**Phylogenetic placement and lectotypification of *Pseudotryblidium neesii* (Helotiales, Leotiomycetes)**

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Abstract: A phylogenetic analysis of combined rDNA LSU and ITS sequence data was carried out to determine the phylogenetic placement of specimens identified as *Pseudotryblidium neesii*. The species forms a distinct clade within Dermateaceae (Helotiales, Leotiomycetes) with Rhizoderma veluwiensis and two *Dermea* species. The geographical distribution of this species, previously known only from Europe on *Abies alba*, is extended to north-western North America where it grows exclusively on *A. grandis*. The name *P. neesii* is lectotypified in order to disentangle the complicated nomenclature of the species. A new, detailed description of *P. neesii* with illustrations is provided after comparison of sequenced specimens with the type material. Furthermore, the new combination *Pseudographis rufonigra* (basionym *Peziza rufonigra*) is made for a fungus previously known as *Pseudographis pinicola*.

Effectively published online: 18 November 2019.

**INTRODUCTION**

*Pseudotryblidium neesii* is a non-lichenized and non-lichenicolous helotialean ascomycete that specifically inhabits bark of *Abies* species (Rehm 1890, Hafellner 2009, Zimmermann 2011). However, it can easily be mistaken for a lichenicolous fungus because the ascomata often break through the thalli of corticolous crustose lichens such as *Loxospora elatina* and *Phlyctis argena* (e.g. Lindsay 1869, van den Boom & Breuss 2002, Hafellner 2009). The species is widespread in Central Europe, with gaps in its distribution probably due to undercollecting. The species was unknown outside of Europe until one of the authors (MH) collected it in Idaho, Montana and Washington (USA), these specimens originally considered as an unknown lichenicolous fungus growing on crustose lichens (*Ochrolechia* species and *Pertusaria carneopallida*). As the material was morphologically similar to *Pseudotryblidium neesii*, we compared internal transcribed spacer (ITS) sequences of North American and European specimens to confirm the morphology-based identification.

For a long time, the phylogenetic position of *Pseudotryblidium* has remained unsettled. Rehm (1890), followed by Bouder (1907), considered *Pseudotryblidium* as belonging to *Patellariaceae*, while in later classifications it was included in *Helotiales incertae sedis* (e.g. Nannfeldt 1932, Jakitsch et al. 2016). We tested the phylogenetic affiliation of this monotypic genus by using two gene loci to identify the position of this fungus within *Helotiales*. As the nomenclature of *Pseudotryblidium neesii* was rather confusing, we have restudied this problem and lectotypify the name.

**MATERIALS AND METHODS**

**Study of specimens**

The examined specimens are deposited in BG, CWU, G, HAL, H, OSC, TU and W and in the private herbaria of P. Diederich, M. Haldeman and E. Zimmermann. External morphology was examined using a Leica MZ 7.5 dissecting microscope. Macroscopic photographs were taken using a Canon 40D camera with a Nikon BD Plan 10 microscope objective, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Microscopic structures were studied using hand-cut sections in water; colour reactions were observed using 5 % KOH (K); Lugol's reagent, both with (K/I) and without (I) pretreatment with K, and Melzer's reagent were used to examine the ascus apical apparatus. Microscopic photographs were prepared using a Leica DMLB microscope with DIC, a Leica EC3 camera and Helicon Focus.

**DNA extraction, PCR amplification and DNA sequencing**

Genomic DNA was extracted from ascomata of *Pseudotryblidium neesii* collected from Switzerland (four specimens) and North America (three), plus from four specimens belonging to the genus *Dermea* (Table 1). DNA extraction was performed using High Pure PCR Template Preparation Kit (Roche Applied Science®) and following the protocol provided by the manufacturer with minor modifications. We amplified the internal transcribed spacer (ITS) using primer pairs ITS0F and ITS1R.
Table 1. NCBI accession numbers of sequences used in molecular phylogenetic analyses. Entries in **bold** (with voucher and lab codes) are newly generated for this study. Detailed information about these vouchers is provided under Specimens examined. ‘na’ indicates information not available.

