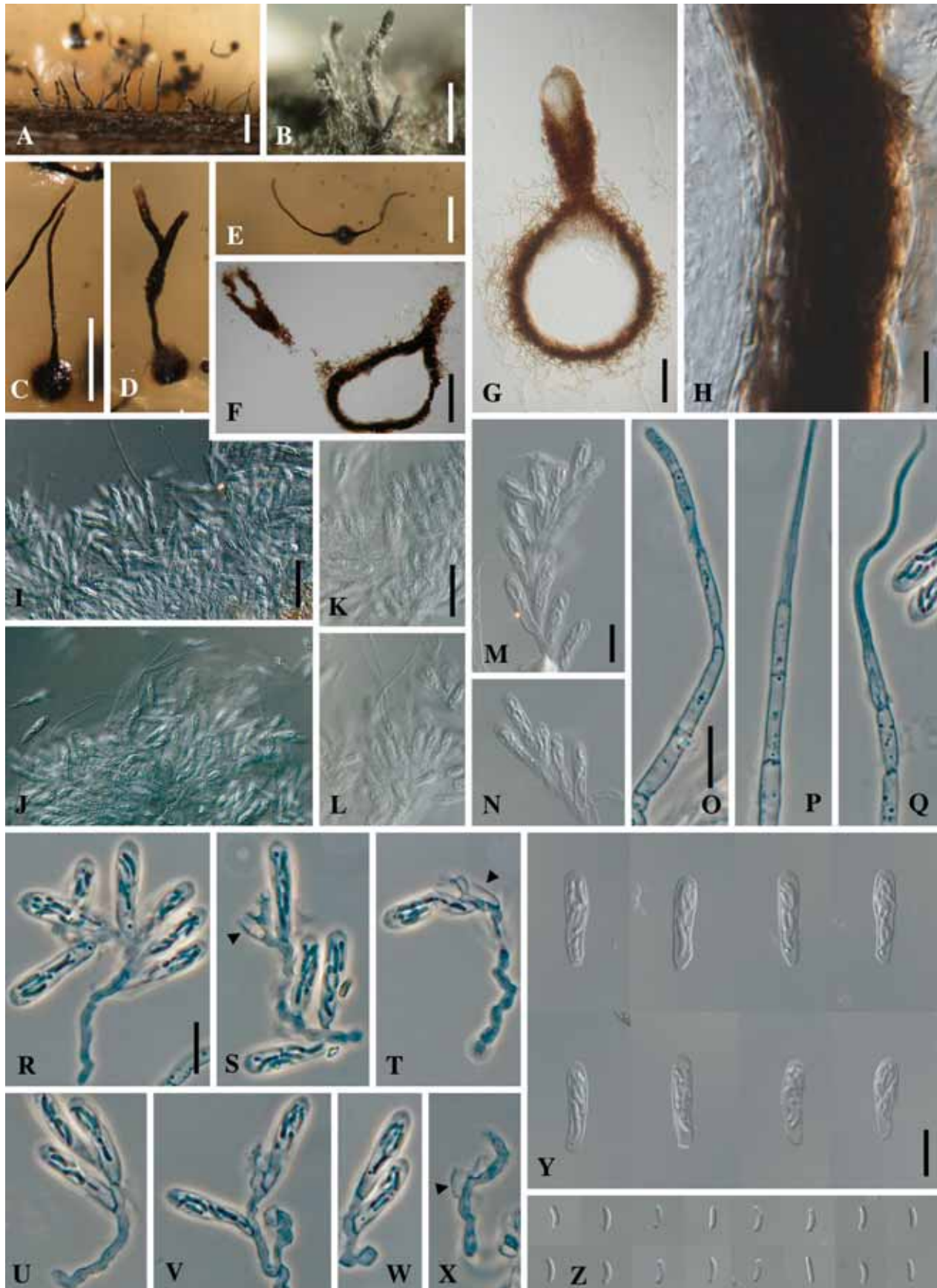


**Fig. 26.** *Togninia parasitica*. A–D. Perithecia on canes of *Vitis vinifera*. E–F. Longitudinal section through perithecia; peridium (F). G. Asci. H–I. Asci intermingled with paraphyses. J. Asci attached to ascogenous hyphae. K–L. Paraphyses, becoming thread-like towards the tips. M–S, U. Ascogenous hyphae with asci attached; remnant bases indicated by arrow heads (P, T). T. Ascogenous hypha with terminal cells. V–W. Asci. X. Ascospores. A–X from CBS 17463 (holotype). A–D: DM; E–I, W–X: DIC; J–V: PC. Scale bars: A–B = 500  $\mu$ m; C–D = 200  $\mu$ m; E = 100  $\mu$ m; G–I = 20  $\mu$ m; F, J–X = 10  $\mu$ m. Scale bar for H applies to I; bar for M applies to N–U; bar for V applies to W–X.



**Fig. 27.** *Phaeoacremonium parasiticum*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–J, N–R. Aerial structures on MEA. D–E. Conidiophores; branched conidiophore (D); mucus structures containing conidia commonly found with this species (indicated with arrow; E and P). F. Mycelium showing prominent exudate droplets observed as warts. G–H. Type III phialides. I. Type II phialide. J. Conidia. K–M. Structures on the surface of and in MEA. Adelophialides with conidia. N–O. Type III phialides. P. Conidiophore. Q–R. Type II phialides. A–R from CBS 860.73. D–M: DIC; N–R: SEM. Scale bars: D–R = 10  $\mu$ m. Scale bar for D applies to E–M.





**Fig. 28.** *Togninia rubrigena*. A–B. Perithecia on canes of *Vitis vinifera*. C–E. Perithecia on adjacent water agar. F–H. Longitudinal sections through perithecia; perithecium with two necks (F); peridium (H). I–L. Asci intermingled with paraphyses. M–N. Asci attached to ascogenous hyphae. O–Q. Paraphyses, becoming thread-like towards the tips. R–W. Ascogenous hyphae with asci attached; remnant bases indicated by arrow heads (S, T, X). X. Ascogenous hypha with remnant bases. Y. Asci. Z. Ascospores. A–Z from CBS 17465 (holotype). A–E: DM; F–N: DIC; O–X: PC. Scale bars: A–E = 500  $\mu$ m; F = 200  $\mu$ m; G = 100  $\mu$ m; I–L = 20  $\mu$ m; H, M–Z = 10  $\mu$ m. Scale bar for C applies to D; bar for I applies to J; bar for K applies to L; bar for M applies to N; bar for O applies to P–Q; bar for R applies to S–X; bar for Y applies to Z.

(2–)4–17 × 1–2 (av. 11 × 1) µm; type II phialides subcylindrical, tapering towards the apex, 14–15 × 1.5–2 (av. 14.5 × 2) µm; type III phialides predominant, mostly cylindrical to subulate, 19–29(–37) × 1.5–2(–2.5) (av. 23 × 2) µm, tapering very gradually and terminating in a narrow neck. *Conidia* mostly oblong-ellipsoidal or obovoid, sometimes allantoid to broadly oblong, 3–4(–4.5) × (1–)1.5–2 (av. 3.5 × 1.5) µm, L/W = 2. *Conidia* remain aggregated in masses when mounted in lactic acid because of copious, tenacious mucus produced.

*On surface or submerged in the agar: Phialides* hyaline, cylindrical, 2–12(–24) × 1 (av. 6 × 1) µm. *Conidia* hyaline, allantoid some oblong-ellipsoidal, (4–)5–7(–9) × 1–1.5 (av. 6 × 1.5) µm, L/W = 4.

*Types: U.S.A.*, California, Stanford University Hospital, isolated from human subcutaneous phaeohyphomycosis, 1971, R.T. Steigbigel, dried specimen at BPI, **holotype** of anamorph, ex-type culture CBS 860.73 = IMI 181115. **South Africa**, tester strains from stems of *Vitis vinifera*, 2000, CBS 113594 and L.M. 461, F. Halleen, 2002, were crossed; single-ascospore subcultures from fertile perithecia were crossed again: CBS 118241 × CBS 118240 on *V. vinifera* canes: Herb. CBS 17463, **holotype** of teleomorph.

*Cultural characteristics:* Colonies reaching a radius of 10.5–11.5 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 30 °C, maximum 40 °C. Colonies on MEA flat, felty with woolly tufts in the centre, with entire edge; after 8 d greyish yellow to brownish grey towards the edge (4B2–D2) above, in reverse greyish beige (4C2); after 16 d olive-brown to greyish beige towards the edge (4D4–4C2) above and in reverse. Colonies on PDA flat, short woolly, with entire edge; after 8 d dark blond to orange-grey towards the edge (5D4–B2) above and in reverse; after 16 d brown (5E4) above, in reverse dark blond (5D4). Colonies on OA flat, felty to powdery, with entire edge; after 8 d brownish grey (4D2) above, after 16 d olive-brown (4D4).

*Substrate:* *Actinidia*, Human, *Phoenix dactylifera*, *Prunus armeniaca*, *Vitis vinifera*.

*Distribution:* Australia, Brazil, Canada, Finland, Iraq, Italy, South Africa, Tunisia, U.S.A.

*Additional cultures examined:* **Australia**, South Australia, Markaranka, *Vitis vinifera*, 2000, I. Pascoe, CBS 113591. **Brazil**, subcutaneous infection in human male, 1999, S.H. Alves, CBS 110033 = FMR 7681. **Canada**, Ontario, Toronto, human, left lower lobe of lung, 10 Aug. 1987, Sunnybrook Medical Centre, CBS 113596 = BB959/NOMH568. **Finland**, human toenail, Univ. of Helsinki, Dept. of Bacteriology and Immunology, 1995, CBS 736.94 = M 547. **Iraq**, *Phoenix dactylifera*, 1975, H.Y. Al-Ani, CBS 184.75. **Italy**, Emilia Romagna, *Actinidia*, 1998, F. Calzarano and S. di Marco,

CBS 101007. **South Africa**, Western Cape, trunk of *Vitis vinifera*, 2001, L. Mostert, CBS 113585; Western Cape, Porterville, De Tuine, trunk of *Vitis vinifera*, 2001, L. Mostert, CBS 113586; Western Cape, from graft union of *Vitis vinifera*, 2000, F. Halleen, CBS 113594. **Tunisia**, root of *Prunus armeniaca*, 1973, B. Jamoussi, CBS 984.73 = CMW 2030 = IMI 192879 = C.P.C. 773. **U.S.A.**, Alabama, Birmingham, human, 2001, S. Moser, CBS 109666 and CBS 109665.

*Notes:* A heterothallic species. Formation of perithecia took 8–12 wk. *Phaeoacremonium parasiticum* is very distinctive, easily recognised by the predominance of long, branched conidiophores, long type II and type III phialides, dark brown hyphae and large hyphal warts of up to 3 µm diam. In different studies, discrepant optimal growth temperatures have been obtained for this species, ranging from 25 °C (Crous *et al.* 1996) to 30 °C (Dupont *et al.* 2002). Both Crous *et al.* (1996) and Du Pont *et al.* (2002) examined the ex-type culture CBS 860.73 and another strain CBS 984.73. These strains, as well as five other strains tested in this study, had an optimum of 30 °C.

**9. *Togninia rubrigena* L. Mostert, W. Gams & Crous, sp. nov.** MycoBank MB500693. Fig. 28A–Z.

*Anamorph:* *Phaeoacremonium rubrigenum* W. Gams, Crous & M.J. Wingf., Mycologia 88: 795. 1996.

*Anamorphe Phaeoacremonium rubrigenum.* Perithecia aggregata vel solitaria, plerumque subepidermalia, saepe etiam in agar formata, subglobosa, nonnulla obpyriformia, (170–)200–460(–470) µm diam; collis atris, plerumque singulis in quoque perithecio, rectis vel curvatis, verrucosis, 515–1300 µm longis. Paraphyses hyalinae, septatae, cylindricae, sursum angustate, filiformes, 46–135 (in medio 83) µm longae. Asci clavati, (12–)16–19 × 4–4.5 (in medio 18 × 4) µm. Ascospores unicellulares, hyalinae, allantoidae vel cylindricae, utrinque rotundatae, 4–6 × 1–1.5 (in medio 5 × 1) µm.

Typus herb. CBS 17465.

*Perithecia* mostly aggregated often solitary, mostly subepidermal but less commonly on the surface of the epidermis, perithecia also formed often on and in the agar, subglobose, sometimes obpyriform, (170–)200–460(–470) µm diam and (225–)235–355(–360) µm tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 5–9 cells (individual cells not visible further outward) and (5–)10–25 µm thick; inner region hyaline at the centrum, pale brown towards the periphery, 5–9 cells and 8–16 µm thick; surface covered with brown, septate hyphal appendages that become hyaline towards their tips (more abundant on older perithecia). *Perithecial necks* black, 1(–2) per perithecium, straight to curved, verrucose, 515–1300 µm long, 40–90 µm wide at the base, and 10–45 µm at the apex, necks sometimes bifurcating near

the apex; necks often proliferating secondarily upon aging and then appearing nodulose; nodules (–123 µm diam) mostly also appearing lower down on the neck. *Paraphyses* hyaline, septate, cylindrical, narrowing and becoming thread-like towards the tip, 46–135 (av. 83) µm long, 2–4 (av. 3) µm wide at the base and 1.5–2.5 (av. 2) µm at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases (12–)16–19 × 4–4.5 (av. 18 × 4) µm; apical region 1–2 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascogenous hyphae* hyaline, branched, smooth-walled, remnant bases 5–6 × 2–2.5 µm. *Ascospores* aseptate, hyaline, mostly allantoid or cylindrical with rounded ends, sometimes containing small guttules at the ends, biseriolate, 4–6 × 1–1.5 (av. 5 × 1) µm.

*Phaeocremonium rubrigenum* W. Gams, Crous & M.J. Wingf., Mycologia 88: 795. 1996. Fig. 29A–R.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 5; hyphae tuberculate with warts up to 1 µm diam, verruculose, orange to pale brown and 1.5–3 µm wide. *Conidiophores* mostly short and usually unbranched, 0–4-septate, often bearing 2 lateral phialides next to the terminal phialide, (20–)23–51(–70) (av. 34) µm long and 1.5–3 (av. 2) µm wide. Percurrent rejuvenation occurring occasionally, each newly proliferated segment swollen at the base. *Phialides* terminal or lateral, mostly monophialidic, verruculose, sparsely tuberculate, rarely smooth, pale brown to hyaline; collarettes 1–3 µm long and 1–2 µm wide; type I phialides cylindrical, (2–)4–8(–14) × 1–1.5(–2) (av. 6 × 1.5) µm, occasionally widened at the base; type II phialides elongate-ampulliform and attenuated at the base, or navicular, (9–)10–15(–16) × 1.5–2(–2.5) (av. 13 × 2) µm; type III phialides predominant, subcylindrical, becoming slightly inflated at or just above the base, (15–)16–24(–28) × 1.5–2(–2.5) (av. 19 × 2) µm narrowing gradually to a long neck. *Conidia* oblong-ellipsoidal, obovoid, or occasionally reniform to allantoid, and prominently biguttulate in one of the two strains examined, 3–5 × 1–2(–2.5) (av. 4 × 1.5) µm, L/W = 2.5.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, (1.5–)2–13(–14) × 1(–2) (av. 6 × 1) µm. *Conidia* hyaline, allantoid, some oblong-ellipsoidal, 4–6(–7) × 1(–1.5) (av. 5 × 1) µm, L/W = 5.

*Types:* U.S.A., Bethesda, National Institute of Health, human patient with pneumonia, 1994, K.J. Kwon-Chung, CBS 498.94 dried colony in herb CBS and ex-type culture, **holotype** of anamorph, dried **isotype** lodged at PREM. CBS 498.94 was compatible with CBS 112046. U.S.A., Illinois, human, infected eye,

vitreous fluid, 2002, C. Conover, CBS 112046; single-ascospore isolates from fertile perithecia were crossed again: CBS 118236 × CBS 118237 on *V. vinifera* canes: Herb. CBS 17465, **holotype** of teleomorph.

*Cultural characteristics:* Colonies reaching a radius of 9.5–10 mm in 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 30 °C, maximum 37 °C. Colonies on MEA flat, felty, with entire edge; after 8 d greyish Magenta above near the centre, purplish white (14D5–14A2) towards the periphery, in reverse greyish Magenta to reddish lilac (14D5–B3); after 16 d greyish ruby to greyish rose towards the edge (12D3–B3) above, in reverse dark ruby to greyish rose towards the edge (12F7–D5). Colonies on PDA flat, short woolly, with entire edge; after 8 d brownish orange (6C3) or dull red (10C3) above, in reverse pale brown to orange-grey (6D4–B2) or greyish brown (10E3); after 16 d greyish brown to reddish brown (9D3–E4) above, in reverse dark brown (9F6) or violet-brown (10F7). Colonies on OA flat, felty to powdery, with entire edge; after 8 d dull red to reddish grey (9C3–9B2) above, after 16 d dark Magenta to purplish grey (13F7–C2) towards the edge.

*Substrate:* Human.

*Distribution:* U.S.A.

*Additional culture examined:* U.S.A., Illinois, human, infected eye, vitreous fluid, 2002, C. Conover, CBS 112046 = UTHSC 00-2395.

*Notes:* A heterothallic species. Formation of perithecia took 4–6 wk. *Phaeocremonium rubrigenum* can be identified by its pink to purplish colony colour on MEA. *Phaeocremonium rubrigenum* and *Pm. griseorubrum*, have longer conidiophores (av. > 30 µm) than the other pink–red-coloured species. *Phaeocremonium rubrigenum* grows faster than *Pm. griseorubrum* with a colony radius of 9.5–10 mm after 8 d in the dark, while *Pm. griseorubrum* only reaches 5–8 mm.

**10. *Togninia viticola*** L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500694. Fig. 30A–Z.

*Anamorph:* *Phaeocremonium viticola* J. Dupont, Mycologia 92: 502. 2000.

*Anamorphe Phaeocremonium viticola.* Peritheciaplurumque aggregata et subepidermalia, globosa vel subglobosa, 225–362(–377) µm diam, collis atris, singulis (ad ternis) in quoque perithecio, rectis vel curvatis, verrucosis, 350–1000 µm longis. Paraphyses hyalinae, septatae, cylindricae, sursum angustatae, 55–115 (in medio 82) µm longae. Asci clavati, (17–)18–24(–26) × (3–)3.5–4(–5) (in medio 21 × 4) µm. Ascosporae unicellulares, hyalinae, oblongo-ellipsoideae vel reniformes, utrinque rotundatae, 3–5 × 1.5–2(–2.5) (in medio 4 × 2) µm.

Typus herb. CBS 17467.

*Perithecia* mostly aggregated sometimes solitary, mostly subepidermal but less commonly on the surface of the epidermis, globose to subglobose, 225–362(–377)  $\mu\text{m}$  diam and (211–)222–324(–328)  $\mu\text{m}$  tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, with cells smaller and more rounded than those of the inner layer, approx. 5–8 cells (individual cells not visible further outward) and 10–25  $\mu\text{m}$  thick; inner region hyaline at the centrum, pale brown towards the periphery, 5–10 cells and 10–20  $\mu\text{m}$  thick; surface covered with brown, septate hyphal appendages that become hyaline towards the tips (more abundant on older perithecia). *Perithecial necks* black, 1(–3) per perithecium, straight to curved, verrucose, 360–1030  $\mu\text{m}$  long, 20–60  $\mu\text{m}$  wide at the base, and 12–32  $\mu\text{m}$  wide at the apex, necks sometimes dividing into two near the apex; necks sometimes proliferating secondarily upon aging with nodules –40  $\mu\text{m}$  diam. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 55–115 (av. 82)  $\mu\text{m}$  long, 1.5–4 (av. 2)  $\mu\text{m}$  wide at the base and 2–4 (av. 2.5)  $\mu\text{m}$  at the apex, persistent. *Asci* appearing spicate when mature, hyaline, clavate, with bluntly rounded apices and with sides parallel or tapering towards the truncate or bluntly obtuse bases, (17–)18–24(–26)  $\times$  (3–)3.5–4(–5) (av. 21  $\times$  4)  $\mu\text{m}$ ; apical region 1–1.5  $\mu\text{m}$  thick, of indistinct structure, with a non-amyloid apical ring. *Ascogenous hyphae* hyaline, branched, smooth-walled, remnant bases 4–5  $\times$  2.5–3  $\mu\text{m}$ . *Ascospores* aseptate, hyaline, oblong-ellipsoidal to reniform with rounded ends, sometimes containing small guttules at the ends, biserial or in a single row, 3–5  $\times$  1.5–2(–2.5) (av. 4  $\times$  2)  $\mu\text{m}$ .

***Phaeoacremonium viticola*** J. Dupont, Mycologia 92: 502. 2000. Fig. 31A–T.

*Aerial structures*: Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 14, tuberculate, with warts up to 2  $\mu\text{m}$  diam, verruculose, medium to pale brown and 1.5–2  $\mu\text{m}$  wide. *Conidiophores* mostly short and usually unbranched, arising from aerial or submerged hyphae, erect, simple, up to 3-septate, often bearing a single phialide as the apical cell, pale brown, paler towards the tip, smooth to verruculose, (15–)18–49(–80) (av. 31)  $\mu\text{m}$  long and 1.5–2(–2.5) (av. 2)  $\mu\text{m}$  wide. *Phialides* terminal or lateral, mostly monophialidic, smooth to verruculose, subhyaline; collarettes 1.5–2.5  $\mu\text{m}$  long, 1–1.5  $\mu\text{m}$  wide; type II and type III phialides most common; type I phialides cylindrical, occasionally widened at the base, 3–9  $\times$  1–1.5(–2) (av. 6  $\times$  1)  $\mu\text{m}$ ; type II phialides either elongate-ampulliform and attenuated at the base or subcylindrical, tapering towards the apex, (8–)9–12(–14)  $\times$  1.5–2 (av. 10  $\times$  1.5)  $\mu\text{m}$ ; type III phialides subcylindrical, some elongate-ampulliform and attenuated at the base, 12–17(–18)  $\times$  1.5–2 (av. 14  $\times$  2)  $\mu\text{m}$ , tapering gradually to a long neck. *Conidia* reniform or obovoid, 2–4  $\times$  1(–1.5) (av. 3  $\times$  1)  $\mu\text{m}$ , L/W = 3.

*On surface or submerged in the agar*: *Phialides* hyaline, cylindrical, (1.5–)2–6(–8)  $\times$  1–1.5(–2) (av. 3  $\times$  1)  $\mu\text{m}$ . *Conidia* hyaline, oblong-ellipsoidal or allantoid, (3–)4–4.5(–5)  $\times$  1–1.5(–2) (av. 4  $\times$  1)  $\mu\text{m}$ , L/W = 4.

*Types*: **France**, Alsace, from stems of *Vitis vinifera*, Aug. 1993, P. Larignon, herb. LCP 933886 **holotype** of anamorph, dried specimen, ex-type culture CBS 101738 = LCP 96.3886; also CBS 118235, P. Larignon, 1999. **Germany**, *Sorbus intermedia*, 1995, K. Weise, CBS 428.95. A crossing of CBS 118235 with CBS 428.95 was fertile *in vitro*: Herb. CBS 17467, **holotype** of teleomorph.

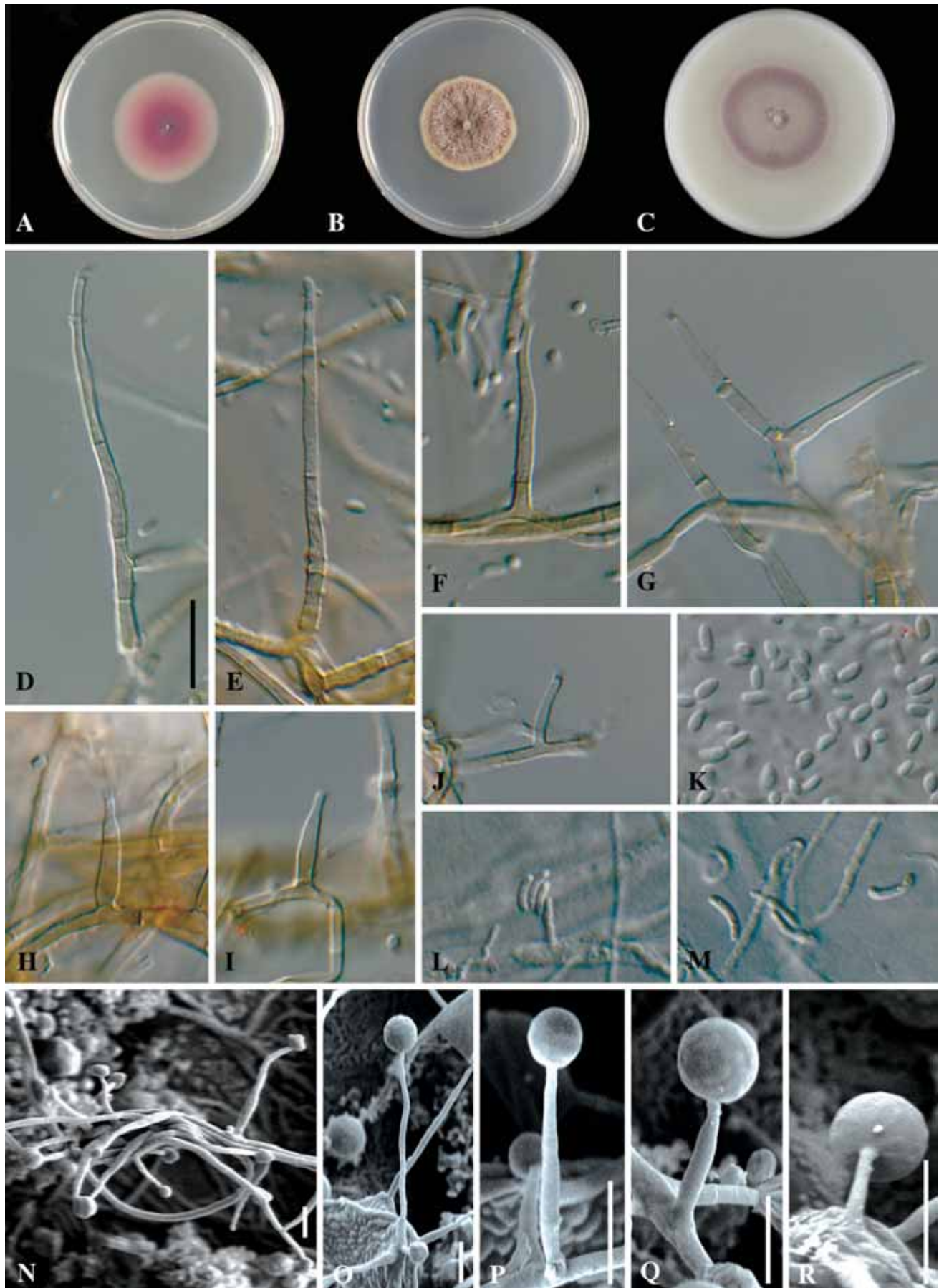
*Cultural characteristics*: Colonies reaching a radius of 6–12 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 25–30 °C, maximum 30–35 °C. Colonies on MEA flat, woolly or felty, with entire edge; after 8 d greyish red (10D5) or grey to white (3B1–A1) above, in reverse violet-brown (10E5) or yellowish white (3A2); after 16 d brownish grey (7C2) with greyish red (10D4) undertone above, in reverse violet brown (11F7). Colonies on PDA flat, woolly or felty, with entire edge; after 8 d greyish brown (8D3) above, reverse reddish brown (8E4); after 16 d grey (3D1) with reddish brown (9E4) undertones above, in reverse violet-brown (10F8). Colonies on OA flat, woolly or felty, with entire edge; after 8 d reddish grey (10B2) or yellowish white (3A2) above, after 16 d dull red (10C3) with brownish grey (10C2) woolly tufts or yellowish white (3A2) with a felty texture. Only two of the strains produced yellow pigment on OA and PDA, namely CBS 101737 and CBS 101739.

*Substrate*: *Vitis vinifera*, *Sorbus intermedia*.

