Cover: A partial plant extracted from a pustule of *Rhipidium cf. interruptum*, on a poplar twig (*Populus nigra*). The figure presents the typical basal cell, filamentous branches, and reproductive organs.
SCOPE AND AIMS

SCOPE
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AIMS
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Cylindromonium dirinariae sp. nov. (Ascomycota, Hypocreales), a new nectrioid lichenicolous species on Dirinaria applanata in Japan

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Key words: lichenicolous fungi, Nectriaceae, new taxon, phylogeny

Abstract: A nectrioid fungus forming a pinkish colony with mainly solitary phialides producing ellipsoid, aseptate conidia in mucoid packets was isolated from Dirinaria applanata. Our taxonomic study based on morphology and phylogenetic analysis using ITS rDNA sequences revealed that the isolates represented a member of the genus Cylindromonium. Based on further morphological examination, nucleotide sequence comparison, and phylogenetic analysis based on LSU rDNA, tef1, and rpb2 in addition to the phylogenetic analysis using the ITS rDNA sequences, the fungus from Dirinaria represents a new species, which is described here as Cylindromonium dirinariae sp. nov. Furthermore, inoculation experiments revealed that this species can also produce perithecia when inoculated on the host lichen in laboratory environments.

INTRODUCTION

Lichenicolous fungi are a term used to circumscribe fungi that grow on lichens. They can interact with their lichen hosts as saprophytes, parasites and commensalistic par symbionts. Lichenicolous fungi usually establish a symbiotic relationship with a single species or genus of lichens, while some species have a wide host range (Diederich et al. 2018). Approximately 2,000 species of lichenicolous fungi have been described on lichens globally and they are classified into 100 families, 55 orders and 10 classes. Ninety-six percent of the fungi are basidiomycetes (Diederich 2018). About 166 lichenicolous fungi have been reported from Japan to date (Frisch et al. 2018, Ohmura & Kashiwadani 2018, Tadome et al. 2018, Zhurbenko & Ohmura 2018, b, Zhurbenko et al. 2018, Zhurbenko & Ohmura 2019, 2020, Frisch et al. 2020, Tadome & Ohmura 2021, 2022, Tadome et al. 2022). In spite of these reports, there has been relatively little research conducted on this fungal group in Japan. Therefore, many species remain to be discovered and described.

The genus Cylindromonium, with the type species C. eugeniicola, was segregated from Acremonium based on analyses of ITS and LSU rDNA sequence data (Summerbell et al. 2011, Crous et al. 2019b). Cylindromonium species are known to be lichenicolous, mycophilic, or saprophytic (Gams 1971, Crous et al. 2019b, 2020, 2021). Cylindromonium was established as a genus to accommodate acremonium-like taxa with unbranched, hyaline, phialidic conidiophores, and cylindrical 1-septate conidia (Crous et al. 2019b). A total of five asexual species have been assigned to Cylindromonium, for which no sexual morph has thus far been reported (Crous et al. 2019b, 2020, 2021). During our research on the diversity of lichenicolous fungi in Japan, a fungus colonising the Physciaceae lichen Dirinaria applanata was found. The purpose of this study is to describe the morphological, physiological, and ecological features of this species, clarify the link to its sexual morph, and discuss its taxonomic placement.

MATERIALS AND METHODS

Collection materials

Field investigations were performed from September 2020 to March 2021 in Tsukuba city, Ibaraki prefecture, Japan. Specimens of the fungus growing on the lichen host D. applanata were found on the bark of Zelkova serrata. A voucher specimen was deposited in the National Museum of Nature and Science (TNS), Tsukuba, Japan. A living ex-type culture was deposited in the Biological Resource Center of the National Institute for Technology and Evaluation (NBRC). Cylindromonium lichenicola strains (CBS 188.70 and CBS 415.70A) were also examined for comparison purposes.
Morphological observations

Samples were observed using a dissecting microscope [M165 C (Leica, Wetzlar, Germany)] and a differential interference contrast compound microscope [BX53 (Olympus, Tokyo, Japan)]. Anatomical examination was performed using hand-cut sections mounted in a drop of water or clear lactophenol. Photographs were taken using a microscope digital camera [Flexacam C3 (Leica, Wetzlar, Germany) or DP23 (Olympus, Tokyo, Japan)]. Dimensions of ascospores, conidia, conidial mass, phialide and hyphal width are given as (minimum–) range of mean ± standard deviation (–maximum) (n = number of measurements). Chemical reactions of the perithecia were observed by using 10 % KOH. To determine if there is a significant difference between each dimension of the present fungus and *C. lichenicola*, the t-test was performed using Microsoft Excel.

Isolation of fungal cultures

Fungal cultures were isolated from freshly collected material. Mycelium or single ascospores were picked up using a flame needle and plated on 1 % malt extract agar (MEA). To confirm differences in colony characteristics on each agar medium, mycelial plugs were subcultured on 1 % MEA, potato dextrose agar (PDA) (Nissui Pharmaceutical, Tokyo, Japan), oatmeal agar (OA) (Becton Dickinson and Co, New Jersey, USA), Sabouraud maltose agar (SMA) (Thermo Fisher Scientific, Massachusetts, USA), malt yeast extract agar (MYA) (Ahmadjian 1961) and Sabouraud glucose agar (SGA) (Stocker-Wörgötter 2002), confirming the recipes of these media according to Crous et al. (2019a). Colour of colonies were determined based on Kornerup & Wanscher (1978).

DNA extraction, PCR amplification and sequencing

Perithecia were sampled from specimen TNS-L-131533, mycelium from specimen TNS-L-131535, and mycelia from a culture derived from specimen TNS-L-131535. For DNA extraction, fungal tissues were suspended in 20 µL of DNA extraction buffer [10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 50 mM KCl, 0.01 % sodium dodecyl sulfate (SDS), 0.01 % Proteinase K], incubated at 37 °C for 60 min, and denatured 90 °C for 10 min; 30 µL of sterile distilled water (SDW) added to the tubes, and stored in a freezer at -20 °C.

Partial sequences of the nuc rDNA ITS1-5.8S-ITS2 (ITS), large subunit (LSU) nuc rDNA regions, elongation factor 1-alpha (tef1), and RNA polymerase II second largest subunit (rpb2) were amplified as these regions are frequently used for phylogenetic analyses of Nectriaceae (Lombard et al. 2015, Crous et al. 2019b, 2020, 2021). The ITS region was amplified using the primers IT5S and ITS4 (White et al. 1990), LSU rDNA using primers LIC24 (Miadlikowska & Lutzoni 2000) and LR7 (Vilgalys & Hester 1990) or LR0R (Rehner & Samuels 1994) and LR6 (Vilgalys & Hester 1990), tef1 using EF1-983F and EF1-1567R (Rehner & Buckley 2005), and rpb2 using RP82-5F2 and RP82-7cR (O’Donnell et al. 2007). PCR was performed in a 15 µL reaction volume containing 1 µL DNA template, 7.5 µL GenRED PCR Mix Plus (Nippon Gene, Tokyo, Japan), 1.5 µL each primer (2 pmol/µL) and 3.5 µL distilled water. The PCR was performed in a TaKaRa PCR Thermal Cycler Dice™ Touch (TaKaRa, Shiga, Japan) as follows for the ITS region; 5 min at 95 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 53 °C, 1 min at 72 °C, and a final step of 8 min at 72 °C. PCR conditions for LSU, tef1 and rpb2 were set according to Frisch et al. (2020), Rehner & Buckley (2005) and O’Donnell et al. (2007), respectively.

PCR products were checked by electrophoresis on a 1.5 % agarose gel stained with Midori Green Direct DNA Stain (Nippon Genetics, Tokyo, Japan) and visualised using WSE-5200 Printgraph 2 M (ATTO, Tokyo, Japan). The PCR products were purified using a FastGene™ Gel/PCR extraction kit (Nippon Genetics, Tokyo, Japan) and ExoSAP-IT™ (Thermo Fisher Scientific, Massachusetts, USA) following the manufacturer’s instructions.

Sequences were obtained via a DNA sequencing service using Applied Biosystems 3730xl DNA analyzer (Thermo Fisher Scientific, Massachusetts, USA) (Eurofins Genomics, Tokyo, Japan). Data and accession number of the voucher specimen, and the obtained sequences from the International Nucleotide Sequence Database (INSD) are shown in Table 1.

Phylogenetic analysis and comparison of sequence data

Both newly generated ITS sequences and reference sequences were used in the phylogenetic analysis. Sequence data of loci other than ITS do not exist for some isolates of the genus *Cylindromonium*. Therefore, only ITS, LSU and rpb2 of four *Cylindromonium* species were used for the phylogenetic analysis (Table 1). ITS sequence data of species with relatively high identity (> 85 %) in the BLAST search and other hypocrean fungi in the *Nectriaceae* were included in the analysis to infer the taxonomic position of the targeted fungi (Table 1). In these analyses, *Stachybotrys chartarum* (KM231858) was chosen as the outgroup (Lombard et al. 2015). Sequences of each locus (ITS, LSU, tef1 and rpb2) were compared with those of *C. lichenicola*. All sequences analysed in this study were deposited in the DNA Data Bank of Japan (DDBJ), a member of International Nucleotide Sequence Database Collaboration (INSDC).

Assembling forward and reverse strands of the sequenced loci were carried out with MUSCLE v. 3.6 (Edgar 2004) in MEGA v. 7 (Kumar et al. 2016) to obtain consensus sequences. DNA sequences were aligned using the online version MAFFT v. 7 (Katoh et al. 2019) (https://mafft.cbrc.jp/alignment/server/) with default settings. MEGA v. 7 (Kumar et al. 2016) was used to truncate sequences up to the determined edge of the dataset.

Phylogenetic analyses were performed with Maximum likelihood (ML) using an online version W-IQ-Tree v. 1.6.12 (Trifinopoulos et al. 2016) (http://iqtree.cibiv.univie.ac.at/). All characters were equally weighted, and gaps were treated as missing data. The ML analysis for the ITS region alignment using the TIM2+F+I+G4 model and for a combined alignment of the three loci, ITS, LSU, rpb2 using the TN93+G (for ITS and LSU) and TN93+I (for rpb2) were performed with 1 000 bootstrap replicates. FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and MEGA X were used for plotting the phylogenetic trees. Sequence alignments were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:530024).

Inoculation experiments

Symptomless thalli of *D. applanata*, the host lichen of *C. dirinariae*, and those of the non-host lichen *Parmotrema tinctorum* were collected in Tsukuba city, Ibaraki prefecture, Japan and confirmed as non-infected via microscopy. Following this step, the lichen surface was cleaned using an ultrasonic cleaner with 0.005 % Aerosol® OT (a surface-active agent) for 1
<table>
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<th>Species</th>
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<th>Host/Substrate</th>
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<th>ITS Accession No.</th>
<th>LSU Accession No.</th>
<th>tef1 Accession No.</th>
<th>rpb2 Accession No.</th>
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<td>LC731274</td>
<td>LC731275</td>
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RESULTS

Taxonomy

Classification: Nectriaceae, Hypocreales, Sordariomycetes.

**Cylindromonium dirinariae** Ohmaki & Okane, sp. nov. MycoBank MB 846061. Fig. 1.

**Etymology:** Name refers to the host genus *Dirinaria* from which it was isolated.

**Diagnosis:** Ascomata perithelial, globose, orange; phialides short; conidia aggregated in mucoid packets in the apex of phialides, ellipsoid, aseptate; the species differs from all other *Cylindromonium* species by its characteristic DNA sequences (ITS, LSU, tef1, rpb2) and from its closest relative *C. lichenicola* also by its shorter phialides.

**Description:** Ascomata occur on the upper surface or soralia of the host lichen thallus; perithelial, scattered, globose, 80–100 µm diam, pociiform when dry, pale orange when young but later becoming dark, KOH negative, ascomatal wall layers of textura globosa, 14–18 µm thick. Asci broadly cylindrical to clavate, non-stipitate, (21.2–24.5 ± 2.3(–29.1) × (3.9–5.0) ± 0.8(–6.5) µm (n = 12), unitunicate, apex simple, 8-spored. Ascospores biseriate, ellipsoid, hyaline, smooth, medially 1-septate, (5.0–7.8 ± 1.1(–10.1) × (1.8–2.6 ± 0.4(–3.7) µm, length/breadth (l/b) = (2.1–2.6 ± 0.4(–3.9) (n = 40). Mycelium consisting of hyaline, smooth, septate, branched, 2 µm diam hyphae. Conidiogenous cells arising directly from aerial hyphae, hyaline, smooth, subcylindrical, 20–35 µm tall, 2 µm wide at the base, tapering to 1 µm at the apex, phialidic, with non-flared collarette. Conidia solitary, adhering in a slimy mass, hyaline, smooth, aseptate, ellipsoid with obtuse ends, (4.1–5.9 ± 0.9(–10.3) × (1.5–2.4 ± 0.4(–3.5) µm, l/b = (1.5–)2.55 ± 0.5(–3.9) (n = 75).

**Culture characteristics:** Colonies flat, circular or irregular, with moderate aerial mycelium and smooth, lobate margin, reaching 20 mm diam on MEA, PDA, OA, SMA and MYA, 30 mm diam on SGA after 3 wk at 23 °C in darkness. On MEA, PDA, SMA, SGA and MYA surface and reverse strong brownish orange; on OA surface grayish orange.

**Host:** *Dirinaria aplannata*.

**Distribution:** Japan.

**Typus:** Japan, Ibaraki, Tsukuba, Tennodai, Univ. of Tsukuba, 36°06′08″N, 140°06′24″E, lichenicolous on *Dirinaria aplannata* on bark of *Zelkova serrata*, 1 Aug. 2020, A. Ohmaki, I. Okane, K. Ohmachi, K. Miyazawa & K. Gibu, FAO 005 (holotype TNS-L-131533, culture ex-type NBRC 115852); DDBJ: ITS = LC731273; LSU = LC731274; tef1 = LC731275; rpb2 = LC744391.
**Cylindromonium dirinariae sp. nov.**

Fig. 1. *Cylindromonium dirinariae*. **A.** *C. dirinariae* colonizing on *Dirinaria applanata*. **B, C.** Colonies on MEA. **B.** Surface. **C.** Reverse. **D.** Growth habit on *Dirinaria applanata*. **E.** Perithecium. **F.** Conidiogenous cells and conidia. **G.** Asci. **H.** Ascospores. **I.** Conidia. **J.** Phialide and conidial mass. Scale bars: **B, C** = 3 cm; **D** = 0.5 mm; **E, F** = 0.25 mm; **G, I, J** = 10 µm; **H** = 5 µm.
Additional materials examined: Japan, Ibaraki, Tsukuba, Tennodai, Univ. of Tsukuba, 36°06′08″N, 140°06′24″E, from Dirinaria planaplanata on the bark of Zelkova serrata, 1 Aug. 2020, A. Ohmaki, I. Okane, K. Ohmachi, K. Miyazawa & K. Gibu, NBCR 115853 = TNS-L-131534 (FAO 004), DDBJ: ITS = LC731276; LSU = LC744402; tef1 = LC744396; rpb2 = LC744390; 6 Nov. 2020, A. Ohmaki, TNS-L-131536 (FAO 090), in an inoculation experiment with isolates on D. planaplanata (Ibaraki: Tsukuba, 1 Aug. 2020, A. Ohmaki, I. Okane, K. Ohmachi, K. Miyazawa & K. Gibu, TNS-L-131533); Tsukuba, Tennodai, Univ. of Tsukuba, 36°06′38″N, 140°06′16″E, from Dirinaria planaplanata on the bark of Zelkova serrata, 15 Mar. 2021, A. Ohmaki, NBCR 115853 = TNS-L-131535 (FAO 006), DDBJ: ITS = LC731277; LSU = LC744401; tef1 = LC744395; rpb2 = LC744392.

Notes: The ex-type strain of C. lichenicola (CBS 425.66) was not available for study, so we conducted morphological observations on other available strains of C. lichenicola (CBS 188.70 and CBS 415.70A) that are similar in terms of their collection sites and hosts. In addition, these strains were also studied when the genus Cylindromonium was established in Crous et al. (2019b).

Morphological features of the Cylindromonium strains (CBS 188.70 and CBS 415.70A) shown in Table 2 correlated well with the original description provided by Gams (1971) [conidia size 5.5–9.8 × 1.5–2.5 µm (l/b = 3.0–4.4), phialide length 30–60 µm, phialide width base 2.0–3.0 µm, apex 0.7–1.5 µm].

As a result of the t-test, there were significant differences between C. dirinariae and C. lichenicola in conidia, phialide length and width (Table 2). As for phialide length, phialides of C. dirinariae were about half as long as those of C. lichenicola.

Materials examined of Cylindromonium lichenicola: Germany, Probsteiherangen, Schüttbrehm, from unnamed apothecia of lichen on tree bark, Oct. 1996 (living strain CBS 188.70), GenBank: LSU = LC744399; tef1 = LC744398; rpb2 = LC744393. Netherlands, Utrecht, Amelisweerd, from aerial algae on tree bark, Oct. 1968 (living strain CBS 415.70A), GenBank: LSU = LC744400; tef1 = LC744397; rpb2 = LC744394.

Phylogeny

The ITS sequences derived from DNA extracted from a peritheciun (specimen TNS-L-131533) and that from mycelia from another specimen (TNS-L-131534) of C. dirinariae were identical. The Phylogenetic analysis based on the ITS region revealed that the two stains were different (Fig. 2). The clade was sister to C. lichenicola and linked with five Cylindromonium species including the type species of the genus Cylindromonium, C. everniae. The clade including the Cylindromonium species also included species of Trichonectria (Bionectriaceae) and Phialoseptomonium (Nectriaceae).

Table 2. Comparison of dimensions (µm) between Cylindromonium dirinariae and C. lichenicola.

<table>
<thead>
<tr>
<th></th>
<th>Conidia*</th>
<th>Phialide length*</th>
<th>Phialide width (Base)*</th>
<th>Phialide width (Apex)*</th>
<th>Hyphae width</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. dirinariae</td>
<td>(4.1–)5.9 ± 0.9 (–10.3) × (1.5–)2.4 ± 0.4 (–3.5)</td>
<td>(14.7–)27.4 ± 8.1 (–56.7)</td>
<td>(1.7–)2.3 ± 0.3 (–3.1)</td>
<td>(1.0–)1.4 ± 0.2 (–2.0)</td>
<td>(1.2–)2.0 ± 0.3 (–2.6)</td>
</tr>
<tr>
<td>C. lichenicola</td>
<td>(4.1–)6.8 ± 1.1 (–10.0) × (1.5–)2.4 ± 0.5 (–3.3)</td>
<td>(33.9–)51.7 ± 7.6 (–69.9)</td>
<td>(2.0–)3.2 ± 0.5 (–4.0)</td>
<td>(1.1–)1.6 ± 0.3 (–2.5)</td>
<td>(1.5–)2.3 ± 0.5 (–3.3)</td>
</tr>
</tbody>
</table>

*Significantly different.
C. rhabdosporum, supported by high bootstrap values (99 %) (Fig. 2). Comparison of other loci sequences also showed that similarity between C. dirinariae and C. lichenicola were 96.7 % in ITS, 98.8 % in LSU, 94.9–95.1 % in pfb2. In addition to phylogeny, C. dirinariae is also morphologically distinct. In our morphological observations and the inoculation experiments, C. dirinariae had 20–35 µm tall phialides and produced perithecia on D. applanata, while C. lichenicola had longer phialides (45–60 µm tall), and failed to produce perithecia on D. applanata and P. tinctorum. Hence, we concluded that C. dirinariae represents a new species. In addition, this is the first report of the sexual morph for the genus Cylindromonium.

The genus Cylindromonium has been reported from Belgium (Gams 1971, Diederich 1989), Germany (Gams 1971, Brackel 2010), Great Britain (Hitch 1995), France (Roux 2012), Luxembourg (Diederich 1989), the Netherlands (Brand et al. 2013, Crous et al. 2021), Czech Republic (Kocourková 2009), India (Joshi et al. 2016), Ukraine (Khodosovtsev et al. 2018), and Australia (Gams 1971, Crous et al. 2020). This is the first report of the genus Cylindromonium from Japan.

Table 3. Colonization rate in inoculation experiments.

<table>
<thead>
<tr>
<th>Lichen species inoculated</th>
<th>Cylindromonium dirinariae</th>
<th>Cylindromonium lichenicola</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dirinaria applanata</td>
<td>16/20* (80 %)**</td>
<td>13/15 (87 %)</td>
<td>3/12 (25 %)</td>
</tr>
<tr>
<td>Parmotrema tinctorum</td>
<td>4/4 (100 %)</td>
<td>2/2 (100 %)</td>
<td>0/5 (0 %)</td>
</tr>
</tbody>
</table>

*Number of colonised lichenicolous fungi/the number of inoculated lichens.

**Perithecia produced.
Inoculation experiments using single ascospore cultures revealed that this fungus is homothallic. The newly described *C. dirinariae* was isolated from *D. applanata*, while the hosts of *C. lichenicola* are diverse, including the lichens *Cladonia*, *Hypogymnia*, *Parmelia saxatilis*, *Tremella cladoniae*, lichens overgrowing *Stereum* species, fungi; *Bulgaria inquinans*, algal-covered bark, *Alnus* bark and *Betula* litter (Gams 1971, Diederich 1989, Hitch 1995, Brackel 2010, Roux 2012, Brand et al. 2013, Tsurykau et al. 2016). Results of our inoculation experiments are suggestive of host specificity; i.e., *C. dirinariae* was able to form perithecia on its original host lichen, *D. applanata*, but not on *P. tinctorum*. *Cylindromonium dirinariae* could therefore be considered host specific.

Glenn et al. (1997) found that *Cylindromonium rhabdosporum* (as *Acremonium rhabdosporum*), occurred on healthy-looking thalli in the field, and perithecia of *Nectriopsis rubefaciens* (as *Nectria rubefaciens*) appeared on the same thalli in the closed plates. Although they mentioned the relationship between *C. rhabdosporum* and *N. rubefaciens*, they did not confirm that they belong to the same holomorph. Further study is therefore needed to determine the homogeneity between *C. rhabdosporum* and *N. rubefaciens*.

Presently there are only a few reports of inoculation experiments of lichenicolous fungi, and they were conducted in the field (Fatma et al. 2019). Glenn et al. (1997) found that continuously moist conditions probably play a pivotal role for lichenicolous fungi to produce perithecia. Inoculation experiments in the moist condition in Petri dishes using axenic cultures of lichenicolous fungi may therefore be a useful technique for studying morphological, physiological and ecological features of lichenicolous fungi. This work has demonstrated the potential of inoculation experiments to investigate the morphological feature of perithecia and host specificity.

Presently the ecology of *C. dirinariae* remains unclear. Other species of *Cylindromonium* were reported to be mycophilic or saprophytic (Crous et al. 2019b, 2020). We expect that inoculation experiments will reveal the interaction between lichenicolous fungi and their host lichens which could help us to better understand the ecological role of lichenicolous fungi.

The ascomycete family *Nectriaceae* includes numerous important plant and human pathogens as well as several facultatively fungicolous or insecticolous species (Rossman 1996, Lombard et al. 2015). Members of *Nectriaceae* are characterised by unicellular ascomata that are white, yellow, orange-red or purple, unitunicate asci and phialidic asexual morphs (Rossman et al. 1999, Lombard et al. 2015). In many cases ascomata show a change of colour when mounted in KOH (Lombard et al. 2015). In our study, perithecia of the *C. dirinariae* were orange in colour and did not react in KOH.

About 400 genera and 2 300 species of lichenicolous fungi are known from lichens, but the actual number of lichenicolous fungal species could be much higher (Diederich et al. 2018). Diederich et al. (2018) estimated 3 000 – 5 000 lichenicolous fungal species will eventually be described based on Hawksworth’s global estimates of fungal diversity (Hawksworth 1991, 2001) and the total number of lichen species (Lücking et al. 2017a, 2017b). Lichenicolous fungi are assumed to be an important source of new species in many groups of fungi, including *Nectriaceae*. 

![Fig. 3. A phylogenetic tree for *Cylindromonium dirinariae* and other *Cylindromonium* spp. constructed from a maximum-likelihood (ML) analysis based on concatenated sequence dataset of ITS, LSU rDNA and rpb2. The outgroup is *Stachybotrys chartarum*. Number above a branch represents the bootstrap value.](image-url)
ACKNOWLEDGEMENTS

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Conflict of interest: The authors declare that there is no conflict of interest.

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Fig. 4. Lesions on the lichen thallii after inoculation. A. Colony of C. dirinariae on D. applanata. B. Colony of C. dirinariae on P. tinctorum. C. Colony of C. lichenicola on D. applanata. D. Colony of C. lichenicola on P. tinctorum. Scale bars = 5 mm.


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New records and barcode sequence data of wood-inhabiting polypores in Benin with notes on their phylogenetic placements and distribution

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Key words: wood-inhabiting fungi, phylogeny, diversity, new taxa, tropical Africa, polypore

Abstract: Wood-inhabiting fungi (WIF), such as polypores, are extremely species-rich and play vital roles in the functioning of forest ecosystems as decomposers. Despite the importance of polypores, our knowledge of the diversity and distribution of these fungi is still poor in general and especially for West Africa. To advance our knowledge we here summarise results from field collections between 2017 and 2021 and present (i) a taxonomic overview, (ii) phylogenetic placements and (iii) an illustrated catalogue of wood-inhabiting polypore fungi with colour pictures. During the field sampling campaigns, we collected 647 specimens. Based on morphological characteristics and molecular barcode data, 76 polypore species belonging to six orders, 15 families and 39 genera were identified. Of the 76 species, 30 are new to the West Africa, 69 new to Benin, and two new combinations Fuscoporia beninensis and Megaspora minuta are proposed. With this summary, we provide new data for further research.

INTRODUCTION

Wood-inhabiting fungi (WIF) such as polypores are ecologically a very diverse group, ranging from saprotrophic, parasitic, to mycorrhizal (Ryvarden & Johansen 1980, Tedersoo et al. 2007). As saprotrophs, they play vital roles in food webs, participate in the recycling of soil carbon and nutrients (Harley 1971), and transform hard-to-digest organic matter (such as lignin and cellulose) into forms usable by other organisms (Stokland et al. 2012). In addition to their ecological importance, polypores have practical importance for human beings as food, medicine and resources for mycoremediation (Gilbertson 1980, Zjawiony 2004, Grienke et al. 2014). Despite the importance of polypores, their diversity and distribution are often overlooked and neglected in the tropics. As result, many species might disappear without been discovered, recorded and identified (Ujang & Jones 2001, Lindenmayer et al. 2013) because of the alarming rate of forest degradation in the tropics and the fact that polypores are partly specialised towards wood from certain plant species (Krah et al. 2018). Likewise, we could not obtain any information on the function of the species in the ecosystem and for human beings (Lindenmayer et al. 2013). Thus, it is important to document the diversity of polypores in less surveyed areas like Benin.

Benin is a country located in West Africa and characterised by a great diversity of landscapes and ecosystems. It abounds with a floristic diversity of 2 807 known plant species out of 3 000 estimated (Akoegninou et al. 2006). Despite the floristic diversity coupled with the dependence of polypores on dead or living trees, only 10 species of polypores have been reported up to 2017. These are Favolus tenuiculus, Ganoderma applanatum, Ganoderma lucidum, Ganoderma mbrekobenum, Lentinus squarrosum, Lentinus tuber-regium, Lentinus velutinus, Nigroporus stipitatus, Pycnoporus sanguineus, and Trametes palisstii (Boa 2004, Ihayere et al. 2010, Eyi-Ndong et al. 2011, Osemwegie et al. 2014, Yorou et al. 2014, Boni & Yorou 2015). These reports were based on field observations and no fungarium material is available for taxonomic revision of some of these species such as G. applanatum and G. lucidum which do not occur in Africa according to recent studies (Cao et al. 2012, Wang et al. 2012). The first mycological investigations in Benin with a focus on basidiomycetes wood-inhabiting polypores started in 2017. From these surveys, 36 species of polypores were fully identified based on morphological examination (Olou et al. 2019a). These species were subsequently listed in the checklist of West African fungi (Piepenbring et al. 2020). Since then, further records and new species of polypores were reported for Benin and West Africa (Olou et al. 2019b, 2020, 2021, Olou et al. 2020).
& Ryvarden 2021). However, as molecular sequence data are lacking, these specimens have never been included in DNA-based studies of polypores. Knowing that fungal identification based on morphology only sometimes yields unreliable results due to the misleading morphological characteristics (Olson & Stenlid 2002, Giraud et al. 2008, Hughes et al. 2013, Perez et al. 2013, Lücking et al. 2014); there is a need to re-examine previously reported polypore species from morpho-anatomical and molecular perspective.

DNA marker sequencing, widely known as barcoding (Hibbett 1992, Bridge et al. 2005, Nilsson et al. 2006, Hibbett et al. 2011, Hibbett & Taylor 2013), has become a popular tool for a variety of studies, including species identification and molecular phylogenetic inference (Hebert et al. 2003, Hebert & Gregory 2005, Savolainen et al. 2005). Different genes or specific DNA regions are used in barcoding application. The ITS region has been widely adopted by the mycological community as the most suitable marker with a high probability of correct identification for species in many groups of fungi (Schoch et al. 2012). Although the ITS region is widely accepted, sequence data for this region are available for less than 1% (Vu et al. 2014) of the estimated 3.8 million species of fungi (Nilsson et al. 2006, Hawksworth & Lücking 2017, Raja et al. 2017). Moreover nearly 70% of the described species have not yet been sequenced (Rossman & Palm-hernández 2008). Knowing that African species have been scantily used in DNA based studies, there is no doubt that many of the species not yet sequenced are from tropical Africa. This lack of DNA sequences from African specimens is a problem in phylogenetic analyses in a global context. Considering these issues, this study aims to summarise results from field collections in Benin between 2017 and 2021 and present (i) a taxonomic overview, (ii) phylogenetic placements and (iii) an illustrated catalogue of wood-inhabiting polypores with colour photos.

MATERIAL AND METHODS

Specimens

A total of 647 specimens of WIF were collected in eight different forests of Benin namely the Pahou forest, semi-deciduous dense forest of Lama, the woodlands of Touï-kilibo, Ouémé supérieur, Trois Rivières, Okpara, National Park W, and the gallery forest of Bassila from July to September each year starting from 2017 to 2021. All wood-inhabiting polypores with a focus on basidiomycetes were photographed in their natural environment before recording using a Sony camera, model DSC-HX400V. The geographic coordinates of occurrence of each specimen were recorded. Small pieces of fresh basidiocarps were placed in plastic bags half-filled with silica gel for a later DNA extraction. The rest of basidiocarps were air- or oven-dried at 45–50 °C for 1–2 d depending on the consistency of the basidiomata. The dried basidiomata were then preserved in plastic bags for morphological investigation. Specimens are deposited at the mycological herbaria of the University of Parakou (UNIPAR) in Benin, with duplicates at the University of Kassel (KAS) in Germany, and at the Institute of Biology, University of Oslo (O) in Norway.

Wood-inhabiting polypore fungi species identification

Macro-morphological descriptions were based on fresh and dried fungarium specimens. Macro-morphological and microstructures descriptions were based on dried fungarium specimens. Macro-morphological characters are described with the aid of a dissecting microscope Leica EZ4 while microstructures are described using a Leica DM500 compound microscope. For the microstructures, fine sections through the basidiomata were prepared for observation using a razor blade under a dissecting microscope Leica EZ4 and mounted in 5% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine. Melzer’s reagent (to test for dextrinoid or amyloid reactions), Cotton Blue (to test for cyanophilic reaction) were used and then examined at a magnification of 1 000× using a Leica DM500 compound microscope. For species identification, we used the identification keys of Ryvarden & Johansen (1980), Gilbertson & Ryvarden (1986, 1987), Bernicchia & Gorjón (2010), Bernicchia & Gorjón (2020).

DNA extraction, amplification, and sequencing

Genomic DNA of all specimens was extracted using mainly the microwave DNA extraction method (Dörnte & Kües 2013). When microwave DNA extraction did not yield good results, the NucleoSpin Plant II DNA extraction kit (Macherey, Nagel, Germany) and the E.Z.N.A.* Fungal DNA Mini kit according to manufacturer’s instructions were used. An Epoch machine was used to measure the amount of DNA before amplification. The extracted genomic DNA was amplified targeting the nuclear ribosomal DNA region spanning both of the internal transcribed spacers (ITS) for all species with the primer pair ITS-1F/ITS4 (White et al. 1990, Gardes & Bruns 1993). The Polymerase Chain Reaction (PCR) procedure was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 52 °C for 30 s, and 68 °C for 1 min, and a final extension of 68 °C for 3 min. For some genera such as *Megasporoporia* and *Microporus*, additional regions namely the large subunit 28S nrDNA (LSU) and the translation elongation factor (Tef) with primer pair LROR/LR5 (Vilgalys & Hester 1990) and EF1-983F/EF1-1567R (Rehner & Buckley 2008) were amplified. The PCR products were further cleaned with a QIAquick PCR Purification Kit according to the manufacturer’s instructions (QIAGEN GmbH, Hilden, Germany) and then sequenced at the company Eurofins Genomics Germany GmbH (https://www.eurofinsgenomics.eu/).

Phylogenetic analysis

Both sequenced DNA strands (forward and reverse) were used to build the consensus sequences using Geneious v. 5.6.7 (Kearse et al. 2012). Thereafter, the names given to each species on the basis of morphological examination were assigned to each consensus sequence. All newly generated sequences were aligned with similar sequences retrieved from GenBank. The resulting alignment was used to construct a global phylogenetic tree for wood-dwelling fungi. Later, more in-depth phylogenetic trees were constructed for the two most represented orders, namely *Hymenochaetales* and *Polyporales*. For *Hymenochaetales*, 27 ITS sequences retrieved from GenBank were aligned together with 10 ITS sequences from Benin specimens. For the analysis on *Polyporales*, 93 ITS sequences retrieved from GenBank were aligned together with 49 ITS sequences from Benin specimens. Sequences were aligned with MAFFT v. 7 (Katoh et al. 2017). Then, the alignments were manually adjusted with AliView v. 1.28 (Larsson 2014) and exported as Phylip format. The best-fit evolutionary model was estimated for each alignment using
the standard model selection (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE v.1.6.12 (Minh et al. 2020, http://www.iqtree.org/) with the command line –m TESTONLY. Following this substitution model, the phylogenetic tree inference of Maximum likelihood (ML) and Bayesian Inference (BI) were performed to verify the phylogenetic position of all newly generated sequences. On the dataset of all orders, a Maximum likelihood analysis was performed. The Branch support was evaluated using the Ultrafast Bootstrap (UFBoot) (Hoang et al. 2018) with 5 000 replicates and approximate likelihood ratio test (SH-ALRT) (Anisimova et al. 2011) with 5 000 replicates. The analysis was performed in IQ-tree v. 1.6.12 (Minh et al. 2020, http://www.iqtree.org/) with the command line mode. On Hymenochaetales and Polyporales datasets, the branch supports were evaluated using two approaches, the Ultrafast Bootstrap (UFBoot) (Hoang et al. 2018) and posterior probability (PP). The approaches were performed using IQ-TREE v. 1.6.12 (Minh et al. 2020, http://www.iqtree.org/) and MrBayes v. 3.2.7 respectively. The ML was run using IQ-TREE v. 1.6.12 with 5 000 replicates. The BI was executed using MrBayes v. 3.2.7 in command line mode (https://github.com/NBISweden/MrBayes) for five million generations until the standard deviation of split frequencies reached 0.01. Chain convergence was determined using Tracer v. 1.7.1 (http://tree.bio.ed.ac.uk/software/tracer/) and the first 25 % (5 000) trees was discarded as burn-in. The remaining trees were used to build the consensus tree using the Phylogenetic Tree Summarization (SumTrees) program within DendroPy v. 4.3.0. (Sukumaran & Holder 2010, https://github.com/jeetsukumaran/DendroPy). The topology of the ML tree is used, and to add the posterior probabilities (PP) of BI on the ML tree, the Phylogenetic Tree Summarization (SumTrees) program within DendroPy v. 4.3.0. (Sukumaran & Holder 2010, https://github.com/jeetsukumaran/DendroPy) was used. Then, the UFBoot values were added to the ML best tree that already has the posterior probabilities using IQ-TREE v.1.6.12 (Trifinopoulos et al. 2016). The resulting tree with (UFBoot / PP) is presented below and the support values of UFBoot and PP are indicated on each node when they are > = 50 %. For some genera with questionable phylogenetic position of some species, a targeted phylogenetic analysis on each genus was performed using the ITS-LSU combination for Megasporoporia and ITS-LSU-Tef for Microporus. The sequences used in the analyses with the GenBank accession numbers, voucher, and origin of the specimens are presented in the supplementary Table S1.

Wood-inhabiting polypore fungi species distribution in Benin

The GPS coordinates from the photos of the species taken in the field were extracted using the DNRGPS (https://gisdata.mn.gov/dataset/dnrgps). In addition to the field data, presence records across Africa with their geographic coordinates were downloaded from Global Biodiversity Facilities (GBIF) for each species reported in this study. Moreover, the check-list of fungi of the West-Africa was used to ensure no omission for this region. Duplicate records were deleted and the World Geodesic System 1984 (EPSG 4326) were used to project the geographic coordinates on Africa continent extent under the QGIS v. 3.22.1. We removed erroneous coordinates like coordinates falling in oceans. In order to make the map easier to read, we aggregate occurrences records by genus level.

RESULTS

Diversity of basidiomycetes wood-inhabiting polypores from Benin

In total, 39 genera, 15 families, and six orders representing 76 species of wood-inhabiting polypores are recorded in Benin. The three most diverse genera are Coriolopsis, Perenniporia and Trametes with five species each. Hymenochaetaeaceae and Polyporaceae are the most dominant families, with respectively 15 and 46 species each. The other 13 families are represented by one, two or three species.

Importance of newly generated sequences

A total of 152 DNA single direction reads were generated, resulting in 76 consensus sequences namely 59 ITS, 10 LSU, 7 Tef. These sequences are mostly the first ever for wood-inhabiting polypores in West Africa. Before this study, sequences of Microporus concinnus, M. incomptus, and Phellinus beninensis were missing in GenBank and are therefore generated here for the first time. The absence of sequences of African wood-inhabiting polypores has always hampered large-scale studies on this group. With these new sequences generated for the West African specimens, further phylogeographic studies integrating sequences from African specimens will be more easily doable.

Phylogenetic placement of Benin wood-inhabiting polypores

Phylogenetic analyses revealed distinct clades corresponding to specific taxonomic orders such as Agaricales, Gloeophyllales, Hymenochaetales, Polyporales, and Russulales. Sequences belonging to the orders of Hymenochaetales and Polyporales are the most abundant in our analyses while the other three orders Agaricales, Gloeophyllales and Russulales are less represented (Supplementary Fig. S1). The phylogenetic tree on Hymenochaetales is composed of Hymenochaetaceae and newly generated sequences are positioned accordingly in the corresponding clades with the exception of sequences named Phellinus beninensis (Fig. 1). Sequences of Ph. beninensis cluster together and fall into the Fuscioporia clade with good branch support (70/1). Further phylogenetic analyses of the genus Fuscioporia confirmed the correct phylogenetic position of Ph. beninensis (Fig. 2). From the phylogenetic tree on Polyporales, newly generated sequences cluster together with other similar sequences retrieved from other studies, except the sequences of Megasporoporia setulosa and sequences of the genus Microporus where species are misplaced (Fig. 3). Sequences of the species identified as Microporus incomptus and M. affinis grouped together with other sequences named M. xanthopus and M. concinnus, while sequences named in this study as Megasporoporia setulosa did not match with other sequences of the same name available in GenBank (Fig. 3). Phylogenetic analyses combining the ITS and LSU regions for Megasporoporia and ITS-LSU-Tef for Microporus resolved the questionable phylogenetic positions of these different species (Figs 4, 5).
Fig. 1. Maximum likelihood (ML) and Bayesian analysis (BI) analyses of Hymenochaetales based on the ITS dataset. Branch support values given as UFBoot / PP. Newly generated and Benin sequences are highlighted in red colour. The sequence names are followed by voucher or strain number and country of origin.
Wood-inhabiting polypores in Benin

Fig. 2. Maximum likelihood (ML) analysis of the genus Fuscoporia with rapid bootstrap values based on the ITS dataset. Newly generated sequences highlighted in red. The sequence names are followed by voucher or strain number and country of origin.
Fig. 2. (Continued).
Wood-inhabiting polypores in Benin

Fig. 3. Maximum likelihood (ML) and Bayesian analysis (BI) analyses of Polyporales based on the ITS dataset. Branch support values given as UFBoot / PP. Newly generated and Benin sequences are highlighted in red. The sequence names are followed by voucher or strain number and country of origin.
Fig. 3. (Continued).
**Annotated and illustrated checklist of wood-inhabiting polypores**

An alphabetical list (by genus name) of wood-inhabiting polypores identified in this study is given below. The current name of each species is checked against Index Fungorum and MycoBank. Whenever there is a difference between the two databases, the phylogenetic position of the species in this study or other published studies is used to select the correct current name. Substrate and collection data are provided for each species wherever possible. At this stage, no generic placement can be given to the genus *Coriolopsis* as the type species (*Coriolopsis*...).
Fig. 4. Maximum likelihood (ML) and Bayesian analysis (BI) analyses of *Megasporoporia sensu lato* based on the combined ITS-LSU dataset. Branch support values given as UFBoot / PP. Newly generated sequences highlighted in red and in blue sequences previously named as *Megasporoporia minuta*. The sequence names are followed by voucher or strain number and country of origin.
Table 1. Newly generated sequences with the GenBank accession numbers, vouchers, and origin of the specimens.

<table>
<thead>
<tr>
<th>Species name</th>
<th>Voucher</th>
<th>Origin</th>
<th>Accession numbers (ITS)</th>
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<td>Amylosporus campbellii</td>
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</table>
polyzona) is currently known as Trametes polyzona. As this study did not focus on the genus that may house the Coriolopsis species, we preferred to keep the name Coriolopsis in inverted commas.

Species recorded for the first time in Benin, i.e. recorded by us through a series of mycological surveys since 2017 are in bold.

**Amylosporus campbellii** (Berk.) Ryvarden – Fig. 6A.

*Substrata*: On the soil in semi-deciduous dense forest.


*Distribution*: Widespread in the tropics. Recorded by us, i.e., recorded by us through a series of mycological surveys since 2017.

**Schizophyllum commune** (Berk. & M.A. Curtis) Nikol. – Fig. 6B.

*Substrata*: On decomposing hardwoods.

*Material examined*: OAB0240, semi-deciduous dense forest of Lama/Zogbodomey (Benin), 6°57'8''N, 2°6'12''E, altitude: 37.8 m a.s.l., 14 Aug. 2018.


**Climacodon pulcherrimus** (Berk. & M.A. Curtis) Nikol. – Fig. 6C.

*Substrata*: On dead tree of Diospyros mespiliformis, but maybe found on other angiosperms.

*Material examined*: OAB0240, semi-deciduous dense forest of Lama (Benin), 6°57'8''N, 2°6'12''E, altitude: 37.8 m a.s.l., 14 Aug. 2018.

*Distribution*: Mainly Tropical, and known from different tropical regions (Moreno et al. 2007). First record in West Africa.

**Coriolopsis” byrsina** (Mont.) Ryvarden – Fig. 6D.

*Substrata*: Found on dead tree of Diospyros mespiliformis, but maybe found on other angiosperms.

*Material examined*: OAB0079, semi-deciduous dense forest of Lama/Zogbodomey (Benin), 6°57'59''N, 2°7'59''E, altitude: 56.1 m a.s.l., 3 Aug. 2017; OAB0225, semi-deciduous dense forest of Lama/Zogbodomey, 6°57'40''N, 2°8'2''E, altitude: 154.3 m a.s.l., 15 Aug. 2018.


**Coriolopsis” caperata** (Berk.) Murrill – Fig. 6E.

*Substrata*: On dead tree of different angiosperm trees.

*Material examined*: OAB0189, woodland of Trois Rivières in Benin, 10°27'30''N, 3° 25'11''E, altitude: 360.9 m a.s.l., 27 Aug. 2017; OAB0194, at the same locality, 10°26'53''N, 3°24'37''E, altitude 360.9 m a.s.l., 27 Aug. 2017.

*Distribution*: Widespread in tropical Africa and seen throughout East Africa (Ryvarden & Johansen 1980). First record in West Africa.

**Coriolopsis” floccosa** (Jungh.) Ryvarden

*Substrata*: On angiosperms of all kinds.


**Coriolopsis” sanguinaria** (Klotzsch) Teng – Fig. 6E.

*Substrata*: On dead angiosperms of all kinds.


**Coriolopsis” strumosa** (Fr.) Ryvarden – Fig. 6F.

*Substrata*: On dead woods.

*Material examined*: OAB0081, semi-deciduous Dense forest of Lama in Benin, 10°27'30''N, 3°25'11''E, altitude: 360.9 m a.s.l., 27 Aug. 2017; OAB0194, at the same locality, 10°26'53''N, 3°24'37''E, altitude 360.9 m a.s.l., 27 Aug. 2017.

*Distribution*: Widespread in the paleotropics from Western Africa to Australia (Ryvarden & Johansen 1980).

**Cubamyces flavidus** (Lév.) Lücking – Fig. 6G.

*Substrata*: On dead wood.
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Material examined: OAB0047, semi-deciduous dense forest of Lama (Benin), 6°57′58″N, 2°90′55″E, altitude 63.5 m a.s.l., 27 Jul. 2017; OAB0090, woodlands of Kilibo (Benin), 8°32′30″N, 2°41′30″E, altitude 334 m a.s.l., 16 Aug. 2017; OAB0196, Trois Rivières (Benin), 10°28′30″N, 3°24′40″E, altitude 364.6 m a.s.l., 28 Aug. 2017.


Cubamyces lactineus (Berk.) Lücking – Fig. 6H.

Substrata: On deciduous wood of many kinds.

Materials examined: OAB0207, National Park W (Benin), 11°28′13″N, 3°3′40″E, altitude 289.7 m a.s.l., 31 Aug. 2017; OAB0232, semi-deciduous dense forest of Lama (Benin), 6°56′40″N, 2°6′20″E, altitude 98.3 m a.s.l., 13 Aug. 2018.
**Distribution:** Rare in Africa (Ryvarden & Johansen 1980). First record in West Africa.

**Diplomitoporus hondurensis** (Murrill) Ryvarden – Fig. 6l.

**Substrata:** On dead hardwood.

**Material examined:** Benin, Province Borgou, OAB0183, Woodland of Trois Rivières (Benin), 10°26'46"N, 3°25'2"E, altitude: 365.1 m a.s.l., 27 Aug. 2017.

**Distribution:** Mainly distributed in Americas (Kout & Vlasák 2010). First record in tropical Africa.

**Earliella scabrosa** (Pers.) Gilb. & Ryvarden – Fig. 6j.

**Substrata:** On dead deciduous trees.


**Distribution:** Pantropical distribution and quite common in tropical Africa (Ryvarden & Johansen 1980).

**Echinochaete brachypora** (Mont.) Ryvarden – Fig. 6k.

**Substrata:** On dead wood.


**Distribution:** Pantropical and quite common in tropical Africa (Ryvarden & Johansen 1980).

**Flavodon flavus** (Klotzsch) Ryvarden – Fig. 6l.

**Substrata:** On fallen and standing dead trunks and branches.


**Distribution:** Throughout tropical Africa (Ryvarden & Johansen 1980).

**Fomitiporia aff. punctata** (P. Karst.) Murrill – Fig. 6m.

**Substrata:** On dead branch of living tree of *Isobstderrnia doka*.

**Material examined:** OAB0208, National parc W, 11°28’14”N, 3°30’40”E, altitude: 288.2 m a.s.l., 31 Aug. 2017.

**Distribution:** The distribution of the species is still unknown even though Ryvarden & Johansen (1980) reported the presence of *Fomitiporia punctata* in East Africa. However, Decock et al. (2007) reported that this species has a distribution restricted to the northern or more temperate areas of the Northern Hemisphere. That means, the specimen identified here as *Fomitiporia aff. punctata* might be a different or new species and therefore additional specimens and other collections named *Fomitiporia punctata* in Africa need to be studied in depth morpho-anatomically and molecularly.

**Fomitopsis ostreiformis** (Berk.) T. Hatt. – Fig. 6n.

**Substrata:** Found on dead trunk of *Mangifera indica*.

**Material examined:** OAB0822, Woodland of Ouémé supérieur (Benin), 9°45’40”N, 2°53’49”E, altitude: 342.9 m a.s.l., 19 Jul. 2021.

**Distribution:** Probably a tropical species. Reported mainly in Asia. In Africa, the specimen was reported from Gabon (Liu et al. 2022). This is the first record in West Africa.

**Fulvifomes fastuosus** (Lév.) Bondartseva & S. Herrera – Fig. 6o.

**Substrata:** On dead and living tree of *Dialium guineense*, and other unidentified angiosperm trees.

**Materials examined:** OAB0015, semi-deciduous dense forest of Pahou/ Ouidah (Benin), 6°23’05”N, 2°09’22”E, altitude: 17.6 m a.s.l., 19 Jul. 2017; OAB0043, semi-deciduous dense forest of Lama/ Zogbodomey (Benin), 6°57’07”N, 2°12’13”E, altitude: 56.8 m a.s.l., 13 Aug. 2018; OAB0235, semi-deciduous dense forest of Lama/ Zogbodomey (Benin), 13 Aug. 2018; OAB0237, semi-deciduous dense forest of Lama/ Zogbodomey (Benin), 6°57’29”N, 2°6’4”E, altitude: 56.9 m a.s.l., 20 Aug. 2018.

**Distribution:** Pantropical and quite common throughout tropical Africa (Ryvarden & Johansen 1980).

**Fulvifomes indicus** (Massee) L.W. Zhou – Fig. 7a.

**Substrata:** On dead and living angiosperm trees.

**Material examined:** OAB0210, National Parc W, 11°28’18”N, 3°30’20”E, altitude: 287.5 m a.s.l., 31 Aug. 2017.

**Distribution:** Reported from Asia and Africa (Ryvarden & Johansen 1980, Zhou 2014). New record in West Africa.

**Fulvifomes rimosus** (Berk.) Fissson & Niemelä – Fig. 7b.

**Substrata:** On dead wood of different kind.

**Materials examined:** OAB0213, National Park W (Benin), 11°28’18”N, 3°30’30”E, altitude 285.2 m a.s.l., 31 Aug. 2017; OAB0214, at the same locality, 11°28’18”N, 3°30’30”E, altitude 285.2 m a.s.l., 31 Aug. 2017;
OAB0215, at the same locality, 11°28'17"N, 3°3'10"E, altitude 284.7 m a.s.l., 31 Aug. 2017.

**Distribution:** Cosmopolitan species, reported from Europe, Asia, Africa, and Australia (Ryvarden & Johansen 1980). In West Africa, reported from Togo, Sierra Leone (Piepenbring et al. 2020) and newly reported here from Benin.

