Mollisia scopiformis, a softcup gets a new name

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Abstract: The soft-cup species *Mollisia scopiformis* is presented from its macro- and microscopy. Its asexual morph was known under the name *Phialocephala scopiformis*. The ecology is described and the new nomenclature is presented. We follow a broad generic concept which includes the recently segregated genus *Phialocephala*.

Introduction

In a two-and-a-half year collaboration with Florian Prell, who was then working at the University of Gießen, numerous ascospore isolates were created in addition to the macro- and microscopic portraits of cup fungi collections that he had made or were sent to him by one of us (I.W.). The rDNA sequences obtained therefrom supplied clearer insights into the species concepts, also within the genus *Mollisia* (Fr.) P. Karst. For a number of years, it has been possible to include genetic data in addition to morphological characteristics when identifying fungi by evaluating relevant areas of the genome, e.g., different areas of ribosomal DNA (rDNA). These sequences permitted to recognize clear species concepts in many cases and may also reflect relationships between the species. To illustrate this, a sequence tree is calculated using a phylogeny program.

Due to possible genetic variability of a given species and the inevitable susceptibility of the method to errors, one can only dare to make first predictions about the taxonomic value of a species when several sequences of a species are at hand. It becomes easier when dealing with a genetically relatively well defined species, i.e., when different finds of the supposedly same species are genetically more or less identical in the ITS barcode region.

The species presented here is a soft cup, which Andreas Gminder, who has dealt with the genus *Mollisia* for many years, provisionally called *Mollisia "pyrenopezizoides"* due to a similarity with the often dark cups from the genus *Pyrenopeziza* Fuckel. Three newly obtained sequences of this species fit well his two existing ones and show also great genetic similarity to the validly described ***Phialocephala scopiformis*** Kowalski & Kehr (1995). It is the anamorph of this soft cup, the species epithet of which means "brush-shaped", referring to its morphology. Brian Douglas (2013) in his dissertation on *Mollisia* and *Pyrenopeziza* species also linked his sample from Wales with *P. scopiformis*. We recently combined this species in Index Fungorum into *Mollisia*, because we favour a broad genus concept due to the extremely great similarity in the teleomorphs of *Mollisia* s.str. and *Phialocephala* (= *Mollisia* with *Phialocephala* anamorph). We have recently transferred this species to *Mollisia* (Index Fungorum 2021), because we follow a broad genus concept based on the very high similarity in the teleomorphs of *Mollisia* s.str. and *Phialocephala* (= *Mollisia* with *Phialocephala* anamorph). According to current knowledge, however, *M. scopiformis* appears to be a genetically variable species, requiring further study.

Materials and methods

The fresh samples were photographed with a Canon Powershot A650 through the MP4400F stereo loupe and microscoped with a NOVEX Holland microscope (I.W.) in water and other microscopy chemicals such as KOH, Congo red SDS, Lugol or cresyl blue. Important microelements were photographed through the trinocular with the microscope

camera MD-300 (I.W.) and later measured on the image. Microphotos of sections were made with Nikon Coolpix 4500 using a Zeiss Standard 14 microscope with 100×/1.25 oil immersion phase contrast achromatic objective and Leitz Periplan 10× ocular (H.B.). Relevant structures on the microphotos were cut to size and put together to form a collage using Adobe PhotoDeluxe Home Edition 4.0 (I.W.) or Adobe Photoshop (H.B.).

The species was initially determined through consultation with *Mollisia* expert ANDREAS GMINDER (undated) and his unpublished key to species, and later by self-acquired knowledge of the species, including its molecular characteristics of the ITS barcode region. Alignment and neighbor joining analysis were carried out with MEGA 7. The sequences of our finds were compared with corresponding sequences from Genbank (NCBI 2021).