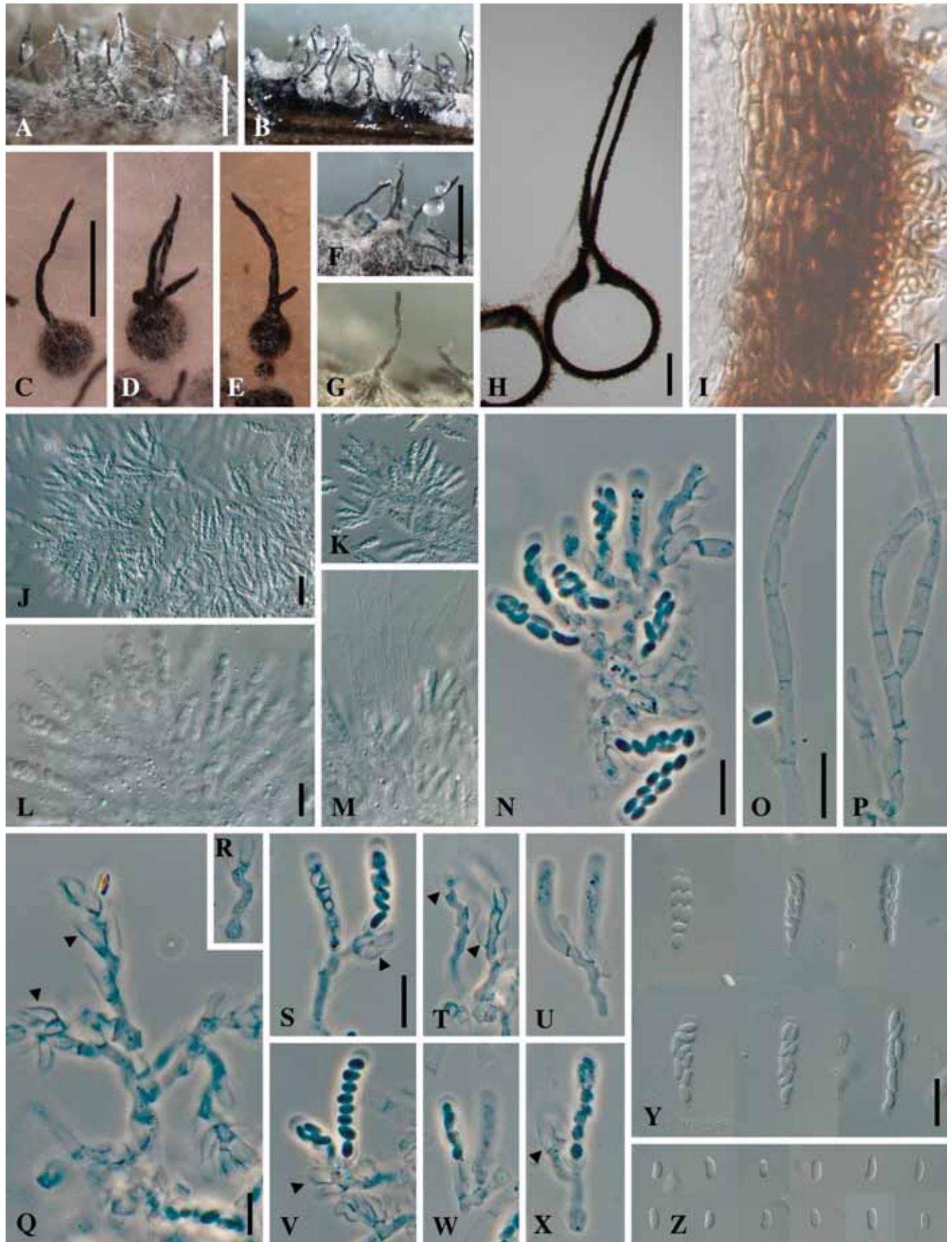
*Distribution*: France, Germany, South Africa.

*Additional cultures examined*: **France**, *Vitis vinifera*, 1996, P. Larignon, CBS 101737 = LCP 97.4014; *V. vinifera*, 1997, P. Larignon, CBS 101739 = LCP 97.4004; **Germany**, *Sorbus intermedia*, 1995, K. Weise, CBS 428.95. **South Africa**, branches of *V. vinifera*, 2001, L. Mostert, CBS 113065.

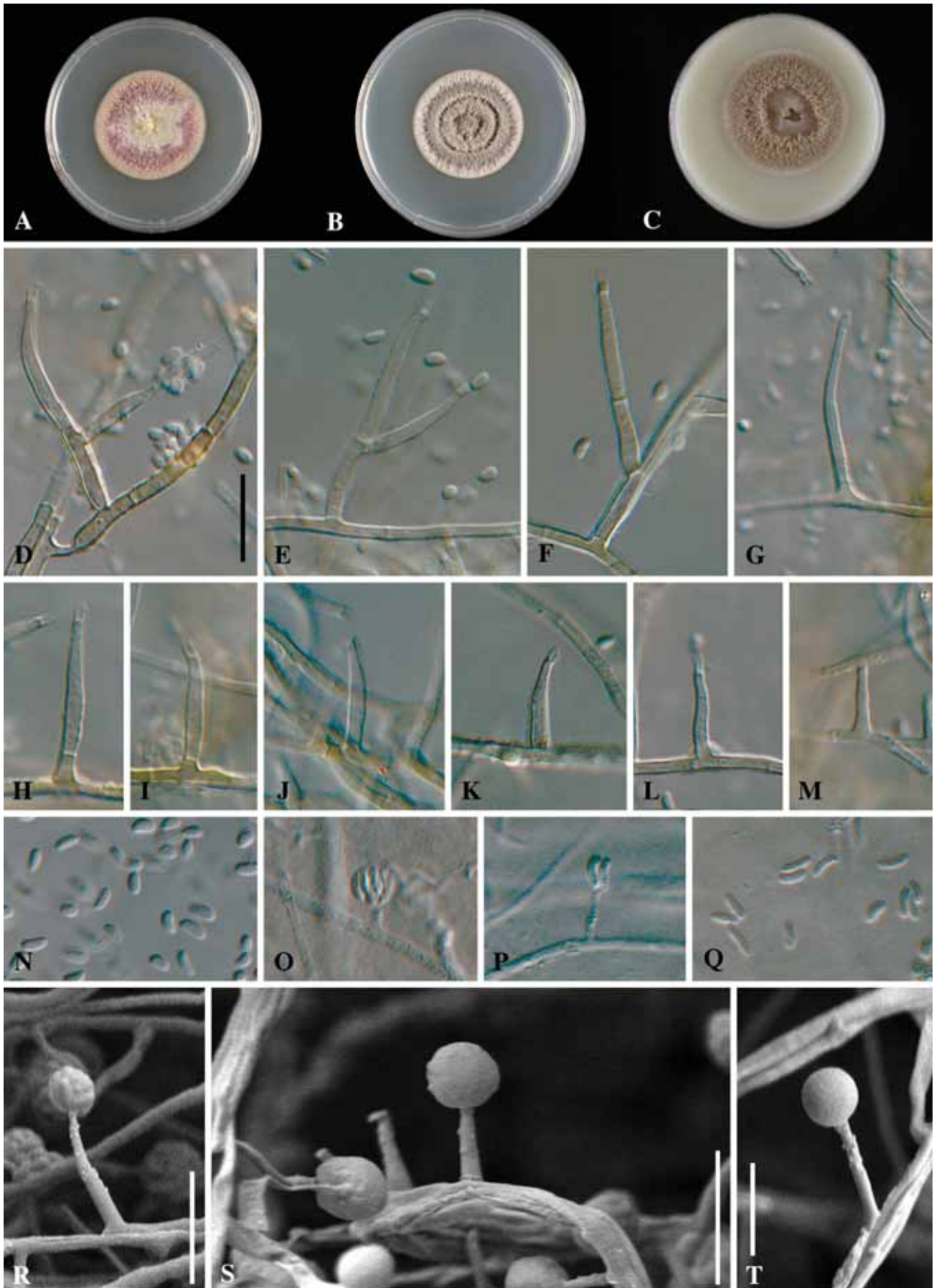
*Notes*: A heterothallic species. Formation of perithecia took 12 wk. *Phaeoacremonium viticola* can be distinguished from most of the other pink or red coloured species by its maximum growth temperature of up to 35 °C. Among members of this group of species, only *Pm. angustius* has a maximum growth temperature as low as 30 °C; that of all other species is 37 or 40 °C (as determined in our system of 3–5 °C increments). Another distinguishing feature of *Pm. viticola* is its long collarettes (up to 2.5  $\mu\text{m}$ ). Cultural variation occurs among the strains of *Pm. viticola*: CBS 101738 produces abundant woolly aerial mycelium, while the other strains have felty-textured colonies. CBS 101738 produces reniform to obovoid conidia rather than the oblong-ellipsoidal or allantoid conidia seen in the other strains.



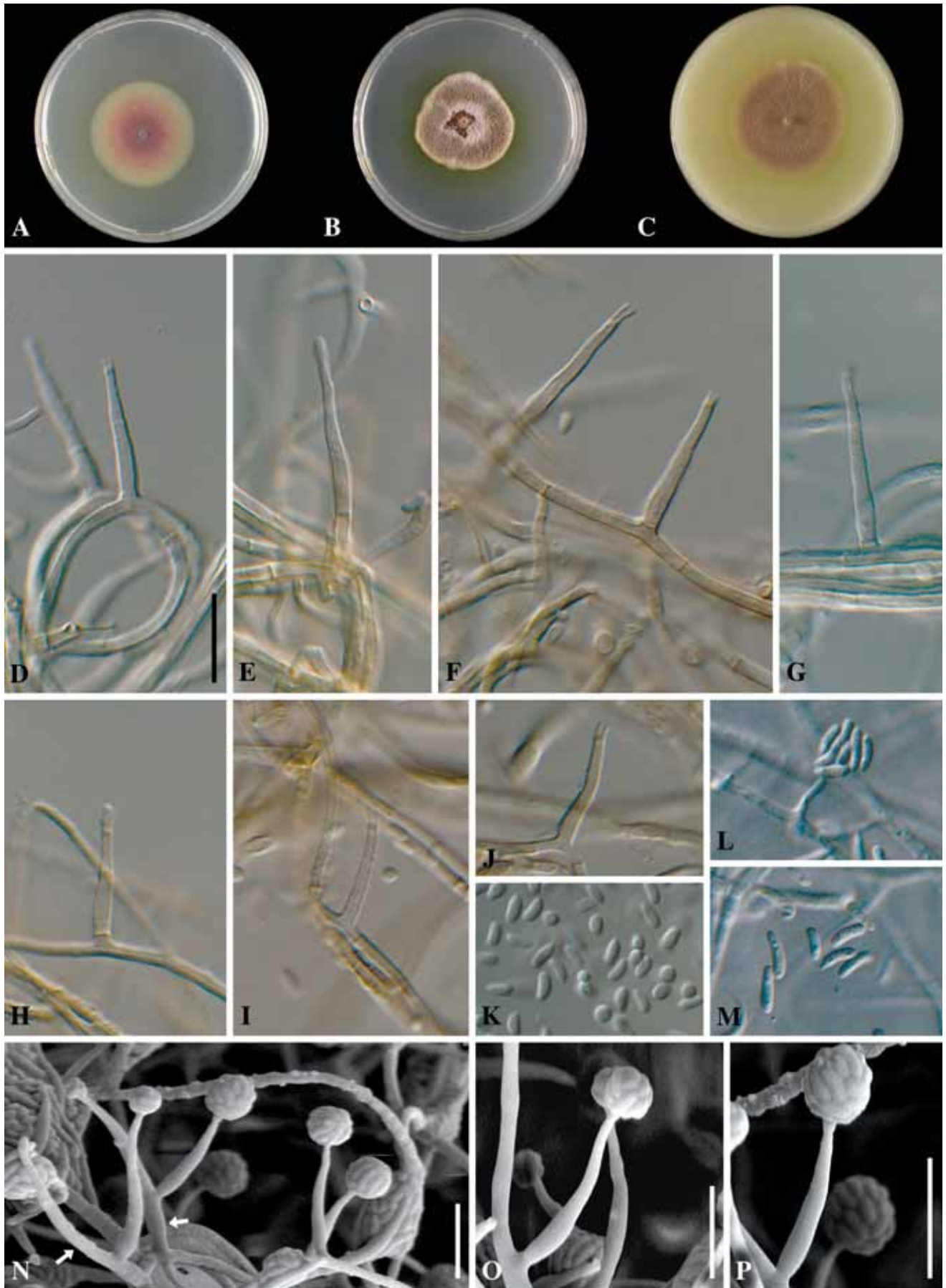
**Fig. 29.** *Phaeoacremonium rubrigenum*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–K, N–R. Aerial structures on MEA. D–G. Conidiophores with Type III phialides; branched conidiophore (G). H–I. Type II phialides. J. Type I phialide. K. Conidia. L–M. Structures on the surface of and in MEA. L. Adelophialide with conidia. M. Conidia. N. Mycelium with conidiophores and phialides. O. Conidiophore. P. Type III phialide. Q. Type II phialide. R. Type I phialide. A–R from CBS 498.94. D–M: DIC; N–R: SEM. Scale bars: D–R = 10  $\mu$ m. Scale bar for D applies to E–M.



**Fig. 30.** *Togninia viticola*. A–B, F–G. Perithecia on canes of *Vitis vinifera*. C–E. Perithecia on adjacent water agar. H–I. Longitudinal sections through perithecia; peridium (I). J–L, N. Asci attached to ascogenous hyphae. M. Asci with paraphyses. O–P. Paraphyses. Q–R, T. Ascogenous hyphae with remnant bases (indicated by arrow heads, Q, S, T, V, X). S, U, V–X. Ascogenous hyphae with asci. Y. Asci. Z. Ascospores. A–Z from CBS 17467 (holotype). A–E: DM; F–N: DIC; O–X: PC. Scale bars: A–G = 500 µm; H = 100 µm; J–K = 20 µm; L–Z = 10 µm. Scale bar for A applies to B; bar for C applies to D–E; bar for F applies to G; bar for K applies to M; bar for O applies to P; bar for S applies to T–X; bar for Y applies to Z.

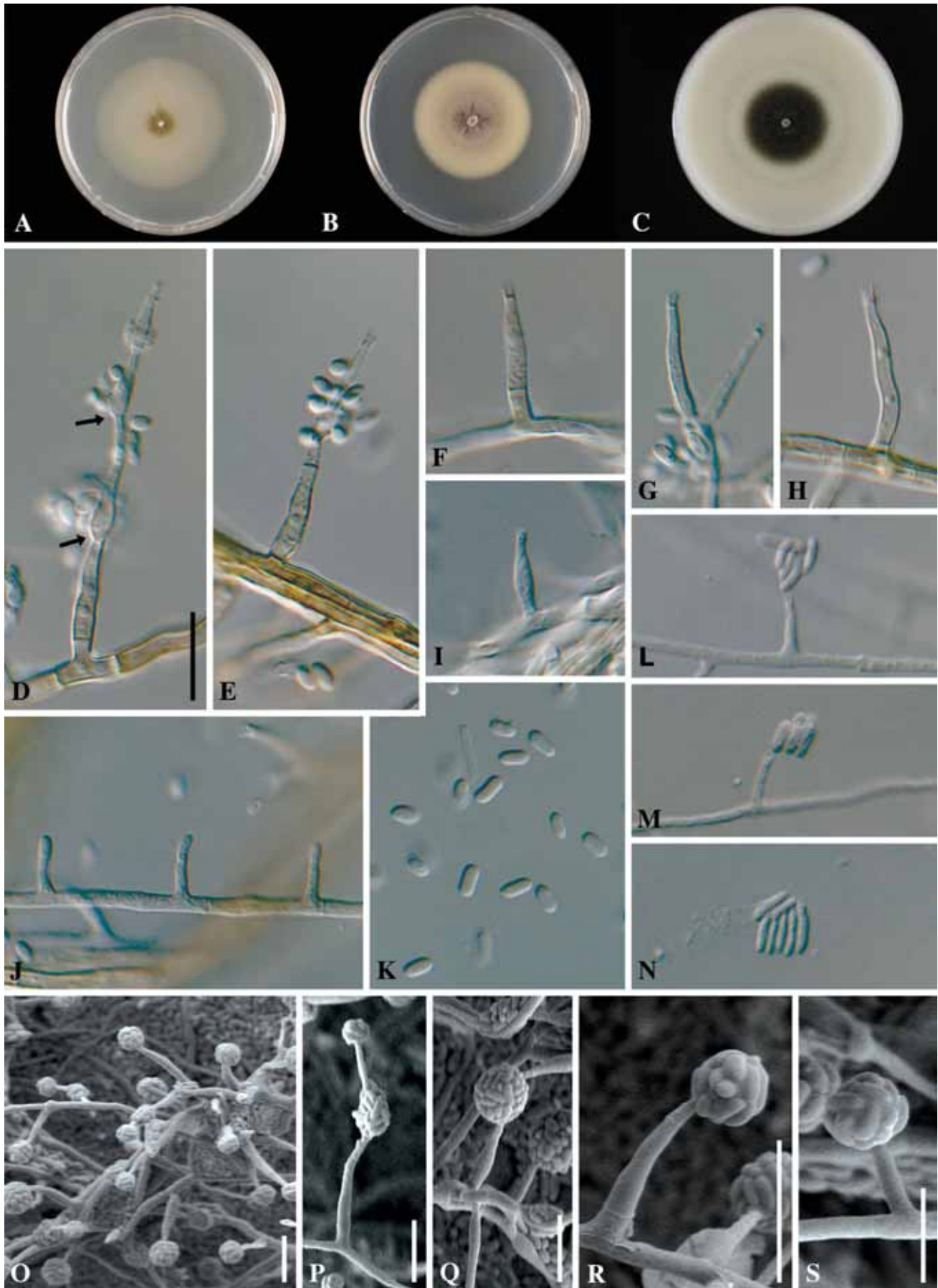


**Fig. 31.** *Phaeoacremonium viticola*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–N, R–T. Aerial structures on MEA. D–F. Conidiophores; branched conidiophore (D); conidiophore with terminal and 1 adjacent lateral phialide (E). G–H. Type III phialides. I–K. Type II phialides. L–M. Type I phialides. N. Conidia. O–Q. Structures on the surface of and in MEA. O–P. Adelophialides with conidia. Q. Conidia. R. Type II phialide. S–T. Type I phialides. A–T from CBS 101738. D–Q: DIC; R–T: SEM. Scale bars: D–S = 10  $\mu$ m; T = 5  $\mu$ m. Scale bar for D applies to E–Q.



**Fig. 32.** *Phaeoacremonium alvesii*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–K, N–P. Aerial structures on MEA. D–E. Conidiophores. F–G. Type III phialides. H. Type II phialide. I–J. Type I phialide. K. Conidia. L–M. Structures on the surface of and in MEA. L. Adelophialide with conidia. M. Conidia. N. Conidiophores (indicated by arrows). O. Type III phialide. P. Type II phialide. A–P from CBS 110034. D–M: DIC; N–P: SEM. Scale bars: D–P = 10  $\mu$ m. Scale bar for D applies to E–M.





**Fig. 33.** *Phaeoacremonium amstelodamense*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–K, O–S. Aerial structures on MEA. D–E. Conidiophores showing percurrent rejuvenation (arrows in D). G–H. Type III phialides. F, I. Type II phialides. J. Type I phialides. K. Conidia. L–N. Structures on the surface of and in MEA. L–M. Adelophialides with conidia. N. Conidia. O. Conidiophores and phialides. P. Conidiophores. Q–R. Type II phialide. S. Type I phialide. A–S from CBS 110627. D–M: DIC; N–R: SEM. Scale bars: D–R = 10  $\mu$ m. Scale bar for D applies to E–N.

**11. *Phaeoacremonium alvesii*** L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43:1758. 2005. Fig. 32A–P.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 8; hyphae tuberculate with warts up to 0.5 µm diam, verruculose, medium to pale brown and 1–2.5 µm wide. *Conidiophores* mostly short and unbranched, occasionally narrower at the base, up to 2-septate, often ending in a single terminal phialide, pale brown, paler towards the tip, (14–)17–43(–50) (av. 27) µm long and 1.5–2 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, sparsely tuberculate, verruculose to smooth, pale brown to hyaline; collarettes, 2–2.5 µm long, 1–1.5 µm wide, type I phialides cylindrical, occasionally widened at the base, (3–)4–12 × 1–1.5 (av. 7 × 1) µm; type II phialides subcylindrical to navicular, rarely swollen at the base, tapering towards the apex, 10–14 × 1.5–2 (av. 13 × 2) µm; type III phialides predominant, navicular to subcylindrical, (13–)14–22 × 1.5–2(–2.5) (av. 17 × 2) µm, tapering gradually to a long neck. *Conidia* mostly obovoid or oblong-ellipsoidal, occasionally reniform to allantoid, 3–4 × 1–1.5(–2) (av. 3 × 1) µm, L/W = 3.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, some subcylindrical, 2–9(–20) × 1(–1.5) (av. 5 × 1) µm. *Conidia* hyaline, allantoid to oblong-ellipsoidal, (4–)4.5–6 × 1–1.5 (av. 5 × 1) µm, L/W = 4.

*Type:* **Brazil**, human subcutaneous infection, 2000, S.H. Alves, dried MEA colony in herb. CBS 7958, **holotype**; ex-type culture CBS 110034 = FMR 7682.

*Cultural characteristics:* Colonies reaching a radius of 9.5–11 mm in 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 30 °C, maximum 37 °C. Colonies on MEA flat, mostly felty textured, with entire edge; after 8 d greyish red to greyish rose (11C4–B3) above or orange-white (5A2), in reverse greyish red becoming paler towards the edge (11D5 to 11D4); after 16 d brownish grey to orange grey (6D2–B2) above, in reverse pale greyish orange to orange-white towards the edge (6B3–A2). Colonies on PDA flat, short woolly-textured, with entire edge; after 8 d brownish orange (7C3) above, in reverse brownish orange to pale brown towards the edge (7C3–D4); after 16 d brownish orange (7C3) above, in reverse dark brown to reddish grey towards the edge (8F5–B2). Colonies on OA flat, felty with a few woolly tufts, with entire edge; after 8 d greyish orange above to orange-white towards the edge (5B4–A2), after 16 d brownish orange (6C3) or chocolate-brown to brown towards the edge (6F4–E4). Yellow pigment produced by strains CBS 110034 and CBS 408.78 on OA, PDA and MEA.

*Substrate:* human, *Dodonaea viscosa*.

*Distribution:* Australia, Brazil, U.S.A.

*Additional cultures examined:* **Australia**, Markaranka, South Australia, stem of *Dodonaea viscosa*, 2000, I. Pascoe, CBS 113590 = VPRI 22409a. **U.S.A.**, Berkeley, human, synovial fluid, 1978, A.A. Padhye, CBS 408.78 = CDC 78-042877; South Carolina, subcutaneous granulomatous lesion of foot of 83-year-old woman, 1997, A.A. Padhye, CBS 729.97.

*Notes:* According to DNA phylogeny, *Pm. alvesii* is most closely related to *Pm. rubrigenum*. These species, however, differ in several aspects. *Phaeoacremonium rubrigenum* has medium to purple-pink colonies, while those of *Pm. alvesii* are medium pink or beige to pale brown. *Phaeoacremonium alvesii*, compared to *Pm. rubrigenum*, has relatively simple, infrequently branched conidiophores and relatively dark brown mycelium. Its type II phialides are more cylindrical than those of *Pm. rubrigenum*. Strains of this species have been misidentified previously as *Pm. aleophilum* (e.g., CBS 110034) and as *Pm. inflatipes* (e.g., CBS 729.97 and CBS 408.78) because of their brownish colony colour.

**12. *Phaeoacremonium amstelodamense*** L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43: 1758. 2005. Fig. 33A–R.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur mostly singly; hyphae tuberculate with warts up to 1 µm diam, verruculose, pale orange-brown, verruculose, and 1.5–2.5 µm wide. *Conidiophores* mostly short and unbranched, constricted at the septa with swollen bases, up to 5-septate, often bearing next to the terminal phialide a lateral one, percurrent rejuvenation often occurring, (15–)16–61(–90) (av. 36) long and 1.5–3 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, sparsely tuberculate, verruculose to smooth and pale brown to hyaline; collarettes, 1–1.5 µm long, 1.5–2 µm wide; type I phialides mostly cylindrical, 2–8 × 1–1.5 (av. 6 × 1) µm; type II phialides predominant, mostly elongate-ampulliform and constricted at the base, tapering towards the apex, (5–)6.5–14 × (1.5–)2–2.5(–3) (av. 10 × 2) µm; type III phialides elongate-ampulliform and attenuated at the base, or subcylindrical, (13–)14–19(–20) × (1.5–)2(–2.5) (av. 17 × 2) µm, tapering towards the apex. *Conidia* mostly oblong-ellipsoidal or obovoid, occasionally allantoid, 2–4 × 1–2 (av. 3 × 1.5) µm, L/W = 2.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, some subcylindrical, 2–13(–15) × 1–1.5 (av. 6 × 1) µm. *Conidia* hyaline, oblong-ellipsoidal, 4–7 × 1–1.5 (av. 5 × 1) µm, L/W = 4.

*Type:* **Netherlands**, Amsterdam, human elbow joint, June 2002, J. Bruins, dried MEA colony in herb. CBS 7960, **holotype**; ex-type culture CBS 110627.

*Cultural characteristics:* Colonies reaching a radius of 11.5–12.5 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 30 °C, maximum 40 °C. Colonies on MEA flat, mostly felty textured, with little aerial mycelium, with entire edge; after 8 d orange-white to dark blond (5A2–5D4), in reverse orange-grey to orange-white (5B2–A2); after 16 d yellowish grey to yellowish white (3D2–A2) above, in reverse olive to yellowish white towards the edge (3F4–A2). Colonies on PDA flat, felty to powdery, with entire edge; after 8 d olive-brown to yellowish white towards the edge (4D3–3A2) above, in reverse brownish grey (4D2); after 16 d brownish grey (4D2) above, in reverse olive-brown to yellowish grey towards the edge (4E3–B2). Colonies on OA flat, felty, with entire edge; after 8 d olive-brown to yellowish white towards the edge (4E4–4A2) above, after 16 d olive (3F4).

*Substrate:* Human.

*Distribution:* The Netherlands.

*Notes:* This strain produced little aerial mycelium, so microscopic observations were made from dense hyphal tufts on the agar. *Phaeoacremonium amstelodamense* can be distinguished by the combination of its beige colonies, its high level of percurrent conidiophore rejuvenation and its elongate-ampulliform type II phialides with strongly constricted bases. Colonies became distinctly olivaceous-green on OA after 16 d.

**13. *Phaeoacremonium angustius*** W. Gams, Crous & M.J. Wingf., *Mycologia* 88: 791. 1996. Fig. 34A–Q.

*Aerial structures:* *Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 9, tuberculate with warts up to 1 µm diam, verruculose, pale brown to hyaline and 1–3 µm wide. *Conidiophores* mostly short and unbranched, arising from aerial or submerged hyphae, erect, simple, up to 1-septate, often ending in a single terminal phialide, pale brown, hyaline towards the tip, smooth to verruculose, (15–)16–42(–60) (av. 25) µm long and 1.5–2 (av. 1.5) µm wide. *Phialides* terminal or lateral, mostly monophialidic, smooth to verruculose, subhyaline; collarettes 0.5–1 µm long, 1–1.5 µm wide; type I phialides most predominant, cylindrical, occasionally widened at the base, (2.5–)3–8.5(–10) × 1(–1.5) (av. 6 × 1) µm; type II phialides either subcylindrical or navicular, tapering towards the apex, (6–)7–12 × 1.5 (av. 11 × 1.5) µm; type III phialides subcylindrical sometimes elongate-ampulliform and attenuated at the base, (11–)12–19.5(–20) × 1.5–2 (av. 15 × 1.5) µm, tapering gradually to a long neck. *Conidia* mostly oblong-ellipsoidal or allantoid, 4–6 × 1 (–1.5) (av. 5 × 1) µm, L/W = 5.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, 2–10(–32) × 1–2 (av. 5 × 1) µm. *Conidia*

hyaline, allantoid to cylindrical with large guttules, 5–8 × 1–1.5 (av. 6 × 1) µm, L/W = 6.

*Types:* U.S.A., California, Salinas, *Vitis vinifera*, 1992, P. Larignon, dried specimen in herb. CBS 249.95, **holotype**; *V. vinifera*, 1992, P. Larignon, dried MEA colony herb. CBS 17447, **epitype designated here**, ex-epitype culture CBS 114992 = LCP 96 3897).

*Cultural characteristics:* Colonies reaching a radius of 9–10 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 25 °C, maximum 30 °C. Colonies on MEA flat, felty, with entire edge; after 8 d colonies white (4A1) or yellowish white (4A2) above, in reverse yellowish white (4A2); after 16 d white (4A1) or yellowish grey (4A2) above, in reverse yellowish white (4A2) or yellowish grey (4A2). Colonies on PDA flat, woolly, felty towards the edge, with entire edge; after 8 d above and in reverse pale yellow (3A3), after 16 d remaining the same except for the reverse becoming a darker yellow (4A3). Colonies on OA flat, felty with few woolly tufts, with entire edge; after 8 d yellowish white (3A2) above, after 16 d yellowish white to greyish red (3A2–9B4) towards the edge. Yellow pigment produced on PDA and OA.

