Stielow, B., Hensel, G., Strobelt, D., Makonde, H.M., Rohde, M., Dijksterhuis, J., Klenk, H.-P., Göker, M. Hoffmannoscypha, a novel genus of brightly coloured, cupulate Pyronemataceae closely related to Tricharina and Geopora

(2013) Mycological Progress, 12 (4), pp. 675-686

Hoffmannoscypha, a novel genus of brightly coloured, cupulate *Pyronemataceae* closely related to *Tricharina* and *Geopora*

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Abstract

The rare apothecial, cupulate fungus *Geopora pellita* (*Pyronemataceae*) is characterized by a uniquely bright yellow-orange excipulum. We here re-examine its affiliations by use of morphological, molecular phylogenetic and ultrastructural analyses. *G. pellita* appears as phylogenetically rather isolated, being the sister group of a clade comprising *Phaeangium*, *Picoa*, the majority of the *Tricharina* species, and the remaining *Geopora* species. Based on its phylogenetic position and its unique combination of morphological characters, we assign *G. pellita* to *Hoffmannoscypha*, gen. nov., as *H. pellita*, comb. nov. As in a previous study, analyses of both large subunit (LSU) and internal transcribed spacer (ITS) ribosomal DNA suggest that the remaining genus *Geopora* is paraphyletic, with the hypogeous, ptychothecial type species more closely related to *Picoa* and *Phaeangium* than to the greyish-brownish cupulate and apothecial *Geopora* spp., indicating that the latter should be reassigned to the genus *Sepultaria.* The current study also shows that ITS confirm LSU data regarding the polyphyly of *Tricharina*.

Keywords: Taxonomy, phylogeny, *Pezizales*, apothecia, ascospores

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Introduction

Ascomycota is the largest fungal phylum and includes approximately 65,000 described species (Kirk et al. 2008). The class *Pezizomycotina* includes the ecologically most specialized and morphologically most diverging species, which contribute to a variety of important ecological processes such as wood and litter decay or are plant pathogens or mutualists in mycorrhizal symbiosis. The largest and most diverse family of *Pezizomycotina*, the *Pyronemataceae*, includes approximately 80 genera and around 660 species (Kirk et al. 2008; Perry et al. 2007). Their ascoma morphology is highly diverse, including cupulate, discoid and pulvinate apothecia as well as hypogeous ptychothecial and stereothecial ascomata (Burdsall 1968; Perry et al. 2007; Tamm et al. 2010). Ecological strategies within the *Pyronemataceae* vary considerably between terricolous, coprophilous, lignicolous, pyrophilous and bryophilous forms (Benkert 1994; Hansen et al. 2001; Krug and Kahn 1991; Perry et al. 2007; Spooner and Butterfill 1999; Vralstad et al. 2002). While most species are saprotrophic, an increasing proportion is identified as ectomycorrhizal symbionts (Laessoe and Hansen 2007; Wei et al. 2010). *Pyronemataceae* have been taxonomically controversial as they are not conjunct by common morphological characters, neither macro- nor microscopically (Perry et al. 2007). While the positioning of many genera has recently been resolved with confidence within *Pyronemataceae* (Hansen and Pfister 2006; Laessoe and Hansen 2007; Perry et al. 2007), the problem of species recognition and, hence, species-diversity estimates, in particular for the sequestrate genera, has attracted much less attention (Guevara-Guerrero et al. 2011; Tamm et al. 2010).

Within apothecial *Geopora,* most species are characterized by a greyish-brownish excipulum such as *G. arenicola, G. sepulta* and *G. tenuis*. However, *Geopora pellita* (Cooke & Peck) T. Schumacher, originally described as Peziza pellita by Cooke and Peck (1875), strongly differs in its macromorphology from its sister species, as it displays a colourful, brightly yellow-orange excipulum. Recent records about G. pellita are rare (Perry et al. 2007; Schumacher 1979; Wells and Kempton 1967) and the species appears in few identification keys only (Dougoud 2007; Hansen and Knudsen 2000). Schumacher (1979) reported the species for the first time outside the USA and reassigned it from *Peziza* into *Geopora.* Perry et al. (2007), using partial 28S rDNA sequences, provided the first molecular evidence that *G. pellita* phylogenetically strongly differs from other apothecial

Geopora species, since it was positioned closer to *Tricharina* than to cupulate and ptychothecial *Geopora* species, which was confirmed by Wei et al. (2010).

In the present study, we analyse a recently collected specimen of *G. pellita* by macromorphological, micromorphological and ultrastructural means as well as phylogenetic analysis using complete ITS and partial D1/D2 LSU (28S) rDNA sequences. The phylogenies suggest to recognize *Geopora pellita* (Cooke & Peck) T. Schumacher as separated, novel genus. This finding is strongly supported by the species' unique yellow-orange apothecium, whose development differs from other *Geopora* species. Accordingly, we propose *Hoffmannoscypha*, gen. nov., to accommodate the species.

