# *Dialonectria favaceae*, a new species from France, and *Dialonectria magnusiana* comb. nov. for *Nectria magnusiana*

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**Abstract:** A new species of *Dialonectria* is proposed, based on a collection occurring on dead stromata of *Diatrypella* cf. *favacea*, on *Alnus incana* in the French Alps. This species is placed in *Dialonectria* based on morphological characters, asexual morph in culture and molecular data. Moreover, the new combination *Dialonectria magnusiana* (Rehm) Lechat & J. Fourn. is proposed to accommodate the basionym *Nectria magnusiana* Rehm. A dichotomous key to the seven currently recognized species of *Dialonectria* is presented.
**Keywords:** *Ascomycota*, *Hypocreales*, *Nectriaceae*, ribosomal DNA, taxonomy.

**Résumé :** une nouvelle espèce de *Dialonectria* est proposée, d'après une récolte effectuée dans les Alpes françaises, sur stromas morts de *Diatrypella* cf. *favacea*, sur *Alnus incana*. Cette espèce est placée dans le genre *Dialonectria* sur la base de ses caractères morphologiques, la forme asexuée produite en culture et les données moléculaires. Par ailleurs, la nouvelle combinaison *Dialonectria magnusiana* (Rehm) Lechat & J. Fourn. est proposée pour le basionyme *Nectria magnusiana* Rehm. Une clé dichotomique des sept espèces de *Dialonectria* actuellement reconnues est présentée.
**Mots-clés :** ADN ribosomal, *Ascomycota*, *Hypocreales*, *Nectriaceae*, taxinomie.

## Introduction

The genus *Dialonectria* (Sacc.) Cooke was reinstated by Gräfenhan *et al.* (2011) for fungicolous *Nectriaceae* formerly placed in *Nectria* "*episphaeria* group" by Booth (1959), *Nectria* subgenus *Dialonectria* by Samuels *et al.* (1991) and *Cosmospora* Rabenh. by Rossman *et al.* (1999). As currently delimited, it is distinguished from other cosmospora-like genera by a fusarium-like asexual morph and parasitism on stromata of *Diatrypaceae*. In contrast, *Cosmospora* and *Pseudocosmospora* C. Herrera & P. Chaverri comprise species with acremonium-like asexual morphs, the former occurring mostly on *Xylariaceae* and polypores, the latter mostly on *Diatrypaceae* (Herrera *et al.*, 2013, 2015). These generic delimitations are primarily based on phylogenetic data and supported by the characteristics of the asexual morph and those of the colony *in vitro*. Due to strong homoplasy of the sexual morphs within these genera, the traditional morphological approach does not allow a sound assessment of generic placement. In many cases, host-specificity appears significant and informative but skilled taxonomists are required to identify the pyrenomycetous hosts, often in poor condition.

As delimited by Gräfenhan *et al.* (2011), *Dialonectria* comprised only *D. episphaeria* (Tode) Cooke and the closely related *D. ullevolea* Seifert & Gräfenhan. Three new species were introduced by Lechat *et al.* (2019), expanding the generic concept to include species with larger ascomata, larger, warted ascospores and having an asexual morph lacking microconidia.

Here we introduce two additional species of *Dialonectria* occurring on *Diatrypella* cf. *favacea* (Fr.) Ces. & de Not., based on phylogenetic, cultural and morphological data. One is an undescribed species collected in the French Alps on effete stromata of *Diatrypella* cf. *favacea* on *Alnus incana* (*Betulaceae*); the other one results from the transfer of the variously interpreted taxon *Nectria magnusiana* Rehm to *Dialonectria*, based on a collection on effete stromata of *Diatrypella* cf. *favacea* on *Betula pendula* (*Betulaceae*). We discuss our results supporting the recognition of the new species and the new combination for *N. magnusiana* based on an analysis of ITS, LSU, RPB2 and acl1 sequences and morphological descriptions with illustrations. A dichotomous key is presented, mostly based on the features of the sexual morphs of the seven currently recognized species of *Dialonectria*.

The taxonomy of polysporous diatrypaceous species occurring on *Betulaceae* and *Fagus* currently assigned to *Diatrypella favacea* in a wide sense is unsettled. These taxa are morphologically highly similar and their segregation based on a supposed host-specificity was not demonstrated (Croxall, 1950; Chlebicki, 1986). This precludes the accurate identification of the fungal hosts of our collections, all the more so since they are overmature.

## Materials and methods

**Morphological studies:** 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 and paratype collections are deposited in LIP herbarium (University of Lille, France) and living cultures are deposited at CIRM (Centre International des Ressources Microbiennes Marseille, France). Cultures of living specimens were made on Difco PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 9 cm diam. incubated at 25°C.

**DNA extraction, PCR and sequencing:** After growth of cultures on PDA medium for 7-10 days, genomic DNA was extracted from a portion of fresh mycelium using the Nucleospin plant II kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. For the cell lysis step, the mycelium was fragmented using FastPrep-24™ 5G Benchtop Homogenizer in a lysing Matrix A tube containing the lysis buffer PL1 and RNAse.

