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ORIGINAL ARTICLE

The neglected hypogeous fungus *Hydnotrya bailii* Soehner (1959) is a widespread sister taxon of *Hydnotrya tulasnei* (Berk.) Berk. & Broome (1846)

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Abstract The neglected false truffle species *Hydnotrya bailii* Soehner (Ascomycetes, Discinaceae) is re-described and separated from its sister taxon *Hydnotrya tulasnei* by morphological and phylogenetic analyses based on internal transcribed spacer rDNA sequences. The most distinct morphological and ecological characters are small globose, rather than kidney-like, ascomata as known from the sister taxon *H. tulasnei*, strictly monoseriate ascospores and montane habitats. Phylogenetic analyses resulted in two clearly separated clusters that revealed the ectomycorrhizal specificity of *H. bailii* to *Picea abies* and that *H. tulasnei* is preferably associated to *Fagus sylvatica*. We also show that *H. bailii* was already present in mycorrhizal samples but until now could not be correctly assigned. Our analyses also indicate cryptic diversity within *H. cerebriformis* and other, morphologically not yet characterized, *Hydnotrya* groups. An emended determination key for all *Hydnotrya* species known from Central Europe is provided.

Keywords Cryptic species · Ectomycorrhizal symbiosis · Soil ecology · False truffles · Taxonomy

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Introduction

The evolution of mycorrhizal mutualism had a profound impact on terrestrial life. Fossil records indicate that mycorrhizae facilitated the colonization of the land by plants, and today over 90% of the plant species form mycorrhizal associations (Smith and Read 2008). Ectomycorrhizal fungi display a great variety in their phenotypic appearance (Hibbett et al. 1997). Mycologists very early noted the morphological similarity between gasteroid hypogeous and epigeous representative fruiting bodies (Hesse 1890; Zeller and Dodge 1924). The shift between these forms is known as gastromycetation (Albee-Scott 2007; Binder and Bresinsky 2002; Thiers 1984). Within the Ascomycetes, this evolutionary process is solely known from the *Morchellaceae-Discinaceae-Helvellaceae-Tuberaceae* clade (Laesso and Hansen 2007).

To date, three genera are known in the family *Discinaceae*: *Discina, Gyromitra* and *Hydnotrya*. Inconsistent species concepts of the hypogeous ectomycorrhizal genus *Hydnotrya* have lead to systematic rearrangements in the past (Montecchi and Sarasini 2000; Moser 1963; Soehner 1959). Identification of *Hydnotrya* species has undergone intensive questioning and controversial descriptions in the last century (Hesse 1890; Hollos 1911; Montecchi and Sarasini 2000; Moser 1963; Soehner 1959; Szemere 1965; Trappe 1975; Zhang 1991). For instance, the type species *Hydnotrya*

tulasnei (Berk.) Berk. & Broome (1846) synonymises *H. carnea* (Corda) Zobel (1854) and *H. intermedia* Buchholtz (1904). The genus is distributed across the Northern hemisphere (Europe, North America and Asia). At present, five European *Hydnotrya* species are accepted, among which *H. cerebriformis* and *H. michaelis* are restricted to mountainous coniferous forests. This indicates that certain *Hydnotrya* species are specific ectomycorrhizal partners of coniferous trees.

In accordance with this hypothesis, the German mycologist Ert Soehner presented a valid Latin description of a novel *Hydnotrya* species, *H. bailii*, in his "Tuberaceen-Studien V" (Soehner 1959). The new species was found in association with *Picea abies* in mountainous forests. Soehner noted that *H. carnea* and *H. intermedia* (synonyms of *H. tulasnei*) can be distinguished from *H. bailii* by lacking strictly monoseriate ascospores. Unfortunately, Soehner's legacy was neglected by later taxonomists (Montecchi and Sarasini 2000; Szemere 1965). Because the valid species diagnosis of *H. bailii* had been overlooked, discriminating characters had not been recognized again. Accordingly, each more recently treated spruce-associated specimen with similarity to *H. tulasnei* was assigned to this species. In contrast, *H. tulasnei* is not associated with spruce but with *Fagus sylvatica*, *Pinus* spp. and *Corylus avellana*.

Here, we reconsider the taxonomical status of the neglected species *Hydnotrya bailii*, based on morphological and molecular characteristics. We re-evaluate its host specificity and provide an overview of the currently described and cryptic *Hydnotrya* species diversity, as well as a morphological key of all Central European species.