*Substrate:* *Vitis vinifera*.

*Distribution:* U.S.A.

*Additional culture examined:* U.S.A., California, Salinas, *Vitis vinifera*, 1992, P. Larignon, CBS 114991 = LCP 93 3551.

*Notes:* Combined ITS/TUB phylogeny confirmed that the original ex-holotype strain of *Pm. angustius* sent to South Africa typified a species differing from *Pm. aleophilum* (Groenewald *et al.* 2001). However, the strain held for some years at CBS as CBS 249.95 was later found through molecular study to contain a contaminant *Pm. aleophilum*; this contaminated culture was then discarded. The synonymy proposed by Dupont *et al.* (2000) for *Pm. angustius* and *Pm. aleophilum* was based on the contaminant. The only remaining subculture of the original ex-type strain from which the initial *Pm. angustius* sequences had been made (Univ. Stellenbosch isolate STE-U) had died in the meantime, and therefore a new epitype was needed. A collection from the original area (CBS 114992 and CBS 114991) yielded material that corresponded with the original *Pm. angustius* in morphology and DNA sequence data and from CBS 114992 the epitype was prepared.

*Phaeoacremonium angustius* and the similar *Pm. austroafricanum* can be distinguished from the other species of *Phaeoacremonium* by their reddish colony colour on OA. *Phaeoacremonium angustius* grows faster and produces colonies with a radius of 9–10 mm after 8 d in the dark on MEA, while *Pm. austroafricanum* reaches only 5–8 mm under the same

conditions. Type I phialides are predominant in *Pm. angustius* while type III phialides predominate in *Pm. austroafricanum*. Conidia of *Pm. angustius* produced on and in the agar are narrower (L/W = 6) than those of *Pm. austroafricanum* (L/W = 3).

**14. *Phaeoacremonium australiense*** L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43: 1759. 2005. Fig. 35A–R.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 6; hyphae tuberculate with warts up to 1 µm diam, verruculose, pale brown and 1.5–3 µm wide. *Conidiophores* mostly short and unbranched, often constricted at the septa, up to 4-septate, sometimes bearing 2 lateral phialides next to the terminal one, (14–)17–50(–64) (av. 26) µm long and 1.5–2.5 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, sparsely and finely tuberculate to verruculose, rarely smooth, subhyaline to hyaline; collarettes slightly flaring, 2–2.5 µm long and 1.5–2.5 µm wide; type I phialides cylindrical, occasionally widened at the base, tapering towards the apex, 3–8 × 1–1.5(–2) (av. 5 × 1.5) µm; type II phialides elongate-ampulliform, attenuated at the base, or navicular, tapering towards the apex, (8–)8.5–14 × 1.5–2(–2.5) (av. 11 × 2) µm; type III phialides subcylindrical to navicular, (12–)13.5–20(–22) × 1.5–2(–2.5) (av. 17 × 2) µm, gradually tapering to a long and narrow neck; all three phialide types occurring in equal proportions. *Conidia* oblong-ellipsoidal to obovoid, occasionally cylindrical or reniform, (2.5–)3–4 × 1–2 (av. 3 × 1.5) µm, L/W = 2.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, some subcylindrical, (2–)3–11(–15) × 1–1.5 (av. 6 × 1) µm. *Conidia* hyaline, oblong-ellipsoidal, (2–)4–7(–9) × 1–1.5 (av. 5 × 1) µm, L/W = 4.

*Type:* **Australia**, Moyhu, Victoria, *Vitis vinifera*, T. Knaggs, dried MEA colony in herb. CBS 7955 **holotype**; ex-type culture CBS 113589.

*Cultural characteristics:* Colonies reaching a radius of 9–10 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 30 °C, maximum 35–37 °C. Colonies on MEA flat, mostly felty textured, with entire edge; after 8 d dark blond above near the centre, orange-white (5D4–A2) towards the periphery, in reverse orange-grey to orange white towards the edge (5B2–A2); after 16 d orange-grey (5B2) or yellowish brown (5D4) above, in reverse brownish orange to orange-white (5C3–A2) or yellowish brown (5D4). Colonies on PDA flat, felty to hairy, with entire edge; after 8 d brownish orange (5C3) above, in reverse yellowish brown (5E4); after 16 d brownish orange (5C3) above, in reverse greyish brown (5E3). Colonies

on OA flat, felty, with entire edge; after 8 d pale yellow (4A3) above with some irregular dark blond (5D4) patches; after 16 d yellowish grey to brownish grey towards the edge (4B2–D2) above. Producing yellow pigment on OA.

*Substrate:* *Vitis vinifera*.

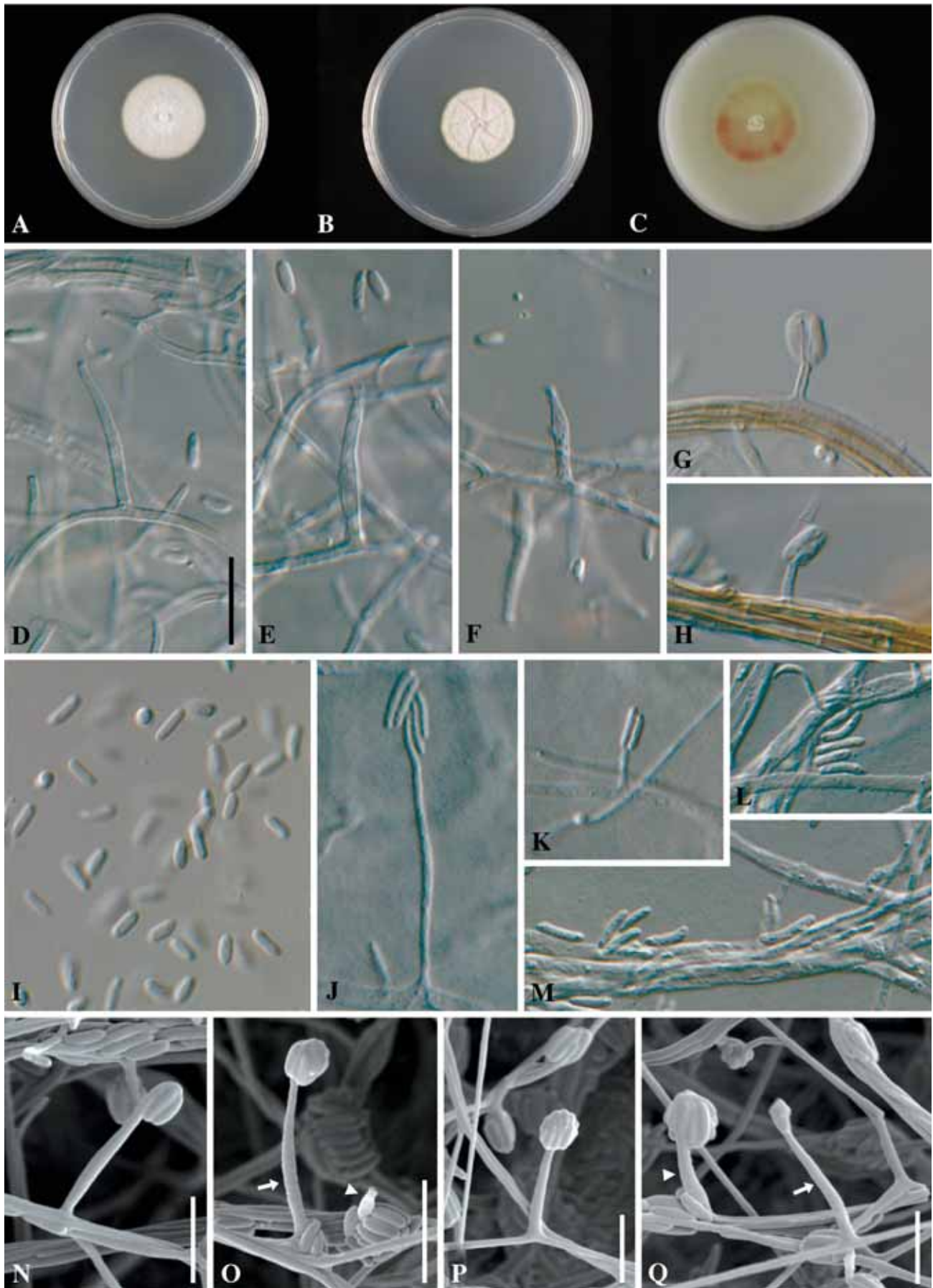
*Distribution:* Australia.

*Additional culture examined:* **Australia**, Victoria, Mildura, *Vitis vinifera*, 2000, J. Edwards, CBS 113592.

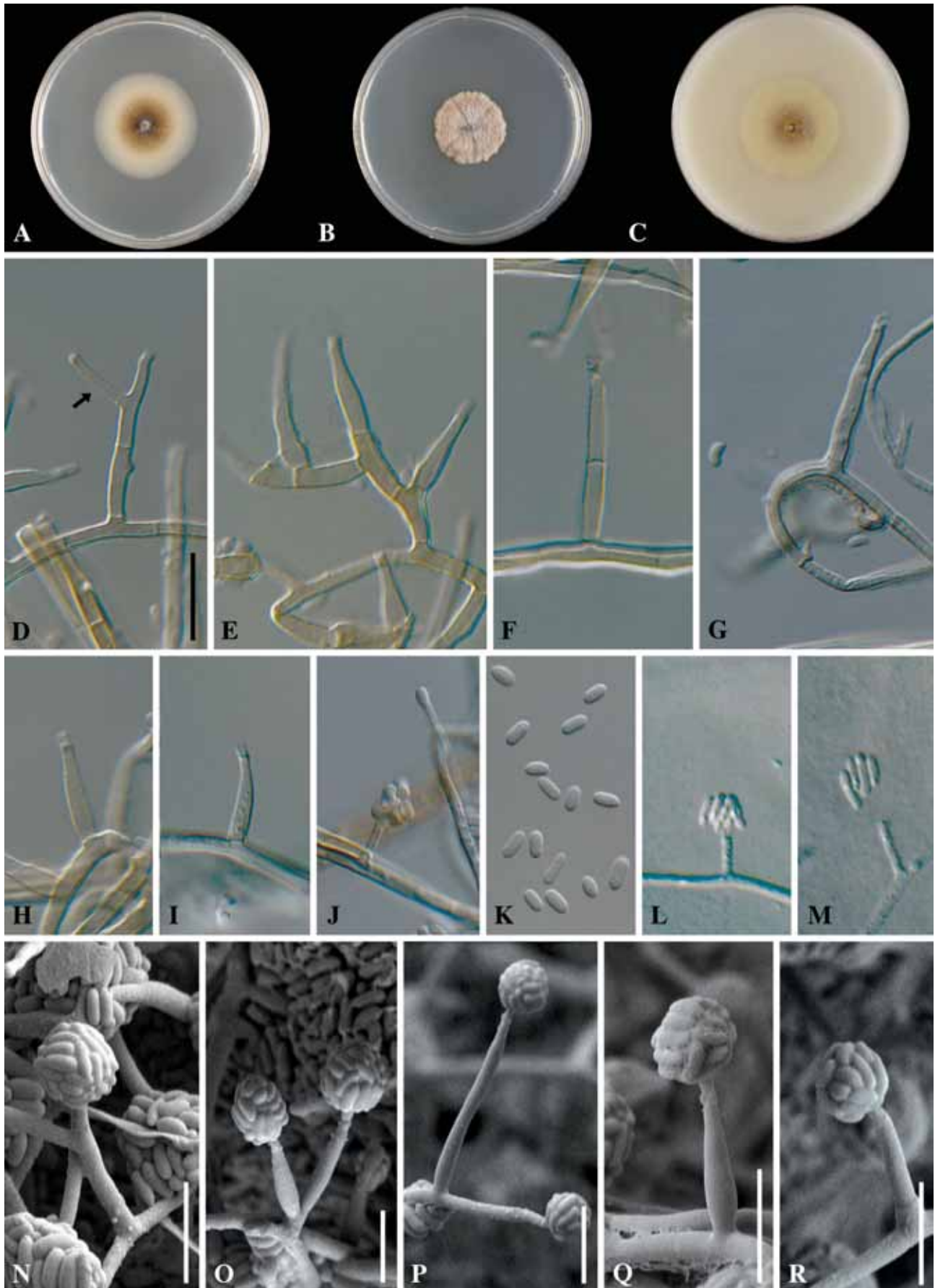
*Notes:* Colonies have a distinct brown centre, with a broad orange-white outer margin that develops after 16 d. The other brown-coloured species *Pm. inflatipes* and *Pm. parasiticum* form long or frequently branched conidiophores, readily distinguishable from the mostly short and unbranched conidiophores of *Pm. australiense*. *Phaeoacremonium australiense* has a verruculose mycelium, distinct from the verrucose mycelium of the three other brown-coloured species, *Pm. krajdenii*, *Pm. sphinctrophorum* and *Pm. tardicrescens*. *Phaeoacremonium australiense* can be distinguished from yet another brown-coloured species, *Pm. aleophilum*, by its failure to form yellow pigment in PDA. The presence of the different phialide types in equal proportions in *Pm. australiense* makes it possible to distinguish this species from *Pm. iranianum* with its predominantly type III phialides.

**15. *Phaeoacremonium griseorubrum*** L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43: 1761. 2005. Fig. 36A–T.

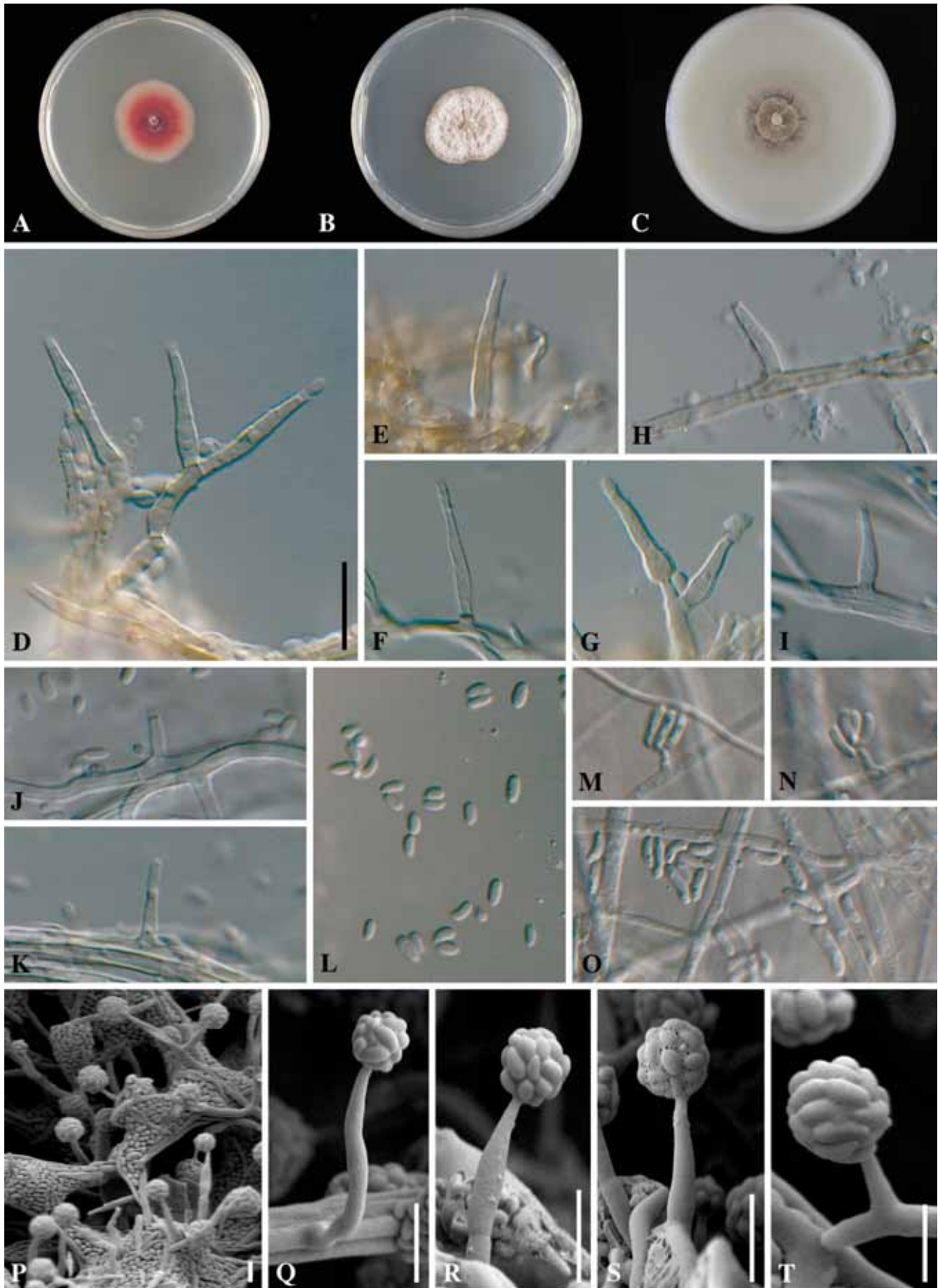
*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 7; hyphae tuberculate with warts up to 1.5 µm diam, verruculose to sometimes verrucose, yellow-brown to hyaline, 1–3 µm wide. *Conidiophores* mostly short, occasionally branched, often constricted at the base, 1–4-septate, next to the terminal phialide often bearing 2 lateral ones, (21–)23–70(–85) (av. 38) µm long and 2–3 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, sparsely and finely tuberculate to verruculose, rarely smooth, pale brown to hyaline; collarettes 1.5–2 µm long and 1–1.5 µm wide; type II and III phialides predominant; type I phialides cylindrical to navicular, occasionally widened at the base, 2–12 × 1–2(–2.5) (av. 7 × 1.5) µm; type II phialides elongate-ampulliform or navicular, tapering towards the apex, (6–)9–15 × 2–2.5 (av. 13 × 2) µm; type III phialides subcylindrical or navicular, (15–)16–24(–25) × 2(–2.5) (av. 19 × 2) µm, gradually tapering towards the apex. *Conidia* mostly obovoid, occasionally oblong-ellipsoidal or globose, (2–)3–4 × (1–)1.5–2 (av. 3 × 1.5) µm, L/W = 2.



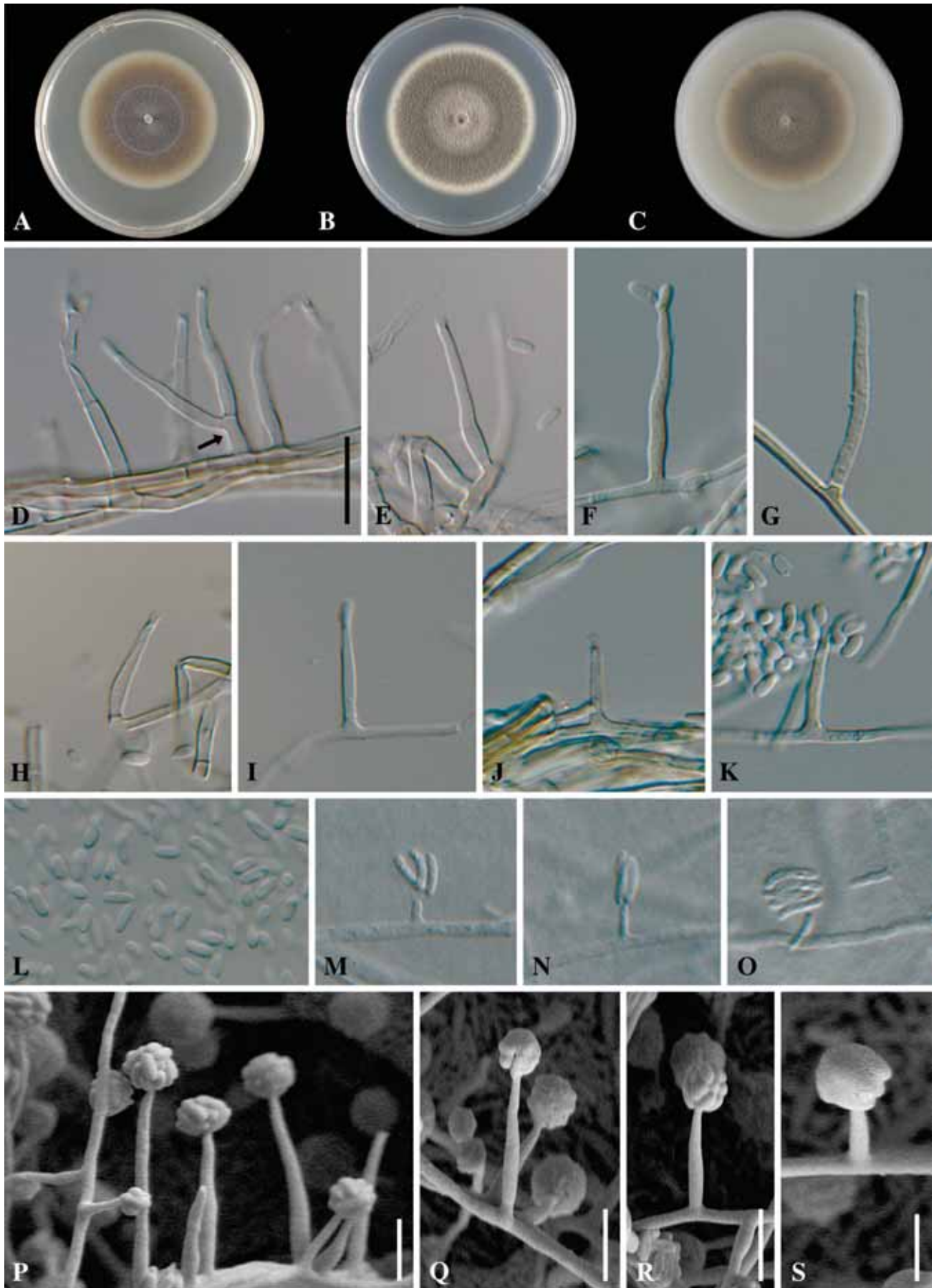
**Fig. 34.** *Phaeoacremonium angustius*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–I, N–Q. Aerial structures on MEA. D. Conidiophore. E. Type III phialide. F. Type II phialide. G–H. Type I phialide. I. Conidia. J–M. Structures on the surface of and in MEA. J. Elongated phialide. K. Adelophialide with conidia. L–M. Conidia. N. Type III phialide. O, Q. Type III (arrows) and type II phialides (arrow heads). P. Type II phialide. A–Q from CBS 114992. D–M: DIC; N–Q: SEM. Scale bars: D–Q = 10  $\mu$ m. Scale bar for D applies to E–M.



**Fig. 35.** *Phaeoacremonium australiense*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–K, N–R. Aerial structures on MEA. D–F. Conidiophores; arrow indicating polyphialide (D). G. Type III phialide. H–I. Type II phialides. J. Type I phialide. K. Conidia. L–M. Structures on the surface of and in MEA. L. Adelophialides with conidia. N–P. Conidiophores. Q. Type II phialide. R. Type I phialide. A–R from CBS 113589. D–M: DIC; N–R: SEM. Scale bars: D–R = 10  $\mu$ m; R = 5  $\mu$ m. Scale bar for D applies to E–M.



**Fig. 36.** *Phaeocremonium griseorubrum*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–L, P–T. Aerial structures on MEA. D. Conidiophore. E–F. Type III phialides. G–I. Type II phialides. J–K. Type I phialides. L. Conidia. M–O. Structures on the surface of and in MEA. M–N. Adelophialides with conidia. O. Conidia. P. Conidiophores and phialides. Q. Type III phialide. R–S. Type II phialides. T. Type I phialide. A–T from CBS 111657. D–O: DIC; P–T: SEM. Scale bars: D–T = 10  $\mu$ m. Scale bar for D applies to E–O.



**Fig. 37.** *Phaeoacremonium inflatipes*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–L, P–S. Aerial structures on MEA. D. Conidiophore (indicated with arrow) with terminal and 1 lateral phialide. E–G. Type III phialides. H–I. Type II phialides. J–K. Type I phialides. L. Conidia. M–O. Structures on the surface of and in MEA. M–N. Adelophialides with conidia. O. Conidia. P, Q. Conidiophores and phialides. R. Type II phialide. S. Type I phialide. A–S from CBS 391.71. D–O: DIC; P–S: SEM. Scale bars: D–T = 10  $\mu$ m. Scale bar for D applies to E–O.



*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical,  $2\text{--}8\text{--}(12) \times 1\text{--}1.5$  (av.  $4 \times 1$ )  $\mu\text{m}$ . *Conidia* hyaline, allantoid some oblong-ellipsoidal,  $5\text{--}7 \times 1\text{--}2$  (av.  $5 \times 1.5$ )  $\mu\text{m}$ , L/W = 3.

*Type:* **U.S.A.**, Maryland, Baltimore, human blood, 2002, D. Sutton, dried MEA colony in herb. CBS 7954, **holotype**; ex-type culture CBS 111657.

*Cultural characteristics:* Colonies reaching a radius of 6–7.5 mm in 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 30 °C, maximum 40 °C. Colonies on MEA flat, mostly felty, with woolly tufts in the centre, with entire edge; after 8 d greyish ruby to greyish rose towards the edge (12D6–B3) above, in reverse greyish ruby to greyish rose towards the edge (12D7–B3); after 16 d greyish ruby (12D5) above, with grey (12B1) and white woolly tufts, in reverse greyish ruby (12E5). Colonies on PDA flat, short woolly, with entire edge; after 8 d dull red (10C3) above, with white woolly tufts, in reverse reddish grey (10B2); after 16 d violet-brown to reddish grey towards the edge (10E5–B2) above, with grey woolly tufts, in reverse violet-brown to reddish grey towards the edge (10E5–B2). Colonies on OA flat, felty to powdery, with entire edge; after 8 d brownish orange to orange-white towards the edge (5C3–A2) above, after 16 d greyish orange (5B3) or reddish grey (8B2).

*Substrate:* human.

*Distribution:* Japan, U.S.A.

*Additional culture examined:* **Japan**, Nagasaki, subcutaneous phaeoophomycosis in man, 1996, K. Nishimoto, CBS 566.97.

*Notes:* *Phaeoacremonium griseorubrum* could be distinguished from the other species producing pink colonies on MEA, namely *Pm. alvesii*, *Pm. rubrigenum*, *Pm. scolyti* and *Pm. viticola*, by its dark pink colonies, dense texture, and slow growth. Colonies reached a radius of only 6–7.5 mm in 8 d at 25 °C on MEA. *Phaeoacremonium viticola* overlaps with *Pm. griseorubrum* in growth rate but has a temperature maximum for growth of 30–35 °C, compared with 40 °C in the latter.

**16. *Phaeoacremonium inflatipes*** W. Gams, Crous & M.J. Wingf., Mycologia 88: 793. 1996. Fig. 37A–S.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 10; some hyphae finely tuberculate with warts up to 0.5  $\mu\text{m}$  diam, verruculose, pale brown to hyaline and 1.5–3  $\mu\text{m}$  wide. *Conidiophores* mostly branched in the basal region, pale brown to hyaline, frequently with a slightly swollen base, up to 5-septate, often bearing next to terminal 2 lateral phialides,  $(14\text{--})18\text{--}40\text{--}(43)$  (av. 28)

$\mu\text{m}$  long and 1.5–2 (av. 2)  $\mu\text{m}$  wide. *Phialides* terminal or lateral, mostly monophialidic, sparsely and finely tuberculate to verruculose, occasionally smooth and mostly hyaline, some pale brown; collarettes, 1.5–3  $\mu\text{m}$  long and 1–1.5  $\mu\text{m}$  wide; type I phialides cylindrical, tapering towards the apex,  $(2\text{--})3\text{--}13\text{--}(16) \times 1\text{--}1.5\text{--}(2)$  (av.  $7 \times 1$ )  $\mu\text{m}$ ; type II phialides elongate-ampulliform and attenuated at the base, or navicular, tapering towards the apex,  $(7.5\text{--})10\text{--}15 \times 1.5\text{--}2$  (av.  $13 \times 2$ )  $\mu\text{m}$ ; type III phialides most common, subcylindrical to navicular,  $(10\text{--})12\text{--}25\text{--}(28) \times 1.5\text{--}2\text{--}(2.5)$  (av.  $18 \times 2$ )  $\mu\text{m}$ , tapering very gradually towards the apex. *Conidia* mostly oblong-ellipsoidal or obovoid, occasionally reniform or allantoid,  $3\text{--}4\text{--}(5) \times 1\text{--}2$  (av.  $4 \times 1.5$ )  $\mu\text{m}$ , L/W = 2.5.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical,  $(1.5\text{--})2\text{--}15\text{--}(19) \times 1$  (av.  $5 \times 1$ )  $\mu\text{m}$ . *Conidia* hyaline, oblong-ellipsoidal to allantoid, some cylindrical,  $(3\text{--})4\text{--}6 \times 1\text{--}(1.5)$  (av.  $5 \times 1$ )  $\mu\text{m}$ , L/W = 5.