The ITS1-5.8S rRNA gene-ITS2 was amplified with the ITS5 and ITS4 primers (White *et al.*, 1990). Two contiguous regions of the second largest subunit of RNA polymerase II (rpb2) were amplified with the PCR primers fRPB2-5F2, fRPB2-7cR and fRPB2-7cF, fRPB2-11aR (O'Donnell *et al.*, 2007). The larger subunit of the ATP citrate lyase (acl1) was amplified using the primers acl1-230up, acl1-1220low (Gräfenhan *et al.*, 2011). The DNA regions were amplified in separate reactions from 1 µl genomic DNA in 50 µl PCR reagent using CloneAmp Hifi PCR Premix (Takara Bio Inc., Japan) in an automated thermal cycler (Mastercycler, Eppendorf, Germany) and processed with the following temperature profile for the ITS region: 35 cycles of 10 s at 98°C, 15 s at 55°C, 5 s at 72°C. The temperature profile for the rpb2 regions was: 5 cycles of 10 s at 98°C, 15 s at 60°C, 10 s at 72°C followed by 30 cycles of 10 s at 98°C, 15 s at 55°C, 10 s at 72°C while the temperature profile for the acl1 sequence was as follows: 5 cycles of 10 s at 98°C, 15 s at 64°C, 10 s at 72°C followed by 5 cycles of 10 s at 98°C, 15 s at 62°C, 10 s at 72°C, followed by 25 cycles of 10 s at 98°C, 15 s at 56°C and 10 s at 72°C. The PCR products were checked on FlashGel™ DNA System (Lonza, Schwitzerland), and sequenced by Genewiz (Leipzig, Germany). Introns of the rpb2 and acl1 gene sequences could not be reliably aligned and were excluded from the final alignment. All the nucleotide sequences generated in this study are deposited in GenBank.

**Table 1 –** Genera, species and GenBank accession numbers of sequences used in the phylogenetic analyses of RPB2 and acl1 sequences.

| Species | GenBank Accession Numbers | |
|---|---|---|
| | RPB2 | acl1 |
| *Albonectria rigidiuscula* | JX171567 | HQ897896 |
| *Atractium stilbaster* | HQ897748 | KM230991 |
| *Clonostachys rosea* | DQ522415 | KX184870 |
| *Cyanonectria cyanostoma* | JX171665 | HQ897895 |
| *Dialonectria episphaeria* | HQ897756 | HQ897892 |
| ***Dialonectria favaceae*** | **MW55805** | **MW55805** |
| ***Dialonectria magnusiana*** | **MW19821** | **MW55805** |
| *Dialonectria ullevolea* | HQ897782 | HQ897918 |
| *Fusarium sambucinum* | HQ897751 | HQ897887 |
| *Fusicolla epistroma* | HQ897765 | HQ897901 |
| *Fusicolla violacea* | HQ897696 | KM231059 |
| *Geejayessia atrofusca* | HQ897775 | HQ897911 |
| *Geejayessia desmazieresii* | HQ897703 | HQ897841 |
| *Macroconia leptosphaeriae* | HQ897755 | HQ897891 |
| *Macroconia papilionacearum* | HQ897776 | HQ897912 |
| *Mariannaea samuelsii* | HQ897752 | HQ897888 |
| *Microcera coccophila* | HQ897705 | HQ897843 |
| *Microcera larvarum* | HQ897717 | HQ897855 |
| *"Cosmospora" flavoviridis* | HQ897702 | HQ897840 |
| *Neocosmospora illudens* | KM232373 | HQ897833 |
| *Stylonectria wegeliniana* | HQ897754 | HQ897890 |

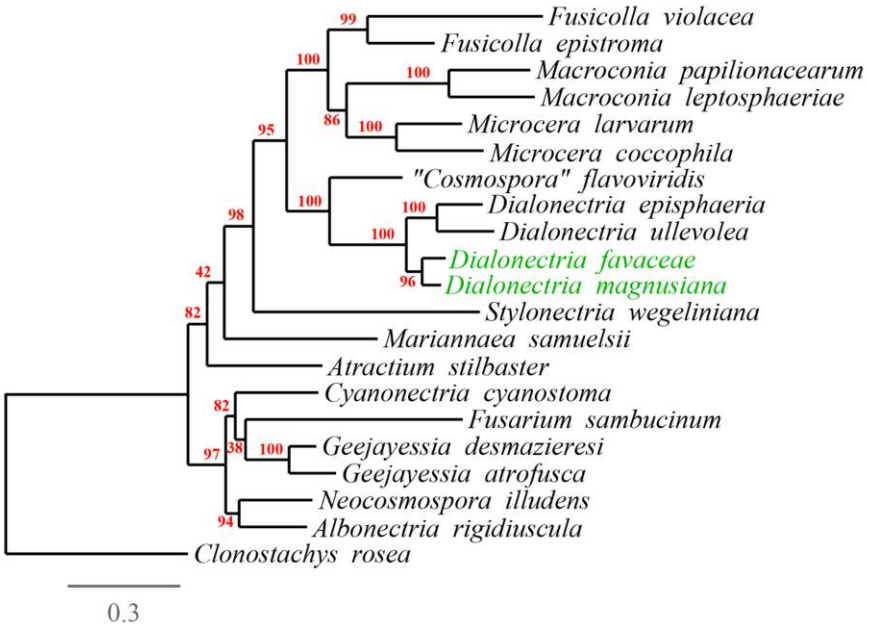


**Fig. 1 –** Maximum likelihood tree (-lnL = 25903.48301) inferred from combined RPB2 + acl1 gene sequences of species having fusarium-like asexual morphs, rooted with *Clonostachys rosea*. ML bootstrap support is given above or below the branches.