Material and methods

Morphological studies

The taxonomic descriptions are based on dried material collected by the authors and original material collected by Ert Soehner deposited at the Botanische Staatssammlung München (Table 1). Microscopic characteristics of eighteen specimens were observed from 10–20 µm thick microtome cross-sections of dried specimens mounted in 5% KOH (w/v) and additionally in cotton blue in lactic acid. Tissue measurements were made with 40x and 100x oil immersion lenses and repeated 20 times.

DNA isolation, PCR and sequencing

Two fruiting body collections of *Hydnotrya bailii*, one collection of *Hydnotrya cerebriformis*, one collection of *Hydnotrya tulasnei* and 14 ectomycorrhizal root tips of *Hydnotrya tulasnei* were sequenced (Table 1). Total genomic DNA was extracted from 50 mg ascomata using the Masterpure® Fungal Genomic DNA Kit following the manufacturer's protocol. The ITS rDNA region was amplified with PCR primers ITS1 and ITS4 (White et al. 1990). The PCR reactions were run on a Biorad thermal cycler with the following settings: initial denaturation for 2 min at 95°C followed by 35 cycles of: 30 s denaturation at 95°C, annealing at 60°C for 30 s, extension for 1 min at 72°C and final extension at 72°C for 10 min. Alternatively, fruiting body tissue and mycorrhized root tips were homogenized with a glass micromortar and a micropestle. DNA isolation, PCR and sequencing were performed as previously described (Münzenberger et al. 2009) with the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990).

Phylogenetic analysis

Sequences obtained as described above were complemented using a nomenclature-based search for *Discinaceae* ITS sequences in Genbank (http://ncbi.nlm.nih.gov/), as well as a search against NCBI's nucleotide collection using Blast (Altschul et al. 1990) and the Genbank sequence EU784276 as query. We further considered all Blast hits with a score larger than the score of the best hit placed within the sister genus, *Gyromitra*. To ensure sufficient sequence overlap between all accessions, sequences comprising only (or almost only) the ITS2 part were removed from the combined dataset (which comprised more ITS1-only than ITS2-only sequences).

The sequences thus obtained were aligned with the fast but accurate POA software (version 2; Lee et al. 2002) in progressive alignment mode. In order to cope with alignment ambiguity caused by the need to include potentially distant outgroup sequences for rooting, we followed a two-step approach. To be able to use the *Gyromitra* sequences as outgroup, the first alignment comprised all selected *Discinaceae* accessions, but was cleaned from ambiguously aligned regions using Gblocks (Castresana 2000). After phylogenetic trees had been inferred from the first alignment as described below, a second alignment was constructed with POA in the same way, but was restricted to those ingroup sequences that appeared sufficiently close to *H. bailii* and *H. tulasnei* in the first phylogenetic trees. Accordingly, the second alignment could be used throughout its entire length in phylogenetic analysis, and rooting of the resulting trees could be done according to the results from the first analysis.

Phylogenetic analysis under the maximum-likelihood (ML) criterion (Felsenstein 1981) was done with RAxML version 7.0.4, using its novel rapid bootstrap option with

Table 1 List of specimens used for sequence and comparative morphological analysis. Specimens without Genbank no. (tilde) were used for morphological study; molecular analysis was tried but specimens did not yield PCR products. Abbreviations (leg.): B (Bail), BB (Ben Bubner), BS (Benjamin Stielow), ES (Ert Soehner), GH (Gunnar Hensel), JO (John), KA (Kallenbach), PR (Peter Reil)