Mollisia scopiformis (Kowalski & Kehr) I. Wagner, Prell & Baral, in Baral & Quijada, Index Fungorum 454: 2 (2020)

≡ ***Phialocephala scopiformis*** Kowalski & Kehr, Can. J. Bot. 73(1): 27 (1995)

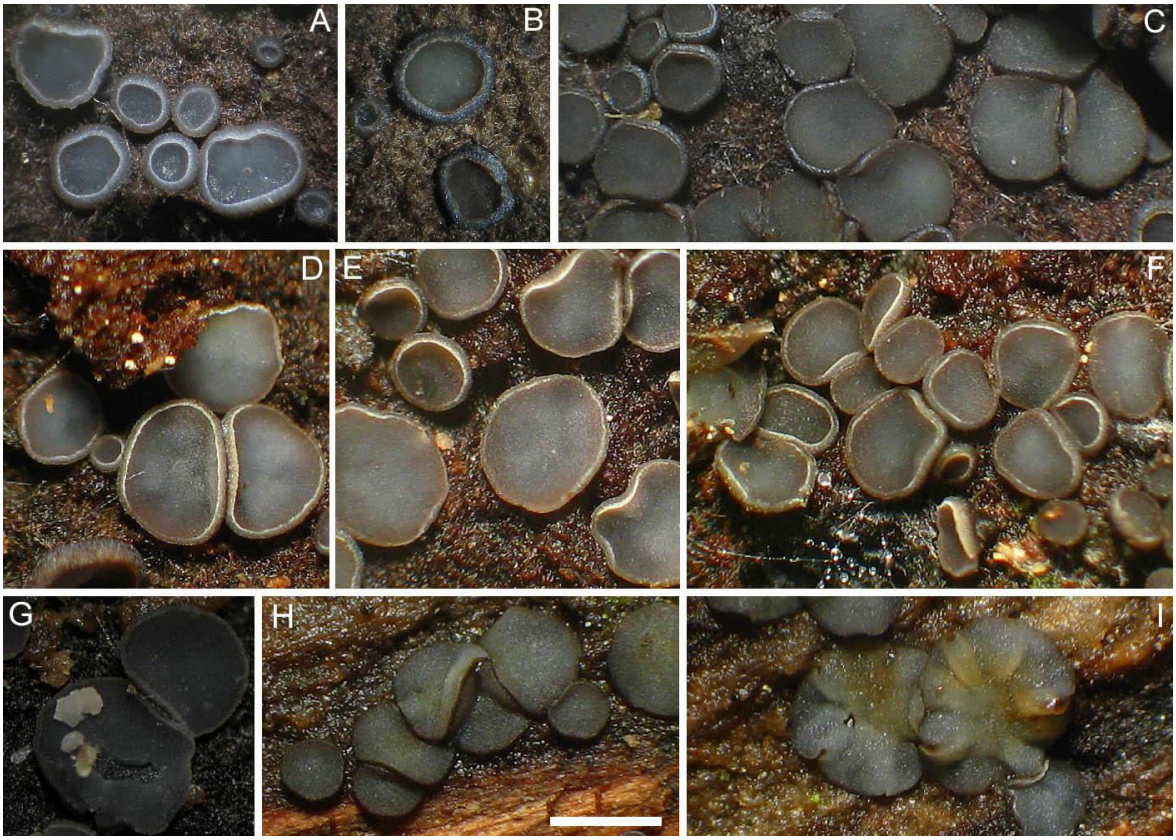

Fig. 1: *Mollisia scopiformis*. Fresh apothecia (diameter approx. 0.5–1.5 mm; G: parts of the hymenium scraped out with a spatula and placed on the disc). Scale bar = 1 mm. Photos: I. Wagner. A-B: IW-120402; C: IW-160220-TR-FP233; D-F: IW-200321; G: IW-090412; H-I: IW-160616-FP294.

Macroscopic characteristics (capital letters in brackets refer to Fig. 1)**:**
Apothecia: 0.5–1.4 (–1.8) mm diam., gregarious to crowded, sessile on a mostly extending, brown to dark brown subiculum (A, B), roundish, also wavy (discinoid) with age, outside brown, disc dark gray with a brownish tinge towards the margin (D, E, F), young with a bulgy margin and finely white-fimbriate (A), later flattening and uniformly dark gray (H), hymenium whitish when drying off or scratched out (G), sometimes ochre-brown at age in damaged areas (I).