## Taxonomy

***Dialonectria favaceae*** Lechat & J. Fourn., *sp. nov.* – Fig. 4 – MycoBank MB 838226

**Diagnosis:** Differs from other species of *Dialonectria* by the combination of a well-developed pseudoparenchymatous stroma, occurrence on *Diatrypella favacea*, pale orange colony on PDA, asexual morph lacking macronidia and nearly smooth-walled ascospores 12–15 × 5.5–6 µm.

**Holotype:** France, Savoie, Parc national de la Vanoise, Pralognan-la-Vanoise, Fontanettes, cirque de l'Arcelin, 45.81328° N 6.747892° E, 1750 m, on dead stromata of *Diatrypella* cf. *favacea* on a dead branch of *Alnus incana*, 20 Jun. 2018, *leg*. C. Lechat, LIP: CLLV18029. Ex-type culture BRFM 2935; Genbank sequences: ITS = MW198213, LSU = MW198211, RPB2 = MW558054 and acl1 = MW558057.

**Etymology:** the specific epithet "*favaceae*" refers to the host *Diatrypella favacea*

**Ascomata** crowded on dead stromata of *Diatrypella* cf. *favacea*, in groups of 5–30, difficult to remove from substratum, subglobose to widely obpyriform, 270–330 µm high, 250–300 µm diam. (Me = 300 × 280 µm, n = 30), smooth, partially embedded in a pseudoparenchymatous stroma arising from ostioles of host, dark red, becoming purple in 3% KOH, yellow in lactic acid, not collapsed or laterally pinched when dry. **Ascomatal surface** composed of cells of undefined shape, forming a *textura epidermoidea*. Apex obtuse, darker than perithecial venter, appearing nearly black when dry, composed of thick-walled, subglobose, ellipsoidal to narrowly clavate cells 3.5–6 µm wide with orange wall. **Ascomatal wall** in vertical section 30–35(–40) µm thick, of two regions; outer region 20–25 µm thick, composed of globose or subglobose to ellipsoidal, thick-walled cells 5–10 × 3–5 µm with orange wall 2–2.5 µm thick; inner region 10–15 µm composed of paler, flattened, thick-walled cells 8–10 × 3–5 µm, gradually becoming thin-walled towards interior, merging with periphyses. **Asci** cylindrical to narrowly clavate, short-stipitate, 80–95 × 8–10(–12) µm, 8-spored, attenuated at rounded to slightly flattened apex with a thickening, ascospores obliquely uniseriate. Filamentous **paraphyses** inserted between asci. **Ascospores** (10–)12–15(–16) × 5.5–6(–6.5) µm (Me = 13.5 × 5.8 µm, n = 30), ellipsoidal, equally 1-septate, constricted at septum, hyaline, smooth or with inconspicuous spinulose ornamentation, difficult to see even in lactic cotton blue.

**Asexual morph** in nature: not observed.

**Cultural characteristics:** After three weeks at 25°C on Difco PDA, colony 2.5–3 cm diam., pale orange in centre, white at margin, slimy, sporulating in white sporodochia. Microconidia fusiform, curved, non-septate 3–7 × 1.5–2 µm. No macroconidia produced after two months.