**Fulviformes yorou** Olou & Langer – Fig. 7C.

**Substrata:** On living tree of *Pseudocedrela kotschyi*.

**Material examined:** OAB0097, Woodland of Toui-Kilibo in Benin, 8°32'30"N, 2°40'49"E, altitude 328.3 m a.s.l., 16 Aug. 2017.

**Distribution:** New to West African mycobiota and presently only reported from Benin (Olou et al. 2019b).

**Funalia leonina** (Klotzsch) Pat. – Fig. 7D.

**Substrata:** On dead wood of different kinds.

**Material examined:** OAB0105, Woodland of Toui-Kilibo in Benin, 8°32'36"N, 2°41'12"E, altitude 313.7 m a.s.l., 17 Aug. 2017; OAB0110, woodland of Toui-Kilibo in Benin, 8°32'37"N, 2°40'13"E, altitude 329.9 m a.s.l., 18 Aug. 2017; OAB0166, woodland of Ouémé supérieur (Benin), 9°46'50"N, 2°12'40"E, altitude 370.5 m a.s.l., 25 Aug. 2017; OAB0181, Trois Rivières (Benin), 10°26'50"N, 3°25'13"E, altitude 372.4 m a.s.l., 27 Aug. 2017; OAB0193, Trois Rivières (Benin), 10°26'53"N, 3°24'37"E, altitude 348.5 m a.s.l., 27 Aug. 2017.

**Distribution:** Widespread in tropical Africa from Senegal in west to Ethiopia in north and south to South Africa (Ryvarden & Johansen 1980).

**Fuscoporia beninensis** (Olou & Ryvarden) Olou, **comb. nov.** MycoBank MB 844735. Fig. 7E. **Basionym:** *Phellinus beninensis* Olou & Ryvarden, *Syn. Fung.* (Oslo) **44:** 10. 2021.

**Substrata:** On an unidentified dead angiosperm tree.

**Material examined:** OAB0132, Woodland of Toui-Kilibo (Benin), 8°37'06"N, 2°37'45"E, altitude: 318.39 m a.s.l., 19 Aug. 2017; OAB0154, at the same locality, 8°32'36"N, 2°37'44"E, altitude: 318.89 m a.s.l., 19 Aug. 2017; OAB0159, at the same locality, 9°46'50"N, 2°38'31"E, altitude: 314.89 m a.s.l., 17 Aug. 2017; OAB0113, at the same locality, 8°37'60"N, 2°38'31"E, altitude: 316.39 m a.s.l., 17 Aug. 2017; OAB0109, at the same locality, 8°32'37"N, 2°41'12"E, altitude: 314.8 m a.s.l., 17 Aug. 2017.

**Materials examined:** OAB0006, semi-deciduous dense forest of Pahou (Benin), 6°23'30"N, 2°9'16"E, altitude: 23.1 m a.s.l., 19 Jul. 2017; OAB0021, dry dense forest of Pahou (Benin), 6°23'30"N, 2°9'17"E, altitude: 30.3 m a.s.l., 21 Jul. 2017; OAB0052, semi-deciduous dense forest of Lama (Benin), 6°57'59"N, 2°9'46"E, altitude: 56.6 m a.s.l., 28 Jul. 2017; OAB0066, at the same locality, 6°57'70"N, 2°6'30"E, altitude: 57.9 m a.s.l., 1 Aug. 2017; OAB0086, at the same locality, 6°56'59"N, 2°8'16"E, altitude: 35.3 m a.s.l., 3 Aug. 2017; OAB0106, Woodland of Toui-Kilibo (Benin), 8°32'36"N, 2°41'12"E, altitude: 312.89 m a.s.l., 17 Aug. 2017.


**Ganoderma aridicola** J.H. Xing & B.K. Cui – Fig. 7H.

**Substrata:** On dead wood.

**Materials examined:** OAB0233, semi-deciduous dense forest of Lama (Benin), 13 Aug. 2018; OAB0241, at the same locality, 6°57'50"N, 2°6'80"E, altitude: 62 m a.s.l., 14 Aug. 2018; OAB0243, at the same locality, 6°57'40"N, 2°6'30"E, altitude: 69.2 m a.s.l., 14 Aug. 2018; OAB0254, at the same locality, 6°57'39"N, 2°6'16"E, altitude: 58.8 m a.s.l., 15 Aug. 2018.

**Distribution:** So far known from the type locality, South Africa (Xing et al. 2016). First record in West Africa.

**Ganoderma enigmaticum** M.P.A. Coetzee et al. – Fig. 7I.

**Substrata:** On stumps, trunks and dead trees.

**Materials examined:** OAB0063, semi-deciduous dense forest of Lama (Benin), 6°58'47"N, 2°5'20"E, altitude: 59.1 m a.s.l., 31 Jul. 2017; OAB0094, Woodland of Toui-Kilibo (Benin), 8°32'30"N, 2°41'30"E, altitude: 333.4 m a.s.l., 16 Aug. 2017; OAB0095, at the same locality, 8°32'30"N, 2°41'30"E, altitude: 333.2 m a.s.l., 16 Aug. 2017; OAB0099, at the same locality, 8°32'37"N, 2°41'12"E, altitude: 314.8 m a.s.l., 17 Aug. 2017; OAB0104, at the same locality, 8°32'36"N, 2°41'13"E, altitude: 314.39 m a.s.l., 17 Aug. 2017; OAB0109, at the same locality, 8°32'33"N, 2°40'53"E, altitude: 316.39 m a.s.l., 17 Aug. 2017; OAB0113, at the same locality, 8°32'37"N, 2°40'12"E, altitude: 328.9 m a.s.l., 18 Aug. 2017; OAB0115, at the same locality, 8°32'36"N, 2°40'48"E, altitude: 332.5 m a.s.l., 18 Aug. 2017; OAB0124, at the same locality, 8°37'60"N, 2°38'11"E, altitude: 310.6 m a.s.l., 19 Aug. 2017; OAB0136, at the same locality, 8°37'60"N, 2°37'45"E, altitude: 318.39 m a.s.l., 19 Aug. 2017; OAB0154, Woodland of Ouémé supérieur (Benin), 9°45'16"N, 2°8'31"E, altitude: 324.89 m a.s.l., 24 Aug. 2017; OAB0159, at the same locality, 9°46'90"N, 2°14'40"E, altitude: 382.3 m a.s.l., 25 Aug. 2017; OAB0170, Trois Rivières (Benin), 10°26'50"N, 3°25'17"E, altitude: 373.7 m a.s.l., 27 Aug. 2017; OAB0174, at the same locality, 10°26'49"N, 3°25'18"E, altitude: 372.5 m a.s.l., 27 Aug. 2017; OAB0175, at the same locality, 10°26'50"N, 3°25'18"E, altitude: 372.4 m a.s.l., 27 Aug. 2017; OAB0219, National Parc W, 11°28'30"N, 3°3'40"E, altitude: 287.5 m a.s.l., 31 Aug. 2017.
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Distribution: Typified with South African material (Coetzee et al. 2015). First record in West Africa and most abundant *Ganoderma* species in Benin.

**Ganoderma aff. lucidum** – Fig. 7J.

**Substrata:** On dead wood.

**Materials examined:** OAB0011, dry dense forest of Pahou (Benin), 6°23′57″N, 2°9′90″E, altitude: 24.7 m a.s.l., 19 Jul. 2017; OAB0016, at the same locality, 6°23′50″N, 2°9′22″E, altitude: 19.7 m a.s.l., 20 Jul. 2017; OAB0017, at the same locality, 6°23′50″N, 2°9′22″E, altitude: 15.9 m a.s.l., 20 Jul. 2017; OAB0029, at the same locality, 6°23′50″N, 2°9′21″E, altitude: 16 m a.s.l., 20 Jul. 2017; OAB0121, Woodland of Toui-Kilibo (Benin), 8°32′34″N, 2°41′12″E, altitude: 321.7 m a.s.l., 18 Aug. 2017; OAB0122, at the same locality, 8°32′34″N, 2°41′13″E, altitude: 320.5 m a.s.l., 18 Aug. 2017; OAB0133, at the same locality, 8°37′60″N, 2°37′45″E, altitude: 343.3 m a.s.l., 19 Aug. 2017; OAB0139, at the same locality, 8°37′60″N, 2°37′44″E, altitude: 317.9 m a.s.l., 19 Aug. 2017; OAB0146, Woodland of Ouémé supérieur (Benin), 9°45′25″N, 2°18′38″E, altitude: 336.5 m a.s.l., 24 Aug. 2017.

**Distribution:** Cosmopolitan species but as the interpretation of the name is very variable, the distribution also is quite variable. Thus, the type species is restricted to Europe and part of China (Cao et al. 2012). While the distribution of the species reported here is still unknown. So far, recorded throughout Benin.

**Ganoderma mbre kobenum** E.C. Otto et al. – Fig. 7K.

**Habitat:** On roots and trunks of living or dead trees of several angiosperms.

**Materials examined:** OAB0083, semi-deciduous dense forest of Lama (Benin), 6°57′32″N, 2°7′42″E, altitude: 50.2 m a.s.l., 3 Aug. 2017; OAB0123, Woodland of Toui-Kilibo (Benin), 8°37′60″N, 2°38′32″E, altitude: 307.89 m a.s.l., 19 Aug. 2017; OAB0143, Woodland of Ouémé supérieur (Benin), 9°45′28″N, 2°19′58″E, altitude: 334.6 m a.s.l., 24 Aug. 2017; OAB0187, Trois Rivières (Benin), 10°27′30″N, 3°25′11″E, altitude 384.5 m a.s.l., 27 Aug. 2017; OAB0218, National Parc W, 11°28′30″N, 3°24′40″E, altitude 369.8 m a.s.l., 28 Aug. 2017; OAB0226, National Parc W, 11°28′14″N, 3°24′49″E, altitude 189.5 m a.s.l., 8 Jun. 2021.

**Distribution:** Typified with West African material. The type locality is Ghana (Crous et al. 2016a, b) and this is a new record for Benin.

**Gloeophyllum striatum** (Fr.) Murrill – Fig. 7L.

**Substrata:** On an unidentified dead angiosperm tree.

**Materials examined:** OAB0129, Woodland of Toui-Kilibo (Benin), 8°37′02″N, 2°38′05″E, altitude: 308.2 m a.s.l., 19 Aug. 2017; OAB0843, Woodland of Ouémé supérieur (Benin), 9°46′41″N, 2°9′21″E, altitude: 16 m a.s.l., 20 Jul. 2017; OAB0140, Woodland of Ouémé supérieur (Benin), 9°45′28″N, 2°19′58″E, altitude: 334.6 m a.s.l., 24 Aug. 2017; OAB0187, Trois Rivières (Benin), 10°27′30″N, 3°25′11″E, altitude 384.5 m a.s.l., 27 Aug. 2017; OAB0218, National Parc W, 11°28′30″N, 3°24′40″E, altitude: 278.7 m a.s.l., 31 Aug. 2017.

**Distribution:** Cosmopolitan species. The type material is Typified with South African material (Coetzee et al. 2015). First record in West Africa.

**Gloeoporus thelephoroides** (Hook.) G. Cunn. – Fig. 7N.

**Substrata:** On dead wood.

**Materials examined:** OAB0230, semi-deciduous dense forest of Pahou (Benin), 6°22′56″N, 2°9′12″E, altitude: 105.9 m a.s.l., 26 Sep. 2017.

**Distribution:** Pantropical, in Africa widespread from Sierra Leone to Rhodesia and Madagascar (Ryvarden & Johansen 1980).

**Grammothele lineata** Berk. & M.A. Curtis – Fig. 7O.

**Substrata:** On deciduous wood of many kinds.

**Materials examined:** OAB0515, semi-deciduous dense forest of Lama (Benin), 20 Aug. 2018.

**Distribution:** Widespread in Africa (Ryvarden & Johansen 1980), first record in West Africa.

**Hexagonia hirta** (P. Beauv.) Fr. – Fig. 8A.

**Substrata:** On hard dead wood.

**Materials examined:** OAB0026, semi-deciduous dense forest of Pahou (Benin), 6°23′20″N, 2°9′14″E, altitude: 23.2 m a.s.l., 19 Jul. 2017; OAB0140, Woodland of Toui-Kilibo (Benin), 8°37′60″N, 2°37′45″E, altitude: 322 m a.s.l., 19 Aug. 2017; OAB0026, semi-deciduous dense forest of Lama (Benin), 6°23′20″N, 2°9′14″E, altitude: 23.2 m a.s.l., 19 Jul. 2017; OAB0026, semi-deciduous dense forest of Lama (Benin), 6°23′20″N, 2°9′14″E, altitude: 23.2 m a.s.l., 19 Jul. 2017; OAB0187, Trois Rivières (Benin), 10°27′30″N, 3°25′11″E, altitude 384.5 m a.s.l., 27 Aug. 2017; OAB0218, National Parc W, 11°28′30″N, 3°24′40″E, altitude: 278.7 m a.s.l., 31 Aug. 2017.

**Distribution:** Seems to be restricted to Africa (Ryvarden & Johansen 1980). In west Africa reported from Ghana, Sierra Leone and Nigeria (Piepenbring et al. 2020). First record in Benin.

**Hexagonia hydnoides** (Sw.) M. Fidalgo – Fig. 8B.

**Substrata:** On hard dead wood.

**Materials examined:** OAB0201, Trois Rivières (Benin), 10°28′90″N, 3°24′40″E, altitude 369.8 m a.s.l., 28 Aug. 2017; OAB0275, semi-deciduous dense forest of Lama (Benin), 6°58′48″N, 2°40′57″E, altitude 369.8 m a.s.l., 28 Aug. 2017; OAB0201, Trois Rivières (Benin), 10°28′90″N, 3°24′40″E, altitude 369.8 m a.s.l., 28 Aug. 2017; OAB0202, Trois Rivières (Benin), 10°28′46″N, 3°25′30″E, altitude 365.3 m a.s.l., 27 Aug. 2017.

**Distribution:** Quite common in Africa (Ryvarden & Johansen 1980). First record in West Africa.

**Hexagonia phellinoides** Ryvarden – Fig. 8C.

**Substrata:** On dead wood.

**Materials examined:** OAB0203, Trois Rivières (Benin), 10°28′11″N, 3°24′49″E, altitude 360.8 m a.s.l., 20 Aug. 2017; OAB0226, National Parc W, 11°28′14″N, 3°3′16″E, altitude 276.8 m a.s.l., 31 Aug. 2017.
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In West Africa, known from Nigeria, Sierra Leone, Burkina Faso and Senegal (Guissou et al. 2008, Kane & Courtecuisse 2013, Olou et al. 2019a, Piepenbring et al. 2020).

**Inonotus pachyphloeus** (Pat.) T. Wagner & M. Fisch. – Fig. 8E.

*Substrata:* Recorded on dead and living angiosperms trees.

**Materials examined:** OAB0246, semi-deciduous dense forest of Lama (Benin), 6°57′30″N, 2°6′80″E, altitude 65.4 m a.s.l., 14 Aug. 2018; OAB0247, at the same locality, 6°57′30″N, 2°6′80″E, altitude 41.1 m a.s.l., 14 Aug. 2018; OAB0260, at the same locality, 6°57′40″N, 2°6′60″E, altitude 119.5 m a.s.l., 15 Aug. 2018.


**Inonotus rickii** (Pat.) D.A. Reid – Fig. 8F.

*Substrata:* On living tree.

**Materials examined:** OAB0253, semi-deciduous dense forest of Lama (Benin); 15 Aug. 2018.

**Distribution:** Probably cosmopolitan species. In Africa, records were from Egypt, Guinea, Morocco, and South Africa (Ouabbou et al. 2012, Shehata & Abd El-Wahab 2013, Tchoumi et al. 2020). First record in West Africa.

**Irpex lacteus** (Fr.) Fr. – Fig. 8G.

*Substrata:* On dead wood.

**Materials examined:** OAB0176, Trois Rivières (Benin), 10°26′49″N, 2°9′21″E, altitude 323 m a.s.l., 11 Sep. 2019.

**Distribution:** Paleotropical, distribution similar to *Lentinus squarrosulus*.

**Lignosus sacer** (Afzel. ex Fr.) Ryvarden – Fig. 8L.

*Substrata:* On ground.

**Materials examined:** OAB0293, Gallery forest of Bassila (Benin), 11 Aug. 2019.

**Distribution:** Tropical Africa from Sierra Leone to Kenya and south to South Africa (Ryvarden & Johansen 1980, Piepenbring et al. 2020).


**Distribution:** So far known from China.

**Megasporoporia setulosa** (Henn.) Rajchenb. – Fig. 8M.

*Substrata:* On fallen and standing dead trunks and branches.

**Materials examined:** OAB0060, semi-deciduous dense forest of Lama (Benin), 6°58′47″N, 2°5′41″E, altitude 55.9 m a.s.l., 31 Jul. 2017; OAB0065, at the same locality, 6°58′48″N, 2°5′60″E, altitude 60 m a.s.l.,
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31 Jul. 2017; OAB0101, Woodland of Toui-KiliBo (Benin), 8°32’36”N, 2°41’12”E, altitude: 315.2 m a.s.l., 17 Aug. 2017; OAB0102, at the same locality, 8°32’37”N, 2°41’12”E, altitude: 314.8 m a.s.l., 17 Aug. 2017; OAB0138, at the same locality, 8°37’60”N, 2°37’45”E, altitude: 314.2 m a.s.l., 19 Aug. 2017.


Mycobonia miquelloi (Mont.) Palacio & Westhalen – Fig. 9C.

Substrata: On dead wood of different kinds.

Materials examined: OAB041, semi-deciduous dense forest of Lama (Benin), 6°57’50”N, 2°10’00”E, altitude 52 m a.s.l., 10 Jul. 2021.


Neonothopanus hygrophanus (Mont.) De Kesel & Degrefeu – Fig. 9D.

Substrata: On dead wood.

Materials examined: OAB0676, semi-deciduous dense forest of Lama (Benin), 6°57’51”N, 2°10’00”E, altitude 52 m a.s.l., 10 Jul. 2021.


Perenniporia beninensis Oluo & Ryvarden – Fig. 9E.

Substrata: On deciduous dead wood.

Materials examined: OAB0050, semi-deciduous dense forest of Lama (Benin), 6°57’59”N, 2°9’46”E, altitude 54.9 m a.s.l., 28 Jul. 2017.

Distribution: So far only known from the type country, Benin (Oluo & Ryvarden 2021).

Perenniporia centrali-africana Decock & Mossebo – Fig. 9F.

Substrata: On deciduous dead wood.

Materials examined: OAB0042, semi-deciduous dense forest of Lama (Benin), 6°57’51”N, 2°10’00”E, altitude 62.3 m a.s.l., 27 Jul. 2017; OAB0042, at the same locality, 6°57’55”N, 2°6’40”E, altitude 54.2 m a.s.l., 2 Aug. 2017; OAB0190, Trois Rivières (Benin), 10°26’59”N, 3°25’12”E, altitude 369.1 m a.s.l., 27 Aug. 2017; OAB0220, National Park W (Benin), 11°28’16”N, 3°25’16”E, altitude 275.1 m a.s.l., 31 Aug. 2017; OAB0282, semi-deciduous dense forest of Lama (Benin), 6°58’00”N, 2°8’80”E, altitude 57.2 m a.s.l., 20 Aug. 2018.

Distribution: Probably a pantropical species. Reported from the type locality, Cameroon (Decock & Mossebo 2001), and also reported from Senegal and Benin in West Africa (Decock & Mossebo 2002, Oluo et al. 2019a).

Perenniporia miniochroleuca Ryvarden – Fig. 9G.

Substrata: On dead wood.
Material examined: OAB0072, semi-deciduous dense forest of Lama (Benin), 6°56’55”N, 2°60’40”E, altitude 72 m a.s.l., 2 Aug. 2017.

Distribution: Specimens have only been seen from Zimbabwe (Ryvarden et al. 2022). First record in West Africa.

**Perenniporia tephropora** (Mont.) Ryvarden – Fig. 9H.

**Substrata:** On deciduous dead wood.


**Perenniporia vanhulleae** Decock & Ryvarden – Fig. 9I.

**Substrata:** Found on dead tree of Diospyros mespiliformis but maybe found on other kind of angiosperms.


**Phanerochaete sordida** (P. Karst.) J. Erikss. & Ryvarden

**Substrata:** On dead wood.


**Phellinus carteri** (Berk. ex Cooke) Ryvarden – Fig. 9J.

**Substrata:** On dead angiosperms.

Material examined: OAB0217, National Park W (Benin), 11°28’30”N, 3°30’30”E, altitude 277.5 m a.s.l., 31 Aug. 2017.

Distribution: Specimens reported from Ghana in Africa (Ryvarden & Johansen 1980), first record in Benin.

**Phellinus purpureogilvus** (Petch) Ryvarden – Fig. 9K.

**Substrata:** On dead wood.


Distribution: Reported from the type locality in Sri Lanka and Tanzania (Ryvarden & Johansen 1980). First record in West Africa.

**Phylloporia beninensis** Olou & Langer – Fig. 9L.

Habitat: On dead wood or dead parts of living trees of woody angiosperms, including Trichilia emetica.


Distribution: Currently known from the type locality in Benin (Olou et al. 2021).

**Phylloporia littoralis** Decock & Yombiyeni – Fig. 9M.

**Substrata:** On living branches, twigs of angiosperm trees.

Material examined: OAB0204, Trois Rivières (Benin), 10°28’50”N, 3°24’33”E, altitude 345.8 m a.s.l., 28 Aug. 2017.

Distribution: Known from the type locality in Gabon (Yombiyeni & Decock 2017) and reported here for the first time outside of the type locality.

**Phylloporia spathulata** (Hook.) Ryvarden

**Substrata:** On dead angiosperm trees.

Material examined: OAB0294.

Distribution: In West Africa, reported from Ghana (Piepenbring et al. 2020) and Benin.

**Piptoporellus baudonii** (Pat.) Tibuhwa, Ryvarden & S. Tibell – Fig. 9N.

**Substrata:** On the ground either from buried roots or from a pseudosclerotium, more rarely on stumps. It attacks many different forest trees and is locally a serious root pathogen in Africa. In Benin, it attacks plant species of the genus Isoberlinia.

Materials examined: OAB0603, woodlands of Okpara; OAB0604, at the same locality; OAB0867, Trois Rivières (Benin), 10°27’90”N, 3°24’53”E, altitude 372.3 m a.s.l., 28 Aug. 2017.


**Podofomes mollis** (Sommerf.) Gorjón – Fig. 9O.

**Substrata:** On dead part of living trees.

Materials examined: OAB0238, semi-deciduous dense forest of

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Lama in Benin, 6°56′41″N, 2°6′11″E, altitude: 84.8 m a.s.l., 13 Aug. 2018; OAB0670, same locality, 10 Jul. 2021; OAB0697, same locality, 6°57′50″N, 2°10′00″E, altitude: 84.8 m a.s.l., 13 Aug. 2018.

Distribution: First record in West Africa.

**Podoscypha bolleana** (Mont.) Boidin – Fig. 10A.

**Substrata:** On dead wood.

**Materials examined:** OAB0683, semi-deciduous dense forest of Lama (Benin), 6°57′51″N, 2°10′00″E, altitude 39.2 m a.s.l., 10 Jul. 2021; OAB0702, at the same locality, 6°57′50″N, 2°10′10″E, altitude 39.2 m a.s.l., 10 Jul. 2021; OAB0722, at the same locality, 11 Jul. 2021. OAB0725, at the same locality, 11 Jul. 2021; OAB0885, National Park W (Benin), 11°28′26″N, 3°3′10″E, altitude 277.1 m a.s.l., 24 Jul. 2021; OAB0885, at the same locality, 11°28′24″N, 3°30′10″E, altitude 275.5 m a.s.l., 24 Jul. 2021.

Distribution: Widespread in tropical regions. In West Africa, reported from Ghana (Piepenbring et al. 2020) and first record in Benin.

**Pycnoporus sanguineus** (L.) Murrill – Fig. 10B.

**Substrata:** On standing and falling trunks of almost every kind of deciduous wood.

**Materials examined:** OAB0088, woodlands of Kilibo (Benin), 8°32′30″N, 2°41′30″E, altitude 333.3 m a.s.l., 16 Aug. 2017; OAB0184, Trois Rivières (Benin), 10°26′46″N, 3°25′30″E, altitude 365.4 m a.s.l., 27 Aug. 2017; OAB0507, woodlands of Okpara (Benin), 9°15′40″N, 2°43′30″E, altitude 336.6 m a.s.l., 11 Sep. 2019; OAB0512, same locality, 9°15′41″N, 2°43′24″E, altitude 360.9 m a.s.l., 11 Sep. 2019.

Distribution: Pantropical and common in tropical Africa (Ryvarden & Johansen 1980). Widespread in dry areas, and recorded mainly in dry forests and Savannahs in Benin.

**Schizophyllum commune** Fr. – Fig. 10C.

**Substrata:** On dead wood.

**Material examined:** OAB0112, woodlands of Kilibo (Benin), 8°32′37″N, 2°40′13″E, altitude 329.4 m a.s.l., 18 Aug. 2017.

Distribution: Cosmopolitan species and widespread in Africa (Piepenbring et al. 2020).

**Schizophyllum umbrinum** Berk. – Fig. 10D.

**Substrata:** On dead wood.

**Material examined:** OAB0507, woodlands of Okpara (Benin), 9°15′39″N, 2°43′47″E, altitude 329.3 m a.s.l., 11 Sep. 2019.

Distribution: Probably a tropical species. First record in West Africa.

**Serpula similis** (Berk. & Broome) Ginns – Fig. 10E, F.

**Substrata:** On a dead stump.

Material examined: OAB0266, dry dense forest of Pahou (Benin), 20 Jul. 2018.

Distribution: Reported from Gambia, Ivory coast, and Nigeria (Piepenbring et al. 2020) and first record from Benin.

**Theleporus calcicolor** (Sacc. & P. Syd.) Ryvarden – Fig. 10G.

**Substrata:** On dead part of living tree of Drypetes floribunda (Müll.Arg.) Hutch.

**Material examined:** OAB0258, semi-deciduous dense forest of Lama (Benin), 6°57′38″N, 2°6′60″E, altitude 67.2 m a.s.l., 15 Aug. 2018.


**Tomophagus colossus** (Fr.) Murrill – Fig. 10H.

**Substrata:** On a completely degraded angiosperm stump.

**Material examined:** OAB0774, woodlands of Kilibo (Benin), 8°32′28″N, 2°41′40″E, altitude 338.6 m a.s.l., 13 Jul. 2021.


**Trametes cingulata** Berk. – Fig. 10I.

**Substrata:** On deciduous wood.

**Materials examined:** OAB0093, woodlands of Kilibo (Benin), 8°32′31″N, 2°41′30″E, altitude 333.1 m a.s.l., 16 Aug. 2017; OAB0114, at the same locality, 8°32′36″N, 2°40′48″E, altitude 330.7 m a.s.l., 18 Aug. 2017; OAB0117, at the same locality, 8°32′35″N, 2°40′48″E, altitude 332.8 m a.s.l., 18 Aug. 2017; OAB0135, at the same locality, 8°37′60″N, 2°37′44″E, altitude 325.1 m a.s.l., 19 Aug. 2017; OAB0155, woodlands of Ouémé Supérieur, 9°46′90″N, 2°14′39″E, altitude 386.1 m a.s.l., 25 Aug. 2017; OAB0161, at the same locality, 9°46′90″N, 2°14′39″E, altitude 380.5 m a.s.l., 25 Aug. 2017; OAB0171, Trois Rivières (Benin), 10°26′50″N, 3°25′17″E, altitude 372.6 m a.s.l., 27 Aug. 2017; OAB0173, at the same locality, 10°26′50″N, 3°25′18″E, altitude 372.3 m a.s.l., 27 Aug. 2017; OAB0178, at the same locality, 10°26′51″N, 3°25′13″E, altitude 373.3 m a.s.l., 27 Aug. 2017; OAB0231, dry dense forest of Pahou (Benin), 6°22′59″N, 2°9′70″E, altitude: 100.5 m a.s.l., 26 Sep. 2017.

Distribution: Widespread in Africa and Asia (Berkeley 1854, Ryvarden & Johansen 1980). Reported from Ghana, Nigeria (Piepenbring et al. 2020) and Benin. It is the most abundant Trametes species in Benin.

**Trametes palisottii** (Fr.) Imazeki – Fig. 10J, K.

**Substrata:** On deciduous wood of all kinds.

**Materials examined:** OAB0118, woodlands of Kilibo (Benin), 8°32′33″N, 2°40′47″E, altitude 332.7 m a.s.l., 18 Aug. 2017; OAB0153, Trois Rivières (Benin), 10°28′80″N, 3°24′40″E, altitude 364.1 m a.s.l., 28 Aug. 2017.
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**Distribution**: Pantropical and very common in areas with seasonal drought. Specimens reported from Ethiopia to Malawi (Imazeki 1952, Ryvarden & Johansen 1980). Widespread in tropical Africa.

**Trametes parvispora** Olou, Yorou & Langer – Fig. 10L.

**Substrata**: On dead part of living angiosperm tree *D. guineense*.

**Materials examined**: OAB0022, dry dense forest of Pahoui/ Ouidah (Benin), 6°23′2.97″N, 2°9′15.90″E, altitude: 33.1 m a.s.l., 21 Jul. 2017; OAB0023 at the same locality, 6°23′3.07″N, 2°9′16.16″E, altitude 18.4 m a.s.l., 21 Jul. 2017; OAB0267, same locality, 6°23′2.49″N, 2°9′16.27″E, altitude 33.1 m a.s.l., 20 Jul. 2018; OAB0268, at the same locality, 26 Aug. 2018.

**Distribution**: Apart from the type locality, Benin (Olou et al. 2020), the species has recently been reported from Nigeria.

**Trametes polyzona** (Pers.) Justo – Fig. 10M.

**Substrata**: On deciduous wood of many kinds.

**Materials examined**: OAB0092, woodlands of Kilibo (Benin), 8°32′31″N, 3°24′38″E, altitude 372.6 m a.s.l., 27 Aug. 2017; OAB0191, Trois Rivières (Benin), 10°26′53″N, 3°24′38″E, altitude 372.6 m a.s.l., 27 Aug. 2017; OAB0195, at the same locality, 10°26′53″N, 3°24′37″E, altitude 346.9 m a.s.l., 27 Aug. 2017.

**Distribution**: Pantropical, in Africa reported from almost all countries south of Sahara (Ryvarden & Johansen 1980, Justo & Hibbett 2011).

**Trametes socotrana** Cooke – Fig. 10N.

**Substrata**: On dead wood.

**Materials examined**: OAB0131, woodlands of Kilibo (Benin), 8°37′20″N, 2°37′44″E, altitude 326.2 m a.s.l., 16 Aug. 2017; OAB0128, at the same locality, 8°37′20″N, 2°38′50″E, altitude 307.4 m a.s.l., 19 Aug. 2017; OAB0165, woodlands of Ouémé Supérieur, 9°46′49″N, 2°12′49″E, altitude 348.6 m a.s.l., 25 Aug. 2017; OAB0191, Trois Rivières (Benin), 10°26′53″N, 3°24′38″E, altitude 372.6 m a.s.l., 27 Aug. 2017; OAB0195, at the same locality, 10°26′53″N, 3°24′37″E, altitude 346.9 m a.s.l., 27 Aug. 2017.


**Tyromyces contractus** Olou & Ryvarden – Fig. 100.

**Substrata**: On dead wood.

**Material examined**: OAB0073, semi-deciduous dense forest of Lama (Benin), 29 Jul. 2017.

**Distribution**: Known so far from the type locality, Benin (Olou & Ryvarden 2021).

**Distribution of identified species in Africa**

Figure 11 shows the distribution of the species collected and identified in this study. The widest distributed genus is *Schizophyllum*, represented in this study mainly by *Schizophyllum commune*. The genus *Microporus* clearly shows a tropical distribution and is distributed from Senegal to Madagascar. Benin, which was the subject of our study, and the Congo seem to be the best covered. Several other countries are still unexplored or occurrence data are not publicly available. Future mycological survey series will be extended to these countries where currently we have very little data on wood-inhabiting fungi. When zooming in on Benin where we collected the specimens, several species and genera of polyopes have a wide distribution from south to north of Benin. However, some genera and species show a geographical pattern. For example, species of the genus *Microporus* were only found in southern Benin, while species of the genera *Gloeophyllum* and *Piptoporellus* were only found in central and northern Benin (dry area of Benin). Within the same genus it is also quite common to observe that although the genus has a wide distribution from the South to the North, some species have narrow distribution and for the moment never recorded in the South. An example is the genus *Trametes* where species are distributed throughout the country. However, some species like *P. sanguineus*, *T. polyzona*, and *T. socotrana* occur mainly in the North of Benin.

**DISCUSSION**

**Phylogenetic position of wood-inhabiting polyopes in Benin**

All investigated specimens were first identified by morpho-anatomical characteristics. Further, we generated DNA sequences for wood-inhabiting polyopes collected in tropical Benin (West Africa) and used them for phylogenetic analyses in order to confirm the morphological identification.

All newly generated sequences fall correctly into the corresponding clades with the exception of the sequences named *Megasporoporia setulosa*, *Microporus concinnus*, *M. incomptus*, *M. affinis* and *M. xanthopus*. For the genus *Microporus*, we performed further phylogenetic analyses involving the ITS, LSU and Tef. The results of these analyses clearly show that *M. affinis*, *M. cocinnus*, *M. incomptus* and *M. xanthopus* are four different species (Fig. 5 and Supplementary Fig. S2). Of all the *M. xanthopus* sequences available on GenBank, only the sequence obtained from a specimen from Nigeria (accession number KT273357) groups with our sequences from Benin (Supplementary Fig. S2). All other *M. xanthopus* sequences available on GenBank are different from the Benin and Nigeria ones. The type locality of *M. xanthopus* is Nigeria, West Africa (MycoBank), so it makes sense that Benin and Nigeria sequences group together and are conspecific. Other specimens outside of West Africa bearing this name need to be carefully examined both phylogenetically and morphologically. Therefore, a thorough taxonomic study of the Chinese specimens currently known as *M. xanthopus* will be crucial to shed light not only on the correct identity of these specimens but also to increase our knowledge of the global distribution of *M. xanthopus*. Phylogenetic analyses of *Microporus* based on the ITS and the combination ITS-LSU-Tef gave a poor and unresolved tree while a phylogenetic analysis based on Tef gave a better tree (Fig. 3, Supplementary Fig. S2 vs Fig. 5). This shows that even though the ITS region is recognised as a universal and suitable region for correct identification (Schoch et al. 2012), this region alone is not sufficient for some genera.
The placement of Rhipidium interruptum (Rhipidiaceae) and cryptic species (Badotti et al. 2017, Lücking et al. 2020) and therefore studies combining different DNA regions are needed and important for accurate identification. Microporus affinis has several varieties (MycoBank). From our analysis, several sequences identified as M. affinis do not group with others of the same name. Besides, the M. affinis sequence from Benin forms a distinct lineage in our analysis. Unfortunately, no spores were found from our specimen despite several attempts. We therefore preferred to keep the name M. affinis for the Benin specimen and further studies on this species will be carried out as soon as additional specimens of this species become available.

Megasporoporia setulosa was originally described from Tanzania, Africa. Our phylogenetic analyses based on the ITS-LSU loci of Megasporoporia and related genera showed the same results like the one from ITS and the specimens from Benin and the one collected by Ryvarden in 1973 from the type locality (Tanzania) group together and form a distinct group far away from sequences from GenBank annotated as M. setulosa from China, Brazil and the USA (Figs 3, 4). The sequence named M. setulosa from China falls in Megasporia clade and grouped together with a sequence of Megasporia hexagonoides. This shows that this sequence was misidentified. Nevertheless, the other sequences named M. setulosa from the USA and Brazil are not actually setulosa but rather a recently described new species, M. neosetulosa (Lira et al. 2021). This confirms that the sequences from our Benin and Tanzania specimens newly generated in this study are the true M. setulosa.

New combinations in the genera Fuscoporia and Megasporia

Our phylogenetic analysis of the genus Fuscoporia places the sequences of the species identified as Ph. beninensis in the Fuscoporia clade. Fuscoporia as well as several other genera such as Fomitiporiella, Fulvifomes, Phylloporia, etc. have been segregated from Phellinus sensu lato (Wagner & Fischer 2001, 2002). These small genera share very similar morpho-anatomical characteristics that complicate their differentiation based on morphology alone. As a result, introducing a new species into one of the small genera based on morphology is very challenging to all polyporologists. Despite above-mentioned consideration, Ph. beninensis was introduced as a new species based on morphological characteristics of a specimen from Benin (Olou & Ryvarden 2021). Here, the sequences of the same species fall in Fuscoporia clade, underlining the importance of combining molecular and morphological data to accurately assign species to genera. Thus, a new combination Fuscoporia beninensis is proposed (see checklist).

Although this study did not focus on the genus Megasporia, our phylogenetic analysis on the genus Megasporoporia and related genera such as Megasporia, and Megasporoporiella showed that the sequences of Megasporoporia minuta cluster together and fall into the clade Megasporia. Megasporia is segregated from Megasporoporia sensu lato and characterised by the acyanophilous, non-dextrinoid hyphae, which are also unbranched to sparingly branched, usually lack hyphal pegs and dendrahyphidia, with a neotropical distribution. However, Megasporoporia is characterised by strongly dextrinoid, cyanophilous hyphae, which are also unbranched to sparingly
branched, lack dendrohyphidia, with the presence of hyphal pegs in most of species, with tropical and subtropical distributions (Yuan et al. 2017). *Megasporoporia minuta* is characterised by an annual to biennial habit, resupinate basidiocarp with a distinct sterile margin, and small, cylindrical to oblong-ellipsoid spores. Unlike most species of *Megasporoporia*, *M. minuta* has small pores, oblong-ellipsoid basidiospores, and lacks hyphal pegs and dendrohyphidia (Zhou & Dai 2008). These characteristics fit well the genus *Megasporia*. *Megasporoporia minuta* was described based on morphological examination only. Given that the genus *Megasporia* is segregated from *Megasporoporia sensu lato*, it can therefore be concluded that in the absence of the molecular data, *M. minuta* has been misassigned, and that in the presence of the molecular data the correct genus for this species is *Megasporia*. Therefore, we propose here a new combination *Megasporia minuta*.

**Diversity and distribution of wood-inhabiting polypores in Benin**

In this study we provide for the first time an illustrated catalogue of wood-inhabiting polypores with colour photos and molecular data as well as species distributions in Africa. A total of 76 species of wood-inhabiting polypores belonging to six orders, 15 families and 39 genera are reported for Benin based on own collections from 2017 to 2021. Of the 76 species, 30 are new to West Africa and 69 are recorded for the first time for Benin.

Of the 10 species reported from Benin before 2017, only five were recollected and identified in this study. The other five species, namely *F. tenuiculus*, *G. applanatum*, *L. lucidum*, *L. velutinus*, and *N. stipitatus*, were not identified during our mycological surveys. Future fieldwork and taxonomic investigation will show whether these species occur in Benin. Several specimens belonging to genera like *Coltricia*, *Favovus*, *Ganoderma*, *Lentinus*, etc. are still awaiting proper identification.

The diversity of wood-inhabiting polypores reported here is very low compared to other regions where the diversity is well explored (Lindblad 2001, Cui & Dai 2007, Robledo & Rajchenberg 2007, Dai et al. 2011, Dai 2012, Niemelä 2013, Ryvenard & Melo 2014, Kunttu et al. 2015, Jang et al. 2016, Ginnis 2017, Bernicchia & Gorjón 2020, Gafforov et al. 2020). This low diversity in Benin is partly because this study only focused on basidiomycetes and did not take into account corticoid and ascomycetous wood-inhabiting fungi such as *Xylariaceae*. In addition, the low sampling effort (only eight partially surveyed forests out of a total of 48 forests existing in Benin), and the relatively short survey period compared to diversity studies in other regions are responsible for the low diversity recorded in Benin. For example, Gafforov et al. (2020) reported 153 species of wood inhabiting fungi based on records from 1950 to 2020. Dai (2012) reported 704 species of polypores in China after 20 years of mycological surveys. Therefore, this checklist on polypores in Benin should be considered as provisional. Considering Benin's floristic diversity of 2,807 species (Akoegninou et al. 2006), and the fact that a high diversity of plant species leads to a high diversity of associated fungal species (Hawksworth 1991, Hawksworth & Lücking 2017) and the scanty knowledge of the mycodiversity in this area (Piepenbring et al. 2020), we are sure that more species of wood-inhabiting polypores can be discovered in Benin in the future.

Most of wood-inhabiting polypores reported in this study are saprotrophs and as such they are key players in wood decomposition, maintenance and functioning of forest ecosystems (Harley 1971, Wei & Dai 2004, Purahong et al. 2018). By decomposing dead plant materials, they increase carbon sequestration below ground in forest ecosystems and thereby reducing greenhouse gas (GHG) emissions. In addition to this, they hold effective mechanisms of lignocellulose-decomposing enzymes useful to attack toxic and non-toxic pollutants (Adaskaveg et al. 1990, Coetzee et al. 2015, Kües 2015). Future studies to evaluate the ability of degradation of plastic waste of new species typified with Benin material will be a new and innovative research application idea to take advantage of the enzymes produced by fungi and decomposers. Apart from saprotrophic species, other species inhabiting living wood such as *Ganoderma*, *Phyllopora*, *Fulvifomes*, *Inonotus*, and *Phellinus* are reported. Some species of these genera can cause enormous damage and important economic losses to forest ecosystems (Ganglo & Maître 2003).

Some wood-inhabiting polypores are edible and/or medicinal mushrooms. Although in Benin the edibility and medicinal effects of polypores are not well known to the public, some species such as *Lentinus squarrosulus* and *Lentinus tuber-regium* are used for food (De Kesel et al. 2002, Boni & Yorou 2015, Fadéyé et al. 2017). Apart from these species, we have other species like *Echinocochaetae brachypora* which is not known by the Beninese public but reported as edible in other areas in Africa (Eyi-Ndong et al. 2011). Unlike plants, the use of mushrooms in traditional medicine is still unknown, yet they are very effective in the treatment of certain diseases in many regions of the world. Among the species used are species of the genus *Trametes* and species of the complex *Ganoderma lucidum* which are used in the treatment of cancer, diabetes, and AIDS (El-Mekkawy et al. 1998, Akbar & Yam 2011). Species of the *G. lucidum* complex are regarded as the most valuable medicinal mushrooms and used to treat various diseases (Paterson 2006, Grienke et al. 2014, Zhu et al. 2016). As these species are reported in Benin, further ethnopharmacological, chemotaxonomic, mycochemical, and pharmacological studies will be important to benefit from these species and achieve the Sustainable Development Goals by directly contributing to human health and well-being (SDG3), decent work and economic growth (SDG8). Indirectly this will also contribute to poverty and hunger eradication (SDG1 and 2) through cash income that will be generated from the cultivation of mushrooms like *Ganoderma* spp.

Hiherto, *Hymenochaetae* and *Polyporales* are the two orders with the highest diversity in Benin. These two orders have also been reported as the most dominant in other studies dealing with the diversity of wood-inhabiting fungi (Dai 2012, Gafforov et al. 2020). This could be justified by the fact that the distribution of species of these two orders would be largely determined by their degree of specificity for host species and broad environmental conditions. Of the 76 species reported here, three species, namely *F. ostreiformis*, *G. striatum*, *P. baudoni* are brown rot fungi. Both species are recorded in the central and northern Benin (drier area of Benin). Previous studies have revealed the effect of climate on the type of rot (Gilbertson 1981, Hibbett & Donoghue 2001). The distribution of some species would require host and habitat preference and thus a particular environmental condition (Gilbert et al. 2008). This could explain why species of some genera like *Microporus*, *Gloeophyllum*, *Piptoporellus* are only present in one area or habitat. Thus, to maintain the diversity of basidiomycetes wood-inhabiting polypore fungi, special attention should be given to
the protection of their habitats. The distribution map provided here is therefore a baseline for good management and decision making on wood-inhabiting fungi.

**ACKNOWLEDGEMENTS**

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**Conflict of interest:** The authors declare that there is no conflict of interest.

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Supplementary Material: http://fuse-journal.org/

**Fig. S1.** Maximum likelihood tree with rapid bootstrap values. The species names are followed by voucher or strain number and country of origin. Species names in red are from Benin.

**Fig. S2.** Maximum likelihood (ML) analysis of the genus *Microporus* with rapid bootstrap values based on the combined ITS-LSU-*Tef* dataset. Newly generated sequences highlighted in red. The sequence names are followed by voucher or strain number and country of origin.

**Table S1.** Taxon names and GenBank accession numbers of some sequences used in this study.
**Phyllosticta rizhaoensis sp. nov. causing leaf blight of Ophiopogon japonicus in China**

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**Abstract:** *Ophiopogon japonicus* (Asparagaceae) is a perennial grass species which can be cultivated as an ornamental and medicinal plant. From April 2021 to September 2022, a serious leaf blight disease of *O. japonicus* was discovered in Rizhao City, Shandong Province, China. The initial disease symptoms were small yellow spots, finally developing as tip blight, often associated with many small, black, semi-immersed pycnidial conidiomata formed in lesions. To obtain isolates of the causal agent for this disease, samples were randomly collected from *O. japonicus* diseased leaves in Rizhao City. In total 97 *Phyllosticta* isolates were obtained from samples, and studied using morphological features and multi-locus phylogenetic analyses of a combined dataset using the internal transcribed spacers (ITS), the 28S large subunit of ribosomal RNA (LSU), and partial translation elongation factor 1-alpha (*tef*) and actin (*act*) and glyceraldehyde-3-phosphate dehydrogenase (*gpdh*) loci. Phylogenetically, these *Phyllosticta* isolates formed a clade in the *P. concentrica* species complex, and clustered with *P. pilospora* and *P. spinarum*. Morphologically, isolates in this clade differed from *P. pilospora* and *P. spinarum* by the size of conidiogenous cells and conidia, and the absence of an apical conidial appendage. As a result, these isolates were described as a novel species *Phyllosticta rizhaoensis*. Pathogenicity was confirmed using Koch’s postulates, which showed that *P. rizhaoensis* could induce leaf blight symptoms on *O. japonicus* in China.

**Key words:** Botryosphaerales leaf blight morphology multigene phylogeny new taxon

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**INTRODUCTION**

*Ophiopogon japonicus* (Asparagaceae), known as dwarf lilyturf or mondo grass, is a perennial ornamental plant that is widely distributed in temperate zones (Lun-Kai & Song-Yun 1991, Lim 2015). *Ophiopogon japonicus* is one of the most popular ground-covering ornamental plants as it is easily cultivated and environmentally durable (Sendo et al. 2010). In addition, roots of *O. japonicus* contain antimicrobial compounds that have been used as a traditional Chinese medicine for the treatment of coughing, phlegm and pneumonia (Liang et al. 2012).

During the growth season some fungal diseases have significantly affected the yield and quality of *O. japonicus*. These include leaf anthracnose caused by *Colletotrichum liriopes* (Wang & Wang 2021), root rot caused by *Fusarium acuminatum* (Tang et al. 2021), leaf blight caused by *Alternaria alternata* (Xu et al. 2022), and leaf blight caused by *P. ophiopogonis* (Kwon et al. 2015).

The genus *Phyllosticta* was established by Persoon (1819) and as a monophyletic genus in *Phyllostictaceae* (Botryosphaerales) (Wijayawardene et al. 2020). Currently, there are 183 species grouped in six species complexes in *Phyllosticta*, and members include plant pathogens, endophytes and saprobes on various host plants (Wulandari et al. 2009, Wikee et al. 2013a, Norphanphoun et al. 2020, Hattori et al. 2020, Bhunjun et al. 2021, Crous et al. 2021, Nguyen et al. 2022, Tan & Shivis 2022, Zhang et al. 2022). Morphologically, species of *Phyllosticta* are characterised by globose pycnidial conidiomata, subcylindrical to ampulliform conidiogenous cells, and hyaline, 1-celled, globose, subglobose, ellipsoidal to ovoid conidia, usually enclosed in a mucoid layer, bearing a single apical mucoid appendage (van der Aa & Vanek 2002), which are the main features in distinguishing *Phyllosticta* from other genera.

During 2021 and 2022, a new leaf blight disease was observed on *O. japonicus* in Rizhao City, Shandong Province, China. The purpose of the present study was therefore to identify the causal organism of *O. japonicus* leaf blight disease in China and confirm its pathogenicity via Koch’s postulates.

**MATERIALS AND METHODS**

**Isolation and morphological studies**

From April 2021 to September 2022, a serious leaf blight disease of *O. japonicus* was observed and recorded during grassland disease surveys in Rizhao City, Shandong Province of China. The initial symptoms of this disease were small circular or ovoid...
chlorotic patches forming on leaves. As the infection advanced, lesions would enlarge and become more reddish in colour. Spots appearing at leaf tips would expand downwards, leading to leaf blight symptoms. Numerous small, black, and semi-immersed pycnidial conidiomata were observed in lesions (Fig. 1). Subsequently, diseased leaf samples were collected from 37 different plants of *O. japonicus* growing in 14 different gardens.

Two different methods were used to isolate fungi from infected leaves. In the first method, segments (0.5 × 0.5 cm) were taken from the margin between the symptomatic and healthy tissues. The segments were surface sterilised for 1 min in 70 % ethanol, 30 s in 1 % NaOCl, subsequently rinsed three times in sterilised water, and placed onto potato dextrose agar (PDA) plates including the antibiotic chloramphenicol (35 μg/L). In the second method, isolates were obtained directly from leaf spots containing pycnidia by using single-spore isolation methods (Choi et al. 1999). The obtained cultures were purified by hyphal-tip methods, and grown at 25 °C for further tests. Specimens were accessioned in the herbarium of the Chinese Academy of Forestry (CAF; http://museum.caf.ac.cn/), Beijing, China. Living cultures were deposited in the China Forestry Culture Collection Center (CFCC; http://cfcc.caf.ac.cn/), Beijing, China.

Isolates were taken from the edge of 7-d-old colonies and then incubated at 25 °C for 7 d on fresh PDA plates. After conidomatal formation, at least 30 fungal structures were measured and captured using an Axio Imager 2 microscope with differential interference contrast (DIC) illumination.

**DNA extraction, PCR, and sequencing**

Five-day-old fungal mycelia were scraped off PDA plates with a sterile scalpel blade. Total DNA was extracted with a CTAB method according to Doyle & Doyle (1990). The PCR amplifications were performed in a 25 μL volume including 1 μL of DNA template, 1 μL of each primer, 12.5 μL 2 × Taq polymerase [KT210831, Tiangen Biotech (Beijing) Co., Ltd.] and 10.5 μL double-distilled water. Five loci, including the internal transcribed spacers (ITS), the large subunit of ribosomal RNA (LSU), partial translation elongation factor 1-alpha (tef), partial partial actin (act) and partial glyceraldehyde-3-phosphate dehydrogenase (gapdh) were amplified and sequenced with primers in suitable amplification conditions (Table 1). PCR products were sequenced by Sangon Biotech (Shanghai) Co., Ltd., (Beijing, China).