Microscopic characteristics ({...} = number of collections, * = living state, † = dead state)**:**

Asci *50–65 (–70) × (5–) 6–7 (–8) µm {7}, protruding 10–12 µm, †43–60 × 4–5 µm {3}, 8-spored, with croziers {9}, apical ring in Lugol's solution strongly blue (bb) {9}. **Ascospores** *(6–) 6.5–9 (–10) × (2–) 2.3–2.8 (–3) µm {9}, mostly elongated ovoid to club- or wedge-shaped, without any noticeable contents (without or with 1–3 tiny drops of oil at the ends), OCI[1] 0(–0.5) {9}. **Paraphyses** cylindrical, *2–3.5 (–4.2) µm wide, apically sometimes somewhat widened or narrowed, from the middle to the base 2–3-septate, at least the upper cell inside with large, long cylindrical, colorless, strongly refractive vacuole (VB) when alive, reacting with KOH (3–10%) in a dark yellow inside the paraphyses {3}, but mostly medium to strong yellow emerging into the medium {7}. **Subhymenium** and **medulla** hyaline. **Ectal excipulum** predominantly of spherical cells, medium reddish brown to brown, turning olive in KOH; **Cortical elements** at the margin sometimes hair-like and 2–3-celled, apical cell mostly somewhat broadened and filled with a refractive vacuole (VB) when alive. **Subiculum hyphae** abundant, *(2.5–) 3.5–5.5 µm wide {6}, bright (reddish-)brown, abundantly septate, with (0.2–) 0.8–1.8 µm thick cell wall {7}.

Habitat: decorticated branches, trunks and stumps, mostly lying on the ground, of *Fagus sylvatica* {1}, *Quercus* sp. {6}, *Prunus* sp. {1}, *Prunus avium* {1}. Associated with *Eriopezia caesia* {1}, *Hyaloscypha daedaleae* {2}, *Mollisia lividofusca* {1}, *Orthodontium lineare* {1}.

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[1] (see Baral & Wagner 2020: 46

Collections examined:

Germany, **Mecklenburg-Western Pomerania**, Rehna, Dechow, Rehna state forest, 70 m, rotten debarked lying branch of *Quercus*, 23.03.2008, leg. T. Richter, det. H.O. Baral (H.B. 8787); – Rehna, Löwitzer Holz, 60 m, little decomposed stumps of *Quercus*, 20.02.2016, leg. T. Richter, det. T. Richter & I. Wagner (IW-160220-TR-FP233, GenBank accession nr. MW843512); – Thuringia, Effelder, "Loch", ca. 465 m, thick lying piece of branch of *Quercus*, on the underside, 12.04.2009, leg. I. Wagner, det. I. Wagner & A. Gminder (IW-090412); – Seltendorf, "Kienberg", ca. 498 m, lying branch of *Prunus*, 2.04.2012, leg. & det. I. Wagner (IW-120402); – Sonneberg, Wehd, "forest recreation", ca. 492 m, rotten stump of *Quercus*, 27.05.2013, leg. & det. Ingo Wagner (IW-130527); – ibid., 486 m, on the underside of an externally heavily weathered branch of *Fagus* in leaf litter, 13.04.2020, leg. & det. I. Wagner (IW-200413-IW066, GenBank accession nr. MW843514); – Sonneberg, Wehd, "Eichberg", ca. 407 m, on lying hidden thick wood of *Quercus* on the ground, 16.06.2016, leg. & det. I. Wagner (IW-160616-FP294, GenBank accession nr. MW843513); – Mönchsberg, "Halbe Maaß", 586 m, thick branch of *Prunus avium* in the litter, 21.03.2020, leg. & det. I. Wagner (IW-200321). – **Baden-Württemberg**, Tübingen, Pfrondorf, Höhberg, 400 m, on the underside of optimally rotten splinterwood of *Quercus*, 03/14/2008, leg. H.O. Baral, det. A. Gminder (H.B. 8779b).

Convincing documentations of the teleomorph form outside Germany are those of S. Tello (on *Quercus faginea*, Jaén, Andalucia, Spain) and J. B. Tanney (on *Picea rubens*, New Brunswick, Canada). These and other German collections can be viewed in the *Mollisia scopiformis* folder at "www.in-vivo-veritas.de" by H.-O. Baral. A detailed photo collage was available to us from the British find of B. Douglas (Wales, Botanical Garden in Carmarthen, ?coniferous wood).