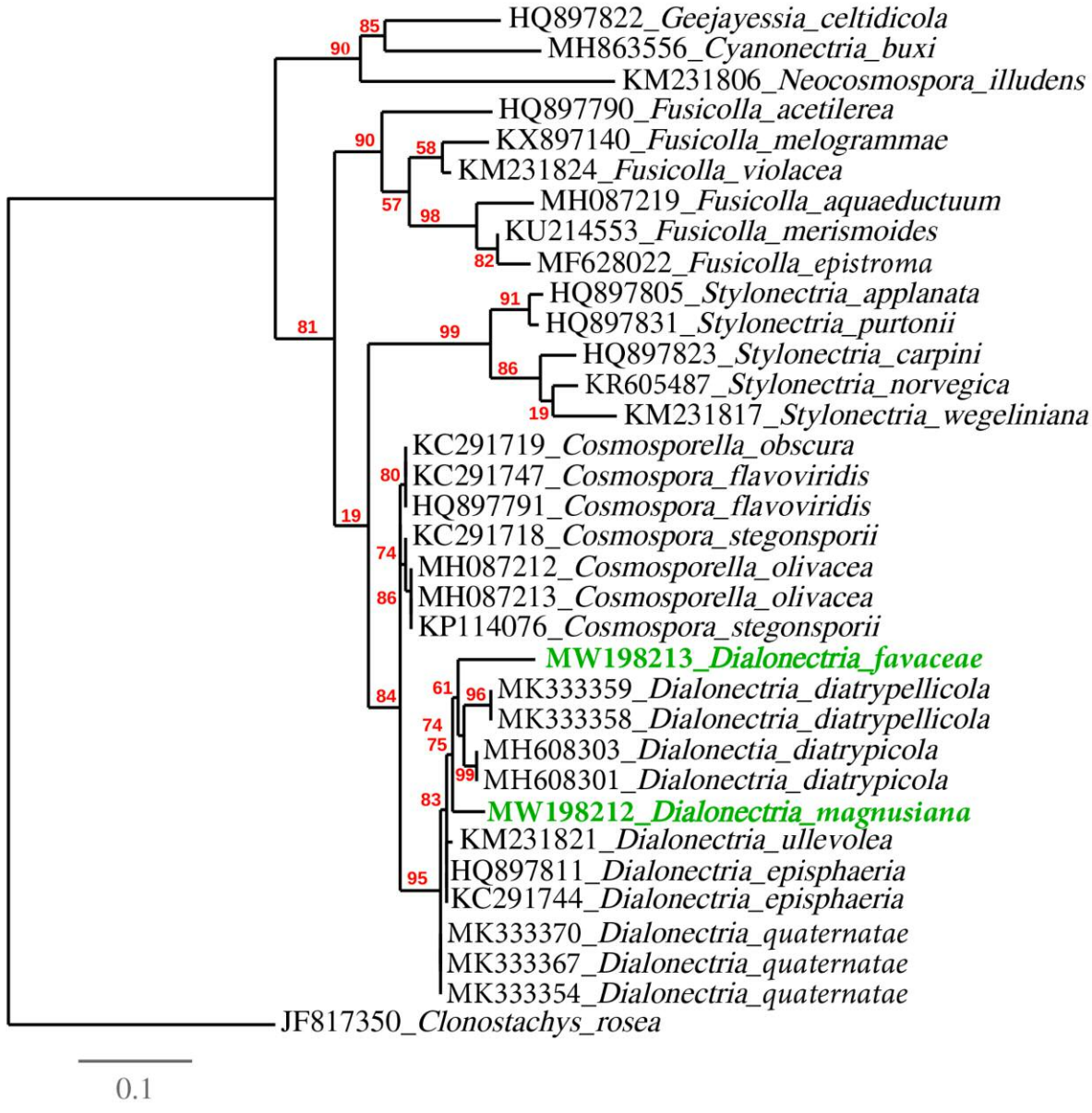


**Fig. 2 –** Maximum likelihood phylogeny (-lnL = 2383.94764) of *Dialonectria* spp. inferred by PhyML 3.0, model hky85 from a 590 bp matrix of ITS sequences, rooted with *Clonostachys rosea*.