**Phylogenetic analyses**

The obtained DNA sequences in this study were used to calculate the sequence similarity using the BLASTn program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Reference sequences are listed in Table 2 (Norphanphoun et al. 2020, Hattori et al. 2020, Nguyen et al. 2022, Tan & Shivas 2022). Sequence alignments were generated with MAFFT v. 7 (https://mafft.cbrc.jp/alignment/server/, Katoh et al. 2019), and edited manually using MEGA v. 7 (Kumar et al. 2016). Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses were conducted individually on each locus (ITS, LSU, tef and act) and a combined dataset including all five loci (ITS, LSU, tef, act and gapdh). The ML analyses were analysed by RAxML-HPC BlackBox v. 8.2.10 (Stamatakis 2014) under the GTR+GAMMA model with 1 000 bootstrap replicates. Bayesian inference analyses were performed by MrBayes v. 3.2.6 (Ronquist et al. 2012). The best-fitted substitution model for the alignments of individual loci with the Akaike information criteria (AIC) using jModelTest v. 2.1.7 (Darriba et al. 2012). Two parallel runs from a random tree topology, each consisting of four Markov chain Monte Carlo (MCMC) chains, running for 1 M generations with sampling every 1 000 generations. The first 25 % of each run was discarded as burn-in and posterior probability values (BPP) were determined using the remaining trees in the majority rule consensus tree. Trees were rooted with *Botryosphaeria stevensi* (CBS 112553) and *B. obtusa* (CMW 8232) and viewed and edited via FigTree v. 1.4 (http://tree.bio.ed.ac.uk/software/figtree), and values of ML bootstraps ≥ 50 % and BPP ≥ 0.9 were c indicated in the phylogenetic trees (ML bootstrap > 94 % and BPP >0.94 were considered significant)..

**Pathogenicity test**

Pathogenicity was tested on leaves of 1-yr-old *O. japonicus* plants in pots. Healthy and unwounded leaves were surface-sterilised by washing with 70 % ethanol for 1 min, with 1 % NaClO for 30 s, and with sterilised water three times. The conidial suspension was obtained from 2-wk-old PDA plates (CFCC 57579) and adjusted to a final spore concentration of 1 × 10⁶ conidia/mL with sterilised water. Leaves were dipped into a 3 mL conidial suspension or sterilised water for 30 s (Denman et al. 2005) and pots placed in transparent plastic bags to induce a high relative humidity. Each inoculation treatment contained six leaves and was repeated twice. All pots were kept at 25 °C (± 2 °C) and over 90 % humidity in a 12 h light/ dark cycle for 1 month. To fulfil Koch’s postulates, the fungal species was re-isolated from the lesions and identified via molecular analyses.

**RESULTS**

**Phylogenetic analyses**

In total 97 isolates exhibited a similar morphology resembling a *Phyllosticta* species. Based on preliminary phylogenetic analyses of three combined regions (ITS, tef and act), isolates resembling *Phyllosticta* clustered with a clade distinct from all other described species. Three isolates (CFCC 57579, CFCC 57580 and CX2) were subsequently chosen for further studies.

BLASTn results of isolate CFCC 57579, showed the highest similarity sequence of 97.64 % with *Phyllosticta spinarum* (CBS 292.90) and *Phyllosticta pilospora* (MUC 2912) in ITS, 99.74 % with *Phyllosticta citribrazilensis* (CBS 100098) in LSU, 91.34 % with *Phyllosticta spinarum* (MUC 2918) and *Phyllosticta pilospora* (MUC 2912) in tef, and 98.19 % in *Phyllosticta spinarum* (MUC 2918) and *Phyllosticta pilospora* (MUC 2912) in act.

The concatenated datasets consisted of 28 species, including two outgroup taxa, *B. stevensii* (CBS 112553) and *B. obtusa* (CMW 8232). The alignment contained 2 399 characters, including gaps (477 for ITS, 763 for LSU, 284 for tef, 245 for act and 630 for gapdh), of which 1 833 were constant, 168 were parsimony-uninformative, and 404 were parsimony-informative. Four single-locus datasets ITS, LSU, tef and act, contained 338, 763, 111 and 135 constant sites, 20, 26, 41, 50 parsimony-uninformative sites, and 119, 30, 132 and 60 parsimony-informative sites, respectively.

In the resulting trees (Figs S1–S4), individual phylogenetic analyses using ITS, LSU, tef and act showed that our isolates
Phyllosticta rizhaoensis sp. nov.

Isolates and GenBank accession numbers of sequences used in this study.

Table 1. Sequences of primer sets targeted for the ITS, LSU, tef1 and act regions.

<table>
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<th>Sequence (5’–3’)</th>
<th>PCR conditions</th>
<th>Reference</th>
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<td>ITS</td>
<td>ITS1</td>
<td>TCCGTAGGTAACCTGGC</td>
<td>Denaturation for 3 min at 94 °C, followed by 30 cycles; 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C, and a final extension of 10 min at 72°C</td>
<td>White et al. (1990)</td>
</tr>
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<td></td>
<td>ITS4</td>
<td>TCTCGGCGATATTGATAT</td>
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<td>White et al. (1990)</td>
</tr>
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<td>LSU</td>
<td>LROR</td>
<td>ACCCGCTGAACCTAGGC</td>
<td>Denaturation for 3 min at 94 °C, followed by 30 cycles; 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C, and a final extension of 10 min at 72°C</td>
<td>Rehner &amp; Samuels (1994)</td>
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<tr>
<td></td>
<td>LRS</td>
<td>TCTCGGCGATATTGATAT</td>
<td>Denaturation for 3 min at 94 °C, followed by 30 cycles; 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C, and a final extension of 10 min at 72°C</td>
<td>Vilgalys &amp; Hester (1990)</td>
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<tr>
<td>tef1</td>
<td>EF1-728F</td>
<td>CATCGAGATTCGGAGAAGG</td>
<td>Denaturation for 3 min at 94 °C, followed by 30 cycles; 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C, and a final extension of 10 min at 72°C</td>
<td>Carbone &amp; Kohn (1999)</td>
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<td></td>
<td>EF1-986R</td>
<td>TACTTGAAGAACCCTTACC</td>
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<td>act</td>
<td>ACT512F</td>
<td>ATGTTGGAAGGCTGTTCGTCG</td>
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<td></td>
<td>ACT783R</td>
<td>TAGAGGTCTGTTCGGGATCAT</td>
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<td>Carbone &amp; Kohn (1999)</td>
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Table 2. Isolates and GenBank accession numbers of sequences used in this study.

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<th>Host</th>
<th>Country</th>
<th>GenBank no.</th>
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<td>China</td>
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<td>Bifrenaria harrisoniae</td>
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<td>P. citribrasilensi</td>
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<td>JF343583</td>
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Table 2. (Continued).

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Notes: NA, not applicable; *ex-type strains.

(CFCC 57579, CFCC 57580 and CX2) represent a phylogenetically distinct species. In the resulting tree (Fig. 2), multi-locus phylogenetic analyses using five loci showed that our isolates were distinct from all other described species, and clustered with *P. pilospora* and *P. spinarum* with high support.

**Taxonomy**

*Phyllosticta rzhaoensis* Ning Jiang & C.B. Wang, *sp. nov.* MycoBank MB 845759. Fig. 1.

**Etymology:** The name refers to the Rizhao City where the fungus was collected.

**Description on PDA:** Conidiomata pycnidial, black, erumpent, separate to aggregated, subglobose to globose, up to 300 μm diam. Conidiogenous 10–30 × 4–6 μm, subcylindrical to amputiform. Conidiogenous cells 7–14 × 2–6 μm, phialidic, subcylindrical, hyaline, thin-walled, smooth. Conidia 8–12 × 5–7.5 μm (av. ± SD = 10 ± 1.1 × 6.5 ± 0.6 μm), hyaline, aseptate, thin- and smooth-walled, granular, ellipsoid to obovoid.

**Culture characters:** Colonies on PDA, flat, spreading, aerial mycelium feathery, grey, slow-growing, reaching 36 mm diam on PDA after 2 wk at 25 °C.


**Notes:** *Phyllosticta rzhaoensis* groups in a distinct clade from the other two *Phyllosticta* species, *P. capitalensis* and *P. ophiopogonis*, that have been reported from *O. japonicus*. Morphologically, *P. rzhaoensis* differs from *P. capitalensis* and *P. ophiopogonis* by its smaller conidia (8–12 × 5–7.5 μm in *P. rzhaoensis* vs 10–14 × 5–7 μm in *P. capitalensis* vs 10–14 × 7–8 μm in *P. ophiopogonis*) (Glenke et al. 2011, Wikee et al. 2013b). *Phyllosticta rzhaoensis* forms an independent sister clade with *P. pilospora* and *P. spinarum* (Fig. 2). *Phyllosticta rzhaoensis* can be distinguished from *P. pilospora* (MUCC 2912) and *P. spinarum* (CBS 292.90) by nucleotide differences, i.e. ITS (8 bp, 7 bp), LSU (7 bp, 2 bp), tef1 (22 bp, 23 bp) and act (5 bp, 4 bp). Morphologically, *P. rzhaoensis* can be distinguished from them by having larger conidiogenous cells (7–14 × 2–6 in *P. rzhaoensis* vs 4–12 × 2–2.5 in *P. pilospora* and 5–12 × 3–5 in *P. spinarum*), smaller conidia (8–12 × 5–7.5 μm in *P. rzhaoensis* vs 9.5–12 × 7–10 μm in *P. pilospora* and 10–15 × 7–8 μm in *P. spinarum*), and the lack of an apical conidial appendage (Wikee et al. 2013b, Hattori et al. 2020).

**Pathogenicity tests**

Five days post inoculation (dpi), small, circular or ovoid, chlorotic lesions appeared at the leaf margins. Leaf spots expanded rapidly at 10 dpi, and turned into white spots surrounded by a chlorotic margin at 20 dpi. After 30 dpi, typical leaf blight symptoms appeared on inoculated leaves. No symptoms were observed on the control inoculations. Re-isolated strains were identified as *P. rzhaoensis* based on phylogenetic analyses.

**DISCUSSION**

A novel leaf blight disease of *O. japonicus* with a high incidence was found in Rizhao City, Shandong Province. *Phyllosticta* isolates were obtained from infected leaves and delineated using multi-locus phylogenetic analyses as a new species, viz. *P. rzhaoensis*. Pathogenicity tests suggest that *P. rzhaoensis* is a pathogen of *O. japonicus* causing leaf blight disease.

*Phyllosticta* contains many plant pathogenic and endophytic species, commonly occurring worldwide on a broad range of host plants (Wikee et al. 2013a). Generally, *Phyllosticta* species cause disease symptoms on leaves, including necrotic lesions often containing many black, globose or subglobose, and semi-immersed pycnidia (Wikee et al. 2011). In this study, disease symptoms on *O. japonicus* leaves and the morphological observation of pycnidia and other fungal structures on lesions showed that this disease was caused by a *Phyllosticta* species. To date two *Phyllosticta* species have been reported causing leaf disease on *O. japonicus*, namely *P. capitalensis* and *P. ophiopogonis* (Farr & Rossman 2022). *Phyllosticta capitalensis* is a cosmopolitan fungus.
found in more than 70 plant families, and is often isolated as an endophyte from healthy plants, or symptomatic plants affected by other *Phyllosticta* species (Guarnaccia et al. 2017). *Phyllosticta ophiopogonis* has been reported causing leaf bight disease of *O. japonicus* in Thailand and Korea, and no records exist of this species on other plant hosts (Wikee et al. 2012, Kwon et al. 2015). However, several *Phyllosticta* species have been associated with other species of Asparagaceae (Farr & Rossman 2022).

Previously, many *Phyllosticta* species were identified based on morphology and host range without elucidating their phylogenetic relationships (van der Aa 1973). To resolve the phylogenetic relationship of *Phyllosticta* species, the ITS locus was first used to define species in this genus (Schoch et al. 2012, Kashyap et al. 2017). Subsequently, multi-locus sequence analyses combining ITS with other housekeeping genes (LSU, tef, act and gapdh) have portrayed clearer evolutionary relationships for species in the genus (Norphanphoun et al. 2020, Zhang et al. 2022). In this research, *P. rizhaoensis* formed a well-resolved clade in the phylogenetic trees which included the *P. concentrica* species complex based on individual loci (ITS, LSU, tef and act) or concatenated gene sequences of five loci (ITS, LSU, tef, act and gapdh).
Fig. 2. Phylogenetic tree obtained by ML analyses using the combined ITS, LSU, tef, act and gapdh sequence alignments of the *Phyllosticta concentrica* species complex. Numbers above the branches indicate ML bootstraps (left, ML ≥ 50 %) and Bayesian Posterior Probabilities (right, BPP ≥ 0.9). The tree is rooted with *Botryosphaeria stevensii* (CBS 112553) and *B. obtusa* (CMW 8232). Ex-type strains are indicated in bold. Isolates from the present study are marked in blue.
Conidia of **Phyllosticta** species are usually enclosed in a mucilaginous sheath with an apical appendage which has been used to distinguish species in the genus (van der Aa 1973, van der Aa & Vaney 2002). However, this character is problematic, due to the mucoid layer and appendage not always being present or visible, e.g., *P. colocasica, P. minima* and *P. sphaeropsoidea*. Furthermore, the conidial appendages of some *Phyllosticta* species might disappear with time or elongate when mounted in water (van der Aa & Vaney 2002, Jin 2011, Wikee et al. 2013b). The various morphological features of *P. rizhaoensis* are compared with those of other species in the *P. concentrica* species complex in Table S1.

In China, *Phyllosticta* species cause diseases on a broad range of host plants, e.g. *Citrus* spp., *Musa* spp. and *Camellia sinensis* (Wu et al. 2014, Zhang et al. 2015, Farr & Rossman 2022). In the present study, a new leaf blight disease of *O. japonicus* is reported. *Phyllosticta* isolates were isolated from diseased leaves and shown to represent a novel species based on morphology and DNA phylogeny. Pathogenicity tests demonstrated that *P. rizhaoensis* could induce leaf blight on *O. japonicus*. To our knowledge, this is the first report about *Phyllosticta* species causing leaf blight symptoms of *O. phiopogon* in China.

**ACKNOWLEDGEMENT**

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**Conflict of interest:** The authors declare that there is no conflict of interest.

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Supplementary Material: http://fuse-journal.org/

Fig. S1. Phylogenetic tree obtained by ML analyses using ITS sequence data of *Phyllosticta concentrica* species complex. Numbers above the branches indicate ML bootstraps (left, ML ≥ 50 %) and Bayesian Posterior Probabilities (right, BPP ≥ 0.9). The tree is rooted with *Botryosphaeria stevensii* (CBS 112553) and *B. obtusa* (CMW 8232). Ex-type strains are indicated in bold. Isolates from the present study are marked in blue.

Fig. S2. Phylogenetic tree obtained by ML analyses using LSU sequence data of the *Phyllosticta concentrica* species complex. Numbers above the branches indicate ML bootstraps (left, ML ≥ 50 %) and Bayesian Posterior Probabilities (right, BPP ≥ 0.9). The tree is rooted with *Botryosphaeria stevensii* (CBS 112553). Ex-type strains are indicated in bold. Isolates from the present study are marked in blue.

Fig. S3. Phylogenetic tree obtained by ML analyses using tef sequence data of the *Phyllosticta concentrica* species complex. Numbers above the branches indicate ML bootstraps (left, ML ≥ 50 %) and Bayesian Posterior Probabilities (right, BPP ≥ 0.9). The tree is rooted with *Botryosphaeria obtusa* (CMW 8232). Ex-type strains are indicated in bold. Isolates from the present study are marked in blue.

Table S1. Morphological comparison of species in the *P. concentrica* species complex.
Two novel endophytic *Tolypocladium* species identified from native pines in south Florida

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Key words: entomopathogens, fungal endophytes, Hypocreales, new taxa, Pine Rocklands

Abstract: This study investigated the incidence and diversity of *Tolypocladium* within trunks of south Florida slash pines (*Pinus densa*). Thirty-five isolates were recovered from trunk tissue including living phloem, cambium, and sapwood. Two novel species of *Tolypocladium* (*T. subtropicale* and *T. trecente*) are described here based on morphological and molecular analysis of concatenated LSU, ITS, tef-1, tub, and RPB1 sequences. Our findings expand our understanding of the distribution, diversity, and ecology of this genus and confirm that it is widely spread as an endophyte across ecosystems and hosts. Strains collected in this survey will be used in future bioassays to determine their potential ecological roles as mycoparasites or entomopathogens.

INTRODUCTION

Research across biomes has demonstrated the importance of fungal endophytes not only in respect to their impact on host fitness but also at the ecosystem level (Hardoim et al. 2008, Gazis et al. 2006, Pujade-Renaud et al. 2022) and bioassays (Photita 2004, Oses et al. 2015, Martin et al. 2015, Gilmartin et al. 2022) and bioassays (Photita et al. 2004, Oses et al. 2006, Pujade-Renaud et al. 2019) connections between the endophytic lifestyle with fungi known as saprotrophic, parasitic, and mutualistic nutritional modes have been established, suggesting that our concepts of the ecological roles of many fungal taxa need revision. Several lineages closely related to entomopathogenic fungi, have been recovered as endophytes from inner leaf, stem, and root tissues (Vega et al. 2008, Gazis et al. 2014, Vidal & Jaber 2015). However, whether these fungal strains harbor the complete version of functional genes involved in entomopathogenicity and protect their host through anti-herbivory mechanisms such as the production of metabolites or direct parasitism is not known. Additional experiments that test the true ecological role or nutritional mode of these strains are needed, such as the study by Wang et al. (2020) which showed that the entomopathogenic caterpillar fungus *Ophiocordyceps sinensis* has an endophytic stage that facilitates infection of the host’s larvae through the consumption of endophytically colonized roots.

Fungal strains recovered as endophytes from cultivated plants and commonly used in biological control (e.g., *Beauveria bassiana*, *Isaria fumosorosea*, *Metarhizium brunneum*, *M. anisopliae*, and *Purpureocillium lilacinum*) often conserve their entomopathogenic capability (Castillo Lopez et al. 2014, Carrillo et al. 2015, Gange et al. 2019, Ramakuwela et al. 2020) and field studies have successfully shown pest suppression as a consequence of endophytic colonization (Canassa et al. 2020, Russo et al. 2021). Conversely, other lineages may have lost the ability to parasitize or antagonize insects and in turn acquired a commensal endophytic habit. So far, direct evidence is lacking in most cases.

*Tolypocladium* (*Ophiocordycipitaeceae, Hypocreales, Sordariomycetes*) currently contains 49 species registered in MycoBank (www.mycobank.org) (MycoBank database, January 2023). *Tolypocladium* species have diverse lifestyles that range from insect and fungal parasites to endolichens and soil-dwelling inhabitants (Bushley et al. 2013, Quandt et al. 2015, Blount 2018, Yu et al. 2021). Several species (i.e., *T. inflatum*, *T. cylindrosporum*, *T. geodes*) are known as prolific producers of secondary metabolites and used widely in medicine and agriculture (Bushley et al. 2013, Li et al. 2015, Kebede et al. 2017). Due to its importance in human medicine, the most studied species is the cyclosporin and efrapeptin metabolite-producing, *T. inflatum* (Hodge et al. 1996, Bushley et al. 2013). Another species with medical importance is *T. cylindrosporum*, which besides being a pathogen of insects and ticks and an endophyte, produces cyclosporin (Matsuda & Koyasu 2000, Scholte et al. 2004, Herrero et al. 2011, Montalva et al. 2019). Multiple nutritional modes have also been found in *T. ophioglossoides*, which genome encodes genes involved in both mycoparasitism


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and entomopathogenicity, differentially expressed depending on substrate utilization (Quandt et al. 2015, 2016).

Using culture-based and metabarcoding approaches, *Tolypocladium* species have been detected as leaf and trunk endophytes of wild and cultivated *Hevea* and closely related species (Gazis et al. 2014, Gazis & Chaverri 2015, Skalsas et al. 2019) and from other hosts (Hanada et al. 2010, Sánchez Márquez et al. 2010). Currently, there are three species (*T. amzonense, T. endophyticum, T. tropicale*) known only as endophytes (Gazis et al. 2014). Species with an endophytic habit form a well-supported clade (Gazis et al. 2014, Yu et al. 2021). However, this clade does not exclusively contain strictly endophytic species as it also comprises *T. album*, a generalist species known from soil and dead and living plant material (Gazis et al. 2014, Yu et al. 2021). Outside the “endophytic clade”, *T. postulatum* has been isolated from wounds of *Pinus contorta* and was originally described from unidentified twigs in an oak forest (holotype BPI 748466, ex-type culture ATCC 74192); however, there is no evidence that these isolates were behaving as true endophytes and not as pathogens or saprotrophs (Bills et al. 2002, Arhipova et al. 2015). Additionally, *T. cylindrosorum* has been isolated from leaves of grasses (Sánchez Márquez et al. 2010) and from woody tissues of cultivated *Theobroma cacao* trees (Hanada et al. 2010).

As *Tolypocladium* becomes more speciose, several studies have made efforts to unveil the evolutionary history of substrate association within the genus. Phylogenetic analyses based on standard fungal markers (LSU, ITS, tef-1, tub, RPB1, RPB2) and genome-scale approaches support the inter-kingdom host jump hypothesis (Nikoh & Fukatsu 2000); however, have resulted in the reconstruction of contradictory ancestral states. Ancestral state reconstruction using multilocus sequence data supports the hypothesis that insect-associated *Tolypocladium* species derived from mycoparasitic ancestors (Gazis et al. 2014, Yu et al. 2021), while phylogenomic approaches suggest a single ecological and nutritional transition from insect to fungi (Quandt et al. 2016). The endophytic clade described by Gazis et al. (2014) still lacks sufficient data to generate robust hypotheses regarding its diversification history; however, it has consistently been recovered as “derived” (Gazis et al. 2014, Wang et al. 2019, Yu et al. 2021, Tehan et al. 2022).

This study represents the first attempt to determine if *Tolypocladium* species are part of the trunk endophytic community of pine trees. Sampled trees were distributed in Pine Rockland habitats in south Florida, a critically endangered tropical dry forest ecosystem (Snyder et al. 1990). This ecosystem is extremely important for animals, birds, reptiles, plants, and microorganisms including mycorrhizae and endophytes (Platt et al. 2010, Lloyd & Slater 2012, Possley et al. 2016, Karlsen-Ayala et al. 2022). Based on morphological and molecular data, we introduce two novel *Tolypocladium* species. In addition, we report few strains that cluster with previously described endophytic *Tolypocladium*, confirming that this genus is widely spread as an endophyte across ecosystems and hosts. Strains collected in this survey will be used in future bioassays to determine their potential ecological roles as mycoparasites or entomopathogens.

**MATERIALS AND METHODS**

**Sample collection and endophyte isolation**

A total of 30 individual *Pinus densa* (south Florida slash pine; Syn: *Pinus elliottii var. densa*) trees were sampled in five different Pine Rockland native forests, (i) University of Florida Tropical Research and Education Center (TREC), Homestead, Florida, USA (n=10), (ii) a Homestead private property (B&P), Homestead, Florida, USA (n=5), (iii) Pine Ridge Sanctuary (PRS), Homestead, Florida, USA (n=5) (iv) a Miami private property located in the Pinecrest area (PCA), Miami, Florida, USA (n=5) and (v) Zoo Miami (ZM), Miami, Florida, USA (n=5) (Table 1).

A sterile increment borer (Mora Borer, Haglöfs Sweden, Bromma, Stockholm, Sweden) was used to sample trunk cores. Before sampling each tree, the increment borer was sterilized by submerging it in 10 % bleach for one minute and brushed with a straw brush to remove any debris. Afterwards, it was rinsed with deionized water, submerged in 95 % ethanol, and flamed. Trees were randomly selected with a minimum distance of 5 m and were sampled from three different parts of their circumference. The increment borer was inserted at a slight upward angle (“30°”) to prevent contamination by water entering the sampling hole (Fig. 1A). The increment borer auger without the handle was attached to an electric drill and used to enter the first 5 cm of the tree (Fig. 1A, B). Once the first layer was bored, the handle was placed in the auger and rotated until the borer penetrated approximately 14 cm of the tree, and then the core was removed with the extractor (Fig. 1C, D). The first core was sampled at an approximate height of 1.5 m and the second and third were taken at 20 cm above and below the initial core. The cores were collected in Ziplock bags and labeled as pine 1 (P1) through pine 30 (P30) and core 1 (C1) through core 3 (C3), (Table 1). Samples were kept in a cooler until brought to the laboratory where they were processed immediately.

**Table 1. Sampling localities and summarized *Tolypocladium* isolation results.**

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1TREC: Tropical Research and Education Center; B&P: Bruce and Pam Forest; PRS: Pine Ridge Sanctuary; PCA: Pinecrest area; ZM: Zoo Miami.
The cores consisted of (i) outer bark, (ii) living phloem, (iii) vascular cambium, (iv) sapwood containing functional xylem, and (v) heartwood composed of old xylem (Fig. 1E). The core size was approximately 14 cm (5.5 inches); 2 cm (0.8 inches) from each end of the core, containing the outer bark and inner heartwood, were discarded (Fig. 1F). Under a biosafety cabinet, the remaining core was dissected into 16–20 pieces of 0.2 cm (0.08 inches), using a flame sterilized scalpel (Fig. 1G) and quickly transferred to 140 mm Petri dishes containing ½ Difco™ potato dextrose agar (PDA) amended with 1 % neomycin-penicillin-streptomycin (Sigma-Aldrich, St Louis, Missouri, USA) (Fig. 1H). Petri dishes were incubated for up to 2 mo at room temperature in darkness (Fig. 1I). Emerging colonies were sub-cultured onto fresh ½ PDA plates to obtain pure isolates (Fig. 1J).

Isolate identification

A total of 35 fungal strains showing tolypocladium-like colony characteristics, such as slow-growth and white cottony appearance (Gams 1971), were recovered. Cultures were purifed either by single-spore or hyphal tip technique and grown in ½ PDA until purity was confirmed. Genomic DNA was extracted from mycelial mats using a GeneJET genomic DNA purification kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA), according to the manufacturer’s instructions with few modifications (Parra et al. 2020). The internal transcribed spacer (ITS) region was amplified using the primers ITS1F (Gardes & Brun 1993) and ITS4 (White et al. 1990) and used to confirm the generic placement of the isolates. Four other loci were amplified: nuclear large subunit (LSU), largest subunit of RNA polymerase II (RPB1), partial β-tubulin gene (tub), and elongation factor 1a.
(tef-1), following protocols described in Gazis et al. (2014) and Quandt et al. (2014). PCR products were cleaned and sequenced at MCLAB laboratories (MCLAB, South San Francisco, California, USA - www.mclab.com). Sequence chromatograms were assessed, and bi-directional reads were trimmed, assembled, and checked for quality using default settings in Geneious v. 9.0.5 (Geneious Computer Software, Newark, New Jersey, USA). Newly generated sequences were deposited in GenBank (Supplementary Table S1).

A combined dataset was constructed by concatenating the newly generated ITS, LSU, tef-1, RPBI, and tub sequences and vouchered sequences retrieved from GenBank (NCBI) (Supplementary Table S1). Taxa included in the analysis represent lineages used in the following systematic studies of the genus: Gazis et al. (2014), Sung et al. (2007), Quandt et al. (2014), Yu et al. (2021), Li et al. (2018), Wijayawardene et al. (2021), Yamamoto et al. (2022), and Wang et al. (2022). A total of 26 Tolypocladium species were included, representing 53% of the currently described species. Each locus was aligned separately using MAFFT v. 7 under default settings (Katoh & Standley 2013). Ambiguously aligned regions were excluded from the alignments using Gblocks v. 0.91b (Talavera & Castresana 2007) under reduced stringency settings. A combined 106-taxa dataset was constructed with five-loci combined LSU (805 bp), ITS (464 bp), tef-1 (825 bp), RPBI (576 bp), and tub (597 bp), for a total base pair length of 3267 nucleotides. Dataset completeness was as follows: 99/106 for ITS, 94/106 for LSU, 84/106 for tef-1, 80/106 for tub, and 83/106 for RPBI (Supplementary Fig. S1, S2). A separate dataset, based only on the ITS marker, included data from 19 species (39% of described species). Maximum Likelihood (ML) phylogenies based on the ITS and on the concatenated dataset were constructed using RaxML v. 8.2.10 (Stamatakis 2014) under the GTRGamma evolutionary model. Branch support values were estimated using 1000 bootstrap (BS) replicates. Ophiocordyceps agritiidis, Ophiocordyceps acicularis, and Ophiocordyceps stylphora were used as outgroup. Alignments were deposited in the Open Science Framework (OSF); the alignments used to construct the phylogenetic trees are available through this URL: https://osf.io/t8uz5/?view_only=6eb775b84a53410aabe9525ddcebecaa.

**Morphological studies**

Four representative strains from each of the new species being described were selected for morphological description. In addition, two isolates of *T. amazonense* (MS308 [type] and LA100) were examined for comparison purposes. Single-spored isolates were grown on PDA, MEA (DifcoTM malt extract agar), Franklin Lakes, New Jersey, USA), and SNA (synthetic nutrient-poor agar) (Nirenberg 1976) and incubated at 25 °C with a photoperiod of 12 h/12 h fluorescent light/dark for 3 wk. Colony characteristics such as shape, size, and color were documented at 14 and 21 d after media inoculation. Colony size measurements were taken in two linear measurements (separated by a 45° angle) of mycelial growth and recorded from each plate at 4, 7 and 10 d after inoculation by measuring the distance between the edge of the inoculum plug and the edge of the colony. Conidia and phialides (length and width) measurements were obtained using a Motic Panthera microscope and Excelis HDS software (Micro Optics of Florida, St. Petersburg, Florida, USA).

Continuous measurements were based on 100 conidia and 30 phialides. Terms used to describe the morphology of the reproductive structures follow Seifert et al. (2011). Images were captured with an Accu-scope digital camera equipped with Excelis HDS HD camera & monitor system (Micro Optics of Florida, St. Petersburg, Florida, USA). A dried culture of the type specimen corresponding to each novel species was deposited in the U.S. National Fungus Collections (BPI) and additional representatives and ex-type cultures were deposited in the Agricultural Research Service Culture Collection. Copies of all isolates are stored at -80 °C as part of Gazis’ laboratory strain collection at the University of Florida, Tropical Research and Education Center.

**RESULTS**

**Isolation of Tolypocladium**

The presence of *Tolypocladium* within trunk tissue of pine trees was confirmed in two of the five locations sampled, Tropical Research and Education Center (TREC) and Pinecrest Area (PCA). From the 30 trees sampled and ~2,000 pieces plated, a total of 35 *Tolypocladium* isolates were recovered. At TREC, *Tolypocladium* was isolated from four of the 10 trees sampled (P4: n = 7; P5: n = 1; P16: n = 2; P19: n = 11) and at PCA, *Tolypocladium* was isolated from all five trees sampled (P21: n = 3; P22: n = 4; P23: n = 4; P24: n = 1; P25: n = 2) (Table 1).

**Identification and phylogenetic placement**

The topology of the phylogenetic tree, based on the combined dataset (ITS, LSU, RPBI, tub, and tef-1), was congruent with previous studies (Gazis et al. 2014, Quandt et al. 2014, Li et al. 2018, Wijayawardene et al. 2021, Yu et al. 2021, Wang et al. 2022) and revealed two novel *Tolypocladium* species (described here as *T. subtropicae* and *T. trecense*). *Tolypocladium subtropicae* (BS = 99) comprised 14 isolates collected from TREC and PCA sites (Fig. 2). A group containing three isolates (JMS361, JMS364, and JMS365) (BS = 98), all recovered from the same tree (PCA: P21), was resolved as sister to *T. subtropicae* and based on molecular and morphological data may represent an undescribed species (here labeled as *Tolypocladium sp. 1*). *Tolypocladium trecense* (BS = 100) comprised 13 isolates collected from TREC and PCA sites. A group composed of three isolates (JMS376, JMS373, and JMS372) (BS = 100), all recovered from the same tree (PCA: P23), formed a sister group to *T. amazonense* (BS = 95) and based on molecular and morphological data may represent a second undescribed species (here labeled as *Tolypocladium sp. 2*). Two other *Tolypocladium* strains recovered in this study (JMS377 and JMS381) grouped with the previously described species *T. tropicale* (BS = 97) (Fig. 2). The phylogeny, based on the ITS sequences, was congruent with the multilocus phylogenetic analysis (Supplementary Fig. S3). Diagnostic morphological characters were scarce, but the four distinctive and previously undescribed clades can be separated from closely related species by differences in one or more of the following characters: color of the underside of colony grown in PDA, conidiophore branching type, and phialide and conidia size and shape (Table 2).
Fig. 2. Phylogenetic tree based on a Maximum Likelihood analysis using five concatenated loci (LSU, ITS, tef-1, tub, and RPB1) showing relationship amongst *Tolypocladium* isolates collected in this study and previously described species. Bootstrap support (BS) values > 75 % are indicated.
**TAXONOMY**

**Tolypocladium subtropicale** JM Soares & Gazis, *sp. nov.* MycoBank MB 845563. Fig. 3.

**Etymology:** Epithet refers to the climatic region where the strains were collected.

**Diagnosis:** *Tolypocladium subtropicale* forms a distinct species strongly supported by molecular data and can be distinguished from other closely related endophytic *Tolypocladium* by a combination of morphological characters, including conidiophore type (unbranched) and phialide (cylindrical) and conidia (ellipsoidal) shape (Table 2).

**Colonies** on MEA circular, forming abundant white pruinose mycelium, forming concentric rings with pale yellow plate reverse. Colonies on PDA forming abundant white floccose mycelium and radially sulcate colonies with bright yellow plate reverse. Colonies on SNA, circular, scarce, colorless to white. *Hyphae* hyaline, branched, smooth-walled (Fig. 3A–F).

**Table 2.** Distinguishing morphological characteristics of previously described endophytic *Tolypocladium*, the two novel species (*T. subtropicale* and *T. trecense*), and the two unidentified independent lineages (*Tolypocladium* sp. 1 & 2).

<table>
<thead>
<tr>
<th>Species</th>
<th>Colony in PDA (F/B)</th>
<th>Conidia Shape</th>
<th>Conidiophore Type</th>
<th>Phialide Shape and Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. album</em>†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS 506 = CBS 136902 W/Y-</td>
<td>Globose to Subglobose</td>
<td>2.3 ± 0.5</td>
<td>T</td>
<td>L/C; 8.2 ± 1.7 × 3.5 ± 1.3</td>
</tr>
<tr>
<td><em>T. amazonense</em>#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS 308 W/Y</td>
<td>Globose</td>
<td>1.4 ± 0.2</td>
<td>T</td>
<td>L; 4.6 ± 1.2 × 1.5 ± 0.3</td>
</tr>
<tr>
<td><em>T. endophyticum</em>#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MX 575 W/P</td>
<td>Globose</td>
<td>1.3 ± 0.2</td>
<td>T</td>
<td>L; 4.1 ± 0.9 × 1.6 ± 0.2</td>
</tr>
<tr>
<td><em>T. subtropicale</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JMS 200* W/Y-</td>
<td>Globose to Ellipsoidal</td>
<td>2.0 ± 0.4</td>
<td>UB</td>
<td>C; 3.5–19 × 1–2.5</td>
</tr>
<tr>
<td>JMS 370 W/W</td>
<td>Ellipsoidal</td>
<td>2.5 ± 0.3 × 1.8 ± 0.2</td>
<td>UB</td>
<td>C; 6.2–15.5 × 1–2.5</td>
</tr>
<tr>
<td><em>Tolypocadium tropicale</em>#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ 214 W/W</td>
<td>Globose</td>
<td>1.5 ± 0.1</td>
<td>T</td>
<td>L; 4.6 ± 1.2 × 1.5 ± 0.3</td>
</tr>
<tr>
<td><em>T. trecense</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JMS 111* W/Y-</td>
<td>Globose</td>
<td>2.0 ± 0.6</td>
<td>UB</td>
<td>C; 6.5–13 × 0.9–2.4</td>
</tr>
<tr>
<td>JMS 196 W/Y-</td>
<td>Globose</td>
<td>2.2 ± 0.1</td>
<td>UB</td>
<td>C; 8.5–21.6 × 0.6–2.8</td>
</tr>
<tr>
<td><em>Tolypocadium</em> sp. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JMS 361 W/Y+</td>
<td>Ellipsoidal</td>
<td>2.8 ± 0.3 × 1.9 ± 0.3</td>
<td>UB</td>
<td>C; 9.2–36 × 2–2.6</td>
</tr>
<tr>
<td>JMS 364 W/Y+</td>
<td>Ellipsoidal</td>
<td>2.6 ± 0.3 × 1.6 ± 0.3</td>
<td>UB</td>
<td>C; no data</td>
</tr>
<tr>
<td>JMS 365 W/Y+</td>
<td>Ellipsoidal</td>
<td>2.6 ± 0.4 × 1.6 ± 0.2</td>
<td>UB</td>
<td>C; 13.9–42 × 1.5–2.7</td>
</tr>
<tr>
<td><em>Tolypocadium</em> sp. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JMS 372 W/P</td>
<td>Ellipsoidal</td>
<td>2.1 ± 0.3 × 1.4 ± 0.3</td>
<td>T</td>
<td>L; 6.3 ± 1.5 × 1.9 ± 0.3</td>
</tr>
<tr>
<td>JMS 373 W/P</td>
<td>Ellipsoidal</td>
<td>2.2 ± 0.2 × 1.7 ± 0.2</td>
<td>T</td>
<td>L; 6.3 ± 1.7 × 2.5 ± 0.2</td>
</tr>
<tr>
<td>JMS 376 W/P</td>
<td>Ellipsoidal</td>
<td>2.3 ± 0.2 × 1.7 ± 0.2</td>
<td>T</td>
<td>L; 6.1 ± 1.5 × 2.3 ± 0.6</td>
</tr>
</tbody>
</table>

1W = white; Y = yellow; Y+ = dark yellow; Y- = pale yellow; P = pink. 2UB = unbranched conidiophore; T = trichodermatoid. 3C = Cylindrical; L = Lageniform. *Ex-type isolate. † Data from Gazis et al. (2014); ‡ Data from Gams (1980).
JMS210, culture accession NRRL 64457; Miami-Dade County, Miami, PCA Pine Rocklands, isolated from the functional sapwood of *P. densa* (south Florida slash pine; Syn: *Pinus elliottii* var. *densa*), 5 May 2021, J.M. Soares strain JMS370 culture accession NRRL 64458.

*Tolypocladium trecense* JM Soares & Gazis, *sp. nov*. MycoBank MB 845562. Fig. 4.

**Etymology:** Epithet refers to the site where the ex-type strain was collected, the University of Florida, Tropical Research and Education Center (TREC).

**Diagnosis:** *Tolypocladium trecense* forms a distinct species strongly supported by molecular data and can be distinguished from other closely related endophytic *Tolypocladium* by a combination of morphological characters such as conidiophore type (unbranched), phialide shape (cylindrical), and lager conidia size (Table 2).

**Colonies** on MEA circular, forming abundant white floccose mycelium and light-yellow plate reverse. Colonies on PDA forming abundant white flat mycelium and radially sulcate colonies and yellow plate reserve. Colonies on SNA, circular, white, floccose (Fig. 4A–F). *Hyphae* hyaline, branched, smooth-walled. *Conidiomata* absent. *Conidiogenous cells* phialidic, unbranched, hyaline, smooth-walled, solitary, intercalary, cylindrical, often with bent necks, broad range in size (6.5–13 x 0.9–2.4 µm). *Conidia* abundant in MEA, PDA, and in SNA, aseptate, mainly globose, hyaline, small (2.0 ± 0.6 µm), single or aggregating in slimy heads at the apex of phialides (Fig. 4G–J). *Chlamydospores* present (Fig. 4K, L), hyaline, intercalary. *Sexual morph* unknown.

Fig. 3. Micrographs showing the morphological characters of *Tolypocladium subtropicale*. A, B. Colony growth in MEA media, (A) upper surface, and (B) the bottom surface of the plate. C, D. Colony growth in PDA media, (C) upper surface, and (D) the bottom surface of the plate. E, F. Colony growth in SNA media, (E) upper surface, and (F) the bottom surface of the plate. G. Slimy heads being produced intercalated to the mycelia where conidia are produced. Photos were taken directly from PDA plates. H–L. Photos showing microscopic structures: (H, I) conidia and phialides, (J, K) conidia and (L) chlamydospore. Scale bars: G = 50 µm; H–L = 10 µm.
Typus: USA, Florida, Tropical Research and Education Center Pine Rocklands, Homestead, Miami-Dade County, isolated from the functional sapwood of *P. densa*, 24 Apr. 2021, J.M. Soares, strain JMS111 (holotype BPI 91123, culture ex-type NRRL 64453). GenBank accessions: ITS: ON490895; LSU: ON495712; *tef*-1 ON512590; *tub*: ON512653; *RPB1*: ON512645.

Additional cultures examined: USA, Florida, Miami-Dade County, Homestead, Tropical Research and Education Center Pine Rocklands, isolated from the functional sapwood of *P. densa* (south Florida slash pine; Syn: *Pinus elliottii var. densa*), 24 Apr. 2021, J.M. Soares, strain JMS196 culture accession NRRL 64454 and strain JMS204 culture accession NRRL 64455.

**DISCUSSION**

In this study, we investigated the incidence of *Tolypocladium* within the functional sapwood of pine trees distributed in the Pine Rocklands ecosystem. Our findings expand our understanding of the distribution, diversity, and ecology of this genus. Multilocus phylogenetic analysis revealed two novel species, here introduced as *T. subtropicale* and *T. trecense*. Both species and six other isolates resolved as two independent undescribed lineages (*Tolypocladium* sp. 1 & 2) clustered within the "endophytic clade", first introduced by Gazis *et al.* (2014). The discovery of *Tolypocladium* as part of the endophytic community of pines expands the host association and geographic distribution of the genus and reinforces the belief that functional sapwood endophytes are a frontier for fungal diversity discovery.
**Tolypocladium** subtropicale was resolved as sister to *Tolypocladium* sp. 1 and even though these two lineages have overlapping morphological characters, molecular data supports their separation. *Tolypocladium* sp. 2 was resolved as sister to *T. amazonense* and can be distinguished from the later by its ellipsoidal conidia and larger phialides. Together, these two lineages, were resolved as sister to the newly described *Tolypocladium trecense* which can be distinguished by its conidiophore type (unbranched vs. trichodermatoid) and the shape of its phialides (cylindrical vs. lageniform). Because all isolates from *Tolypocladium* sp. 1 and from *Tolypocladium* sp. 2 were recovered from single trees, and therefore could be clonal, we decided to leave these clades as unnamed independent putative species. Future sampling and recovery of additional conspecific strains may warrant their description as a novel species.

In general, *Tolypocladium* was recovered in low incidence and abundance. Isolates were only recovered from trees at the TREC (5/10 trees) and PCA (5/5 trees) sites. The low incidence and abundance of *Tolypocladium* is likely a reflection of the culture dependent isolation technique used in this study which favors fast growing endophytes (*i.e.*, Pestalotiopsis, Phomopsis, Trichoderma). Multiple studies have shown that culture and molecular (*i.e.*, high throughput sequencing-HTS) based approaches are needed to achieve a realistic characterization of the endophytic community. For instance, HTS analysis of tall grass *Brachypodium rupestre* (shoots, rhizomes, and roots) identified abundant and rare species of endophytes which were 5.8 times more enriched in number of taxa when compared to the traditional culture-based method. However, metabarcoding approaches were unable to detect the most abundant endophyte, *Ommiendematus graminis*, recovered through culturing (Durán *et al.* 2021). In addition, the number of OTUs recovered through HTS approaches may vary depending on the primer employed in the assays. GilMartin *et al.* (2022) used HTS to examine the functional sapwood of beech trees and detected more OTUs when using LSU than when using ITS and culture-based approaches; however, unique OTUs were detected in each approach emphasizing the importance in the employment of more than one method to characterize endophyte communities. The need for multiple sampling approaches is especially important when working with long-lived hosts (*i.e.*, hardwoods) in tropical and subtropical areas (Skaltsas *et al.* 2019, Fonseca *et al.* 2022).

*Tolypocladium* has been reported in high incidence from wild and cultivated Hevea (rubber trees), through culture-dependent (Gazis & Chaverri 2015) and a combination of culture and culture-independent approaches (Skaltsas *et al.* 2019). In fact, in Skaltsas *et al.* (2019), *Tolypocladium* was classified as a core endophyte, being present in more than 50 % of the individual trees of each species sampled (*H. guianensis*, *H. nitida*, *H. pauciflora*, and *Micrandra spruceana*). Furthermore, *Tolypocladium* was the most abundant genus recovered from adult trees through culture-dependent methods (123 isolates out of 2,061 recovered from 125 trees) and the fourth most abundant genus recovered through culture-independent approaches (14 % of sequence reads, 9 % incidence frequency from a total of 1,086,242 reads from 91 trees).

*Tolypocladium* has been isolated as endophyte in other hosts such as *Theobroma cacao*, *Scapania verrucosa*, and *Nothofagus dombeyi* (Hanada *et al.* 2010, Zeng *et al.* 2011, Molina *et al.* 2020). Although the number of endophyte studies focusing on this genus is low, a search in the Global Fungi Database (short sequence reads database, https://globalfungi.com) revealed that surveys based on high throughput sequencing have recovered this genus as an endophyte of roots and above-ground tissues. Currently, a total of 53 species hypothesis (SH) are identified as *Tolypocladium*, associated with multiple functional guilds (GlobalFungi, October 2022). *Tolypocladium* SHs data originated from studies targeting fungi from forest soil systems in different geographic regions across several continents such as North and South America, Asia, and Australia (Větrovský *et al.* 2020). This information suggests that species of the *Tolypocladium* endophytic clade are widely distributed.

*Tolypocladium* is a diverse genus with species ranging from tree endophytes, mycoparasites, and entomopathogens. Formulating a robust hypothesis to explain the evolutionary history of host association within the genus is hindered by the incompleteness of available molecular data. Among the 49 described *Tolypocladium* species, only 35 have sequence data available, including eight species with one marker sequenced (either ITS or LSU) and 27 species with more than one ribosomal marker or protein coding gene (RPB1, tub and tef-1) or a combination. The remaining fourteen species have only morphological data available. While the number of available *Tolypocladium* genomes has increased in the last few years, the number of genomes available is only fourteen (Tehan *et al.* 2022).

To fill some of the aforementioned knowledge gaps in regard to the ecology of *Tolypocladium* strains with an endophytic habit, strains collected in this study will be used in future bioassays to address the following pending questions: (i) are the *Tolypocladium* isolates recovered strict endophytes (commensal nutritional mode) or are they capable of parasitizing other species of fungi, nematodes, and/or insects and (ii) are the *Tolypocladium* isolates recovered capable of producing industrially promising metabolites.

**ACKNOWLEDGMENTS**

We thank Dr. Bruce Schaffer, Pamela Moon, Raul Moas, and Terry Glancy for allowing us to sample trees in their private Pine Rocklands forests. We thank Miami-Dade County (Parks, recreation, and Open Spaces division) for issuing research permits. Funding was provided by IFAS 2020 Early Career Seed Grant and Hatch project 005765, both given to RG.

**Conflict of interest:** The authors declare that there is no conflict of interest.

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**Supplementary Material**: http://fuse-journal.org/

Fig. S1. Alignment used to construct the concatenated phylogenetic tree.

Fig. S2. Alignment used to construct the ITS phylogenetic tree.

Fig. S3. Phylogram of *Tolypocladium* species collected in this study and previously described generated from Maximum likelihood analysis of ITS sequence alignment. Bootstrap support (BS) values > 75 % are indicated.

Table S1. Strains used to construct the multilocus phylogeny. Information on isolation source and GenBank accession numbers.
**Hydnum atlanticum**, a new species from Eastern North America

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**Key words:** Hydnaceae, new taxon, systematics, taxonomy

**Abstract:** A new species of *Hydnum* subgenus *Rufescentia* is described based on collections made in Canada (New Brunswick, Newfoundland and Labrador) and the USA (New York). The new species is found in conifer dominated forests (e.g. *Abies, Picea*) and occurs in bryophyte-covered (*Sphagnum, Bazzania*) soil. It differs from the ecologically similar *H. quebecense* in the duller brown colors of the basidiomes, the smaller basidiospores and the basidia predominantly with three or four sterigmata. Phylogenetic analysis of the ITS region place *H. subconnatum* and *H. oregonense* as the closest relatives of *H. atlanticum*, but these taxa differ in the larger basidiospores, number of sterigmata per basidium, caespitose growth and/or geographic distribution.


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**INTRODUCTION**

*Hydnum* is a widespread genus of ectomycorrhizal fungi commonly known as “hedgehog mushrooms” and collected as edibles in many parts of the world. Phylogenetic work in Europe, Asia and North America has revealed considerably higher levels of species diversity than initially estimated based on morphological characters. While European names (e.g. *H. repandum, H. rufescens*) have been widely applied to species in other geographic areas, phylogenetic data have shown that most species are endemic at the continental level (Greben et al. 2009, Olariaga et al. 2012, Vizzini et al. 2013, Feng et al. 2016, Niskanen et al. 2018, Swenie et al. 2018, Cao et al. 2021, Sugawara et al. 2022a, b).

The diversity of the genus in Eastern North America has been the focus of a recent monographic study (Swenie et al. 2018) that recognized and described 17 phylogenetic species occurring in the area. One of the species included in this monograph was not formally described, as only one collection was available at the time of publication, and was given the provisional code “Hydnum sp. AS30”. During a one-year intensive study of the diversity of *Hydnum* in the province of New Brunswick in Canada, 32 collections of this taxon were sequenced, and the species is formally described here as *Hydnum atlanticum sp. nov*.

**MATERIALS & METHODS**

**Collections and morphological analyses**

Collections of *Hydnum* studied here were made during weekly field work carried out in New Brunswick, between July and November 2021, with the exception of the NY and NL collections of *H. atlanticum*, that were studied by one of us (R. Swenie) from fungarium material. Specimens were photographed in the field and annotations were made before drying. Color codes used in the description are from Kornerup & Wanscher (1978). Basidiospores and other microscopic features were observed and measured from dried tissue mounted in water or a mixture of KOH and Congo Red. The notation [412/33] indicates the total number of basidiospores measured, and the number of collections studied.