Species	Collection data	Leg.	Material	Host	Genbank	Laboratory label	Deposited (Herbarium)	Herbarium label
Hydnotrya bailii	Kesslermoos, Hinterzarten, Germany	PR	fruiting body	*Picea abies*	GQ140238	*IT10*	PR	030803PR
Hydnotrya bailii	Kesslermoos, Hinterzarten,Germany	PR	fruiting body	*Picea abies*	GQ140239	*IT11*	PR	030803PR
Hydnotrya bailii	Schierke, Harz, Germany	GH	fruiting body	*Picea abies*	GQ140237	*IT12*	GH	110808GH
Hydnotrya bailii	Kesslermoos, Hinterzarten, Germany	PR	fruiting body	*Picea abies*	GQ149465	*997*	PR	030803PR
Hydnotrya bailii	Schierke, Harz, Germany	GH	fruiting body	*Picea abies*	GQ149464	*979*	GH	110808GH
Hydnotrya bailii	Holotypus, wodospad kamieńczyka (Zackenfall), Poland	B	fruiting body	*Picea abies*	~	~	Botanische Staatssammlung Munich	~
Hydnotrya bailii	Simmerberg, Germany (Herb. Soehner 2064)	ES	fruiting body	*Picea abies*	~	~	Botanische Staatssammlung Munich	2064
Hydnotrya bailii	Zuckermantel, Germany (Herb. Soehner)	ES	fruiting body	*Picea abies*	~	~	Botanische Staatssammlung Munich	~
Hydnotrya cerebriformis	Rensberg, Rensberger Moor, Germany	PR	fruiting body	*Picea abies*	GQ140234	*IT5*	PR	980813
Hydnotrya cerebriformis	Rensberg, Rensberger Moor, Germany	PR	fruiting body	*Picea abies*	GQ140235	*IT6*	PR	980813
Hydnotrya cerebriformis	Rensberg, Rensberger Moor, Germany	PR	fruiting body	*Picea abies*	GQ140236	*IT7*	PR	980813
Hydnotrya cerebriformis	Sklene, Slowakia	BS	fruiting body	*Picea abies*	~	~	BS	855HBS
Hydnotrya intermedia	Harz, Germany, on *Picea abies,*	KA	fruiting body	*Picea abies*	~	~	Botanische Staatssammlung Munich	~
Hydnotrya intermedia	Germany(Herb. Soehner 1564)	JO	fruiting body	*Picea abies*	~	~	Botanische Staatssammlung Munich	1564
Hydnotrya michaelis	Stolberg	GH	fruiting body	*Picea abies/ Pseudotsuga sp.*	~	~	GH	180508GH
Hydnotrya michaelis	Sklene, Slowakia	BS	fruiting body	*Picea abies*	~	~	BS	856HBS
Hydnotrya tulasnei	Alte Göhle, Freyburg, Germany	GH	fruiting body	*Carpinus betulus*	GQ140240	*IT8*	GH	250704GH
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149458	*462*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149454	*8*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149455	*10*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149456	*450*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149457	*460*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149459	*620*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149460	*656*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149461	*657*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylvatica*	GQ149462	*895*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Fagus sylavtica*	GQ149463	*896*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Pinus sylvestris*	GQ215698	*766*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Pinus sylvestris*	GQ215699	*419*	~	~
Hydnotrya tulasnei	Sandkrug, Brandenburg, Germany	BB	mycorrhized root	*Pinus sylvestris*	GQ215700	*712*	~	~
Hydnotrya tulasnei	Eisenach, Hohe Sonne, Germany	GH	fruiting body	*Fagus sylvatica*	~	~	GH	260904GH

Fig. 1 *Hydnotrya bailii* (Picture: G. Hensel)

subsequent search for the best tree under the GTRMIX approach (Stamatakis et al. 2008). GTRMIX uses the fast but accurate GTRCAT model approximation during heuristic search but the full GTR+GAMMA model for the final likelihood computation (Stamatakis 2006). Bootstrapping under the maximum-parsimony (MP) criterion (Fitch 1971) was 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 both ML and MP bootstrapping, 1000 replicates were conducted. Trees were inferred from the two alignments in exactly the same manner. To quantify the separation between *H. tulasnei* and *H. bailii* observed in the inferred trees, we calculated pairwise uncorrected distances with PAUP* (treating gaps as missing data) and determined the maximum within-cluster and minimum between-cluster distances (the "barcoding gap") for these two clades using OPTSIL (Göker et al. 2009) in input cluster quality mode.

Sequence alignments and phylogenetic trees are included in the online supplementary material available at http://dx.doi.org/10.1007/s11557-009-0625-1.

Fig. 2 *Hydnotrya tulasnei* (Picture: G. Hensel)

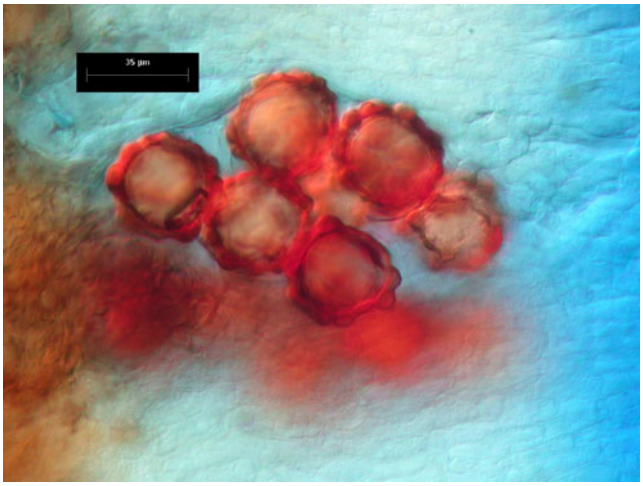

Fig. 3 Single biseriate ascus of *Hydnotrya tula*snei (Scale bar represents 35 µm (DIC 40x)