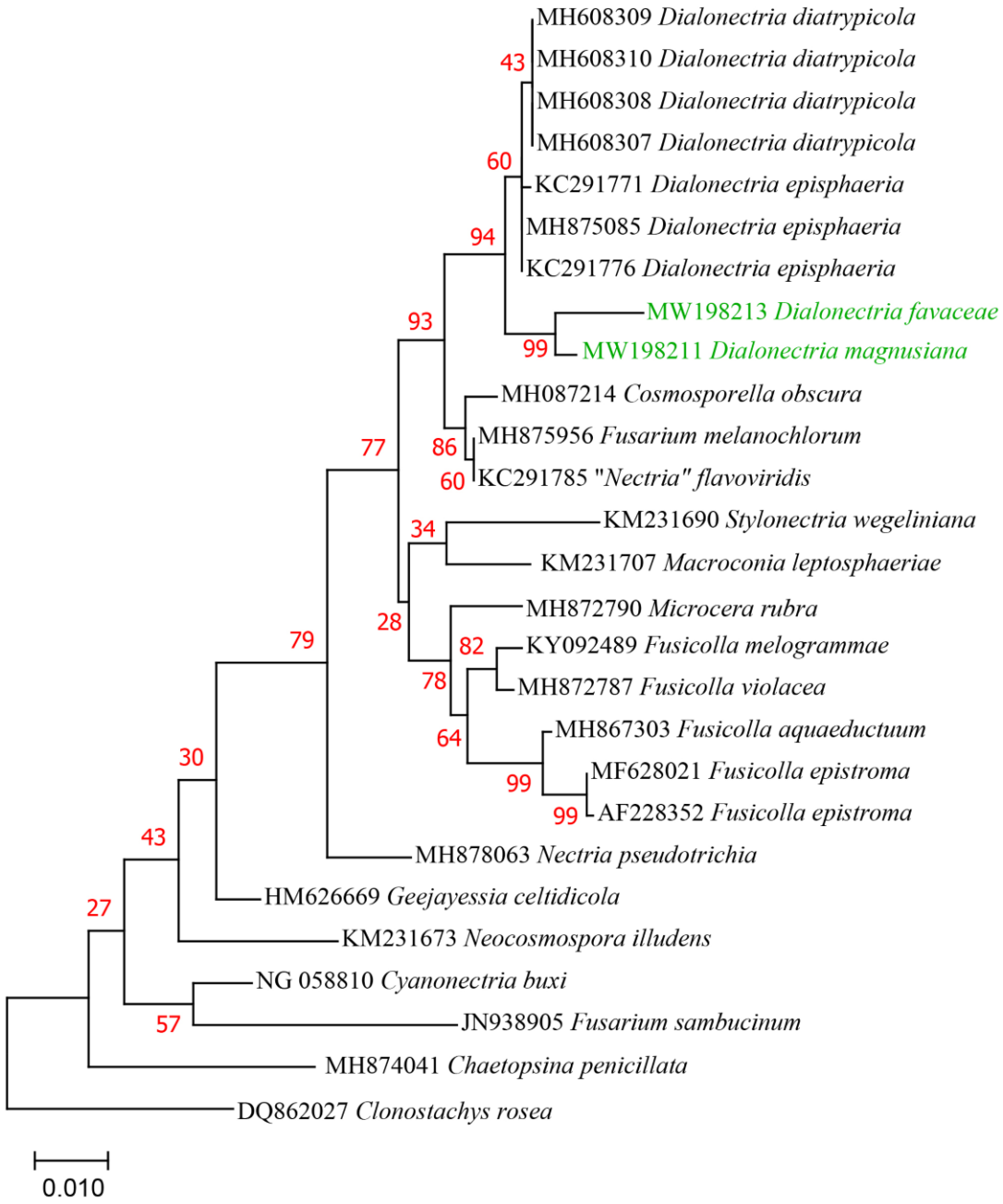


**Fig. 3 –** Maximum likelihood phylogeny (-lnL = 2668.17683) of *Dialonectria* spp. inferred by PhyML 3.0, model hky85 from a 840 bp matrix of LSU rRNA sequences, rooted with *Clonostachys rosea*.

***Dialonectria magnusiana*** (Rehm) Lechat & J. Fourn., *comb. nov.* – Fig. 5 – MycoBank MB 838227

Basionym: *Nectria magnusiana* Rehm, *in* Saccardo, *Michelia*, 1 (3): 294 (1878).

**Ascomata** crowded on dead stromata of *Diatrypella* cf. *favacea*, in groups of 4–60, basally embedded in a weakly developed prosenchymatous stroma arising from ostioles of host, subglobose, 270–350 µm high, 250–320 µm diam. (Me = 320 × 290 µm, n = 30), smooth-walled, brownish orange to pale reddish brown, turning dark reddish brown in 3% KOH, yellow in lactic acid, slightly cupulate, dark brown to blackish brown when dry. **Ascomatal surface** composed of cells of undefined shape, forming a *textura epidermoidea*. **Apex** obtuse, composed of palisadic, cylindrical to narrowly clavate cells, vertically arranged 6–12 × 2–3 µm with pale brownish orange wall. Basal stroma cream-coloured to pale yellow, prosenchymatous, composed of hyphae of asexual morph. **Ascomatal wall** 50–55 µm thick, composed of two regions; outer region 22–30 µm thick, composed of subglobose to ellipsoidal, thick-walled cells 3.5–10 × 4–6.5 µm, with orange to reddish brown wall, 2–2.8 µm thick; inner region 18–25 µm thick, composed of ellipsoidal, elongate cells 6–12 × 2.5–4.5 µm, becoming subhyaline, thin-walled towards interior. **Asci** cylindrical to narrowly clavate, short-stipitate, 80–100 × 8–12 µm (Me = 90 × 10 µm, n = 20), apex simple, ascospores obliquely uniseriate. Evanescent, narrowly moniliform **paraphyses**, up to 6 µm diam. at base, inserted between asci. **Ascospores** ellipsoidal, rounded to slightly attenuated at ends, equally 1-septate, (12–)13–14(–15) × (5–)5.5–6.5(–7) µm (Me = 14 × 6.4 µm, n = 30), hyaline to faintly rosy, pale orange en masse when mature, finely spinulose, not constricted at septum.

**Asexual morph** in natural environment: Sporodochia arising from ostioles of fungal host, pulvinate, pale yellow to pale orange, not changing colour in 3% KOH or lactic acid. Conidiophores branched, each branch bearing 2–5 terminal phialides 20–36 × 2.5–