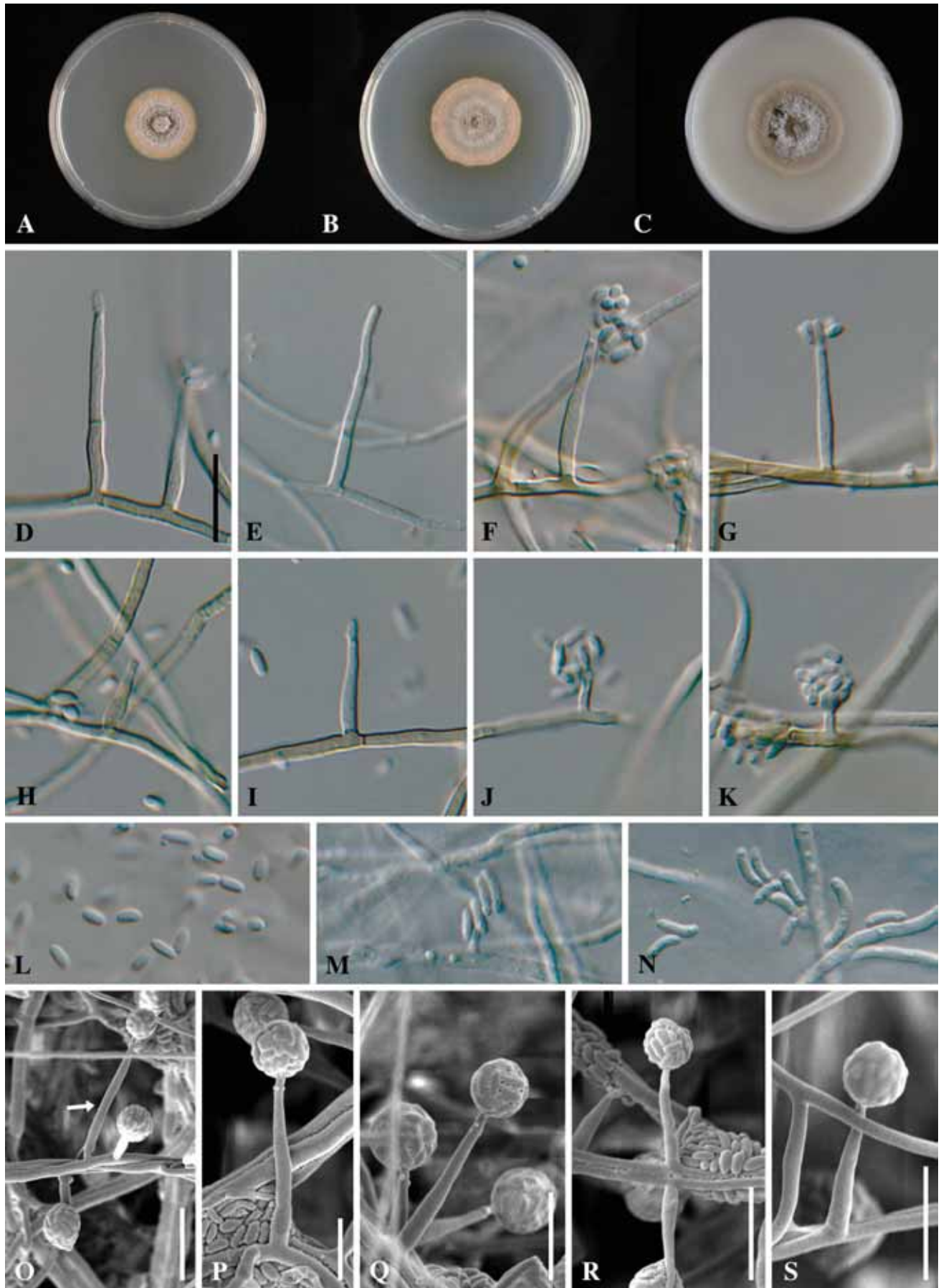
*Types:* **U.S.A.**, Texas, on stems of *Quercus virginiana*, 1966, R.S. Halliwell, dried colony in herb. CBS 391.71, **holotype**; ex-type culture CBS 391.71 = IMI 192880 = CMW 2027 = C.P.C. 770; dried **isotype** lodged at PREM.

*Cultural characteristics:* Colonies reaching a radius of 12.5–13 mm in 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 25–30 °C, maximum 35 °C. Colonies on MEA flat, mostly felty to powdery, with entire edge; after 8 d brown to orange-grey towards the edge (6E4–5B2) above or olive-brown to yellowish grey towards the edge (4E3–4B2), in reverse teak-brown to brown (6F5–E4); after 16 d orange-grey (5B2) or brownish grey (4D2) above, in reverse orange-greyish (5B3) or olive-brown (4F4). Colonies on PDA flat, felty, with entire edge; after 8 d colonies greyish beige (4C2) above, in reverse olive-brown to pale yellow towards the edge (4E4–A3); after 16 d grey to brownish grey (4D1–E2) above, in reverse brownish grey (4F2) or olive (3E4). Colonies on OA flat, felty to powdery, with entire edge; after 8 d brownish beige (6D3) or brownish orange (5C3) above, after 16 d brownish grey (4E2).

*Substrate:* *Nectandra* sp., *Quercus virginiana*, *Vitis vinifera*.

*Distribution:* Costa Rica, U.S.A.

*Additional cultures examined:* **Costa Rica**, Turrialba, on stems and roots of *Nectandra* sp., 7 Nov. 1974, I.A.S. Gibson, CBS 166.75 = IMI 190668. **U.S.A.**, Georgia, Chehaw Park, *Hypoxylon truncatum* on dead hardwood branches, 28 Apr. 2000, B. Horn, CBS 113273 = NRRL 32148.



**Fig. 38.** *Phaeoacremonium iranianum*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–L, O–S. Aerial structures on MEA. D–E. Conidiophores. F–G. Type III phialides. H–I. Type II phialides. J–K. Type I phialides. L. Conidia. M–N. Structures on the surface of and in MEA. M. Adelophialide with conidia. N. Conidia. O. Type III (arrow) and type I phialides. P. Conidiophore. Q. Type III phialide. R. Type II phialides. S. Type I phialide. A–S from CBS 101357. D–N; O–S: SEM. Scale bars: D–R = 10 µm; S = 5 µm. Scale bar for D applies to E–N.

*Notes: Phaeoacremonium inflatipes* can be identified based on its branched conidiophores, combined with a brown colony colour. Sequences of *Pm. inflatipes* strains were somewhat heterogeneous. One isolate, CBS 166.75, from woody plant material, was distant from the ex-type strain (CBS 391.71) based on ACT and TUB data (Fig. 10), and also differed by having a growth optimum of 25 °C in contrast to the 30 °C optimum observed for CBS 391.71. No conclusive cultural or morphological differences were found, however, to support segregating this isolate as a distinct species. The three strains studied here are therefore considered to represent *Pm. inflatipes*.

**17. *Phaeoacremonium iranianum*** L. Mostert, Gräfenhan, W. Gams & Crous, **sp. nov.** MycoBank MB 500227. Fig. 38A–S.

*Etymology:* Named after the country, Iran, from which the majority of strains were collected.

In mycelio aërio hyphae singulae vel ad 27 fasciculatae, tuberculatae, verruculosae, medio brunneae vel dilute brunneae. Conidiophora plerumque brevia et simplicia, saepe iuxta phialidem terminalem 1–2 phialides laterales portantia, (17–)20–50 (in medio 30) µm longa. Phialides terminales vel laterales, praecipue typi III; phialides typi I cylindricae, (2–)3–12(–13) (in medio 6) µm longae; phialides typi II subcylindricae vel nonnumquam elongato-ampulliformes, ad basim attenuatae, 8–11 (in medio 10) µm longae; phialides typi III subcylindricae vel nonnullae naviculares, (13–)14–22(–26) (in medio 18) µm longae. Conidia hyalina, oblongo-ellipsoidea, 3–4(–5) × (1–)1.5(–2) (in medio 4 × 1.5) µm, long./lat. = 3. In superficie vel submersa in agar, phialides hyalinae, cylindricae, 2–17(–20) (in medio 5) µm; conidia hyalina, cylindrica vel allantoidea, 5–7(–10) × 1–1.5(–2) (in medio 6 × 1.5) µm, long./lat. = 4

Typus herb. CBS 17450.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 27, tuberculate with warts up to 1 µm diam, verruculose, medium to pale brown and 1–2.5 µm wide. *Conidiophores* mostly short and usually unbranched, arising from aerial or submerged hyphae, erect, simple, up to 3-septate, often bearing next to the terminal phialide 1–2 lateral ones, pale brown, paler towards the tip, smooth to verruculose, (17–)20–50 (av. 30) µm long and 1–2 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monopodial, smooth to verruculose, subhyaline to hyaline; collarettes 1.5 µm long, 1–2 µm wide; type III phialides most common; type I phialides cylindrical, occasionally widened at the base, (2–)3–12(–13) × 1–1.5 (av. 6 × 1.5) µm; type II phialides subcylindrical some elongate-ampulliform and attenuated at the base, tapering towards the apex, 8–11 × 1.5–2 (av. 10 × 2) µm; type III phialides subcylindrical some navicular, (13–)14–22(–26) × 1–

2 (av. 18 × 2) µm, tapering gradually to a long neck. *Conidia* oblong-ellipsoidal 3–4(–5) × (1–)1.5(–2) (av. 4 × 1.5) µm, L/W = 3.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, 2–17(–20) × 1–2 (av. 5 × 1.5) µm. *Conidia* hyaline, cylindrical to allantoid, 5–7(–10) × 1–1.5(–2) (av. 6 × 1.5) µm, L/W = 4.

*Type:* **Italy**, *Actinidia chinensis*, 1998, F. Calzarano & S. Di Marco, dried MEA colony in herb. CBS 17450, **holotype**; ex-type culture CBS 101357.

*Cultural characteristics:* Colonies reaching a radius of 5–9 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 30 °C, maximum 37 °C. Colonies on MEA flat, felty to cottony, with entire edge; after 8 d brownish orange to orange white towards the edge (5C3–A2) above, in reverse brownish orange (5C4); after 16 d greyish brown to greyish orange towards the edge (5D3–B3) or olive-brown (4E5–D4) above, in reverse greyish brown (5D3) or olive-brown (4E5). Colonies on PDA flat, felty, with entire edge; after 8 d brown to reddish brown (7E5–B2) above, in reverse dark brown (9F6); after 16 d brownish grey (7D2) above, in reverse dark brown (9F6). Colonies on OA flat, felty to woolly, with entire edge; after 8 d greyish brown to yellowish white (5D3–3A2) above; after 16 d brownish grey to dark blond towards the edge (5C2–D4) above. Colonies producing yellow pigment on OA.

*Substrate:* *Actinidia chinensis*, *Vitis sylvestris*, *V. vinifera*.

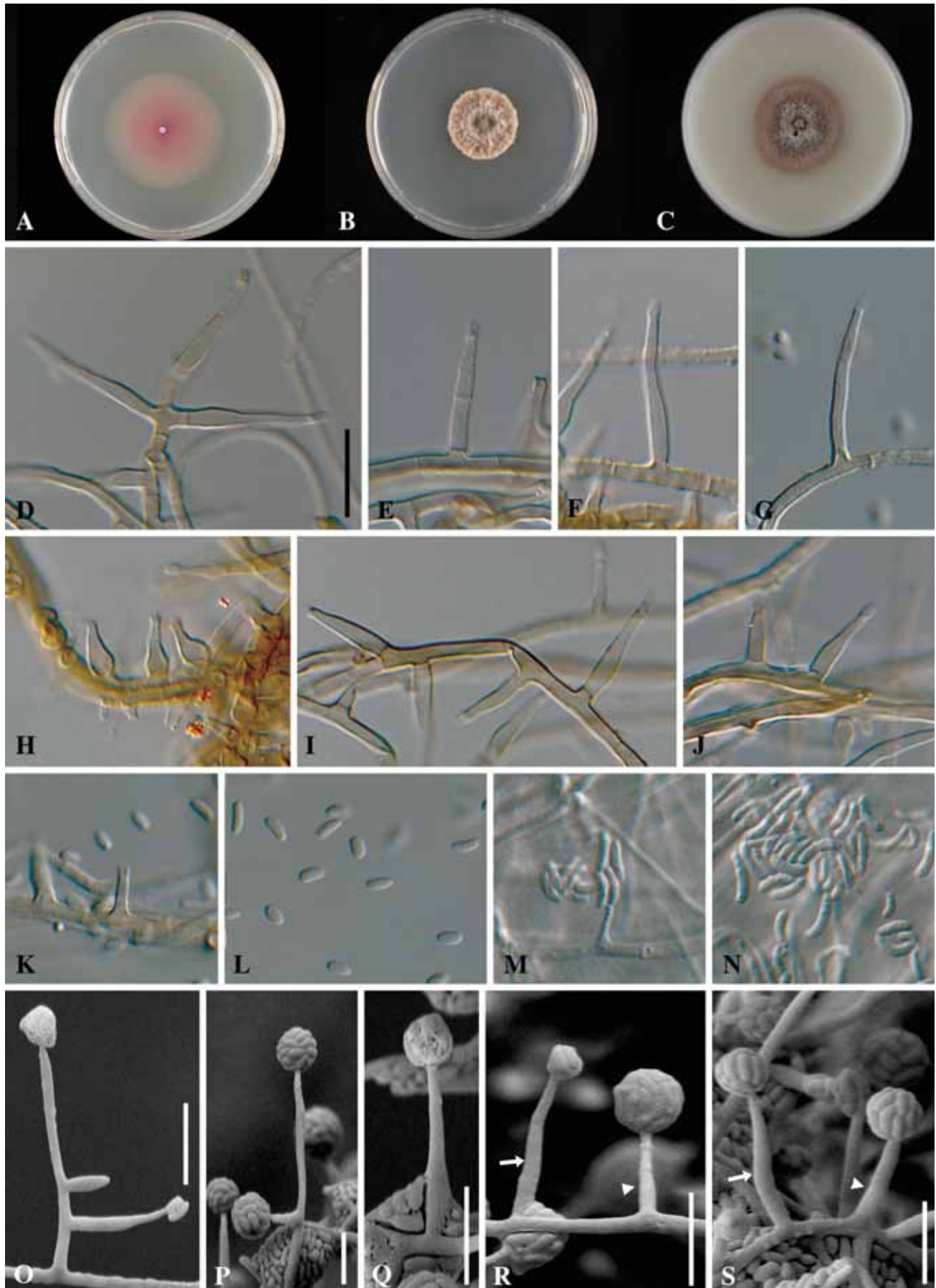
*Distribution:* Iran, Italy.

*Additional cultures examined:* **Iran**, East Azerbaijan, Arasbaran, Veinagh, stems of *Vitis sylvestris*, 2004, T. Gräfenhan, CBS 117114; South Iran, 25 km east of Firuzabad, Maymand, *V. vinifera*, 2003, T. Gräfenhan, CBS 117112; East Iran, 10 km south of Shahrud, *V. vinifera*, 2003, T. Gräfenhan, CBS 117113.

*Notes:* *Phaeoacremonium iranianum* is phylogenetically and morphologically close to *Pm. aleophilum*. It can be distinguished from it by the predominance of type III phialides and by its subcylindrical type II phialides.

**18. *Phaeoacremonium scolyti*** L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43: 1763. 2005. Fig. 39A–S.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 7; hyphae tuberculate with warts up to 1 µm diam, verruculose occasionally verrucose, medium brown to pale brown and 1–2 µm wide. *Conidiophores* mostly short and usually unbranched, subcylindrical to navicular, up to 3-septate, besides the terminal phialide often bearing 1–2 lateral ones, (15–)17–35(–39) (av. 26) µm long and 1.5–2.5 (av. 2) µm wide.



**Fig. 39.** *Phaeoacremonium scolyti*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–L, O–S. Aerial structures on MEA. D–E. Conidiophores; conidiophore with terminal and 2 adjacent lateral phialides (D). F–G. Type III phialides. H–J. Type II phialides. K. Type I phialides. L. Conidia. M–N. Structures on the surface of and in MEA. M. Adelophialide with conidia. N. Conidia. O–P. Conidiophores. Q. Type III phialide. R–S. Type III (arrows) and type II (indicated with arrow heads) phialides. A–S from CBS 113597. D–N: DIC; O–S: SEM. Scale bars: D–R = 10  $\mu$ m. Scale bar for D applies to E–N.

*Phialides* terminal or lateral, occasionally polyphialidic, tuberculate to verruculose, rarely smooth, pale brown to hyaline; collarettes 1.5–2 µm long and 1–1.5 µm wide; type I phialides cylindrical, occasionally swollen at the base, (2–)3–7 × 1–1.5 (av. 5 × 1) µm; type II phialides predominant, elongate-ampulliform, attenuated or constricted at the base, or navicular, tapering towards the apex, 7–14 × 1.5–2(–2.5) (av. 10 × 2) µm; type III phialides subcylindrical, subulate to elongate-ampulliform, (10–)14–20 × 1.5–2(–2.5) (av. 16 × 2) µm, tapering gradually to the apex. *Conidia* oblong-ellipsoidal or obovoid, occasionally reniform or allantoid, 2.5–4(–4.5) × 1–2 (av. 3 × 1.5) µm, L/W = 2.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, 2–11(–15) × 1–1.5 (av. 5 × 1) µm. *Conidia* hyaline, allantoid, 4–7 × 1–1.5 (av. 5.5 × 1) µm, L/W = 5.

*Type:* **South Africa**, Western Cape, *Vitis vinifera*, 1999, M. Groenewald, dried MEA colony in herb. CBS 7952, **holotype**; ex-type culture CBS 113597 = C.P.C. 3092.

*Cultural characteristics:* Colonies reaching a radius of 10.5–12 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, temperature 25–30 °C, maximum 37 °C. Colonies on MEA flat, felty, with entire edge; after 8 d pinkish white (12A2) to hyaline/translucent above, in reverse reddish grey (12B2); after 16 d rose (12A3) above, in reverse greyish rose (12B3). Colonies on PDA flat, felty to woolly, with entire edge; after 8 d reddish grey (8B2) above, in reverse the same; after 16 d greyish brown (8D3) above, in reverse violet-brown (10E5). Colonies on OA flat, felty, with entire edge; after 8 d pale brown to greyish red (7D4–9C4) above, after 16 d reddish grey to greyish brown (9B2–8D3).

*Substrate:* *Vitis vinifera*, *Quercus robur* with *Scolytus intricatus*.

*Distribution:* Czech Republic, France, South Africa.

*Additional cultures examined:* **Czech Republic**, Bacov near Velky Osek in Polabi region, from larva of *Scolytus intricatus*, on branch of *Quercus robur*, 1998, A. Kubátová, CBS 112585 = CCF 3266. **France**, Pyrénées atlantiques, Domaine de Grouseilles, *Vitis vinifera*, 1997, P. Larignon, CBS 113593.

*Notes:* *Phaeoacremonium scolyti* can be distinguished by the combination of medium pink to translucently pale colonies on MEA and elongate-ampulliform type II phialides, which are often strongly constricted at the base.

**19. *Phaeoacremonium sphinctrophorum*** Mostert, Summerb. & Crous, **sp. nov.** MycoBank MB500231. Fig. 40A–Q.

*Etymology:* Greek *sphincter* = constriction; referring to the constrictions present at the conidiophore septa.

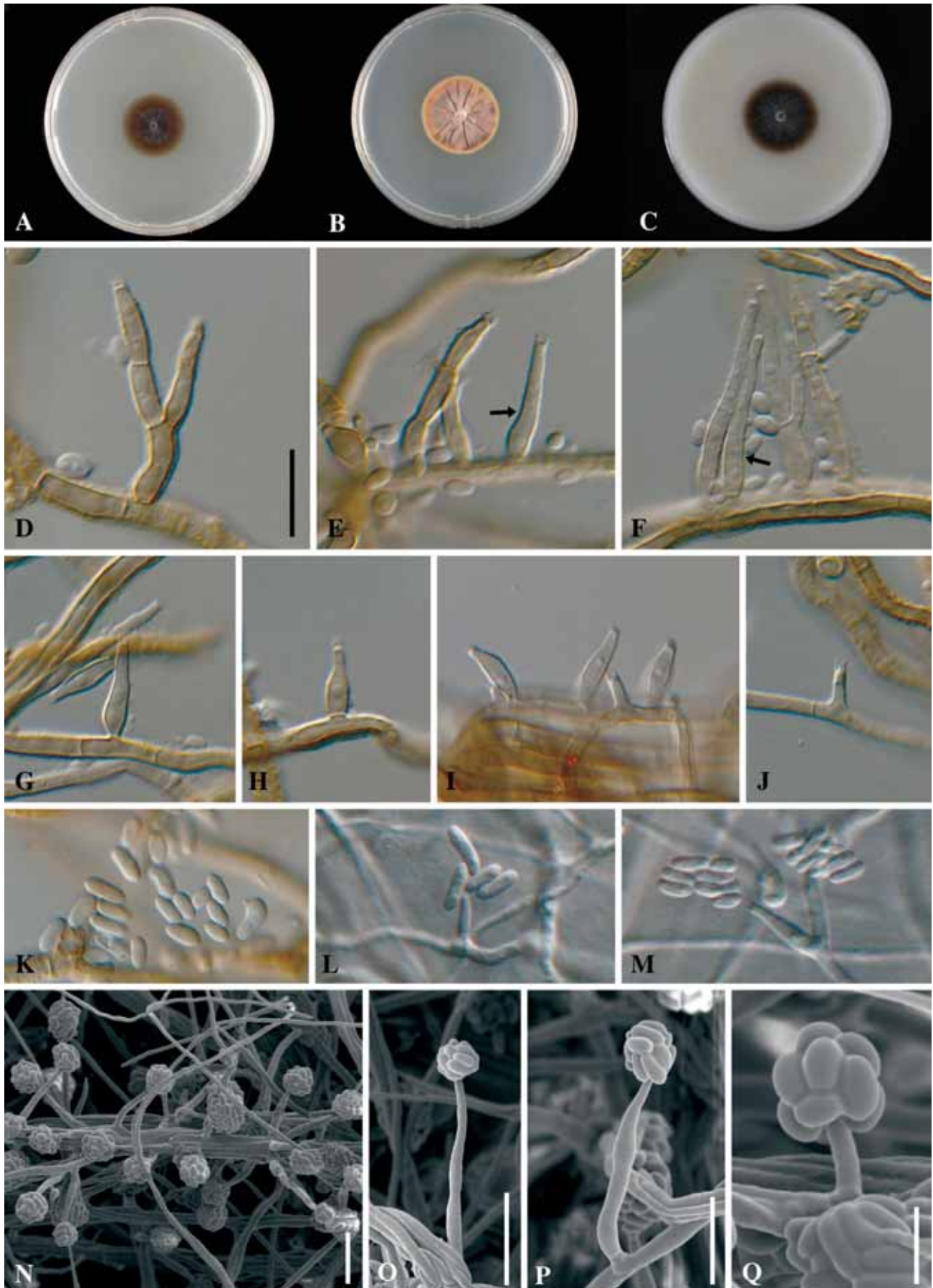
In mycelio aereo hyphae singulae vel ad 4 fasciculatae, verrucosae, fuscae vel medio brunneae. Conidiophora plerumque brevia et ramosa, saepe ad septa conspicue constricta, iuxta phialidem terminalem nonnumquam lateralem portantia, (11–)13–40(–50) (in medio 23) µm longa. Phialides terminales vel laterales, praecipue typi II; phialides typi I cylindricae, 2–7(–10) (in medio 4) µm longae; phialides typi II elongato-ampulliformes, ad basim attenuatae, vel elongato-ampulliformes, ad basim constrictae, (5–)7–13(–14) (in medio 9) µm longae; phialides typi III subcylindricae vel elongato-ampulliformes, ad basim attenuatae, 14–21(–25) (in medio 17) µm longae. Conidia hyalina, oblongo-ellipsoidea vel obovoidea, (2.5–)3–4 × 1.5–2 (in medio 3 × 1.5) µm, long./lat. = 2. In superficie vel submersa in agar, phialides hyalinae, cylindricae, nonnullae in medio inflatae, (2–)3–13(–15) (in medio 6) µm; conidia hyalina, oblongo-ellipsoidea, conspicue guttulatae, 4–6 × 1–2 (in medio 5 × 1.5) µm, long./lat. = 3.

Typus herb. CBS 17452.

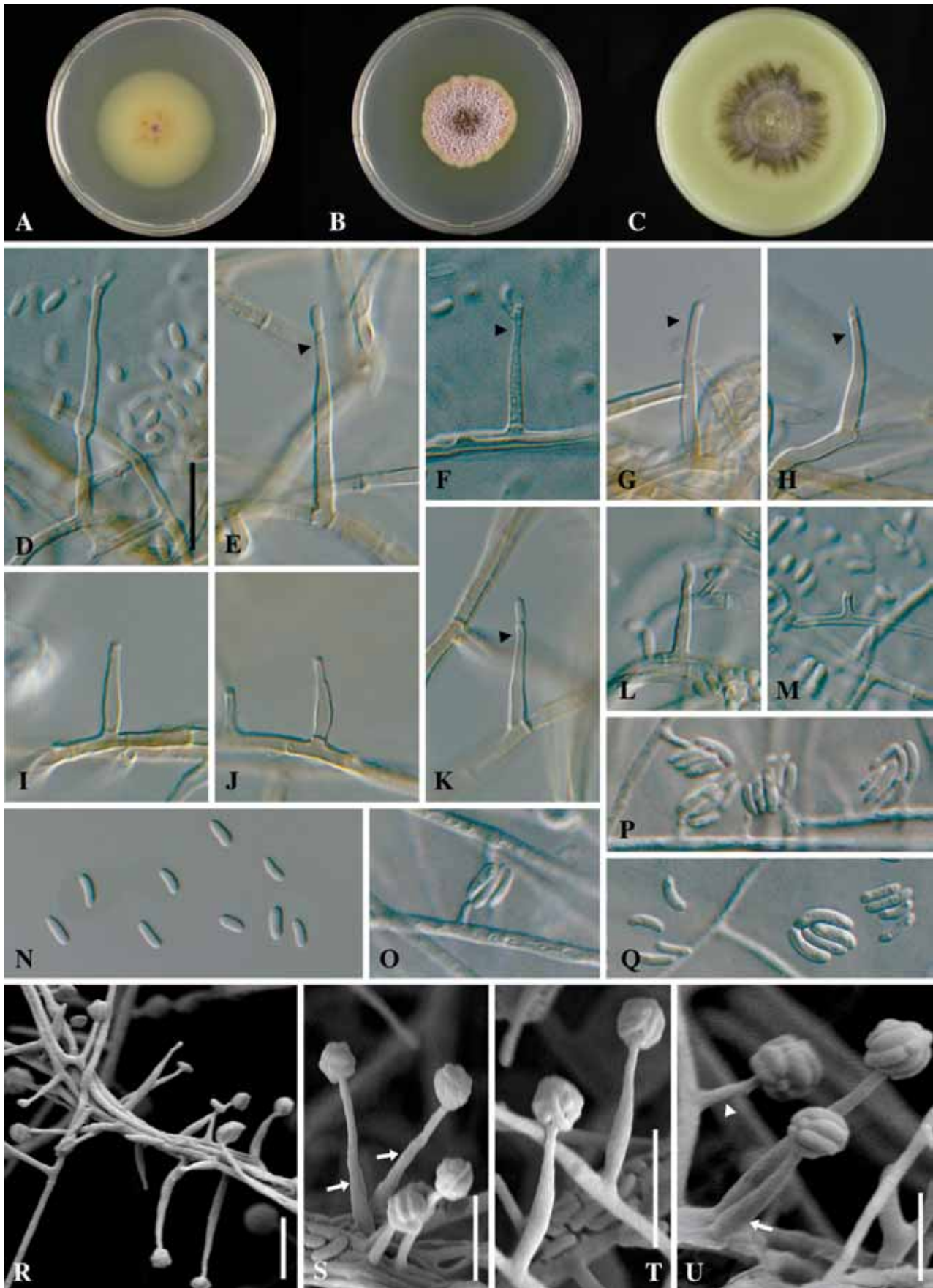
*Aerial structures:* *Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 4, verrucose, dark to medium brown and 1.5–3.5 µm wide. *Conidiophores* mostly short and often branched, arising from aerial or submerged hyphae, erect, simple, up to 4-septate, often prominently constricted at the septa, besides the terminal phialide sometimes bearing an additional lateral one, brown, paler towards the tip, smooth to verrucose, (11–)13–39(–50) (av. 23) µm long and 2–3(–3.5) (av. 2.5) µm wide. *Phialides* terminal or lateral, mostly monophaialidic, verrucose to verruculose, brown to pale brown; collarettes 0.5–1.0 µm long, 1.5–2 µm wide; type II phialides most common; type I phialides cylindrical, occasionally widened at the base, 2–7(–10) × 1–1.5(–2) (av. 4 × 1.5) µm; type II phialides elongate-ampulliform attenuated at the base or elongate-ampulliform and constricted at the base, tapering towards the apex, (5–)7–13(–14) × 2–3(–4) (av. 9 × 2) µm; type III phialides subcylindrical or elongate-ampulliform and attenuated at the base, 14–21(–25) × 1.5–2(–2.5) (av. 17 × 2) µm, tapering gradually to a long neck. *Conidia* mostly oblong-ellipsoidal or obovoid, (2.5–)3–4 × 1.5–2 (av. 3 × 1.5) µm, L/W = 2.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, some inflated in the middle, (2–)3–13(–15) × 1–2(–2.5) (av. 6 × 2) µm. *Conidia* hyaline, oblong-ellipsoidal and prominently guttulate, 4–6 × 1–2 (av. 5 × 1.5) µm, L/W = 3.