| Species name         | Voucher / Lab code | LSU acc. no. | ITS acc. no. |
|----------------------|--------------------|--------------|--------------|
| Coleophoma caliginosa| GU973598           | KR889090     |              |
| Cryptosporiopsis sp. | GU973599           | GU973506     |              |
| Dermea acerina       | DQ2478011          | AF141164     |              |
| Dermea acerina       | MH867440           | MH855942     |              |
| Dermea acerina       | TU104990 / DE364   | MK894288     | MK894299     |
| Dermea balsamea      | MH871804           | na           |              |
| Dermea bicolor       | MH867659           | na           |              |
| Dermea cerasi        | JN086690           | JN033387     |              |
| Dermea cerasi        | MH870721           | MH854868     |              |
| Dermea cerasi        | TU104988 / DE367   | MK894290     | MK894301     |
| Dermea cerasi        | TU104987 / DE368   | MK894291     | MK894302     |
| Dermea hamamelidis   | MH867660           | AF141157     |              |
| Dermea libocedri     | MH867661           | MH856142     |              |
| Dermea mollissacula  | MH868355           | MH856839     |              |
| Dermea mollissacula  | MH867662           | na           |              |
| Dermea padi          | MH867663           | na           |              |
| Dermea persica       | MH104720           | MH104719     |              |
| Dermea piceina       | MH867664           | MH855942     |              |
| Dermea pinicola      | MH867665           | MH856144     |              |
| Dermea prunastri     | MH867666           | na           |              |
| Dermea tulasnei      | MH867667           | MH856145     |              |
| Dermea sp.           | TU104991 / DE369   | MK894289     | MK894300     |
| Fabrella tsugae      | AF356694           | U92304       |              |
| Fungal endophyte isolate 4073 | DQ979436 | DQ979592 | |
| Fungal endophyte isolate 4510 | DQ979445 | DQ979647 | |
| Glutinomyces inflatus| LC189052           | LC218289     |              |
| Helotiales sp.       | JX507673           | JX507672     |              |
| Helotiales sp.       | JX535103           | JX535102     |              |
| Monilinia laxa       | MH868237           | MH856718     |              |
| Neofabraea illicii   | KF137617           | KF137635     |              |
| Neofabraea sp.       | KF137612           | KF137630     |              |
| Neofabraea sp.       | KF137619           | KF137633     |              |
| Parafabraea eucalypti| GQ303310           | KR859091     |              |
| Pezicula californiae| GU973597           | GU973504     |              |
| Pezicula corylina    | KR858959           | KR859167     |              |
| Pezicula frangulæae  | GU973600           | KR859208     |              |
| Pezicula heterochroma| KR859002           | KR859210     |              |
| Pezicula neocinnamomea| KR859007         | KR859215     |              |
| Pezicula radicicola  | KR859028           | KR859236     |              |
| Pezicula rubi        | KR859039           | KR859247     |              |
| Phlyctema vagabunda  | KR859069           | KR859275     |              |
| Polyphillus sieberi  | MG719706           | na           |              |
| Polyphillus sieberi  | MG719704           | na           |              |
| Polyphillus sieberi  | MG719703           | na           |              |
| Pseudofabraea citricarpa| KR859075       | na           |              |
| Pseudostrybidium neesii | TU86401 / HE299   | MK894285     | MK894292     |
| Pseudostrybidium neesii | TU86402 / HE300   | MK894286     | MK894293     |
Phylogeny and lectotypification of Pseudotryblidium neesii