**DNA extraction, amplification and sequencing**

For DNA extraction small fragments of dried basidiomes were used. Molecular work was carried out at ALVALAB (http://www.alvalab.es/) using standard methods for the study of basidiomycete fungi (e.g. Justo & Hibbett 2011). The following primer combinations were used for amplification and sequencing: ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) for ITS; HEF1F and HEF1R (Feng et al. 2016) for part of the translation elongation factor 1-alpha (TEF1-α); and RPB2-6F2 and RPB2-7R2 (Matheny et al. 2007, Tanaka et al. 2020) for the RNA polymerase II second largest subunit (RPB2). Raw data were edited and assembled in ChromasPro v. 2.1.10 (Technelysium).

**Phylogenetic analyses**

The ITS dataset included 132 new sequences generated from New Brunswick collections and reference sequences for other species of *Hydnum*, mostly from the studies of Swenie et al.
(2018) and Niskanen et al. (2018), with a particular focus on subgenus *Rufescencia*. The dataset included 180 *Hydnum* sequences and two *Sistotrema* sequences as outgroups.

Sequences were aligned using MAFFT v. 7 under the FFT-NS-i strategy (Katoh et al. 2019). The alignment was inspected and manually corrected in AllView v. 1.28 (Larsson 2014). Two phylogenetic analyses were run: (i) maximum likelihood (ML) analyses using RAXML v. 8.2.10 (Stamatakis 2014) under a GTR+GAMMA substitution model with 1 000 bootstrap (BS) replicates; (ii) Bayesian inference (BI) analyses using MrBayes v. 3.2.7 (Ronquist et al. 2012) for 10 M generations under a GTR+GAMMA model with four chains, and trees sampled every 1 000 generations. The burn-in phase was set to 2.5 M generations, and this value was confirmed to be adequate by checking the graphic representation of the likelihood scores of the sampled trees, the standard deviation of split frequencies (values < 0.01), and the potential scale reduction factor (values close to 1). All analyses were run using resources at the CIPRES Science Gateway (Miller et al. 2010). All phylogenetic trees were initially visualized using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). Species-level clades represented by multiple sequences were collapsed to facilitate graphical representation. Trees were exported from FigTree as SVG files and edited in Adobe Illustrator v. 27.3.1 for final presentation.

**RESULTS**

**Molecular phylogeny**

The topologies of the best tree from the ML analysis and the consensus tree for the BI analysis are essentially identical, and there is no strongly supported conflict between both, so only the ML tree is presented here (Fig. 1) with support values from both analyses. The ITS phylogeny recovers *Hydnum atlanticum* as a well-supported clade in subgenus *Rufescencia*, subsection *Tenuiformia* (Fig. 1). The grouping of *H. subconnotatum*, *H. oregoneense* and *H. atlanticum* is also well-supported.

In our phylogeny the subgenera recognized in Niskanen et al. (2018) and Sweenie et al. (2018) are also recovered (*Hydnum*, *Alba*, *Palida* and *Rufescencia*). The subsections *Tenuiformia*, *Multicoloria* and *Rufescencia* are not recovered as part of a single group, viz. section *Rufescencia* in the phylogeny of Niskanen et al. (2018). In the analyses performed here these three groups are well-supported, but they appear intermixed with other lineages in subgenus *Rufescencia*: /Ovoidespora, sect. Magnorufescencia, and taxa of uncertain placement within the subgenus.

**Hydnum atlanticum** Justo, A. Hood & Sweenie, sp. nov. MycoBank MB 847209. Figs 2, 3.

**Etymology:** atlanticum, for its distribution in Atlantic North America.

**Typus:** Canada, New Brunswick, Saint John County, Little Salmon River Protected Natural Area, Dustin Brook access trail to Fundy Footpath (45.500016, -65.277409), on bryophyte-covered soil in conifer dominated forest (*Picea, Abies*) with some scattered *Betula*, 1 Sep. 2021, A. Justo, AJ1597 (holotype NBM-F-009401).

Pileus 20–77 mm wide, convex to plano-convex, becoming depressed at center in mature specimens, often distinctly umbilicate; surface smooth, or minutely squamulose-fibrillose, hygrophanous, often distinctly zonate; predominantly brown, with rather muted dull tones (6C8, 6C7, 6C6, 6C5, 6D8, 5C8, 5C7), rarely with more vivid bright brown yellow or brown orange tones (68B, 68B, 58B); margin incurved and entire when young, becoming wavy to eroded in mature specimens, sometimes slowly staining orange after touch or damage. Spines crowded, acute, sometimes becoming flattened, adnate to slightly decurrent, pale cream to pale brown, up to 7–15 mm long in mature specimens. Stipe 24–67 mm × 4–15–(20) mm, cylindrical or slightly widening towards the base, central, sometimes fused in groups of two or three specimens; surface smooth, white, with orange staining when bruised or in damaged places; often with spines at the apex. Flesh white, very slowly becoming cream to pale orange after cut. *Smell* pleasant but not very distinct. *Taste* pleasant, sometimes with a nutty aftertaste, not bitter. *Basidiospores* [412/33] (6.0–)6.5–8.0(–8.6) × 5.4–7.6(–8.0) μm, Q = 1.00–1.17 (1.41), Qav = 1.08–1.13; aw × avw = 7.7–7.7 × 6.9–6.9 μm, mostly globose to subglobose, some broadly ellipsoid, very rarely ellipsoid, smooth, hyaline in KOH. *Basidia* 37–55 × 7.6–10.5 μm, narrowly clavate or suburniform, with (2–)3–4 stigmata. *Pileipellis* an interwoven cutis, hyphae smooth, cylindrical, thin-walled, hyaline, mostly 5–8 μm wide. *Clamp-connections* common in all tissues.

**Distribution:** Canada (New Brunswick, Newfoundland and Labrador) and USA (New York).

**Ecology:** Fruiting gregariously, in patches of 3–10 basidiomes. In conifer dominated forests. All New Brunswick collections were made under *Abies balsamea*, *Picea glauca* and/or *Picea* spp., sometimes with *Betula* and *Pinus banksiana* in the mix; in humid, bryophyte-covered areas, often including *Sphagnum* spp. but also *Bazzania trilobata*. The NY collection was made under *Tsuga* and *Larix* in a bog area in *Sphagnum*-covered soil. The Labrador collection was made under *Alnus*, *Pinus*, and *Picea* and was recorded as “on soil” without specific mention of the presence of moss. July–October.

**Additional collections examined:** Canada, New Brunswick, Queens County, Grand Lake Protected Natural Area, off Pondstream Road (45.9448, -66.11180), in somewhat dense, young stand of *Abies balsamea*, 17 Aug. 2021, A.W. Hood, AH90 (NBM-F-009413); ibid., (45.94503, -66.11151), AH91 (NBM-F-009414); ibid., (45.94514, -66.11031), under *Abies balsamea*, A. Justo, AJ1534, (NBM-F-009418); ibid., (45.94514, -66.11031), under *A. balsamea*, AJ1535 (NBM-F-009419); New Brunswick, Saint John County, City of Saint John, Black Beach trail (45.15611, -66.22657), under *A. balsamea* and *Picea glauca*, 21 Oct. 2021, A.W. Hood, AH183 (NBM-F-009410); ibid., (45.16483, -66.22390), AH198 (NBM-F-009411); ibid., (45.16245, -66.22726), AH199 (NBM-F-009412); New Brunswick, Saint John County, Fundy Footpath, Rapidly Brook access trail (45.48583, -66.261667), under *Picea* and *A. balsamea*, 25 Aug. 2021, A. Justo, AJ1566 (NBM-F-009422); ibid., (45.48461, -65.26389), under *P. glauca*, A.W. Hood, AH97 (NBM-F-009415); New Brunswick, Saint John County, Fundy Trail Parkway, McLeod Brook Falls Loop trail (45.49555, -65.30972), under *Abies*, *Picea* with some *Betula*, 22 Aug. 2021, A. Justo, AJ1557 (NBM-F-009420); ibid., (45.495556, -65.309722), 1 Sep. 2021, A. Justo, AJ1624 (NBM-F-009428); ibid., (45.49557, -65.30446), under *P. glauca* near stream, 1 Sep. 2021, A.W. Hood, AH124 (NBM-F-009407); New Brunswick, Saint John County, Fundy Trail Parkway, Road to Walton Glen Reception Center (45.490564, -65.311361), under *Abies* and *Picea*, 25 Sep. 2021, A. Justo, AJ1732 (NBM-F-009429); New Brunswick, Saint John County, Fundy
Fig. 1. Best tree from the ML analysis of the ITS dataset. Bootstrap support values from the ML analysis (BS ≥ 70 %) and Posterior Probabilities form the BI analysis (PP ≥ 0.90) are given on or below the branches. The first part of the tree is a detailed view of the top subsection from the second part of the tree.
Fig. 1. (Continued).
Hydnum atlanticum sp. nov.

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Trail Parkway, Walton Glen Gorge trail (45.489694, -65.307883), upland forest on sandy soil, mostly Picea and Pinus banksiana, with scattered Abies and Betula, 13 Oct. 2021, A. Justo, AJ1826 (NBM-F-009430); ibid., AJ1828 (NBM-F-009431); ibid., (45.49213, -65.30287), under A. balsamea and P. glauca, A.W. Hood, AH173 (NBM-F-009408); ibid., (45.49210, -65.30271), under Abies balsamea and P. glauca, A.W. Hood, AH174 (NBM-F-009409); New Brunswick, Saint John County, Little Salmon River Protected Natural Area, Dustin Brook access trail to...
Fundy Footpath (45.500016, -65.277409), in conifer dominated forest (Picea, Abies), with some scattered Betula, on moss covered soil, 1 Sep. 2021, A. Justo, AJ1596 (NBM-F-009423); ibid., AJ1602 (NBM-F-009424); ibid., AJ1603 (NBM-F-009443); ibid., AJ1609 (NBM-F-009425); AJ1614 (NBM-F-009426); ibid., AJ1615 (NBM-F-009427); ibid., (45.49910, -65.2774), under A. balsamea, Betula papyrifera and P. glauca, in very damp mossy area, A.W. Hood, AH111 (NBM-F-009402); ibid., (45.49494, -65.27791), under P. glauca and B. papyrifera, A.W. Hood, AH117 (NBM-F-009405); ibid., (45.49799, -65.27793), under P. glauca, with ferns along trail, A. W. Hood, AH116 (NBM-F-009404); ibid., (45.49489, -65.27769), under P. glauca, B. papyrifera and A. balsamea, A.W. Hood, AH118 (NBM-F-009406); ibid., (45.49923, -65.27658), under P. glauca, A.W. Hood, AH115 (NBM-F-009403); New Brunswick, Sunbury County, Grand Lake Protected Natural area, Coy Road (45.97962, -66.18163), under A. balsamea, 17 Aug. 2021, A. Justo, AJ1527 (NBM-F-009416); ibid., AJ1528 (NBM-F-009417); Newfoundland and Labrador, Labrador, Division No. 10, Goose Bay, Tranquility Trail (53.533461, -60.1395), on soil under Alnus, Pinus, Picea, 10 Sep. 2016, E. Michelin, GBHV16A-344 (TENN-F-078322). USA, New York, Hamilton County, Raquette Lake, Silver Beach Bog, Sphagnum substrate under Tsuga, Larix, 550 m a.s.l., 28 Jul. 1982, A. Sabol, AS30 (CORT 007356).

**DISCUSSION**

*Hydnum atlanticum* was initially recorded in Svenie et al. (2018) as "Hydnum sp. AS30", but it was not formally described as only one collection was available at the time. In the present paper we study 33 recent Canadian collections of this taxon, 32 from New Brunswick and one from Newfoundland and Labrador, and formally describe the species. The New Brunswick collections were part of an intensive 1-year study of *Hydnum* in the province, where a total of 162 collections of *Hydnum* were made, and subsequently 132 collections were selected for sequencing. All newly generated ITS sequences are included in the phylogeny presented here (Fig. 1), including three species newly recorded in Canada as part of this study: *Hydnum alboaurantiacum*, *H. cuspidatum* and *H. vagabundum*.

The external morphology of *H. atlanticum* is very similar to other species in subgenus *Rufescentia*, with small to medium basidiomes and brown to orange brown or yellow brown colors. Colors of the basidiomes tend to be a bit more dull brown than other species of this subgenus, but slightly more brightly colored basidiomes can occur. *Hydnum atlanticum* has only been collected so far in humid, moss-covered areas. It shares this character with *H. quebecense*, which occurs in the same habitats, often at the same time as *H. atlanticum*. In the field *H. quebecense* has brighter, more intense orange brown or reddish brown colors. The difference in color of the basidiomes was clear enough in the field that specimens of *H.atlanticum* and *H. quebecense* were recognized as different species whenever they were collected together in the same area. Microscopically, basidiospore size and number of sterigmata per basidium separate the species well: *H. atlanticum* has smaller basidiospores (av. 7.2–7.7 × 6.5–6.9 μm), and predominantly three or four sterigmata per basidium, while *H. quebecense*...
has larger basidiospores (av. 8.4–8.9 × 7.8–8.1 μm; cf. Niskanen et al. 2018 and Swenie et al. 2018), and predominantly two sterigmata per basidium. Color differences might not be clear or consistent enough to separate *H. atlanticum* from other species in subgenus *Rufescenta*. In these cases, habitat can offer important clues for identification, with *H. atlanticum* fruiting in humid, bryophyte-covered soil in conifer-dominated forests. As with other taxa in subgenus *Rufescenta*, sequence data might be needed for confident identification.

Based on the ITS phylogeny presented here (Fig. 1), *H. atlanticum* belongs in subgenus *Rufescenta*, section *Rufescenta* characterized by small to medium basidiomes with deeply colored (brown, orange) pilei and globose to ellipsoid basidiospores (Niskanen et al. 2018). Phylogenetically, *H. atlanticum* is recovered as part of subsection *Tenuiformia* together with *H. umbilicatum*, *H. ellipsosporum*, *H. cuspidatum*, *H. quebecens*, *H. subconnatum* and *H. oregonense* (Fig. 1). Niskanen et al. (2018) characterized subsection *Tenuiformia* morphologically by the combination of small to medium basidiomes (pileus 20–45 mm), non-subcurrent spines and rather large basidiospores that are ovoid-subglobose (av. > 8.5 × 7.5 μm) or ellipsoid (av. 10.0 × 6.6 μm). *Hydnum atlanticum* deviates from this morphological characterization by the larger basidiomes (pileus up to 77 mm), often subcurrent spines, and smaller basidiospores (av. 7.2–7.7 × 6.5–6.9 μm). Subsection *Tenuiformia* does receive good phylogenetic support in the ITS analysis.

According to the ITS phylogeny the closest relatives of *H. atlanticum* are *H. oregonense* and *H. subconnatum* (Fig. 1). *Hydnum oregonense* differs by the slightly smaller basidiomes (up to 45 mm), more yellow-orange tones in the pileus, bigger basidiospores (av. 9.5 × 9.0 μm), and predominately 2-spored basidia (Niskanen et al. 2018). This species occurs only in Western North America associated with conifers (*Pinus*, *Tsuga*, *Pseudaugasus*). *Hydnum subconnatum* differs from *H. atlanticum* by the often caespitose basidiomes, the bigger basidiospores (av. 8.9 × 8.5 μm), and the habitat in hardwood dominated forests of the Southeastern and Midatlantic USA (North Carolina, Tennessee, Georgia, Pennsylvania). We did not collect *H. subconnatum* as part of our 1-year survey of the New Brunswick species, but during the preparation of this article one collection of this species has been confirmed in New Brunswick (https://www.inaturalist.org/observations/14031527). The NB collection, a first record for Canada, was collected in a section of an urban park dominated by old *Tsuga canadensis* trees, with some *Fagus* in the mix.

In addition to ITS sequences, we selected 4 collections of *H. atlanticum*, including the holotype, to generate partial TEF1-α and rpb2 sequences (GenBank OQ236551–OQ236558). We did not perform a phylogenetic analysis based on these loci, as the sampling across *Hydnum* is much more limited than for ITS. We did BLAST our newly generated sequences to search for possible matches in GenBank, but no match was found for either loci. The closest match for the TEF1-α sequences of *Hydnum atlanticum* is *H. ellipsosporum* (GenBank KU612773; 97.4 % sequence similarity, 13 individual nucleotide differences). The closest match for the rpb2 sequences of *Hydnum atlanticum* is *H. umbilicatum* (GenBank LC717869; 98.4 % sequence similarity, nine individual nucleotide differences).

**ACKNOWLEDGEMENTS**

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**Conflict of interest:** The authors declare that there is no conflict of interest.

**REFERENCES**


Additions to the knowledge of the genus *Helvella* in Europe. New records and *de novo* description of five species from the Nordic region

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**Key words:**
cryptic species
geographic distribution
molecular phylogeny
new taxa

**Abstract:** *Helvella* is a species-rich genus, forming a large variation of astounding ascocarps in many different habitats. During the last decade, molecular markers and morphological characters have been combined to delimit and identify cryptic species in this genus. We report on a list of 54 species of *Helvella* s.s. in the Nordic region and describe five new species, *i.e.* *H. bresadolae*, *H. convexa*, *H. japonica*, *H. nordlandica* and *H. oroarctica*. The morphological and molecular characteristics of the new species and the emended / hypocreatoriformis, / fibrosa-macropus, and / fallax-pezioides lineages of *Helvella* s.s. are shortly commented upon. Further we include a discussion of the distribution of species in the Nordic region based on a large set of studied collections. The ecological versatility and variable geographic patterns of these species indicate that cryptic species may have contrasting ecology in their local habitats.

**INTRODUCTION**

*Helvella* is a speciose genus of the order *Pezizales* of the *Pezizomycetes* (*Ascomycota*) that comprises a polymorphic group of larger apothecial fungi. Species of the genus range in shape from subsessile to stipitate, with a cupulate or lobed capitate apothecium, seated on a cylindrical, ribbed and/or furrowed stipe.

In a series of biosystematic studies of the genus *Helvella* in Europe, we used multiple gene genealogies, single gene sequences and morphological characters to clarify species limits and assess species-level relationships in this group of ascomycetous fungi (Skrede et al. 2017, 2020, Hansen et al. 2019, Løken et al. 2020). Species names and distribution maps of *Helvella* spp. in literature and on the internet reflects an underestimated of the true diversity of recognisable *Helvella* species in Europe.

The ignorance of species diversity also distorts the impression of how widespread these species are and which ecological needs they may have. Species that seem widespread may instead be species complexes of many species with very specific niches.

The current study is a follow-up of the biodiversity and biogeography of this species-rich group of fungi in the Nordic region (Skrede et al. 2017, Hansen et al. 2019, Løken et al. 2020), with emphasis on collections from Denmark, Norway and Sweden. We provide a list of species including information about the countries where fresh and dried specimens of the pertinent species have been barcoded and determined, followed by descriptions and phylogenetic placement of five newly described species in Norway and Sweden. We also include a discussion about the distribution patterns and possible ecological role of *Helvella* spp.

**MATERIALS AND METHODS**

We have investigated most collections of *Helvella* in the fungaria C, S, UPS, TRH, TROM and O. All collections we have investigated from the Nordic region and obtained a sequence from are summarised in Supplementary Table S1. As we have worked in the fungaria in Norway, Sweden and Denmark, collections from these countries are overrepresented in our data.

In order to understand the biogeographical pattern of a well-resolved species complex we made detailed maps of all collections of the *Helvella corium* species aggregate, including the species *H. corium*, *H. alpina*, *H. pseudoalpina*, *H. nannfeldtii*, *H. alpestris*, *H. macrospora* and *H. alpicola* (Løken et al. 2020). For this group a high number of collections were available, increasing the probability that the species are collected from most of their distribution areas. Distribution maps were produced using ArcMap v. 10.8.1 (http://www.esri.com). The elevation raster was downloaded via NOAA (NOAA National Geophysical Data Center 2009; Amante & Eakins 2009) and the Arctic Circle via Natural Earth.

A subset of all collections was carefully examined morphologically, using the methodology below. Microscopic examinations of fresh specimens, when available, and from squash mounts of fresh and rehydrated (dried) specimens...
mounted in water and methylene blue in lactic acid (Cotton blue) were undertaken. In addition, a selection of fresh apothecia was fixed in formalin-acetone-alcohol (5 mL formalin, 5 mL glacial acetic acid, 90 mL 70 % ethanol-alcohol pr 100 mL solution), then dehydrated in a graded butyl alcohol series, embedded in paraffin, sectioned at 8–10 μm thickness, and stained in safranin-fast-green, following the protocol of Johansen (1940). Photomicrographs were taken with a Canon 35 mm camera mounted on a Zeiss WL microscope. Microanatomical terms follow Starbäck (1895) and Korf (1973).

For most collections we have produced at least one DNA sequence as a barcode. For new species and closely related species included in the phylogenetic analyses we have amplified and sequenced several genetic markers. For all samples we have followed the same protocols. DNA from individual specimens was extracted using the E.Z.N.A.*HP Fungal DNA Kit (Omega Biotek D3195), following the slightly modified procedure from Skrede et al. (2017). The three genetic markers, heat shock protein 90 (hsp), RNA polymerase II second largest subunit (rpb2) and nuclear ribosomal large subunit DNA (LSU) were amplified using PuReTaq Ready-To-Go PCR Beads (GEhealthcare, Waukesha, WI), and purified with 10 % ExoSAP-IT (GEhealthcare, Waukesha, WI). For primer sequences, detailed PCR conditions, and sequencing techniques, see the procedures in Skrede et al. (2017). In addition to sequences produced in the present study, sequences from a representative selection of Helvella species were downloaded from GenBank and included for reference. The sequences were automatically aligned using the MUSCLE v. 3.8.425 (Edgar 2004) plugin in Geneious Prime v. 2019.2.3 (Biomatters, Auckland), followed by manual inspection. Alignments of each marker are available in the Dryad data repository (https://doi.org/10.5061/dryad.h44j0zpqm).

The collection information and Genebank accession numbers of specimens included in the phylogeny are summarised in Table 1. Alignments of each marker were analysed individually by the Maximum Likelihood (ML) method implemented in RAxML v. 8.2.11 (Stamatakis 2006), using the GTRCAT approximation. A concatenated alignment was made, allowing for the inclusion of some missing data, we allowed for the inclusion of some missing data by permitting individuals lacking one or two of the markers (one individual lack hsp, five lack rpb2 and 20 lack LSU). Bootstrap analyses using 1 000 pseudo-replications were included in all ML analyses. The concatenated alignment is available in Dryad data repository (https://doi.org/10.5061/dryad.h44j0zpqm). The ML analyses were the basis for species delimitation, using a simplified genealogical concordance phylogenetic species recognition (Avise & Ball 1990, Taylor et al. 2000, Dettman et al. 2003).

RESULTS

Distribution of species in Norden

Specimens collected in the Nordic region are summarised in Table 2. Detailed information about origin, collection data, collector and available DNA sequences of each collection are found in Supplementary Table 5.1. Some species are found in many countries in the Nordic region, e.g. Helvella acetabulum, H. corium and H. fallax are found in Denmark, Norway, Sweden, Svalbard and Greenland, and others have a more restricted distribution, e.g. H. semiobrata, H. retinervis and H. ephippoides found only in Sweden, H. queletiana and H. platypodia only in Denmark and H. macroperma only in Norway. This indicates that there is a large diversity in biogeographical distribution patterns in Helvella.

For the more detailed distribution of the H. corium species aggregate, it can be observed that H. corium has a wide distribution in both the lowland and high mountain areas (Fig. 1). This contrasts the other species of this aggregate, with alpine/arctic distributions. However, also among these, there are large variations, e.g. H. nanfeldtii is found widespread in alpine and arctic areas, while H. alpicola is only found in a restricted calcareous mountain area close to the arctic circle in Norway (but also recorded from a single mountain locality in Graubünden, Switzerland, see Skrede et al. 2017).

Phylogenetic placement of five new species from Norden

For the phylogenetic analyses, 45 individuals of the new species and their close relatives were included. A total of 19 hsp, 17 rpb2 and six LSU sequences were newly produced for this study and were submitted to GenBank. All GenBank accession codes of included sequences can be found in Table 1. A concatenated 3-gene alignment of 1 361 bp, including 347 bp of rpb2, 294 bp of hsp, and 720 bp of LSU, submitted to ML analysis identified three major evolutionary lineages and a number of clades and subclades, all with a bootstrap support above 90 % (Fig. 2). Five independent clades/ subclades were discerned as new species to science. Two of these, i.e. the stipitate-cupulate H. oroarctica and H. nordlandica, are nested in the / hypocrateriformis lineage, where H. oroarctica is a sister species to H. scyphoides, H. platypodia and H. nordlandica, a sister to H. scyphoides and H. platypodia. All species of this lineage have stipitate-cupulate apothecia. Two new species with a convex (capitate) apothecium, i.e. H. convexa and H. japonica, are circumscribed and phylogenetically assembled to the / fibrosa-macropus lineage, which now consists of five species. This lineage exhibits a broad spectrum of apothecial shapes, from regularly stipitae-cupulate to bi- to tri-lobed stipitate-capitate. Helvella convexa, H. macropus and H. ephippoides constitute one clade, and H. fibrosa and H. japonica a sister clade. The fifth new species, i.e. H. bresadolae, is nested in the / fallax-pezizoides lineage, in which four stipitate-capitate European species now are accommodated in two clades: one clade with H. pulla and H. fallax, and the other with H. pezizoides and H. bresadolae (cf. Fig. 2).

Taxonomy

Hypocrateriformis lineage sensu Skrede et al. (2017):

Helvella nordlandica Skrede & T. Schumach., sp. nov. – MycoBank MB 848109. Fig. 3A, B.

Etymology: Pertaining to its discovery and restricted distribution in Nordland County of Norway.

### Table 1. Locality, identification, type and sequence information for Helvella and Dissingia specimens included in the phylogeny in Figure 2. The columns LSU, hsp, rpb2 and ITS are GenBank accession numbers. Accession numbers in italics are new accessions from this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>ID</th>
<th>LSU</th>
<th>hsp</th>
<th>rpb2</th>
<th>ITS</th>
<th>Locality</th>
<th>Coll. date</th>
<th>Collector – Collector ID</th>
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<td>KY784242</td>
<td>KY772489</td>
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<td>Switzerland, Graubünden, Las Palüs</td>
<td>29.8.1984</td>
<td>H. Dissing</td>
</tr>
<tr>
<td><em>H. bresadólae</em></td>
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<td>Q6633417</td>
<td>—</td>
<td>—</td>
<td>France, Nice</td>
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<td><em>H. macropus</em></td>
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### Table 2. List of molecularly identified Helvella specimens from the Nordic countries. Total number of sequenced specimens are shown in parenthesis; number of new, fresh specimens from field work in 2015–2018 is given as (-fx).

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<tr>
<th>Species</th>
<th>Author</th>
<th>Geographic origin of collections in Norden</th>
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<td>(Malencon) Van Vooren &amp; Frund</td>
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<td>(Scop.) Fr.</td>
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<td>S.B. Løken &amp; Skrede &amp; T. Schumach.</td>
<td>Norway (7-f4), Greenland (3), Svalbard (1)</td>
</tr>
</tbody>
</table>
Table 2. (Continued).

<table>
<thead>
<tr>
<th>Species</th>
<th>Author</th>
<th>Geographic origin of collections in Norden</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helvella pubescens</em></td>
<td>Skrede, T. Carlsen &amp; T. Schumach.</td>
<td>Norway (1), Denmark (6), Sweden (1)</td>
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<td><em>Helvella pulla</em></td>
<td>Holmsk.</td>
<td>Norway (3), Denmark (19)</td>
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<tr>
<td><em>Helvella queletiana</em></td>
<td>Sacc. &amp; Trav.</td>
<td>Denmark (3)</td>
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<td>Skrede &amp; T. Schumach.</td>
<td>Sweden (2)</td>
</tr>
<tr>
<td><em>Helvella rivularis</em></td>
<td>Dissing &amp; Sivertsen</td>
<td>Norway (47-f3), Sweden (4), Finland (3), Iceland (1)</td>
</tr>
<tr>
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<td>Skrede, T. Carlsen &amp; T. Schumach.</td>
<td>Norway (10)</td>
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<td><em>Helvella semiobruta</em></td>
<td>Donadini &amp; Berthet</td>
<td>Sweden (1)</td>
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<td>Norway (46-f4), Svalbard (2-f1), Denmark (3), Greenland (16), Sweden (11), Iceland (3)</td>
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<td>Afz.</td>
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<td><em>Balsamia aestivalis</em></td>
<td>(R. Heim &amp; L. Remy) K. Hansen, Skrede &amp; T. Schumach.</td>
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<tr>
<td><em>Dissingia confusa</em></td>
<td>K. Hansen &amp; X.H. Wang</td>
<td>Norway (25), Denmark (6), Sweden (16)</td>
</tr>
<tr>
<td><em>Dissingia leucomeleoaena</em></td>
<td>(Pers.) K. Hansen &amp; X.H. Wang</td>
<td>Norway (1), Denmark (4), Sweden (13)</td>
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<tr>
<td><em>Dissingia oblongispora</em></td>
<td>(Harmaja) T. Schumach. &amp; Skrede</td>
<td>Norway (3), Sweden (3)</td>
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Fig. 1. Map of the distribution of the *Helvella corium* species aggregate (species defined according to Løken et al. 2020) from Sweden, Norway, Denmark, Svalbard and Greenland based on the collections in the fungaria C, O, S, TRH, TROM and UPS.
Helvella spp. from Europe

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Fig. 2. Maximum likelihood tree made using the GTRCAT approximation in RAxML of new and representative species of Helvella. Dissingia confusa is used as outgroup. The tree is based on a partitioned alignment of the three molecular markers hsp, rpb2 and LSU.
Apothecium regularly stipitate-cupulate, 0.4–1.2 cm broad, stipe 1.5–2.6 cm tall, 0.1–0.3 cm wide; hymenium brown, drying dark chocolate brown; receptacle paler, greyish, densely pubescent, with conspicuous tufts of fascicled hyphae deeply staining in Cotton blue; stipe terete, solid, sub-pubescent, yellowish white along the whole length, thus contrasting the greyish receptacle and brownish hymenium above. Medullary excipulum of densely interwoven textura intricata, hyphae short-celled, 3–6 µm broad. Outer excipulum of textura angularis, single cells 10–20 µm diam, towards the outer surface drum-shaped to club-shaped, forming densely packed cell rows extending into tufts of fascicled hyphoid hairs, 70–150 µm long, by 6–12 µm broad. Asci pleurorhynchous, 245–270 × 13–15 µm, 8-spored. Ascospores ellipsoid, 16.2–16.8–17.8 × 10.5–11.2–12.2 µm, with one large internal oil drop (when mature).

Paraphyses straight, unbranched, cell walls brownish along the whole length, 3.0–4.2 µm broad below, gradually enlarged to 6–7 µm at the clavate tips.

Specimens examined/sequenced: See Table 1.

Notes: Helvella nordlandica belongs in the / hypocrateriformis lineage, as delineated by Skrede et al. (2017). We have selected a specimen in C (ex TRH), collected and labelled H. cupuliformis, as the holotype specimen of this new species (Fig. 3). Helvella nordlandica resembles H. scyphoides in size, shape and colours. However, the two species are separated based on larger asci and ascospores in the latter (cf. Skrede et al. 2017).

Helvella oroarctica S.B. Løken & T. Schumach., sp. nov. MycoBank MB 848112. Fig. 4A–C.

Etymology: Refers to its preference of mountainous areas of the Arctic (oroarctic).

**Fibrosa – macropus lineage sensu Skrede et al. (2017):**

*Helvella convexa* Skrede & T. Schumach., *sp. nov.* MycoBank MB 848113. Fig. 5A, B.

*Etymology:* Pertaining to its convex stipitate-capitate apothecia.


*Illustration:* Dissing (1966: fig. 32.b, as *H. pezizoides*).

*Apothecia* stipitate-capitate, involute, 1.0–2.0 cm broad, solitary, brownish black, with edge permanently deflexed; hymenium black, receptacle surface densely pubescent, brownish black stipe slender, terete, solid, pubescent, 3.8–6.4 cm tall, 0.3–0.7 cm wide. *Medullary excipulum* of a loosely interwoven *textura intricata*; hyphae multisepate, light brown-walled, 3.5–5 µm broad. *Outer excipulum of textura angularis – globosa,* cells 15–30 µm diam, brown-walled, towards surface cells in rows turning out perpendicularly to receptacle surface, outermost cells giving rise to brown-walled, multisepate, short-segmented hyphoid hairs in dense fascicles, hairs 60–180 µm long, individual hair cells 12–25 µm long, by 8–15 µm broad, cells constricted at septa. *Asci* pleurorhynchous, 260–295 × 10.2–13.0 µm, 8-spored. *Ascospores* ellipsoid, smooth, slightly pointed towards the poles, 17.0–19.3–20.8 × 8.4–9.0–9.6 µm, with one large internal oil drop. *Paraphyses* straight, brown-coloured along the whole length, 2.5–3.2 µm broad below, enlarged to 4–5 µm broad in upper third, clavately enlarged to 8–13 µm at the tips.

*Specimens examined/sequenced:* See Table 1.

*Notes:* *Helvella convexa* is sister species to *H. macropus* and *H. ephippioides* in our phylogeny (Fig. 2). The sequenced specimen from Gästrikland, Sweden, selected as the holotype specimen by us (Fig. 5), was photographed and identified as *H. pezizoides* by Dissing (1966: 117, fig. 32.b.). This specimen, with a long and slender stipe, is probably representative of Dissing’s misconception of *H. pezizoides* when stating “one more detail to illustrate *H. pezizoides* absolute precise: sometimes the stipe is longer and more slender than shown by Afzelius and Boudier” (Dissing 1966: 119). The long and slender stipe typically accords to *H. convexa* and may probably be helpful in the field in discriminating against the look-alike species *H. pezizoides*.
**Helvella japonica** Skrede, S.B. Løken & T. Schumach., sp. nov. MycoBank MB 848114. Fig. 6A, B.

**Etymology:** Pertaining to Japan; i.e. the place of its first discovery by the senior author in Nikko National Park, Honshu.


**Apothecia** stipitate-capitate, saddle-shaped, at first cupulate, then expanding to bi-to tri-lobate with a recurved margin, 1.2–2.8 cm broad, hymenium brownish grey, receptacle greyish, densely pubescent to villose; stipe 3.0–10.5 cm tall, 0.35–0.60 cm wide, terete, solid, villose, concolourous with receptacle. **Medullary excipulum** of dense textura intricata, hyphae 3–7 µm broad. **Outer excipulum** of textura angularis, cells unordered (not in parallel rows), individual cells to 20–30 µm long, by 10–20 µm broad, outermost cells smaller, building tufts of hyphoid hairs to 150 µm in length. **Asci** cylindrical, with a stout base, 200–240 × 14–17 µm, 8-spored. **Ascospores** broadly ellipsoid, 16.8–18.1–19.5 × 10.3–11.0–11.8 µm, with one large internal oil drop. **Paraphyses** straight, 3–4.5 µm broad below, gradually enlarged to 6.5–8 µm at the tips.

**Specimens examined/sequenced:** See Table 1.

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**Fig. 5.** Helvella convexa, coll. UPS-F-14567 – **holotype;** Sweden, Gästrikland, Valbo, Lindesnäs, 10 Aug. 1957, leg. J. A. Nannfeldt. A. Dried specimens. B. Envelope, originally labelled *H. atra*, later annotated *H. pezizoides.*

**Fig. 6.** Helvella japonica, coll. O-F-253389 – **holotype;** Japan, Honshu, Tochigi, Okunikko, Chuuenjikohan, 22 Aug. 1983, leg. T. Schumacher J33/83. A. Dried specimens. B. Vertical median section of apothecium stained in safranin – fast green.
Notes: *Helvella japonica* is sister species to *H. fibrosa* in our phylogeny (Fig. 2). The species was included as *Helvella* sp. “JAPAN 2” in Skrede *et al.* (2017). We have selected a specimen from Japan in the fungarium of Oslo as the holotype specimen (Fig. 6). Additional specimens of *H. japonica* have also turned up in recent sequenced material from Sweden and Norway. Gross morphology resembles *H. pezizoides* and *H. sublicia* of the / fallax-pezizoides and the / rivularis-sublicia lineages, respectively. The greyish brown stipitate-capitate, relatively tall and folded apothecia are characteristic features of *H. japonica*. It contrasts *H. pezizoides* which has smaller and darker (blackish) apothecia and also differ micro anatomically by larger asci, (260–300 × 12–15 µm) and smaller, narrower ascospores, (14.5–15.3–16.5 × 8.5–9.0–9.6 µm). A vertical median section of a typical apothecium of *H. japonica* and other “hairy” *Helvella* spp., is shown in Fig. 6B (coll. O-F-253389). The description of *H. pezizoides* ss. Weber (1972) reminds of the present species.

**Etymology**: Pertaining to the provisional name used by Rehm after having received the Austrian specimen from its collector Giacomo Bresadola.

**Typus**: Norway, Nordland, Saltdal, Junkerdalen, 10 Aug. 2016, S. Bua Løken & T. Schumacher (holotype TROM-F-610068)

**Apothecia** stipitate-capitate, saddle-shaped with recurved lobes when mature, hymenium dark brownish black, receptacle naked, pale to dark greyish black, cap 1–2.8 cm broad; stipe 1.5–3.5 cm tall, 0.25–0.40 cm wide, terete, solid, concolourous with receptacle or slightly paler towards base. *Medullary excipulum* of a dense textura intricata, hyphae 3–5 µm broad. *Outer excipulum* of textura angularis, cells intermixed (not in parallel rows), outermost cells club-shaped, individual cells to 20–30 µm long, by 10–15 µm broad. *Asci* subclavate to cylindrical, with a stout base, 200–240 × 14–17 µm, 8-spored. *Ascospores* ellipsoid, 16.8–17.5–18.5 × 10.8–11.3–12.2 µm, with one large internal oil drop and several small droplets towards poles, premature ascospores occasionally with external pustules. *Paraphyses* straight, 3–4.5 µm broad below, gradually enlarged to 6.5–8 µm at the clavate tips.

**Specimens examined/sequenced**: See Table 1.

Notes: A newly collected fresh specimen from Nordland, Norway, is selected as the holotype specimen (Fig. 7). This collection was made in a calcareous schistose rock area in Nordland County, Northern Norway. Two old and well-preserved specimens from the Austrian and French Alps ex herb. Rehm in the fungarium of Stockholm (S) have been molecularly linked to the newly collected material from Northern Norway (see Table 1). The small-sized asci and medium-sized ascospores discriminate *H. bresadolae* from morphologically lookalike species, *e.g.* *H. convexa*, *H. pezizoides*, *H. pulla* and *H. sublicia* (cf. Skrede *et al*. 2017, also providing a key to the three latter species). Judging from the original label of the Austrian specimen, Rehm seemingly acknowledged its status as a new species in a letter to the collector G. Bresadola, for which he provided the provisional name *H. bresadolae* (see collection label in Fig. 7C). Later he apparently changed his opinion and referred the specimen to *H. pezizoides* Afzelius (1783), a disposition later concurred by Dissing (1966) (cf. label in Fig. 7C).

**DISCUSSION**

Although easily distinguished from other macro-fungi by apothecial shapes, it is surprisingly difficult to morphologically distinguish species of *Helvella*. Large phenotypic plasticity as a response to ecological and biogeographic features in many species, and the lack of distinctive (species-specific) microscopic characters to discriminate among them are probably the reasons for these difficulties. This led previous mycologists to adopt a rather broad morphological species concept, which has turned out to be mostly incompatible with the present-day phylogenetic species concept that is to be preferred.

Early infrageneric subdivisions of *Helvella* based solely on gross morphology, hairiness of receptacular surface and stipe, and ascocarp ontogeny (Le Gal 1947, Dissing 1966, Weber 1972, Abbott & Currah 1997) have gained little support from recent studies that use genetic markers to infer monophyletic groups as basis for classification (Landeros *et al.* 2015, Skrede *et al*. 2017, 2020, Løken *et al*. 2020). This also accords to the three infrageneric lineages of *Helvella*, i.e. the / hypocrateriformis, the / fibrosa – macropus and the / fallax – pezizoides lineages under study (cf. Fig. 2).

In a first study by Skrede *et al*. (2017), species limits and naming of 55 European (phylogenetic) species were re-assessed and a number of morphospecies aggregates phylogenetically resolved, based on a sample of 432 specimens deposited in the fungaria of C and O. Five major clades (A–E) and 18 intrinsic evolutionary lineages were phylogenetically inferred from a *hsp* and *rpb2* and LSU dataset of 55 European and 27 extra-European species of *Helvella s.l.* In the present work, we add five new species that belong in the/ hypocrateriformis, / fibrosa – macropus and / fallax - pezizoides lineages of *Helvella s.l.* (Fig. 2; cf. Skrede *et al*. 2017, Hansen *et al*. 2019).

Two species, *i.e.* *H. nordiniaca* and *H. ororaartica*, are phylogenetically adhered to the / hypocrateriformis lineage (Skrede *et al*. 2017), which based on additions of one new species in Skrede *et al*. (2020), *i.e.* *H. platypodia*, and the two new species in the present work, now encompasses five species in Europe. Species of this lineage all have stipitate-cupulate apothecia.

The two new species added to the / fibrosa – macropus lineage, *i.e.* *H. convexa* and *H. japonica*, expand this lineage to include a wider spectrum of apothecial shapes, from regularly stipitate-cupulate to bi- to tri-lobed stipitate-capitate. Out of the five European species now adhered to this lineage, four species, *i.e.* *H. macropus*, *H. ephippioideae*, *H. japonica* and *H. fibrosa*, also have a known distribution outside of Europe.

The stipitate-capitate *H. bresadolae* occupies a position as sister species to *H. pezizoides*, thus expanding the / fallax – pezizoides lineage to include *H. pulla*, *H. fallax*, *H. pezizoides* and *H. bresadolae*. Species of this lineage all have stipitate-capitate apothecia.
Skrede et al. (2017) reported on 55 European Helvella species, of which seven were new to science, based on materials from one or more of the Nordic countries. In the follow-up study focusing on evolution and generic limits in the family Helvellaceae, three Helvella species were transferred to a new genus Dissingia, one species (i.e. H. silvicola) to Midotis, one species (i.e. H. terrestris) re-installed in Pindara, and one species (i.e. H. aestivalis) accommodated in the formerly putatively pure underground genus Balsamia (Hansen et al. 2019). Thus, 49 species remained in the restricted circumscription of the genus (i.e. Helvella s. str.). Løken et al. (2020) published a taxonomic review of the Helvella corium species aggregate, including one newly described species, i.e. H. pseudoalpina, providing biogeographic and ecological notes on six species from the Nordic countries. A subsequent study of Skrede et al. (2020) recorded on 30 spp. of Helvella from Southern Europe (Spain), adding seven new species to the list of European Helvella spp.

With the addition of five new species of Helvella from the present work, the list of European Helvella spp. now amounts to 62 species, of which altogether 54 occur in the Nordic countries (Table 2). In addition, three species of Dissingia, one species of Pindara and one of Balsamia, formerly placed in Helvella s.l., are parts of the diversity of Helvellaceae in the Nordic region (cf. Table 2).

Biogeography and ecology

Through this expanded survey of old and newly collected fresh specimens of Helvella from the Nordic countries, we have gained a much better understanding of the occurrence and biogeographic patterns of some species and subgroups of Helvella within the Nordic region. However, it should be noted that we have mainly collected in Norway and investigated the Helvella collections in the fungaria C, S, UPS, TRH, TROM and O. Some Helvella species have a restricted distribution in the Nordic region. Helvella semiobruta has a single record from Gotland, Sweden, a distribution assumed to be governed by
local climatic and ecological features on this island of the Baltic Sea. This species, which is common in the Mediterranean region (Filippa et al. 2013, Skrede et al. 2020), finds similar climatic and ecological conditions in the Nordic region only in the hemiboreal islands of Gotland and Öland. Three species are known from Denmark only, probably due to preference for a temperate European climate, not much abundant in the rest of the Nordic region. This applies to H. phlebophoroides, H. platypodia and H. queletiana. Four species, in addition to H. semiobrunta, are known from Sweden only, viz. H. hypocrateriformis, H. ephippioides, H. japonica and H. retinervis. Helvella hypocrateriformis, commonly recorded under its synonymous name H. cupuliformis, is a relatively short-stipitate, cupulate species that has been poorly understood in the past. Judging from published descriptions and illustrations of this apparently uncommon species in Europe, most records in the literature are suggested to represent the more common look-alike species H. scyphoides and H. rivularis (Dissing & Sivertsen 1980, Peric 2011, Van Vooren 2014). Eleven species are exclusively found in the arctic-alpine biome of Norway and Sweden, and partially in Greenland, Iceland and Svalbard, i.e., H. alpestris, H. alpina, H. arctoalpina, H. bresadolae, H. capucina, H. dryadophila, H. macroserpa, H. norlandica, H. oroarctica, H. philonotis, H. pseudoalpina. Further, three species are so far known only from Northern (arctic) Norway, i.e., H. bresadolae, H. norlandica and H. oroarctica.

The knowledge of distribution of a single species is dependent on sufficient collections and correct species delimitation. As we observed for the Helvella corium species aggregate, where we have done detailed morphological and molecular examinations of all material in the larger extant Nordic fungaria supplemented with additional field collection efforts from unexplored areas (Skrede et al. 2017, Løken et al. 2020), the discerned species have highly variable distribution patterns. A distribution map based on H. corium s.l. would have indicated one widespread species that occur in all habitats. Cryptic species with highly divergent distribution patterns and habitat choices have been documented for a number of fungal morphospecies in other genera as well; e.g., in Serpula himantioides (Carlson et al. 2011), Amanita muscaria (Geml 2006), Skeletocutis nivea (Korhonen et al. 2018), Heterobasidion annosum s.l. (Garbelotto et al. 1998, Dalman et al. 2010) and the lichen forming Parmelia mayi (Molina et al. 2011). The distribution map presented here may indicate some climatic demands of the species in question. However, the ecological roles of the species in the local ecosystems are largely unknown and needs further investigation. In the study by Løken et al. (2020), where molecular sequences of old, preserved and newly collected specimens were used to define species limits in the H. corium aggregate, one of the new species, H. alpestris, produced an ITS sequence that was identical to a sequence obtained earlier in a root tip of Salix reticulata taken on the same spot (Weidemann 1998). With the data available at that time, Weidemann (1998) could not match the sequence from the root tip to the sequence she had obtained from an apothecium of H. corium collected at another locality. Thus, she suggested that it had to be a close relative of H. corium that formed the observed ectomycorrhizal root tip of S. reticulata. With the current data available we can conclude that H. alpestris is an ectomycorrhizal associate with S. reticulata, and represents the unknown Helvella species in the work of Weidemann (1998). Whether the other species of the H. corium complex also associates with plant roots, is highly suggestive as they often occur with specific plant species as well. However, whether they are ectomycorrhizal or exhibit other biotrophic relationships with their “hosts” needs to be investigated.

Many Helvella species have been suggested to have an ECM lifestyle (e.g. see Tedersoo et al. 2006, Nguyen et al. 2013, Hwang et al. 2015). Both Hwang et al. (2015) and Tedersoo et al. (2006) found Helvella ITS sequences in plant root tips, similarly as we have done when comparing to Weidemann’s (1998) study. Nevertheless, Hwang et al. (2015) also found clades with no evidence of ECM associations, e.g. the elatica group [clade VI in Hwang et al. (2015), clade E in Skrede et al. (2017)]. Thus, it appears that the ecology is highly variable within the genus Helvella. Further studies of the ecology of Helvella species are urgently needed as these species may have important ecosystem functions in a number of natural habitats.

ACKNOWLEDGEMENTS

We acknowledge all the collectors, who deposited specimens of Helvella in the fungaria of O, TRH, BG, TROM, S, UPS, GB, UME, and C, for their dedicated efforts in collecting valuable material, as well as the curators and staff for their help in supplying material. The curators Å. Kruys and C. Lange have taken photographs of the holotype specimens of Helvella con vexa and H. norlandica, deposited in the fungaria of UPS and C. Finally, we acknowledge the University of Oslo and the Norwegian Biodiversity Information Centre (grant no. 45/15) for funding.

Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES


Supplementary Material: http://fuse-journal.org/

Supplementary table. All Helvella specimens investigated for this study where least one Sanger sequence is available. All specimens are from the nordic countries. Which sequences that are available for each speciemens is marked with GenBank number or marker name. All sequences are available in alignments submitted to DataverseNO repository. The sequences with GenBank accession numbers can also be found in GenBank. 
INTRODUCTION

Freshwater fungi are abundant and diverse in rivers, streams and lakes around the world (Jones et al. 2014). They include saprophytes, plant parasites, animal parasites, endophytes and some form mycorrhizae (Jones et al. 2014). Fungi form a rich tapestry of interactions with other organisms within freshwater ecosystems, playing critical roles such as decomposition of organic matter (Calabon et al. 2020).

A major impediment to full understanding of fungal ecology and conservation is the lack of taxonomic information (May et al. 2019). With only 3–8 % of fungal species described (Hawksworth & Lücking 2017), it is imperative to accurately describe and delimit many more fungi to allow ecologists, conservation, and evolutionary biologists to accurately study them and their interactions. During a survey of freshwater fungi on wood in streams in southern Australia, two distinctive and novel ascomycetes were collected.

The first ascomycete species found during our survey was morphologically similar to many species within the Diaporthomycetidae by having wide, septate paraphyses, unitunicate, cylindrical asci with a distinctive non-amyloid apical apparatus (Maharachchikumbura et al. 2015), but we were unable to assign it any known taxa. Maharachchikumbura et al. (2015) described the subclass Diaporthomycetidae (Sordariomycetes) and outlined the orders and families included in this new subclass. Hyde et al. (2021) further refined the phylogeny of this subclass including 21 orders.

The second species had morphological characters consistent with the genus Pleurothecium but did not fit the description of any of the previously described species. Höhn (1919) established *Pleurothecium* for *P. recurvatum*, a species that is commonly found in freshwater habitats. Réblová et al. (2016) introduced *Pleurotheciales* and *Pleurothecia* to accommodate *Pleurothecium* within the Sordariomycetes. There are currently eight accepted species of *Pleurothecium*, six of which occur in fresh water (Réblová et al. 2012, Hyde et al. 2017, Luo et al. 2018, 2019, Shi et al. 2021).

In this study, we provide morphological descriptions and phylogenies for these two novel taxa based on multigene analyses.

MATERIALS AND METHODS

Collection details and examination

Samples of submerged wood less than 5 cm diam were collected in Scott Creek Conservation Park, South Australia from two streams, approximately 50 cm deep with a muddy base, that flow only during winter. Samples were sealed into plastic bags for transport to the laboratory. The riparian vegetation is a mixture of native vegetation and invasive weeds.

Samples were incubated in sterile plastic containers and regularly examined for fungi over 6 months using a Leica MZ7s dissecting microscope. Any fungi observed were photographed, described, and transferred to a microscope slide with a drop of distilled water. A cover slip was added and the slide was examined using a Nikon Eclipse Ni with differential interference contrast. Photographs were taken using either a Sony RX-100 or Canon D6 camera.
Single spore isolation

Potato dextrose agar (PDA, BD micro) was autoclaved, cooled to 60 °C, 100 mg/L streptomycin and 70 mg/L of penicillin added as filter-sterilised stock solutions and 10 mL poured into each 60 mm diam plate.

