

Three new species of *Dialonectria* (Nectriaceae) from France

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Abstract: Three species of *Dialonectria* collected in France on *Diatrypaceae* are described and illustrated as new species. Their taxonomic position is based on morphological characteristics and phylogenetic analysis of the ITS sequences. Phylogenetic comparison with genera belonging to the *Nectriaceae* and having an acremonium- or fusarium-like asexual morph places the three new species in *Dialonectria*.

Keywords: Ascomycota, *Diatrype*, *Diatrypella*, fusarium-like, *Hypocreales*, *Quaternaria*, ribosomal DNA, taxonomy.

Résumé : Trois espèces de *Dialonectria* récoltées en France sur *Diatrypaceae* sont décrites et illustrées comme espèces nouvelles. Leur position taxinomique est fondée sur les caractéristiques morphologiques et l'analyse phylogénétique des séquences ITS. La comparaison phylogénétique avec les genres appartenant aux *Nectriaceae* ayant un stade asexué de type acremonium ou fusarium place les trois nouvelles espèces dans le genre *Dialonectria*.

Mots-clés : ADN ribosomal, Ascomycota, *Diatrype*, *Diatrypella*, type-fusarium, *Hypocreales*, *Quaternaria*, taxinomie.

Introduction

In continuing the survey of the *Nectriaceae* in temperate areas (LECHAT & APLIN, 2016; LECHAT & FOURNIER, 2015; 2016; 2017a; LECHAT & ROSSMAN, 2017b; LECHAT *et al.*, 2010; 2014; 2015; 2018), several specimens of cosmospora-like species were repeatedly collected on dead or effete stromata of *Diatrypaceae*, which enabled a detailed morphological characterization and successful single ascospore isolations. These fungi were assigned to the *Nectriaceae* based on ascomata changing colour in 3% KOH and lactic acid and a fusarium-like asexual morph. Phylogenetic analysis of ITS sequences place these species in *Dialonectria* close to *D. episphaeria* (Tode : Fr.) Cooke and *D. ullevolea* Seifert & Gräfenhan, from which our fungi morphologically differ in having larger ascospores and thicker ascomatal walls.

Materials and methods

Dry specimens were rehydrated and examined using the method described by ROSSMAN *et al.* (1999). Microscopic observations and measurements were made in water. The holotype specimen and paratypes were deposited in LIP herbarium (University of Lille, France) and living cultures in the CBS Collection of the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands), and at CIRM-CF (Centre International des Ressources Microbiennes, Marseille, France). Cultures of the living specimen were made on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 9 cm diam incubated at 25°C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Santander, Spain): Total DNA was extracted from dry specimens blending a portion of them using a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65°C. A similar volume of chloroform:isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13 000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL ddH₂O. PCR amplification was performed with the primers ITS1F and ITS4 (WHITE *et al.*, 1990; GARDES & BRUNS, 1993) for ITS. Chromatograms were checked searching for putative reading errors, and these were corrected.

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (TAMURA *et al.*, 2013). Nomenclature

follows MycoBank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

Taxonomy

Dialonectria diatrypellicola Lechat & J. Fourn., *sp. nov.* Fig. 2
MycoBank MB 829215

Diagnosis: Differs from all known *Dialonectria* species in having the largest ascospores (13–)14–18(–19) × 9–10 µm and occurrence on effete or dead stromata of *Diatrypella quercina*.

Holotype: FRANCE, Ariège, Rimont, Saurine, on effete or dead stromata of *Diatrypella quercina* on *Quercus* bark, 24 Feb. 2009, *leg.* J. Fournier, JF09006 (LIP JF09006), ex-type culture CBS 126102. GenBank ITS sequence MK333359.

Etymology: The epithet "*diatrypellicola*" refers to *Diatrypella quercina* on which this species consistently occurs.

Ascomata superficial, not obviously stromatic but seated on a minute, pseudoparenchymatous basal stroma, gregarious in groups of 10–50, widely obpyriform to subglobose, 300–380 µm high, 250–360 µm diam (Me = 340 × 300 µm, n = 30), collapsing laterally or not collapsing when dry, red, turning purple in 3% KOH and yellow in lactic acid. Ascomatal surface composed of globose to ellipsoidal angular cells, finely roughened with some yellowish scurf. **Apex** obtusely rounded to discoidal, concolorous, made of a palisade of parallel, vertically arranged, cylindrical to clavate cells with orange wall. **Basal stroma** orange, 10–50 µm high, penetrating host tissues, turning purple in 3 % KOH, yellow in lactic acid, composed of globose to ellipsoidal, thick-walled cells 5–14 × 5–11 µm with wall 2–2.5 µm thick evolving upwards to form ascomatal wall. **Ascomatal wall** in vertical section 35–60 µm thick, composed of two regions: outer region 25–40 µm thick, composed of globose or subglobose to ellipsoid thick-walled cells 4–8 × 2.5–4 µm with orange wall 2–3 µm thick; inner region 10–20 µm thick, composed of hyaline, flattened, thick-walled cells 9–12 × 3–5 µm becoming thin-walled towards interior. **Asci** cylindrical to slightly clavate, 110–130 × 12–14 µm, 8-spored, ascospores obliquely uniseriate or biseriate above and uniseriate below, apex simple; evanescent moniliform paraphyses inserted between asci, up to 10 µm diam at base. **Ascospores** (13–)14–18(–19) × 9–10 µm, ellipsoidal, rounded at ends, 1-septate, constricted at septum, with one large droplet in each cell, hyaline, becoming pale brownish yellow when mature, conspicuously warted.