Material and Methods

Collection and morphological studies

In general, the methods of collection, macroscopic and microscopic studies were those of Castellano et al. (1989) and Pegler et al. (1993). Fresh and dried specimens were cut by hand and mounted in water or alternatively in 5% KOH for microscopic observation. The newly collected fungal specimen was deposited under the accession number M-0156529 at the Botanische Staatssammlung München (M) (Agerer et al. 2000) and at the Fungarium Gunnar Hensel (FUNGH). Specimens designated as *Geopora pellita* deposited at New York Botanical Garden Herbarium (NYBG) and at Harvard University Herbarium (FH) (Table 1) were used for comparative morphological analysis. Additionally, the collections from the pyrophilus genus *Tricharina* deposited at NYBG and M were analysed with light microscopy and field-emission scanning electron microscopy (FESEM), like the *Geopora* collections from Guevara-Guerrero et al. (2011) and the novel ones (Table 1). Tissue measurements were made with 40x and 100x oil immersion lenses (Zeiss Axiophot) and repeated 20 times. For FESEM, spores were harvested by scratching on a cross-sectioned *G. pellita* apothecium, seated onto conductive carbon adhesive tabs and covered with a gold film by sputter coating (SCD 500, Bal-Tec, Liechtenstein) before being examined in a field-emission scanning electron microscope (Zeiss DSM 982 Gemini) using the Everhart Thornley SE detector and the in-lens detector in a 50:50 ratio at an acceleration voltage of 5 kV. Images were recorded onto MO-disk, and contrast and brightness were adjusted with Adobe Photoshop CS3 and Illustrator CS5.

DNA isolation, PCR, cloning and sequencing

Total genomic DNA was extracted from approximately 100 mg of dried apothecium material using the Masterpure® Yeast Genomic DNA Kit following the manufacturer's protocol. DNA extraction from ancient specimens obtained from NYBG and BSM (Table 1) followed a modified protocol based on the EZNA Forensic DNA kit. Between 5 and 30 mg of apothecia, depending on age and condition of the herbarium specimens, were homogenized in 1.2 ml lysis buffer containing 1% SDS, 10 mM Tris pH 8.0, 5 mM NaCl, 50 mM molecular biological grade DTT, 100 µg/ml proteinase K, 10 mM EDTA and 2.5 mM PTB (N-Phenacylthiazoliumbromide) based on a modification from Erickson et al. (2005). Microtubes were incubated in a water bath at 37 °C for 24 h following centrifugation at 9000 g for 10 min and transfer of 1 ml supernatant into a new microtube, precipitation with 600 µl 2-propanol and 60 µl 3 M sodium acetate at 4 °C for 48 h, following the EZNA forensic DNA manufacturer's instructions with the exception of the last washing step being performed four times. The ITS nrDNA region was amplified with PCR primers ITS1/ITS4 and ITS1F/ITS4 under semi-nested conditions (Gardes et al. 1993, White et al. 1990, Stielow et al. 2010). PCR conditions for amplifying the partial 28S rDNA using the standard primers LR0R and LR3 only differed in their annealing temperature (55 °C instead of 60 °C). PCR for ancient specimens was performed with ITS5/ITS4 and followed by reamplification under semi-nested conditions with ITS3/ITS2 paired with ITS4/ITS5 or by direct amplification under standard conditions. PCR products were cut out or directly purified using Macherey-Nagel NucleoSpin Extract II kit (740609.50). The cycle-sequencing reaction was set up using the Beckman Coulter GenomeLab DTCS Quick Start Kit or the ABI big dye terminator v3.1 following the manufacturers' protocols, followed by bidirectional sequencing with a Beckman Coulter Genome lab capillary electrophoresis system or the Lifetechnologies (ABI) 3730XL DNA analyser. PCR products from ancient specimens that resulted in poor trace quality were cloned using the TOPO TA 2.1 cloning kit (LifeTech). Sequences were manually corrected for sequencing artefacts and forward and reverse sequences assembled using Invitrogen Vector NTI 11 or Lasergene Seqman.

Phylogenetic inference

The ITS and LSU nrDNA alignments were the ones used in Guevara-Guerrero et al. (2011). These were carefully compiled, extensively tested regarding the sensitivity of the resulting phylogenies to alignment ambiguity (which was negligible), and already used to draw taxonomic conclusions in the group. The newly obtained *G. pellita* and *Tricharina*

sequences that comprised both ITS1 and ITS2 were added to the ITS alignment using the POA software (version 2; Lee et al. 2002) in profile alignment mode. As in our previous study (Guevara-Guerrero et al. 2011), phylogenetic analysis under the maximum-likelihood (ML) criterion (Felsenstein 1981) was conducted with RAxML version 7.2.7, using its novel rapid bootstrap option combined with the autoMRE bootstopping criterion (Pattengale et al. 2009) with subsequent search for the best tree under the GTRMIX approach (Stamatakis et al. 2008). Bootstrapping under the maximum-parsimony (MP) criterion (Fitch 1971) was again done with PAUP* version 4.0b10 (Swofford 2002), treating gaps as missing data, collapsing branches of zero minimum length, and using 10 rounds of random sequence addition followed by TBR branch swapping per bootstrap replicate. In MP bootstrapping, 1000 replicates were conducted. As before (Guevara-Guerrero et al. 2011), rooting of the resulting trees was done using the midpoint rooting method (Farris 1972; Hess and Russo 2007). Sequence alignments and phylogenetic trees are included in the online supplementary material. For depicting the trees, clades comprising at least three sequences were collapsed if they were either taxonomically homogeneous or contained only environmental samples. If a clade contained environmental samples some of which had a genus annotation, these genera were indicated.

The additional ITS sequences from which only ITS1 or ITS2 could be amplified due to the insufficient preservation of the material were analysed separately. Here we focussed on the identity of the biological material deposited as *Geopora pellita* and thus only calculated pairwise similarities from *exact* pairwise sequence alignments using the Smith-Waterman algorithm as implemented in the EMBOSS suite (Rice et al. 2000).