Ascospores or conidia were transferred to a sterile 1.5 mL microtube with 20 µL of sterile water using a sterile micro needle. The mixture was agitated for several seconds, then transferred to a PDA plate using a pipette. Plates were incubated at room temperature and checked over 5 d for germinating spores using a Leica MZ7s dissecting microscope. Germinated spores were picked off the agar surface using a sterile needle and transferred to individual PDA plates which were incubated at room temperature.

DNA extraction, amplification, and sequencing

**Standard method**

Approximately 50 mg of fungal mycelium was scraped from the surface of agar cultures with a sterile scalpel and the genomic DNA was isolated using a Qiagen DNeasy Plant Mini kit following the manufacturer’s protocols. The final DNA extracts were eluted into 100 µL of buffer.

Primers LROR/LRS (Vilgalys & Hester 1990, Renhner & Samuels 1994) were used to amplify the sequences from the 28S nrRNA gene (28S), while ITS1/ITS 4 (White et al. 1990) were used to amplify the internal transcribed spacer regions and the intervening 5.8S nr RNA gene (ITS), and NS1/NS4 (White et al. 1990) were used to amplify the 18S nrRNA gene. Sequences from protein-coding gene, translation elongation factor 1-alpha (TEF1), were amplified with primers were EF1-983F and EF1-2218R (Renhner & Buckley 2005).

Reaction mixtures contained 5 µL buffer, 1 µL (10 mM each) dNTPs, 1 µL (10 µM) of each primer, 0.25 µL hotStart Taq DNA polymerase (New England Biolabs), 1 µL DNA template, and 16.75 µL sterile milliQ water.

PCR amplification was performed in an Applied Biosystems 2720 Thermo Cycler. Cycling conditions for PCR were initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C (ITS) or 54 °C (28S) for 50 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min. Cycling conditions for TEF1 was 95 °C for 1 min; 35 cycles of 95 °C for 30 s, 57 °C for 50 s, and 68 °C for 1 min; followed by 68 °C for 5 min. PCR products were observed on a 1.5 % agarose electrophoresis gel stained with Gel Red (Gene Target Solutions).

The resulting amplicons were purified using a Qiagen QIAquick PCR Purification Kit and sequenced in both directions using the respective primers by the Australian Genome Research Facility. Raw sequence reads were assembled, examined, and edited using Sequencher v. 5.3 (Gene Codes Corporation). Newly generated sequences were submitted to NCBI GenBank under the accession numbers listed in Tables 1 and 2.

**Direct PCR method**

Numerous attempts were made to culture specimens. When cultures were not obtained, conidiophores or ascomata were used for direct PCR. In these cases, Phire Plant Direct PCR Master Mix (Thermo Fisher Scientific) was used. Ascomata or conidiophores were placed into 20 µL of Phire dilution buffer at 4 °C, heated in a dry block to 98 °C for 10 min, then on ice for 5 min, vortexed briefly, then centrifuged for 1 min at 11 000 rcf.

Reaction mixtures for the Phire kit contained 7 µL sterile milliQ water, 10 µL master mix, 1 µL of each primer, 1 µL DMSO, and 1 µL DNA template. PCR cycling conditions for Phire kit extracts was 98 °C for 5 min; 35 cycles 98 °C for 5 s, 60 °C (ITS), 56.8 °C (28S) or 55.5 °C (18S) for 5 s, 72 °C for 20 s; followed by a final extension at 72 °C for 1 min.

For some samples using the Phire kit, a second band was persistent on electrophoresis gels, despite optimisation of PCR conditions. In these cases, the band-stab and re-amplification technique of Bjouson & Cooper (1992) was used. Re-amplification used the standard PCR protocol and primers described above. PCR primers, product clean up and sequencing were as described above for the standard method.

Phylogenetic analyses

The generated sequences for each gene were used in megablast searches (Zhang et al. 2000) to identify closely related sequences in NCBI’s GenBank nucleotide database. Other sequences used in this study were derived from GenBank. Sequences were aligned in Geneious Prime v. 2023.0.4 (https://www.geneious.com) using MUSCLE. Ambiguously aligned regions were removed from the alignments using GBLOCKS v. 0.91b (Castresana 2000, Talavera & Castresana 2007) on the Phylogeny.fr platform (Dereeper et al. 2008). Alignments were imported into Mega X v. 10.2.6 (Stecher et al. 2020) to find the best substitution models for phylogenetic analyses. The best substitution models were K2+G (ITS) and TN93+G (28S and TEF1) for the first species and K2+G (ITS and 28S), K2 (18S) for the second species. However, RaXML and MrBayes do not use these models, so GTR+G+I was used for all analyses as recommended by Abadi et al. (2019). Genes were then concatenated and maximum-likelihood phylogenetic trees were constructed with partitions for each gene region using RaXML v. 8.2.11 ( Stamatakis 2014) within Geneious using the GTR + I + G substitution model and branch support values were calculated with 1 000 rapid bootstrap inferences. The same alignment was analysed using Bayesian analysis with MrBayes using the GTR GAMMA I substitution model (v. 3.2.6; Huelsenbeck & Ronquist 2001) with partitions for each gene region within Geneious. All resulting trees were formatted in Geneious, then further edited in Adobe Illustrator v. 27.0.

RESULTS

Phylogenetic analyses

Phylogenetic trees based on multi-locus analyses (Figs 1, 2) show the relationships between the new species and other related taxa. Branch supports of Maximum Likelihood bootstrap ≥ 70 % and Bayesian PP value ≥ 0.90 are indicated above the branches.

Despite numerous attempts, we were unable to recover high-quality sequence data from the ITS region of either of the specimens of the first species. To place this undescribed perithecial species (AD291710 and AD219607) from our survey, the 28S and TEF1 sequences were analysed with ITS, 28S and TEF1 sequences of 30 species in five families in the Sordariomycetes (Fig. 1). The dataset comprised 1 670 characters: 321 bp for ITS, 506 bp for 28S, 843 bp for TEF1. The 18S sequences were mostly uninformative and were not included. The tree is rooted to Cancellidium appplanatum, C. cinereum and Obliquiminima.
Freshwater ascomycetes from southern Australia

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hyalina. The undescribed ascomycete nested within the Diaporthomycetidae and formed a well supported clade (100/1) with Proliferophorum thailandicum and Paraproliferophorum hyphaene, which together form a new family (Fig. 1). For the undescribed Pleurothecium (AD291629 and AD291640) the combined ITS, 18S and 28S sequences were assessed with the eight previously recognised species in Pleurothecium (Fig. 2). The dataset comprised 2 161 characters: 511 bp for ITS, 814 bp for 28S, 836 bp for 18S. The tree is rooted to Phaeoisaria clematidis. The two new specimens form a sister clade to Pleurothecium aquaticum and Pleurothecium guttulatum, representing a new species, Pleurothecium brunius (Fig. 2).

Taxonomy

Melanascomaceae Fryar & D.E.A. Catches, fam. nov. Index Fungorum IF 900400.

Table 1. GenBank accession numbers of selected taxa from Diaporthomycetidae used for phylogenetic analyses. Newly generated sequences are shown in bold.

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Asexual morph: Hyphomycetous. Conidiophores macronematous, mononematous, sub-cylindrical to cylindrical, unbranched or branched, erect, olivaceous brown to dark brown, light brown at the apex, septate, smooth or ornamented. Conidiogenous cells holoblastic, polyblastic, terminal, subhyaline to brown. Conidia fusiform to cylindrical, subhyaline to brown, 0–3-septate when mature, guttulate, smooth.

Sexual morph: Ascomata perithecial, non-stromatic, subglobose with a straight neck. Ascomatal wall textura angularis, dark brown. Paraphyses persistent, cylindrical, septate, unbranched. Asci unitunicate, eight-spored, apex with a non-amyloid apical ring. Ascospores ellipsoid, septate, ornamented, without appendages or sheath.

Type genus: Melanascoma Fryar & D.E.A. Catches
Table 2. GenBank accession numbers of selected taxa from Pleurothecium used for phylogenetic analyses. Newly generated sequences are shown in bold.

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Fig. 1. Phylogram generated from maximum likelihood analysis based on combined ITS, 28S and TEF1 sequence data of Melanascoma panespora and closely related taxa. Bootstrap values equal to or great that 70 % and Bayesian posterior probabilities equal to or greater than 0.90 are given above the nodes. Ex-type strains are shown in bold and newly generated sequences shown in blue.
Melanascoma Fryar & D.E.A. Catches, *gen. nov.*, Index Fungorum IF 900396.

*Etymology*: The prefix “Melan-” means darkly coloured. *Melanascoma* refers to the darkly coloured ascomata.


*Type*: *Melanascoma panespora* Fryar & D.E.A. Catches

**Melanascoma panespora** Fryar & D.E.A. Catches, *sp. nov.* Fig. 3. Index Fungorum IF 900395.

Typos: Australia, South Australia, Scott Creek Conservation Park (S35°5′45.90", E138°40′59.16), on submerged decaying wood in an ephemeral stream, 30 Aug. 2020, S.C. Fryar (*holotype* AD219607; GenBank sequences: 28S - OQ799385; 18S - OQ799375).

*Etymology*: The specific epithet refers to the appearance of the ascospores, like Vienna bread loaves.

Fig. 4. *Pleurothecium brunius* (holotype AD291640). A. Conidiophores on wood from fresh water. B–D. Conidiophores and conidiogenous cells. E–J. Conidia. Scale bars: A = 100 µm; all others = 5 µm.
Additional material examined: Australia, South Australia, Scott Creek Conservation Park (53°5′45.90′′, E138°40′59.16′′), on submerged decaying wood in an ephemeral stream. S. Fryar (paratype AD219607; GenBank sequences: 28S - OQ789909; 18S - OQ789908; TEF1 - OQ870569).

Notes: Melanascoma panespora forms a clade with Proliferophorum thailandicum and Paraproliferophorum hypnaeones (Fig. 1) both of which form monotypic genera. They are described from asexual morphs, with sexual morphs unknown, therefore morphological comparisons with these genera are not possible at this stage. This clade is sister to Populosaceae and forms a larger clade with Pseudostanjeniheughesiacaeae and Acrodictyaceae. Further analysis and data will be required to decide which of these clades represents a new order within the Diaporthomycetidae.

Morphologically M. panespora resembles other species within the subclass Diaporthomycetidae in having globose to subglobose brown to black ascomata, wide, septate paraphyses, unisuturate, cylindrical asci with a distinctive non-amyloid apical apparatus (Maharachchikumbura et al. 2015). Rhamphoria is similar to Melanascoma but has dictyosporous ascospores (Réblóvá et al. 2018). Atractospora is also similar but has fusiform ascospores and lateral necks (Réblóvá et al. 2016).

Melanascoma panespora shares some characteristics with Rivulicola species (Hyde et al. 1997, Raja et al. 2009, Ranghoo et al. 2000). The asci of M. panespora resemble those of Rivulicola species having uniseriate asci with large inamyloid apical rings and a pedicel. The ascospores of Rivulicola are ellipsoid, hyaline, and septate; as in M. panespora but Rivulicola has ascospores with a mucilaginous sheath and without constrictions at the septa. In addition, the ascomata and necks of Rivulicola are hyaline to pale brown compared with the dark brown ascomata and necks of Melanascoma. There are currently no available sequences for Rivulicola in GenBank for phylogenetic comparison.

Hyde et al. (2021) noted that, in their analyses, Proliferophorum diverged from Platyrachelon around 76 MYA, which falls within the family range (50–130 MYA). In our analysis the clade including Melanascoma, Proliferophorum and Paraproliferophorum is sister to Platyrachelon, forming a distinct lineage. We therefore introduce the new family Melanascomaceae to accommodate these three genera.

**PLEUROTHECUM BRUNIUS** Fryar & D.E.A. Catches, sp. nov., Index Fungorum IF 900397. Fig. 4.

Etymology: The specific epithet refers to the brown colour of the conidia.

**Typus:** Australia, South Australia, Scott Creek Conservation Park (53°5′45.90′′, E138°40′59.16′′), on submerged decaying wood in an ephemeral stream, S.C. Fryar (holotype AD291640; GenBank sequences: ITS - OQ799373; 28S - OQ799347; 18S - OQ799346).

Asexual morph: Conidiophores macronematous, mononematous, straight or slightly flexuous, septate, smooth, unbranched, dark brown, paler towards the apex, apex hyaline, (60–)80–100(–115) × 3–3.5 μm. Conidiogenous cells integrated, terminal, polyblastic, denticulate, hyaline, swollen, with 6–8 cylindrical denticles, swollen part 3–7 × 3–5 μm, denticles (2–)2.5–4 μm long, 1 μm wide. Conidia hyaline to dark brown, ellipsoid, 1–3-septate with conspicuous septa, not constricted at the septa, straight to slightly curved, smooth-walled (14–)16–19 × 5–6 μm. Sexual morph undetermined.

**Distribution:** Found in South Australia, Australia.

Additional material examined: Australia, South Australia, Scott Creek Conservation Park (53°5′45.90′′, E138°40′59.16′′), on submerged decaying wood in an ephemeral stream, S.C. Fryar (paratype AD291629; GenBank sequences: ITS - OQ799378; 28S - OQ799377; 18S - OQ799376; TEF1 - OQ784578).

Notes: Pleurothecium brunius forms a clade with *P. aquaticum* and *P. guttulatum* (Fig. 2). Morphologically it is different to *P. aquaticum* in having dark brown, shorter conidia, and longer conidiophores. Pleurothecium brunius is different to *P. guttulatum* by having dark brown, septate conidia, and a bulbous apex.

**ACKNOWLEDGEMENTS**

Financial support from the Australian Biological Resources Survey (ABRS) is gratefully acknowledged.

**Conflict of interest:** The authors declare that there is no conflict of interest.

**REFERENCES**


Freshwater ascomycetes from southern Australia


Adding a missing piece to the puzzle of oomycete phylogeny: the placement of *Rhipidium interruptum* (*Rhipidiales*)

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**Abstract:** Oomycetes are a group of fungus-like organisms, which phylogenetically comprise early diverging lineages that are mostly holocarpic, and two crown classes, the Peronosporomycetes and Saprolegniomycetes, including many well-investigated pathogens of plants and animals. However, there is a poorly studied group, the Rhipidiales, which placement amongst the crown oomycetes is ambiguous. It accommodates several taxa with a sophisticated vegetative and reproductive cycle, as well as structural organisation, that is arguably the most complex in the oomycete lineage. Despite the remarkable morphological complexity and their notable perseverance in the face of faster-growing saprotrophic oomycetes and fungi, the knowledge on *Rhipidiales* is limited to date, as the most complex members are not easily cultured, even by targeted approaches. This also leads to inadequate sequence data for the order, which was sourced from only the two least complex out of seven introduced genera, *i.e.* *Saprohycess* and *Salispina*. In the present study, *ex-situ* baiting was done using various fruit substrates, and naturally-shed twigs or fruits acquired from water bodies were examined. As a result of these efforts, the species *Rhipidium interruptum* was obtained and gross cultivation was accomplished using *Populus nigra* twigs as substrate, which allowed further documentation of both asexual and sexual reproduction. This enabled phylogenetic and detailed morphological study, as well as an epitypification of the species. Phylogenetic analyses based on cox2 and nrLSU sequences revealed *Rhipidium* as the sister genus of *Saprohycess*. The morphological studies done support a conspecificity of *R. interruptum* and *R. continuum*, which might in turn be conspecific with *R. americanum*. Though several further studies will be required to fit the scattered missing pieces of knowledge on *Rhipidiales* together revealing a more complete picture of oomycete evolution, we hope that the current study can serve as a cornerstone for future investigations in the group.

**INTRODUCTION**

Oomycetes are eukaryotic, fungus-like, heterotrophic microorganisms that are ubiquitous in marine, brackish, freshwater, as well as terrestrial habitats, and comprise both saprotrophs and pathogens of various hosts (Sparrow 1960, Howard & Johnson 1969, Choi et al. 2008, Thines 2014, Bennett et al. 2018, Bennett & Thines 2019, Hassett et al. 2021). Destructive oomycete pathogens are responsible for several devastating diseases (Erwin & Ribeiro 1996, Bruno et al. 2011), the most notorious being *Phytophthora infestans*, which causes potato late blight and led to the historical Great Famine in Ireland (Yuen 2021). On animal hosts, saprolegniosis caused by *Saprolegnia* species can cause ecological damage by infesting and killing amphibians, fish, crustaceans, and several other aquatic beings, and cause enormous economic losses in aquaculture (Bly et al. 1992, Czeczuga et al. 1999, Hussein & Hatai 2002, Costa & Lopes 2022). Apart from these rather well-known groups, some holocarpic oomycetes have high ecological relevance, as they have been found to fatally infect diverse species of diatoms and algae that play a vital role by forming the basic energy source of the aquatic food web (Jensen 1993, Serôdio & Lavaud 2020). For example, in Iceland, *Pontisma blauvikense* was found parasitic to brown algae (Buaya et al. 2023), *Lagena ausuennarstadhirensis* and *Miracula einbuurlaekurica* were reported as endoparasitoids in peninate freshwater diatoms (*Buaya & Thines 2022a*, *Thines & Buaya 2022*), and *M. blauvikense* and *M. islandica* were found to parasitise marine diatoms (*Buaya et al. 2021a*, *Buaya & Thines 2022b*). Other holocarpic oomycetes are obligate parasites of other oomycetes, *e.g.* *Olpidiopsis verrucosa* was found parasitic to *Achlya glomerata* (Johnson 1955), and *O. incrassata* pathogenic to several *Saprolegniaceae* species (Slifkin 1961).
addition to the pathogenic species, a multitude of saprotrophic oomycetes are omnipresent, both in *Peronosporales* and *Saprolegniales* (Marano et al. 2016, Beakes & Thines 2017). Also, there are a variety of saprotrophic oomycetes not belonging to either order and that have attained much less attention despite their widespread occurrence. These are organisms previously included in one order, the *Leptomitaes* (Sparrow 1960), but are now placed in separate orders. The first order is *Leptomitaes*, which includes *Leptomitus lacteus* (*Leptomitaceae*), commonly found in polluted water, thus called “sewage fungus” (Dix & Webster 1995). The second order is *Rhipidiales*, which is the subject of the present study. Examples from the latter order are *Salispinia hoi* (*Salispinaceae*), isolated from decaying mangrove leaf litter (Bennett et al. 2018), and *Aquilinderella fermentans* (*Rhipidiaceae*), which was acquired from various species of juicy fruits or nuts as baiting substrates in stagnant water, and was notably observed thriving in anaerobic conditions (Emerson & Weston 1967, Emerson & Held 1969, Czeczuga et al. 2004).

The most complex thallus structure in *Rhipidiales* is found amongst *Rhipidium* species. *Rhipidium* includes the species *R. americanum*, *R. compactum*, *R. interruptum* (*eupoeaenum*), *R. parthenosporum*, and *R. thaxteri*, which were recovered from a number of submerged plant substrates (Cornu 1871, Thaxter 1896, Kolkwitz et al. 1915, von Minden 1916, Kanouse 1927b, Matthews 1936, Sparrow 1960). Compared to the study on pathogenic oomycetes, saprotrophs are generally less studied, even though they are increasingly gaining attention in recent years (Nakagiri 2000, Hulvey et al. 2010, Marano et al. 2016, Bennett & Thines 2020).

The phylum *Oomycota* is usually sorted into three groups – the early diverging lineages with several holocarpic species, and the two “crown clades” that comprise the majority of known oomycete diversity, namely the classes *Peronosporomycetes* and *Saprolegniomycetes* (Beakes et al. 2014, Thines 2014). The order *Rhipidiales* is probably sister to the clade of all other taxa in *Peronosporomycetes* (Thines 2014) and comprises two families, *Rhipidiaceae* and *Salispinaceae* (Bennett et al. 2018, Bennett & Thines 2020), with six genera included in the former, *Rhipidium*, *P. salpinges*, *Aquilienderella*, *Mindienella*, *Nellymyces*, *Araiospora* (Sparrow 1960, Emerson & Weston 1967, Batko 1971), and only *Salispinia* in the latter (Bennett et al. 2018). Amongst the seven genera of *Rhipidiales*, sequence data are exclusively available from two of them, viz. *Somatosporum* (*Somatosporomyces elongatus* of the *Rhipidiaceae*) and *Salispinia* (*Salispinia hoi*, *Salispinia intermedia*, *Salispinia lobata*, and *Salispinia spinosa* of the *Salispinaceae*) (Petersen & Rosendahl 2000, Jesus 2015, Bennett et al. 2018).

There are only few phylogenetic studies involving rhipidialean members based on either single or concatenated loci (Hudspeth et al. 2000, Petersen & Rosendahl 2000, Riethmüller et al. 2000, Hudspeth et al. 2003, Beakes et al. 2012). In these studies, contradicting or poorly resolved topologies based on different loci were observed. Riethmüller et al. (2000) analysed the large ribosomal subunit DNA (nrLSU) of several oomycetes, and inferred that the subclass *Rhipidiomycetidae* was closer related to *Saprolegniomycetidae* than to *Peronosporomycetidae*, with maximum bootstrap support. In contrast, Petersen & Rosendahl (2000), also based on nrLSU sequences they studied, could not explicitly resolve the placement of *Rhipidiales* due to low bootstrap support (74 % and 67 %, depending on the analysis). Hudspeth et al. (2000) inferred a phylogeny based on partial mitochondrial cytochrome c oxidase subunit II (cox2) sequences of representative species from *Peronosporomycetidae* and *Saprolegniomycetidae* (currently recognised as *Peronosporomycetes* and *Saprolegniomycetes*, respectively), and found strong bootstrap support for a clade accommodating *Peronosporomycetes* and *Rhipidiales*. In all of these studies, it was emphasised that *Somatosporum elongatus* was the only species representing *Rhipidiales*, thus, additional representatives were required to validate the monophyly of *Rhipidiales*. Furthermore, the conflicting topologies found highlighted the need for including more sequence data and taxa (Hudspeth et al. 2000, Petersen & Rosendahl 2000, Riethmüller et al. 2000, Dick 2001). Bennett et al. (2018) reconstructed the peronosporomycete phylogeny based on a concatenation of nrLSU, cox1 and cox2, thereby inferring a monophyletic *Rhipidiales*, including both *Salispinaceae* and *Rhipidiaceae*. However, the phylogenetic reconstruction did not contain an outgroup outside the crown groups, thus not clarifying if *Peronosporomycetes* or *Saprolegniomycetes* should include *Rhipidiales*. In addition, none of the studies mentioned above included a member of *Rhipidium*. Due to the lack of sequence data for *Rhipidium*, the placement of this group has remained obscure.

Studies into the morphologically more complex members of the *Rhipidiaceae*, such as members of the genus *Rhipidium*, which feature a well-developed, branched rhizoid, a well-differentiated basal cell, and filamentous branches carrying reproductive organs, have been hampered by the difficulty in cultivating them. Though short gross or pure culture maintenance of species with restricted thallus growth (*Rhipidium*, *Araiospora*, and *Aquilinderella*) was achieved, they could not be sustained as growth ceased after some time (von Minden 1916, Emerson 1950, Emerson & Weston 1967). Detailed studies regarding physiology, life cycle, ecological interaction, and other experiments requiring a constant supply of vital material are limited as a result of difficulties in long-term axenic cultivation. Considering that *Rhipidiales* are arguably the structurally most complex group of oomycetes, and appears to occupy a peculiar ecological niche that renders them competitive in the face of faster-growing substrate competitors, this seems to be a critical obstacle towards understanding the evolutionary plasticity of oomycetes. In addition, such cultures would also enable the sequencing of multiple loci or even high-quality genomes that could help understanding the evolution of complex thallus structures from simple mycelial forms. An increased knowledge on *Rhipidiales* would also enable in-depth ecological and evolutionary comparisons to the equally diverse but also understudied *Leptomitaes*, which often occur in similar environments and have some similar features, such as constricted hyphae.

Considering the research gaps mentioned above, the aim of the present study was to isolate the type species of *Rhipidium* and achieve gross culture for at least one strain over prolonged time to obtain sequence data for a phylogeny based on both nuclear and mitochondrial sequences that might resolve the ambiguous placement of *Rhipidiales*.

**MATERIALS AND METHODS**

The species discussed in this study was acquired with various approaches to sampling from different water bodies in three European countries (Fig. 1), with submerged plant substance collection (natural baits), *ex-situ* mud-baiting, multi-pustule sub-baiting, and gross cultivation.
Sampling and baiting

**Denmark**

**Sampling date:** 16 Nov. 2021. **Location:** The sampled ditch was near the intersection of Vesterhavsvej and Gammelgabvej, in Nørre Nebel (55.795356°N, 8.233995°E). **Description of the environment:** The ditch had a shallow, reddish brown muddy bed, with some leaf litter (Malus sylvestris and Quercus petraea) (Fig. 1A), and was surrounded with pastures and fields. Above the sampling site, trees arranged into a hedge, which included Malus sylvestris and Quercus petraea were observed, and along the ditch several additional tree species were present. **Natural bait collection:** Fallen apples submerged in the ditch were collected, with one of them (fruit code GVap01 for Gammelgabvej-Vesterhavsvej apple 01) conspicuously covered by loosely distributed whitish grey or light greyish brown pustules on parts of its surface (Fig. 2A, appearance of the fruit GVap01 on the third day after collection). The fruits were kept in tap water in a plastic bucket when not examined. **Multi-pustule sub-baiting:** The sub-baiting was carried out on 19 Nov. 2021, by picking 10 pustules with fine-end tweezers from GVap01, to a container with tap water and three apples (collected from one of the fruiting apple trees nearby the sampled ditch, and stored at room temperature before use). The baiting set was incubated at room temperature in Blåvand, Denmark, without a fixed light period, with daylight through windows for around eight hours, subsequently indoor fluorescent lamp light for roughly another eight hours, and darkness for the rest of the day. The baiting set was incubated under this condition for 4 d, transported by car for approx. 8 h, to be again incubated at room temperature (circa 20 °C) in a laboratory room at the Biodiversity and Climate Research Centre in Frankfurt am Main, Germany, again without specific settings of the light cycle.

**Italy**

**Sampling date:** 31 Dec. 2021. **Location:** The sampled ditch was on the side of the street Via Pralboino, which connects the towns Pralboino and Gottolengo, in the Province of Brescia (45.275659°N, 10.241645°E). **Description of the environment:** The ditch had a shallow, greyish brown muddy bed, with a large amount of leaf litter and shedded twigs from Populus nigra, both in the water and on the banks of the ditch. Living and decaying grasses and herbs covered the banks on both sides of the ditch, and plants of Arum maculatum were growing along. Some widely spaced trees (Populus nigra var. italica) were growing above the ditch. A wide area of durum wheat fields was surrounding the ditch that was next to an unpaved road. **Ex-situ mud-baiting:** About 500 g of wet mud was collected from the ditch bed into a BPA-free, plastic food storage container (Lock & Lock, Seoul, South Korea), and brought to the laboratory after the end of the sampling trip. The mud baiting was carried out 6 d after sampling, on 6 Jan. 2022. A selection of various fruits, namely apples, olives, tomatoes (small fruit variety), and Cotoneaster spp., was employed as baiting substrates in water above the mud. The baiting set was incubated at 10 °C with 12/12 h light/dark period to simulate the natural conditions at the time of sampling. On 16 Feb. 2022, after 40 d of baiting, one of the olive fruits had developed pustules that were used for sub-baiting on other fruits, two tomatoes were heavily infested by fungi thus removed, and the remaining set including the olive showing pustules (simplified fruit code given: VPolv01) were transferred (with another two olives from the original container added 6 d later that had been overlooked during the first transfer) into a 370 mL Weck jar (J. WECK GmbH & Co. KG, Wehr-Öffingen, Germany) containing fresh tap water mixed with some muddy water from the original container, and incubated without a sealing ring. The water was replaced whenever turbid since then. All following examinations, on fruits apart from VPolv01, were carried out based on this set of baits.

**Germany**

**Sampling date:** 28 May 2022. **Location:** Samples were taken from the river Lahn close to its bank near a platform reaching into the river, where the water was running slower in comparison to the main stream (50.572778°N, 8.641806°E), and from the Lake Silbersee at its southern shore (50.575694°N, 8.640194°E), both near Gießen (Hesse). **Natural bait collection:** Submerged twigs were collected from the water, with some of them already showing small, yet suspicious, pustules on the surface. The sampled twigs were from several tree species, including Salix sp. and Populus sp., while some of them already showing small, yet suspicious, pustules on the surface. The sampled twigs were from several tree species, including Salix sp. and Populus sp., while some of them already showing small, yet suspicious, pustules on the surface. The sampled twigs were from several tree species, including Salix sp. and Populus sp., while some of them already showing small, yet suspicious, pustules on the surface. The sampled twigs were from several tree species, including Salix sp. and Populus sp., while some of them already showing small, yet suspicious, pustules on the surface.
of various species, trees were growing around the shore of Silbersee, a shallow lake derived from the extraction of gravel.

**Material maintenance:** The sampled twigs were rinsed and placed in 142 mm (diam) × 20 mm (depth) polystyrene Petri dishes (Sarstedt, Nümbrecht, Germany) with fresh tap water after having been brought to the laboratory, and incubated at 10 °C with a 12/12h light/dark rhythm. The twigs were rinsed once per week (roughly), meanwhile the water in dishes was renewed, to avoid an overly flourishing population of protists and their food, to slow down the speed of decay of the twigs.

**Gross cultivation**

The gross cultivation was performed on twig samples collected from the river Lahn and the lake Silbersee, by co-incubating the sampled twigs with new twigs in 142 mm × 20 mm polystyrene Petri dishes (Sarstedt, Nümbrecht, Germany) in tap water. For this, twigs of different tree species were employed. Twigs of alder (*Alnus glutinosa*) and poplar (*Populus nigra*) were successively applied on 13 and 20 Jun. 2022 to the dishes and co-incubated with the sampled twigs.

The alder twigs were artificially wounded at multiple positions with sterile surgical blades, after serendipitously observing pustules growing from a natural bark opening close to the end of one twig. However, the pustules on alder twigs did not progress as well as those thriving on poplar. Therefore, the sub-cultivation on alder twigs was discontinued after a few rounds of successive weekly addition of twigs.

Due to the quick development of various decay-associated organisms on natural poplar twigs, twigs added subsequently were subjected to a mild pasteurisation in a hot water bath from respectively the 23rd and 25th of Jun. 2022 for gross cultures from Lahn and Silbersee. The hot bathing at 60 to 65 °C for 2–3 h drastically reduced potential contaminants from the twig materials, and was fixed to 2 h at 65 °C after a few experiments. When many twigs were bathed at once, twigs not directly used for baiting were stored at -20 °C until needed. Additional hot-bathed poplar twigs were applied to the gross cultivation in an interval of 1–2 wk, depending on the condition of pustule growth, meanwhile all the new and old twigs were rinsed and water replaced as mentioned before.

**Material examination and specimen preparation**

The plant substrates were screened, and suspicious pustules growing on the substrate surface were carefully extracted from the plant tissue and dissected by using fine-tipped tweezers, assisted by a fine iron needle, under a dissecting microscope (Zeiss SteREO Discovery). The pustules were then mounted onto microscopic slides using tap water and covered with coverslips for further documentation using differential interference contrast light microscopy on compound microscope (Zeiss Axio Imager2) equipped with Zeiss Axiocam MRc5 camera operated by AxioVision Rel. 4 for photography and measurements. All the microscopy-related equipment and software were acquired from Carl Zeiss, Oberkochen, Germany.
The processing of materials differed between the initial phase of the investigation and the later phase, as outlined below. The initial phase encompasses the handling of samples from Denmark and Italy. For this, a single pustule was dissected into two halves, one for specimen preparation, and the other collected into a drop of 10 μL sterile deionized distilled water in a 2 mL SafeSeal microtube (Sarstedt, Nümbrecht, Germany), and frozen at -20 °C for later DNA extraction and sequence analyses. Another one or more pustules on the same fruit with similar appearance, occurring adjacent to the previously dissected one, was/were subsequently detached from the substrate and used for microscopy. However, if the pustule selected for specimen preservation and DNA extraction is distinct from the one used for microscopy, this method always leads to an indirect connection between the morphological and phylogenetic conclusions. Therefore, this practice was abandoned after the authors realised the issue. This means that only the sequence data were considered unambiguous for samples derived from the earlier method, while morphological features, even though in line with the results from the later method, were not considered for the morphological characterisation. To solve the matter, in the later phase the procedure from the initial phase was modified and applied to the samples from Germany (Lahn and Silbersee). Instead of cutting single pustule into halves, it was divided into three parts, one for obtaining a specimen, a second for microscopy, and a third for molecular analyses. Thereby, all the materials used for a single isolate could be directly connected.

The specimens of single pustule isolates were stored in approximately 1 mL of TE-buffered ethanol solution (94–96 % ethanol, 0.3–0.5× TE), in 1.8 mL screw-capped CryoPure tubes (Sarstedt, Nümbrecht, Germany). Specimens of the isolates included in the phylogenetic analyses of this study were deposited in the Herbarium Senckenbergianum (Frankfurt am Main), with the Herbarium codes: FR-0046161, FR-0046163 to FR-0046166 (Table 1).

DNA extraction and sequence analyses

Genomic DNA was extracted using the innuPREP Plant DNA Kit (Analytikjena AG, Jena, Germany), following the manufacturer’s instruction. Polymerase chain reaction (PCR) was performed to amplify nrLSU and cox2 of the obtained rhipidiaceous isolates, using a Mango™ DNA polymerase kit (Meridian Bioscience, Inc., Cincinnati, Ohio, USA), with each single reaction performed in a 25 µL volume containing 1× colourless reaction buffer, 2 mM of MgCl₂, 0.8 mg/mL of bovine serum albumin, 200 μM of dNTPs, 400 μM of each forward and reverse primers, 0.15 μL of Mango™ polymerase, 11.35 μL of sterile deionised distilled water, plus 2 μL of DNA template. To amplify nrLSU, the primers LR0R-O and LR6-O (Moncalvo et al. 1995, Riethmüller et al. 2002), and for cox2, cox2-F and cox2-RC4 were used (Hudspheth et al. 2000, Choi et al. 2015). The PCR cycling programs were run on an Eppendorf Mastercycler proS equipped with a vapoprotect lid (Eppendorf AG, Hamburg, Germany). For amplifying nrLSU, PCR was initiated with a denaturation at 95 °C for 2 min, followed by 40 or 36 cycles at 95 °C for 20 s, 53 or 54.5 °C for 20 s, and 72 °C for 2 min, and terminated with a final elongation at 72 °C for 7 min; for cox2, PCR started with an initial denaturation at 96 °C for 6 min, followed by 36 cycles at 96 °C for 20 s, 50 °C for 40 s, and 72 °C for 40 s, and a final elongation at 72 °C for 6 min. The PCR amplicons were subsequently mixed with home-made DNA loading buffer (AG Thines, Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Frankfurt am Main, Germany) and loaded on an 1 % agarose gel stained with ethidium bromide, flanked by the HyperLadder™ 1kb standard (Meridian Bioscience, Inc., Cincinnati, Ohio, USA), and visualised after electrophoresis by UV illumination. Each of the successfully amplified products were diluted to slightly lower than 4 ng/µL with sterile water and sent with the fitting forward and reverse primer solution (diluted freshly from 100 mM to approximately 5 mM with molecular grade water) for sequencing at the laboratory centre of SBiK-F (Frankfurt am Main, Germany).

Consensus sequences were obtained via Geneious Pro v. 5.6.7 (Biomatters, Inc., Auckland, New Zealand) by an editing based on forward and reverse sequences, assisted by a sequence chromatography visualisation in Chromas v. 2.6.6 (Technelysium Pty. Ltd., South Brisbane QLD, Australia) for judging on the reliability of basecalls. The final consensus sequences were aligned after the addition of sequences for the loci investigated from previous publications (cited in Table 1) and from an unpublished genome of the Lagenisca coscinodis strain LgC2 (IsL, Buaya et al. 2019a) using the MAFFT v. 7 online server (Kato et al. 2019; https://mafft.cbrc.jp/alignment/server/). Accessed on 20 Feb. 2023). The aligned sequence datasets of the single loci were trimmed to remove leading and trailing gaps via MEGA v. 7.0.26 (Kumar et al. 2016), followed by concatenating both loci via FASconCAT, v. 1.11 (Kück & Meusemann 2010). The phylogenetic analyses of the concatenated dataset were performed using the TREase webserver (Mishra et al. unpublished; http://thines-lab.senckenberg.de/trease/). Accessed on 20 Feb. 2023), executing FastTree2 (Price et al. 2010) for minimum evolution (ME) trees with the generalised time-reversible (GTR) model and 1 000 bootstrap replicates, RAxML v. 8 (Stamatakis 2014) for maximum likelihood (ML) reconstruction applying the GTRAGGMA model and 1 000 bootstrap replicates, MVRayes v. 3.2 (Ronquist et al. 2012) for Bayesian inference (BI) with the 6 GTR model run for 1 000 000 generations, sampling every 1 000th tree, and discarding the 30 % of the trees for ensuring sampling of trees from the stationary phase. The phylogenetic trees were displayed, rooted and adjusted to stepwise-up with FigTree v. 1.4.0 (Rambaut 2012) and MEGA v. 7.0.26 (Kumar et al. 2016), and subsequently annotated in MS PowerPoint 2019 (Microsoft Inc., Redmond, U.S.A.).

The sequences obtained in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/), with accession numbers given in Table 1. The alignments used for generating the trees can be retrieved from Supplementary File 1.

RESULTS

Baiting and cultivation

Danish samples — multi-pustule sub-baiting

White pustules were discovered after 12 d of baiting (on 1 Dec. 2021) on the surface of one of the three apples that served as baiting substrate (Fig. 2B, photographed on the first day when the pustules were found, before they grew more). A specimen (FR-0046164) was made with one of the halves of a pustule, and the other half from the same pustule was used for the phylogenetic inference of this study.
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<td>MJ654164</td>
<td>Hulvey &lt;i&gt;et al.&lt;/i&gt; (2010) Bennett &lt;i&gt;et al.&lt;/i&gt; (2017a)</td>
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<tr>
<td>Salispina hoi</td>
<td>USTCMS 1611&lt;sup&gt;mc&lt;/sup&gt;</td>
<td></td>
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<td>MF991430</td>
<td>Bennett &lt;i&gt;et al.&lt;/i&gt; (2018)</td>
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<td>F4257</td>
<td>= AR6 = DNA isolation No. MG 53-4</td>
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<td>Thines &lt;i&gt;et al.&lt;/i&gt; (2009)</td>
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<td>CBS 223.65</td>
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<td>HQ665165</td>
<td>NW012157837</td>
<td>Robideau &lt;i&gt;et al.&lt;/i&gt; (2011) Jiang &lt;i&gt;et al.&lt;/i&gt; (2013)</td>
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**Italian samples — in-situ mud-baiting**

Suspicious pustules were found after 24 d of baiting (on 30 Jan. 2022), growing on the olive VPolv01 and some tomatoes. Pustules on fruit VPolv01 already grew large by 14 Feb. 2022, each roughly 1–2 mm diam, densely covering the fruit surface. They appeared somewhat slimy, with a colour ranging from very light grey to light yellowish brown (Fig. 2C). The specimen FR-0046165 was made from VPolv01 from one half of a pustule with the other half serving as material for sequence analyses.

Pustules appeared on the other olive and tomato fruits, and were collected 9 and 10 d, respectively, after the container was re-baited as described earlier in the methods section. The specimen FR-0046166 was made in the same way as FR-0046165, from one of the olive fruits (fruit code: VPolv05) co-incubated with VPolv01.

**Lahn and Silbersee samples — gross cultures**

Pustules appearing to have been formed by a *Rhipidium* species were first seen on a natural wound located at one of the ends of an alder twig. Though the alder twigs were artificially wounded subsequent to this observation, and more pustules were found on those wounds, the pustules on alder twigs did not thrive as those on the poplar twigs. Therefore, the cultivation on alder twigs was terminated, and the pustules emerging on poplar twigs, which were first found on 30 Jun. 2022 (7 and 5 d after addition of hot-water-treated twigs to Lahn and Silbersee cultures), were subcultured for further study.

These gross cultures were later used for documentation of asexual and sexual reproduction of the obtained *Rhipidium* species. The specimens, FR-0046161, FR-0046162, and FR-0046163, were derived from this culture as described before, and enabled a direct correlation of sequences and morphology. The gross cultures of Lahn could be sustained to date (late May, 2023), while the cultivation of the Silbersee isolate was as first considered not sustainable due to an absence of newly appearing pustules over a few months. However, shortly after the initial manuscript draft was submitted, *Rhipidium* pustules were again found from the Silbersee cultures, with successive growth and reproduction until today (9 Jun. 2023).

**Growth habit and morphology**

The description here is based on the isolates FR-0046161 to FR-0046163, and the two isolates without preserved fungarium specimens LNunk-sub-pn03 and LNunk-sub-pn04, all from Lahn or Silbersee sub-cultures using poplar twigs as substrate. All the isolates measured in the morphological description were confirmed having identical cox2 sequences. Their specific characteristics are given below, and microscopic features presented in Fig. 3.

*Pustules* thrived on the surface of poplar twigs, were usually widely spaced, usually emerging from natural cracks of the twigs, or right at or near the budding points. Well-developed pustules were nearly semi-spherical (Fig. 2D), with a refractive granular surface, colour white, off-white, to light orange-pink, or very pale brown, like seashell and bisque (hexadecimal colour code #fff5ee and #2d2d2b, respectively), when observed with naked eyes or under low magnification through a dissecting microscope (Fig. 2D, E). Pustules were formed by several (often about 30) individual plants (i.e. structure consisting of rhizoid, basal cell, and filamentous branches with or without reproductive organs) in a cluster (Fig. 3N–Q). *Rhizoids* were arbuscular, branched monopodially, hyaline or stained with the colours of the consumed plant substrates, often with thicker “main roots” (Fig. 3S), and decreasing strongly in diameter at the second or third branching order, followed by fine branches in similar width, with blunt or round distal ends (Fig. 3U); occasionally a single “main root” from which distinctly thinner “side roots” branched was formed (Fig. 3T). *Basal cells* were cylindrical, trumpet-like, or clavate, varying in size, 463–992 μm long × 43–110 μm diam (n = 19, as basal cells were difficult to detach in a way that their shape would remain unspoiled), hyaline, normally carrying granules containing consumed plant substances in the respective colours. The basal cells were swollen towards the top, or doubly to quintuply lobed (Fig. 3N, O), but sometimes also branched (Fig. 3P, Q), rarely with secondary or higher order lobes/branches. In case of simple swellings these were often flattened like a platform. The wall of the basal cells was refractive and thickened throughout, but generally thicker towards the apical ends. A constricted site at the basal cell joint towards the rhizoid was often observed, but this feature was also frequently absent. *Filamentous branches* were tubular, cylindrical or clavate, slightly swollen near the distal ends, not tapering towards the bases, with primary filamentous branches varying in size, 39–609 μm long × 10–27 μm diam (n = 100), colourless, sometimes containing granules of plant substances in the colour of the substrate. The filamentous branches emerged from the distal ends of lobes or branches of the basal cell, or irregularly at the crown of the swollen section (or platform), sometimes with extreme differences in length (very long and short) observed on the same basal cell, growing singly (Fig. 3C) or with a second or more proliferations (often once, sometimes twice, Fig. 3D, E), constricted at both the base, beneath reproductive organs, and the distal ends, but not at the budding point of proliferating branches (Fig. 3D, E). Secondary (proliferated) filamentous branches 36–305 μm long × 11–19 μm diam (n = 11, as secondary branches tended to fold, rendering them difficult to measure unambiguously). Though in some cases long primary filamentous branches were observed, often they were rather short and stout, while secondary filamentous branches arising from them were rather long and slender. Without a clear-cut pattern, zoosporangia or oogonia were formed before the proliferation of filamentous branches and at their distal ends. However, at proliferation sites mostly zoosporangia were formed, while at the most distal ends zoosporangia or oogonia were produced. *Zoosporangia* were prolate ellipsoidal (majority), prolate spheroidal, obovoid, globose, ovoid, narrowly obovoid, perprolate ellipsoidal, or obpyriform, 42–90 μm long × 28–61 μm diam (n = 100), hyaline, smooth-walled, mostly solely (Fig. 3C), or doubly (Fig. 3F), rarely triply at the interval or distal ends of filamentous branches, each always sitting on a constriction site (Fig. 3C–F), with a wide distal opening when empty, either with the earlier shape remaining or displaying a partially wrinkled wall. In that case empty zoosporangia displayed multiple fine streaks along the longitudinal axis. Discharge tubes or extending sheaths were not observed, but presumably formed and evanescent, as occasionally short and collapsed residues were observed at the orifice of the sporangia (Fig. 3D). *Zoosporangial tubules* were not observed, as no stimulation method was found to trigger release. *Oogonia* were globose or subglobose, 38–69 μm × 40–68 μm (n = 53), hyaline when immature, hyaline, golden or copper when containing an oospore, with a smooth wall on the outer surface, while the inner surface had an undulate contour due to an uneven
thickening of the oogonial wall (Fig. 3G). Oospores (Fig. 3G–J) were globose or oblate spherical, singly in each oogonium, not filling the oogonium completely. The oospore wall was unevenly thickened leading to sculptured surface with irregular ridges forming a broad reticulum (Fig. 3J), strongly reflective, leading to an asterisk-like (Fig. 3H) or undulate (Fig. 3G) appearance in mid-section view, with a reticulate pattern (Fig. 3I) when focussing between the outer and the inner wall. The size of the oospores was 31–55 μm × 34–55 μm (n = 50) when including the outer wall. Due to the refractive nature the distinction

The placement of *Rhipidium interruptum* (Rhipidiaceae)

The cell lumen of the oospores was globose or oblate spherical, with a smooth outline, 21–35 × 22–34 μm (n = 50), and appeared colourless. Tiny granules were sometimes observed outside the centre in a section-view (Fig. 3H). *Antheridia* were clavate, prolate ellipsoidal, obovoid, or reniform, 19–41 μm long × 14–18 μm diam (n = 7, as antheridia mostly had a deformed appearance after fertilisation), often with granular contents (colour cupper, not filling the entire antheridium), generally adhering to the lower part of oogonium close to the constriction site. Antheridial filaments were stout (Fig. 3M), slender and irregularly bending (Fig. 3L), or sometimes branching (Fig. 3K). The antheridia appeared to be both monoclinous and diclinous (Fig. 3K–M), but the origins were mostly obscure, as antheridial filaments were rather thin-walled and did not retain shape or integrity well after fertilisation. *Constrictions* were located at the base of reproductive organs between them and their supporting branches, wall strongly thickened at the constrictions, refractive two ends of the channel.

**Phylogeny and species delimitation**

Only moderately supported conflicting topologies were observed when comparing phylogenetic reconstructions based on nrlSU and cox2 (Supplementary File 2). This is due to the well-known fact that cox2 is too variable to reveal higher level-relationships by itself (e.g. Choi et al. 2015, Buaya & Thines 2022b). However, the signal in the more conserved first and second codon positions can be harnessed when including a second, more conserved locus. Thus, a concatenated alignment of cox2 and nrlSU was used for inferring the phylogenetic tree shown in Fig. 4. Apart from the five *Rhipidium* isolates derived from this study, 23 reference species, representative of the main orders of *Peronosporomycetes* and *Saprolegniomycetes*, were adopted in the phylogenetic analyses (14 members of *Peronosporales*, one of the *Albuginiales*, two of the *Saprolegniales*, one of the *Leptomatales*, and three of the *Rhipidiales*), while two additional species, *Haliphthoros milfordensis* NJM 0131 and *Haptoglossa zoospora* LEV6507, representing the early-diverging oomycete lineages, served as outgroup. The phylogenetic tree shown in Fig. 4 is based on the topology of the ME reconstruction, with support values of all analyses (ME, ML, BI) added on the branches next to the nodes. The accession numbers of all sequences used in this study are listed in Table 1.

The five *Rhipidium* isolates, FR-0046161, FR-0046163–FR-0046166, formed a monophyletic clade without internal sequence variation in the concatenated tree, with maximum support in all analyses (Fig. 4). This clade was sister to *Sapromyces elongatus* (CBS 213.82). The clade accommodating *Rhipidium* and *Sapromyces* species was also highly supported in the three phylogenetic reconstructions (100 % bootstrap support (BS) in ME, 94 % BS in ML, and 1.0 posterior probability (PP) in BI). The *Rhipidiaceae*, with currently the above-mentioned two representatives of a genus, included, can, thus, be interpreted as monophyletic.

The two *Salispinia* species, with sequences of *Salispinia spinosa* (CBS 591.85) and *Salispinia hoi* (USTCMS 1611) as representatives of the *Salispinaceae*, formed a clade that received maximum support values in all three reconstructions, and was placed sister to the clade representing *Rhipidiaceae*, thereby forming a monophyletic *Rhipidiaceae*. The monophyly of the *Rhipidiales* was strongly supported by all three reconstructions, with 100 % BS in ME, 99 % BS in ML, and 1.0 PP in BI.

**TAXONOMY**

Based on the strongly supported monophyletic clade formed by the *Rhipidium* isolates, with no internal variation, and considering the morphological similarity of the isolates, they are interpreted as belonging to the same species. Comparing to the systematic description of several *Rhipidium* species (Sparrow 1960), the species showed similarities with both *R. interruptum* and *R. americanum*. However, according to Thaxter (1896) these two species can be differentiated by the antheridial characteristics, as the former is usually diclinous, with branching or contorted stalks, while the latter is monoinous (or androgynous) (Thaxter 1896, Sparrow 1960). Thus, the species found in this study has been interpreted as *R. interruptum*, due to the observation of branching antheridial threads (Fig. 3K), and the sometimes-twisted antheridial stalk (Fig. 3L). As a first step towards stabilising the taxonomic treatment of *Rhipidium*, which species and morphological differentiation have been interpreted divergently, an epitype for its type species, *Rhipidium interruptum*, is given below.


*Specimens examined:* Denmark, Nørre Nebel, ditch, on an apple fruit, collected on 16 Nov. 2021, I. Tsai & M. Thines, sub-baiting on 19 Nov. 2021, on an apple fruit, isolated on 14 Jan. 2022, fungarium number FR-0046164. *Italy*, Province of Brescia, Pralboino, ditch, mud, collected on 31 Dec. 2021, I. Tsai & M. Thines, mud-baiting on 6 Jan. 2022, on an olive fruit, isolated on 14 Feb. 2022, fungarium number FR-0046165. Isolates derived from subculturing of the aforementioned source: from an olive fruit, isolated on 25 Feb. 2022, fungarium number FB-0046166; as well as from a tomato fruit, isolated on 26 Feb. 2022, laboratory isolate code VPtm01. *Germany*, Gießen, river Lahn, on a twig, collected on 28 May 2022, M. Thines, gross culture established on 20 Jun. 2022, on a poplar twig, isolates obtained from the gross culture on 6 Jul. 2022, fungarium number FR-0046161, FR-0046162; as well as on 15 Sep. 2022, laboratory isolate codes LNunk-sub-pn03, LNunk-sub-pn04; Gießen, lake Silbersee, on a twig of *Salix sp.*, collected on 28 May 2022, M. Thines, gross culture established on 20 Jun. 2022, on a poplar twig,
isolate obtained from the gross culture on 6 Jul. 2022, fungarium number FR-0046163.