Cultural characteristics: Colony 2.5–3 cm diam after three weeks, producing fusarium-like asexual morph, pale orange in centre, crimson in middle area, white at margin, sporulating in bright orange

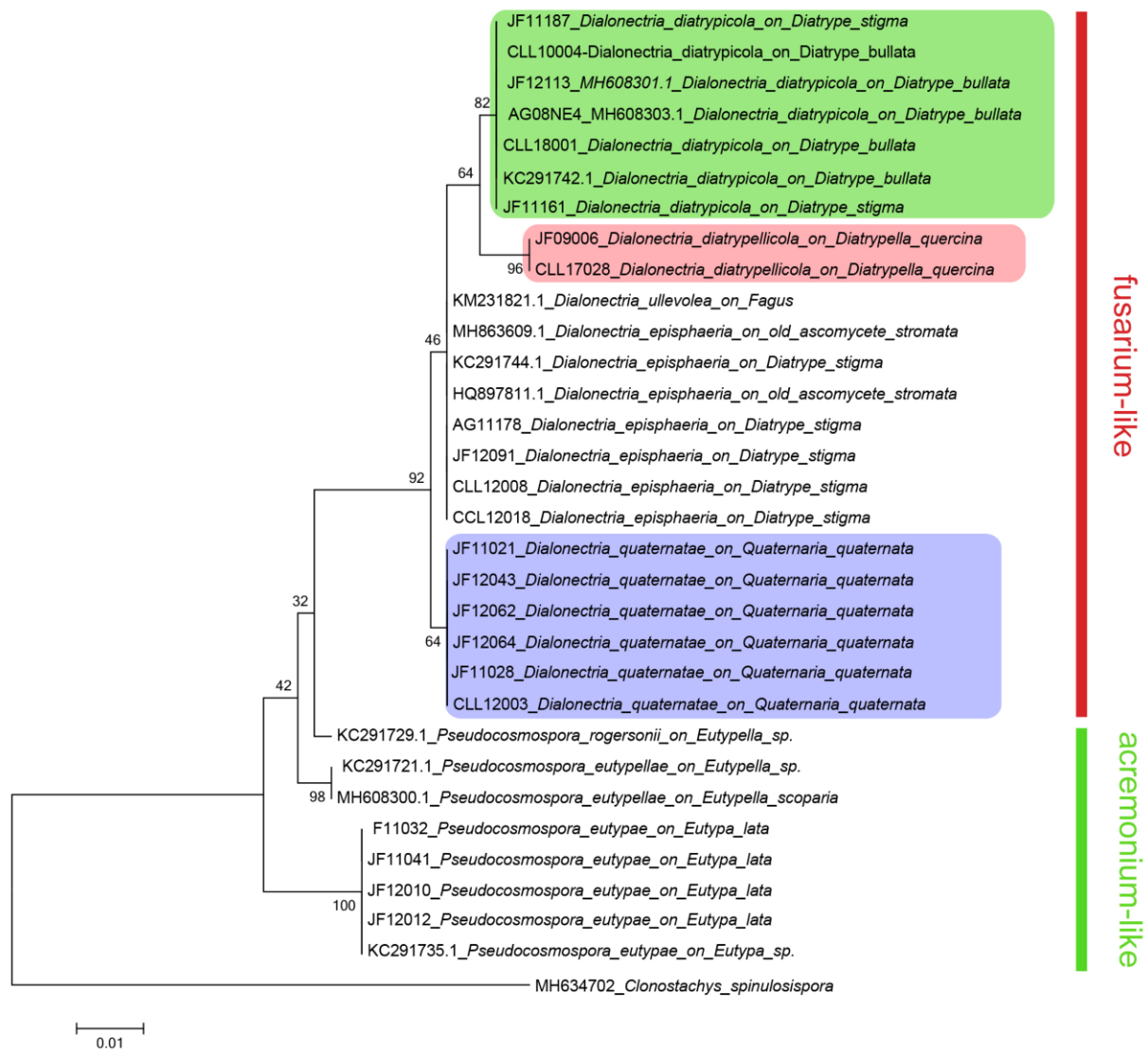


Fig. 1 – Maximum likelihood phylogeny (-lnL = 1826.22624) of *Dialonectria* spp. inferred by PhyML 3.0, model HKy85 from a 704 bp matrix of ITS sequences, rooted with *Clonostachys spinulosispora*.

sporodochia. Microconidia fusiform, curved, non-septate 4.5–9.5 × 1.5–2 µm. Macroconidia cylindrical to fusiform, curved, rounded at tip, acute at base with a foot cell, 1-septate (35–)40–52(–56) × 2.8–4 µm (Me = 47 × 3.6 µm, n = 30).