Ecology:

The species has a preference for dead wood, which lies or stands relatively well protected from drying out. The apothecia are often aggregated on the underside of half-embedded thicker branches or logs in the litter, preferably of oak (*Quercus*). Other recognized or reported substrates were *Prunus*, *Fraxinus*, *Fagus*, *Carpinus*, and *Picea*. The evaluation of the phenology of around 20 collections from Europe (including data from the mapping project of mushrooms in Germany) reveals a prevalence for the period from December to June.

Our findings question Tanney et al.'s (2016) view that the species was restricted to coniferous wood (*Picea* spp.). However, the observed small genetic differences between American and European finds and their correlation with differences in characteristics of the teleomorph could indicate a necessary species separation (see below).

Molecular results:

The ITS sequences of the six European apothecial samples differ by three constant nucleotide deviations from previous sequences in GenBank (counting starts after the SSU motif ATCATTA): In ITS1 position 39 (G/A), in 5.8S position 307 (T/C) and in ITS2

position 427 (T/C), respectively. They are responsible for the formation of two groups in our phylotree (Fig. 4). However, there is also variation within the groups (0.2–1.3% in the European group and 0–0.4% in the predominantly North American material in GenBank). The p-distance between the two groups is 1–1.9%. Whether this is sufficient to distinguish two species remains to be investigated. Noteworthy is that one of the three constant deviations lies in the conservative 5.8S region. A slightly higher distance is observed in the S1506 intron (2–2.3%). One GenBank strain under the name *M. scopiformis* (DO34) deviates more strongly (ITS: 2.5–3.3%, S1506 intron: 4–5.5%). Other species from the *Phialocephala* relationship such as *Phialocephala dimorphospora* W.B. Kendr. differ by about 7–12% in the ITS region.