*Cultural characteristics:* Colonies reaching a radius of 6–15 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 25–30 °C, maximum 30–37 °C. Colonies on MEA flat, felty or cottony, with an entire edge; after 8 d brown (5E5) or greyish brown (6D3) above, in reverse soot-brown (5F5) or greyish brown (6D3); after 16 d brown (7E5) or orange-grey (5B2) above, in reverse dark brown (7F5) or olive-brown (4F6).



**Fig. 40.** *Phaeoacremonium sphinctrophorum*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–K, N–Q. Aerial structures on MEA. D. Branched conidiophore. E–F. Conidiophores and type III phialides (arrows). G–I. Type II phialides. J. Type I phialide. K. Conidia. L–M. Structures on the surface of and in MEA. L–N. Adelophialides with conidia. N. Mycelium with conidiophores and phialides. O–P. Conidiophores. Q. Type I phialide. A–Q from CBS 337.90. D–M: DIC; N–Q: SEM. Scale bars: D–P = 10 µm; Q = 5 µm. Scale bar for D applies to E–M.



**Fig. 41.** *Phaeocremonium subulatum*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–N, R–U. Aerial structures on MEA. D–E. Conidiophores. F–H. Type III phialides. I–L. Type II phialides. Arrow heads indicate subulate apex of phialides (E–H, K). M. Type I phialide. N. Conidia. O–Q. Structures on the surface of and in MEA. O–P. Adelophialides with conidia. Q. Conidia. R. Mycelium with conidiophores and phialides. S. Type III phialides (arrows). T. Type II phialides. U. Type II (arrow) and type I (arrow head) phialides. A–U from CBS 113584. D–Q: DIC; R–U: SEM. Scale bars: D–U = 10  $\mu$ m. Scale bar for D applies to E–Q.

Colonies on PDA flat, felty, with entire edge; after 8 and 16 d brown to brownish orange (7E4–C5) or white (5A1) above, in reverse brown (7E4). Colonies on OA flat, felty, with entire edge; after 8 d hair-brown, with mouse-grey undertone (5E4–4E3) or pale brown (6D5) above, after 16 d brown to brownish orange towards the edge (6E4–6C4).

*Type:* **Canada**, Ontario, Toronto, phaeohyphomycotic cyst of patient from Laos, 1988, S. Krajden & R.C. Summerbell, dried MEA colony in herb. CBS 17452, **holotype**; ex-type culture CBS 337.90.

*Substrate:* Human.

*Distribution:* Canada, U.S.A.

*Additional culture examined:* **U.S.A.**, Hawaii, man, subcutaneous cyst, 1998, A.A. Padhye, CBS 694.88 = CDC 88-023023.

*Notes:* *Phaeoacremonium sphinctrophorum* can be distinguished by its verrucose mycelium lacking warts, and by its short conidiophores (av. 23 µm) with often constricted septa. Although the two strains placed in this species are phylogenetically closely related (Fig. 10), a few morphological and cultural differences were noted. CBS 694.88 has relatively pale, cottony colonies, an optimum growth temperature of 25 °C, and a growth rate twice that of CBS 337.90.

**20. *Phaeoacremonium subulatum*** L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43: 1763. 2005. Fig. 41A–U.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 10; hyphae tuberculate with warts up to 0.8 µm diam, verrucose, orange to pale brown and 1.5–2.5 µm wide. *Conidiophores* mostly short and usually unbranched, 1–7-septate, often with an additional phialide next to the terminal one, (17–)18–32(–45) (av. 25) µm long and 1.5–2.5 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, sparsely to finely tuberculate to verrucose, rarely smooth and subhyaline to hyaline; all three phialide types occurring in equal proportions; collarettes 1 µm long and 1–1.5 µm wide; type I phialides cylindrical, occasionally widened at the base, tapering towards the apex, 3–9 × 1–1.5 (av. 6 × 1) µm; type II phialides subcylindrical to subulate, occasionally elongate-ampulliform and attenuated at the base, tapering towards the apex, (7–)9–13 × 1.5–2 (av. 11 × 2) µm; type III phialides subcylindrical to subulate, (12–)12.5–20(–21) × 1.5–2 (av. 16 × 2) µm, tapering gradually into a long, narrow neck. *Conidia* oblong-ellipsoidal, cylindrical, occasionally reniform, 3–5 × 1–1.5(–2) (av. 4 × 1) µm, L/W = 3.

*On surface or submerged in the agar:* *Phialides* hyaline, cylindrical, 2–17 × 1–1.5 (av. 5 × 1) µm. *Conidia* hyaline, allantoid to oblong-ellipsoidal with large guttules, (4–)5–7(–8) × 1–2 (av. 6 × 1.5) µm, L/W = 4.

*Type:* **South Africa**, Western Cape, Paarl, Zandrif, trunk of *Vitis vinifera*, 2001, L. Mostert, dried MEA colony in herb. CBS 7956, **holotype**; ex-type culture CBS 113584.

*Cultural characteristics:* Colonies reaching a radius of 8.5–11.5 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 25–30 °C, maximum 37 °C. Colonies on MEA flat, felty, with entire edge; after 8 d pale yellow (4A3) to translucent above, in reverse the same; after 16 d pale brown to orange-white (6D4–5A2) above, in reverse brown to pale orange towards the edge (5E4–A3). Colonies on PDA flat, woolly, with entire edge; after 8 d brownish orange (5C3) above, reverse greyish brown (5E3) and remaining the same after 16 d. Colonies on OA flat, felty with few woolly tufts and entire edge; after 8 d pale brown to pale yellow (4B4–A3) above, after 16 d olive-brown to greyish yellow towards the edge (4E3–4B3). Colonies producing yellow pigment in the agar on MEA, OA and PDA.

*Substrate:* *Vitis vinifera*.

*Distribution:* South Africa.

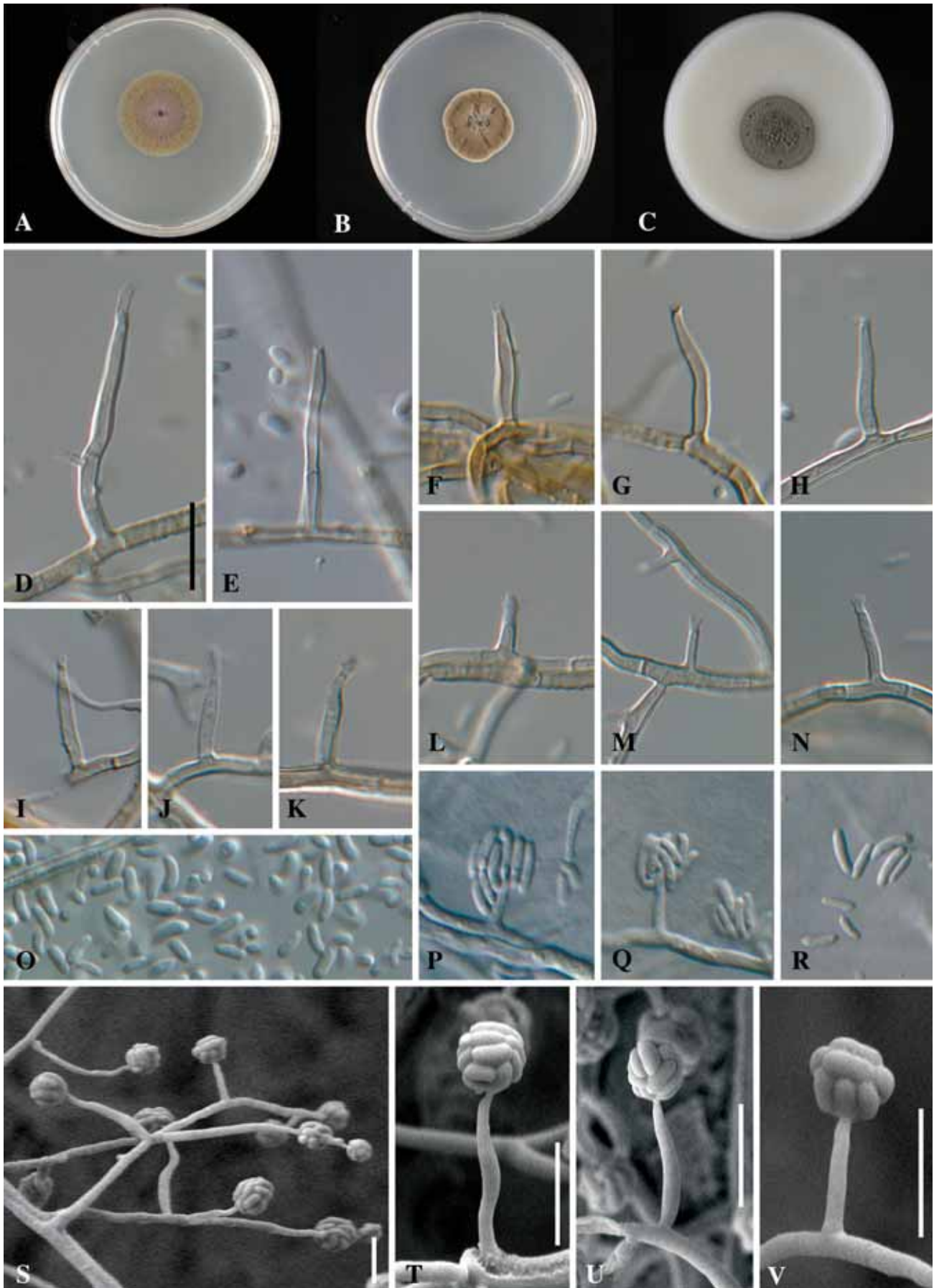
*Additional culture examined:* **South Africa**, Western Cape, Stellenbosch, Nietvoorbij, trunk of *Vitis vinifera*, 2002, L. Mostert, CBS 113587.

*Notes:* *Phaeoacremonium subulatum* can be distinguished by its strong production of yellow pigment on MEA, OA and PDA and by its subcylindrical to subulate type II and type III phialides.

**21. *Phaeoacremonium tardicrescens*** L. Mostert, Summerb. & Crous, J. Clin. Microbiol. 43: 1764. 2005. Fig. 42A–V.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 8; hyphae tuberculate with warts up to 0.5 µm diam, verrucose to strongly verrucose, medium brown and 1.5–2.5 µm wide. *Conidiophores* mostly short and usually unbranched, up to 7-septate, often bearing a single phialide as the apical cell, (13–)16–52(–67) (av. 31) µm long and 1–2 (av. 1.5) µm wide. *Phialides* terminal or lateral, mostly monophialidic, sparsely to finely tuberculate to verrucose, often smooth, subhyaline to hyaline; collarettes 1.5–2.5 µm long and 1.5–2 µm wide; type I and type III phialides predominant; type I phialides mostly cylindrical, tapering towards the apex, 2–12 × 1–1.5 (av. 5 × 1) µm; type II phialides subcylindrical to subulate or





**Fig. 42.** *Phaeoacremonium tardicrecens*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–O, S–V. Aerial structures on MEA. D–E. Conidiophores. F–H. Type III phialides. I–K. Type II phialides. L–N. Type I phialide. O. Conidia. P–R. Structures on the surface of and in MEA. P–Q. Adelophialides with conidia. R. Conidia. S. Mycelium with conidiophores and phialides. T. Conidiophore. U. Type II phialides. V. Type I phialide. A–V from CBS 110573. D–R: DIC; S–V: SEM. Scale bars: D–U = 10 µm; V = 5 µm. Scale bar for D applies to E–R.

occasionally elongate-ampulliform and attenuated at the base, tapering towards the apex, (7–)9–14(–15) × 1–2(–2.5) (av. 11.5 × 1.5) µm; type III phialides subcylindrical to navicular, (10–)14.5–24(–34) × 1–2 (av. 17.5 × 1.5) µm and tapering gradually to a long neck. *Conidia* oblong-ellipsoidal to allantoid, (3.5–)4–6 × 1–1.5(–2) (av. 5 × 1) µm, L/W = 3.

*On surface or submerged in the agar: Phialides* hyaline, subcylindrical to cylindrical, some also elongate-ampulliform, (2–)3–16(–17) × 1–2 (av. 8 × 1.5) µm. *Conidia* hyaline, allantoid and some oblong-ellipsoidal, with large guttules, 4–7(–8) × 1–2 (av. 5 × 1.5) µm, L/W = 4.

*Type: U.S.A.*, Texas, Dallas, human, 2000, Levi, dried MEA colony in herb. CBS 7953, **holotype**; culture ex-type CBS 110573 = UTHSC 00-146.

*Cultural characteristics:* Colonies reaching a radius of 8–9 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 30 °C, maximum 40 °C. Colonies on MEA flat, felty, with entire edge; after 8 d brownish grey (4D2) above, in reverse olive-brown (4F4); after 16 d pale olive-brown (4E4) above, in reverse olive-brown (4F5). Colonies on PDA flat, felty, with entire edge; after 8 d brown to orange-grey (5E5–B2) above, reverse brown (5F5); after 16 d brown to brownish grey (5E5–E2) above, in reverse brown (5F5). Colonies on OA flat, felty, with entire edge; after 8 and 16 d olive-brown (4D3–4F4) above.

*Substrate:* human.

*Distribution:* U.S.A.

*Notes:* *Phaeoacremonium tardicrescens* is difficult to distinguish from other brown-coloured species that have verrucose mycelium, such as *Pm. krajdenii*, *Pm. parasiticum* and *Pm. sphinctrophorum*. It has a growth rate slower than *Pm. krajdenii* and is also distinct in its ability to grow at 40 °C. It is distinct from *Pm. parasiticum* in producing only small hyphal warts up to 0.5 µm diam. *Phaeoacremonium sphinctrophorum* has constricted septa in the conidiophores, and also more strongly inflated phialides and shorter conidia.

**22. *Phaeoacremonium theobromatis*** Mostert, H.C. Evans, Summerb. & Crous, **sp. nov.** MycoBank MB500229. Fig. 43A–U.

*Etymology:* Named after its host, *Theobroma gileri*.

In mycelio aërio hyphae singulae vel ad 16 fasciculatae, tuberculatae, verrucosae, medio brunneae vel dilute brunneae. Conidiophora plerumque brevia et simplicia, in phialidem singulam exeuntia, 18–40(–42) (in medio 24) µm longa. Phialides terminales vel laterales, praecipue typi I; phialides typi I cylindricae, (1.5–)2–9(–15) (in medio 5) µm longae; phialides typi II seu subcylindricae,

nonnullae elongato-ampulliformes, ad basim attenuatae, seu naviculares, 5–13 (in medio 11) µm longae; phialides typi III subcylindricae, 14–19(–21) (in medio 16) longae. Conidia hyalina, plerumque oblongo-ellipsoidea, nonnumquam reniformia, 3–4(–5) × 1–1.5 (in medio 4 × 1) µm, long./lat. = 4. In superficie vel submersa in agar, phialides cylindricae, 2–10(–41) (in medio 4) µm longae; conidia hyalina, allantoida vel oblongo-ellipsoidea, 4–6(–6.5) × 1(–1.5) (in medio 5 × 1) µm, long./lat. = 5.

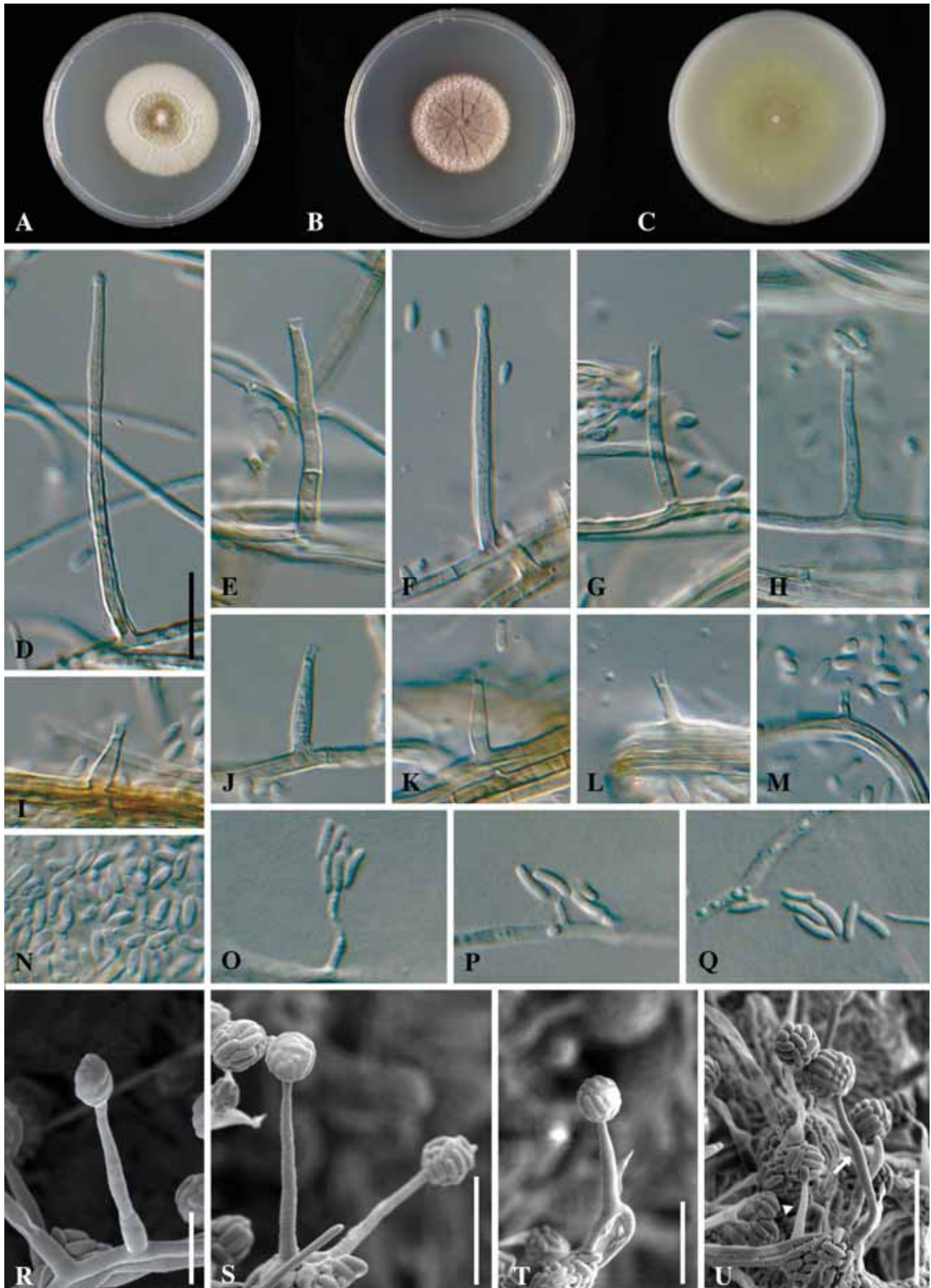
Typus herb. CBS 17453.

*Aerial structures: Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 16, tuberculate with warts up to 1 µm diam, verrucose, medium to pale brown and 1–2 µm wide. *Conidiophores* mostly short and usually unbranched, arising from aerial or submerged hyphae, erect, simple, up to 2-septate, usually bearing a single terminal phialide, pale brown, paler towards the tip, smooth to verrucose, 18–40(–42) (av. 24) µm long and 1.5–2 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, smooth to verrucose, subhyaline to hyaline; collarettes 1–1.5 µm long, 1.5 µm wide; type I phialides most common; type I phialides cylindrical or becoming widened at the base, (1.5–)2–9(–15) × 1–1.5 (av. 5 × 1) µm; type II phialides either subcylindrical some elongate-ampulliform and attenuated at the base or navicular, tapering towards the apex, 5–13 × 1.5–2 (av. 11 × 2) µm; type III phialides subcylindrical, 14–19(–21) × 1.5–2 (av. 16 × 2) µm, tapering gradually to a long neck. *Conidia* mostly oblong-ellipsoidal occasionally reniform, 3–4(–5) × 1–1.5 (av. 4 × 1) µm, L/W = 4.

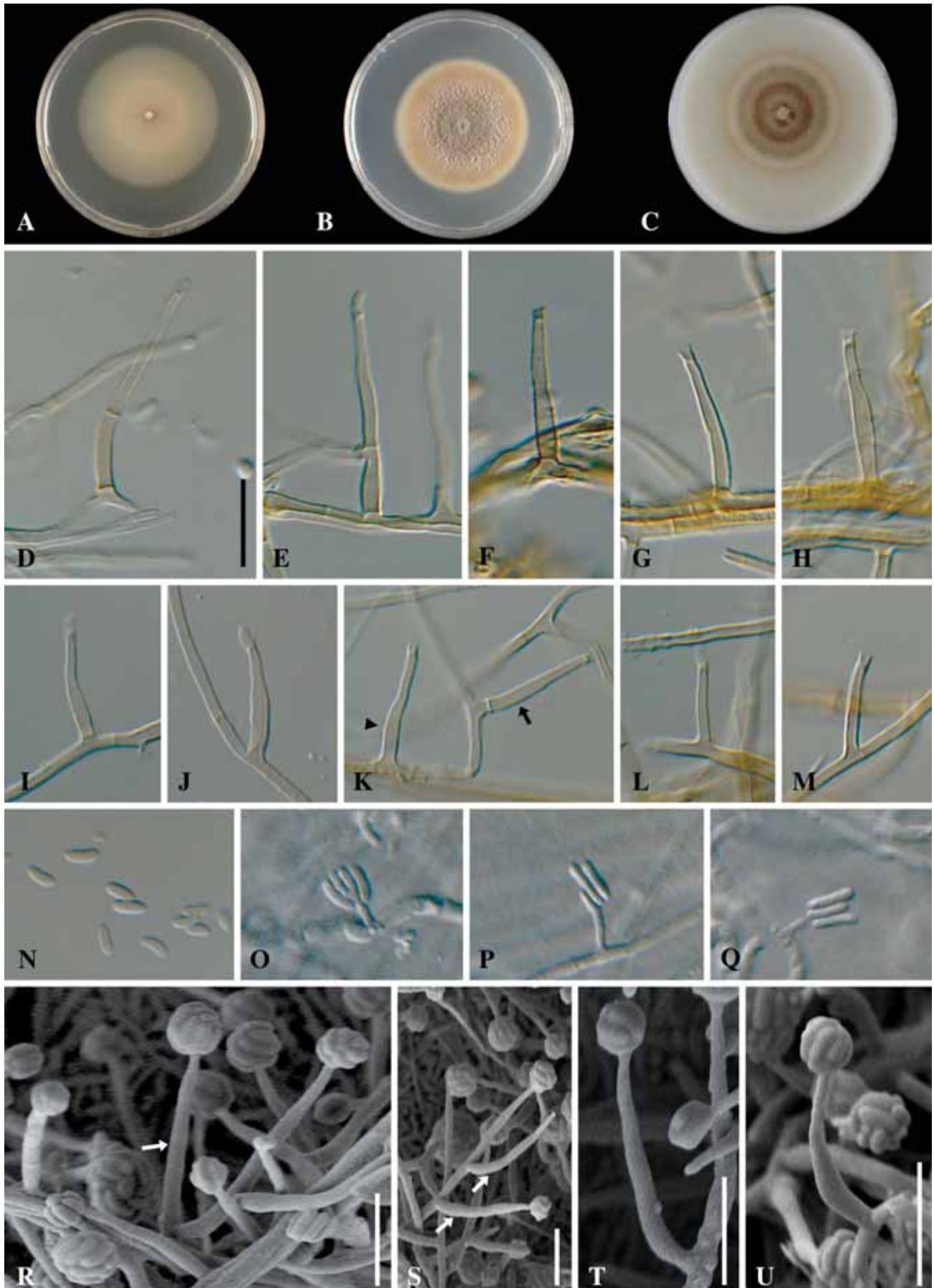
*On surface or submerged in the agar: Phialides* hyaline, cylindrical, 2–10(–41) × 1(–1.5) (av. 4 × 1) µm. *Conidia* hyaline, allantoid or oblong-ellipsoidal, 4–6(–6.5) × 1(–1.5) (av. 5 × 1) µm, L/W = 5.

*Type: South America*, Ecuador, Pichincha Province, Rio Caoni, Vicente Maldonado, isolated as an endophyte from stem of *Theobroma gileri*, 8 May 2000, H.C. Evans, dried MEA colony herb. CBS 17453, **holotype**; culture ex-type CBS 111586.

*Cultural characteristics:* Colonies reaching a radius of 11 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 30 °C, maximum 30 °C. Colonies on MEA flat, felty to cottony, with entire edge; after 8 and 16 d brownish orange to orange-white (5C3–A2) above, in reverse greyish brown to orange-white towards the edge (5D3–A2). Colonies on PDA flat, felty, with entire edge; after 8 d pale yellow (4A3) above and in reverse; after 16 d brownish grey (6C2) above, in reverse pale brown (7D5). Colonies on OA flat, smooth to yeast-like, with entire edge; after 8 d yellowish white (3A2) above, after 16 d above, pale yellow (3A3). Colonies producing yellow pigment on OA.



**Fig. 43.** *Phaeoacremonium theobromatis*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–N, R–U. Aerial structures on MEA. D–F. Conidiophores. G–H. Type III phialides. I–K. Type II phialides. L–M. Type I phialides. N. Conidia. O–Q. Structures on the surface of and in MEA. O–P. Adelophialides with conidia. Q. Conidia. R. Conidiophore. S. Type III phialides. T. Type II phialide. U. Conidiophore (arrow) and type I phialide (arrow head). A–U from CBS 111586. D–Q: DIC; R–U: SEM. Scale bars: D–U = 10  $\mu$ m. Scale bar for D applies to E–Q.



**Fig. 44.** *Phaeoacremonium venezuelense*. A–C. Sixteen-day-old colonies on MEA (A), PDA (B) and OA (C). D–N, R–U. Aerial structures on MEA. D–E. Conidiophores. F–H. Type III phialides. I–J. Type II phialides. K. Type II (arrow) and type I (arrow head) phialides. L–M. Type I phialides. N. Conidia. O–Q. Structures on the surface of and in MEA. Adelophialides with conidia. R. Branched conidiophore (indicated by arrow). S–T. Type III phialides (arrows in S). U. Type II phialide. A–U from CBS 651.85. D–Q: DIC; R–U: SEM. Scale bars: D–U = 10 μm. Scale bar for D applies to E–Q.

*Substrate: Theobroma gileri.*

*Distribution:* Ecuador.

*Notes:* *Phaeoacremonium theobromatis* was first identified as an unknown species of *Acremonium* ("sp. 5") (Evans *et al.* 2003). It can be distinguished from the other pale brown *Phaeoacremonium* species (*Pm. amstelodamense*, *Pm. argentinum*, *Pm. austroafricanum*, *Pm. subulatum* and *Pm. venezuelense*) by yellow pigment production, phialide types and maximum growth temperature. Of the species listed, only *Pm. theobromatis*, *Pm. subulatum* and *Pm. austroafricanum* produce yellow pigment in OA. *Phaeoacremonium theobromatis* and *Pm. austroafricanum* both have a maximum growth temperature of 30 °C, contrasting with the 37 °C maximum recorded for *Pm. subulatum*. *Phaeoacremonium austroafricanum* produces pale yellow colonies on OA, distinct from the red colonies of *Pm. theobromatis*, and also tends to produce type III phialides, while *Pm. theobromatis* produces a significantly higher proportion of type I phialides.