Additional specimens examined: FRANCE, Ariège, Rimont, Peyrau, on dead stromata of *Diatrypella quercina* on *Quercus* bark, 17 Jan. 1999, *leg.* J. Fournier, JF99007 (LIP); *ibid.*, same host, 2 Jun. 2002, *leg.* J. Fournier, JF02101 (LIP); Ariège, Rimont, Saurine, on dead stromata of *Diatrypella quercina* on *Quercus* bark, 8 Jan. 2003, *leg.* J. Fournier, JF03003 (LIP); *ibid.*, same host, 10 Feb. 2009, *leg.* J. Fournier, JF09005 (LIP); *ibid.*, same host, 17 Jun. 2010, *leg.* J. Fournier, JF10082 (LIP); Dordogne, Lalinde, Courcelles, on dead stromata of *Diatrypella quercina* on *Quercus* bark, 26 Dec. 2001, *leg.* J. Fournier, JF01279 (LIP); Ille-et-Vilaine, Acigné, on dead stromata of *Diatrypella quercina* on *Quercus* bark, 30 Oct. 2017, *leg.* J. Esnault, CLL17028 (LIP).

Dialonectria diatrypicola Lechat, J. Fourn. & Gardiennet, *sp. nov.* MycoBank MB 829216 Fig. 3
 Diagnosis: Similar to *Dialonectria diatrypellicola* but differs by smaller ascospores and occurrence on effete stromata of *Diatrype bullata* and *D. stigma*.

Holotype: FRANCE, Côte-d'Or, Is-sur-Tille, on dead or effete stromata of *Diatrype bullata* (*Diatrypaceae*), 5 Nov. 2008, *leg.* A. Gardiennet, AG08NE4 (LIP), ex-type culture: CBS 124395, GenBank ITS sequence: MH608303.

Etymology: The epithet "*diatrypicola*" refers to *Diatrype* spp., on which this species occurs.

Ascomata superficial, not obviously stromatic but seated on a minute, pseudoparenchymatous basal stroma, gregarious in groups of 2–10(–15), widely obpyriform to subglobose, 270–320 µm high, 250–280 µm diam (Me = 290 × 270 µm, n = 30), collapsing laterally or not collapsing when dry, orange when immature, becoming bright red when mature, turning dark purple in 3% KOH, yellow in lactic acid, with a darker ostiolar region 40–50 µm high × 120–140 µm diam. **Ascomatal surface** composed of globose to ellipsoidal, angular cells up to 16 µm in greatest dimension, smooth but may appear finely roughened due to a network of pale orange hyphal elements 2–2.5 µm diam covering partially some ascomata. **Apex** obtusely rounded to discoidal, concolorous or darker than perithecial wall, sometimes appearing nearly black when dry, made of a palisade of parallel, vertically arranged, cylindrical to clavate cells. **Basal stroma** dark orange, 45–70 µm high, penetrating host tissues, turning purple in 3% KOH, yellow in lactic acid, composed of globose to ellipsoidal, thick-walled cells 5–10 × 5–11 µm with wall

2–3 μm thick evolving upwards to ellipsoidal cells 8–12 \times 6–8 μm with thinner wall 1.5–2 μm thick to form ascomatal wall. **Ascomatal wall** in vertical section 30–45 μm thick, made of two regions: outer region 20–30 μm thick, composed of thick-walled globose, subglobose to ellipsoidal cells 5–10 \times 5–8 μm with orange wall 1.5–2 μm

thick; inner region 10–15 μm thick, composed of flattened cells 7–12 \times 3–5 μm , hyaline, with wall 1.5 μm thick. **Asci** unitunicate, cylindrical, short-stipitate, 75–90 \times 6.5–8 μm , with 8 uniseriate ascospores, apically rounded with a faintly refractive ring; evanescent moniliform paraphyses interspersed between the asci, up to