Results

Comparative morphological study

The original specimens collected by Soehner and Bail (Table 1) and deposited as *H. bailii* at the Botanische Staatssammlung München were, regarding their micro- and macromorphology, nearly identical to our recent collections. The most striking morphological differences to the sister taxon *H. tulasnei* are the small globose fruiting body (Figs. 1 and 2) and the monoseriate assembly of the slightly smaller ascospores (Figs. 3 and 4). Most specimens of *H. tulasnei* compared to have larger fruiting bodies, and their ascospores do not strictly follow monoseriate but often biseriate assembly. These morphological characteristics were also present in the original material collected by

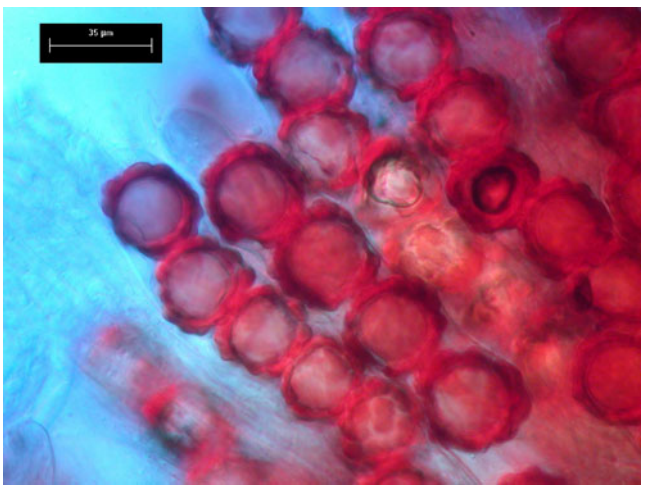

Fig. 4 Several monoseriate asci arranged in parallel of *Hydnotrya bailii (Scale* bar represents 35 µm (DIC 40x)

Soehner and determined as *H. intermedia*, which is now regarded as a synonym of *H. tulasnei*.

A dichotomous key was developed and tested by using specimens from *H. cerebriformis, H. michealis, H. tulasnei* and *H. bailii*. Specimens of the two other European species *H. confusa* and *H. cubispora* known only from the British islands were not included.

Phylogenetic hypothesis

The first alignment comprised 56 sequences and 2237 characters, most of its length being caused by few sequences containing insertions within the ITS1 and others comprising parts of the large subunit rDNA. Gblocks reduced this alignment to 395 unambiguously aligned characters. The resulting best ML tree had a log likelihood of −2547.675 and is shown in Fig. 5 together with ML and MP bootstrap values. While the separation of ingroup (*Hydnotrya*) and outgroup (*Gyromitra*) sequences was moderately to well supported (99/70%), strong support (96–100%) was achieved for a basal split of *Hydnotrya* in a clade comprising Genbank sequences from *H. michaelis* and one from "*H. variiformis*" and another clade comprising the remaining specimens (*H. tulasnei*, *H. bailii*, *H.*