**23. *Phaeoacremonium venezuelense*** L. Mostert, Summerb. & Crous, *J. Clin. Microbiol.* 43: 1764. 2005. Fig. 44 A–U.

*Aerial structures:* Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 6; hyphae strongly tuberculate with warts up to 1 µm diam, verruculose, orange-brown to pale brown and 1–3 µm wide. Conidiophores short and occasionally branched, 1–4-septate, sometimes bearing next to the terminal phialide a lateral one, (20–)28–34(–52) (av. 31) µm long, 1–2.5 (av. 2) µm wide. Percurrent rejuvenation occasionally occurring, with the newly proliferated segment markedly swollen at the base. Phialides terminal or lateral, mostly monophialidic, sparsely tuberculate to verruculose, occasionally smooth and pale brown to subhyaline; collarettes 1–3 µm long, 1–2 µm wide; type I phialides cylindrical, tapering towards the apex, (4.5–)5–14(–16) × 1–1.5(–2) (av. 9 × 1.5) µm; type II phialides mostly subcylindrical to navicular, tapering towards the apex, 12–14 × 1.5–2(–2.5) (av. 13 × 2) µm; type III phialides predominant, subcylindrical, navicular to subulate, (14–)15–23(–24) × (1–)1.5–2 (av. 18 × 1.5) µm, very gradually tapering towards the apex. *Conidia* oblong-ellipsoidal or fusiform-ellipsoidal, occasionally reniform-allantoid, 3–4(–5) × 1–1.5(–2) (av. 4 × 1) µm, L/W = 3.

*On surface or submerged in the agar:* Phialides hyaline, subcylindrical to cylindrical and a few elongate-ampulliform, (2–)3–29(–36) × 1–2 (av. 11 × 1) µm. *Conidia* hyaline, allantoid and some oblong-ellipsoidal with large guttules, 5–8 × 1–1.5 (av. 6 × 1) µm, L/W = 6.

*Type: Venezuela*, human mycetoma in foot, 1985, M.B. de Alborno, dried MEA colony in herb. CBS 7957, **holotype**; culture ex-type CBS 651.85 = ATCC 32628.

*Cultural characteristics:* Colonies reaching a radius of 9–16 mm in 8 d at 25 °C. Minimum temperature for growth 15 °C, optimum 30 °C, maximum 40 °C. Colonies on MEA flat, felty to powdery, with entire edge; after 8 d orange-white (5A2) or greyish orange (6C3) above, in reverse orange-white (5A2) or brownish orange (6C4); after 16 d brownish orange to orange-grey towards the edge (5C3–5B2) above, in reverse greyish brown to orange grey towards the edge (5D3–B2). Colonies on PDA flat, felty to short woolly, with entire edge; after 8 d brownish orange (6C4) or pale brown (6D4) above, in reverse brown (5F5–6E5); after 16 d brownish grey (7D2) or brown (5F5) above, in reverse brown (6E5–F4). Colonies on OA flat, felty, with entire edge; after 8 d orange-grey to orange white towards the edge (5B2–A2) above, after 16 d pale brown to brownish grey towards the edge (6D4–6C2).

*Substrate:* Human, *Vitis vinifera*.

*Distribution:* Canada, South Africa, Venezuela.

*Additional cultures examined:* **Canada**, Ontario, human, tissue from right ankle, 26 Nov. 2002, S. Krajden, St. Joseph's Health Centre, CBS 113595 = SF 9587 (02). **South Africa**, Western Cape, Paarl, Zandrift, trunk of *Vitis vinifera*, 2001, L. Mostert, CBS 110119. **Unknown**, CBS 113598 = C.P.C. 3697.

*Notes:* *Phaeoacremonium venezuelense* and the similar *Pm. amstelodamense* and *Pm. argentinense* can be distinguished from other pale brown species by the absence of yellow pigmentation on OA. *Phaeoacremonium amstelodamense* can easily be recognised by the olive-green colonies formed on OA. Conidiophores of *Phaeoacremonium venezuelense* are more often branched than those of *Pm. argentinense*. The type II phialides of *Pm. venezuelense* are subcylindrical, whereas those of *Pm. argentinense* are more often elongate-ampulliform. The small phylogenetic differences observed among the strains of *Pm. venezuelense* (Fig. 10) are also reflected in minor phenotypic differences. Strains CBS 110119 and CBS 113595 had prominent orange-brown mycelium. CBS 110119 had more warts than the other strains and had woolly tufts on the colonies, whereas the other strains were felty to powdery in texture. These phenotypic and genetic differences among the strains appear to be of minimal taxonomic value and cannot be considered a basis for proposing separate species.

### Species excluded from *Togninia*

Species of *Togninia* that have been re-examined as part of this study include *T. inconspicua*, *T. crataegi*, *T. vasculosa*, *T. cornicola* and *T. villosa*. Species described

as *Erostella* that were re-examined were *E. transversa* and *E. rhododendri*. No holotype specimens could be obtained for the other *Togninia* species described by Berlese (1900), namely *T. ambigua*, *T. jungens*, *T. salicis-babylonicae*, *T. reniformis*, *T. tetraspora* and *T. quaternarioides*, as well as *E. minutissima* A.I. Romero & Samuels (Romero & Samuels 1991). Berlese (1900) illustrated various species of which two, *T. ambigua* and *T. quaternarioides*, have stipitate asci. He suggested that these species probably do not belong to *Togninia*, which has asci with truncate bases. *Erostella minutissima* closely resembles the recognised species of *Togninia* in that the asci are sympodially arranged on the ascogenous hyphae and are also oblong-clavate with truncate bases. Romero & Samuels (1991) placed their specimen in *Erostella*, since Barr (1990) had reduced *Togninia* to synonymy under *Erostella*.

*Togninia* can be distinguished from genera in the *Calosphaeriales* by having asci with truncate bases and thickened apices, ascogenous hyphae that proliferate in an acropetal succession so that the asci appear spicate at maturity, and ascospores that are aseptate and hyaline. Genera of the *Calosphaeriales* generally have 8-spored, stipitate asci and often one- or more-septate ascospores which can be pigmented. However, in the case of *Pleurostoma* the asci are polysporous and subglobose with truncate bases.

**24. *Calosphaeria cornicola*** Ellis & Everh., Proc. Acad. Philadelphia: 342. 1894.

≡ *Togninia cornicola* (Ellis & Everh.) Berl., Icon. Fung. 3: 11. 1900.

*Anamorph*: Unknown.

*Perithecia* scattered or loosely collected in subvalsiform groups of 3–5, 200–250 µm diam, buried in the inner bark, covered by the epidermis, which is raised into little pustules and pierced by the papilliform ostiole. *Asci* clavate, 27–32 × 6–6.5 µm, rounded above and gradually narrowed towards the base. *Paraphyses* linear, nucleate, much longer than the asci. *Ascospores* biseriata, 0–3-septate, subhyaline, allantoid or oblong-ellipsoidal, smooth, 10–13(–14) × 2–3 (av. 12 × 2.5) µm.

*Substrate*: *Cornus asperifolia*.

*Distribution*: U.S.A.

*Types*: U.S.A., Kansas, Rockport, on dead branch of *Cornus asperifolia*, collection of E. Bartholomew No. 1470, May 1894, herb. NY, **lectotype** designated here (best material of sample No. 1470), herb. FH, **isotype**.

*Notes*: The description is partly based on that of Ellis & Everhart (1894). The holotype material is poor and only ascospores were observed. The pigmentation, septation and large ascospores of this specimen indicate that it is not a species of *Togninia*. Hence we use its original name in *Calosphaeria*.

**25. *Calosphaeria rhododendri*** (Rehm) L. Mostert, **comb. nov.** MycoBank MB500695, Fig. 45A–T.

*Basionym*: *Togninia rhododendri* Rehm, Ann. Mycol. 5: 536. 1907.

≡ *Erostella rhododendri* (Rehm) Sacc. & Trotter, Syll. Fung. 22: 353. 1913

*Anamorph*: Unknown.

*Perithecia* gregarious or valsoid in groups of 2–3, immersed, subepidermal, subglobose to flat, 140–490 µm tall and 320–620 µm diam (n = 7). Peridium consisting of two layers of *textura angularis*: outer region brown, cells often of the same shape as inner layer, approx. 4–6 cells and 14–20 µm thick; inner region hyaline (centrum), 3–6 cells and 6–19 µm thick. *Perithecial necks* black, curved, of medium length, 170–350 µm long, 40–45 µm wide at base and ca 50 µm at the apex. *Paraphyses* not seen. *Ascogenous hyphae* hyaline, smooth-walled, with short branches, constricted at the septa, producing subtending cells, up to 10 µm tall and up to 2 µm wide, each giving rise to an ascus. *Asci* unitunicate, in fascicles, clavate with obtuse to rounded apices, tapering towards a broadly stipitate base, short hair-like structure present at the base, 8-spored, 24–39(–44) × (5–)6–8 (av. 30 × 7) µm, stipe (2–)4–15(–20) (av. 10) µm long; apical region 1.5–4 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascospores* 0(–1)-septate, hyaline, oblong-ellipsoidal to slightly curved, smooth, biseriata, 7–10 × 2 (av. 9 × 2) µm.

*Substrate*: *Rhododendron hirsutum*.

*Distribution*: Germany.

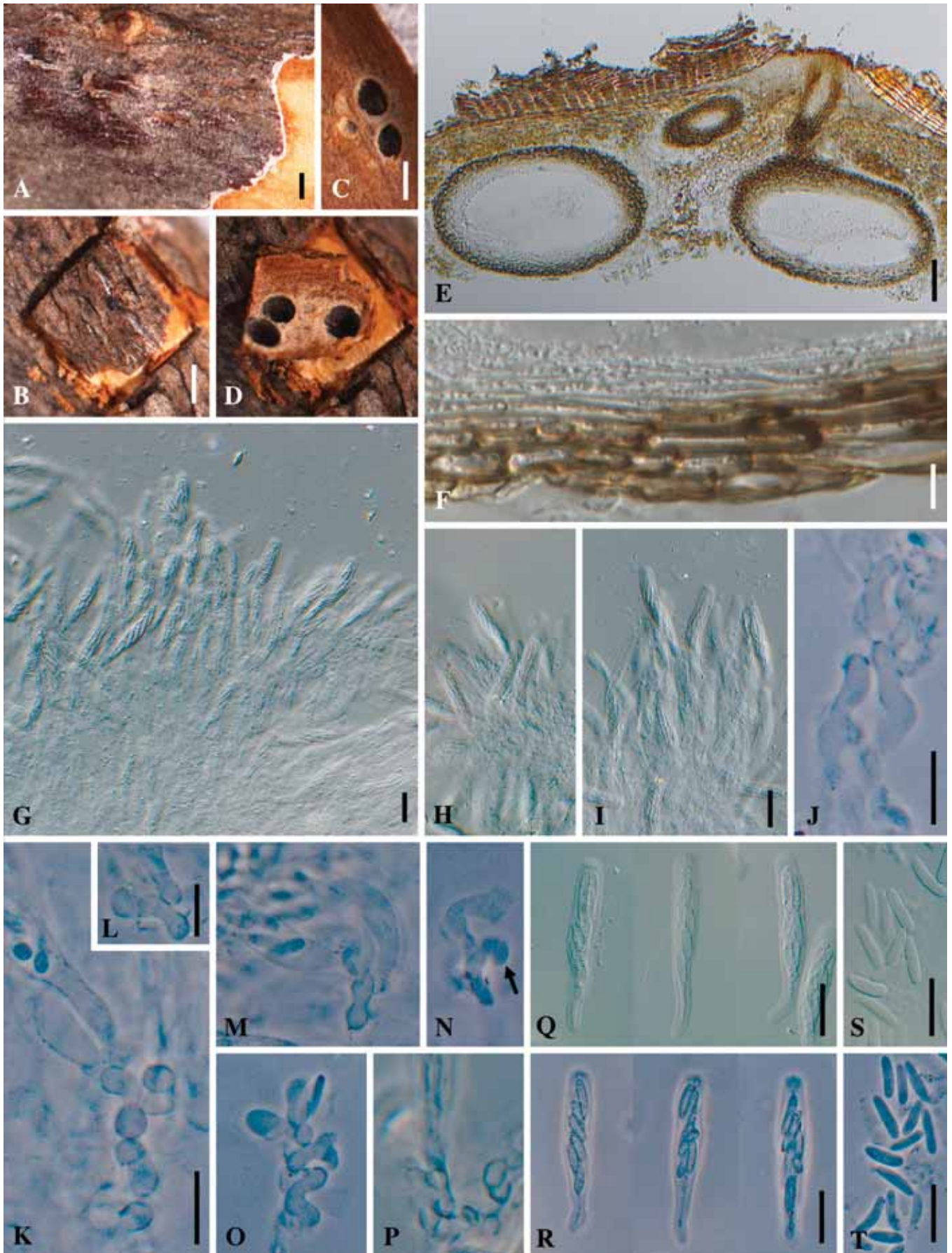
*Type*: **Germany**, Bavarian Alps, between Schlehdorf and Herzogenstand Mountain, on a branch of *Rhododendron hirsutum*, 1905, Rehm, herb. S F6218, **holotype**.

*Notes*: The specimen resembles *Calosphaeria aurata* Nits. in that the asci and necks are subepidermal and the basal part of the perithecium is flattened. Also, the ascospores are in the same size range as *C. aurata*, 8–10 µm long. *Calosphaeria rhododendri*, however, differs in having fewer perithecia (2–3) clustered together than the 5–15 aggregated in *C. aurata*. The ascospores of *C. aurata* are lunate, unlike the oblong-ellipsoidal ascospores of *C. rhododendri*. *Calosphaeria aurata* has septate paraphyses whereas no paraphyses were observed in *C. rhododendri*.

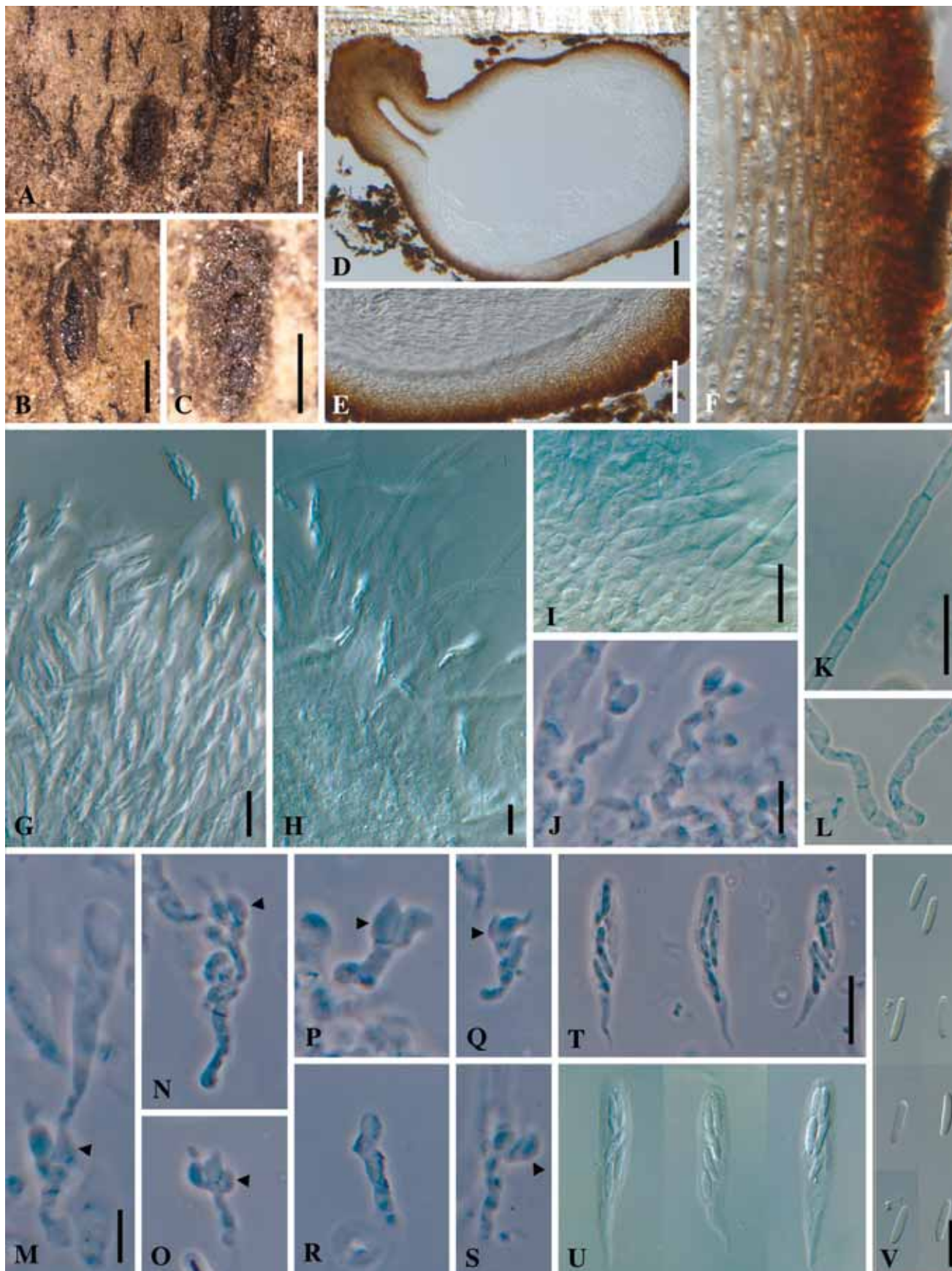
**26. *Calosphaeria transversa*** (Sacc. & Fairm.) L. Mostert, **comb. nov.** MycoBank MB500696. Fig. 46A–V.

*Basionym*: *Erostella transversa* Sacc & Fairm., J. Mycol. 12: 48. 1906.

≡ *Togninia transversa* (Sacc & Fairm.) House, Bull. N.Y. State Mus. 233/234: 24. 1921.

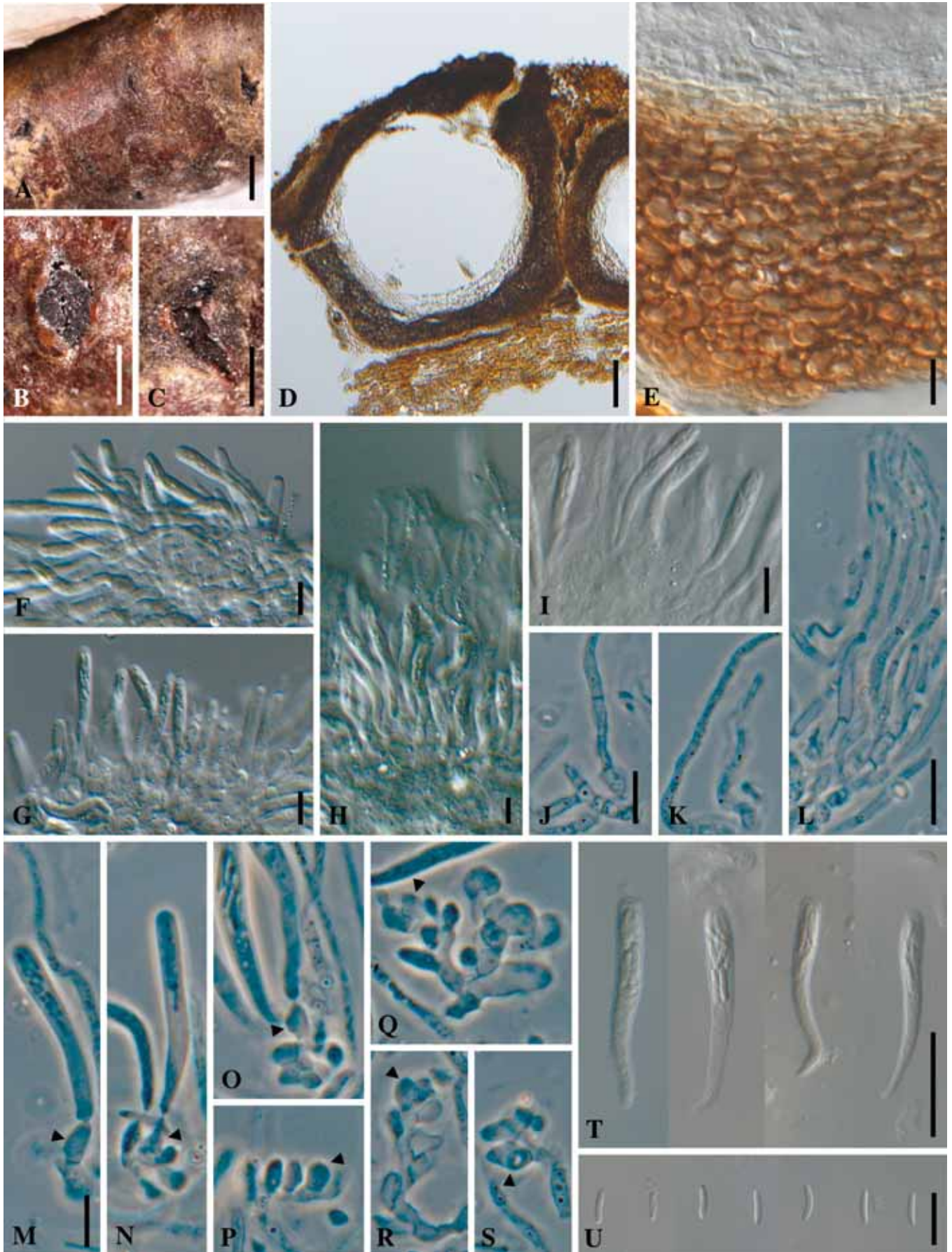


**Fig. 45.** *Calosphaeria rhododendri*. A–B. Perithecia beneath the bark of *Rhododendron hirsutum*. C–D. Perithecia sunken into the turned-over bark. E–F. Longitudinal section through perithecia; peridium (F). G–I. Asci. J–P. Ascogenous hyphae; crozier formation on apex of ascogenous hyphae (O). Q–R. Asci. S–T. Ascospores. A–T from F6218 (holotype). A–D: DM; E–I, Q, S: DIC; J–P, R, T: PC. Scale bars: A–D = 500 µm; E = 100 µm; G–I = 20 µm; F, J–T = 10 µm. Scale bar for C applies to D; bar for G applies to H; bar for L applies to M–P.



**Fig. 46.** *Calosphaeria transversa*. A–C. Lenticells on *Betula* sp. with perithecia breaking through the openings. D–F. Longitudinal sections through perithecia; asci attached to the inner wall of the peridium (E); peridium with inner cells having pale brown walls (F). G–I. Asci and paraphyses. J. Ascogenous hyphae. K–L. Paraphyses. M–S. Ascogenous hyphae; immature ascus attached to subtending cell on ascogenous hypha (M); subtending cell becoming ‘pointed’ at the apex (P); subtending cells indicated by arrow heads. T–U. Asci. V. Ascospores. A–V from holotype (PAD). A–C: DM; D–I, U, V: DIC; J–T: PC. Scale bars: A–D = 100  $\mu$ m; E = 50  $\mu$ m; G–I = 20  $\mu$ m; J–V = 10  $\mu$ m. Scale bar for K applies to L; bar for M applies to N–S; bar for T applies to U.





**Fig. 47.** *Calosphaeria tumidula*. A–C. Perithecia breaking through lenticells and cracks in the bark of *Fagus sylvatica*. D–E. Longitudinal section through perithecia; peridium (E). F–I. Asci; asci and paraphyses (H). J–L. Paraphyses. M–S. Ascogenous hyphae; immature asci attached to subtending cells on ascogenous hyphae (M–O); subtending cells indicated by arrow heads. T. Asci. U. Ascospores. A–U from holotype (PAD). A–C: DM; D–I, T, U: DIC; J–S: PC. Scale bars: A = 1000  $\mu$ m; B–C = 500  $\mu$ m; D = 100  $\mu$ m; E–U = 10  $\mu$ m. Scale bar for J applies to K; bar for M applies to N–S.

*Anamorph*: Unknown.

*Perithecia* valsoid, in groups of 2–7, immersed, subepidermal, breaking through lenticels, globose to subglobose, 441–784 µm tall and 392–735 µm diam ( $n = 9$ ); apex rounded, papillate. Peridium consisting of two layers of *textura angularis*: outer region dark brown, cells more rounded and smaller than those of the inner layer, approx. 10–11 cells and 20–30 µm thick; inner region hyaline (centrum) to pale brown, 6–10 cells and 17–23 µm thick. *Perithecial necks* black, 196–564 µm long and 132–269 µm wide. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 95–205 µm long, 4–6 µm wide at the base and 1.5–2 µm at the apex, persistent, arising from the basal cells of each fascicle of asci. *Ascogenous hyphae* hyaline, smooth, elongated in the direction of growth, with short branches, sympodially producing a series of subtending cells, 5–12 × 3–5 µm, each giving rise to an ascus. *Asci* unitunicate, in fascicles, clavate, apex obtuse to bluntly rounded, stipitate, with a short hair-like structure at the base, 8-spored, 20–29 × (5–)6–8 (av. 24 × 7) µm, stipe 11–25 (av. 17) µm long; apical region 2–3 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascospores* 0–1-septate, subhyaline, oblong-ellipsoidal, smooth, small guttulate, biseriata, 8–10(–11) × 1.5–2 (av. 8.5 × 1.5) µm.

*Substrate*: *Betula* sp.

*Distribution*: U.S.A.

*Type*: U.S.A., New York, in forest near Lyndonville, on bark of *Betula* sp., 30 Sep. 1905, C.E. Fairman, herb. PAD, **holotype**.

*Notes*: *Calosphaeria transversa* is most similar to *C. acerina* Ellis & Everh. The two species have clavate asci as well as paraphyses, and their ascospores are similar in size and shape. However, they differ in that the perithecial aggregations of *C. acerina* include 4–12 perithecia, in contrast to the 2–7 perithecia observed in aggregations of *C. transversa*. The sporiferous part of the ascus of *C. acerina*, 35–40 × 6 µm, is longer than that of *C. transversa*, 20–29 µm. Furthermore, ascospores of *C. acerina* are aseptate and allantoid, while those of *C. transversa* are 0–1-septate, and oblong-ellipsoidal.

**27. *Calosphaeria tumidula*** Sacc., Atti Soc. Veneto-Trent. Sci. Nat. Padova 4: 77–100 (Fungi ven. novi, Ser. 4: 20). 1875. Fig. 47A–U.  
= *Togninia minima* var. *tumidula* (Sacc.) Berl., Icon. Fung. 3: 11. 1900.

*Anamorph*: Unknown.

*Perithecia* gregarious or in valsoid groups of 3–6, immersed, subepidermal, immersed in bark, globose to subglobose, 368–480 µm tall and 368–612 µm diam ( $n = 6$ ); apex rounded, papillate. Peridium consisting

of two layers of *textura angularis*: outer region brown, with cells smaller and more rounded than those of the inner layer, approx. 11–12 cells and 50–53 µm thick; inner region hyaline (centrum) to pale brown, 7–8 cells and 15–20 µm thick. *Perithecial necks* black, 94 µm long and 140 µm wide. *Paraphyses* hyaline, septate, cylindrical, narrowing towards a thread-like tip, 40–120 µm long, 2–3 µm wide at the base and 1–1.5 µm at the apex, persistent, arising from the basal cells of each fascicle of asci. *Ascogenous hyphae* hyaline, smooth-walled, with short branches, producing a sympodial succession of subtending cells, 5–10 × 3 µm, each giving rise to an ascus. *Asci* unitunicate, in fascicles, oblong with obtuse to rounded apex, stipitate, 8-spored, 15–20 × 4–5 (av. 17 × 4) µm, stipe 17–25 (av. 21) µm long. Apical region 1–2 µm thick, of indistinct structure, with a non-amyloid apical ring. *Ascospores* aseptate, hyaline, cylindrical to allantoid, smooth, biseriata when young, becoming pluriseriate, (4)5–8 × 1–1.5 (av. 6 × 1) µm.

*Substrate*: *Fagus sylvatica*

*Distribution*: Italy.