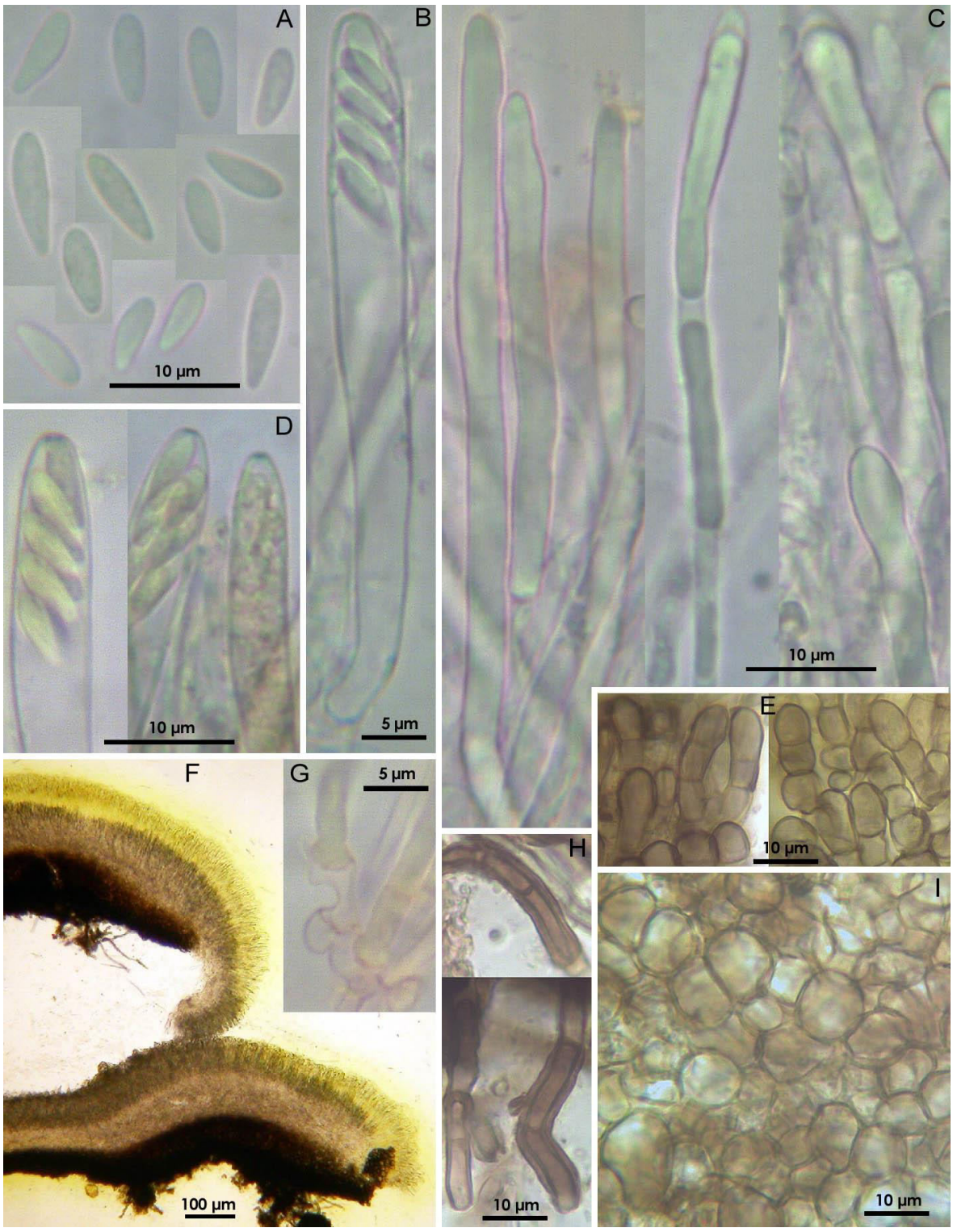

Fig. 2: Microstructures of *Mollisia scopiformis*. **A:** vital ascospores in H_2O; **B:** vital ascus with 8 biseriate spores, in H_2O; **C:** vital paraphyses with refractive vacuoles, in H_2O; **D:** ascus apices with blue reacting apical rings, in IKI (Lugol); **E:** hair-like end cells of the cup flank near the margin in surface view, in H_2O; **F:** yellow KOH reaction of the vacuoles (VBs) in the hymenium; **G:** base of young asci with croziers, in Congo red-SDS; **H:** thick-walled subiculum hyphae, in H_2O; **I:** cells of the more basal ectal excipulum viewed from a squash mount, in H_2O. A,C,H: IW-160220-TR-FP233; B,D(right),I: IW-160616-FP294; D(left),G,E: IW-130527; F: H.B. 8787. – Photos: I. Wagner, except F: H.-O. Baral