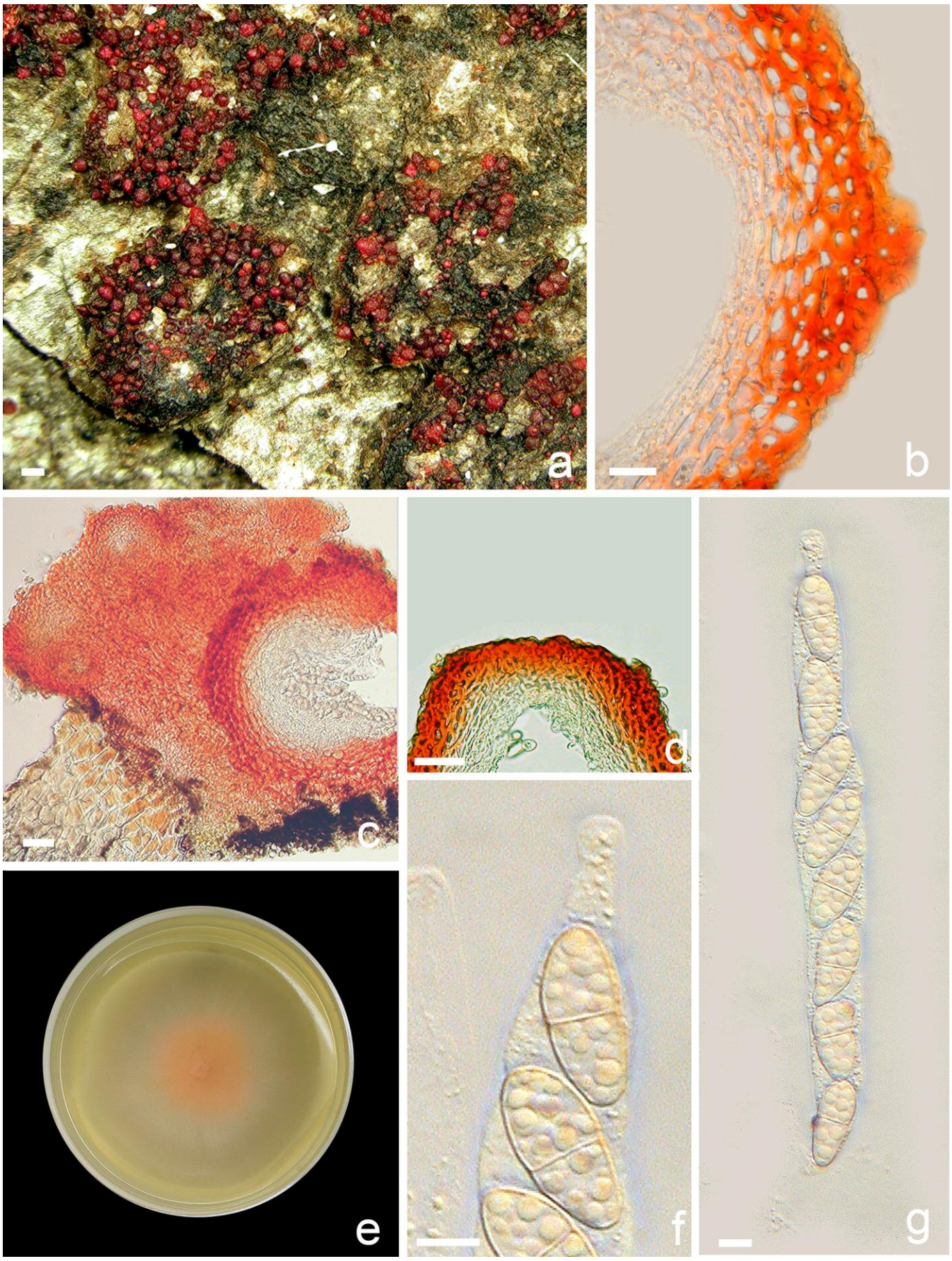


**Fig. 4 – *Dialonectria favaceae*** (Holotype CLLV18029). a: Habit of ascomata on dead stromata of *Diatrypella*; b: Vertical section of lateral ascomatal wall; c: Vertical section through ascoma and stroma; d: Vertical section of ascomatal apex; e: Culture at three weeks; f: Apex of ascus and ascospores; g: Ascus and ascospores. Scale bars: a = 500 µm; b = 10 µm; c, d = 20 µm; f, g = 5 µm.