**Typification:** **Germany,** M. von Minden, Mykolog. Untersuch. Berichte, 1916; plate 2, fig. 9–20, **lectotype** (iconotype) designated by Cejp (1959). **Germany,** Gießen, Lahn, May 2022, I. Tsai & M. Thines, LNunk-sub-pn01 **(epitype) designated here,** MBT10012614, voucher deposited in the Fungarium Senckenbergianum, accession number FR-0046161).

**Notes:** The observed characteristics of the isolates LNunk-sub-pn01 (FR-0046161), LNunk-sub-pn02 (FR-0046162), LNunk-sub-pn03, and LNunk-sub-pn04, matched the depiction of von Minden (1916), although zoospores release and germination could not be observed in the current study. However, based on all the accessible features, these isolates can be considered as representative for the species *R. interruptum*. As LNunk-sub-pn01 provided the best collection of characters amongst all the four isolates, its specimen (FR-0046161) was designated as the epi-type of the species.

**DISCUSSION**

In several previous studies, it was suggested that there is a need to include more representatives of the *Rhipidiales* in future phylogenetic reconstructions (Riethmüller et al. 2000, Hudspeth et al. 2000, Petersen & Rosendahl 2000, Dick 2001). However, it took more than 15 yr, until Li et al. (2016) and Bennett et al. (2018) included *Salispina* species (*Salispinaceae*) into the oomycete phylogeny, and Bennett et al. (2018) could reclassify them to the *Rhipidiales* in their own family, *Salispinaceae*. However, *Sapromyces elongatus* remained the sole taxon representing *Rhipidiaceae*. With the acquisition of documentable *Rhipidium* isolates in this study, the *Rhipidiaceae* were finally represented by taxa of *Rhipidiaceae* that span the entire spectrum of the family, from the genus *Sapromyces* that features only weakly differentiated basal cells to the genus *Rhipidium*, which arguably forms the most pronounced differentiation between rhizoids, basal cells, filamentous branches, and reproductive organs. *Sapromyces* and *Rhipidium* together formed a well-supported monophyletic clade in this study, sister to another well-supported lineage, the *Salispinaceae*. In line with Bennett et al. (2018) the *Rhipidiales*
was strongly supported as monophyletic. However, as Bennett et al. (2018) did not include early-diverging oomycete lineages that could serve as an outgroup, and no member of the Leptomitales was included, the question of whether or not Rhipidiales can be considered members of the Peronosporomycetes (Beakes & Thines 2017) could not be resolved in that study.

The phylogeny shown in this study is overall consistent with previous studies (Thines et al. 2009, Thines 2014, Li et al. 2016, Bennett et al. 2018), though based on a distinct or partially different dataset. The combination of nrLSU and cox2 seems to be highly suited to clarify oomycete phylogenetic relationships on various levels (Thines et al. 2009). Bennett et al. (2018) additionally included coxl in their phylogenetic study, with a similar resolution as Thines et al. (2009). In line with Chet et al. (2015) we assume that both cox1 and cox2 show a high discriminatory power towards species level, while the resolution on higher levels still seems to be satisfactory. However, the wider phylogenetic sampling available for cox2 sequences renders them more suitable to resolve global phylogenetic patterns. Also nrSSU that was previously used, e.g. in the study of Li et al. (2016), and resulted in a reconstruction that inferred the monophyly of Rhipidiales with moderate to high support. Though the locus seems to provide a good resolution in delimitation of species of early diverging holocarpic oomycetes (Buaya et al. 2019b, 2021b, Buaya & Thines 2020), nrLSU sequences seem to be better suited to resolve relationships in the “crown group” of oomycetes, the Peronosporomycetes and Saprolegniomycetes, which is the reason they were used in the current study. In the present study, the closely related species Salisapila sapoeloensis and Salisapila tartarea, as well as Saprolegnia ferox and Saprolegnia parasitica could be differentiated, while at the same time, no intraspecific variation was observed within Rhipidium interruptum, supporting that all isolates reported on in this study are representatives of a single species.

Rhipidium interruptum was first introduced by Cornu (1871), described with "L’une [espèce] présente des filaments munis de nombreux étranglements (the one [species] has filaments with numerous constrictions)”, which was distinguished from another species, Rhipidium continuum, stating "L’autre n’en a jamais qu’un seul [étranglement] à la base de chaque filament (the other has but a single [constriction] at the base of each filament)”. Apart from this, both species resembled each other, with a stellate oospore in mid-section-view. Thus, the two species were exclusively differentiated based on the formation of constrictions. Some years later, van Tieghem provided a sketch of R. interruptum (van Tieghem 1884, fig. no. 617, p. 1024). Subsequently, Thaxter (1896) found Rhipidium in North America and assigned his samples to a new species, Rhipidium americanum, based on non-branched monoclinous antheridia that attached to the base of the oogonium below which they originated. After two decades in which Rhipidiales did not receive much attention, von Minden (1916) scrutinised Rhipidium in Germany and proposed Rhipidium europaeum as a new name to accommodate and replace both R. interruptum and R. continuum, since the specimen he had found at that time featured filamentous branches both with and without multiple constructions. Thus, both names were no longer representative to the species, and the character was assumed to be influenced by environmental conditions (Kolkwitz et al. 1915, von Minden 1916). Kanouse (1927a, 1927b) and Sparrow (1936) reported the occurrence of R. europaeum in Michigan and Cambridge, respectively, picking up the revision of von Minden (1916). Paradoxically, by reporting R. europaeum in North America, they rendered this name as ambiguous as von Minden (1916) had found R. interruptum ambiguous. A few decades later, Sparrows (1960) gave a general description of R. interruptum in his account Aquatic Phycomycetes, in which R. europaeum and its varieties as well as R. continuum were synonymised with R. interruptum. Cejpr (1959) designated a lectotype (iconotype) for R. interruptum, based on von Minden's illustration (1916, plate 2), which was also recognised by Dick (2001) in his systematic account Straminipilous Fungi. However, heretofore no actual specimen had been designated as an epitype, leaving the interpretation of the reportedly highly variable type species of Rhipidium (Sparrow 1960) ambiguous. However, it is essential for the interpretation of variation and potentially existing similar species to pinpoint R. interruptum to a specimen for which sequence data are available, which is the reason why an epitype for R. interruptum was designated in this study.

It is noteworthy to mention that – when considering the observed variation in R. americanum and R. interruptum, the two species differ only in one character to distinguish them, which is in the development of antheridia. In the former they are monoclinous, and the latter diclinous with bending, twisting, or often branched stalks (Thaxter 1896, Kanouse 1927b, Sparrow 1936, 1960). In this study, the deterioration of antheridial structures after fertilisation rendered the unambiguous identification of the origin of antheridia difficult. Still, both openly branching (Fig. 3K) and unbranched bending (Fig. 3L) antheridial stalks were observed, necessitating the interpretation of the species as R. interruptum. However, it is noteworthy that Kanouse (1927b) illustrated a Rhipidium species she interpreted as R. americanum due to the formation of monoclinous antheridia arising directly below the oogonium. However, she showed that antheridia could also be branched instead of forming the typical short-stalked and unbranched form. Considering the high degree of variation observed in previous studies (e.g. von Minden 1916) and in the current one, it seems both possible that R. americanum is an independent species capable of shifting some characters, e.g. to cope with environmental conditions, or that R. americanum is conspecific with R. interruptum, with the formation of diclinous vs monoclinous antheridia being responses to different environmental conditions of the same species. Thus, it seems to be advisable to carry out further molecular and morphological studies including more isolates from both Europe and North America as well as testing variation incited by various environmental conditions (von Minden 1916), to investigate the intraspecific variation and environmental plasticity of Rhipidium species.

However, an investigation of Rhipidiaceae is still challenging due to the difficulty of gross and pure cultivation, which is additionally hampered by the difficulty of triggering zoospore release, which was rarely observed in the laboratory (Matthews 1936). Also, the low number of reports for some species and genera, e.g. Mindeniella (Kanouse 1927a, Sparrow & Cutter 1941), renders targeted sampling difficult, especially, as in many areas, land use has changed dramatically over the past century. Thus, while this study marks a first step into investigating Rhipidiaceae in more details by providing sequence data of the type of Rhipidium, R. interruptum, designating an epitype for it, and establishing a procedure for maintaining gross cultures with poplar twigs now surviving for almost 1 yr, it is clear that the current study is only the beginning of collecting the missing pieces of the puzzle of rhipidialean species evolution and their phylogenetic placements in the oomycete tree of life.
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Conflict of interest: The authors declare that there is no conflict of interest.

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The placement of *Rhipidium interruptum* (**Rhipidiaceae**)


**Supplementary Material:** http://fuse-journal.org/

**Supplementary File 1.** Sequence alignment of concatenated dataset. The full length of dataset is 1153 bp. Sections: *cox2* positions 1–437, *nrLSU* positions 438–1153.

**Supplementary File 2.** Phylogenetic reconstructions (in Minimum Evolution) for the individual loci. Bootstrap support values from Minimum Evolution and Maximum Likelihood, and posterior probabilities from Bayesian Inference are given on the branches next to the nodes in the respective order. A minus sign denotes an alternate but not highly supported topology (BS < 80 % or PP < 0.98). An “x” represents alternate highly supported topology (BS > 80 % or PP > 0.98). The isolates of *Rhipidium interruptum* derived from this study are marked in bold. The scale bars represent the number of substitutions per site.
INTRODUCTION

Although the Fungi are highly diverse and estimated to represent between 2.2 and 3.8 million species, only around 150,000 species have been described to date. Fungi are essential for ecosystem processes, and are of economic importance as plant, human or animal pathogens, or as agents for industrial or pharmaceutical industries (Lücking et al. 2021). In spite of their importance, only 2,000–2,500 species of fungi are described annually, illustrating a great challenge to understand and preserve this important biodiversity resource. Resolving this problem relies on increased efforts to collect, preserve and describe novel species (Cheek et al. 2020). In order to facilitate the description of novel species, the New and Interesting Fungi (NIF) series is published annually.

Key words: biodiversity; ITS barcodes; multi-gene phylogeny; new taxa; systematics; typification.

Abstract: Three new genera, six new species, three combinations, six epitypes, and 25 interesting new host and/or geographical records are introduced in this study. New genera: Neoleptodontium (based on Neoleptodontidium aquaticum), and Nothoramularia (based on Nothoramularia ragnhildianicola). New species: Acremonium aquaticum (from cooling pad water, USA), Cladosphialophora lariicola (on dead wood of Larix sp., Netherlands), Cyphellophora neerlandica (on lichen on brick wall, Netherlands), Geonectria muralis (on moss growing on a wall, Netherlands), Harposporium illinoisense (from rockwool, USA), and Neoleptodontium aquaticum (from hydroponic water, USA). New combinations: Cyphellophora deltoidea (based on Anthopis deltoidea), Neoleptodontidium aciculare (based on Leptodontidium aciculare), and Nothoramularia ragnhildianicola (based on Ramularia ragnhildianicola). Epitypes: Cephaliophora trapaica (from water, USA), Miricatena prunuclea (on leaves of Prunus serotina, Netherlands), Nothoramularia ragnhildianicola (on Ragnhildiana ferruginea, parasitic on Artemisia vulgaris, Germany), Phyllosticta multicorniculata (on needles of Abietis balsamea, Canada), Thyronecchia caraganae (on twigs of Coragana arborescens, Ukraine), and Trichosphaeria pilosa (on decayed Salix branch, Netherlands). Furthermore, the higher order phylogeny of these genera regarded as incertae sedis is resolved, namely Cephaliophora (Ascoscleridinae, Pezizales), Miricatena (Helotiales, Leotiomycetes), and Trichosphaeria (Trichosphaericae, Trichosphaeriales), with Trichosphaeriacae being an older name for Plectosphaerellaceae.


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in the journal *Fungal Systematics and Evolution*. Papers include the description of new species, report new host or geographical records, and new sexual-asexual connections. These and the present study also include validations (typifications) of fungal taxa and list interesting observations relating to fungi and their biology.

**MATERIALS AND METHODS**

**Isolates**

Samples (see Table 1) were treated as previously detailed (Crous et al. 2019c). Single conidial colonies were established on Petri dishes containing 2% malt extract agar (MEA) as described by Crous et al. (1991), and single ascospore cultures were established following the method described by Crous (1998). Colonies were sub-cultured on 2% potato dextrose agar (PDA), oatmeal agar (OA), MEA (Crous et al. 2019c), or autoclaved pine needles on 2% tap water agar (PNA) (Smith et al. 1996), and incubated at 25°C under continuous near-ultraviolet light to promote sporulation. Reference strains and specimens of the studied fungi are maintained in the culture collection and fungarium (CBS) of the Westerdijk Fungal Biodiversity Institute (WI), Utrecht, the Netherlands.

**DNA extraction, amplification (PCR) and phylogeny**

Fungal mycelium (Table 1) was scraped from the surface of agar cultures with a sterile scalpel and the genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturers’ protocols. All loci were amplified following previously published protocols. The first part of the 28S nrDNA gene (LSU) and complete internal transcribed spacer regions with intervening 5.8S nrDNA gene (ITS) of the nrDNA operon were sequenced for all the isolates included in this study (for amplification conditions, see Fan et al. 2018). Other loci were sequenced for various species or genera using primers and conditions specific for those groups of fungi. Amplification of the partial DNA-directed RNA polymerase II second largest subunit gene (*rpb2*), the partial translation elongation factor 1-alpha gene (*tef1*, first part) and the partial beta-tubulin gene (*tub2*) followed Klaubauf et al. (2018), while amplification of the partial actin gene (*actA*), the partial glyceraldehyde-3-phosphate dehydrogenase gene (*gapdh*) and the partial histone H3 gene (*his3*) followed Videira et al. (2016). Amplification of the partial DNA-directed RNA polymerase II largest subunit gene (*rpb1*) followed Klabauf et al. (2014), and the partial translation elongation factor 1-alpha gene (*tef1*, second part) followed Réblova et al. (2020). The first part of the 18S nrDNA gene (SSU) was amplified as described by Hernández-Restrepo et al. (2020). The resulting fragments were sequenced in both directions using the respective PCR primers and the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA, USA); DNA sequencing amplicons were purified through Sephadex G-50 Superfine columns (Sigma-Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were analysed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA, USA). The DNA sequences were analysed and consensus sequences were computed using Geneious Prime v. 2022.0.2 (http://www.geneious.com, Kearse et al. 2012).

The sequences for each gene region were subjected to megablast searches (Zhang et al. 2000) to identify closely related sequences in the NCBI’s GenBank nucleotide database. The results are provided as part of the species notes or as selected phylogenetic trees. Maximum-likelihood (ML) phylogenetic trees were constructed generated using IQ-TREE v. 2.1.3 (Nguyen et al. 2015) and branch support values were calculated with 1,000 non-parametric bootstrap replicates and optimal model finding using the TESTNEW option using ModelFinder (Kalyaanamoorthy et al. 2017) as implemented in IQ-TREE. Bayesian analyses were performed with MrBayes v. 3.2.7a (Ronquist et al. 2012) as explained in Braun et al. (2018), while RAXML v. 8.0.0.0 (Stamatakis 2014) was used with default parameters to provide additional ML support values for selected trees, while parsimony analyses using PAUP* v. 4.0a build 168 (Swofford et al.) were performed as explained in Videira et al. (2016). All resulting trees were printed with Geneious Prime v. 2022.0.2 and the layout of the trees was done using Adobe Illustrator 2022 v. 26.3.1. Sequences derived in this study were submitted to GenBank (Table 1) and the alignments and phylogenetic trees in figshare. (doi: 10.6084/m9.figshare.23447330). The optimal identity thresholds to discriminate filamentous fungal species followed Vu et al. (2019).

**Morphology**

Slide preparations were mounted in lactic acid, Shear’s mounting fluid, Melzer’s solution, or water, from colonies sporulating on MEA, PDA, PNA or OA. Observations were made with a Nikon SMZ25 dissection microscope, and with a Zeiss Axios Imager 2 light microscope using differential interference contrast (DIC) illumination and images recorded on a Nikon DS-Ri2 camera with associated software. Colony characters and pigment production were noted after 2–4 wk of growth on MEA, PDA and OA (Crous et al. 2019c) incubated at 25°C. Colony colours (surface and reverse) were scored using the colour charts of Rayner (1970). Taxonomic novelties were submitted to MycoBank (www.MycoBank.org; Crous et al. 2004).

**RESULTS**

**Phylogeny**

Phylogenetic trees were generated for the taxonomic novelties, or to better clarify the position of a taxon in a broader context where needed. These trees are discussed in the species notes and the statistics associated with the phylogenetic analyses presented in this study are provided in supplementary Table S1.

**Taxonomy**

*Acremonium aquaticum* Crous & Jurjević, *sp*. nov. MycoBank MB 848820. Fig. 1.

**Etymology:** Name refers to the fact that it was isolated from water.

*Mycelium* consisting of hyaline, smooth, branched, septate, 1.5–2 µm diam hyphae. *Conidiophores* reduced to conidiogenous cells, solitary, erect, subcylindrical with apical taper, hyaline, smooth, phialidic, 20–30 × 1.5–2 µm. *Conidia* in long, unbranched...
Table 1. Collection details and GenBank accession numbers of isolates treated in this study, and associated ex-type strains where available. Species for which additional sequences were generated during the course of this study are also listed here. Novel GenBank accession numbers are indicated in bold font.

<table>
<thead>
<tr>
<th>Species</th>
<th>Culture or voucher accession number(s)</th>
<th>Locality and Substrate</th>
<th>Collector(s) and collection date</th>
<th>GenBank accession number(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acremonium aquaticum, sp. nov.</strong></td>
<td>CBS 149454 = CPC 42867, ex-type</td>
<td>USA: Cooling pad water from greenhouse</td>
<td>Z. Jurjević, 4 Oct. 2021</td>
<td>OQ990087, OQ990041, OQ989208, OQ989251</td>
</tr>
<tr>
<td><strong>Acrostalagmus luteoalbus</strong></td>
<td>CBS 149685 = CPC 43187</td>
<td>South Africa: <em>Portulacaria afr</em> , leaf</td>
<td>P.W. Crous, 27 Feb. 2022</td>
<td>OQ990088, OQ990042, OQ989209, OQ990238</td>
</tr>
<tr>
<td><strong>Biscogniauxia anceps</strong></td>
<td>CBS 149687 = CPC 43197</td>
<td>Spain: <em>Eucalyptus</em> sp., bark</td>
<td>M.A. Delgado, 25 Mar. 2022</td>
<td>OQ990096, OQ990050, OQ989210, OQ990239</td>
</tr>
<tr>
<td><strong>Cephalophora tropica</strong></td>
<td>CBS 149457 = CPC 42877, ex-epitype</td>
<td>USA: Pan water of crocodile farm</td>
<td>Z. Jurjević, 24 Nov. 2021</td>
<td>OQ990097, OQ990051, OQ989211, OQ989258</td>
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<tr>
<td><strong>Ceratocystis ficiola</strong></td>
<td>CBS 149669 = CPC 44213</td>
<td>Italy (Sicily): Trunk necrosis in <em>Ficus carica</em></td>
<td>G. Polizzi, 2022</td>
<td>OQ990098, OQ990051, OQ989211, OQ989258, OQ989240, OQ990099</td>
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<td><strong>Chloridium caudigerum</strong></td>
<td>CBS 149688 = CPC 42899</td>
<td>Netherlands: <em>Ulmus</em> sp., branch</td>
<td>E.R. Osieck, 19 Feb. 2022</td>
<td>OQ990100, OQ990052, OQ989259, OQ989241</td>
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<tr>
<td><strong>Cladophialophora laricicola, sp. nov.</strong></td>
<td>CBS 149944 = CPC 41384, ex-type</td>
<td>Netherlands: <em>Larix</em> sp., dead wood</td>
<td>J. Boers, 16 Mar. 2021</td>
<td>OQ990101, OQ990053, OQ989242</td>
</tr>
<tr>
<td><strong>Cylindromonium eugeniicola</strong></td>
<td>CBS 149607 = CPC 37170, ex-type</td>
<td>South Africa: <em>Eugenia capsensis</em>, leaf litter</td>
<td>M.J. Wingfield, 2010</td>
<td>OQ990102, OQ990054, OQ989259, OQ989229</td>
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<tr>
<td><strong>Cyphellophora deltoidea, comb. nov.</strong></td>
<td>CBS 149669 = CPC 43126</td>
<td>Spain (Gran Canaria): <em>Eucalyptus</em> sp., dead twig</td>
<td>A.L. van Iperen, 1 Apr. 2022</td>
<td>OQ990103, OQ990055, OQ989259, OQ989229</td>
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<td><strong>Cyphellophora neerlandica, sp. nov.</strong></td>
<td>CBS 149512 = CPC 42634, ex-type</td>
<td>Netherlands: Lichen on brick wall</td>
<td>J. Boers, 12 Nov. 2021</td>
<td>OQ990089, OQ990043, OQ989252</td>
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<td>Species</td>
<td>Culture or voucher accession number(s)</td>
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<td><strong>Didymella brevipilosa</strong></td>
<td>CPC 42641</td>
<td>Netherlands: Lichen on brick wall</td>
<td>J. Boers, 12 Nov. 2021</td>
<td>OQ990090, OQ990044 – OQ989253</td>
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<tr>
<td></td>
<td>CBS 149049 = CPC 41600</td>
<td>Canada: <em>Abies balsamea</em>, buds</td>
<td>D. Malloch, 4 May 2021</td>
<td>OQ990105, OQ990057 – – –</td>
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<tr>
<td><strong>Drepanopeziza populii-albae</strong></td>
<td>CBS 149510 = CPC 42336</td>
<td>Russia: <em>Populus alba</em></td>
<td>T.S. Bulgakov, 26 Jun. 2021</td>
<td>OQ990106, OQ990058 – – –</td>
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<tr>
<td></td>
<td>CBS 149070 = CPC 41602</td>
<td>Canada: <em>Abies balsamea</em>, buds</td>
<td>D. Malloch, 4 May 2021</td>
<td>OQ990110, OQ990062 – – –</td>
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<td></td>
<td>UAMH 10297, ex-type</td>
<td>USA: <em>Populus tremuloides</em>, twig</td>
<td>A. Tsuneda, 7 Aug. 2001</td>
<td>NR_121303, NG_059198 – – –</td>
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<tr>
<td><strong>Fusariella atrovirens</strong></td>
<td>CBS 149690 = CPC 43304</td>
<td>Namibia: Lichenicolous on unknown lichen growing on rock</td>
<td>P.W. Crous, 4 Apr. 2022</td>
<td>OQ990107, OQ990059 – OQ989213 tef1 (second part): OQ989243</td>
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<tr>
<td><strong>Fusariella hughesii</strong></td>
<td>CBS 149074 = CPC 41594</td>
<td>Ukraine: <em>Adonis vernalis</em>, overwintered stems</td>
<td>A. Akulov, 11 Apr. 2021</td>
<td>OQ990108, OQ990060 – – –</td>
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<tr>
<td><strong>Geonectria muralis, sp. nov.</strong></td>
<td>CBS 149515 = CPC 42404, ex-type</td>
<td>Netherlands: Moss growing on the bottom part of wall</td>
<td>J. Boers, 7 Sep. 2021</td>
<td>OQ990109, OQ990061 – – –</td>
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<tr>
<td></td>
<td>CPC 42405</td>
<td>Netherlands: Moss growing on the bottom part of wall</td>
<td>J. Boers, 7 Sep. 2021</td>
<td>OQ990110, OQ990062 – – –</td>
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<tr>
<td></td>
<td>CPC 42406</td>
<td>Netherlands: Moss growing on the bottom part of wall</td>
<td>J. Boers, 7 Sep. 2021</td>
<td>OQ990111 – – – –</td>
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<tr>
<td><strong>Harposporum illinoisensis, sp. nov.</strong></td>
<td>CBS 149456 = CPC 42872, ex-type</td>
<td>USA: Rockwool</td>
<td>Z. Jurjević, Oct. 2021</td>
<td>OQ990112, OQ990063 – OQ989214 – OQ989262 actA: OQ989191, tef1 (second part): OQ989244</td>
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<tr>
<td><strong>Microcera physciae</strong></td>
<td>CBS 148283 = CPC 41284, ex-type</td>
<td>Netherlands: <em>Physcia tenella</em></td>
<td>J. Boers, 10 Mar. 2021</td>
<td>NR_175225, NG_081335, OK651168, OK651208</td>
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<tr>
<td><strong>Mircatena prunicola</strong></td>
<td>CBS 149448 = CPC 42627, ex-epitype</td>
<td>Netherlands: <em>Prunus serotina</em>, leaves</td>
<td>E. Slootweg, 7 Nov. 2021</td>
<td>OQ990115, OQ990066 – – –</td>
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<tr>
<td></td>
<td>CBS 149455 = CPC 42868, ex-type</td>
<td>USA: Hydroponic water in greenhouse</td>
<td>Z. Jurjević, 4 Oct. 2021</td>
<td>OQ990116, OQ990067 – – – –</td>
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<tr>
<td></td>
<td>CPC 42875</td>
<td>USA: Greenhouse peet</td>
<td>Z. Jurjević, 4 Oct. 2021</td>
<td>OQ990117, OQ990068 – – – –</td>
</tr>
<tr>
<td><strong>Neoleptodontidium aquaticum, gen. et sp. nov.</strong></td>
<td>CBS 123.86, ex-type</td>
<td>India: <em>Rotten wood</em></td>
<td>V. Rao, Jan. 1984</td>
<td>MH861931, MH873620</td>
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<td></td>
<td>CBS 149075 = CPC 42463</td>
<td>Germany: On <em>Ragnhildiana ferruginea</em>, parasitic on <em>Artemisia vulgaris</em></td>
<td>J. Kruse, 7 Sep. 2021</td>
<td>OQ990119, OQ990070 – – – –</td>
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<tr>
<td>Species</td>
<td>Culture or voucher accession number(s)¹</td>
<td>Locality and Substrate</td>
<td>Collector(s) and collection date</td>
<td>GenBank accession number²</td>
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<tr>
<td><em>Ophiognomonia setacea</em></td>
<td>CBS 149076 = CPC 42462, ex-epitype</td>
<td>Germany: On <em>Ragnhildiana ferruginea</em>, parasitic on <em>Artemisia vulgaris</em></td>
<td>J. Kruse, 7 Sep. 2021</td>
<td>OQ990118 OQ990069 – – –</td>
</tr>
<tr>
<td></td>
<td>CBS 859.79, ex-epitype</td>
<td>Switzerland: Quercus sp.</td>
<td>M. Monod, 8 May 1979</td>
<td>AY818958 AY818962 – – tef1 (second part): JQ414154</td>
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<tr>
<td><em>Paracutypella citricola</em></td>
<td>CBS 149693 = CPC 43208</td>
<td>Spain: Bark of unknown tree</td>
<td>26 Jan. 2022, J. Castillo</td>
<td>OQ990122 OQ990073 OQ989217 OQ989264 –</td>
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<tr>
<td></td>
<td>CBS 149078 = CPC 41919</td>
<td>Canada: <em>Abies balsamea</em>, buds</td>
<td>D. Malloch, 4 May 2021</td>
<td>OQ990124 OQ990075 – –</td>
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<tr>
<td></td>
<td>CBS 149077 = CPC 41921, ex-epitype</td>
<td>Canada: <em>Abies balsamea</em>, buds</td>
<td>D. Malloch, 4 May 2021</td>
<td>OQ990125 OQ990076 – –</td>
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<tr>
<td><em>Phlylosticta multicorculata</em></td>
<td>CBS 149664 = CPC 44105</td>
<td>UK: <em>Allium schoenoprasum</em></td>
<td>P.W. Crous, May 2022</td>
<td>OQ990126 OQ990077 OQ989219 – –</td>
</tr>
<tr>
<td></td>
<td>MFLUCC 17-0464, ex-type</td>
<td>China: Saprobic on decaying wood submerged in Jinsha River</td>
<td>H.Y. Su, Apr. 2015</td>
<td>– – – –</td>
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<tr>
<td><em>Pleurothecilla aquatica</em></td>
<td>CBS 145564 = CPC 35443, ex-type</td>
<td>Italy: <em>Pistacia lentiscus</em>, leaves</td>
<td>P.W. Crous, 13 Apr. 2018</td>
<td>SSU: MF399220</td>
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<tr>
<td></td>
<td>CBS 149695 = CPC 44110</td>
<td>Netherlands: <em>Juncus effusus</em>, stems</td>
<td>P.W. Crous &amp; S. Denman, 14 May 2022</td>
<td>– – – –</td>
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<tr>
<td><em>Ruptoseptoria unedonis</em></td>
<td>CBS 149697 = CPC 44069</td>
<td>UK: <em>Arbutus unedo</em>, leaf spot</td>
<td>P.W. Crous &amp; S. Denman, 14 May 2022</td>
<td>– – – –</td>
</tr>
<tr>
<td><em>Schizothecium conicum</em></td>
<td>CBS 149695 = CPC 44110</td>
<td>Netherlands: <em>Juncus effusus</em>, stems</td>
<td>E.R. Osiack, 28 Apr. 2022</td>
<td>– – – –</td>
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<tr>
<td><em>Sporidesmiella pini</em></td>
<td>CBS 148302 = CPC 40067, ex-type</td>
<td>Netherlands: <em>Pinus sylvestris</em>, dead culms</td>
<td>A.L. van Iperen, 1 Nov. 2020</td>
<td>– – – –</td>
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<td></td>
<td>CBS 149045 = CPC 41495</td>
<td>Netherlands: <em>Juncus effusus</em>, dead culms</td>
<td>E.R. Osiack, 9 Mar. 2021</td>
<td>– – – –</td>
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<tr>
<td></td>
<td>CPC 41494</td>
<td>Netherlands: <em>Juncus effusus</em>, dead culms</td>
<td>E.R. Osiack, 9 Mar. 2021</td>
<td>– – – –</td>
</tr>
</tbody>
</table>

¹ accession number(s) for the type strain. ² GenBank accession numbers for the ITS, LSU, rpb2, tub2, and actA loci.
Crous et al.

Notes: Acremonium and allied genera were recently revised by Hou et al. (2023). Acremonium aquaticum is phylogenetically (92% bootstrap support; Fig. 2) closely related to A. charticola (conidia in mucoid heads, 3.2–4.5 × 1.4–2.0 μm; Gams 1971) but is morphologically distinct in having larger conidia that are formed in chains.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Acremonium sp. from indoor plaster in Russia (strain tk2, GenBank LT549084.1; Identities = 537/537 (100 %), no gaps), Acremonium charticola (strain CBS 881.73, GenBank A621774.1; Identities = 472/506 (93 %), 11 gaps (2 %)), and Acremonium alternatum (strain NIOSN M-120, GenBank MG589592.1; Identities = 412/446 (92 %), six gaps (1 %)). It is also identical to isolates from a pine tree in South Korea (GenBank MK848676.1), a tempea painting on canvas in Slovenia (GenBank MZ687371.1), soil collected in a 40-year-old Pinus merkusii forest in Viet Nam (GenBank MW504687.1), from Ageratina adenophora in China (GenBank MK304178.1), and marine sediment in China (GenBank KX098125.1). Closest hits using the LSU sequence were Acremonium charticola (strain CBS 881.73, GenBank MH872552.1; Identities = 817/823 (99 %), two gaps (0 %)), Acremonium sordidulum (strain SP17, GenBank MZ269296.1; Identities = 814/822 (99 %), one gap (0 %)), and Acremonium alternatum (strain MUT<ITA> 6246, GenBank MN947574.1; Identities = 792/800 (99 %), one gap (0 %)). Closest hits using the actA sequence had highest similarity to Tilachlidium brachiatum (strain CBS 505.67, GenBank KM231249.1; Identities = 543/637 (85 %), 20 gaps (3 %)), Acremonium chrysogenum (no strain number specified, GenBank AF056976.1; Identities = 558/658 (85 %), 35 gaps (5 %)), and Acremonium sp. from Leymus chinensis in China (strain 324, GenBank JN836732.1; Identities = 587/633 (93 %), 15 gaps (2 %)). Closest hits using the rbp2 (first part) sequence had highest similarity to Acremonium alternatum (strain AFLCIL 1396, GenBank F1238366.1; Identities = 573/676 (85 %), 11 gaps (1 %)), and Caespitomonium euphorbiace (culture CPC 39083, GenBank OK651157.1; Identities = 627/866 (72 %), 42 gaps (4 %)). Closest hits using the tef1 (second part) sequence had highest similarity to Acremonium charticola (strain 06700, GenBank KT878367.1; Identities = 722/753 (96 %), no gaps), Amphichorda guana (strain LCS519, GenBank KX855212.1; Identities = 696/748 (93 %), no gaps), and Acremonium sclerotogenum (strain 06239, GenBank KT783358.1; Identities = 712/769 (93 %), five gaps (0 %)). No significant hits were obtained using the tub2 sequence.

Authors: P.W. Crous, J.Z. Groenewald, Z. Jurjević & S. Balashov

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Acrostalagmus luteoalbus (Link) Zare et al. [as ‘luteo-albus’], *Mycol. Res.* **108**: 581. 2004. Fig. 3.

**Description and illustration:** Zare et al. (2004).

**Material examined:** South Africa, Western Cape Province, Kirstenbosch, on leaf of Portulacaria afra (*Portulacaceae*), 27 Feb. 2022, PW. Crous, HPC 3862, culture CPC 43187 = CBS 149685.

**Notes:** Acrostalagmus luteoalbus was recently reported as a major constituent of the mixed mycobiota in the wet cork liner of a water-damaged outdoor wall, and also from indoor dust in Finland (Andersson et al. 2021). The present record represents a new report from South Africa, where it was isolated from a leaf of *Portulacaria afra*.

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to numerous sequences of *Acrostalagmus luteoalbus* (e.g. EF_332, GenBank MT528981.1; Identities = 502/502 (100 %), no gaps), and *Nectria inventa* (e.g. strain CBS 388.65, GenBank MH858627.1; Identities = 502/502 (100 %), no gaps). Closest hits using the LSU sequence were *Nectria*.
inventa (strain CBS 236.55, GenBank MH869007.1; Identities = 855/855 (100 %), no gaps), Acrostalagmus luteoalbus (strain MUT<ITA> 4778, GenBank KP671745.1; Identities = 855/855 (100 %), no gaps), and Acrostalagmus annulatus (strain CBS 121213, GenBank LR025806.1; Identities = 855/855 (100 %), no gaps). Closest hits using the rpb2 (first part) sequence had highest similarity to Acrostalagmus luteoalbus (strain CBS 112.16, GenBank LR026101.1; Identities = 742/743 (99 %), no gaps), and Acrostalagmus annulatus (strain CBS 121213, GenBank LR026108.1; Identities = 738/743 (99 %), no gaps). Closest hits using the tef1 (second part) sequence had highest similarity to Acrostalagmus luteoalbus (strain CBS 388.65, GenBank LR026372.1; Identities = 787/788 (100 %), no gaps), Acrostalagmus annulatus (strain CBS 121213, GenBank LR026378.1; Identities = 782/789 (99 %), no gaps), and Verticillium zaregamsianum (strain V202, GenBank LR026378.1; Identities = 782/786 (99 %), no gaps). On dead culms of Juncus effusus (Juncaceae), 28 Apr. 2022, E.R. Osieck, HPC 3962 = WI-55#4461, cultures CPC 44107, 44106; Utrecht Province, Nieuw-Wulten, north of Houten, 1.5 m a.s.l., 52°08'25”N, 05°10'34”E, on dead culms of J. effusus, 9 Dec. 2021, E.R. Osieck, HPC 3812 = WI-42#4355, culture CPC 42686.

Notes: Appendopyricularia, based on A. juncicola, was introduced as a new hyphomycete genus occurring on culms of Juncus effusus in the Netherlands (Crous et al. 2022b). This is the first record of this taxon also occurring on culms of Carex elongata.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence of CPC 42686 had highest similarity to Appendopyricularia juncicola (strain CPC 41278, GenBank NR_182605.1; Identities = 500/501 (99 %), one gap (0 %)), Thyridium pluriloculosum (strain GZU1FR21.876, GenBank OK493561.1; Identities = 402/481 (84 %), 25 gaps (5 %)), and Phialemonium dimorphosporum (strain SLE, GenBank DQ403199.1; Identities = 412/495 (83 %), 30 gaps (6 %)). The ITS sequence of CPC 42686 is identical to those of CPC 44053, 44055, 44106 and 44107 (508/508, 508/508, 502/502 and 508/508 nucleotides, respectively). Closest hits using the LSU sequence of CPC 42686 were Appendopyricularia juncicola (strain CPC 41278, GenBank NG_149075.1; Identities = 808/808 (100 %), no gaps), Paradiplococcium singularare (strain CBS 126091, GenBank NR_158721.1; Identities = 797/839 (95 %), three gaps (0 %)), and Barbatosphaeria varioseptata (strain CBS 137797, GenBank NG_058674.1; Identities = 798/840 (95 %), five gaps (0 %)).

Notes: Appendopyricularia, based on A. juncicola, was introduced as a new hyphomycete genus occurring on culms of Juncus effusus in the Netherlands (Crous et al. 2022b). This is the first record of this taxon also occurring on culms of Carex elongata.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence of CPC 42686 had highest similarity to Appendopyricularia juncicola (strain CPC 41278, GenBank NR_182605.1; Identities = 500/501 (99 %), one gap (0 %)), Thyridium pluriloculosum (strain GZU1FR21.876, GenBank OK493561.1; Identities = 402/481 (84 %), 25 gaps (5 %)), and Phialemonium dimorphosporum (strain SLE, GenBank DQ403199.1; Identities = 412/495 (83 %), 30 gaps (6 %)). The ITS sequence of CPC 42686 is identical to those of CPC 44053, 44055, 44106 and 44107 (508/508, 508/508, 502/502 and 508/508 nucleotides, respectively). Closest hits using the LSU sequence of CPC 42686 were Appendopyricularia juncicola (strain CPC 41278, GenBank NG_149075.1; Identities = 808/808 (100 %), no gaps), Paradiplococcium singularare (strain CBS 126091, GenBank NR_158721.1; Identities = 797/839 (95 %), three gaps (0 %)), and Barbatosphaeria varioseptata (strain CBS 137797, GenBank NG_058674.1; Identities = 798/840 (95 %), five gaps (0 %)). The LSU sequence of CPC 42686 is identical to those of CPC 44053, 44055, 44106 and 44107 (792/792, 825/825, 804/804 and 804/804)

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Appendopyricularia juncicola Crous & Osieck, Persoonia 48: 265. 2022. Fig. 4.

Description and illustration: Crous et al. (2022b).

Materials examined: Netherlands, Overijssel Province, Reutum, Reutumerveen, 22.5 m a.s.l., 52°23'43”N, 06°49'24”E, on dead culms of Carex elongata (Cyperaceae), 20 Mar. 2022, E.R. Osieck, HPC 3952 = WI-55#4449, cultures CPC 44053 = CBS 149686, CPC 44055; Overijssel Province, Witte Veen, Haaksbergen, 39 m a.s.l., 52°02'46”N, 05°10'34”E, on dead culms of J. effusus, 9 Dec. 2021, E.R. Osieck, HPC 3812 = WI-42#4355, culture CPC 42686.
838/838 nucleotides, respectively). Closest hits using the tef1 (first part) sequence had highest similarity to Appendopyricularia juncicola (strain CPC 41278, GenBank ON605627.1; Identities = 338/345 (98 %), one gap (0 %)), Madurella fahalii (strain CBS 129176, GenBank MN078441.1; Identities = 141/149 (95 %), no gaps), and Podospora comata (strain Wa139-, GenBank CP071493.1; Identities = 151/165 (92 %), four gaps (2 %)). The tef1 sequence of CPC 42686 is 98 % similar to those of CPC 44053, 44055 and 44106 (338/344, 337/344, and 338/345 nucleotides, respectively; all including one gap). The closest hits using the tub2 sequence of CPC 42686 had highest similarity to Appendopyricularia juncicola (strain CPC 41278, GenBank ON605635.1; Identities = 671/690 (97 %), three gaps (0 %)); while the tub2 sequence of CPC 44055 is identical to that of Appendopyricularia juncicola (strain CPC 41278, GenBank ON605635.1; Identities = 690/690 (100 %), no gaps). The tub2 sequence of CPC 42686 is 97 % similar to those of CPC 44055, 44106 and 44107 (677/696, 672/691 and 677/696, nucleotides, respectively; all three includes an indel of three nucleotides and are identical to Appendopyricularia juncicola strain CPC 41278, GenBank ON605635.1).

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Biscogniauxia anceps (Sacc.) J.D. Rogers et al., Mycol. Res. 100: 669. 1996. Fig. 5.

Description and illustration: Rogers et al. (1996).

Material examined: Spain, Pontevedra, O Grove, on bark of Eucalyptus sp. (Myrtaceae), 25 Mar. 2022, M.A. Delgado, HPC 3867 = RKS 1164, culture CPC 43197 = CBS 149687.
Notes: Species of *Biscogniauxia* are found as endophytes and opportunistic pathogens on old and stressed trees (Bahmani et al. 2021). *Biscogniauxia aniceps* is known to occur on bark of various tree hosts in Europe and is reported here from *Eucalyptus* bark in Spain.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Biscogniauxia nummularia* (strain 123, GenBank EF026132.1; identities = 481/481 (100 %), no gaps), *Biscogniauxia aniceps* (strain MUCL 51395, GenBank NR_153649.1; identities = 534/585 (91 %), 16 gaps (2 %)), and *Digitodochium amoenum* (strain LA, GenBank KC774569.1; identities = 534/585 (91 %), 16 gaps (2 %)).

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**Cephaliophora tropica** Thaxt., *Bot. Gaz.* 35: 158. 1903. Fig. 6.

**Classification:** Pezizomycetes, Pezizales, Ascodesmidaceae.

**Description and illustration:** Ruszkiewicz-Michalska et al. (2017).


**Notes:** *Cephaliophora tropica* is a pantropical and occasionally temperate species (Seifert et al. 2011). It is commonly isolated from dung, soil, and water (Ruszkiewicz-Michalska et al. 2017). Because no type was indicated in the original description, a lectotype and epitype are designated here to fix the application of the name. The genus *Cephaliophora* is considered incertae sedis in MycoBank and Index Fungorum, but was shown by Hansen et al. (2013) and confirmed here to belong to *Ascodesmidaceae*.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Cephaliophora tropica* (strain CBS 133.33, GenBank MH855385.1; identities = 529/529 (100 %), no gaps), and *Cephaliophora irregularis* (strain YG-C22, GenBank KX683420.1; identities = 504/514 (98 %), no gaps). Closest hits using the LSU sequence were *Cephaliophora tropica* (strain CBS 315.66, GenBank MH870444.1; identities = 830/830 (100 %), nogaps), *Cephaliophora irregularis* (strain CBS 218.62, GenBank KO126668.1; identities = 826/830 (99 %), no gaps), and *Ascodesmis rosicola* (voucher GUCC 190035.1, GenBank MZ221605.1; identities = 815/830 (98 %), no gaps). Closest hits using the SSU sequence were *Cephaliophora tropica* (strain JCM 6019, GenBank AB001112.1; identities = 1000/1000 (100 %), no gaps), *Cephaliophora irregularis* (strain IFO 6778, GenBank AB001109.2; identities = 998/1000 (99 %), no gaps), and *Eleutherascus lectardii* (strain CBS 626.71, GenBank NG_062685.1; identities = 994/1000 (99 %), no gaps). Closest hits using the rpb1 sequence had highest similarity to *Cephaliophora tropica* (strain CBS 133.33, GenBank JX943656.1; identities = 727/729 (99 %), no gaps), *Cephaliophora irregularis* (strain CBS 218.62, GenBank JX943655.1; identities = 674/719 (94 %), five gaps (0 %)), and *Ascodesmis nigricans* (strain CBS 428.91, GenBank JX943653.1; identities = 582/690 (85 %), nine gaps (1 %)). Closest hits using the rpb2 (first part) sequence had highest similarity to *Cephaliophora tropica* (strain CBS 133.33, GenBank JX943763.1; identities = 598/600 (99 %), no gaps), *Cephaliophora irregularis* (strain CBS 218.62, GenBank JX943762.1; identities = 686/732 (94 %), two gaps (0 %)), and *Ascodesmis rosicola* (voucher GUCC 190204.1, GenBank MZ333140.1; identities = 728/882 (83 %), three gaps (0 %)). Closest hits using the tef1 (second part) sequence had highest similarity to *Cephaliophora tropica* (strain CBS 133.33, GenBank KC109224.1; identities = 901/903 (99 %), five gaps (0 %)), and *Ascodesmis rosicola* (voucher GUCC 190204.1, GenBank MZ333140.1; identities = 728/882 (83 %), three gaps (0 %)).

**Fig. 6. Cephaliophora tropica** (CPC 42877). A–G. Conidiophores with conidiogenous cells giving rise to conidia. H. Conidia. Scale bars = 10 µm.
no gaps), *Cephaloihora irregularis* (strain CBS 218.62, GenBank KC109223.1; Identities = 899/958 (94 %), 11 gaps (1 %)), and *Ascodesmis nigricans* (strain CBS 389.68, GenBank KC109221.1; Identities = 781/887 (88 %), 24 gaps (2 %)).

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*Ceratocystis ficicola* Kajitani & Masuya, *Mycoscience* 52: 351. 2011. Fig. 7.

Ascomata perithecial, solitary with brown to black, globose base, 180–220 µm diam, with erect, brown neck, 1 000–1 300 µm long, becoming paler brown toward apex, 40–50 µm diam at base, 18–20 µm diam at apex. Ostiolar hyphae divergent, subhyaline, 130–200 µm long. Asci not observed. Ascospores hyaline, galeate, aseptate, 7–8 × 6–7 µm in top view, 4–5 µm high in side view, accumulating in creamy mucoid masses at apices of perithecia.

*Thielaviopsis* asexual morph: endoconidiophores solitary on mycelium, pale brown to brown, smooth, tapering to truncate apex, 80–160 µm long, 5–7 µm diam at base, 1–4-septate. Conidiogenous cells phialidic, cylindrical, 500–75 µm long, 4–5 µm diam at base, 4 µm diam at apex. *Endoconidia* hyaline, becoming pale brown, smooth, guttulate, aseptate, subcylindrical with obtuse to truncate ends, (11–)15–17–(20) × 4–5(–6) µm, occurring in unbranched chains.

*Ceratocystis ficicola* causes vascular wilt of fig trees in Japan, and has also recently been reported from Greece (Tsopelas et al. 2021). This is the first record of the pathogen from Italy. Based on ITS alone, the present collections might represent a novel species. However, this was not supported by the secondary barcodes (see below). A closer inspection of the ITS blast alignment revealed a similarity of 577/600 nucleotides, with the 19 of the 23 mismatches being accounted for by gaps caused mainly by differences in repeat length repeats in T- or A-rich parts of the sequences.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence of CPC 44213 had highest similarity to *Ceratocystis ficicola* (strain MAFF 625119, GenBank NR_119410.1; Identities = 577/600 (96 %), 19 gaps (3 %)), *Ceratocystis cercfabiensis* (strain CMW 42512, GenBank KP727589.1; Identities = 541/598 (90 %), 20 gaps (3 %)), and *Ceratocystis uchidae* (strain CBS 115164, GenBank NR_164012.1; Identities = 560/620 (90 %), 23 gaps (3 %)). The ITS sequences of CPC 44213 and 44214 are identical (603/603 nucleotides). Closest hits using the LSU sequence

![Fig. 7. Ceratocystis ficicola (CPC 44213). A. Colony on PDA. B. Colony on SNA. C. Ascoma exuding ascospores. D, E. Ostiolar hyphae. F. Subcylindrical endoconidia. H. Aleuroconidia. I, J. Ascospores. Scale bars: C = 300 µm, all others = 10 µm.](image-url)
of CPC 44213 were Ceratocystis ficicola (strain CMW38543, GenBank KM495342.1; Identities = 808/809 (99 %), one gap (0 %)), Ceratocystis polychroma (strain CMW11424, GenBank KM495368.1; Identities = 805/808 (99 %), no gaps), and Ceratocystis obpyriformis (strain CBS 122511, GenBank MH874746.1; Identities = 817/821 (99 %), one gap (0 %)). Closest hits using the rpb2 (first part) sequence had highest similarity to Ceratocystis ficicola (strain CMW38543, GenBank KM495342.1; Identities = 808/809 (99 %), one gap (0 %)), Ceratocystis polychroma (strain CMW11424, GenBank KM495368.1; Identities = 805/808 (99 %), no gaps), and Ceratocystis obpyriformis (strain CBS 122511, GenBank MH874746.1; Identities = 817/821 (99 %), one gap (0 %)). Closest hits using the tef1 (second part) sequence of CPC 44213 had highest similarity to Ceratocystis ficicola (strain C1355, GenBank KY982680.1; Identities = 892/897 (99 %), no gaps), Ceratocystis fimbriata (strain C3372, GenBank KY982688.1; Identities = 892/897 (99 %), no gaps), and Ceratocystis uchidae (strain C1714, GenBank KY982680.1; Identities = 892/897 (99 %), no gaps). Closest hits using the tub2 sequence of CPC 44213 had highest similarity to Ceratocystis huliohia (strain B, GenBank KU043266.1; Identities = 1 241/1 327 (94 %), 27 gaps (2 %)), Ceratocystis uchidae (strain CBS 114720, GenBank KU043266.1; Identities = 1 241/1 327 (94 %), 27 gaps (2 %)), and Ceratocystis populicola (strain CBS 114725, GenBank KC589392.1; Identities = 522/621 (84 %), 35 gaps (5 %)). There was no overlap between our tub2 sequence and the two sequences available on GenBank for Ceratocystis ficicola (GenBank KY685077 and KY685078).

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Chloridium caudigerum (Höhn.) S. Hughes, Canad. J. Bot. 36: 748. 1958. Fig. 8.

Description and illustration: Réblová et al. (2022).

Material examined: Netherlands, Utrecht Province, Houten, Nieuw Wulven, 1.5 m a.s.l., 52°02’53”N, 05°09’42”E, on branch of Ulmus laevis (Ulmaceae), 28 Jan. 2022, E.R. Osieck, HPC 3827 = WI-46#4391, culture CPC 42899 = CBS 149688.