Results

Phylogenetic inference from ITS rDNA sequences

The alignment comprised 250 ITS rDNA sequences and had a total length of 1813 positions. The resulting best ML tree had a log likelihood of -20,735.83 and is shown in Fig. 1 together with ML (left) and MP (right) bootstrap values on each branch. The separation of the outgroup clades on the one hand, comprising operculate apothecial discomycete genera such as *Aleuria*, *Cheilymenia*, *Pseudaleuria*, *Scuttelinia*, and *Wilcoxina*, but also one of the newly generated *Tricharina* sequences (JQ824118), and the

ingroup clades on the other hand was strongly supported (100/97%). Note, however, that the comparison of annotations such as “fungal sp. ARIZ AZ0886” with the LSU tree (Fig. 2) indicated yet another *T. gilva* cluster, separate from all newly sequenced *Tricharina* samples. The ingroup clades included apothecial *Geopora* spp. (= *Sepultaria* spp.; Guevara-Guerrero et al. 2011), ptychothecial *Geopora* spp., *Phaeangium* spp., *Picoa* spp. and three of the newly generated *Tricharina* sequences. Strong support was achieved for the clade comprising these three sequences together with some environmental samples (96/98%) as well as for its sister group, comprising all remaining ingroup sequences (99/97%). Within the latter, *Geopora pellita* branched first, followed by environmental sequence GQ281480, whose annotation indicates the ectomycorrhizal morphotype of *Pinirhiza daqingensis*. Moderate support (78/73%) was obtained for the monophyly of all remaining ingroup sequences. The monophyly of apothecial *Geopora* spp. was strongly supported (100/99%), as well as clade comprising *Phaeangium* and *Picoa* sequences (100/99%). Moderate support was obtained for the monophyly of the hypogeous, apothecial *Geopora* spp. (92/66%) and for their sister-group relationship with *Phaeangium* and *Picoa* (72/77%).

Phylogenetic inference from 28S (LSU) rDNA sequences

The alignment comprised 630 LSU rDNA sequences and had a total length of 6711 positions. The resulting best ML tree had a log likelihood of -50,155.98 and is shown in Fig. 2 together with ML (left) and MP (right) bootstrap values on each branch. Again, outgroup clades were collapsed due to their size; for the complete tree see the supplementary files. Very strong to moderate support (99/82%) was achieved for the monophyly of a group comprising the genera *Geopora*, *Phaeangium*, *Picoa* and *Tricharina* with the exception of *Tricharina gilva*, which belonged to the sister group of that clade. Strong to low (89/61%) support, depending on the phylogenetic optimality criterion (ML/MP), indicated the monophyly of a cluster comprising *Phaeangium*, *Picoa* and *Geopora* except for *G. pellita.* The sister-group relationship of hypogeous ptychothecial *Geopora*, *Phaeangium* and *Picoa* was also highly to weakly supported (95/60%), whereas the monophyly of the clade containing the majority of accessions annotated as *Tricharina*, obtained strong to moderate support (96/84%). The Genbank 28S sequence DQ220343, annotated as a *G. pellita* specimen collected in Michigan (USA) by Pfister in 1969 (Perry et al. 2007), was almost identical to the sequence obtained from our specimen (Fig. 2).

Sequence and morphological identity of herbarium specimens

The best hit of partial ITS sequence obtained from the NYBG 114 specimen (JQ062972) was to our complete *G. pellita* sequence (HQ913564), yielding 96.1% Smith-Waterman similarity. The second best hit (HM123158) corresponded to only 83.7% sequence identity. Identical results were obtained for the NYBG 301 specimen (JQ062973). The NYBG 228 specimen (accession number JQ062974), however, yielded FM206460 as best hit (99.0%), followed by hits to other *Geopora arenicola* sequences. A photograph (supplementary material, image 20) attached to specimen NYBG 228 (collected in 1906), confirmed that this specimen should not be assigned to *G. pellita,* since for some of the apothecia it was obviously shown that they started in a hypogeous state, instead of developing superficially on the substrate as in all other examined specimens annotated as *G. pellita*. (The term “hypogeous” is used here as defined by Kirk et al (2008); see also Weber et al (1997) p. 156, schemes C–D and F–I, for illustrations of strictly hypogeous sporocarps in Ascomycetes. “Superficially on the substrate” refers to the appearance of sporocarps as given by Yang and Korf (1985) on p. 470, in schemes A to D). For this reason, the morphological description of *G. pellita* given below excluded NYBG 228. LSU data revealed the identity of our newly collected *G. pellita* specimen with the DHP 297 specimen (DG220343), as apparent from Fig. 2.

Unfortunately, not all specimens could be sequenced, and in some cases the condition of the deposits was so poor that they could hardly be examined microscopically. This is particularly apparent for the holotype material (NYBG 00914741) collected by Cooke and Peck (1872), deposited at the NYBG. The type specimen of *G. pellita* available to us for comparison would most likely not allow for molecular sampling and exists, at the NYBG, as a single microscopical glass slide. Any other isotype material deposited in other institutions was not available to us for this study. The description and the drawing (supplementary material, image 21) given by Cooke and Peck, however, shows apothecium and spore characteristics identical to those of the other examined *G pellita* specimens except NYBG 228, as detailed below.

The partial ITS sequences obtained from herbarium deposits annotated as *Tricharina* yielded the following best hits. Specimen M-0178316 (“Tricharina praecox”, JQ824119) yielded as best hit HM123089 ("fungal sp. ARIZ AZ0347"), which according to the comparison with the LSU data (Fig. 2) belongs to a *Tricharina gilva* cluster. But the similarity was only 87.2%; M-0178316 was thus judged as an unknown *Tricharina* species.