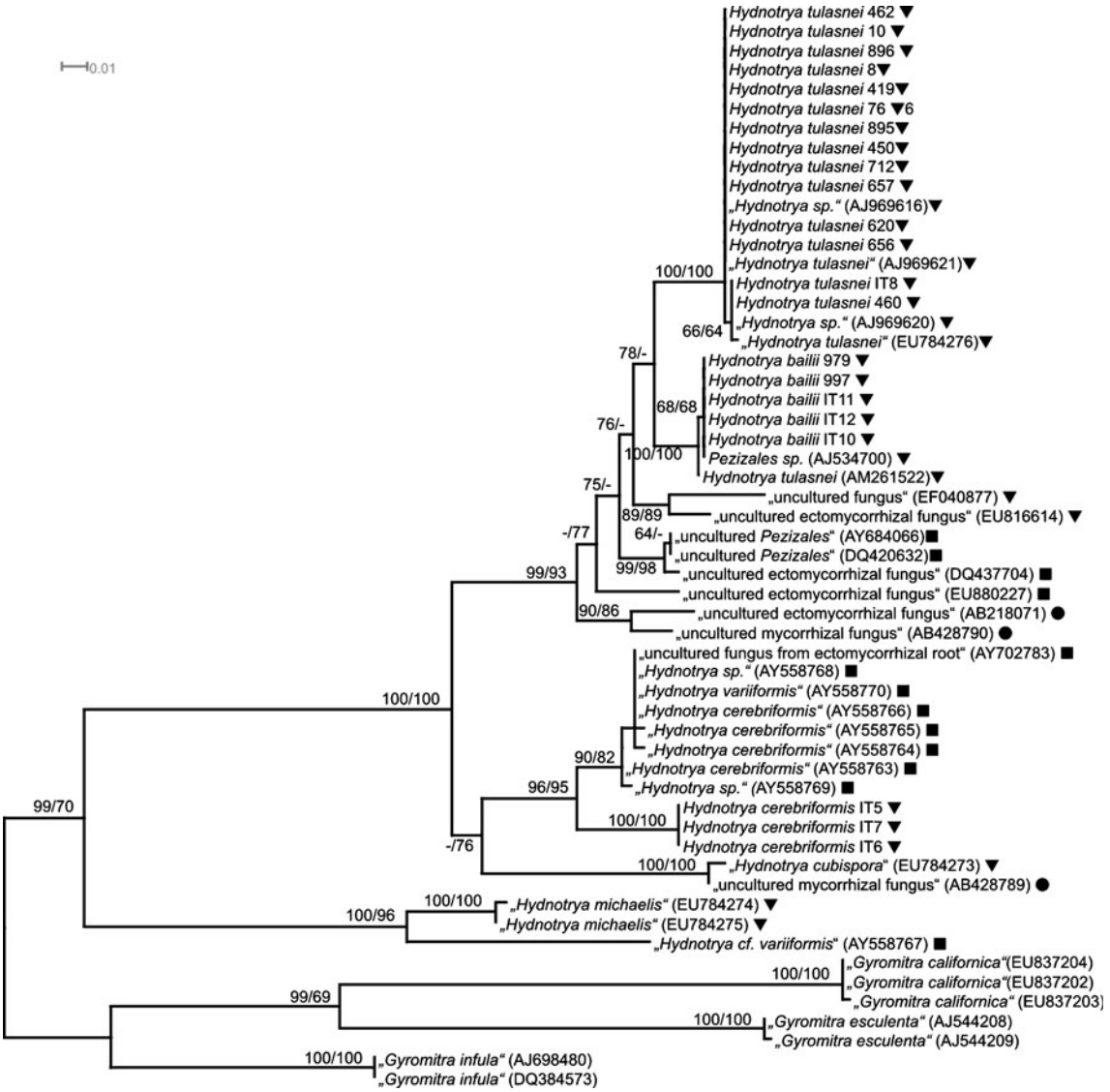

Fig. 5 Phylogenetic tree inferred under the maximum-likelihood (ML) criterion from 395 unambiguously aligned characters and rooted with the sister genus *Gyromitra* (Discinaceae). Numbers on the branches represent support values from 1,000 replicates under the ML (left) and maximum-parsimony (right) criterion. The branches are scaled in terms of the expected number of substitutions per site. Sequences from Genbank are indicated by their accession number in parentheses after their "organism" modifier as found in the Genbank flatfiles. Labels of sequences newly obtained in the course of this study include the assigned taxon name and the isolation number. Note the considerable distance between *H. michaelis*/"*H.* cf. *variiformis*" and the other *Hydnotrya* collections. Triangles indicate collections from Europe, squares indicate collections from North America and dots indicate collections from Asia

cerebriformis, "*H. variiformis*", *H. cubispora* and various environmental and undetermined samples). Because the *H. michaelis* clade was almost as distant to the clade containing the remaining *Hydnotrya* as the outgroup, the second alignment was restricted to this large *Hydnotrya* subclade, and rooting of the second tree was done according to this subclade's basal split into *H. tulasnei*, *H. bailii* and some environmental samples on the one hand and *H. cerebriformis*, "*H. variiformis*", *H. cubispora* and other undetermined samples on the other hand.

The best ML tree inferred from the taxonomically restricted but full-length second alignment had a log likelihood of −4381.652 and is shown in Fig. 6 together with ML and MP bootstrap values. The topology is nearly identical to the corresponding subtree of the tree shown in Fig. 5, but, as expected, the support values are significantly higher for some groupings. *H. bailii* and *H. tulasnei* are revealed as well-separated and well-supported (100%) sister groups of each other (90/68%). Both include undetermined Genbank samples; the *H. bailii* cluster also includes one Genbank sequence (AM261522) identified as *H. tulasnei*. Pairwise uncorrected distances within the *H. tulasnei* cluster were at most 1.03%; within the *H. bailii* cluster, at most 0.27%; between the two clusters, a minimum of 4.33%. Further sequences from undetermined samples form a grade at the base of the *H. bailii*/*H. tulasnei* clade, most of them being also quite distant to each other and forming highly supported (95–100%) subclades. Within the outgroup, our three European collections of *H. cerebriformis* appear as even more distant from their North American counterparts (represented by GenBank accessions) than *H. bailii* from *H. tulasnei*, also hinting at cryptic diversity within *Hydnotrya*.