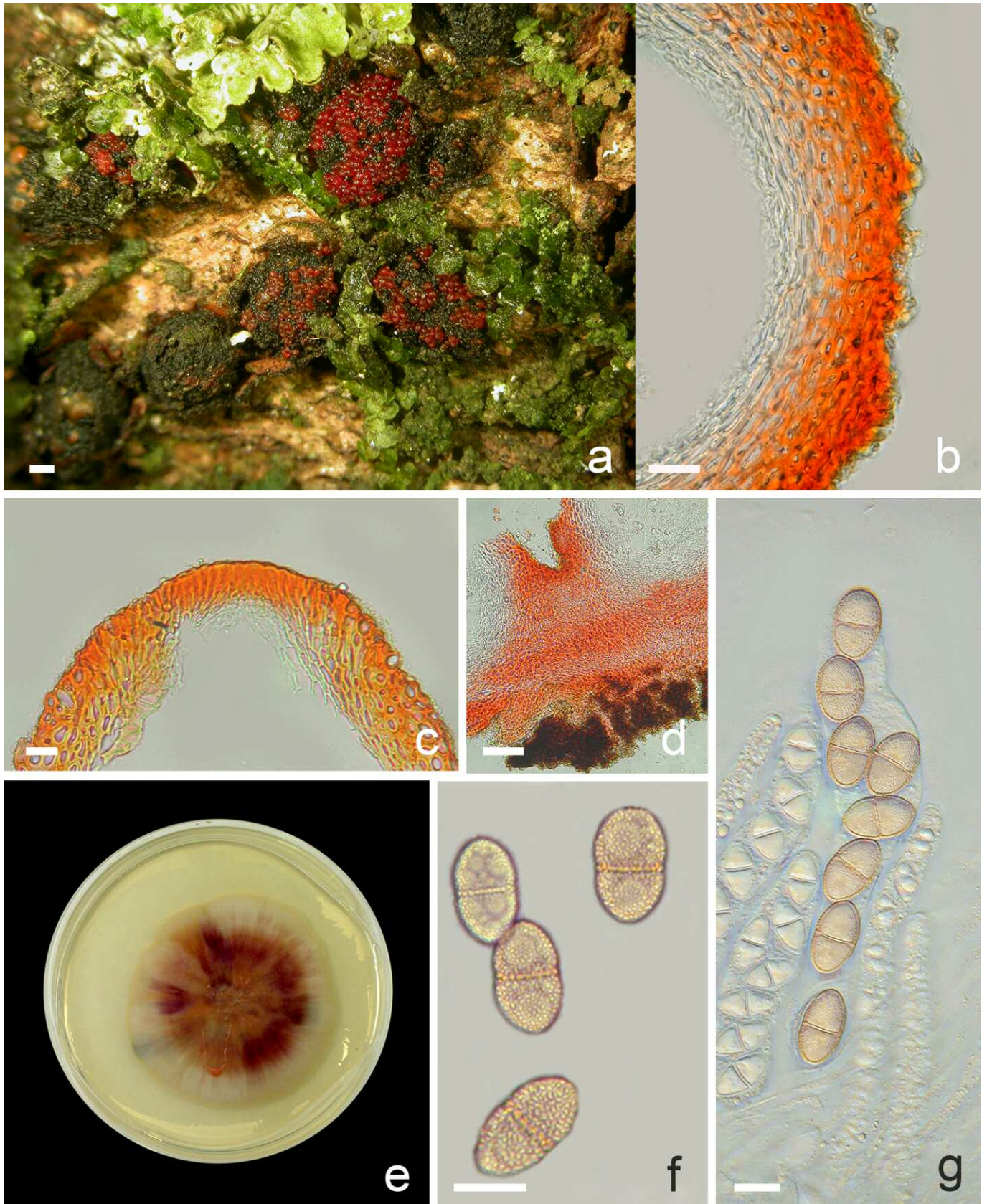


Fig. 2 – a–g: *Dialonectria diatrypelicola* (Holotype JF09006); a: Ascomata in natural environment; b: Vertical section of lateral ascomatal wall; c: Vertical section through ascomatal apex; d: Vertical section through ascoma, stroma and host; e: Culture at three weeks; f: Ascospores in water; g: Asci and ascospores in water. Scale bars: a = 1 mm; b, c = 20 μm ; d = 50 μm ; f = 10 μm ; g = 10 μm .