LA-W (Tedesco et al. 2008), and the large subunit ribosomal RNA gene (LSU) using LR0R and LR7 (Hopple & Vilgalys 1994). The PCR reaction mix (25 μL) consisted of 5 μL 5× HOT FIREPol Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.5 μL of both primers (all 20 μM), 3–5 μL of target-DNA and the rest of distilled water. The temperatures and time for each cycle of polymerase chain reaction (PCR) were as follows: denaturation was set 95 °C for 30 s, annealing 57 °C 30 s and extension 72 °C 60 s. In total 36 cycles were run. The PCR products were visualized on a 1 % agarose gel stained with ethidium bromide, and for the purification of PCR products, 1 μL of FastAP and 0.5 μL of Exonuclease I (Thermo Scientific, Waltham, Massachusetts, USA) were added to each tube per 20 μL of the product. Both complementary strands were sequenced in Macrogen Inc. (Amsterdam, the Netherlands) with primers ITS4 and ITS5 (White et al. 1990) and CTB6 (Garbelotto et al. 1997) and LR7. Sequencher v. 4.10.1. (GeneCodes Corp., Ann Arbor, MI, USA) was used to check, assemble and manually adjust the resulting sequence fragments. The consensus sequences were compared with those publicly available in NCBI (https://www.ncbi.nlm.nih.gov) and UNITE (https://unite.ut.ee) databases using blastn (Altschul et al. 1990) comparison. The ITS sequences of North American and European specimens (two and four respectively) were compared using SeaView v. 4.6 software (Gouy et al. 2010).

Phylogenetic analyses

The closest match of rDNA ITS and LSU sequences belonged to Dermateaceae (Helotiales) according to blastn comparisons of DNA sequences. Following that, we compiled separate alignments for ITS and LSU sequences including newly generated (eight LSU and ten ITS) and NCBI downloaded (Table 1) sequences. In all alignments, Fabrella tsuage (Cenangiaceae) and Monilinia laxa (Sclerotiniaceae) were chosen to root phylogenetic trees.

ITSx (Bengtsson-Palme et al. 2013) was used for extraction of neighbouring parts of conservative rDNA regions in the ITS alignment. The matrix of newly generated and obtained sequences was aligned using MUSCLE (Edgar 2004) with default options and checked visually and corrected manually with SeaView v. 4.6 (Gouy et al. 2010). The online version of Gblocks v. 0.91b (Talavera & Castreana 2007) run at http://molevol.cmima.csic.es/castresana/Gblocks_server.html was used to eliminate poorly aligned positions and divergent regions of the LSU alignment but allowing smaller final blocks and gap positions within the final blocks. The resulting ITS (44 sequences) alignment consisted of 514 nucleotide positions, of which 156 variable (37.8 %) and 128 (31 %) informative, and the LSU (50) alignment of 819 nucleotide positions, of which 124 variable (23.3 %) and 86 (16.1 %) informative.

Both DNA regions were analysed separately, then combined to LSU + ITS matrix, as no obvious topological conflict was found in statistically supported clades (posterior probabilities (PP) ≥ 0.95 and bootstrap values (BS) ≥ 75 %; data not shown). DNA alignments were analysed using Maximum Likelihood (ML) applied with RAxML v. 8.2.10 (Stamatakis et al. 2008) and Bayesian Markov Chain Monte Carlo (MCMC, later BI) applied with MrBayes v. 3.2.6. (Ronquist et al. 2012) methods. Except BI of the combined LSU + ITS alignment, the rest of the analyses were implemented at the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The best-fit nucleotide substitution model according to the lowest value of AIC criterion calculated over 56 possible models using jModeltest v. 2.1.6. (Darriba et al. 2012) was TIM3 + I + G for LSU and GTR + I + G for ITS. The data was partitioned accordingly in the two-marker analysis. For all BI analyses, two parallel simultaneous runs with four-chains run starting from the random tree were applied. The number of generations (ngen) for single marker analyses was set 2 000 000 and for two-marker analysis 9 000 000. Samplefreq and printfreq were set to 500 and diagfreq 2 000. The analyses were run until the convergence of the chains was confirmed by the standard deviation of split frequencies that reached below 0.01. Moreover, the potential scale reduction factor (PSRF) for all models and factors of combined analysis remained below 1.008. The first 25 % of saved data was discarded as ‘burn-in’; a 50 % majority-rule consensus tree and posterior probabilities (PP) were calculated from the rest.