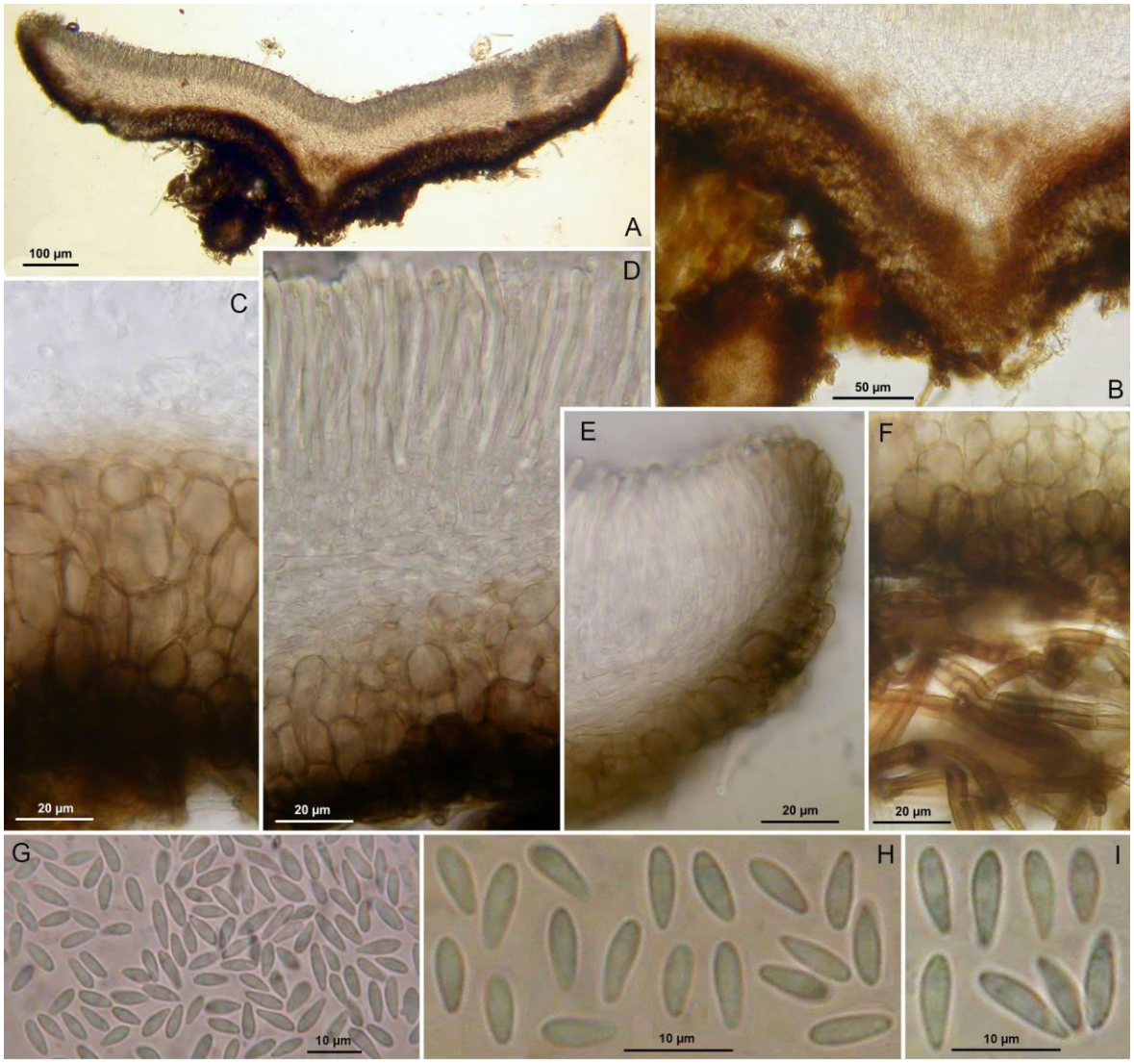

Fig. 3: Median section of an apothecium of *Mollisia scopiformis* (vital in H_2O). **A:** overview; **B:** basal area of the excipulum, with a spherical ?apothecium initial below left; **C:** ectal excipulum (brown) and medulla (hyaline) at lower flank; **D:** do., with hymenium; **E:** marginal region; **F:** ectal excipulum at lower flank, with subiculum hyphae; **G-I:** ascospores. A-B, G-H: H.B. 8787; C-F, I: H.B. 8779b. – Photos: H.-O. Baral