Notes: Chloridium caudigerum represents a common European species, found especially on decaying wood of deciduous trees. This species closely resembles Chl. chlamydosporum and Chl. virescens (Réblová et al. 2022). The sexual morph of Chl. virescens is also known as Melanopsammella vermicularioides. Melanopsammella is characterised by ascospores already fragmenting in the ascus. The sexual morph of Chl. caudigerum (also present in the collection) is similar but differs in having setose ascomata, which are glabrous in Chl. virescens (Réblová loc. cit.).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Chloridium caudigerum (strain ICMP 22547, GenBank OP455384.1; Identities = 491/491 (100 %), no gaps), Chloridium virescens (strain CBS 127310, GenBank MH864519.1; Identities = 498/520 (96 %), one gap (0 %)), and Chloridium jilinense (strain NN046507, GenBank OL627659.1; Identities = 457/469 (97 %), two gaps (0 %)). Closest hits using the LSU sequence were Chloridium caudigerum (strain ICMP 22547, GenBank OP455491.1; Identities = 844/845 (99 %), one gap (0 %)), Chloridium jilinense (strain NN046507, GenBank OL655058.1; Identities = 834/840 (99 %), no gaps), and Chloridium virescens (strain CBS 127627, GenBank MH876080.1; Identities = 836/845 (99 %), no gaps).
(99 %), one gap (0 %)). Closest hits using the **tef1** (second part) sequence had highest similarity to *Chloridium caudigerum* (strain CBS 145490, GenBank OP464953.1; Identities = 859/860 (99 %), no gaps), *Chloridium moratum* (strain FMR 11343, GenBank OP464997.1; Identities = 838/861 (97 %), two gaps (0 %)), and *Chloridium detriticola* var. *detriticola* (strain ICMP 15144, GenBank OP464977.1; Identities = 836/860 (97 %), no gaps). Closest hits using the **tub2** sequence had highest similarity to *Chloridium caudigerum* (strain FMR 12411, GenBank OP465062.1; Identities = 702/703 (85 %), 12 gaps (1 %)), and *Chloridium bellum* var. *luteum* (strain CBS 141.54, GenBank OP465041.1; Identities = 613/718 (85 %), 28 gaps (3 %)).

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*Chloridium gamsii* Réblová & Hern.-Restr., *Stud. Mycol.* **103**: 143. 2022. Fig. 9.

**Description and illustration:** Réblová et al. (2022).

*Mycelium* consisting of hyaline, smooth, branched, septate, 1.5–2 µm diam hyphae, which become brown and verruculose adjacent to conidiophores, up to 3 µm diam. *Conidiophores* solitary, erect, straight, flexuous, 1–3-septate, subcylindrical, medium brown, smooth, 35–80 x 3–3.5 µm. *Conidiogenous cells* integrated, terminal, subcylindrical, medium brown, smooth, with flared collarette; apex incl. collarette 3–5 µm diam, 20–30 x 2–3 µm. *Conidia* solitary, aggregated in mucoid mass, emerging in sympodial arrangement at apex, hyaline, smooth, guttulate, ellipsoid to ovoid, asceptate, 3.5–6 x 3–3.5 µm. *Chlamydospores* solitary, terminal on hyphae, medium brown, smooth, thick-walled, asceptate, guttulate, ellipsoid to ovoid, 5–7 x 4–5 µm.

**Culture characteristics:** Colonies erumpent, spreading, with moderate aerial mycelium and smooth, even margin, reaching 15 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface olivaceous grey and reverse iron-grey.

**Material examined:** Netherlands, Friesland Province, Terschelling, on *Cladonia portentosa* (*Cladoniaceae*), 7 Jun. 2021, J. Boers, HPC 3646 = CBS H-24942, culture CPC 41933 = CBS 149043.

**Notes:** *Chloridium gamsii* was recently described from decaying wood collected in Belgium and is reported here from a lichen in the Netherlands. Based on published data, this species appears to be common in Europe, Australasia, with a few records from Canada and the USA (Réblová et al. 2022).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the **ITS** sequence had highest similarity to *Chloridium gamsii* (strain CBS 667.75, GenBank OP455415.1; Identities = 481/484 (99 %), no gaps), *Chloridium virescens* (strain CBS 127310, GenBank MH864519.1; Identities = 489/508 (96 %), two gaps (0 %)), and *Chloridium biforme* (strain ICMP 23429, GenBank OP455363.1; Identities = 472/485 (97 %), two gaps (0 %)). Closest hits using the **LSU** sequence were *Chloridium gamsii* (strain CBS 667.75, GenBank OP455522.1; Identities = 808/809 (99 %), no gaps), *Chloridium virescens* var. *chlamydosporum* (strain CBS 126074, GenBank MH875525.1; Identities = 802/809 (99 %), no gaps), and *Chloridium peruense* (strain CBS 126074, GenBank OP455531.1; Identities = 802/809 (99 %), no gaps). Closest hits using the **tef1** (second part) sequence had highest similarity to *Chloridium gamsii* (strain CBS 667.75, GenBank OP464990.1; Identities = 814/833 (98 %), no gaps), *Chloridium biforme* (strain ICMP 23429, GenBank OP464937.1; Identities = 813/833 (98 %), no gaps), and *Chloridium caudigerum* (strain FMR 12411, GenBank OP464956.1; Identities = 807/834 (97 %), two gaps (0 %)).

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*Cladophialophora laricicola* Crous & Boers, **sp. nov.** MycoBank MB 848823. Fig. 10.

**Etymology:** Name refers to the host genus *Larix* from which it was isolated.

*Mycelium* consisting of medium brown, smooth, septate, branched, 2.5–5 µm diam hyphae. *Conidiophores* erect,
flexuous, subcylindrical, medium brown, smooth, with terminal and at times intercalary conidiogenous cells. Conidiogenous cells medium brown, subcylindrical, smooth, holoblastic, 10–15 × 3–4 µm. Conidia occurring in cylindrical chains (5–12), brown, smooth to roughened, aseptate, broadly ellipsoid, guttulate, becoming thick-walled, encased in mucoid sheath, individual conidia (6–)8–9(–10) × (4–)5(–6) µm; at time cylindrical conidial chains form lateral chains, and in extreme cases arranged in hand-like, penicillate configuration.

Culture characteristics: Colonies erumpent, spreading, with folded surface, lobate, feathery margin, and medium aerial mycelium, reaching 10 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface and reverse iron-grey.

Typus: Netherlands, Drenthe Province, Dwingelderveld National Park, 52.829188, 6.432495, on dead wood of Larix sp. (Pinaceae), 16 Mar. 2021, J. Boers, HPC 3608 (holotype CBS H-24954 culture ex-type CPC 41384 = CBS 148944).

Notes: Cladophialophora laricicola is phylogenetically distinct from other species presently known from their DNA sequence data. In the maximum parsimony phylogenetic tree (Fig. 11), it clustered sister to Cl. tortuosa and Cl. floridana but with no support. A maximum likelihood analysis conducted with IQ-TREE placed it basal to all ingroup species (data not shown). All sequenced loci indicated some affinity with Cladophialophora but not a tight association with any other sequenced species (see below).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Cladophialophora aff. parmeliae (strain KoLRI_053928, GenBank MZ855385.1; Identities = 497/546 (91 %), 12 gaps (2 %)), Cladophialophora chaetospira (strain CBS 114747, GenBank EU035403.1; Identities = 551/607 (91 %), 14 gaps (2 %)), and Capronia rubiginosa (strain BBB 536, GenBank NR_165891.1; Identities = 534/589 (91 %), 17 gaps (2 %)). Closest hits using the LSU sequence were Capronia semiimmera (strain AFTOL-ID 658, GenBank FJ358226.1; Identities = 773/782 (99 %), no gaps), Phialophora americana (strain MUCL 40613, GenBank AF050280.1; Identities = 773/782 (99 %), no gaps). Closest hits using the tef1 (first part) sequence had highest similarity to Cladophialophora sp. (strain SYPF 8340, GenBank MF588932.1; Identities = 282/339 (83 %), 17 gaps (5 %)), Exophiala bergeri (strain RBG7236, GenBank OP066900.1; Identities = 252/303 (83 %), 15 gaps (4 %)), and Cladophialophora carrionii (strain CBS 114399, GenBank KJ609515.1; Identities = 228/269 (85 %), 12 gaps (4 %)). Closest hits using the tub2 sequence had highest similarity to Cladophialophora chaetospira (strain CBS 114747, GenBank KF928578.1; Identities = 308/377 (82 %), ten gaps (2 %)), Cladophialophora carrionii (strain CBS 114393, GenBank KF928580.1; Identities = 306/382 (80 %), 21 gaps (5 %)), and Phialophora americana (strain CBS 221.97, GenBank KU306350.1; Identities = 283/355 (80 %), ten gaps (2 %)).

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**Cylindromonium eugeniicola** Crous, *Persoonia* **43**: 313. 2019.

**Fig. 12.**

*Description and illustration:* Crous et al. (2019c).

*Material examined:* Spain, Gran Canaria, on dead twig of Eucalyptus sp. (Myrtaceae), 1 Apr. 2022, A.L. van Iperen, HPC 3904, culture CPC 43326 = CBS 149689.

*Notes:* The hyphomycete genus *Cylindromonium* (based on *Cy. eugeniicola*) was described from leaf litter of *Eugenia capensis* collected in South Africa (Crous et al. 2019c). This is the first record of the fungus occurring on twigs of an *Eucalyptus* sp. in Spain, which is interesting as both host genera are members of Myrtaceae.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Cylindromonium eugeniicola* (strain CPC 37170, GenBank NR_166338.1; Identities = 563/567 (99 %), one gap (0 %)), *Cylindromonium lichenicola* (strain CBS 188.70, GenBank MH859549.1; Identities = 514/570 (90 %), 14 gaps (2 %)), and *Cylindromonium dirinariae* (strain FA006, GenBank LC731277.1; Identities = 513/569 (90 %), 11 gaps (1 %)). Closest hits using the LSU sequence were *Cylindromonium eugeniicola*.
(strain CPC 37170, GenBank NG_068337; Identities = 828/830 (99 %), no gaps), Trichonectria setadpressa (voucher A.F.28886, GenBank MT154012; Identities = 819/842 (97 %), no gaps), and Cylindrocladiella lanceolata (strain CBS 129565, GenBank MH876849; Identities = 833/861 (97 %), no gaps).

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Cyphellophora neerlandica Crous & Boers, sp. nov. MycoBank MB 848821. Fig. 13.

Etymology: Name refers to the Netherlands where it was collected.

Sporulating poorly on SNA. Mycelium consisting of pale brown, smooth, branched, septate, 1.5–2 µm diam hyphae. Conidiophores reduced to conidiogenous cells aggregated in clusters, pale brown, smooth, ampulliform to ellipsoid, phialidic, 4–6 × 3–4 µm, with cylindrical collarette, 1–2 µm long. Conidia solitary, pale brown, smooth, subcylindrical, straight to slightly curved, apex subobtuse, base truncate, 3-septate, (27–)30–33(–36) × 2 µm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and smooth, lobate margin, reaching 30 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface and reverse iron grey.

Typus: Netherlands, Limburg Province, Eys, brick wall, on lichen, 12 Nov. 2021, J. Boers, HPC 3805 (holotype CBS H-25164, culture ex-type CPC 42634 = CBS 149512); culture CPC 42641.


Notes: Cyphellophora neerlandica is related to Cyp. clematidis (conidia aseptate, ellipsoid, (3–)4–5(–6.5) × (1.5–)2(–2.5) µm; Crous et al. 2019b), and Cyp. jingdongensis (only known from its sexual morph; Yang et al. 2018), but is phylogenetically and morphologically distinct. In the phylogenetic tree (Fig. 14), Cyp. neerlandica clusters in a lineage containing sequences from the ex-type cultures of Cyp. clematidis and Anthopsis deltoidea (conidia aseptate, deltoid) as well as several unnamed species. Anthopsis deltoidea is the type species of the genus Anthopsis (Marchisio et al. 1977) and should be reduced to synonymy.

Fig. 12. Cylindromonium eugeniicola (CPC 43326). A. Colony on SNA. B–D. Conidiophores with conidiogenous cells giving rise to conidia. E. Conidia. Scale bars = 10 µm.

Fig. 13. Cyphellophora neerlandica (CPC 42634). A, B. Conidiogenous cells giving rise to conidia. C. Conidia. Scale bars = 10 µm.
under *Cyphellophora* (De Vries 1962), as this clade now contains species with aseptate, as well as septate conidia. *Cyphellophora clematidis* and *Cyp. deltoidea* also form a well-supported basal lineage in *Cyphellophoraceae* in the phylogeny of Quan et al. (2020) (clade 2 in fig. 3). Two additional species of *Anthopsis* are known, namely *A. catenata* and *A. microspora*. Only the former is known from molecular data, and the ITS and LSU sequences of its ex-type culture (GenBank NR_159623 and MH873124)
blast distant to Dactylospora, indicating an association with Dactylosporaceae (Sclerococcoles, Eurotiomycetes).

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence of CPC 42634 had highest similarity to Cyphellophora ‘pauciseptata’ (voucher INBio 481A, GenBank KU204581.1; Identities = 540/601 (90 %), 16 gaps (2 %)), Cyphellophora clematidis (strain CBS 144983, GenBank NR_163356.1; Identities = 469/545 (86 %), 39 gaps (7 %)), and Anthopsis deltoidea (strain CBS 263.77, GenBank NR_153555.1; Identities = 398/452 (88 %), 22 gaps (4 %)). The ITS sequence of CPC 42634 is identical to that of CPC 42641 (591/591 nucleotides). Closest hits using the LSU sequence of CPC 42634 were Cyphellophora clematidis (strain CBS 144983, GenBank NG_068614.1; Identities = 818/850 (96 %), 11 gaps (1 %)), Xenobotrys acaducospora (strain CBS 219.95, GenBank NG_067437.1; Identities = 816/848 (96 %), eight gaps (0 %)), and Cyphellophora jingdongensis (strain IFRDCC 2659, GenBank MF285236.1; Identities = 796/830 (96 %), ten gaps (1 %)). The LSU sequence of CPC 42634 differs with a single substitution from that of CPC 42641 (814/815 nucleotides). Closest hits using the tub2 sequence of CPC 42634 had highest similarity to Cyphellophora oxysora (strain CBS 698.73, GenBank KC455232.1; Identities = 309/403 (77 %), 22 gaps (5 %)), and Cyphellophoraceae sp. (strain not specified, GenBank MN913418.1; Identities = 269/338 (80 %), 22 gaps (6 %)). The tub2 sequence of CPC 42634 is identical to that of CPC 42641 (495/495 nucleotides).

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Didymella brevipilosa Magaña-Dueñas et al., J. Fungi 7: 4. 2021. Fig. 15.

Description and illustration: Magaña-Dueñas et al. (2021).

Conidiomata pycnidial, solitary, eustromatic, brown, 200–250 µm diam, with one to several apical ostioles; wall of 3–4 layers of brown texture angularis. Conidiophores reduced to conidiogenous cells, hyaline, smooth, ampulliform, phialidic, 4–6 × 4–5 µm. Conidia solitary, aseptate, fusoid-ellipsoid, apex subobtuse, base truncate, hyaline, smooth, guttulate, (4.5–)5–6(–8) × (2–)2.5–3(–3.5) µm.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium and smooth, lobate margin, reaching 45 mm diam after 2 wk at 25 °C. On MEA surface amber, reverse ochreous; on PDA surface and reverse isabelline; on OA surface isabelline.

Material examined: Canada, New Brunswick, Charlotte Co., 1.5 km SW of Little Lepreau, 45.135614 °–66.492269 °, on buds of Abies balsamea (Pinaceae), 4 May 2021, D. Malloch, HPC 3633 = CBS H-24968, culture CPC 41600 = CBS 149049.

Notes: Didymella brevipilosa was recently described from submerged plant debris collected in freshwater in Spain (conidia aseptate, hyaline, smooth, bacilliform to kidney-shaped, 4–5 × 2–3 µm; Magaña-Dueñas et al. 2021). This is the first record of this fungus from Canada.

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Didymella brevipilosa (as Didymella sp.; strain FMR 17415, GenBank OU612373.1; Identities = 498/499 (99 %), no gaps), Didymella americana (strain 8907, GenBank MK646045.1; Identities = 524/531 (99 %), one gap (0 %)), Didymella keratinophila (strain UTHSC DI16-200, GenBank NR_158275.1; Identities = 524/531 (99 %), one gap (0 %)), and Peyronellaea pomorum (strain F115, GenBank KM979827.1; Identities = 522/529 (99 %), one gap (0 %)). Closest hits using the LSU sequence were Didymella brevipilosa (as Didymella sp.; strain FMR 17415, GenBank OU612372.1; Identities = 812/812 (100 %), no gaps), Ascochyta medicaginicola (strain CBS 111.53, GenBank MH868649.1; Identities = 812/812 (99 %), no gaps), Didysimulans mezzonensis (strain MFLUCC 15-0067, GenBank KY496733.1; Identities = 810/812 (99 %), no gaps), and Didysimulans italica (strain MFLUCC 15-0059, GenBank KY496730.1; Identities = 810/812 (99 %), no gaps). Closest hits using the actA sequence had highest similarity to Are Didymella finnmarkica (strain CBS 145572, GenBank MK876458.1; Identities = 573/607 (94 %), no gaps), Didymella combreti (strain CBS 137982, GenBank KJ869228.1; Identities = 559/600 (93 %), no gaps), and Didymella rabei (strain AR628, GenBank KM244530.1; Identities = 559/610 (92 %), five gaps (0 %)). Closest hits using the rpb2 sequence had highest similarity to Are Didymella brevipilosa (as Didymella sp.; strain FMR 17415, GenBank OU612359.1; Identities = 482/489 (99 %), no gaps), Didymella aliena (strain JZB380013, GenBank

Fig. 15. Didymella brevipilosa (CPC 41600). A. Conidiomata on SNA. B. Conidiomata on OA. C. Conidioma with ostiole. D. E. Conidiogenous cells. F. Conidia. Scale bars = 10 µm.

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MH645899.1; Identities = 524/575 (91%), no gaps), *Didymella microchlamydospora* (strain CBS 140543, GenBank MN988314.1; Identities = 458/504 (91%), no gaps), and *Didymella subrosea* (strain CBS 733.79, GenBank MT018174.1; Identities = 457/504 (91%), no gaps). Closest hits using the *tub2* sequence had highest similarity to are *Didymella brevipilosa* (as *Didymella sp.*; strain FMR 17415, GenBank no gaps; Identities = 450/454 (99%), no gaps), *Didymella glomerata* (strain ATCC MYA-2373, GenBank MZ073910.1; Identities = 447/483 (93%), four gaps (0%)) and *Didymella combreti* (strain CBS 137982, GenBank KJ869246.1; Identities = 448/488 (92%), six gaps (1%)).

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*Drepanopeziza populii-albae* (Kleb.) Nannf., *Nova Acta R. Soc. Scient. Upsal.*, Ser. 4 **8**(no. 2): 170. 1932. Fig. 16.


**Typus**: Russia, Rostov region, Shakhty, Donetsk, on *Populus alba* (Salicaceae), 26 Jun. 2021, T.S. Bulgakov, HPC 3708 = CBS H-25157, culture CPC 42336 = CBS 149510.

**Notes**: *Drepanopeziza populii-albae* is a common foliar pathogen of *Populus alba* (Spiers 1998).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Drepanopeziza populii-albae* (strain CBS 152.66, GenBank MH858754.1; Identities = 547/548 (99%), one gap (0%)), *Erysiphe adunca* (strain 3_23, GenBank KY660741.1; Identities = 546/548 (99%), one gap (0%)), and *Drepanopeziza brunnea f. sp. ‘monogermutbi’* (as *Marssonina brunnea f. sp. ‘monogermutbi’*, strain RBHB1, GenBank KM246347.1; Identities = 532/548 (97%), one gap (0%)). Closest hits using the LSU sequence were *Drepanopeziza populii-albae* (strain CBS 153.66, GenBank MH870387.1; Identities = 836/836 (100%), no gaps), *Drepanopeziza tremulae* (strain CBS 408.64, GenBank MH870103.1; Identities = 812/824 (99%), three gaps (0%)), and *Mastigosporium rubricosum* (strain CBS 405.66, GenBank MH870478.1; Identities = 829/845 (98%), no gaps).

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*Endoconidioma populii* Tsuneda et al., *Mycologia* **96**: 1129. 2004. Fig. 17.

**Description and illustration**: Tsuneda et al. (2004).

On OA forming immersed sclerotium-like structures, 70–250 µm diam, brown, ellipsoid to globose, remaining sterile. On SNA mycelium brown, covered in mucoid layer, roughened, 3–5 µm diam. Conidiophores reduced to solitary or aggregated conidiogenous loci on hyphae, denticulate, 2–3 µm diam, blastic, giving rise to solitary conidia. *Conidia* fusoid-ellipsoid, apex subobtuse, base truncate, 0(–1)-septate, hyaline, smooth, becoming pale brown, covered in mucoid layer, (13–)16–18(–22) x (5–)6–6.5(–7) µm.

**Culture characteristics**: Colonies flat, spreading, with sparse aerial mycelium and feathery, lobate margin, reaching 40 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface and reverse iron-grey.

**Material examined**: Canada, New Brunswick, Charlotte Co., 1.5 km SW of Little Lepreau, 45.135614°-66.492269°, on buds of *Abies balsamea* (Pinaceae), 4 May 2021, D. Malloch, HPC 3633 = CBS H-24972, culture CPC 41602 = CBS 149070.

![Fig. 16. Drepanopeziza populii-albae (CPC 42336). A. Colony on MEA. B–G. Conidiophores with conidiogenous cells giving rise to conidia (note germinating conidia in D). Scale bars = 10 µm.](image-url)
Notes: *Endoconidioma populi* was originally described from twigs of *Populus tremuloides* collected in Canada (Tsuneda *et al.* 2004). It produces a yeast-like morph in culture, as well as endoconidia, and a coelomycetous, coniothyrium-like morph (Crous *et al.* 2020).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to “*Hormonema carpetanum*” (strain 235J14, GenBank KU51685.1; Identities = 570/570 (100 %), no gaps), *Endoconidioma populi* (strain IRAN2350C, GenBank KX180155.1; Identities = 563/563 (100 %), no gaps), *Endoconidioma leucospermi* (as *Coniozyma leucospermi*; strain CBS 111289, GenBank EU552113.1; Identities = 578/581 (99 %), no gaps), and *Endoconidioma populi* (strain UAMH 10297, GenBank NR_121303.1; Identities = 553/556 (99 %), no gaps). Closest hits using the LSU sequence were “*Hormonema carpetanum*” (strain ATCC 74360, GenBank MF611880.1; Identities = 826/830 (99 %), no gaps), *Endoconidioma leucospermi* (as *Coniozyma leucospermi*; strain CBS 111289, GenBank EU552113.1; Identities = 825/830 (99 %), no gaps), *Endoconidioma euphorbiae* (strain CPC 38583, GenBank MW175391.1; Identities = 823/830 (99 %), no gaps), and *Endoconidioma populi* (strain UAMH 10297, GenBank NG_059198.1; Identities = 823/830 (99 %), no gaps).

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**Fusariella atrovirens** (Berk.) Sacc., *Atti dell´Istituto Veneto Scienze* 2: 463. 1884. Fig. 18.

Description and illustration: Lin *et al.* (2016).

Material examined: Namibia, Gobabeb Namib Research Institute, salt pan, lichenicolous on unknown lichen growing on rock, 4 Apr. 2022, P.W. Crous, HPC 3888, culture CPC 43304 = CBS 149690.

Notes: *Fusariella atrovirens* is cosmopolitan, occurring on various plant hosts, leaf litter, dung and in soil (Seifert *et al.* 2011). It is here recorded as lichenicolous, growing on an unknown lichen in Namibia.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Fusariella atrovirens* (strain CBS 311.73, GenBank MH860688.1; Identities = 536/536 (100 %), no gaps), *Hydropisphaera erubescens* (strain I-10, GenBank KF813068.1; Identities = 455/456 (99 %), no gaps), and *Fusariella sinensis* (strain OUCMBI110148, GenBank KP269058.1; Identities = 533/536 (99 %), no gaps). Closest hits using the LSU sequence were *Fusariella bizzozeriana* (strain CBS 306.73, GenBank MH878365.1; Identities = 810/810 (100 %), no gaps), *Fusariella cinccina* (strain CBS 312.73, GenBank MH878376.1; Identities = 853/857 (99 %), no gaps), and *Fusariella atrovirens* (strain...
CBS 311.73, GenBank MH872395.1; Identities = 841/845 (99 %), two gaps (0 %)). Closest hits using the rpB2 (first part) sequence had highest similarity to *Fusariella atrovirens* (strain AK18-21, GenBank OX335003.1; Identities = 857/868 (99 %), no gaps), *Hydropisphaera peziza* (strain CBS 102038, GenBank DQ522444.1; Identities = 716/838 (85 %), two gaps (0 %)), and *Heleococcus aurantiacum* (strain CBS 201.35, GenBank JX158463.1; Identities = 734/863 (85 %), two gaps (0 %)). Closest hits using the tef1 (second part) sequence had highest similarity to *Fusariella* sp. (strain MFLUCC 15-0844, GenBank KX025155.1; Identities = 899/915 (98 %), no gaps), *Hydropisphaera erubescens* (strain AF20-LD 186, GenBank DQ181741.1; Identities = 867/916 (95 %), no gaps), and *Heleococcus japonense* (strain CBS 397.67, GenBank JX158398.1; Identities = 859/916 (94 %), no gaps). No tef1 sequences of *Fusariella atrovirens* are available for comparison.

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**Fusariella hughesii** Chab.-Frydm., *Canad. J. Bot.* **42**: 1485. 1964. Fig. 19.

**Description and illustration**: Lin et al. (2016).

*Mycelium* consisting of hyaline, smooth, branched, septate, 2.5–3 μm diam hyphae, forming hyphal swellings (up to 10 μm diam) in older hyphae on MEA and PDA. *Conidiophores* solitary or aggregated, erect, arising from superficial hyphae, subcylindrical, branched, up to 5-septate, 100 μm tall. *Conidiogenous cells* terminal and intercalary, subcylindrical with apical taper, hyaline, smooth, 20–35 × 2.5–3 μm, phialidic, giving rise to bisepitodal conidial chains. *Conidia* subcylindrical, 3-septate, guttulate, hyaline to pale greenish, smooth, apex obtuse, base obconically truncate, hilum 2–2.5 μm diam, straight, occurring in long, unbranched chains, (8–)16–19(–22) × (2.5–)3(–3.5) μm.

**Culture characteristics**: Colonies erumpent, spreading, surface folded, with sparse aerial mycelium and smooth, lobate margin, reaching 20 mm diam after 2 wk at 25 °C. On MEA surface ochreous, reverse umber; on PDA surface and reverse pale luteous; on OA surface pale luteous.

**Material examined**: Ukraine, Dvorichna district, Kharkiv region, Krasne Pershe village, National Park Dvorichanskyi, on overwintered stems of *Adonis vernalis* (Ranunculaceae), 11 Apr. 2021, A. Akulov, CWU (MYC) AS 8121 = HPC 3630 = CBS H-24976, culture CPC 41594 = CBS 149074.

**Notes**: *Fusariella hughesii*, which was originally isolated from seeds of *Trigonella arabica* and *Phalaris minor* in Israel (Lin et al. 2016), is reported here from dead stems of *Adonis vernalis* in Ukraine.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Fusariella hughesii* (strain CBS 435.70, GenBank MH859784.1; Identities = 569/576 (99 %), no gaps), *Fusariella sinensis* (strain OUCMB110131, GenBank KP269041.1; Identities = 545/569 (96 %), four gaps (0 %)), and *Fusariella atrovirens* (strain CBS 311.73, GenBank MH860688.1; Identities = 554/579 (96 %), four gaps (0 %)). Closest hits using the LSU sequence were *Fusariella hughesii* (strain CBS 435.70, GenBank MH715471.1; Identities = 722/724 (99 %), no gaps), *Hydropisphaera erubescens* (strain CBS 128364, GenBank MH876356.1; Identities = 721/724 (99 %), no gaps), and *Fusariella concinna* (strain CBS 312.73, GenBank MH878376.1; Identities = 720/724 (99 %), no gaps).

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**Geonectria muralis** Crous & Boers, *sp. nov.* MycoBank MB 848824. Fig. 20.

**Etymology**: Name refers to the phycoparasitic habit of the fungus on an old church wall.

*Perithecia* globose, 150–180 μm diam, orange, arising from substral hyphae, not changing colour in KOH, with central ostiole, 15 μm diam; wall 15–18 μm thick, of 3–4 layers of textura angularis; outer wall smooth, with hyphal outgrowths; hyphae smooth, branched, septate, 3–4 μm diam. *Asci* 8-spored, stalked, subcylindrical, unicellular, with apical mechanism, 40–65 × 9–11 μm. *Ascospores* bi- to triseriate, hyaline, becoming pale brown with age, smooth, guttulate, broadly ellipsoid, medianly 1-septate, constricted at septum, (12–)14–16(–17) × (5–)8–9 μm. *Asexual morph* not seen.

**Culture characteristics**: Colonies flat, spreading, with sparse to moderate aerial mycelium and smooth, lobate margin, reaching...
35 mm diam after 2 wk at 25 °C. On MEA and PDA surface and reverse sienna; on OA surface apricot. Homothallic, with perithecia also forming in culture.

**Typus:** Netherlands, Gelderland Province, Dodewaard, church, on algae growing on the bottom part of wall, 7 Sep. 2021, J. Boers, HPC 3749 (holotype CBS H-25161, culture ex-type CPC 42404 = CBS 149515); cultures CPC 42405, 42406.

**Notes:** *Geonectria muralis* is related to *Nectria pyrospheara* (CBS 165.26), *Nectria zonata* (AR 1612) and *Geonectria subalpina* (CBS 143540). In the phylogenetic tree (Fig. 21), it clustered with 97 % ML bootstrap support and 93 % parsimony bootstrap support with *Nectria zonata*, a *Hydropisphaera* sp. and the LSU sequence of the ex-type strain of *Geonectria subalpina*, the type species of the monotypic genus *Geonectria*. *Geonectria*, based on *G. subalpina*, was described from bare soil collected in the subalpine region, and is characterised by orange perithecia that do not change colour in 3 % KOH, striae, 1-septate, hyaline, finely striae ascospores, and an acremonium-like asexual morph (Lechat et al. 2018).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence of CPC 42404 had highest similarity to *Hydropisphaera* sp. BGL-2019a (voucher BDNA-L-0000095, GenBank MN187059.1; Identities = 501/554 (90 %), 28 gaps (5 %)), *Nectria pyrospheara* (strain CBS 165.26, GenBank MH854877.1; Identities = 476/569 (84 %), 41 gaps (7 %)), and *Heleococcum aurantiacum* (strain CBS 201.35, GenBank MH855645.1; Identities = 472/568 (83 %), 36 gaps (6 %)). The ITS sequence of CPC 42404 is identical to those of CPC 42405 and CPC 42406 (both 552/552 nucleotides). Closest hits using the LSU sequence of CPC 42404 were *Hydropisphaera* sp. BGL-2019a (voucher BDNA-L-0000095, GenBank MN181431.1; Identities = 828/842 (98 %), three gaps (0 %)), *Geonectria subalpina* (strain CBS 143540, GenBank NG_067817.1; Identities = 808/842 (96 %), four gaps (0 %)), and *Nectria zonata* (strain AR 1612, GenBank U17424.1; Identities = 809/845 (96 %), 11 gaps (1 %)). The LSU sequence of CPC 42404 differs from that of CPC 42405 at three nucleotide positions (796/799 nucleotides, all in repeat motifs).

**Authors:** P.W. Crous, J.Z. Groenewald & J. Boers

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**Harposporium illinoisense** Crous & Jurjević, *sp. nov.* MycoBank MB 848825. Fig. 22.

**Etymology:** Name refers to Illinois, the state in the USA where it was isolated.

*Mycelium* consisting of hyaline, smooth, branched, septate, 2–3 µm diam hyphae, constricted at septa with age. *Conidiophores* reduced to conidigenous cells; phialides solitary on hyphae, or in clusters, ampulliform, 5–7 × 2.5–3 µm, with cylindrical necks, 1.5–2 × 1 µm. *Conidia* solitary, aggregating in mucoid mass, hyaline, smooth, aseptate, sickle-shaped, widest in middle, apex subobtuse, base truncate, 6–10 × 1–2 µm.

**Culture characteristics:** Colonies erumpent, spreading, with moderate aerial mycelium and feathery, lobe margin, reaching 20 mm diam after 2 wk at 25 °C. On MEA surface amber, reverse sienna; on PDA surface amber, reverse sienna; Czapek Yeast Autolysate Agar (CYA) surface amber, reverse sienna; on OA surface hazel. It also shows antibacterial properties on CYA and MEA. No growth at 37 °C, on CYA.


**Notes:** Species of *Harposporium* parasitise free-living nematodes and rotifers. Most species of *Harposporium* infect nematodes via ingested conidia (Glockling 1998). *Harposporium illinoisense* was isolated from rockwool in a greenhouse, and was probably associated with nematodes. Phylogenetically, *H. illinoisense* is quite distinct from other presently knowed from DNA data (Fig. 23) and also the blast results (see below) are insufficiently conclusive to provide a proper placement for this species.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence of CPC 42405 had highest similarity to *Purpureocilium takamizusanense* (strain PT3, GenBank CP086360.1; Identities = 476/554 (86 %), 46 gaps (8 %)), *Harposporium helicoides* (strain CBS 944.70, GenBank MH860014.1; Identities = 490/558 (88 %), 29 gaps (5 %)), and *Drechmeria balanoides* (as *Haptocladium balanoides*, strain Harp5, GenBank EF546660.1; Identities = 475/540 (88 %), 27 gaps (5 %)). Closest hits using the LSU sequence were *Harposporium harposporiferum* (strain CBS 213.86, GenBank MH873635.1;
Fig. 21. Consensus phylogram (50 % majority rule) obtained from the maximum likelihood analysis with IQ-TREE v. 2.1.3 of the Hypocreales LSU nucleotide alignment. Maximum likelihood (> 74 %) and maximum parsimony (> 74 %) bootstrap support values from 1000 non-parametric bootstrap replicates are shown at the nodes. Culture collection or voucher numbers and GenBank accession numbers (superscript) are indicated for all species. Sequences derived from material with a type status are indicated with a culture or voucher number highlighted with bold face. The tree was rooted to Neocosmospora rubicola (culture CBS 101018; GenBank NG_069232) and the species treated here is highlighted with bold face. The families, order and class are shown in coloured blocks to the right of the tree. The scale bar indicates the expected number of changes per site.


Hysterobrevium rosae


Description and illustration: Jayasiri et al. (2018).

Material examined: Netherlands, Utrecht Province, Nieuw Wulven, near Houten, 1.5 m a.s.l., 52°02'53"N, 05°14'04"E, on bamboo stick (used as support post, not locally grown), 11 Feb. 2022, E.R. Osiack, HPC 3837 = WI-48/ #4404, culture CPC 42948 = CBS 149699.

Notes: The genus Hysterobrevium is characterised by hysteriaceous ascomata and muriform spores, which are less than 25 µm in length (Boehm et al. 2009). Hysterobrevium rosae is morphologically similar to H. mori (Boehm loc. cit.), but distinguished in having smaller hysterothecia, shorter asci and hyaline ascospores (Jayasiri et al. 2018).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Hysterobrevium mori (strain A1045A, GenBank MT230464.1; Identities = 710/710 (100 %), no gaps), Hysterobrevium constrictum (strain JCM 2753, GenBank NR_175063.1; Identities = 676/716 (94 %), four gaps (0 %)), and Hysterobrevium sp. MR-2017a (strain MFLUCC 16-2163, GenBank MZ467050.1; Identities = 393/422 (93 %), six gaps (1 %)). No ITS sequence of Hysterobrevium rosae was available for comparison.

Closest hits using the LSU sequence were Hysterobrevium rosae (strain MFUC 14-0551, GenBank MH535897.1; Identities = 844/844 (100 %), no gaps), Hysterobrevium mori (strain CBS 123335, GenBank FJ161202.2; Identities = 866/869 (99 %), no gaps), and Gloniopsis praelonga (strain SMHS280, GenBank GQ221912.2; Identities = 865/869 (99 %), no gaps). Closest hits using the rp2b (first part) sequence had highest similarity to Hysterobrevium similacis (as Gloniopsis similacis, strain CBS 114601, GenBank FJ161114.1; Identities = 833/882 (94 %), no gaps), and Hysterobrevium constrictum (strain HKAS 121127, GenBank OK506220.1; Identities = 808/876 (92 %), no gaps).

Closest hits using the rpb1 (second part) sequence had highest similarity to Hysterobrevium similacis (strain SMHS280, GenBank GQ221912.2; Identities = 865/869 (99 %), no gaps), Hysterobrevium constrictum (strain HKAS 121127, GenBank OK506220.1; Identities = 808/876 (92 %), no gaps).

Authors: P.W. Crous, J.Z. Groenewald & E.R. Osiack

Microcera physciae

Crous & Boers, Persoonia 47: 233. 2021. Fig. 24.

Description and illustration: Crous et al. (2021b).

Material examined: Netherlands, Limburg Province, Eys, brick wall, on lichen, 12 Nov. 2021, J. Boers, HPC 3805, culture CPC 42638.

Notes: Microcera physciae is a lichenicolous fungus described from Physcia tenella in the Netherlands (Crous et al. 2021b), while M. lichenicola was described from Parmelia sulcata, also in the Netherlands (Crous et al. 2022a).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Microcera lichenicola (strain CBS 149169, GenBank ON811502.1; Identities = 530/530 (100 %), no gaps), Microcera
**Fig. 23.** Consensus phylogram (50 % majority rule) obtained from the maximum likelihood analysis with IQ-TREE v. 2.1.3 of the *Ophiocordycipitaceae* LSU nucleotide alignment. Bootstrap support values (> 75 %) from 1 000 non-parametric bootstrap replicates are shown at the nodes. Culture collection or voucher numbers and GenBank accession numbers (superscript) are indicated for all species. Sequences derived from material with a type status are indicated with a culture or voucher number highlighted with bold face. The tree was rooted to *Falcocladium eucalypti* (culture CPC 38019; GenBank NG_068318) and the species treated here is highlighted with bold face. The family, order and class are shown in coloured blocks to the right of the tree. The scale bar indicates the expected number of changes per site.

*physciae* (strain CBS 148283, GenBank NR_175225.1; Identities = 496/497 (99 %), no gaps), and *Microcera larvarum* (strain CBS 738.79, GenBank KM231825.1; Identities = 519/531 (98 %), three gaps (0 %)). Closest hits using the LSU sequence were *Microcera physciae* (strain CBS 148283, GenBank NG_081335.1; Identities = 814/815 (99 %), no gaps), and *Microcera rubra* (strain CBS 638.76, GenBank NG_058100.1; Identities = 805/816 (99 %), no gaps). Closest hits using the *rpb1* sequence had highest similarity to *Microcera physciae* (strain CPC 41284, GenBank OK651153.1; Identities = 717/720 (99 %), two gaps (0 %)), and *Microcera lichenicola* (strain CBS 148313, GenBank ON803533.1; Identities = 690/720 (96 %), two gaps (0 %)), and *Microcera coccophila* (strain MAFF 241482, GenBank KC291895.1; Identities = 629/689 (91 %), two gaps (0 %)). Closest hits using the *rpb2* (first part) sequence had highest
similarity to *Microcera physciae* (strain CPC 41284, GenBank OK651168.1; Identities = 871/874 (99%), no gaps), *Microcera lichenicola* (strain CBS 148313, GenBank ON803543.1; Identities = 840/876 (96%), one gap (0%)), and *Microcera larvarum* (strain NRRL 20473, GenBank JX171587.1; Identities = 766/850 (90%), one gap (0%)). Closest hits using the *tef1* (first part) sequence had highest similarity to *Microcera physciae* (strain CPC 41284, GenBank OK651190.1; Identities = 460/470 (98%), two gaps (0%)), *Microcera lichenicola* (strain CBS 148313, GenBank ON803570.1; Identities = 449/483 (93%), six gaps (1%)), and *Microcera larvarum* (strain CBS 169.30, GenBank AB587032.1; Identities = 375/470 (80%), 24 gaps (5%)). Closest hits using the *tub2* sequence had highest similarity to *Microcera physciae* (strain CPC 41284, GenBank OK651190.1; Identities = 526/526 (100%), no gaps), *Microcera lichenicola* (strain CBS 149169, GenBank KM231957.1; Identities = 375/470 (80%), 24 gaps (5%)). Closest hits using the *tef1* (first part) sequence had highest similarity to *Microcera physciae* (strain CPC 41284, GenBank OK651190.1; Identities = 460/470 (98%), two gaps (0%)), *Microcera lichenicola* (strain CBS 148313, GenBank ON803570.1; Identities = 449/483 (93%), six gaps (1%)), and *Microcera larvarum* (strain CBS 169.30, GenBank AB587032.1; Identities = 374/304 (90%), no gaps).

**Authors:** P.W. Crous, J.Z. Groenewald & J. Boers

*Miricatena prunicola* Punith. & Spooner, *Kew Bull.* **66**: 638. 2011. Fig. 25.

**Classification:** Leotiomycetes, Helotiales, incertae sedis.

**Description and Illustration:** Punithalingham & Spooner (2011).

The compound conidia and conidiogenesis of *M. prunicola* was discussed and fully described by Punithalingham & Spooner (2011). In this paper they comment on the terminal cells being ampulliform to lageniform to obpyriform. What they did not observe, however, is that these terminal cells become fertile, acting as conidiogenous cells, or give rise to 2–4 smaller ampulliform phialides, 5–10 × 3–4.5 µm, that again give rise to solitary conidia, ellipsoid, aseptate, hyaline, smooth, guttulate, 3–4 × 2.5–3 µm, aggregating in a mucoid conidial mass.

**Culture characteristics:** Colonies erumpent, spreading, with sparse to moderate aerial mycelium and even, lobate margin, reaching 35 mm diam after 2 wk at 25 °C. On malt extract agar (MEA) surface and reverse isabelline; on potato dextrose agar (PDA) surface cinnamon, reverse isabelline; on oatmeal agar (OA) surface sienna to orange.

**Typus:** Netherlands, Gelderland Province, Ede, Kreelsche Zand, on leaves of *Prunus serotina* (Rosaceae), 7 Nov. 2021, E. Slootweg, HPC 3800 (epitype designated here CBS H-25163, MBT 10013416, culture ex-epitype CPC 42627 = CBS 149448). UK, Surrey, Frensham, Little Pond (near), on living leaves of *Prunus serotina*, 8 Jul. 2007, B.M. Spooner (holotype K(M) 155328).

**Notes:** The monotypic genus *Miricatena* was introduced for *M. prunicola*, a foliar pathogen of *Prunus serotina* in the UK (Punithalingam & Spooner 2011). Due to the lack of cultures and DNA data, the phylogenetic position of *Miricatena* has remained unknown. The present record of *M. prunicola* from the Netherlands made it possible to designate an epitype for this pathogen, and also resolve its placement in the Helotiales, Leotiomycetes based on the high similarity of the ITS and LSU sequences to species of Cadophora and Pyrenopeziza.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Cadophora prunicola* (voucher JKI-Cad64, GenBank MW960107.1; Identities = 490/547 (90%), 16 gaps (2%)), *Cadophora malorum* (strain CBS 101359, GenBank DQ404350.1; Identities = 488/548 (89%), 17 gaps (3%)), and *Cadophora orientoamericana* (voucher JKI-Cad62, GenBank MW804716.1;
Identities = 417/448 (93 %), six gaps (1 %)). Closest hits using the LSU sequence were Cadophora luteo-olivacea (strain CBS 128576, GenBank MH876422.1; Identities = 836/850 (98 %), no gaps), Cadophora fastigiata (strain CBS 872.69, GenBank MH871250.1; Identities = 836/850 (98 %), no gaps), and Pyrenopeziza lonicerae (strain CBS 332.58, GenBank MH869339.1; Identities = 836/850 (98 %), no gaps). No significant hits were obtained using the tef1 (first part) sequence.

Authors: P.W. Crous, J.Z. Groenewald & E. Slootweg

Neoleptodontidium Crous & Jurjević, gen. nov. MycoBank MB 848826.

Etymology: Name refers to its morphological similarity to Leptodontidium.

Classification: Sordariomycetes, Xylariomycetidae, Xylariales, incertae sedis.

Mycelium consisting of hyaline, smooth, branched, septate hyphae. Conidiophores solitary, erect, subcylindrical, medium brown, thick-walled, lower part finely roughened, 1–3-septate, frequently rejuvenating through terminal phialide, forming a new phialide above the older phialide, where a rosette of conidia remains attached in a mucoid mass, 30–60 × 2.5–3.5 µm. Conidiogenous cells terminal, subcylindrical, medium brown, somewhat paler to the rest of the conidiophore, smooth, terminal phialidic opening with flared collarette, 1.5–2 µm diam, at times also with lateral phialidic openings on conidiogenous cell, 10–30 × 2–2.5 µm. Conidia solitary, hyaline, smooth, guttulate, aseptate, subcylindrical, apex obtuse, straight to slightly curved, tapering to subobtuse hilum, 3–4 × 1.5 µm, aggregating in mucoid mass.

Type species: Neoleptodontidium aquaticum Crous & Jurjević

Neoleptodontidium aquaticum Crous & Jurjević, sp. nov. MycoBank MB 848827. Fig. 26.

Etymology: Name refers to the fact that it was isolated from water.

Mycelium consisting of hyaline, smooth, branched, septate, 2.5–3 µm diam hyphae. Conidiophores solitary, erect, subcylindrical, medium brown, thick-walled, lower part finely roughened, 1–3-septate, frequently rejuvenating through terminal phialide, forming a new phialide above the older phialide, where a rosette of conidia remains attached in a mucoid mass, 30–60 × 2.5–3.5 µm. Conidiogenous cells terminal, subcylindrical, medium brown, somewhat paler to the rest of the conidiophore, smooth, terminal phialidic opening with flared collarette, 1.5–2 µm diam, at times also with lateral phialidic openings on conidiogenous cell, 10–30 × 2–2.5 µm. Conidia solitary, hyaline, smooth, guttulate, aseptate, subcylindrical, apex obtuse, straight to slightly curved, tapering to subobtuse hilum, 3–4 × 1.5 µm, aggregating in mucoid mass.

Culture characteristics: Colonies flat, spreading, with sparse to moderate aerial mycelium and feathery, lobate margin, reaching 25 mm diam after 2 wk at 25 °C. On MEA surface isabelline, reverse honey; on PDA surface and reverse honey; on OA surface olivaceous grey.


Additional isolate examined: USA, greenhouse, peet, Z. Jurjević 5683, culture CPC 42875.


*Typus:* India, Karnataka, Dt. Bidar, Maniknagar, on rotten wood, Jan. 1984, V. Rao, culture ex-type CBS 123.86.

*Notes:* *Neoleptodontidium* is reminiscent of the genus *Leptodontidium* (based on *L. trabinellum*), in having erect conidiophores and conidiogenous cells with a long rachis with denticles (Hernández-Restrepo *et al.* 2017). However, *Neoleptodontidium* is distinct in that it forms minute, terminal and lateral exosphiala-like phialides. Phylogenetically it is allied to *Leptodontidium aciculare*, which also forms similar phialides (Rao & Hoog 1986), and is therefore also placed in *Neoleptodontidium* (Fig. 27). *Neoleptodontidium* clusters in the *Xylariales*, but with unclear familial association; the closest families are *Oxydothidaceae*, *Castanediellaceae* and *Barmaeliaceae*.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the *ITS* sequence of CPC 42868 had highest similarity to *Leptodontidium aciculare* (strain CBS 123.86, GenBank MH861931.1; Identities = 498/513 (97 %), one gap (0 %)), *Castanediella cognizarii* (strain CBS 542.96, GenBank MH862597.1; Identities = 376/418 (90 %), nine gaps (2 %)), and *Linteromyces quintiniae* (strain CBS 146792, GenBank NR_171989.1; Identities = 389/433 (90 %), 11 gaps (2 %)). The *ITS* sequences of CPC 42868 and 42875 are identical (510/510 nucleotides). Closest hits using the *LSU* sequence of CPC 42868 were *Leptodontidium aciculare* (strain CBS 123.86, GenBank MH873620.1; Identities = 817/824 (99 %), no gaps), *Entosordaria quercina* (strain RO, GenBank MF488994.1; Identities = 794/826 (96 %), two gaps (0 %)), and *Entosordaria perfidiosa* (strain EPE, GenBank MF488993.1; Identities = 791/826 (96 %), two gaps (0 %)). The *LSU* sequences of CPC 42868 and 42875 are identical (822/822 nucleotides).


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*Etymology:* Name refers to the fact that it resembles *Ramularia* in morphology, but is phylogenetically distinct from that genus.

*Classification:* *Lecanoromycetes*, *Acarosporomycetidae*, *Acarosporales*, *Acarosperaceae*.

*Colonies* hyperparasitic on colonies caused by the leaf-spotting *Ragnhildiana ferruginea*. Mycelium hyaline, composed of conidiophores usually aseptate, i.e., reduced to conidiogenous cells, rarely with a single septum, subcylindrical to usually conical, straight to somewhat geniculate-sinuous caused by sympodial proliferation, unbranched, hyaline, smooth or almost so, with a single or 2–3 minute conidiogenous loci, sometimes almost denticule-like, slightly thickened and darkened, or conidia arising from minute peg-like protuberances of the hyphae, somewhat attenuated towards a truncated tip. *Conidia* solitary or usually in simple or branched acropetal chains, ellipsoid-ovoid, fusiform, subcylindrical, usually straight, 0–1-septate, thin-walled, hyaline, smooth or almost so to finely rough-walled, ends rounded to attenuated, with a single basal hilum and 1–2 hila at the apex, minute, barely thickened and darkened (adapted from Braun & Kruse 2021).


*Nothoramularia ragnhildianicola* (J. Kruse & U. Braun) Crous, J. Kruse & U. Braun, *comb. nov.* MB 848830. Fig. 28.


*Description and illustration:* Braun & Kruse (2021).

*Culture characteristics:* Colonies erumpent, spreading, with moderate aerial mycelium and feathery, lobate margin, reaching 6 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface dirty white, reverse luteous with patches of ochreous.