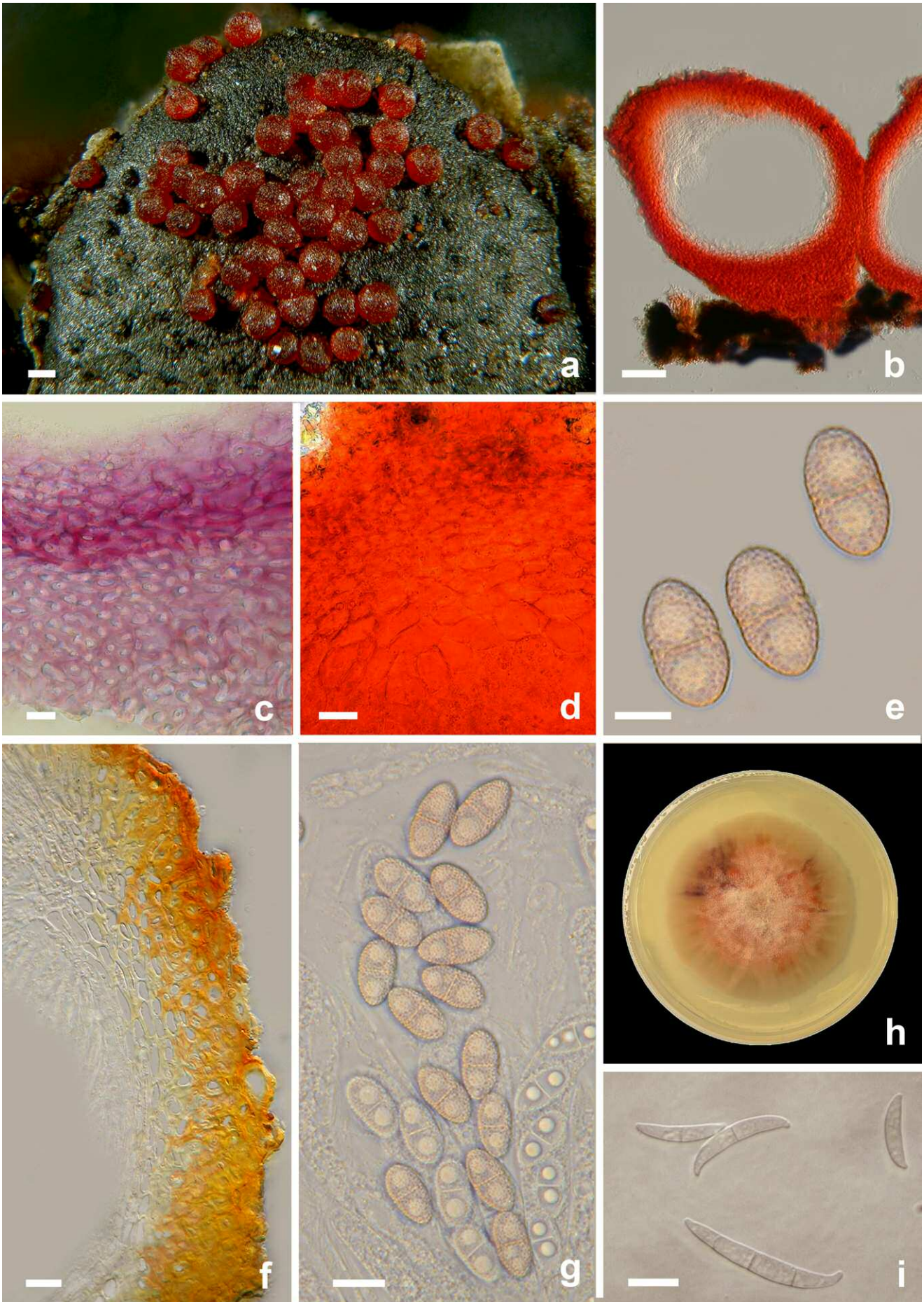


Fig. 3 – a–i: *Dialonectria diatrypicola* (Holotype AG08NE4). a: Ascomata in natural environment. b: Vertical section through ascomata, stroma and host; c: Cells of the stroma in 3% KOH, evolving to form ascomatal wall; d: Cells of ascomatal wall in surface view; e: Ascospores in water; f: Vertical section of lateral ascomatal wall; g: Asci and ascospores in water; h: Culture after three weeks; i: Macroconidia from culture. Scale bars: a = 200 μ m; b = 50 μ m; c, d, f, g, i = 10 μ m; e = 5 μ m.

10 µm wide at base. **Ascospores** (11–)12–13(–14) × 6–7.2 µm (Me = 12.5 × 6.5 µm, n = 50), ellipsoidal, equally two-celled with one large drop in each cell, slightly constricted at the septum, pale brownish–yellow when mature, with low obtuse warts.

Cultural characteristics: Colony slow growing, 5–6 cm diam after three weeks, producing a fusarium-like asexual morph, slimy, white at centre, with conspicuous dark reddish radiating strands, with yellow to pale orange sporodochia sporulating in middle area; microconidia aseptate, narrowly ellipsoidal to subcylindrical, 4.5–7(–9) × 2–3 µm, hyaline, smooth-walled; macroconidia slightly curved with pedicellate foot–cell, 0–3-septate, 14–35 × 3–4 µm, smooth-walled.

Additional specimens examined: FRANCE, Ariège, Rimont, Grand Bois, Binette, on *Diatrype stigma*, 8 Dec. 2011, leg. J. Fournier, JF11187 (LIP), GenBank ITS sequence MK333357; *ibid.*, on *Diatrype bullata*, 22 Oct. 2012, leg. J. Fournier, JF12113 (LIP), GenBank ITS sequence MH608301; Ariège, Rimont, Peyrau, on dead stromata of *Diatrype bullata*, 23 Aug. 2011, leg. J. Fournier, JF11109 (LIP); *ibid.*, same host, 7 Jul. 2012, leg. J. Fournier, JF12079 (LIP); Aude, Roquefeuil, Bois du Pinet, on dead stromata of *Diatrype bullata*, 21 Oct. 2011, leg. J. Fournier, JF11141 (LIP); Deux-Sèvres, Le Vanneau, on *Diatrype bullata*, 17 Jan. 2010, leg. C. Lechat, CLL10004 (LIP), GenBank ITS sequence MH608305; Granzay, on *Diatrype bullata*, 18 Sept. 2010, leg. C. Lechat, CLL10043 (LIP); Eure, Martot, Carrière SPS, on *Diatrype bullata*, 20 Jan. 2018, leg. A. Delannoy, CLL18001 (LIP), GenBank ITS sequence MH608302.