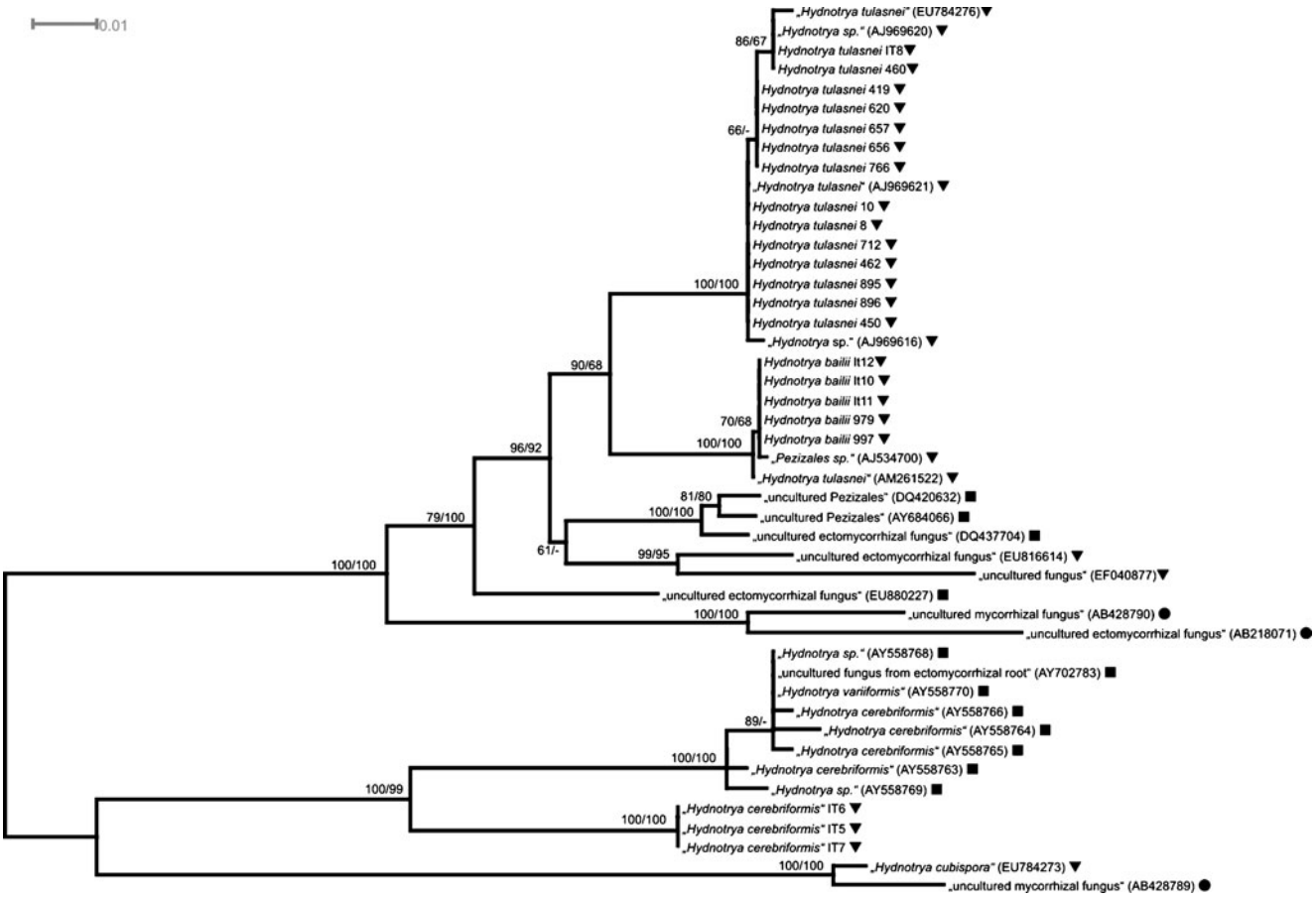

Fig. 6 Phylogenetic tree inferred under the maximum-likelihood (ML) criterion from a full ITS alignment including closely related *Hydnotrya* sequences selected and rooted according to the tree in Fig. 5. Numbers on the branches represent support values from 1,000 replicates under the ML (left) and maximum-parsimony (right) criterion. The branches are scaled in terms of the expected number of substitutions per site. Sequences from Genbank are indicated by their accession number in parentheses after their "organism" modifier as found in the GenBank flatfiles. Labels of sequences newly obtained in the course of this study include the assigned taxon name and the isolation number. Triangles indicate collections from Europe, squares indicate collections from North America and dots indicate collections from Asia

Taxonomic description

Because Soehner's description was neglected by more recent studies, we here repeat the Latin diagnosis given in 1959, and provide the first English diagnosis.