The nucleotide substitution model for ML was set GTR + G. Branch support was provided by bootstrap analysis (1 000 pseudoreplicates), and all other parameters were set to default values. The phylogenetic trees were visualised and edited using FigTree v. 1.4.4 (Rambaut et al. 2014), and Adobe Illustrator CS3® was used for artwork. The alignment files used for the analyses is available in TreeBASE repository under reference number TB25257 (http://purl.org/phylo/treebase/phylows/study/TB2:525257).

RESULTS

European and North American populations

The one-by-one comparison of ITS sequences supported the morphology-based identification that North American specimens belong to Pseudotryblidium neesii. The variability of the ITS alignment of six sequences (470 bp), two from North American and four from European specimens, is 1.1 %, i.e. five informative nucleotide positions. These single nucleotide mutations (SNP) do not correspond to the geographical origin of the material (see file in TreeBASE repository under reference number TB25257).
In total we saw 15 collections of *P. neesii* from Idaho, Montana, Oregon and Washington, all made exclusively on *Abies grandis*. One of us (MH) inspected many *A. lasiocarpa* trees, as well as other conifers in Idaho, but did not find *P. neesii* on those substrates. The examined European material was exclusively growing on *A. alba*.

**Phylogenetic position of Pseudotryblidium**

The ITS, LSU and combined LSU + ITS based Maximum Likelihood (ML) and Bayesian trees had no topological conflicts in the supported clades (Fig. 1), all indicating the placement of *Pseudotryblidium neesii* within the family Dermateaceae, Helotiales (PP = 1; BS = 99).
However, the internal relationships between larger clades within the Dermateaceae clade remained unsupported.

In all analyses, the genus Dermea was paraphyletic. The core group of Dermea species including the type species D. cerasii formed a well-supported clade (PP = 1, BS = 82 %; Fig. 1), while Pseudotryblidium neesii together with Dermea piceina, D. acerina and Rhizoderma veluwiensis formed a distinct though unsupported clade within Dermateaceae. Dermea balsamea was distant from the core of the Dermea-clade and proved to be close to Polypilus sieberi (Hyaloscyphaceae, Helotiaceae).

Nomenclature and taxonomy

The nomenclature of Pseudotryblidium neesii is intricate and confusing. Nees (1836) introduced the name Peziza lecanorae, which is a younger homonym of Peziza lecanora J.C. Schmidt & Kunze, 1817. The two epithets are confusable (see Art. 53.2) and had been regarded as homonyms by Flotow (in Rabenhorst, Klotzschii Herb. Viv. Mycol., Cent. 15: no. 1419, 1850), who introduced the replacement name Peziza neesii Flot., which is, however, a homonym as well (non Peziza neesii Saut., 1841).

Körber (1865) intended to introduce a new combination (“Leciographa neesii (Flot.) Körb.”), based on Peziza neesii Flot., but an illegitimate name cannot be used as a basionym (Art. 6.10). Therefore, Leciographa neesii would represent a name only ascribable to Körber, either as a replacement name based on Peziza neesii Flot. with the same type as this illegitimate name or as a name of a new taxon with a different type (Art. 58.1, and Ex. 1). However, Leciographa neesii is also an illegitimate name, according to Art. 52.1 (nom. superfl.), because Körber (1865) cited Leciographa zwackhii Massal. (“Cat. Graph. 6792”), a species validated by Zwackh (1862: 571), as a synonym. Names illegitimate by being superfluous, according to Art. 52.1, are automatically typified by the types of the names that ought to have been adopted. Therefore, Körber’s (i.e.) name is formally a homotypic synonym of Phaeographa zwackhii (Massal. ex Zwackh) Hafellner (= Leciographa zwackhii Massal. ex Zwackh) and has to be regarded as a misapplied name in the context of Pseudotryblidium neesii. Arnold (1874) introduced Dactylospora neesii, intended to be a new combination based on Peziza neesii Flot., which is, however, de facto a new name only attributable to Arnold (according to Art. 58.1, and Ex. 1), either as a replacement name based on Peziza neesii Flot. with the same type as this illegitimate name or as a name of a new taxon with a different type. Arnold (i.e.) referred to several previously published descriptions and cited several examined exsiccatea, including Körber, Lich. Sel. Germ. 420, but he realized that Leciographa zwackhii and L. neesii represent two different species and excluded L. zwackhii from the synonymy of L. neesii, which he reallocated to Dactylospora. In any case, Dactylospora neesii represents the first valid name for the species concerned.