M-0178317 (“Tricharina praecox”, JQ824119) yielded a much higher similarity to this cluster (99.7% to "fungal sp. ARIZ AZ0886") and was thus regarded as a misidentification of *T. gilva*. This was confirmed by M-0178315, annotated as “Tricharina gilva”, also matching this cluster (99.0–100.0%). M-0178313 (“Tricharina gilva”) had as best hit "Geopora cf. cooperi SOC1051" (FJ789595), but only with a similarity of 82.4%, and thus was judged as misidentified and of uncertain affinity.

The size of all apothecia, as well as the size and shape of the spores of all examined specimens annotated as *Tricharina* (examples are given in the supplementary material, images 23–30) were in accordance with previously published descriptions by Yang and Korf (1985) and Ellis and Ellis (1998), e.g., much smaller in size than those of *Geopora* (details are given below). This also holds for the NYBG 1968 specimen (apothecia 1.0–1.5 mm in diameter), even though its ITS sequence indicates a taxonomic affiliation to other discoid *Pyronemataceae* like *Pyronema*, *Trichopheae* and *Wilcoxina* spp. (Fig. 1). Its black excipulum, the more elongated, entirely smooth spores (supplementary material, image 14, 30) containing black guttules (observed unstained in water), as well the substrate (“flat sand within a greenhouse”), do not match the description of *Tricharina* species in the literature. For this reason, ITS and morphological or ecological data appear to be in agreement regarding the investigated herbarium specimens of *Tricharina*. The following comparison between *Geopora pellita* and *Tricharina* collections thus only relies on the *Tricharina* collections that either could be approved using ITS sequencing or were collected from burned soil, even though the morphological differences to *G. pellita* would be the same for the other specimens annotated as *Tricharina*.

Morphological comparison of *Geopora* spp. *and Tricharina* spp. with *G. pellita*

Results from macro- and micromorphological as well as ultrastructural examinations of the target specimens are shown in Figs. 3 and 4; further Nomarski interference contrast and SEM pictures of the investigated reference specimens are given in the supplementary material (images 1–16, 23–30).

The apothecia of *G. pellita* (Fig. 3, image 1) were yellow-orange and superficially attached to the substrate. They might be slightly sunken into the substrate but did not emerge from a hypogeous development. The star-like shape of the mature apothecia (Fig. 3, image 1) was similar to the one typical for other *Geopora* spp. (Fig. 3, image 5; *G. sepulta*) but

these nevertheless strongly differ in color. The holotype drawing of *P. pellita* Cook & Peck (1872), given in the supplementary material (image 21), shows exactly the same type of apothecium as given in Fig. 3, image 1. Mature apothecia of *Tricharina* (supplementary material, images 17–19) were considerably smaller than the ones of *G. pellita*, commonly less than 10 mm in diameter (mature apothecia are 1–5 mm in diameter for most species), but similar in color to *G. pellita*.

Juvenile apothecia of *Geopora* spp. tended to expand their exoperidium to very distinct lobes, thereby pulling the apothecium from a hypogeous to a position superficially on the substrate (Fig. 3, image 5). But *G. pellita* did not appear to develop in this way, even though the lobes of its exoperidium were obvious (Fig. 3, image 1). Already its juvenile apothecia were superficially attached to the substrate and its development thus appeared more like the one of *Tricharina* spp., even though the apothecia of the latter were not lobed (supplementary material, images 17–19).

Multiseptate, finely warted and cylindrical setae with blunted to pointed apices were found in all investigated species. *G. pellita* showed a broad basal cell connected to the cells of the ectal excipulum, from where the finely warted setae emerge (Fig. 3, images 4, 6). The setae were always arranged in fascicles nonetheless their density became narrower towards the apothecial base. We did not detect differences to the setae of other *Geopora* spp., or *Tricharina* spp. The setae of *G. pellita* did not contain globose inclusions, as known from pyrophilus genera such as *Wilcoxina* spp (Yang and Korf 1985).

Spore sizes were in the range of 21–27 x 10–12 µm in *G. pellita*. Similarly, the examined *Geopora* specimens never showed spores less than 20 µm in length. In contrast, spore sizes were 12–17 x 5–11 µm for the investigated *Tricharina* species. Asci of all three genera were of similar shape and size, and always cylindrical, apically operculate with a narrowing base, non-amyloid, uniseriate and eight-spored. In *Tricharina* they were always less than 200 µm in length but in *G. pellita* and the other *Geopora* spp. always longer than 200 µm (e.g., Figure 3, image 2). The paraphyses were usually slender, multiseptate and slightly clavate at the apex. In those species with a colorful hymenium, the paraphyses contained small inclusions of pigments that apparently gave rise to the overall color of the apothecium (Figure 3, images 2, 4). This character could mostly only observed on fresh, recently collected apothecia. All findings are supported by pictures assembled in the supplementary material, obtained from the examined herbarium specimens (Table 1).

The ascospores of *G. pellita* and *Geopora* spp. appear, when observed with Nomarski interference contrast microscopy, entirely smooth and guttulate (Fig. 3, image 2). The guttulate spores of *G. pellita* have already been indicated by Cooke & Peck in their drawing of the type specimen (supplementary material, image 21). Likewise, the spore surfaces of *Tricharina* spp. appeared smooth (supplementary material, images 23–29).