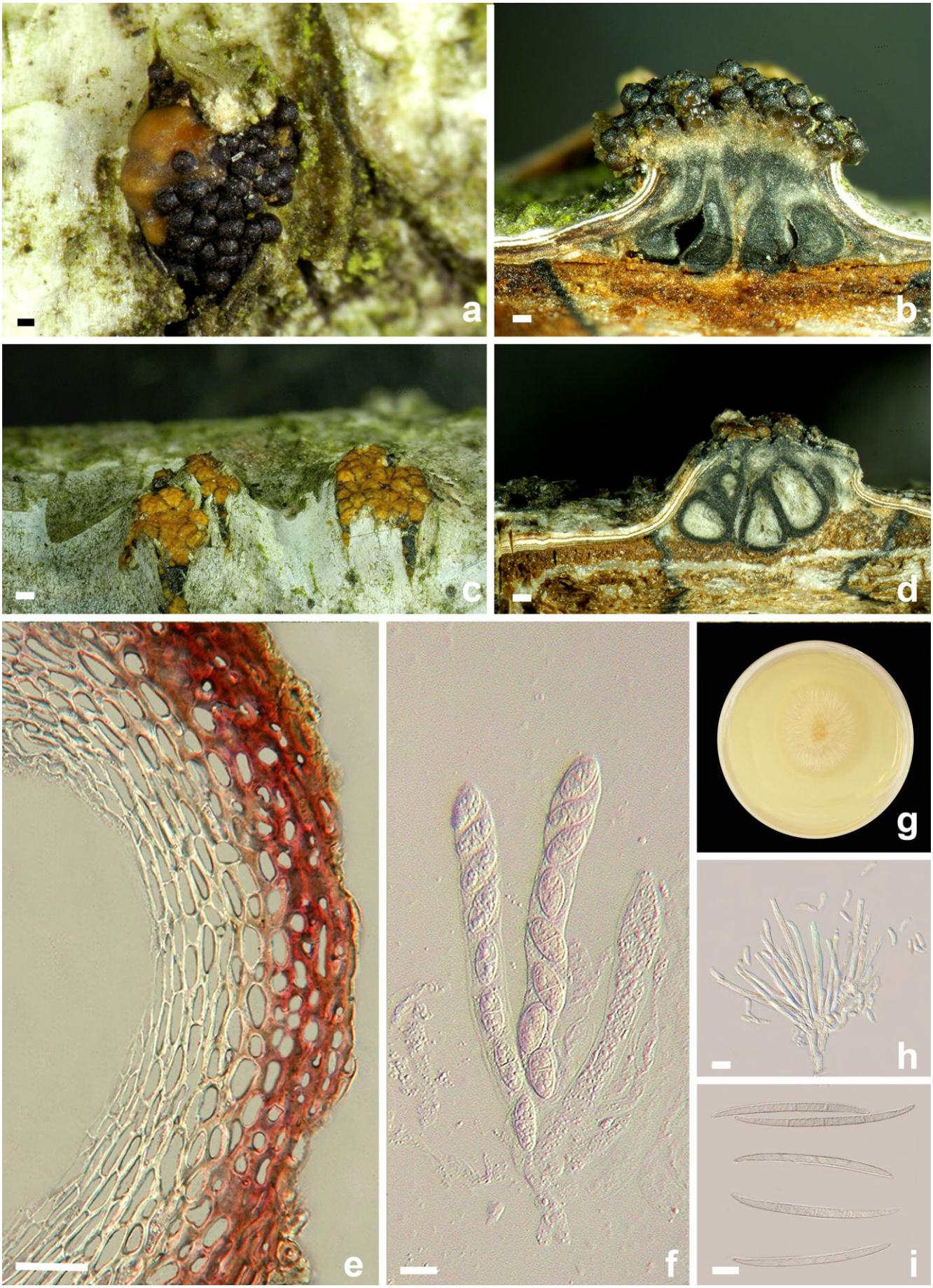


**Fig. 5 – *Dialonectria magnusiana*** (CLL10029) a: Habit of blackish ascomata associated with their asexual morph on dead stromata of *Diatrypella*; b: Vertical section through ascomata and stroma of host; c: Asexual morph in natural environment; d: Vertical section through sporodochia of asexual morph as well as stroma of host; e: Vertical section of ascomatal wall in KOH 3%; f: Asci and ascospores in water; g: Culture at three weeks; h: Primary conidiophores and microconidia from culture; i: Macroconidia from nature. Scale bars: a-d = 200 µm; e = 20 µm; f, i = 10 µm; h = 5 µm.

3 µm, producing cylindrical to slightly fusiform, curved, not septate, microconidia 4.5–6.5 × 1.5–2 µm. Secondary conidiophores bearing subulate conidiogenous cells producing fusiform, slightly curved, acute at ends, 3-septate macroconidia (26–)40–70 × 3–3.5 µm.

**Cultural characteristics:** After three weeks, colony 2.5–3 cm diam., producing a fusarium-like asexual morph, white to cream, slimy, sporulating at middle area. Microconidia fusiform, curved, non-septate 4.5–7(–7.5) × 1.5–2 µm. Macroconidia not produced.

**Specimen examined:** France, Aube, Saint-Pouange, La Coloterie, 48°13'59.5"N 4°02'13.1"E, 122 m, on *Diatrypella* cf. *favacea* on a dead branch of *Betula pendula*, 24 Oct. 2010, *leg*. A. Gardiennet, LIP: CLL10029, culture BRFM 1591, Genbank ITS, LSU, RPB2 and acl1 sequences: ITS = MW198212, LSU = MW198210, RPB2 = MW558055 and acl1 = MW558056.

## Discussion

The placement of these recent collections in *Dialonectria* is suggested by their occurrence on effete stromata of *Diatrypaceae* and a fusarium-like asexual morph in culture. This was unambiguously supported by our phylogenetic analyses based on four markers showing them nested in this subclade with strong support (Figs. 1–3).

Both fungi occur on effete stromata of *Diatrypella favacea* on *Betulaceae* and lack macroconidia in culture, which sets them apart from the known species in this genus; moreover, the combined RPB2 + acl1 phylogeny (Fig. 1) and the LSU-based phylogeny (Fig. 3) suggest a close relationship, which is less clearly supported by the ITS-based phylogeny (Fig. 2) with only 91% of similarity.

The collection CLL10029 described above (Fig. 5) matches well with the protologue of *N. magnusiana* issued by Saccardo (1878), its occurrence on *Diatrypella favacea* on *Betula*, crowded, blackish brown perithecia, and spinulose ascospores 13–15 × 7 µm with a faint rosy tone. Based on its fusarium-like asexual morph and its strong phylogenetic affinities with *Dialonectria*, the new combination *Dialonectria magnusiana* is therefore proposed for this taxon.

As reported by Booth (1959), the name *Nectria magnusiana* was introduced by Rehm (1878) in his *Ascomycetes Exsiccatae* for a species occurring on *Diatrypella favacea* on *Betula* sp. from Germany, but without a diagnosis, which was done by Saccardo (1878) and later by Rehm (1881).

For a long time, many collections of nectriaceous fungi parasitizing dead stromata of *Diatrypella* spp. have been assigned to *N. magnusiana*, leading to some confusion around this name, as discussed by Samuels *et al.* (1991).