*Typus:* Germany, Rheinland-Pfalz, Landkreis Bad Dürkheim, Birkenheide, 1.6 km northwest of Eyersheimer Hof, meadow, 49°29‘41”N, 8°15‘29”E.
<table>
<thead>
<tr>
<th>Genus/Species</th>
<th>Culture/Clinical Accession Numbers</th>
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<tr>
<td><em>Leptodontidium</em></td>
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<tr>
<td><em>obscurum</em> CBS 405.85</td>
<td>MFl 873582</td>
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<tr>
<td><em>camptobactrum</em> CBS 237.53</td>
<td>MFl 886712</td>
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<tr>
<td><em>irregulare</em> CBS 152.60</td>
<td>MFl 89480</td>
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<tr>
<td><em>eliatius</em> var. <em>ovalisporum</em> CBS 394.76</td>
<td>MFl 877222</td>
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<td><em>eliatius</em> CBS 329.53</td>
<td>MFl 868765</td>
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<tr>
<td><em>trabinellum</em> SH17-39</td>
<td>ON241825</td>
</tr>
<tr>
<td><em>Neoleptodontidium</em></td>
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<tr>
<td><em>aciculare</em> CBS 123.86</td>
<td>MFl 873620</td>
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<tr>
<td><em>biseptatus</em> CBS 10037</td>
<td>NG_067443</td>
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<td><em>Dactylaria</em></td>
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<td><em>zapatensis</em> CBS 429.93</td>
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<td><em>monticola</em> CBS 188.95</td>
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<td><em>acaciae</em> CPC 29771</td>
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**Fig. 27.** Consensus phylogram (50 % majority rule) obtained from the maximum likelihood analysis with IQ-TREE v. 2.1.3 of the *Xylariales* LSU nucleotide alignment. Maximum likelihood (> 74 %) bootstrap support values from 1 000 non-parametric bootstrap replicates are shown at the nodes. Culture collection or voucher numbers and GenBank accession numbers (superscript) are indicated for all species. Sequences derived from material with a type status are indicated with a culture or voucher number highlighted with bold face. The tree was rooted to *Ramularia endophylla* (culture CBS 113265; GenBank AY490776) and the species treated here are highlighted with bold face. The families, orders and classes are shown in coloured blocks to the right of the tree. The scale bar indicates the expected number of changes per site.

**Additional material examined:** Germany, Rheinland-Pfalz, Landkreis Bad Dürkheim, Birkenheide, 49°29'41"N, 8°15'29"E, on *Ragnhildiana ferruginea*, parasitic on *Artemisia vulgaris*, 7 Sep. 2021, J. Kruse, POLL 9802 = HPC 3779, culture CPC 42463 = CBS 149075.

**Notes:** In culture, conidial loci are somewhat thickened, darkened, but not refractive. Ramoconidia commonly have numerous loci, giving rise to branched conidial chains. Conidiophores are erect, subcylindrical, straight, with terminal and intercalary conidiogenous cells, up to 150 µm tall, or reduced to conidiogenous loci on hyphae. Phylogenetically (Fig. 29), *No. ragnhildianicola* forms a lineage basal to *Neoacrodontiella*, *Neospermospora* and *Cytosporella*, with which it clusters with moderate support (87 % maximum parsimony and 74 % maximum likelihood).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence of CPC 42462 had highest similarity to *Neoacrodontiella eucalypti* (strain CBS 145561, GenBank NG_067885.1; Identities = 622/651 (96 %), six gaps (0 %)), *Neospermospora avenae* (strain CBS 886.68, GenBank MH878416.1; Identities = 607/652 (93 %), eight gaps (1 %)), and *Cytosporella juncicola* (strain CPC 38040, GenBank NG_068344.1; Identities = 597/651 (92 %), six gaps (0 %)). The ITS sequence of CPC 42462 is identical to that of CPC 42463 (739/739 nucleotides).

**Authors:** P.W. Crous, J.Z. Groenewald, U. Braun & J. Kruse


**Description and illustration:** Sogonov et al. (2008).

**Material examined:** Spain, Pontevedra, O Grove, on leaves of *Quercus robur* (Fagaceae), 21 Jan. 2022, J. Castillo, HPC 3874 = RKS 1161b, culture CPC 43206 = CBS 149691.

**Notes:** Known from overwintered leaves of *Fagaceae* in Canada, Europe, and the USA (Sogonov et al. 2008).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Ophiognomonia setacea* (strain CBS 859.79, GenBank AY818958.1; Identities = 534/535 (99 %), no gaps), *Ophiognomonia asiatica* (strain CBS 131351, GenBank NR_120057.1; Identities = 501/512 (98 %), five gaps (0 %)), and *Ophiognomonia sogonovii* (strain CBS 145561, GenBank NG_067885.1; Identities = 534/535 (99 %), no gaps), *Ophiognomonia asiatica* (strain CBS 131351, GenBank NR_120057.1; Identities = 501/512 (98 %), five gaps (0 %)), and *Ophiognomonia sogonovii* (strain CBS 145561, GenBank NG_067885.1; Identities = 534/535 (99 %), no gaps), *Ophiognomonia asiatica* (strain CBS 131351, GenBank NR_120057.1; Identities = 501/512 (98 %), five gaps (0 %)), and *Ophiognomonia sogonovii* (strain CBS 145561, GenBank NG_067885.1; Identities = 534/535 (99 %), no gaps), *Ophiognomonia asiatica* (strain CBS 131351, GenBank NR_120057.1; Identities = 501/512 (98 %), five gaps (0 %)).
Fig. 29. The first of two equally most parsimonious trees obtained from the Lecanoromycetes LSU sequence alignment. The scale bar indicates the number of changes and the numbers at the nodes represent maximum parsimony (>70%) and maximum likelihood (> 70%) bootstrap support values from 1 000 non-parametric bootstrap replicates. Branches that appear in the strict consensus tree are highlighted by thickened lines. Culture collection or voucher numbers and GenBank accession numbers (superscript) are indicated for all species. Sequences derived from material with a type status are indicated with a culture or voucher number highlighted with bold face. The tree was rooted to Xylaria hypoxylon (AFTOL-ID 51; GenBank AY544648) and the species treated here is highlighted with bold face. The families, orders and class are shown in coloured blocks to the right of the tree.
Ophiognomonia alni-cordatae (strain CBS 131353, GenBank JQ414175.1; Identities = 226/237 (95 %), no gaps). Closest hits using the tef1 (second part) sequence had highest similarity to Ophiognomonia setacea (strain CBS 859.79, GenBank JQ414154.1; Identities = 308/308 (100 %), no gaps), Melanconis stilbostoma (strain AFTOL-ID 936, GenBank DQ836910.1; Identities = 864/925 (93 %), two gaps (0 %)), Cryptodiaporthe aesculi (strain AFTOL-ID 1238, GenBank DQ836914.1; Identities = 861/924 (93 %), no gaps), and Gnomonia gnomon (strain AFTOL-ID 952, GenBank DQ471094.1; Identities = 859/924 (93 %), no gaps).


Paradissoconium narthecii Crous & Boers, Persoonia 47: 211. 2021. Fig. 30.

Description and illustration: Crous et al. (2021b).

Material examined: Netherlands, Drenthe Province, Dwingeloo, on dead leaves of Narthecium ossifragum (Melanthiaceae), 26 Sep. 2021, J. Boers, HPC 3781, culture CPC 42494 = CBS 149692.

Notes: Paradissoconium narthecii was described from dead leaves of Narthecium ossifragum in the Netherlands (Crous et al. 2021b), for which this represents a second collection of this fungus.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Paradissoconium narthecii (strain CBS 148449, GenBank NR_156213.1; Identities = 462/507 (91 %), 17 gaps (3 %)). Closest hits using the LSU sequence were Parasdissoconium narthecii (strain CBS 148449, GenBank NG_081323.1; Identities = 804/804 (100 %), no gaps), Ramichloridium eucleae (strain CBS 138000, GenBank NG_058086.1; Identities = 813/827 (98 %), two gaps (0 %)), and Ramichloridium indicum (strain CBS 171.96, GenBank EU041856.1; Identities = 795/811 (98 %), no gaps). Closest hits using the actA sequence had highest similarity to Paradissoconium narthecii (strain CPC 41970, GenBank OK651125.1; Identities = 539/539 (100 %), no gaps), Cladosporium ramotenellum (strain CBS 145592, GenBank MT223748.1; Identities = 382/407 (94 %), no gaps), and Davidiellomyces australiensis (strain CBS 142165, GenBank KY979853.1; Identities = 382/407 (94 %), no gaps). The rpb1 sequence is identical to that of Paradissoconium narthecii (strain CPC 41970, GenBank OK651151.1; Identities = 518/518 (100 %), no gaps).

Authors: P.W. Crous, J.Z. Groenewald & J. Boers


Description and illustration: Dissanayake et al. (2021).

Material examined: Spain, Cambrils, on bark of unknown tree, 26 Jan. 2022, J. Castillo, HPC 3869 = RKS 1158, culture CPC 43208 = CBS 149693.

Notes: Paraeutypella citricola was originally described (as Eutypella citricola) from twigs of Citrus in Argentina, and

Fig. 30. Paradissoconium narthecii (CPC 42494). A. Conidiophore. B–H. Conidiophores and conidiogenous cells giving rise to primary and secondary conidia. I. One-septate primary and aseptate secondary conidia. Scale bars = 10 µm.
recently reported from dead twigs of Acer palmatum in China (Dissanayake et al. 2021). It is recorded here from bark of a dead, unknown tree in Spain. This species has inoperculate asci that are distinctly amyloid (mounted in Lugol).

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Paraeutypella citricola (strain ICMP 17420, GenBank MH497577.1; Identities = 529/529 (100 %), no gaps), and Paraeutypella vitis (strain 59, GenBank KU320620.1; Identities = 513/530 (97 %), three gaps (0 %)). Closest hits using the LSU sequence were Paraeutypella citricola (as Eutypella citricola, strain CBS 128334, GenBank MH876333.1; Identities = 867/867 (100 %), no gaps), Libertia flaginea f. minor (strain CBS 196.30, GenBank MH866558.1; Identities = 862/868 (99 %), one gap (0 %)), and Eutypella microtheca (strain CBS 128336, GenBank NG_064278.1; Identities = 830/837 (99 %), no gaps). Closest hits using the rpb2 (first part) sequence had highest similarity to Paraeutypella citricola (strain GMBC0053, GenBank MW814998.1; Identities = 898/899 (99 %), no gaps), Allocryptovalsa sichuanensis (voucher HAKS 107017, GenBank MW658624.1; Identities = 770/886 (87 %), eight gaps (0 %)), and Eutypella quaternata (voucher MFLU 15-2605, GenBank MT432247.1; Identities = 715/860 (83 %), 14 gaps (1 %)). Closest hits using the tub2 sequence had highest similarity to Paraeutypella citricola (voucher HUEFS 131041, GenBank KT175565.1; Identities = 666/671 (99 %), no gaps), Diatrypella longisca (strain KUMCC 20-0021, GenBank MW239658.1; Identities = 564/663 (85 %), 26 gaps (3 %)), and Eutypella microtheca (strain AD53, GenBank KR822701.1; Identities = 548/667 (82 %), 39 gaps (5 %)).


Phomatospora endopteris W. Phillips & Plowr., Grevillea 13 (no. 67): 76. 1885. Fig. 31.

Description and illustration: Senanayake et al. (2016).

Cultures derived from single ascospores shot from a perithecium onto agar, but material inadequate for description. Ascospores aseptate, fusoid-ellipsoid, pale brown, guttulate, longitudinally striate, ends bluntly rounded, somewhat flattened, 9–10 (–11) × 3 μm. Mycelium consisting of hyaline, smooth, branched, septate, 1–1.5 μm diam hyphae. Conidiophores erect, straight to flexuous, arising from superficial hyphae, subcylindrical, 1–2-septate, or reduced to conidiogenous cells, pale brown, smooth, branched or not, 20–50 × 3–3.5 μm. Conidiogenous cells terminal and intercalary, pale brown, smooth, subcylindrical to obclavate, 7–20 × 3–3.5 μm, with terminal rachis of subdenticulate loci, 0.5–1 × 0.5 μm, slightly thickened and darkened. Conidia solitary, aseptate, hyaline, smooth, guttulate, slightly curved, narrowly fusoid, apex subobtuse, base truncate, (11–)13–15 (–18) × 1.5 μm.

Culture characteristics: Colonies erumpent, spreading, with sparse to moderate aerial mycelium and smooth, even margin, reaching 18 mm diam after 2 wk at 25 °C. On MEA surface dirty white, reverse ochreous; on PDA and OA surface and reverse dirty white.

Material examined: Netherlands, Utrecht Province, Bilthoven, on stems of Pteridium aquilinum (Dennstaedtiaceae), 24 May 2021, P.W. Crous, HPC 3644 = CBS H-24975, culture CPC 41832 = CBS 149073.

Notes: Phomatospora species are known from marine, aquatic and terrestrial habitats, where they are usually saprobic on submerged wood or decaying twigs (Senanayake et al. 2016). Phomatospora endopteris was isolated from single ascospores that shot onto agar from decaying stems of Pteridium aquilinum. Although the immersed ascomata could not be located, the ascospores were fusoid-ellipsoid and longitudinally striate, which fit well with the circumscription of the genus. Ellis & Ellis (1997) list ascospores of Phomatospora endopteris as 10–11 × 2.5–3 μm, thus fitting with those of the present collection. Phomatospora endopteris is currently not known from sequence data and the blast results below confirm its association with the genus Phomatospora. Although the genus contains more than 100 species names, only a few are currently known from sequence data, namely P. bellaminuta, P. biseriata, P. striatigera, P. uniseriata and P. vitifera. The lectotype species, P. berkeleyi, is currently not known from molecular data. See Phukhamsakda et al. (2020) for a recent phylogenetic treatment of the genus.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Phomatospora biseriata (strain MFLUCC 14-0832A, GenBank NR_154640.1; Identities = 478/522 (92 %), 11 gaps (2 %)), Phomatospora sp. (strain UBOCC-A-118152,
Phyllosticta multicorniculata Bissett & M.E. Palm, Canad. J. Bot. 67: 3382. 1989. Fig. 32.

Description and illustration: Bissett & Palm (1989).

Culture characteristics: Colonies erumpent, spreading, with moderate aerial mycelium and feathery, lobate margin, reaching 30 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface olivaceous grey, reverse iron-grey.

Typus: Canada, Prince Edward Island, Milton Station, on needles of Abies balsamea (Pinaceae), 19 Jul. 1966, C.D. MacCall (holotype DAOM 114552); New Brunswick, Charlotte Co., 1.5 km SW of Little Lepreau, 45.135614° -66.492269°, on buds of A. balsamea, 4 May 2021, D. Malloch, HPC 3633 (epitype designated here CBS H-24979, MBT 10013419, culture ex-epitype CPC 41921 = CBS 149077).

Additional material examined. Canada, New Brunswick, Charlotte Co., 1.5 km SW of Little Lepreau, 45.135614° -66.492269°, on buds of A. balsamea, 4 May 2021, D. Malloch, HPC 3633, culture CPC 41919 = CBS 149078.

Notes: Conidia of P. multicorniculata are (10–)12–15(–17) × (7–) 10–11(–13) μm, enclosed in a mucilaginous sheath, 1.5 μm thick, with 2–5 conoidal or cylindrical apical appendages, thus closely matching those of the holotype (Bissett & Palm 1989). Within the genus Phyllosticta, P. multicorniculata is peculiar in that it has several apical appendages, whereas most species have only a single, or no apical appendage. In the phylogenetic tree (Fig. 33), P. multicorniculata had a basal position in Phyllostictaceae (76 % / 95 % / 1). Neither the Bayesian nor the RAxML analyses resolve this species as a basal sister lineage; only in the most parsimonious trees does it take up a position as a basal sister lineage but without any support in the parsimony bootstrap consensus tree. Whether this species could represent a novel genus in the future remains to be seen as the phylogenetic trees obtained from the different analyses were inconclusive to clearly separate it from the other species in Phyllosticta. The similarities of the different loci seem to fit within the genetic diversity of the genus (blast results filtered for Phyllostictaceae): ITS (based...
Fig. 33. Consensus phylogram (50 % majority rule) obtained from the maximum likelihood analysis with IQ-TREE v. 2.1.3 of the Phyllostictaceae and related families LSU nucleotide alignment. Maximum likelihood (> 74 %) and maximum parsimony (> 74 %) bootstrap support values from 1 000 non-parametric bootstrap replicates, and Bayesian posterior probabilities (> 0.84), are shown at the nodes. Thickened lines represent nodes which received full support (100 % / 100 % / 1) from all three analyses. Culture collection or voucher numbers and GenBank accession numbers (superscript) are indicated for all species. Sequences derived from material with a type status are indicated with a culture or voucher number highlighted with bold face. The tree was rooted to *Diaporthe perjuncta* (voucher BPI 748437; GenBank NG_059064) and the species treated here is highlighted with bold face. The families, orders and class are shown in coloured blocks to the right of the tree. The scale bar indicates the expected number of changes per site.
on the top 2 531 blast hits using GenBank FJ824766) = 82.08 % – 100 %; LSU (based on the top 282 blast hits using GenBank KF766342) = 90.24 % – 100 %; actA (based on the top 345 blast hits using the first 240 nucleotides of GenBank JX025029) = 77.13 % – 100 %; gapdh (based on the top 355 blast hits using GenBank MN556783) = 86.39 % – 100 %; and tef1 (based on the top 249 blast hits using GenBank MN556818) = 79.34 % – 100 %.

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence of CPC 41921 had highest similarity to *Phyllosticta parthenocissi* (deposited in GenBank as *Guignardia bidwellii*; strain CBS 111645, GenBank JF824766.1; Identities = 445/536 (83 %), 27 gaps (5 %)), *Phyllosticta foliorum* (deposited in GenBank as *Guignardia philiprina*; strain CBS 447.68, GenBank AF312014.1; Identities = 448/514 (83 %), 32 gaps (5 %)), and *Phyllosticta citrina* (strain PD 010 04421662, GenBank MZ416911.1; Identities = 329/367 (90 %), 15 gaps (4 %)). The ITS sequences of CPC 41921 and 41919 are identical (528/528 nucleotides). Closest hits using the LSU sequence of CPC 41921 were *Phyllosticta philiprina* (strain CBS 901.69, GenBank KF766342.1; Identities = 765/793 (96 %), no gaps), *Phyllosticta styriacola* (strain JFRL 03-770, GenBank OQ195355.1; Identities = 759/793 (96 %), no gaps), and *Phyllosticta miniama* (strain CBS 585.84, GenBank KF666382.1; Identities = 759/793 (96 %), no gaps). The LSU sequences of CPC 41921 and 41919 are identical (793/793 nucleotides). Closest hits using the actA sequence of CPC 41921 had highest similarity to *Phyllosticta ependalarticola* (strain CBS 146014, GenBank MN556783.1; Identities = 462/504 (92 %), nine gaps (1 %)), *Phyllosticta astroafricana* (strain CBS 144593, GenBank MK442640.1; Identities = 445/485 (92 %), six gaps (1 %)), and *Phyllosticta hagahagoensis* (strain CBS 144592, GenBank MK442641.1; Identities = 458/504 (91 %), nine gaps (1 %)). The actA sequences of CPC 41921 and 41919 are identical (533/533 nucleotides). Closest hits using the gapdh sequence of CPC 41921 had highest similarity to *Phyllosticta hubeiensis* (strain LC1654, GenBank JX025029.1; Identities = 304/336 (90 %), no gaps), *Phyllosticta rhizophora* (strain NCUVCC 19-0358, GenBank MT363251.1; Identities = 301/336 (90 %), no gaps), and *Phyllosticta capitans* (strain IPN20.1, GenBank KX280619.1; Identities = 300/335 (90 %), no gaps). The gapdh sequences of CPC 41921 and 41919 are identical (545/545 nucleotides). Closest hits using the tef1 (first part) sequence of CPC 41921 had highest similarity to *Phyllosticta encephalarticola* (strain CBS 146014, GenBank MN556818.1; Identities = 290/332 (87 %), 14 gaps (4 %)), *Phyllosticta hagahagoensis* (strain CBS 144592, GenBank MK442705.1; Identities = 290/332 (87 %), 14 gaps (4 %)), and *Phyllosticta carissioca* (strain CPC 25665, GenBank KT950879.1; Identities = 290/332 (87 %), 14 gaps (4 %)). The tef1 sequences of CPC 41921 and 41919 are identical (409/409 nucleotides).

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**Pleurotheciella aquatica** Z.L. Luo et al., *Mycol. Progr.* 17: 517. 2018. Fig. 34.

Description and illustration: Huang et al. (2022).


Notes: *Pleurotheciella aquatica* was described from submerged wood collected in China (Huang et al. 2022). This is the first report of this fungus from the UK.

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Pleurotheciella aquatica* (voucher MFLU 17-0911, GenBank NR_160591.1; Identities = 499/500 (99 %), no gaps), *Pleurotheciella fusiformis* (strain MFLUCC 16-1356, GenBank MF399235.1; Identities = 494/514 (96 %), nine gaps (1 %)), and *Pleurotheciella unisepata* (strain KAS 4459, GenBank KT278729.1; Identities = 500/534 (94 %), 13 gaps (2 %)). Closest hits using the LSU sequence were *Pleurotheciella aquatica* (voucher MFLU 17-0911, GenBank NG_066193.1; Identities = 799/800 (99 %), one gap (0 %)), *Pleurotheciella fusiformis* (strain MFLUCC 17-0115, GenBank MF399249.1; Identities = 755/762 (99 %), one gap (0 %)), and *Pleurotheciella unisepata* (voucher MFLUCC 17-0913, GenBank NG_066195.1; Identities = 790/800 (99 %), one gap (0 %)). Closest hits using the rpb2 (first part) sequence had highest similarity to *Pleurotheciella aquatica* (strain MFLUCC 17-0464, GenBank MF401405.1; Identities = 655/655 (100 %), no gaps), *Pleurotheciella fusiformis* (strain MFLUCC 17-0113, GenBank MF401403.1; Identities = 661/699 (95 %), no gaps), and *Pleurotheciella unisepata* (strain KUMCC 15-0407, GenBank MF401401.1; Identities = 541/599 (90 %), no gaps).

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**Fig. 34. Pleurotheciella aquatica** (CPC 44105). **A–C.** Conidiophores and conidiogenous cells giving rise to conidia. **D.** Conidia. Scale bars = 10 μm.
Ramularia pistaciae Crous, Persoonia 42: 353. 2019. Fig. 35.

Description and illustration: Crous et al. (2019a).


Notes: Ramularia pistaciae was recently described from leaves of Pistacia lentiscus collected in Rome, Italy (Crous et al. 2019a). This is the first record from the UK, where it is associated with leaf spots of Arbutus unedo.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Ramularia pistaciae (strain CBS 145564, GenBank NR_165576.1; Identities = 491/491 (100 %), no gaps), Ramularia glennii (strain SFI-D21, GenBank MT535837.1; Identities = 480/492 (98 %), two gaps (0 %)), and Ramularia eucalypti (strain SFI-D26, GenBank MT535835.1; Identities = 480/492 (98 %), two gaps (0 %)). Closest hits using the LSU sequence were Ramularia alangiicola (strain CPC 10299, GenBank KX287023.1; Identities = 825/828 (99 %), no gaps), Ramularia eucalypti (strain CBS 120726, GenBank NG_069156.1; Identities = 812/815 (99 %), no gaps), and Ramularia rumicicola (strain CPC 11296, GenBank KX287206.1; Identities = 824/828 (99 %), no gaps (0 %)). Closest hits using the actA sequence had highest similarity to Ramularia pistaciae (strain CBS 145564, GenBank MK876462.1; Identities = 571/575 (99 %), no gaps), Ramularia gaultheriae (strain CBS 299.80, GenBank KX288569.1; Identities = 532/577 (92 %), six gaps (1 %)), and Ramularia unterseheri (strain CBS 124852, GenBank KP894376.1; Identities = 530/576 (92 %), three gaps (0 %)). Closest hits using the gapdh sequence had highest similarity to Ramularia pistaciae (strain CBS 145564, GenBank MK876473.1; Identities = 525/536 (98 %), one gap (0 %)), Ramularia viziellae (strain CPC 25731, GenBank KP894644.1; Identities = 417/457 (91 %), seven gaps (1 %)), and Ramularia lamigera (strain IRAN 3985C, GenBank MW272941.1; Identities = 335/423 (91 %), seven gaps (1 %)). Closest hits using the his3 sequence had highest similarity to Ramularia tricherae (strain CBS 108973, GenBank KP894799.1; Identities = 346/359 (96 %), no gaps), Ramularia abscondita (strain CBS 114727, GenBank KX288753.1; Identities = 341/356 (96 %), two gaps), and Ramularia variabilis (strain CPC 16866, GenBank KP894829.1; Identities = 345/362 (95 %), no gaps). Closest hits using the rpb2 (first part) sequence had highest similarity to Ramularia gaultheriae (strain CBS 299.80, GenBank KX288569.1; Identities = 620/731 (85 %), no gaps), Ramularia neodeusta (strain CPC 13567, GenBank KX288638.1; Identities = 618/735 (84 %), six gaps (0 %)), and Ramularia cyclaminicola (strain CBS 399.51, GenBank KX288571.1; Identities = 617/736 (84 %), four gaps (0 %)). Closest hits using the tef1 (first part) sequence had highest similarity to Ramularia malicola (strain CBS 119227, GenBank KX288036.1; Identities = 301/308 (98 %), one gap (0 %)), Ramularia rumicicola (strain CPC 11296, GenBank KX288065.1; Identities = 274/330 (83 %), 17 gaps (5 %)), and Ramularia lamii var. lamii (strain CBS 108971, GenBank KX288023.1; Identities = 272/313 (87 %), 13 gaps (4 %)).

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Fig. 35. Ramularia pistaciae (CPC 44067). A–H. Conidiophores and conidiogenous cells giving rise to branched conidial chains. I. Conidia. Scale bars = 10 µm.
Ruptoseptoria unedonis (Roberge ex Desm.) Quaedvlieg et al., Stud. Mycol. 75: 357. 2013. Fig. 36.

Description and illustration: Quaedvlieg et al. (2013).


Notes: Ruptoseptoria unedonis is known to cause prominent leaf spotting of Arbutus unedo, and is also known from the UK (Quaedvlieg et al. 2013).

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Ruptoseptoria unedonis (strain CBS 355.86, GenBank KF251228.1; Identities = 494/494 (100%), no gaps), Chuppomyces handelii (strain CBS 113302, GenBank EU167581.1; Identities = 471/496 (95%), four gaps (0%), and Neosonderdenia eucalypti (strain CBS 145081, GenBank NR_165602.1; Identities = 460/499 (92%), 13 gaps (2%)). Closest hits using the LSU sequence were Ruptoseptoria unedonis (strain CBS 755.70, GenBank KF251732.1; Identities = 825/826 (99%), one gap (0%)), Chuppomyces handelii (strain CBS 113302, GenBank GU214437.1; Identities = 811/828 (98%), five gaps (0%)), and Pruniphilomyces circumscissus (strain CPC 36434, GenBank MT223926.1; Identities = 795/827 (96%), five gaps (0%)). Closest hits using the rpb2 (first part) sequence had highest similarity to Ruptoseptoria unedonis (strain CBS 755.70, GenBank MF951659.1; Identities = 768/771 (99%), no gaps), Chuppomyces handelii (strain CBS 113302, GenBank MF951475.1; Identities = 677/773 (88%), no gaps), and Neopendiella nectandreae (strain ATCC 200932, GenBank MF951546.1; Identities = 580/740 (78%), 16 gaps (2%)).

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Description and illustration: Bell & Mahoney (1995).


Notes: Schizothecium conicum is a common species occurring on dung of herbivores, and is here recorded from culms of Juncus effusus in the Netherlands.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Podospora conica (strain CBS 128.94, GenBank AY515366.1; Identities = 519/521 (99%), one gap (0%)), Schizothecium carpinicola (strain CBS 228.87, GenBank NR_103589.1; Identities = 515/523 (98%), two gaps (0%), and Podospora vesticola (strain NZ196A5, GenBank AY515365.1; Identities = 514/523 (98%), two gaps (0%). Closest hits using the LSU sequence were Schizothecium vesticola (strain SMH3187, GenBank AY780076.1; Identities = 823/823 (100%), no gaps), Podospora tetraspora (strain CBS 262.70, GenBank MH871362.1; Identities = 821/823 (99%), no gaps), and Podospora minicauda (strain CBS 227.87, GenBank MH873757.1; Identities = 818/823 (99%), no gaps). No LSU sequence of Podospora conica was available for comparison in GenBank.

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Sporidesmiella pini Crous, Persoonia 47: 259. 2021. Fig. 38.

Description and illustration: Crous et al. (2021b).

Mycelium consisting of hyaline, smooth, branched, septate, 1.5–2 µm diam hyphae. Conidiophores solitary, erect, macroanomatocephalous, straight to slightly flexuous, brown, smooth- and thick-walled, base swollen, 7–10 µm diam, 3–6-septate, 50–150 × 3.5–4.5 µm. Conidiogenous cells integrated, terminal, subcylindrical, scars inconspicuous, proliferating percurrently with delayed succession, rejuvenating sympodially, pale brown, smooth, 45–65 × 3.5–4 µm. Conidia solitary, dry, obovoid, medium brown, smooth, guttulate, apex obtuse, base slightly darkened, 2.5–4 µm diam, 3–4 distoseptate, with central pore in each septum, (17–)19–21(–24) × (8–)9–10 µm.

Culture characteristics: Colonies erumpent, spreading, with moderate aerial mycelium and smooth, lobate margin, reaching 30 mm diam after 2 wk at 25°C. On MEA and PDA surface bay with vinaceous pigment, reverse dark brick; on OA surface ochreous.


Notes: Sporidesmiella pini was recently described from needles of Pinus sylvestris collected in Utrecht Province of the Netherlands (Crous et al. 2021b). This represents a second collection from the Netherlands, namely from Juncus effusus culms collected in Overijssel Province. This represents the 3rd species of Sporidesmiella collected from Juncus in the Netherlands. Their conidial morphology is similar (3–4-distoseptate, obovoid) but Sp. junci and Sp. juncicola have (slightly) longer conidia: viz. (20–)22–25(–27) × (9–)10–12 and (20–)28–32(–35) × (8–)9–10 µm (Crous et al. 2021b, 2022a).
Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence of CPC 41494 had highest similarity to *Sporidesmiella pini* (strain CPC 40067, GenBank OK664747.1; Identities = 559/569 (98 %), five gaps (0 %; four of which involve T-repeats at three positions)), *Sporidesmiella hyalosperma* (strain CPC 37552, GenBank MT223845.1; Identities = 552/572 (97 %), ten gaps (1 %)), and *Sporidesmiella obovoidia* (strain MFLUCC 17-2372, GenBank MW286492.1; Identities = 546/570 (96 %), 15 gaps (2 %)). The ITS sequences of CPC 41494 and 41495 are 98 % identical (551/561 nt, including five gaps all involving different numbers of T-repeats at four positions). Closest hits using the LSU sequence of CPC 41494 were *Sporidesmiella pini* (strain CPC 40067, GenBank NG_081347.1; Identities = 642/642 (100 %), no gaps), *Sporidesmiella obovoidia* (strain MFLUCC 17-2372, GenBank NG_075412.1; Identities = 639/642 (99 %), no gaps), and *Sporidesmiella hyalosperma* (strain S-1518, GenBank MK849842.1; Identities = 636/642 (99 %), no gaps). The LSU sequences of CPC 41494 and 41495 are 100 % identical (642/642 nt). Closest hits using the *rpb2* (first part) sequence

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**Fig. 37.** *Schizothecium conica* (CPC 44110). A. Ascomata on SNA. B–G. Asci with ascospores. Scale bars: A = 180 µm, all others = 10 µm.

**Fig. 38.** *Sporidesmiella pini* (CPC 41495). A. Colony on SNA. B–E. Conidiophores and conidiogenous cells giving rise to conidia. F. Conidia. Scale bars = 10 µm.
of CPC 41494 had highest similarity to *Sporidesmiella pini* (strain CPC 40067, GenBank OK651177.1; Identities = 746/747 (99 %), no gaps), *Sporidesmiella hyalosperma* (strain MFLUCC 18-1013, GenBank MW504070.1; Identities = 695/745 (93 %), no gaps), and *Sporidesmiella novae-zelandiae* (voucher MFLU 18-2332, GenBank MN124525.1; Identities = 656/747 (88 %), no gaps). Closest hits using the *tef1* (second part) sequence of CPC 41494 had highest similarity to *Sporidesmiella* sp. (strain JAUCC 3436, GenBank OK323223.1; Identities = 338/356 (95 %), no gaps), *Sporidesmiella hyalosperma* (voucher MFLU 18-2330, GenBank MN194033.1; Identities = 327/342 (96 %), no gaps), and *Sporidesmiella aquatica* (voucher MFLU 18-1602, GenBank MN194034.1; Identities = 323/342 (94 %), no gaps). No *tef1* sequence of *Sporidesmiella pini* is available for comparison.

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**Thyronectria caraganae** Voglmayr et al., *Mycol. Progr.* 15: 924. 2016. Fig. 39.

**Description and illustration:** Voglmayr et al. (2016).

*Mycelium* consisting of hyaline, smooth, branched, septate, 1.5–2 µm diam hyphae. *Conidiophores* reduced to conidiogenous cells, 2–10 × 2–3.5 µm, forming directly on hyphae, varying from hyphal pegs to ampulliform cells, phialidic, solitary or aggregated, giving rise to sporodochia. *Conidia* allantoid, hyaline, smooth, slightly curved, aseptate, guttulate, ends obtuse, (4–)5–8(–12) × 1.5–2(–3) µm.

**Culture characteristics:** Colonies flat, spreading, with sparse aerial mycelium, covering dish after 2 wk at 25 °C. On malt extract agar (MEA) surface and reverse sienna; on potato dextrose agar (PDA) surface and reverse pale luteous; on oatmeal agar (OA) surface ochreous.

**Typus:** Ukraine, Mykolaiv district, Berezansky area, Tashine, on *Caragana arborescens* (Leguminosae), 16 May 1990, L.V. Smyk (*holotype* WU 35938); Dvorichna district, Kharkiv region, Krasne Pershe village, National Park Dvorichanskyi, on twigs of *C. arborescens*, 11 Apr. 2021, A. Akulov, ex CWU (MYC) AS 8120 = HPC 3629 (*epitype* designated here CBS H-24959, MBT 10013420, culture ex-epitype CPC 41504 = CBS 148949).

**Additional material examined:** Ukraine, Kharkiv region, Arseniivskyi skyt, Nnat. Park Sviati hory, on dead branch of *Caragana arborescens*, 1 Aug. 2021, A. Akulov, HPC 3729, culture CPC 42342 = CBS 149509.

**Notes:** When Voglmayr et al. (2016) described *Thyronectria caraganae*, no asexual morph was observed, and DNA was extracted directly from the specimen due to the absence of a culture. In this study, we isolated the fungus from a fresh collection, and also observed an asexual morph to develop in

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**Fig. 39.** *Thyronectria caraganae* (CPC 41504). A. Ascomata erumpent from host tissue. B, C. Asci. D, E. Ascospores. F–I. Conidiophores and conidiogenous cells giving rise to conidia. J. Conidia. Scale bars: A = 180 µm, all others = 10 µm.
vitro, making this an appropriate candidate for epitypification. The two cultures examined here were not genetically identical to each other. For the present, we have chosen not to introduce an additional species pending the collection of more specimens.

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence of CPC 41504 had highest similarity to *Thyronectria caraganae* (voucher WU 35399, GenBank NR_155911.1; Identities = 560/560 (100 %), no gaps), *Thyronectria rhodochlora* (strain NP10, GenBank KM225684.1; Identities = 534/565 (95 %), 12 gaps (2 %)), and *Thyronectria abieticola* (strain THYA, GenBank OL439231.1; Identities = 532/563 (94 %), 12 gaps (2 %)). The ITS sequences of CPC 41504 and 42342 are 98 % identical (531/542 nt, including three gaps). Closest hits using the LSU sequence of CPC 41504 were *Thyronectria caraganae* (strain TCA1, GenBank KXS14385.1; Identities = 757/757 (100 %), no gaps), *Thyronectria aurigera* (strain THAU1, GenBank OL439233.1; Identities = 738/757 (97 %), no gaps), and *Thyronectria berolinensis* (strain CBS 127382, GenBank MH875990.1; Identities = 736/756 (97 %), no gaps). The LSU sequences of CPC 41504 and 42342 are 99 % identical (752/757 nt, no gaps). A manual comparison using the actA sequence of CPC 41504 revealed a similarity of 91 % (224/246 nt) with *Thyronectria caraganae* (strain TCA1, GenBank KXS14382.1). Closest hits using the rpb1 sequence of CPC 41504 had highest similarity to *Thyronectria caraganae* (strain TCA1, GenBank KXS14390.1; Identities = 487/488 (99 %), no gaps), *Thyronectria austroamericana* (strain GG, GenBank KJ570717.1; Identities = 414/490 (84 %), five gaps (1 %)), and *Thyronectria rhodochlora* (strain NP1, GenBank KJ570727.1; Identities = 411/490 (84 %), five gaps (1 %)). The rpb1 sequences of CPC 41504 and 42342 are 98 % identical (479/488 nt, no gaps). Closest hits using the rp22 (first part) sequence of CPC 42342 had highest similarity to *Thyronectria rhodochlora* (strain NP2, GenBank KJ570751.1; Identities = 722/839 (86 %), six gaps (0 %)), *Thyronectria xanthoxyli* (strain NP12, GenBank OL440145.1; Identities = 720/837 (86 %), two gaps (0 %)), and *Thyronectria berolinensis* (strain CBS 127382, GenBank HM534883.1; Identities = 716/835 (86 %), four gaps (0 %)). Closest hits using the tef1 (second part) sequence of CPC 42342 had highest similarity to *Thyronectria caraganae* (strain TCA1, GenBank KXS14396.1; Identities = 720/728 (99 %), no gaps), *Metapochonia rubescens* (strain CBS 110436, GenBank KJ398795.1; Identities = 800/864 (93 %), no gaps), and *Metapochonia bulbillosa* (strain P6656, GenBank MT701577.1; Identities = 800/864 (93 %), no gaps). Closest hits using the tub2 sequence of CPC 41504 had highest similarity to *Thyronectria caraganae* (strain TCA1, GenBank KXS14399.1; Identities = 359/359 (100 %), no gaps), *Metapochonia bulbillosa* (strain CBS 145.70, GenBank KJ398549.1; Identities = 201/224 (90 %), eight gaps (3 %)), and *Metapochonia goniodes* (strain CBS 891.72, GenBank KJ398551.1; Identities = 202/226 (89 %), seven gaps (3 %)). The tub2 sequences of CPC 41504 and 42342 are 97 % identical (348/359 nt, no gaps).

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**Description and illustration:** Réblová & Gams (2016).

Ascomata in densely aggregated clusters in substrate, superficial, non-stromatic, globose with papillate apex and central ostiole, dark brown to black, 180–250 μm diam, sparsely setose; setae up to 20 μm long, 6–7 μm diam near base, dark brown, 0–1-septate, tapering to subobtuse apex. Ostiole periphysate. *Paraphyses* hyaline, septate, 2.5–3 μm diam, trabeclae-like. Asci 35–55 × 4–6 μm, cylindrical, short stipitate, apex obtuse, without visible discharge mechanism (in Melzer), 8-spored. *Ascosporae* 7–(8.5) × (2.5)–3–3.5 μm, broadly ellipsoid, hyaline, aseptate, becoming mildly septate, guttulate, verruculose, at times with lateral pegs on some ascospores. *Mycelium* consisting of hyaline, smooth, branched, septate, 1.5–2 μm diam hyphae. *Conidiophores* erect, solitary, branched below, subverticillately, or unbranched, subcyllindrical, hyaline, smooth, 1–3-septate, 60–100 × 3–4 μm. *Conidigenous cells* integrated, terminal and lateral, hyaline, smooth, phialidic, subcyllindrical to aculeate, apex with cylindrical collarette, 1–2 μm long, 25–50 × 2.5–3 μm. *Conidia* aggregating in mucoid mass, hyaline, smooth, aseptate, broadly ellipsoid, guttulate, apex subobtuse, tapering to truncate hilum, 0.5 μm diam or base slightly rounded, (4.5–) 5–(6–) × 2.5–3 μm.

**Culture characteristics:** Colonies flattened, spreading, folded, with moderate aerial mycelium and smooth, lobate margin, reaching 40 mm diam after 2 wk at 25 °C. On malt extract agar (MEA), potato dextrose agar (PDA) and oatmeal agar (OA) surface and reverse olivaceous grey.


**Notes:** Barr (1990) circumscribed the *Trichosphaeriaceae* to include *Acanthostigma*, *Eriosphaeria*, *Rhamphoria* and *Trichosphaeria*, although Barr & Cannon (1994) agreed that the family was heterogeneous. Several taxa were subsequently relocated to *Nisssliaeae*, *Lasiosphaeriaceae* and *Sordariales*. Due to these problems, Réblová & Winka (2001) suggested that no additional genera be accepted in the family, until the phylogeny of *T. pilosa* could be resolved.

Réblová & Gams (2016) examined several collections from Persoon's fungarium preserved in L under the name *S. pilosa*. However, these were shown to represent different fungi not corresponding with the protologue. Furthermore, the original illustration [Persoon 1800: tab. 10: 9–10; cited as fig. 2 in Réblová & Gams (2016)] was regarded as insufficient to serve as a nomenclatural type, and therefore a neotype was designated, which correlates well with the epitype we designate in this study.

Because of the uncertain higher-level phylogeny of *Trichosphaeria*, Réblová & Gams (2016) recommended accepting *Trichosphaeria* as the only member of the *Trichosphaeriaceae*, pending further collections of *T. pilosa*. Currently, Wijayawardene et al. (2022) list 11 genera as belonging to *Trichosphaeriaceae* (*Trichosphaeriaceae*). Based on the phylogenetic tree presented in this study (Fig. 41), *T. pilosa* is embedded within what was previously recognised as *Plectosphaerellaceae* (see for example Giraldo & Crous 2019), with *Fuschoypha expansa* as closest phylogenetic neighbour (97 % / 83 % / 1). *Trichosphaeriaceae* predates *Plectosphaerellaceae* (1885 vs 2007), and the latter is
therefore reduced to synonym. The family clade is fully supported in all three phylogenetic analyses. On the other hand, the lineage containing Australiascaceae, Glomerellaceae, Malaysiaascaceae and Reticulasceae had low to full support (54% / 90% / 1), while the node connecting these families to the Trichosphaeriaceae node was poorly supported in the maximum likelihood analyses (68% and 38%). Furthermore, this node was absent in the Bayesian phylogeny as these former families formed a sister lineage to Falcocladiaceae, Microascales and Torpedosporales with a Bayesian posterior probability value of 0.84 (data not shown).

We have therefore retained the use of Glomerellales for those families, while resurrecting Trichosphaeriaceae for the former family Plectosphaerellaceae. In the phylogenetic analysis of Hyde et al. (2020), "Trichosphaeriaceae" (based on "Brachysporium" groenendalenis; see pages 357 and 364 of Hyde et al. 2020) is genetically distinct from families associated with Glomerellales (see pages 359 and 388 of Hyde et al. 2020). Genera previously considered to belong to Trichosphaeriaceae need to be re-evaluated as it is clear that some of these, e.g. Brachysporium, need to be assigned to a different order and family.

With the designation of an epitype in this study, this matter is now resolved, although the recently introduced family Plectosphaerellaceae (2007) is reduced to synonymy. Based on the phylogeny of T. pilosa, the Trichosphaeriaceae presently contains 25 genera.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to Verticillium albo-atrum (strain CBS 103.95, GenBank LR026714.1; Identities = 476/496 (96%), three gaps (0%)), Plectosphaerella kunmingensis (strain KUMCC 18-0181, GenBank MK993014.1; Identities = 481/511 (94%), 11 gaps (2%)), and Plectosphaerella cucumerina (strain ZMXR22 GenBank MT446127.1; Identities = 486/518 (94%), ten gaps (1%)). Closest hits using the LSU sequence were Plectosphaerella kunmingensis (voucher KUMCC 18-0181, GenBank MK993015.1; Identities = 838/848 (99%), no gaps), Gibellulopsis nigrescens (strain CBS179.40, GenBank MH867573.1; Identities = 836/848 (99%), no gaps), and Cephalosporium serratim (strain CBS 290.30, GenBank LR025872.1; Identities = 804/816 (99%), no gaps). No significant hits were obtained using the actA and his3 sequences. Closest hits using the tef1 (first part) sequence had highest similarity to Verticillium albo-atrum (strain RgVa1, GenBank MZ710737.1; Identities = 641/694 (92%), no gaps), Plectosphaerella slobbergiarum (strain NL1930002, GenBank MW890074.1; Identities = 510/557 (92%), no gaps), and Plectosphaerella cucumerina (strain CBS 137.37, GenBank LR026199.1; Identities = 629/691 (91%), no gaps). Closest hits using the tef1 (first part) sequence had highest similarity to Plectosphaerella ramisepata (strain CBS 138161, GenBank KY421317.1; Identities = 144/148 (97%), no gaps), Gloeocladium cibotii (strain CBS 299.70H, GenBank EF543807.1; Identities = 147/152 (97%), no gaps), and Plectosphaerella alismatis (strain CBS 113362, GenBank KY421328.1; Identities = 143/148 (97%), no gaps). Closest hits using the tef1 (second part) sequence had highest similarity to Plectosphaerella kunmingensis (strain KUMCC 18-0181, GenBank MK993017.1; Identities = 834/894 (93%), no gaps), Plectosphaerella cucumerina (strain CBS 144925, GenBank LR594767.1; Identities = 837/903 (93%), no gaps), and Plectosphaerella plurivora (strain GZUIFR-H26-5.1, GenBank MK930456.1; Identities = 834/901 (93%), no gaps). Closest hits using the tub2 sequence had highest similarity to Plectosphaerella populi (strain CBS 139623, GenBank KY421311.1; Identities = 261/331 (79%), 15 gaps (4%)), and Pleurotheciella rivulata (strain CBS 125237, GenBank KT278760.1; Identities = 259/332 (78%), 17 gaps (5%).

![Image](image-url)

Fig. 40. Trichosphaeria pilosa (CPC 42927). A. Ascomata on host tissue. B. Ascomatal wall with setae. C, D. Asci. E. Ascospores. F. Colony on OA. G–I. Conidiophores and conidiogenous cells giving rise to conidia. J. Conidia. Scale bars: A = 180 µm, all others = 10 µm.
Fig. 41. Consensus phylogram (50 % majority rule) obtained from the maximum likelihood analysis with IQ-TREE v. 2.1.3 of the Sordariomycetes ITS/LSU/tef1 nucleotide alignment. Maximum likelihood (> 74 %) and RAxML (> 74 %) bootstrap support values from 1 000 non-parametric bootstrap replicates, and Bayesian posterior probabilities (> 0.84), are shown at the nodes. Thickened lines represent nodes which received full support (100 % / 100 % / 1) from all three analyses. Culture collection or voucher numbers are indicated for all species. Sequences derived from material with a type status are indicated with a culture or voucher number highlighted with bold face. GenBank accession numbers of the sequences used in the alignment are listed in supplementary Table S2. The tree was rooted to Savoryella lignicola (strain NF00204) and the species treated here is Savoryella lignicola (strain NF00204) and the species treated here is

Classification: Sordariomycetes, Hypocreomycetidae, Trichosphaeriales, Trichosphaeriaceae.

Order

Family

Currently accepted genera
Acrostalagnus Corda, Icones fungorum hucusque cognitorum 2: 15. 1838.
Chordomyces Bilanen et al., Fungal Diversity 76: 55. 2015.

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Fig. 41. (Continued).


**Musicillium** Zare & W. Gams, *Nova Hedwigia* **85**: 482. 2007.


**Plectosphaerella** Kleb., *Phytopathol. Z.* **1**: 43. 1929.


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**Zaanenomyces versatilis** Crous & Osieck, *Persoonia* **47**: 235. 2021. Fig. 42.

**Mycelium** consisting of pale brown, smooth, guttulate, branched, septate, 1.5–2 μm diam hyphae, forming hyphal coils. **Conidiophores** erect, solitary, arising from superficial hyphae, terminal or intercalary, pale brown, smooth, subcylindrical, 15–40 2–3 μm. **Conidiogenous cells** terminal, pale brown, smooth, 5–25 × 2–3 μm, developing a rachis of denticles, 1–3 × 1–2 μm, not thickened nor darkened, with truncate apices. **Conidia** solitary, dry, fusoid, widest in middle, hyaline, smooth, guttulate, apices obtuse, hilum truncate, 1–1.5 μm diam, unthickened, not darkened, 3–6(–8)-septate, (35–)50–60(–70) × (2.5–)3 μm.

**Culture characteristics:** Colonies erumpent, spreading, with moderate aerial mycelium and smooth, lobate margin, reaching 15 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface and reverse olivaceous grey.


**Notes:** *Zaanenomyces* (*Tubeufiaceae*) was recently introduced for three cercosporoid-like hyphomycetes occurring on culms of *Juncus* in the Netherlands (Crous et al. 2021b). *Zaanenomyces versatilis* was described as having conidia that are (3–)7–10(–12)-septate, (16–)43–50(–55) × (2.5–)3(–3.5) μm. The present collection has slightly longer conidia, with less septa, but fits well based on its DNA phylogeny.

Based on a megablast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Zaanenomyces versatilis* (strain CBS 148312, GenBank NR_175227.1; Identities = 556/557 (99 %), no gaps), *Zaanenomyces moderatricis-academiae* (strain CBS 148315,

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**Fig. 42. Zaanenomyces versatilis** (CPC 42831). **A.** Colony on SNA. **B–E.** Conidiophores and conidiogenous cells giving rise to conidia. **F.** Conidia. Scale bars = 10 μm.
GenBank NR_175222.1; Identities = 485/505 (96 %), six gaps (1 %)), and Camporesiomyces vaccini (strain CBS 216.90, GenBank NR_156202.1; Identities = 490/559 (88 %), 15 gaps (2 %)). Closest hits using the LSU sequence were Zaenonemycetes versatilis (strain CBS 148312, GenBank NG_081336.1; Identities = 820/820 (100 %), no gaps), Zaenonemycetes moderaticris-academicae (strain CBS148315, GenBank_NG_081331.1; Identities = 789/793 (99 %), no gaps), and Helicosporium luteosporum (voucher MFLU 16-2871, GenBank NG_059773.1; Identities = 811/836 (97 %), no gaps). Closest hits using the tef1 (second part) sequence had highest similarity to Helicosporium flavum (strain MFLUCC 16-1230, GenBank KX873285.1; Identities = 765/825 (93 %), two gaps (0 %)), Tubefusia cylindrotheca (strain MFLUCC 17-1792, GenBank MH550968.1; Identities = 755/825 (92 %), two gaps (0 %)), and Helicosporium viridisporum (strain GZCC 22-2008, GenBank OP698087.1; Identities = 754/824 (92 %), no gaps).

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Conflict of interest: The authors declare that there is no conflict of interest.

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Supplementary Material: http://fuse-journal.org/

**Table S1.** Summary of phylogenetic information for the different analyses in this study

**Table S2.** GenBank accession numbers of sequences used to generate the alignment for the placement of *Trichosphaeria pilosa*.
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