Hydnotrya bailii Soehner 1959

Latin diagnosis Ascomata hypogaea, primum subglobosa, subtomentosa, cum 1–3 cavernis, postea tuberose excavate, carnosa, 1–2(−2,5) cm in diam. metientia. Gleba canaliculis et vinculis gyroso-labyrintheis composite, cubicula hymenio ascisque subhymenialibus crebris et paraphysibus vestita. Parietes 1 mm crassi foris subalbidis. Asci cylindracei apice rotundati, 250–300×35–40 µm longi et lati, 8-spori, paraphyses ascos superantes, Sporae exacte uniseriatae, sphaericae, verrucosae, (27.5-)30–34(−37.5) µm cum sculptura in diam metientes.

Holotype Zackenfall, Sudeten mountain range (1880, leg. Bail: Rabenhorst, Hb. Mycol. ed. 2. no. 321-M: Holotypus, Botanische Staatsammlung München).

Etymology and diagnosis The name of the species refers to the collector of the holotype, Bail. Young ascomata ochre, later magenta reddish, when ageing dark brown to brown-black, irregularly wrinkled and elongated, bulbous, uneven, with deep furrows often with multiple lobes, with one or many irregular vent-like disruptive openings of elliptic to crater-like shape or many smaller ones leading to the inner space, openings usually found at the apex, seldom at basal site, surface waxy velvety, lacking a basal hole. Peridia 150–200 µm thick.

Gleba ochre to magenta reddish to dark brown; depending on ascomata age, strongly convoluted cavities, with white velvety coating: Paraphyses small, cylindrical (250–300 µm). Asci cylindrical, 250–300×30–40 µm, eight-spored, numerous asci in subhymenium. Ascospores strictly monoseriate, (27.5-) 30–34 (−37.5) µm (with ornamentation), globose, ripened spores brown-reddish, blistered warty. Ascomata 1–2(−2.5) cm in size with pleasant aromatic smell.

Ecology The holotype is known from montane spruce (*Picea abies*) forests. The present collections, which confirm the host specificity postulated by Soehner (1959), were made near Schierke (Harz; Saxony-Anhalt; Germany) and near Hinterzarten (Black Forest; Baden-Württemberg, Germany).

Specimens studied for comparisons Holotype *Hydnotrya bailii* (Soehner) (leg. Bail 1880), *Hydnotrya bailii* (Soehner) 2064 Simmerberg (Herb. Soehner), *Hydnotrya bailii* Zuckermantel (1905). All specimens are deposited at the Botanische Staatsammlung München (corresponding curator Dr. D. Triebel).

Discussion

Based on morphological data and ITS sequence analysis we here confirm the neglected taxon *Hydnotrya bailii* (Soehner 1959) as a distinct species. *H. bailii* is morphologically differentiated from *Hydnotrya tulasnei* by its smaller and globose fruiting body, strictly monoseriate ascospores, specificity to spruce and to a montane-boreal habitat. We do not consider the lack of an ITS sequence from the holotype or other material determined by Soehner as *Hydnotrya bailii* (which would clarify the issue of sequence identity with the present material) as an obstacle for recognizing the two species as distinct. Morphological comparison confirmed the identity of our recently collected specimens and those investigated by Soehner (1959) regarding the characteristics listed by Soehner as typical for *H. bailii*.

Hydnotrya species such as *Hydnotrya cerebriformis* Harkn. (1889), *Hydnotrya michaelis* (E. Fisch) Trappe (1975), *Hydnotrya cubispora* (E.A. Bessey & B.E. Thomps.) Gilkey (1939) (Lack 2003) and *Hydnotrya confusa* Spooner (1992) can be easily distinguished by their ascospore ornamentation. In contrast, *H. tulasnei* and *H. bailii* are more difficult to distinguish due to their similar tissue anatomy. This might explain the complete absence of *H. bailii* in the current literature and databases (e.g. Mycobank, Index Fungorum). Difficult access to the original publication (Soehner 1959) and the small number of mycologists in Europe collecting hypogeous fungi may also account for this problem.