Mature ascospores of *Geopora pellita* showed a finely warted ornamentation when observed with FESEM (Fig. 3, image 7; Fig. 4, images 1, 2; supplementary material, images 5–9). This ornamentation was not observed in juvenile spores, which were entirely smooth (Fig. 3, image 7; Fig. 4, image 2). The same ornamentation of juvenile, intermediate and mature spores was observable on the specimens obtained from NYBG and FH (supplementary material, images 5–9), indicating the biological identity of this *G. pellita* material. Immature spores of *G. arenosa* and *G. sepulta* were also smooth, whereas mature ones showed a rough but not warted spore surface (supplementary material). *Tricharina* sp. M-0178316 showed juvenile smooth spores in addition to mature spores that were even more pronouncedly warted than the ones of *G. pellita* (supplementary material, images 11–13, 15–16). The same pronounced ornamentation was observed for specimen M-0178317, but not for specimen Rehm. Ascom. 456 / 1878 that showed a rough but not warted surface (supplementary material, image 10 and 11–12).

Discussion

Identity of the investigated specimens

There is ample evidence that our specimen corresponds to the *G. pellita* from the literature. First, the unique macro- and micromorphology of *G. pellita*, which is not found in any closely related apothecial genera, makes a misidentification rather unlikely. Second, with the exception of the specimen annotated as *G. pellita* (NYBG 228), which turned out to be affiliated to *G. arenicola*, both morphologically and regarding its ITS rDNA, the morphology and the ITS sequences were almost to entirely identical between the newly collected *G. pellita* specimen and the herbarium material. This was confirmed by observations on the ultrastructure of the spores, which revealed a finely warted ornamentation in all proper *G. pellita* specimens that was somewhat distinct from the other *Geopora* species examined, as well as from the *Tricharina* species under study. This finding is not in conflict with the smoothness of the ascospores of *G. pellita* reported by

Cooke and Peck (1872), Dougoud (2007), Schumacher (1979) and Wells and Kempton (1967) because the warts could not be seen in light microscopy. Accordingly, the ascospore surface of other *Geopora* spp. has also been described as entirely smooth by a variety of authors (Breitenbach and Kränzlin 1981; Dennis 1981; Hansen and Knudsen 2000; Tamm et al. 2010), even though the mature ascospores are not smooth when visualized by FESEM.

Some herbarium deposits were too old and scarce to extract DNA. This is particularly evident for the type specimen (*Peziza pellita* Cooke & Peck 1872, Grevillea 1: 5, NYBG specimen ID 00914741). Accordingly, epitypification appears to be the best way to address the critical issue of a representative specimen for this species. The epitype, our novel collection, is deposited at the Botanische Staatssammlung München under the accession number described below (corresponding curator Dr. D. Triebel). Despite its overall poor condition, however, the type deposit contains a drawing, which unambiguously indicates a micro- and macromorphology identical to the proposed epitype, and thus the biological identity of the investigated specimens. The description of the *G. pellita* habitats found in the literature (Schumacher 1979), “growing in a sand accumulation in the upper inundation zone of the river on coarse sand among *Pohlia gracilis* and *Bryum* spp.”, also corresponds well to the collection site of our novel specimen, which was found in a sand pit in association with *Pinus* sp. and embedded in unidentified mosses.

Dried ascomata of many small discoid *Pyronemataceae* are known to be very brittle, and often cells cannot be properly hydrated again. Even later tissue observations made from herbarium specimens of our own collections of *G. pellita*, whose apothecia are much larger in size than those of *Tricharina*, were almost impossible. Thus it is difficult to identify herbarium specimens of *Tricharina* based on apothecial macromorphology due to the age of the specimens. A cross-comparison of the sequence affiliation of those specimens from which ITS sequences could be obtained with the LSU data, however, allowed us to conclude that two distinct but real *Tricharina* clades exists, one harboring the type species, *T. gilva*, and a second one containing at least *T. ochroleuca* (which occurs in a clade of comparable positioning in both the ITS and LSU trees; see also Perry et al. 2007) but probably also *T. hiemalis* (this study), *T. groenlandica* (this study) and *T. praecox* (Perry et al. 2007). Moreover, we have shown that *Tricharina* species reported to develop smooth ascospores when viewed with the light microscope (Yang and Korf 1985) have obviously warted spores when examined by scanning-electron microscopy. Our examination also

indicates that quite a few herbarium specimens of *Tricharina* are misidentified, and that a revision of the genus is needed.

Classification of *G. pellita* relative to *Geopora* and *Tricharina*

Recently, phylogenetic studies on major genera of *Pyronemataceae* have been conducted by Perry et al. (2007). The results of this study revealed *Geopora* (except *G. pellita*) as a monophyletic group using maximum-parsimony and Bayesian analyses, but neither *Phaeangium* nor *Picoa* were included in the dataset. Using either 28S or ITS rDNA sequence data, our analyses place these two genera within a paraphyletic *Geopora* with high confidence, at least under the maximum-likelihood criterion, in agreement with the results obtained by Guevara-Guerrero et al. (2011) and Sbissi et al. (2010), and highlighting the importance of sufficient taxon sampling. Because *Sepultaria* already exists as a validly published name for the apothecial *Geopora* spp., there is little reason for not using it once again for these fungi. As shown in the present study, the other necessary measure to obtain a monophyletic *Geopora* is to exclude *G. pellita*. This species is neither phylogenetically placed within the genus nor does its macromorphology agree with the other *Geopora* species.