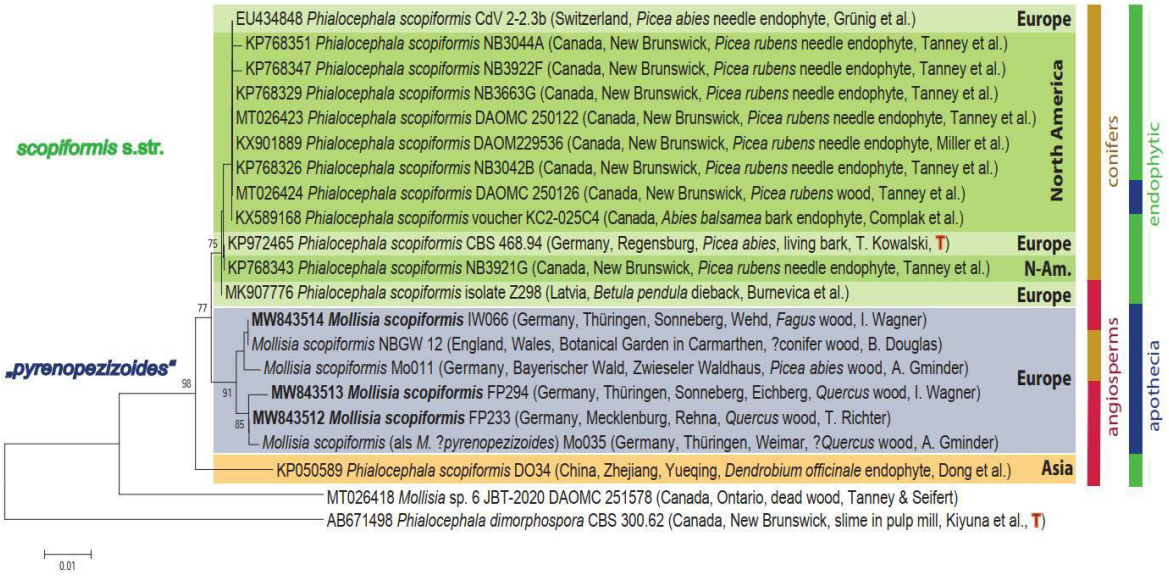

Fig. 4: Neighbor-joining analysis of the ITS region of *Mollisia scopiformis*, with *Phialocephala dimorphospora* and *Mollisia* sp. as outgroup. Sequences in GenBank (mostly from spruce) clustered separately from our sequences on both angio- and gymnosperms. While the second clade only includes European finds, the first clade contains mainly Canadian isolates but also three European ones. T = type. – Phylogramm: H.-O. Baral

Discussion

Mollisia scopiformis is a species of wide distribution, for which, however, no convincing name could be found except for Andreas Gminder's *Mollisia* key.

The clearest feature of *M. scopiformis* are the predominantly elongated ovoid to club-shaped, almost wedge-shaped ascospores with an oil content of 0(–0.5) (OCI scale 0–5) and average vital dimensions of 8 × 2.5 µm, with their length remaining always below 10 µm and their width with a maxinum of at least 2.5 µm. Another characteristic is the up to three-celled cortical cells at the margin, which in most wood-inhabiting *Mollisia* spp. without clear hairs only contain a maximum of two cells.

Microscopically similar wood-dwelling *Mollisia* species with the combination of characteristics "KOH+, crozier+, ascus apical ring amyloid, spore length less than 10 µm" have a different spore shape and / or different spore widths.

On the basis of the genetic deviations observed, one could infer the existence of two small species with geographical and ecological ties. However, the American sequences in GenBank come from a narrowly defined region in New Brunswick (Canada), where either apothecia were found on rotten spruce branches or mostly mycelium was isolated from living needles, whereas the six European sequences of the second clade come apothecia collected in different regions of Europe, predominantly on hardwood (oak and beech). Examples deviating from this scheme are the type of *P. scopiformis*, which was isolated by the author of the species, T. Kowalski, from from living spruce bark (*Picea abies*) near Regensburg, and the sequences from the Bavarian Forest (A. Gminder) and Wales (B. Douglas), which were obtained from apothecia on softwood (uncertain for Wales).

It should be noted, however, that the three finds on oak form a separate group in the phylotree and those two on conifers and one on beech a sister group to them (with the restriction that the substrate has not been ascertained in all of them). In addition, our apothecial finds on hardwood differ in an intensely yellow KOH reaction and the ± complete absence of oil droplets in the ascospores from the Canadian apothecial finds, for which Tanney et al. (2016) reported a negative KOH reaction and described the spores as often guttulate. These authors have illustrated only a few tiny drops at the spore ends, but alternatively also indicated in their description 2–3 (–4) drops with a diameter of 1–2.5

µm. In addition, they stated that there was no clear subiculum. The large spore guttules appear a bit implausible and cannot be assessed due to the lack of illustration. The statement "hemiamyloid" for the asci is a mistake, as well as the much too small ascus dimensions (J.B. Tanney pers. comm.). However, the British find by B. Douglas (pers. comm.) showed also only a very weak KOH reaction and the spores occasionally contained 1–3 tiny drops at the ends.

Acknowledgements

We thank Brian Douglas (London, Great Britain) for making his dissertation on *Mollisia* and *Pyrenopeziza* species available, him and Andreas Gminder (Goslar) for sending their sequences, and Torsten Richter (Rehna) for making his findings available. We are grateful to Florian Prell (Bomlitz) for his work at the Institute for Food Chemistry and Food Biotechnology at the Justus Liebig University in Giessen, which enabled him to carry out molecular studies on sample material from various helotialean ascomycetes. We thank Joey Tanney (Victoria, Canada) for valuable discussions and for reviewing the manuscript.

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