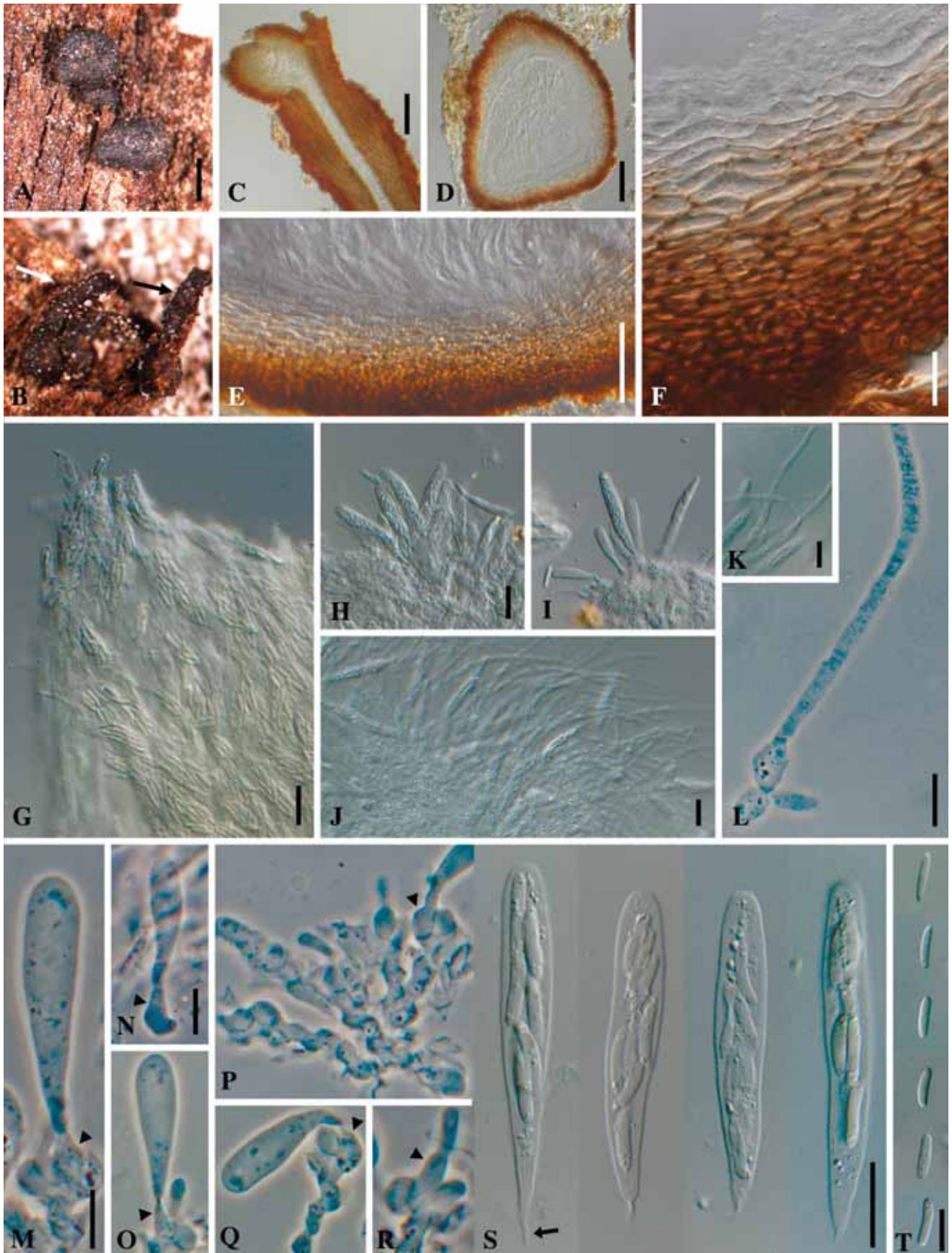
*Type*: **Italy**, Treviso, in a forest near Cansiglio, branches of *Fagus sylvatica*, with bark, Saccardo, herb. PAD, **holotype**.

*Notes*: Our examination of the type specimen showed that the perithecia were larger than the range of 250–330 µm diam reported by Saccardo. The classification in *Calosphaeria* is probably correct.

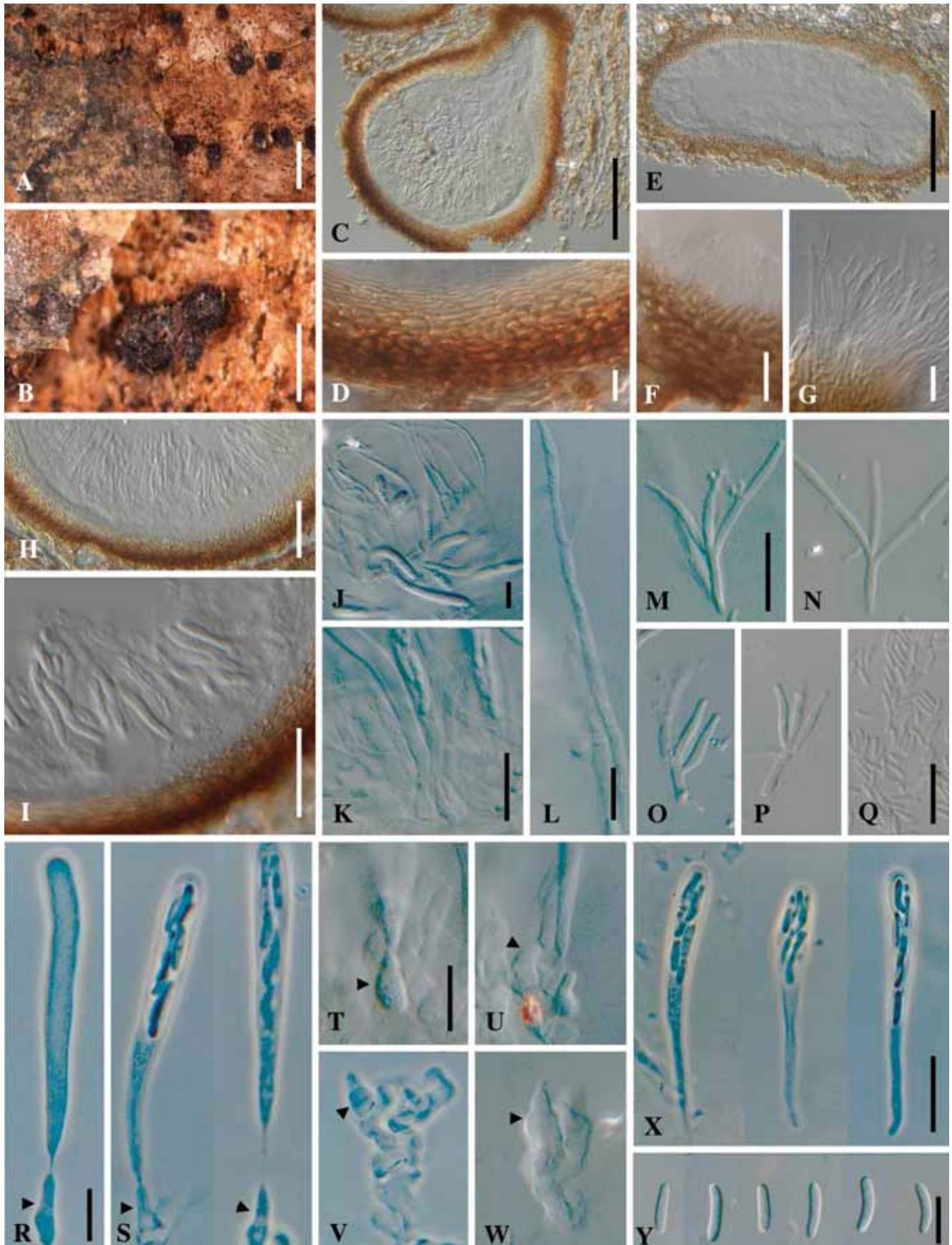
**28. *Calosphaeria vasculosa*** Sacc., Syll. Fung. 1: 101. 1882. Fig. 48A–U.  
= *Togninia vasculosa* (Sacc.) Berl., Icon. Fung. 3: 9. 1900.

*Anamorph*: Unknown.

*Perithecia* in valsoid groups of 3–8, immersed, subepidermal, subglobose to obpyriform, 392–657 µm tall and 343–515 µm diam ( $n = 3$ ); apex rounded, papillate or short-necked. Peridium consisting of two layers of *textura angularis*: outer region brown, with cells smaller and more rounded than those of the inner layer, approx. 8–10 cells and 22–30 µm thick; inner region hyaline (centrum) to pale brown, 9–12 cells and 30–35 µm thick. *Perithecial necks* straight to curved, black, 635–880 µm long and 110–155 µm wide at the base and 80–125 µm wide at the apex. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 110–225 µm long, 2–3.5 µm wide at the base and 1.5–2 µm at the apex, not persistent, arising from the basal cells of each fascicle of asci. *Ascogenous hyphae* hyaline, smooth-walled, with short branches, producing a sympodial sequence of subtending cells, up to 7 × 4 µm, each giving rise to an ascus. *Asci* unitunicate, in fascicles, clavate and stipitate, with a short hair-like



**Fig. 48.** *Calosphaeria vasculosa*. A–B. Perithecia underneath the bark of *Betula alba*; neck of perithecium indicated by arrow (B). C–F. Longitudinal section through perithecia; apex of a neck (C); asci attached to the inner wall of the peridium (E); peridium (F). G–J. Asci; asci and paraphyses (J). J–L. Paraphyses. M–R. Ascogenous hyphae; immature asci attached to subtending cell on ascogenous hyphae (M–O); subtending cells indicated by arrow heads. S. Asci; short hair-like structure indicated by arrow. T. Ascospores. A–U from holotype PAD, no. 782. A–B: DM; C–K, S, T: DIC; L–R: PC. Scale bars: A–B = 500  $\mu$ m; C–E = 50  $\mu$ m; G–K = 20  $\mu$ m; F, L–T = 10  $\mu$ m. Scale bar for A applies to B; bar for H applies to I; bar for N applies to O–R.



**Fig. 49.** *Jattaea villosa*. A–B. Perithecia exposed from underneath the bark of *Carpinus* sp. C–D. Longitudinal section through perithecia; peridium (D). E–G, M–Q. Spermatogonia. E–F. Longitudinal sections through spermatogonia. G. Spermatophores. H–I. Asci attached to peridium. J–K. Asci; asci and paraphyses (J). L. Paraphyses. M–P. Spermatophores with phialides. Q. Spermatia. R–S. Subtending cells with immature asci. T–W. Ascogenous hyphae with subtending cells; subtending cells indicated by arrow heads. X. Asci. Y. Ascospores. A–Y from holotype B 700009127. A–B: DM; C–Q, Y: DIC; R–X: PC. Scale bars: A = 1000  $\mu$ m; B = 500  $\mu$ m; C, E = 100  $\mu$ m; H–I = 50  $\mu$ m; J–K = 20  $\mu$ m; D, F–G, L–Y = 10  $\mu$ m. Scale bar for M applies to N–P; bar for R applies to S; bar for T applies to U–W.

structure at the base, 8-spored, (24–)30–43(–45) × 7–10(–11) (av. 35 × 8) μm, stipe (13–)15–26(–29) (av. 21) μm long; apical region 4–5 μm thick, of indistinct structure, with a non-amyloid apical ring. *Ascospores* 0–1-septate, hyaline or subhyaline, cylindrical to slightly curved, smooth, minutely guttulate, biseriate, 10–13 × 2–2.5 (av. 11 × 2) μm.

*Substrate*: *Betula alba*, *Prunus spinosa*.

*Distribution*: France.

*Type*: **France**, Rouen, on branches of *Betula alba*, Saccardo, herb. PAD no. 728, **holotype**.

*Additional specimen examined*: Unknown, *Prunus spinosa* with *Cucubitararia*, June 1875, A.C. Bruinsma, herb. M 0098799.

*Notes*: Specimen M 0098799 differs from the type in that the asci are shorter, 22–28 × 7–9 (av. 27 × 7.5) μm, and have longer stipes, 16–30 μm (av. 26) μm. The ascospores, however, are similar in morphology.

**29. *Jattaea villosa*** (Nits.) L. Mostert, **comb. nov.** MycoBank MB500697. Fig. 49A–Y.

*Basionym*: *Calosphaeria villosa* Nits., Pyrenom. Germ.: 98. 1867.

≡ *Togninia villosa* (Nits.) Berl., Icon. Fung. 3: 10. 1900.

*Anamorph*: Unknown.

*Perithecia* separate, often in rows, sometimes in groups of 2–5, immersed, subepidermal, subglobose, 330–380 μm tall and 310–360 μm diam (n = 4); apex rounded, papillate, rarely short-necked. Peridium consisting of two layers of *textura angularis*: outer region brown, with cells smaller and more rounded than those of the inner layer, approx. 5–8 cells and 14–16 μm thick; inner region hyaline (centrum) to pale brown, 6–10 cells and 12–22 μm thick; surface covered with brown, septate, hyphal appendages. *Perithecial necks* black, 122 μm long and 49 μm wide. *Paraphyses* hyaline, septate, cylindrical, narrowing towards the tip, 47–100 (av. 88) μm long, 2–3 μm wide at the base and 1–2 μm at the apex, persistent, arising from the basal cells of each fascicle of asci. *Ascogenous hyphae* hyaline, smooth, reduced to subglobose cells, often mucronate at the apex, 5–8 μm tall and 3–5 μm wide, arranged alongside each other. *Asci* unitunicate, in fascicles, clavate and stipitate, 8-spored, 20–47(–55) × 5–7(–10) (av. 34 × 6) μm, stipe 7–30(–35) (av. 14) μm long; apical region 2–2.5 μm thick, of indistinct structure, with a non-amyloid apical ring. *Ascospores* 0(–3)–septate, hyaline, allantoid or oblong-ellipsoid, smooth, biseriate when young, becoming crowded in the ascus, 8–11(–12) × (1–)1.5–2 (av. 9 × 2) μm. *Spermatogonia* occur together with perithecia, subglobose to flattened, subepidermal, ostiolate, ca 220 μm tall and 145–565 μm diam, wall consisting of compacted hyphal growth, 18–20 μm thick; centrum lined with *spermatophores*,

hyaline, branched, 20–40 long and 1–1.5 wide at the base. *Phialides* hyaline, subcylindrical or elongate-ampulliform, 5–15 μm long and 1–1.5 μm wide at the base. *Spermatia* hyaline, cylindrical to slightly curved with rounded ends, 3–4 × 1 μm.

*Substrate*: *Carpinus* sp.

*Distribution*: Germany.

*Type*: **Germany**, Westfalen, Münster, Münster-Nienberge, on *Carpinus* sp., 1867, Nitschke, herb. B 700009127, **holotype**.

*Additional specimens examined*: **Germany**, Brandenburg, Landkreis Havelland, Rathenower Stadtforst, on *Carpinus*, herb. B 700010035.

*Notes*: The second specimen examined differed somewhat from the type: it had larger perithecia (ranging from 400–630 in diam) that were relatively frequently arranged in groups, and also had asci with relatively long stipes (av. 30 μm). *Jattaea villosa* resembles *J. berlesiana* Sacc. & Trav. in that both species have the same ascospore shape and size, and both have spermatia associated with the perithecia. *Jattaea villosa* is distinct, however, having broad perithecia 310–360 μm diam, while those of *J. berlesiana* are approximately 200 μm diam; the varying ascus size, [20–47(–55) × 5–7(–10)] μm, contrasts with the relatively uniform asci of *J. berlesiana*, (38–42 × 6–7 μm). The ascospores of *J. villosa* can be up to 3-septate, while those of *J. berlesiana* are aseptate.

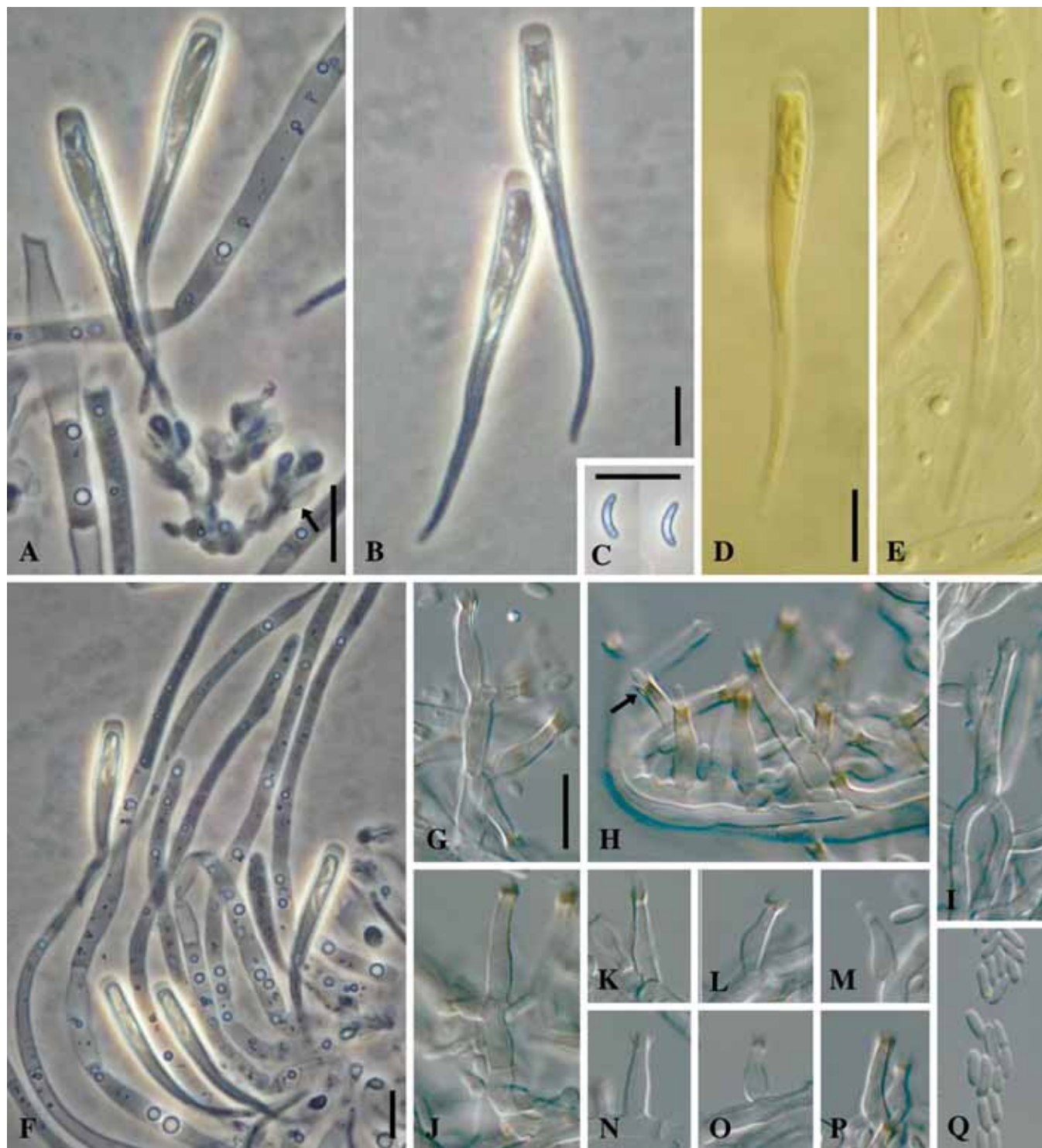
#### Genera of the *Calosphaeriales* resembling *Togninia*

The current concept of the *Calosphaeriales* includes the families *Calosphaeriaceae* and *Pleurostomataceae*. Detailed morphological study of the centrum and specifically of the type of ascogenous hyphae led Réblová *et al.* (2004) to include *Calosphaeria*, *Togniniella*, *Jattaea* and *Wegelina* in the *Calosphaeriaceae*. What these fungi have in common is that the asci are arranged in fascicles arising singly from short branches, i.e. lateral and terminal subtending cells, of the ascogenous hyphae, produced in a sympodial succession. Of these genera, only *Calosphaeria* and *Togniniella* have had the phylogenetic relationship confirmed with sequence data (Réblová *et al.* 2004). The genus *Pleurostoma* has been placed in the *Pleurostomataceae* (Réblová *et al.* 2004). This family is currently characterised by having short proliferating ascogenous hyphae with asci arising from a crozier system. The asci are arranged in a short spicate formation with a bulbous base that remains attached to the ascogenous hyphae after ascus dehiscence. The *Togniniaceae* (*Diaporthales*), including the genera *Togninia* and *Romellia*, have ascogenous hyphae that proliferate and continue to elongate and branch in an acropetal succession, giving rise to asci directly in a spicate arrangement. The placement of the

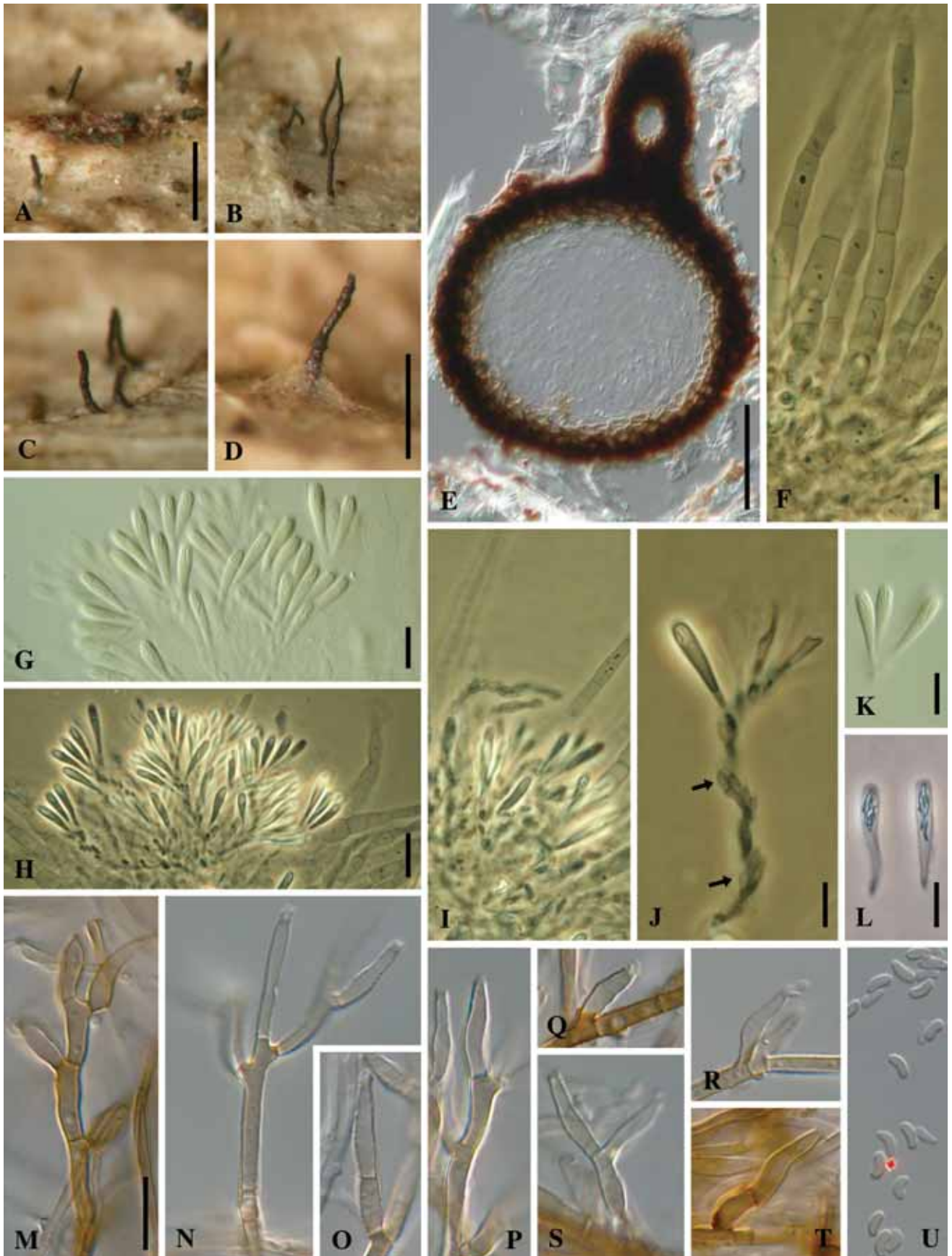
two stromatic genera *Pachytrype* and *Enchnoa* remains uncertain. *Pachytrype* has been described as having spicate asci, characteristic of the *Calosphaeriales* and the *Togniniaceae*. Re-examination of type specimens as well as collection of culturable specimens will be necessary to understand the position of this genus.

The genera of the *Calosphaeriales* and *Togniniaceae* can be distinguished on various characters. *Calosphaeria* as typified by *Calosphaeria*

*pulchella* (Fig. 50A–F) is the only genus that has perithecia in a valsoid arrangement. The anamorph, *Calosphaeriophora*, typified by *Calosphaeriophora pulchella* (Fig. 50G–Q), has phialides with distinctly pigmented necks. *Togniniella* features long-necked immersed perithecia and spicately arranged asci. This combination of features can be seen in *Togniniella acerosa* (Fig. 51A–L).



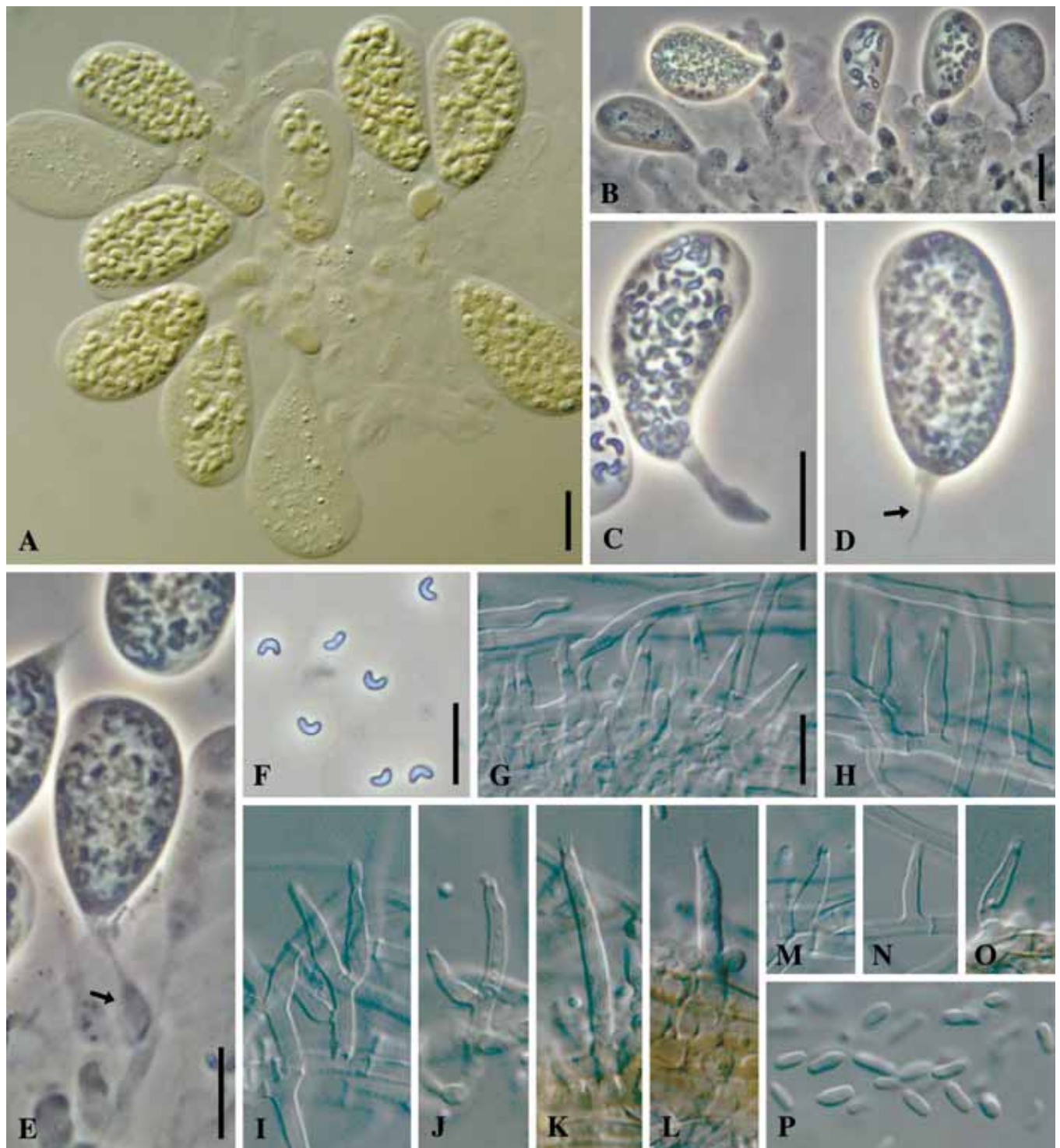
**Fig. 50.** A–F. *Calosphaeria pulchella*, teleomorph. A. Ascogenous hyphae with asci. B, D–E. Asci. C. Ascospores. F. Paraphyses intermingled with asci. G–Q. *Calosphaeriophora pulchella*, anamorph. G, J. Conidiophores. H–I, K–P. Phialides with pigmentation around the necks (arrow in H). Q. Conidia. A–F from PRM 901842 and G–Q from ex-type culture CBS 115999 (MEA, 14 d old). D–E, G–Q: DIC; A–C, F: PC. Scale bars: A–Q = 10 µm. Scale bar for E applies to E; bar for G applies to H–Q. Photographs A–F by Réblová.