There are strong indications that *Hydnotrya tulasnei* is ectomycorrhizal with preference for broad leafed trees. Tedersoo et al. (2006) analyzed beech ectomycorrhizae whose ITS sequences were identical to sporocarp sequences of *Hydnotrya tulasnei* found in a Danish beech forest (AJ969616 and AJ969620 from mycorrhized root tips, AJ969621 from a sporocarp). Another sporocarp sequence (EU784276) of original material collected by Hawker (1954) was morphologically determined as *Hydnotrya tulasnei* (Brock et al. 2009). These four sequences fall into our *Hydnotrya* cluster (Figs. 5 and 6). The sequences of the *H. tulasnei* cluster are prevalently from broad-leafed trees but never from spruce. Sequences of specimens reported to be spruce-associated *Hydnotrya* species, do not fall into our *H. tulasnei* cluster. Vohnik et al. (2007) identified a spruce-associated sporocarp from a mountainous area of Southern Bohemia in the Czech Republic (AM261522) as *H. tulasnei* based on 99% sequence identity with Genbank entry

AJ534700. This sequence stems from a mycorrhized root tip collected in an Estonian mixed forest also containing spruce (Tedersoo et al. 2003). Both sequences fall unambiguously into our *H. bailii cluster*. We have to conclude that (Vohnik et al. 2007) and (Tedersoo et al. 2003) collected specimens of H. *bailii*. This supports our observation that *H. bailii* is separated from *H. tulasnei* not only by morphological characters and ITS-sequence but also by preference for coniferous trees and a montane to boreal distribution.

Regarding other *Hydnotrya* species, our results reveal that *H. cerebriformis* requires a taxonomic revision, as our European collections are clearly differentiated from the North American ones (represented by the Genbank sequences in Figs. 5 and 6) in our phylogenetic reconstructions using the ITS locus. The branches separating the two clades are even longer than those separating *H. bailii* from *H. tulasnei* (Figs. 5 and 6). Cryptic *Hydnotrya* species from distinct and biogeographically diverse sampling sites across Europe (Peintner et al. 2007, EF040877 unpublished: EU816614) North America (Dickie et al. 2009, EU880227 unpublished: AY684066, DQ420632, DQ 437704) and Japan (Ishida et al. 2007, AB218071, Ogura-Tsujita and Yukawa 2008, AB428790) and forming several clades within a grade next to the *H. tulasnei*/*H. bailii* cluster also indicate that the species diversity of *Hydnotrya* is far greater than previously assumed. Morphologically well-characterized species such as *Hydnotrya confusa* that were not yet sequenced may account for some of the observed "cryptic" diversity. Most likely some *Hydnotrya* species still remain undiscovered. We therefore recommend intensifying the search for fruiting bodies at these sampling sites to clarify the presence of such unknown taxa most likely morphologically similar to *H. tulasnei*. Furthermore, the investigation of collections from other areas is required, other continents as well as other regions of Europe such as the Mediterranean, the Balkans and the Carpathian areas. For now, the 50th anniversary of *Hydnotrya bailii* should be dedicated to the resurrection of this valid species whose discriminating characters were not recognized during the past 50 years.

Dichotomous key to the central European *Hydnotrya* species

- 1. Ascospores in general ellipsoid, 24–35×13–26 µm, (quotient: 1.3–1.8) brown, irregularly warty, hyaline when younger. Ptycothecium strongly folded inwards, brown to brownish-yellow, sometimes carmine, hollow, with reddish gleba and white hymenial surface. So far only reported from montane coniferous habitats.....*Hydnotrya michaelis*
- 1′. Ascospores in general globose to cubic.....2
- 2. Ascospores brown to yellow-brown with dense spiny ornamentation, 28–35 µm in diameter*. Ptycothecium dark brown-carmine coloured, strongly folded inwards. Gleba uncoloured, with white hymenial surface. Associated with montane conifers.....*Hydnotrya cerebriformis*
- 2′. Ascospores, cubic, 20–45 µm in diameter*.....*Hydnotrya cubispora*
- 2″. Ascospores ornamented with large warts and coarse harsh surface, mono and biseriate, 21–38 µm in diameter*.....3
- 3. Ascospores biseriate, rarely monoseriate, 25–38 µm in diameter*. Ptycothecium uneven, reddish-ochre with brown notes, kidney-like, lobated, surface not much folded inwards, 1–6 cm in width. Gleba rose-whitish, later dark red with white hymenial surface. Broadest host range of all *Hydnotrya* species, but in general associated with *Fagus, Corylus* and *Pinus* in lowlands and montane habitats.....*Hydnotrya tulasnei*
- 3′. Ascospores strictly monoseriate (when young rarely biseriate), 21–36 µm in diameter*. Ptycothecium uneven, reddish-ochre-brown, lobated, infolded on the surface, in general rather globose than wide, 1–1.5 cm, gleba rose-whitish, later dark red with white hymenial surface. Strictly associated with *Picea abies* in montane habitats.....*Hydnotrya bailii*

*Including ornamentation

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