G. pellita forms a grade in the LSU tree together with *Tricharina*, which appears polyphyletic in the tree, subdivided into a *T. gilva* and a *T. ochroleuca/praecox* clade. The latter is more closely related to *Geopora*, *Phaeangium*, *Picoa* and *Sepultaria* than *G. pellita*, whereas the *T. gilva* clade is the sister group of all these taxa (Fig. 2). Apparently, including *G. pellita* into *Tricharina* would at most change the status of the latter from polyphyletic to paraphyletic (see Farris 1974 for formal definitions of these terms) and would not be an acceptable solution either. The ITS tree (Fig. 1) shows the same relationships, the main difference being the position of the *T. gilva* cluster; but this is just an issue of rooting. That only monophyletic groups can be accepted in modern taxonomic classifications can hardly be denied (Farris 1979; Hennig 1965; Wiley and Lieberman 2011).

The presence of the prominent hyaline to brownish fascicular hairs in *Tricharina,* arising from the ectal excipulum, which are also found in *G. pellita* or other *Geopora* species (Yang and Korf 1985), cannot be used as morphological character delimiting the three genera. Clear differences in septation, size, tip shape or inclusions have neither been found. Since the apothecium and excipulum micromorphologies are not useful for the

delimitation of *Geopora* species, too, spore characters and excipulum macromorphology have been used instead (Dougoud 2007; Hansen and Knudsen 2000; Tamm et al. 2010; Yao and Spooner 1996). Schumacher (1979) renamed *Peziza pellita* to *Geopora pellita* based on a description in accordance with the ones given by Cooke and Peck (1872) and by Wells and Kempton (1967), which referred to a similar excipulum and spore morphology. The lobes of the excipulum fulfill an important function in *Geopora* spp. by pulling the mature apothecium above the substrate to disperse the spores; they should not be compared to possible rifts, which may occur in many small *Discomycetes* such as *Tricharina*.

Prominent lobes are seen in *G. pellita*, too, but cannot have the same function as in other *Geopora* species because already the juvenile apothecia of *G. pellita* are superficially attached to the substrate. Another obvious distinction of *G. pellita* from *Geopora* species such as *G. arenicola*, *G. arenosa*, *G. cervina*, *G. sepulta* and *G. sumneriana* is that these have a brownish-grayish pigmented excipulum in common, whereas the yellow-orange apothecia of *G. pellita* resemble the ones of *Tricharina* regarding their color.

But morphologically *G. pellita* does neither fit to *Tricharina*. Both *G. pellita* and the other *Geopora* species are characterized by a strong lobation of mature apothecia, which is neither known from *Tricharina* nor other pyrophilus genera. This is also supported by the size of the ascospores, which are within *Geopora* (and *G. pellita*) always longer than 20 µm and in *Tricharina* always shorter than 20 µm. The specimens observed in this study analogously differed regarding the length of their asci. Apothecia of *Tricharina* are strictly cupulate, in some species even discoid (Yang and Korf 1985). The size of their apothecia is usually between 1–10 mm, only in a single species, *T. fibrillosa* (Currey) Yang & Korf, up to 20 mm, which is similar to the size of *G. pellita* (Yang and Korf 1985). Ecological differences separating *G. pellita* from *Tricharina* are less certain at the moment, since *G. pellita* is not definitively known to form ectomycorrhizal associations. At least, this species has not yet been found on pyrophilous sites or on decaying wood, which is typical for most *Tricharina* species (Yang and Korf 1985).

Based on our results, we suggest the novel, so far monotypic genus *Hoffmannoscypha* to accommodate *G. pellita* as *H. pellita*, comb. nov. An additional splitting of *Tricharina*, which is, of course, beyond the scope of the present study, might be an acceptable future solution for the remaining non-monophyly of *Tricharina* apparent in phylogenetic analyses.

Taxonomy

Hoffmannoscypha Stielow, Göker & Klenk, gen. nov.

Mycobank number: MB 561770.

English description: APOTHECIA superficially attached to the substrate, often slightly sunken into it, without stipe, roundish when juvenile and nearly entirely closed at the apex, cupulate when mature, at full maturity with ripped edges; lobes erect when juvenile, effused when mature. ECTAL EXCIPULUM (epicutis) dark yellow-orange, seldom orange-brown, pseudoparenchymatous, with angular or isodiametric, thick-walled cell agglomerates (textura angularis), cells giving rise to dark brown, thick-walled, multiple septate cylindrical finely warted setae, single or cespitose, dark brown in water and 5% KOH. ASCI inoperculate, cylindrical, eight-spored, monoseriate. PARAPHYSES slender, single, septate, with gentle orange pigmentation giving rise to the colour of the hymenium, slightly thickened and bent at the tip, arranged in palisade order.

Type species: *Hoffmannoscypha pellita* (Cooke & Peck) Stielow, Hensel, Göker & Klenk

Etymology: “*Hoffmannoscypha*” = “Hoffmann's cup”. Named in honour of the German mycologist and DSMZ curator Dr. Peter Hoffmann who dedicated the over 40 years of his working life to collection, preservation and identification of fungi.

Anamorphs: The species is unknown from pure axenic culture.

Hoffmannoscypha pellita (Cooke & Peck) Stielow, Hensel, Göker & Klenk, comb. nov.

= *Peziza pellita* Cooke & Peck 1872, Grevillea 1: 5

= *Lachnea pellita* (Cooke & W. Phillips) Sacc. 1889, Syll. fung. (Abellini) 8: 169

= *Sepultaria pellita* (Cooke & Peck) Seaver 1928, North American Cup-fungi, (Operculates), New York 152

= *Geopora pellita* (Cooke & Peck) T. Schumacher 1979, Norw. J. Bot. 26 (1): 56

Mycobank number: MB 561771.