Dialonectria quaternatae Lechat & J. Fourn. sp. nov. Fig. 4
Mycobank MB 829217

Diagnosis: Differs from other known species of *Dialonectria* by smooth-walled ascospores (10–)11–12.5(–13) × 4.5–5.5 µm and occurrence on *Quaternaria quaternata*.

Holotype: FRANCE, Aude, Belcaire, Clos de la Plaine, on dead or effete stromata of *Quaternaria quaternata* on *Fagus sylvatica*, 26 Apr. 2012, leg. J. Fournier, JF12064 (LIP), ex-type culture BRFM 1762, Genbank ITS sequence MK333370.

Etymology: The epithet “*quaternatae*” refers to *Quaternaria quaternata*, on which this species occurs.

Ascomata superficial, not obviously stromatic but seated on a minute, pseudoparenchymatous basal stroma, in groups of 2–8(–10), subglobose to globose, 230–290(–300) high, 190–280 µm diam (Me = 270 × 240 µm, n = 30), collapsing laterally or not collapsing when dry, smooth, bright red, becoming purple in 3% KOH, yellow in lactic acid. **Ascomatal surface** composed of cells of undefined shape, forming a *textura epidermoidea*. **Apex** obtusely rounded to subconical, composed of subglobose to elongate thick-walled cells with pale orange wall. **Basal stroma** pale orange to orange, 5–20 µm high, penetrating host tissues, turning purple in 3% KOH, yellow in lactic acid, composed of narrowly ellipsoidal cells 8–12 × 5–12 µm with wall 1.5–2 µm thick, evolving upwards to subglobose, subangular cells 6–14 × 5–12 µm with wall 1.5–2 µm thick to form ascomatal wall. **Ascomatal wall** in vertical section 30–35 µm thick, of two regions: outer region 20–25 µm thick, composed of globose or subglobose to ellipsoid thick-walled cells 5–10 × 3–5 µm with orange wall 2–2.5 µm thick; inner region 10–15 µm composed of hyaline, flattened, thick-walled cells 9–14 × 3–6 µm, becoming hyaline and thin-walled towards interior, merging with periphyses at apex. **Asci** cylindrical, apex flat to rounded with a ring-like apical thickening, 80–95 × 6–8 µm, 8-spored, ascospores obliquely uniseriate. **Paraphyses** not seen. **Ascospores** (10–)11–12.5(–13) × 4.5–5.5 µm (Me = 12 × 5 µm, n = 30), ellipsoidal, 1-septate, slightly constricted at septum, subhyaline, eventually very pale brown, smooth-walled.

Cultural characteristics: Colony 2.5–3 cm diam after three weeks, aerial mycelium floccose, white in centre, bright yellow in middle area and white at margin, producing a fusarium-like asexual morph. No microconidia produced. Macroconidia curved, cylindrical to

fusiform, with pointed foot-cell, 1–3-septate (52–)65–70 × 4–4.5 µm (Me = 67 × 4.2 µm, n = 50), smooth.

Additional specimens: FRANCE, Ariège, Rimont, Grand Bois, on dead stromata of *Quaternaria quaternata* on *Fagus sylvatica*, 25 Mar. 2011, leg. J. Fournier, JF11021 (LIP), culture BRFM 1584, GenBank ITS sequence MK333360; Loumet, Prat Communal, on dead stromata of *Q. quaternata* on *F. sylvatica*, 17 Sept. 2011, leg. J. Fournier, JF111121 (LIP); *ibid.*, same host, 20 Apr. 2012, leg. J. Fournier, JF12043 (LIP), culture BRFM 1740, GenBank ITS sequence MK333367; Aude, Belcaire, Clos de la Plaine, on dead stromata of *Q. quaternata* on *F. sylvatica*, 26 Apr. 2012, leg. J. Fournier, JF12062 (LIP), culture BRFM 1742, GenBank ITS sequence MK333369; *ibid.*, on dead stromata of *Q. quaternata* on *F. sylvatica*, 27 Apr. 2012, leg. J. Fournier, JF12065 (LIP); Deux-Sèvres, Villiers-en-Bois, forêt de Chizé, on dead stromata of *Q. quaternata* on *F. sylvatica*, 27 Jan. 2012, leg. C. Lechat CLL12003 (LIP), culture BRFM 1748, GenBank ITS sequence MK333354; Hautes-Pyrénées, Castillon, Moulin de Sarthe, on dead stromata of *Q. quaternata* on *F. sylvatica*, 9 Apr. 2011, leg. J. Fournier, JF11028 (LIP), culture BRFM 1587, GenBank ITS sequence MK333361.