**Fig. 51.** A–L. *Togniniella acerosa*, telomorph. A–D. Necks breaking through bark of *Nothofagus* sp. E. Longitudinal section through perithecium. F. Paraphyses. G–I. Asci attached to ascogenous hyphae intermingled with paraphyses. J. Ascogenous hypha with subtending cells (arrows) from which single asci arise. K–L. Asci. M–U. *Phaeocrella acerosa*, anamorph. M–P, S–T. Conidiophores. Q–R. Phialides. U. Conidia. A–F, I–L from PDD 81431 (holotype); G, H, PDD 81432 and M–U from ex-type culture CBS 113648 (MEA, 14 d old). A–D: DM, E, G, K, M–U: DIC; F–J, L: PC. Scale bars: A–D = 500  $\mu$ m; E = 100  $\mu$ m; H–I = 20; D, F–G, J–U = 10  $\mu$ m. Scale bar for A applies to B–C; bar for H applies to I; bar for M applies to N–U. Photographs F–L by Réblová.

Its anamorph, *Phaeocrella acerosa* (Fig. 51M–U) can be recognised by its branching conidiophores that are basally pigmented. The genus *Pleurostoma* is distinct in having perithecial necks that are often lateral, as well as asci that are subglobose or obpyriform and arranged in a short spicate arrangement (*Pleurostoma ootheca*, Fig. 52A–F). Its anamorph *Pleurostomophora* includes three species that differ in morphology.

*Pleurostomophora ootheca* (Fig. 52G–P) has hyaline and mostly discrete phialides and dimorphic conidia. *Pleurostomophora richardsiae* has pigmented phialides and flaring collarettes, and it can produce two conidial types, i.e. brown, (sub)globose conidia and hyaline, ellipsoidal conidia. *Pleurostomophora repens* produces (sub)hyaline, complex conidiophores and cylindrical conidia. According to Barr (1985), *Jattaea* can be

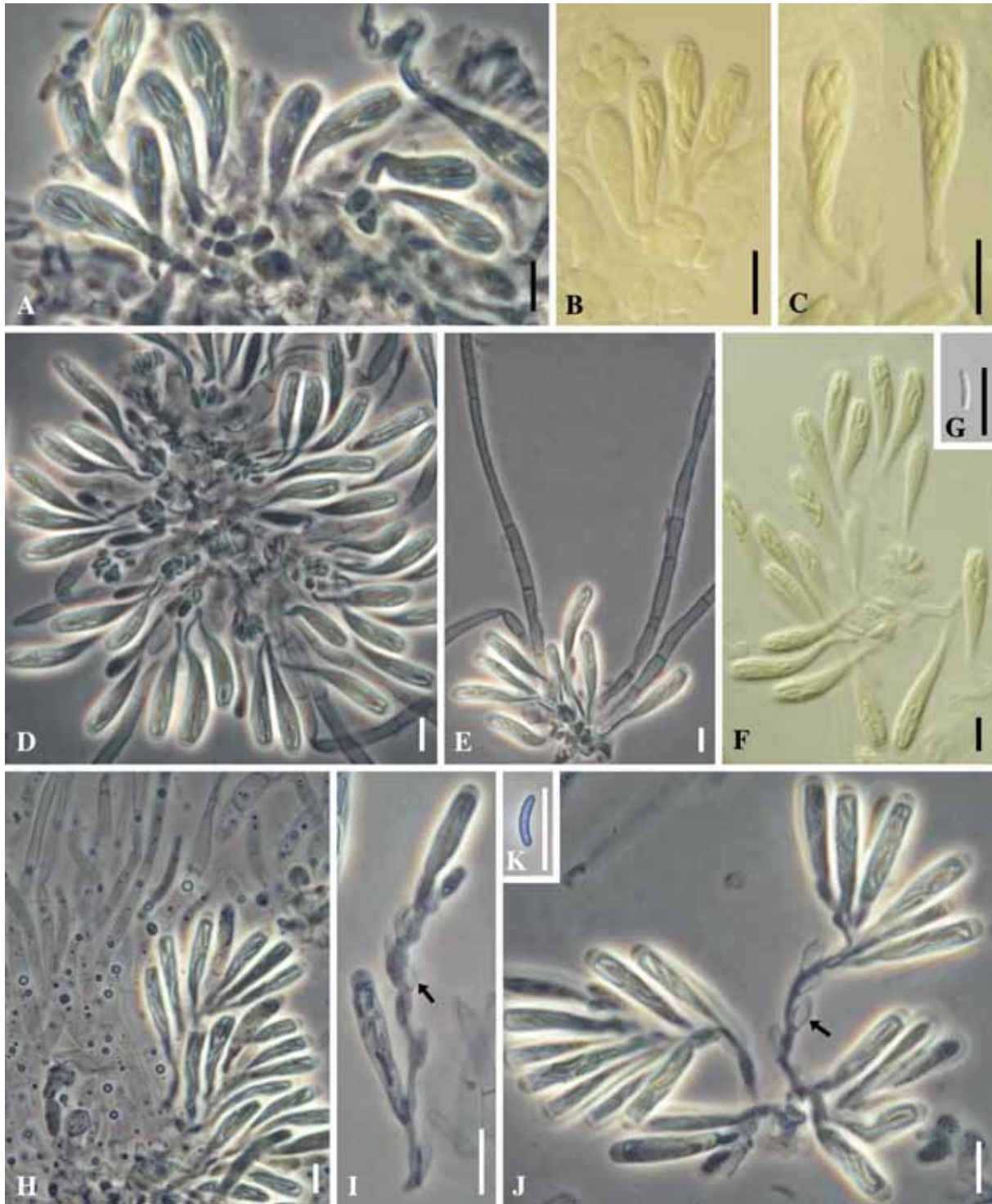


**Fig. 52.** A–F. *Pleurostoma ootheca*, teleomorph. A–B. Ascogenous hyphae with asci. C–D. Asci; asci often have a hair-like structure at the base (indicated by arrow, D). E. Ascus attached to subtending cell (arrow). F. Ascospores. G–P. *Pleurostomophora ootheca*, anamorph. G–H. Phialides. I–L. Conidiophores. M–O. Phialides. P. Conidia. A–F from K 122385 and G–P from ex-type culture CBS 115329 (MEA, 14 d old). A, G–P: DIC; B–F: PC. Scale bars: A–P = 10 µm. Scale bar for C applies to D; bar for G applies to H–P. Photographs A–F by Réblová.



distinguished from *Calosphaeria* by short papillae or necks and by perithecia not forming circinate groups. The ascogenous hyphae are short and the asci are arranged in fascicles, as can be seen in *Jattaea microtheca* (Fig. 53A–C). The genus *Wegelia* can be distinguished by having long necks, short ascogenous hyphae and asci arranged in fascicles. Representative short ascogenous hyphae are illustrated for *Wegelia*

*discreta* (Fig. 53D–G). Species of *Romellia* can be recognised by short, broad necks and a spicate arrangement of the asci. This spicate arrangement is seen in *Romellia vibratilis* (Fig. 53H–K). *Enchnoa* has perithecia with a diameter larger than 500 µm, usually surrounded by a pigmented hyphal tomentum. The species of *Pachytrype* are stromatic, with long necks and spicately arranged asci.



**Fig. 53.** A–C. *Jattaea microtheca*. A–B. Ascogenous hyphae with asci. C. Asci. D–G. *Wegelia discreta*. D–F. Asci attached to ascogenous hyphae, in E. intermingled with paraphyses. G. Ascospore. H–K. *Romellia vibratilis*. H. Asci and paraphyses. I–J. Asci attached to ascogenous hyphae; sympodially arranged subtending cells (arrows). K. Ascospore. A–C from NY (isotype), D–G from DAOM 35410, H–K from K. & L. Holm 3848a in NY. B–C, F–G: DIC; A, D–E, H–K: PC. Scale bars: A–K = 10 µm. Photographs A–K by Réblová.

## The genera of the *Calosphaeriales*

*Calosphaeria* Tul. & C. Tul., Sel. Fung. Carpol. 2: 108. 1863.

*Anamorph*: *Calosphaeriophora* Réblová, L. Mostert, W. Gams & Crous, Stud. Mycol. 50 (2004): 542. 2005.

*Ascomata* immersed, small to medium sized, globose or sphaeroid, usually oblique and in circinate groups, in valsoid configuration with necks converging, at times separate and scattered; surface glabrous and smooth, verrucose, or with hyphal coating; peridium of compressed rows of reddish brown cells; necks elongate, at times emerging through the stromatic discs. *Asci* unitunicate, clavate, stipitate, in fascicles, 8-spored. *Paraphyses* broad, elongate, tapering. *Ascospores* hyaline, allantoid, occasionally straight and oblong or ellipsoid, aseptate or septate. Description taken from Barr (1985).

*Type species*: *C. pulchella* (Pers.: Fr.) Schroeter

*Calosphaeriophora* Réblová, L. Mostert, W. Gams & Crous, Stud. Mycol. 50 (2004): 542. 2005.

*Mycelium* smooth, hyaline, similar to that of *Acremonium*. *Conidiophores* micronematous, arising from aerial or submerged hyphae, erect, simple, mostly unbranched and subcylindrical. *Phialides* terminal or lateral, often aggregated in dense clusters on bundles of hyphae; elongate-ampulliform and attenuated at the base; hyaline with a finely pigmented apical region below the collarette; collarettes deep and flaring; adelophialides commonly occurring. *Conidia* aggregated in round, slimy heads at the tips of the phialides, hyaline, oblong-ellipsoidal or cylindrical with a tapered base.

*Type species*: *Calosphaeriophora pulchella* Réblová, L. Mostert, W. Gams & Crous

*Notes*: The genus *Calosphaeria* has 80 species and nine varieties listed in MycoBank (2005). Re-examination of many specimens identified as *Calosphaeria* has revealed that a high proportion represent other genera such as *Calyculosphaeria*, *Coronophora*, *Diaporthe*, *Jattaea*, *Pleurostoma*, *Quaternaria*, *Togninia*, *Valsa* and *Wegelia*. All names in *Calosphaeria* need to be examined in detail to assess their taxonomic status.

*Enchnoa* Fr., Summa Veget. Scand. 393. 1849.

*Anamorph*: Unknown.

*Ascomata* beneath periderm, in slight or conspicuous, blackish subiculum, gregarious, large-sized, sphaeroid; apex papillate, upright; surface bearing a hyphal tomentum; peridium of compressed rows of cells. *Asci* unitunicate, clavate, stipitate, in fascicles, 8-spored. *Paraphyses* sparse, broad, elongate, tapered.

*Ascospores* hyaline or light reddish brown or olive or greyish brown in mass, allantoid, aseptate. Description taken from Barr (1985).

*Type species*: *E. lanata* (Fr.) Fr.

*Notes*: The genus *Enchnoa* has 15 species and two varieties according to MycoBank (2005).

*Jattaea* Berl., Icon. Fung. 3: 6. 1900.

*Anamorph*: Unknown.

*Ascomata* gregarious, often in rows or small groups, immersed beneath the periderm, but often appearing superficial when the periderm is sloughed, small to medium-sized, globose, with a papilla or short narrow neck (equal to the diameter of the ascoma), often bent or curved, central or eccentric; surface of ascoma usually roughened by short hyphae; peridium of several rows of compressed cells. *Asci* unitunicate, clavate, stipitate, arranged in fascicles, 8-spored. *Paraphyses* broad, elongate, tapered. *Ascospores* hyaline, at times becoming light greyish brown, allantoid, aseptate to multi-septate.

Description taken from Barr (1985).

*Lectotype species*: *J. algeriensis* Berl. (Clements & Shear 1931).

*Note*: The genus *Jattaea* currently has 13 species listed in MycoBank (2005).

*Pachytrype* Berl. ex M.E. Barr, J.D. Rogers & Y.M. Ju, Mycotaxon 48: 530. 1993.

*Anamorph*: *Cytospora*-like.

*Stromata* shallow, rounded or irregular, or well-developed as pulvinate or irregular masses, composed of interwoven hyphae and cells, greenish to brownish. *Ascomata* monostichous or polystichous, beaks becoming elongate. *Asci* unitunicate, numerous, oblong, sessile, in spicate configuration. *Ascospores* hyaline, oblong to allantoid, aseptate.

*Type species*: *P. princeps* (Penzig & Sacc.) M. E. Barr, J. D. Rogers & Y.M. Ju

*Anamorph*: Colonies forming pycnidia of ca 1 mm diam, single or confluent, internally convoluted, lined with short conidiophores terminating in two or more phialidic conidiogenous cells. *Conidia* variable in shape and size, ellipsoid, allantoid, rod-shaped, individually hyaline, extruded from pycnidium in yellowish drops. Description taken from Barr (1993).

*Notes*: Only two species of *Pachytrype* have been described (Barr 1993).

*Pleurostoma* Tul. & C. Tul. Sel. Fung. Carpol. 2: 247. 1863.

*Anamorph*: *Pleurostomophora* D. Vijaykrishna, L.

Mostert, R. Jeewon, W. Gams, K.D. Hyde & Crous, Stud. Mycol. 50 (2004): 390. 2005.

*Ascomata* gregarious, superficial, black, small to medium-sized, globose, with eccentric or lateral papilla; peridium with smooth surface, composed of several rows of compressed reddish brown cells. *Asci* unitunicate, subglobose or broadly oblong, in spicate clusters from proliferating ascogenous hyphae, polysporous. *Paraphyses* not seen. *Ascospores* hyaline, allantoid, aseptate.

Description adapted from Barr (1985).

*Type species*: *P. candollei* Tul. & C. Tul.

***Pleurostomophora*** D. Vijaykrishna, L. Mostert, R. Jeewon, W. Gams, K.D. Hyde & Crous, Stud. Mycol. 50 (2004): 390. 2005.

*Mycelium* branched, septate, hyaline when young, becoming brown with age. *Conidiophores* single, separate, resembling those of *Phialophora*, hyaline to pigmented. *Phialides* mostly monophialidic and short; collarettes inconspicuous or flaring. *Conidia* aggregated in slimy masses at the apices of conidiogenous cells, at least partly hyaline, smooth, mostly dimorphic, being either straight to allantoid and hyaline, or shorter, subglobose to ellipsoid, and often brown.

*Type species*: *P. ootheca* D. Vijaykrishna, R. Jeewon & K.D. Hyde

*Notes*: Four species of *Pleurostoma* has been described (Mycobank 2005). The genus *Pleurostomophora* currently consists of three species (Vijaykrishna *et al.* 2004).

***Romellia*** Berl., Icon. Fung. 3: 5. 1900.

*Anamorph*: Unknown.

*Ascomata* immersed to superficial, gregarious or ± circinate or solitary, globose, with short broad necks reaching to or extending slightly beyond substrate surface; peridium surface slightly or strongly tomentose, with hyphae extending into the wood in upper regions as a thin stromatic layer; peridium of several rows of compressed cells, reddish brown. *Asci* unitunicate, oblong-clavate, sessile, in spicate arrangement, 8-spored. *Paraphyses* broad, elongate, tapering. *Ascospores* hyaline, allantoid or oblong, aseptate.

Description adapted from Barr (1985).

*Type species*: *R. vibratilis* (Fr.) Berl.

*Notes*: The generic description is based on *R. vibratilis*, the type species. Barr (1985) accepted three species in *Romellia*, viz. *R. vibratilis*, *R. cornina* and *R. tympanoides*. Later, Barr *et al.* (1993) referred *R. vibratilis* to *Pleurostoma* and the other two species to *Jattaea* Berl. The genera *Romellia* and *Jattaea* are

currently under revision by M. Réblová (pers. comm.). Excluding *Romellia tympanoides* with its atypical polysporous asci, four species of *Romellia* are currently known (Mycobank 2005).

***Togniniella*** Réblová, L. Mostert, W. Gams & Crous, Stud. Mycol. 50 (2004): 543. 2005.

*Anamorph*: *Phaeocrella* Réblová, L. Mostert, W. Gams & Crous, Stud. Mycol. 50 (2004): 545. 2005.

*Perithecia* solitary, nonstromatic, dark brown to black, glabrous; venter globose to subglobose, entirely immersed; neck protruding beyond the substratum, central, elongate; ostiole periphysate. *Perithecial* wall two-layered; outer wall of *textura prismatica*, consisting of brown, thin-walled, brick-like cells with opaque walls; inner layer of thinner-walled, subhyaline to hyaline, elongated and compressed cells. *Ascogenous hyphae* persistent, proliferating sympodially, branched, forming a sympodial succession of short ellipsoidal subtending cells along a side. *Asci* arising singly, in acropetal succession, separating from the basal cells when mature and floating freely within the centrum, with no distinct discharge mechanism, 8-spored; unitunicate, clavate, truncate to broadly rounded at the thickened apex, narrowly tapering towards the base from the sporiferous portion. *Paraphyses* persistent, abundant, not branching, septate, hyaline, more or less cylindrical, tapering near the tip, apically free, longer than the asci. *Ascospores* suballantoid, hyaline, aseptate, smooth, arranged in a fascicle in the upper part of the ascus.

Description taken from Réblová *et al.* (2004).

*Type species*: *Togniniella acerosa* Réblová, L. Mostert, W. Gams & Crous

***Phaeocrella*** Réblová, L. Mostert, W. Gams & Crous Stud. Mycol. 50 (2004): 545. 2005.

*Mycelium* consisting of branched, separate, septate hyphae; tuberculate, medium- to pale brown. *Conidiophores* morphologically similar to *Phaeoacremonium*, though more regularly branched, with prominent constrictions at the septa. *Phialides* subhyaline becoming hyaline towards the tip; having distinct, shallow, flaring collarettes; adelophialides occurring rarely. *Conidia* aggregated in round, slimy heads at the apices of the phialides, hyaline, mostly obovoid, oblong-ellipsoidal or reniform.

*Type species*: *P. acerosa* Réblová, L. Mostert, W. Gams & Crous

*Notes*: The genus *Togniniella* contains a single species, *Togniniella acerosa*, with its anamorph *Phaeocrella acerosa* (Réblová *et al.* 2004).

*Wegelina* Berl., Icon. Fung. 3: 8. 1900.

Anamorph: Unknown.

*Perithecia* mostly scattered or loosely grouped, venter globose to subglobose or convex, usually submerged in the substratum, lacking stromatic tissues; neck cylindrical, strongly elongated, lateral, horizontal or oblique. *Paraphyses* persistent, elongate, septate, tapering. *Asci* clavate, 4 or 8-spored. *Ascospores* allantoid, hyaline, aseptate or multi-septate.

Type species: *W. discreta* Berl.

Description translated from Berlese (1900) and adapted according to Barr (1998) and Réblová (2006).

Notes: *Wegelina* was reinstated by Barr (1998) in the *Calosphaeriales* to include species with stout, sulcate necks and lightly pigmented, occasionally delicately septate ascospores. The species complying with this redefinition were *W. polyporina* M.E. Barr and *W. subdenudata* (Peck) M.E. Barr. Réblová (2006) found *W. polyporina* to be a member of *Ceratostomella*. If *W. polyporina* is excluded, the genus *Wegelina* contains five species (MycoBank 2005).

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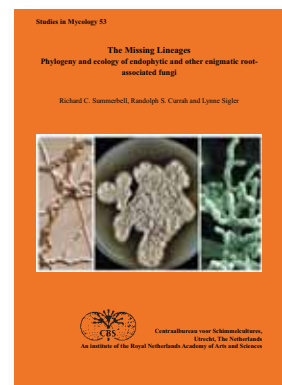
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**Studies in Mycology 53: The Missing Lineages. Phylogeny and ecology of endophytic and other enigmatic root-associated fungi.**

Richard C. Summerbell, Randolph S. Currah and Lynne Sigler.

This volume helps to bring the molecular biological revolution to some of these "missing lineages." Included are some fungi fitting the classic ecological category of 'sterile root fungus,' as well as others that do sporulate, but only after very long incubation. Also, the conidial soil- and root-associated fungal genus *Oidiodendron*, which shares with 'sterile root fungi' the problem of having morphology so reduced that reliable species distinction has been next to impossible, is reexamined with advanced phenotypic methodologies to find distinctions reflecting the molecular genetic distinctions that are now known within the group. The first part of the volume, then, consists of biosystematic studies elucidating either the primary phylogenetic systematics, or the post-molecular phenotypic reinterpretation, of previously intractable groups of root-associated fungi and their relatives. The volume consists of 11 contributions.

**252 pp., fully illustrated with colour pictures (A4 format), paperback, 2005. € 65**



**Studies in Mycology 52: Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (*Ascomycota*, *Diaporthales*, *Valsaceae*) from *Eucalyptus***

Edited by Pedro W. Crous

Gerard C. Adams, Michael J. Wingfield, Ralph Common and Jolanda Roux

*Cytospora* species and their *Valsa* teleomorphs are commonly found on *Eucalyptus* trees and some of these have been associated with stem canker diseases. This study was based on extensive collections of *Cytospora* species and their teleomorphs from *Eucalyptus* trees in Africa, Australia, Central and South America, Southeast Asia and California. Sixty-two *Cytospora* and three *Cytospora*-like isolates from *Eucalyptus*, Phylogenetic analysis clustered isolates of *Cytospora* from *Eucalyptus* into at least 15 unrelated groups. 28 taxa are fully described and illustrated with full colour micrographs.

**147 pp., fully illustrated with colour pictures (A4 format), paperback, 2005. € 55**

**Studies in Mycology 51: Fungi of the Antarctic: Evolution under Extreme Conditions**

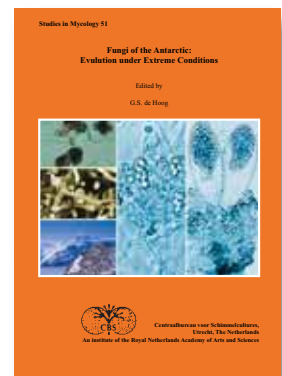
Edited by G.S. de Hoog

L. Selbmann, G.S. de Hoog, A. Mazzaglia, E.I. Friedmann and S. Onofri: **Fungi at the edge of life: cryptoendolithic black fungi from Antarctic deserts**

G.S. de Hoog, E. Göttlich, G. Platas, O. Genilloud, G. Leotta and J. van Brummelen: **Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica**

Twenty-six strains of black, mostly meristematic fungi isolated from cryptoendolithic lichen dominated communities in the Antarctic were described by light and Scanning Electron Microscopy and sequencing of the ITS rDNA region. In addition, cultural and temperature preferences were investigated. The phylogenetic positions of species recognized were determined by SSU rDNA sequencing. Most species showed affinity to the order Dothideales and constitute two main groups referred to under the generic names *Friedmanniomyces* and *Cryomyces* (gen. nov.), each characterized by a clearly distinct morphology. Two species could be distinguished in each of these genera. Six strains could not be assigned to any taxonomic group; among them strain CCFEE 457 belongs to the Hysteriales, clustering together with Mediterranean marble-inhabiting *Coniosporium* species in an approximate group with low bootstrap support. All strains proved to be psychrophiles with the only exception for the strain CCFEE 507 that seems to be mesophilic- psychrotolerant. All had very thick melanized cell walls, the ability to produce exopolysaccharides and to grow meristematically. They are thought to be well adapted to the harsh environment of the Antarctic cold Desert. Hypotheses concerning their origin and evolution are put forward.

**82 pp., (A4 format) paperback, 2005. € 40**



**CBS Biodiversity Series.**



**No. 4: Hypocreales of the Southeastern United States: An Identification Guide**

Gary J. Samuels, Amy Y. Rossman, Priscila Chaverri, Barrie E. Overton and Kadri Põldmaa

An illustrated guide is presented to the members of the ascomycete order Hypocreales that are known to occur in the southeastern states of the United States, including North and South Carolina, Tennessee and Georgia. Species were selected mainly based on records in the United States National Fungus Collections (BPI). These states include or surround the Great Smoky Mountains National Park. Species of the Hypocreales are among the most numerous, and certainly most conspicuous, of the microfungi. The order also includes some of the most economically important fungi. This guide is intended for individuals who are participating in All Taxa Biological Diversity studies of the Great Smoky Mountains National Park as well as other interested professionals and amateurs. Short descriptions and colour illustrations of one-hundred and one species and two varieties in twenty genera are provided. Keys to genera and species are included. The new combination *Neonectria ditissima* is proposed.

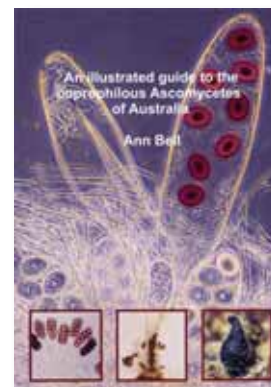
**145 pp., over 120 colour pictures (A4 format), paperback with spiral binding, 2006. € 70**

### No. 3: An illustrated guide to the coprophilous Ascomycetes of Australia

Ann Bell

Descriptions, keys and illustrations (many in colour). Ann Bell's observations of her own collections and some 2,000 microscope slides and assorted notebooks on Australian coprophilous fungi made by the late Major Harry Dade during his retirement years in Victoria.

173 pp., 115 plates (A4 format), paperback with spiral binding, 2005. € 55

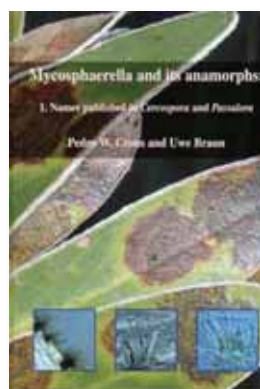


### No. 2: Cultivation and Diseases of Proteaceae: Leucadendron, Leucospermum and Protea

Pedro W. Crous, Sandra Denman, Joanne E. Taylor, Lizeth Swart and Mary E. Palm

The Proteaceae represent one of the Southern Hemisphere's most prominent flowering plant families, the cultivation of which forms the basis of a thriving export industry.

Diseases cause a loss in yield and also limit the export of these flowers due to strict phytosanitary regulations. In this publication the fungi that cause leaf, stem and root diseases on Leucadendron, Leucospermum and Protea are treated. Data are provided pertaining to the taxonomy, identification, host range, distribution, pathogenicity and control of these pathogens. Taxonomic descriptions and illustrations are provided and keys are included. Disease symptoms are illustrated with colour photographs. 510 pp. (17 x 25 cm), paperback, 2004. € 55



### No. 1: Mycosphaerella and its anamorphs: 1. Names published in Cercospora and Passalora

Pedro W. Crous and Uwe Braun

This book contains a compilation of more than 3000 names that have been published or proposed in *Cercospora*, of which 659 are presently recognised in this genus, with a further 281 being referred to *C. apii* s.lat. Approximately 550 names of *Passalora* emend. (incl. *Mycovellosiella*, *Phaeoramularia*, *Tandonella* and *Phaeoisariopsis* p.p.) are treated in a second list. In total 5720 names are treated. 553 taxonomic novelties are proposed.

571 pp., 31 figures (17 x 25 cm), hard cover, 2003. € 80

### Selection of other CBS publications.



### Identification of Common Aspergillus Species

Maren A. Klich

Descriptions and identification keys to 45 common *Aspergillus* species with their teleomorphs (*Emericella*, *Eurotium*, *Neosartorya* and *Sclerocleista*). Each species is illustrated with a one page plate and three plates showing the most common colony colours.

116 pp., 45 black & white and 3 colour plates (Letter format), paperback, 2002. € 45



### Introduction to food- and airborne fungi

Robert A. Samson, Ellen S. Hoekstra and Jens C. Frisvad

Seventh edition with updated taxonomy and addition of some important species. The keys to the taxa were improved. The taxonomy and the nomenclature of *Fusarium* is revised and the number of *Penicillium* species has been increased because they are frequently encountered on food and indoor environments. The identification of *Penicillium* based on morphological characters remains difficult and therefore synoptic keys and tables are added to assist with the identification.

389 pp., 120 plates (A4 format), paperback, 2004. € 55



### Atlas of Clinical Fungi

G.S. de Hoog, J. Guarro, J. Gené and M.J. Figueras (eds)

The second fully revised and greatly expanded edition of the Atlas of Clinical Fungi appeared in 2000. The modest and very competitive price of this standard work has certainly contributed to the popularity of the first edition. In recent years the application of molecular biology has become within reach for many routine laboratories. The new Atlas will provide ample molecular data for the majority of clinically relevant fungi. It will set a standard for innovative techniques in medical mycology. In addition, antifungal susceptibility data will be given for most species, which will provide essential knowledge for the clinician in view of adequate therapy.

1126 pp., fully illustrated with line drawings and black & white photo plates (A4 format), hard cover, 2004. € 140.

Interactive CD-ROM version of the Atlas € 65, Book plus CD-ROM € 180