English description: With the features of the genus. APOTHECIA 0.5–2 cm in diameter. ODOUR not distinct. ECTAL EXCIPULUM (epicutis) 325–400 µm wide, cells 14–45 µm wide; setae on average 60–140 µm in length, at most 500 µm long, 4–6 µm thick. MEDULLA 400–600 µm wide, with prostrated interwoven hyphae (intermediate between textura intricata and textura globulosa), several hyphae forming large globose inflations; hyphae 3–14 µm wide; globose inflations 15–30 µm wide; hyaline, slightly orange in water when fresh; hyaline, colourless in 5% KOH. ASCOSPORES ellipsoid, smooth and hyaline when juvenile; guttulate with a large central guttule, two smaller guttules adjacently beside, spores with guttules of the same size rarely observable; entirely mature spores finely warted-punctuated (ornamentation approximately 300–800 nm in height), walls approximately 0.5–1 µm thick, in water and 5% KOH. Ascospore size (23–)23.5–27.5(–28) x (10.5–)11–13(–13.5) µm, on average 25 x 12 µm, length/width quotient 1.9–2.3, on average 2.1. ASCI 220–280 x 14–19(–24) µm in size. PARAPHYSES in water and 5% KOH 3.5–5.5 µm in diameter at the tips, 6–7 µm in diameter at the centre.
Specimens examined: M-0156529, Botanische Staatssammlung Munich = GH20100409, Fungarium Gunnar Hensel; Germany, Freihufen-Großräschen, sand pit, approximate location 51°34'31.22” N 13°57'49.42” E, 130 m above sea level, in sandy mineral soil, beneath pine trees (*Pinus* sp.), Dirk Strobelt & Gunnar Hensel, 4th September 2010. Four additional specimens of *G. pellita* that were investigated in this study are given in Table 1.

Epitypification: Investigated holotype of *Peziza pellita* (Cooke & Peck) T. Schumacher 1979, New York Botanical Garden, specimen ID 00914741. We here designate the specimen of *Hoffmannoscypha pellita* (Cooke & Peck) Stielow, Hensel, Göker & Klenk, deposited at Botanische Staatssammlung Munich as epitype of the genus, specimen ID M-0156529.

Key to the investigated genera

1 Mature apothecia not lobed, usually less than 10 mm in diameter, brightly orange-red to orange-brown pigmented, growing superficially on or slightly sunken the substrate; ascospores less than 20 µm in length; asci less than 200 µm in length; occurring on burnt substrate or

decaying organic material *Tricharina*

1* Mature apothecia lobed, usually more than 10 mm in diameter, either brightly orange-yellow or greyish to (light) brownish pigmented; ascospores more than 20 µm in length; asci more than 200 µm in length; neither occurring on burnt ground nor on decaying organic material.

2 Apothecia always with pronounced orange-yellow pigments; juvenile apothecia growing superficially on, at most slightly sunken into, the substrate *Hoffmannoscypha*

2* Apothecia never with orange, red or yellow pigments, but greyish to (light) brownish pigmented; juvenile apothecia strictly hypogeous, when mature growing superficially on the substrate *Geopora*

Acknowledgments

We kindly thank Dr. Ellen Bloch, New York Botanical Garden Herbarium, for the straightforward loans of *Sepultaria pellita* (*G. pellita*) and *Tricharina* spp. specimens, as well giving permission for nucleic acid extraction from specimens and digitalization of the drawings by G. Massee and F. Seaver (included in the supplementary material). Dr. Dagmar Triebel, Botanische Staatssammlung München, is kindly acknowledged for giving permission for nucleic acid extraction from *Tricharina* spp. specimens and the kind cooperation.

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genus, *Wilcoxina* (*Pezizales*). Mycotaxon 24:467–531.

Tables

Table 1. List of herbarium specimens and fungal strains used for molecular sequence and comparative morphological analysis; specimens without Genbank accession number were used for studying the morphology only. Species names given in square brackets are likely to be misidentified (see the main text for details). Accession numbers marked with stars are LSU sequences; the other represent the ITS. Abbreviation used in accordance with the Index Herbariorum where applicable: CBS, Centraalbureau voor Schimmelcultures; FUNGH, Fungarium Gunnar Hensel; FH, Harvard University Herbarium; ITCV, Instituto Tecnológico de Ciudad Victoria; M, Botanische Staatssammlung München; NYBG, New York Botanical Garden; S, Swedish Museum of Natural History.

Species	Collection data	Habitat details	Deposit	Accession number
Geopora arenosa (Fuckel) S. Ahmad	Stolberg, Hainfeld; Germany; 2006; G. Hensel & U. Täglich	Next to walking path on diabase	FUNGH: GH20090606	
Geopora cf. *cooperi* Harkn.	Santibanez de Valcorba, Spain	?	S: F23212	FR694203
Geopora cf. *cooperi* Harkn.	Gotland, Sweden	?	S. F48895	FR694202
Geopora cf. *cooperi* Harkn.	Sachsen-Anhalt, Germany; 2010; G. Hensel & U. Täglich	?	FUNGH: GH GH20100807	HQ184958, HQ184959
Geopora pellita (Cooke & Peck) T. Schumacher	USA; 1872; Cooke & Peck	?	NY: NYBG 00914741	
Geopora pellita (Cooke & Peck) T. Schumacher	Colorado, Peaceful Valley; USA; 1929; F. Seaver & P.F. Shope	Soil, in humus	NY: NYBG 159	JQ062972
Geopora pellita (Cooke & Peck) T. Schumacher	Colorado, Kingston Peak; USA; 1935; P. & V. Shope	Under aspens	NY: NYBG 301	JQ062973
[*Geopora pellita* (Cooke & Peck) T. Schumacher]	Canada, Ontario, Toronto, High Park; 1906; J. H. Faull & J. H. Jackson	Sandy soil	NY: NYBG 228	JQ062974
Geopora pellita (Cooke & Peck)	Freienhufen; Germany; 2010; D.	On sandy mineral soil, embedded in	M: GH20100409	HQ913564, HQ993571*