Discussion

ROSSMAN *et al.* (1999) gave a detailed account of the taxonomic history of nectriaceous fungicolous species. They reinstated the genus *Cosmospora* Rabenh., mostly for those with fusarium-like asexual morph, comprising species belonging to the *Nectria episphaeria* group recognized by BOOTH (1959), and those placed in *Nectria* subgenus *Dialonectria* by SAMUELS *et al.* (1991).

Cosmospora sensu ROSSMAN *et al.* (1999) was subsequently revised based on a phylogenetic reassessment of *Fusarium sensu lato* by GRÄFENHAN *et al.* (2011), revealing that *Cosmospora* was not monophyletic. This led to the segregation of *Cosmospora* Rabenh., restricted to species with an acremonium-like asexual morph, along with *Dialonectria* (Sacc.) Cooke, *Fusicolla* Bonord., *Macroconia* (Wollenw.) Gräfenhan, Seifert & Schroers, *Microcera* Desm. and *Stylonectria* Höhn., all of the latter genera having a fusarium-like asexual morph yielding macroconidia. HERRERA *et al.* (2013) introduced *Pseudocosmospora* C. Herrera & P. Chaverri for ten cosmospora-like species occurring on stromata of *Diatrypaceae*, having acremonium-like asexual morphs and greenish yellow to dark green colonies *in vitro*. HERRERA *et al.* (2015) demonstrated that *Cosmospora villiuscula* (Samuels, Yoshim. Doi & Rogerson) Rossman & Samuels represents a species complex and introduced seven new species occurring on *Xylariaceae*. The current generic concepts are still provisional because many cosmospora-like species have not yet been documented phylogenetically, but these generic concepts strongly suggest that host specificity and asexual state morphology are key characteristics.

Cosmospora-like fungi occurring on *Diatrypaceae* belong to *Dialonectria* and *Pseudocosmospora*, and are primarily distinguished by their asexual morph, respectively fusarium-like and acremonium-like. This is well illustrated in Fig. 1, where both genera appear on two separate clades. The combination of morphological characters and phylogenetic analysis of the three species described above unambiguously points toward the genus *Dialonectria* as defined by GRÄFENHAN *et al.* (2011) and LOMBARD *et al.* (2015).

Only two species are currently recognized in *Dialonectria*, viz. *D. episphaeria*, the type species, and *D. ullevolea*. The former name was long applied to most red nectriaceous fungi occurring in temperate regions on *Diatrypaceae* and *Xylariaceae*, making its definition confusing. According to BOOTH (1959), who revised the type specimen on *Diatrype stigma*, *D. episphaeria* features a thinner ascomatal wall than our three fungi (18–22 µm thick vs. 30–60 µm thick) and significantly smaller ascospores 7–11 × 3.5–5 µm vs. 12–18 × 4.5–7.2 µm.

The sexual morph of *D. ullevolea* is only known from ascomata obtained in culture on CMA, that are said to resemble those of *D. epis-*

phaeria in gross morphology and in producing ascospores in the same size range $9.7\text{--}11 \times 4\text{--}4.5 \mu\text{m}$ (GRÄFENHAN *et al.*, 2011), thus differs from the three species described above. These morphological differences are clearly supported by our phylogenetic analysis

(Fig. 1), and lead us to introduce *Dialonectria diatrypellicola*, *D. diatrypicola* and *D. quaternatae* as new species.

Dialonectria diatrypellicola and *D. diatrypicola* share similar pale brown and conspicuously warted ascospores. The former has larger ascospores $(13\text{--})14\text{--}18\text{--}(19) \times 9\text{--}10 \mu\text{m}$ vs. $(11\text{--})12\text{--}13\text{--}(14) \times 6\text{--}$