Booth (1959) described a collection with a fusarium-like presumed asexual morph, producing only microconidia on the natural substrate, that he referred to as *N. magnusiana*. The morphological characteristics of the sexual and asexual morphs that he reported are not sufficient to link it to *D. magnusiana* more than to any other species. Later, Booth (1971) described a specimen of *N. magnusiana* that he linked to *Fusarium epistromum* (Höhn.) Booth, a synonym of *Dendrodochium epistromum* Höhn., which differs significantly from what he described in 1959. It also differs from *F. magnusianum* as described by Samuels *et al.* (1991) and illustrated by Wollenweber (1930) and Gerlach & Nirenberg (1982), in having macroconidia 1–3-septate but not producing microconidia.

*Dendrodochium epistromum*, the supposed asexual morph of *N. magnusiana*, was shown by Gräfenhan *et al*. (2011) to be phylogenetically unrelated to *Dialonectria*. They proposed the new combination *Fusicolla epistroma* (Höhn.) Gräfenhan & Seifert to accommodate the basionym. The sexual morph of *F. epistroma* differs morphologically from *N. magnusiana* in having pale yellow to pale orange, setose ascomata not changing colour in 3% KOH, as defined by Lechat & Aplin (in Crous *et al*., 2016) and Lechat & Rossman (2017). Accordingly, *F. epistroma* could not be the asexual morph of *Nectria magnusiana* as proposed by Booth (1971).

Finally, based on morphological characteristics (Fig. 5) and phylogenetic analyses (Figs. 1–3), the new combination *Dialonectria magnusiana* (Rehm) Lechat & J. Fourn. is proposed to accommodate *Nectria magnusiana* Rehm.

The collection CLLV18029 described above (Fig. 4) represents the third species in this genus occurring on *Diatrypella*. Our phylogenetic analyses place it in *Dialonectria* on a sister branch to *N. magnusiana* (Fig. 1), from which it differs by having bright red ascomata crowded on a pseudoparenchymatous stroma, while *N. magnusiana* has reddish brown to blackish brown ascomata not obviously stromatic, as defined by the protologue (Saccardo, 1878). Based on morphological features of asexual-sexual morphs, cultural characteristics and phylogenetic analyses, *Dialonectria favaceae* Lechat & J. Fourn. is therefore proposed as a new species. These new additions raise to three the number of *Dialonectria* spp. occurring on *Diatrypella*, namely *D. favaceae* and *D. magnusiana*, both on *Diatrypella favacea* (this paper), and *D. diatrypellicola* Lechat & J. Fourn., on dead stromata of *Diatrypella quercina* (Pers.) Cooke on *Quercus* (Lechat *et al*., 2019). It is interesting to note the absence of other records of cosmospora-like fungi on *Diatrypella* hosts in the literature, despite it is a speciose genus with 157 taxa worldwide listed in MycoBank at this date. For instance, we never recorded cosmospora-like fungi on *Diatrypella* spp. commonly occurring in France on *Alnus*, *Corylus* and *Fagus*, usually referred to *Diatrypella nigro-annulata* (Grev.) Nitschke, *D. tocciaeana* De Not. or *D. verruciformis* (Ehrh.) Nitschke, all synonymized with *D. favaceae* by Croxhall (1950), nor on *D. placenta* Rehm saprobic of *Alnus*. Thus, currently known cosmospora-like fungi on *Diatrypella* spp. from temperate Europe all belong to *Dialonectria*.

### Key to the species of *Dialonectria*

**1** Ascomata less than 150 µm diam; ascospores 7–11 × 3.5–5 µm ..... **2**
**1** Ascomata more than 200 µm diam; ascospores more than 11 µm long ..... **3**
**2** On *Diatrype stigma*; colony on PDA white to pale brown ..... ***D. episphaeria***
**2** On unidentified pyrenomycetes on *Fagus* bark; colony on PDA pale orange ..... ***D. ullevolea***
**3** Ascospores conspicuously warted ..... **4**
**3** Ascospores smooth to finely verrucose ..... **5**
**4** Ascospores 14–18 × 9–10 µm; on *Diatrypella quercina* ..... ***D. diatrypellicola***
**4** Ascospores 12–13 × 6–7 µm; on *Diatrype bullata* (and occasionally *D. stigma*) ..... ***D. diatrypicolla***
**5** Ascospores 11–12.5 × 4.5–5.5 µm; on *Quaternaria quaternata* ..... ***D. quaternatae***
**5** Ascospores larger, 12–15 × 5.5–6.5 µm; on *Diatrypella favacea* ..... **6**
**6** Ascomata bright red, on a pseudoparenchymatous stroma; ascospores smooth-walled or nearly so ..... ***D. favaceae***
**6** Ascomata reddish brown to blackish brown, lacking pseudoparenchymatous stroma; ascospores finely spinulose .. ***D. magnusiana***

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## Authors' contributions

Christian Lechat was responsible for the study conception, morphological studies, phylogenetic analyses, figures and plates design, registrations in MycoBank. The first draft of the manuscript was written by Christian Lechat and was critically reviewed by Jacques Fournier who proposed an enhanced version. Delphine Chaduli, Laurence Lesage-Meessen and Anne Favel were responsible of sequencing and registration of sequences in Genbank. All authors read and approved the final manuscript.

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