T. Schumacher	Strobelt & G. Hensel	moss close to Pinus sp.		
Geopora pellita (Cooke & Peck) T. Schumacher	USA; 1969; D. Pfister	?	GH: DHP 297	DQ220343*
Geopora sepulta (Fr.) Korf & Burds.	Belzig; Germany; 2009; G. Hensel & U. Täglich	City centre Belzig, lawn on sand	FUNGH: GH20091122	
Geopora toluquensis Guevara, Göker & Stielow	Parque Nacional Nevado de Toluca, Mexico; 2009; G. Guevara	?	ITCV: ITCV 1081	HQ184960, HQ184961
Tricharina gilva (Boud.) Eckblad	Bavaria, Augsburg; Germany; 1878; Britzelmeyer	On calcareous soil between ash in a backyard	NY: Rehm. Ascom. 456	
[*Tricharina gilva* (Boud.) Eckblad]	North Carolina, Durham; USA; 1968; F.A. Wolf	On flat sand in greenhouse, New York Bot. Gard. Herb. Specimen	NY: NYBG 1968	JQ824118
Tricharina gilva (Boud.) Eckblad	Berlin; Germany; 1885; P. Sydow	Lake shore	NY: NYBG 775	
Tricharina gilva (Boud.) Eckblad	USA; 1915; F. Seaver	?	NY: NYBG FJ1915	
Tricharina gilva (Boud.) Eckblad	USA; 1904	On sandy soil among moss	NY: NYBG 1905	
Tricharina gilva (Boud.) Eckblad	Hechendorf, Breitbrünn; Germany; 1963; Th. Kupka	Burned soil	M: M-0178315	JQ824122, JQ824123
Tricharina gilva (Boud.) Eckblad	Tirol, Nederjoch; Austria; 1948; M. Moser	Burned soil	M: M-0178314	
[*Tricharina gilva* (Boud.) Eckblad]	Lappland, Kiruna; Sweden; 1965; A. Bresinsky	Burned soil	M: M-0178313	JQ824121
Tricharina groenlandica Chin S. Yang & Korf	Greenland; 1983; H. Dissing	?	CBS: CBS 237.85	JQ824125
Tricharina hiemalis Chin S. Yang & Korf	USA; H.K. Saksena	?	CBS: CBS 263.60	JQ824124
Tricharina mikolae Yang & Wilcox	Oregon; USA; 1982; Yang	Ascocarps collected on soil of a pot culture of red pine seedlings in greenhouse, Syracuse	NY: NYBG 914971	

Tricharina ochroleuca (Bers.) Eckblad	Norway; 1979; S. Silvertsen & H. Dissing	?	CBS: CBS 238.85	JQ824126
[*Tricharina praecox* (Karst.) Boudier]	Holzkirchen, MTB 8136; Germany; 1980; A. Einhellinger	Burned soil	M: M-0178317	JQ824119
[*Tricharina praecox* (Karst.) Boudier]	Tirol, Nederjoch; Austria; 1948; M. Moser	Burned soil	M: M-0178316	JQ824120

Figures

Fig. 1. Phylogenetic tree inferred under the maximum-likelihood (ML) criterion from the ITS rDNA alignment. Numbers on the branches represent support values from 1000 bootstrap replicates under the ML (left) and the maximum-parsimony criterion (right) if at least 60%. Branches are scaled in terms of the expected number of substitutions per sites. Leaf names are from their original annotations; corrections, if any, can be inferred from the group names on the right side of the vertical bars. The sequences newly generated in the course of our study are listed in Table 1. See the electronic supplementary material for the complete tree.

Fig. 2. Phylogenetic tree inferred under the ML criterion from the 28S (LSU) rDNA alignment. Numbers on the branches represent support values from 1000 bootstrap replicates under the ML (left) and the maximum-parsimony criterion (right) if at least 60%. Branches are scaled in terms of expected number of substitutions per sites. Leaf names are from their original annotations; corrections, if any, can be inferred from the group names on the right side of the vertical bars. The sequences newly generated in the course of our study are listed in Table 1. See the electronic supplementary material for the complete tree.

Fig. 3. 1: Mature yellow-orange apothecia of *H. pellita (*M: M-0156529*)*. 2: Monoseriate asci arranged in parallel as well as the orange-yellow pigmented paraphyses causing the characteristic colour of the excipulum; bar = 10 µm. 3: Juvenile apothecium of *H. pellita*. 4: Cross-section through the excipulum of *H. pellita*; bar = 1000 µm. 5: Apothecia of *Geopora sepulta (*FUNGH: GH20091122*)*. 6: Cells and setae of the ectal excipulum of *H. pellita*, bar = 10 µm. 7: FESEM image of a single juvenile spore in a broken ascus; bar = 10 µm.

Fig. 4. 1: FESEM image of a mature, finely warted ascospore of *H. pellita;* bar = 10 µm. 2: FESEM image of juvenile *H. pellita* spores (smooth surface) and a single mature spore (warted surface; central position); bar = 10 µm.