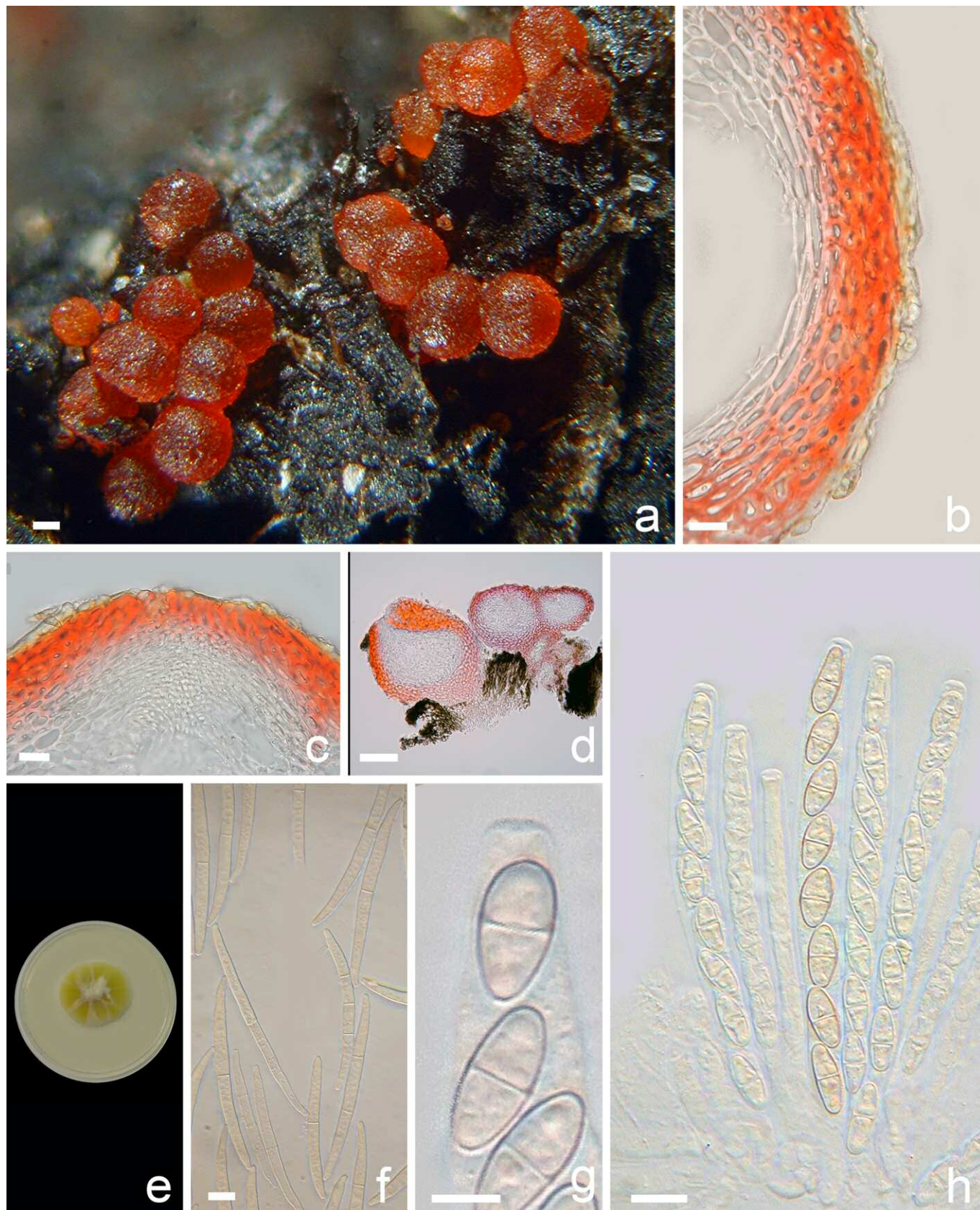


Fig. 4 – a–h: *Dialonectria quaternatae* (Holotype JF12064); a: Ascomata in natural environment; b: Vertical section of lateral ascomatal wall; c: Vertical section through ascomatal apex; d: Vertical section through ascomata, stroma and host; e: Culture at three weeks; f: Macroconidia from culture; g: Apex of ascus and ascospores in water; h: Asci and ascospores in water. Scale bars: a = 100 μm ; b, c = 10 μm ; d = 100 μm ; f–h = 10 μm .

7.2 µm and longer, 1-septate macroconidia (35–)40–52(–56) × 2.8–4 µm vs. 1–3-septate 16–27 × 2.5–4 µm; their colonies in culture both feature vivid red to orange tones that are lacking in *D. episphaeria* and *D. quaternatae*, colonies of *D. ullevolea* being pale orange to greyish orange. They can likewise be distinguished by their host specificity, respectively on *Diatrypella quercina* and *Diatrype*, mostly *D. bullata*.

Dialonectria quaternatae is easily distinguished from all known *Dialonectria* species by smooth-walled ascospores, longer macroconidia 1–3-septate (52–)65–70 × 4–4.5 µm and its occurrence on *Quaternaria quaternata*, a common saprobe on *Fagus sylvatica* bark on which it is most often present.

Although *D. diatrypicola* occurs on two different hosts (mostly *Diatrype bullata* but occasionally *D. stigma*), we think that a high specificity prevails between cosmospora-like fungi and their hosts. *Dialonectria diatrypicola* appears as an exception and accurate identification of the pyrenomycetous hosts is an important step in identifying cosmospora-like species. This is regrettably not the case for most newly described species like *D. ullevolea* whose type collection was made on “branch of *Fagus sylvatica*” without further mention of a possible fungicolous lifestyle; two additional collections of this species mention the presence of an associated pyrenomycete stroma, which remains fairly vague (GRÄFENHAN *et al.*, 2011). Identification of effete stromata of *Diatrypaceae* and *Xylariaceae* is obviously a challenging task for non-specialists but implementing it would substantially contribute to disentangling the taxonomy of cosmospora-like fungi.

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