Original material of the name Peziza lecanorae Nees (1836), non Peziza lecanora J.C. Schmidt & Kunze, 1817, available for lectotypification purposes (Art. 9.3, 9.4), could not be traced and is probably not preserved, although it would be necessary if Dactylospora neesii would be considered a replacement name for Peziza neesii Flot. In order to disentangle the complicated nomenclature of Pseudotryblidium neesii and to stabilize the application of this name, we prefer to use the second option and treat D. neesii as the name of a new taxon with a new type (according to Art. 58.1). “Körber, Lich. Sel. Germ. 420” was cited in Arnold (1874) in the protologue of D. neesii and represents syntype material. A duplicate of this exsiccata deposited at herb. H is designated here as lectotype.

Peziza rufonigra Saut. (Sauter 1841) is another name that has been considered a synonym of Pseudotryblidium neesii (Keissler 1916). We re-examined three specimens in W collected by Sauter and annotated as Peziza rufonigra by him. Two of them (from Taunus) have been studied by Keissler (1916) and annotated as Pseudotryblidium neesii. The third specimen (no. 1217) has been annotated by Keissler as “P. n. versimiliter”, thus only doubtfully the same species. It is therefore clear that the two first specimens (nos. 1238 and 1276) could be used for a lectotypification of the name Peziza rufonigra, both macroscopically and microscopically very similar to each other and possibly duplicates from the same collection. The two specimens (1238 and 1276) do not resemble Pseudotryblidium neesii: the ascomata are not roundish, but elongate; the ascomatal margin is not striate; the ascomata are black and not brown; the asci are very long and narrow; the ascospores are different in shape (ratio L/B larger), more regularly septate and react I+ dark violet brown; and the typical K+ purplish reaction of the exiple of P. neesii is missing (Fig. 4). This material clearly represents the species presently called Pseudographis pinicola (Nyl.) Rehm. Because Peziza rufonigra is the oldest known name for this species, it is combined in Pseudographis below to replace P. pinicola.

Pseudotryblidium neesii (Arnold) Rehm (as “(Flot.) Rehm”), Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.3 (Lief.. 33): 370. 1890 (“1896”). Figs 2, 3.

Basionym: Dactylospora neesii Arnold (as “(Flot.) Arnold”), Flora, Regensburg 57(7): 108, 1874 [Art. 58.1 (Ex. 1)]. Type: Poland (Silesia), forest near Rybnik, amongst the thallus of Phlyctis argena, on Abies, undated, Stein & Körber, Körber, Lich. Sel. Germ. 420 (H 9218 699, lectotype of Dactylospora neesii designated here, MycoBank MBT389275); CWU!, M non vid., BG non vid., isotypelectotypes.

Synonyms: Peziza lecanorae Nees, Flora 19(1), Beibl.: 24. 1836, nom. illeg. [Art. 53.1], non Peziza lecanora J.C. Schmidt & Kunze, 1817.

Peziza neesii Flot., in Rabenhorst, Klotzschii Herb. Viv. Mycol., Cent. 15: no. 1419. 1850, nom. illeg. [Art. 53.1], non Peziza neesii Saut., 1841.

Misapplied name: Leciographa neesii Körb. (as “(Flot.) Körb.”), Parerga lichenol. (Breslau) 2: 463. 1865 [Art. 58.1 (Ex. 1)], nom. illeg. [Art. 52.1].

Ascomata dark reddish brown to almost black, erumpent, later sessile, narrowed below to substipitate, ascomatal margin roundish to somewhat undulate, often radially striate when young; ascomata hard, leathery to almost horny in consistency, solitary or more rarely in groups. Epiphymenium dark brown, course granular. Hymenium yellowish to brownish. Subhymenium of interwoven hyphae, yellowish. Excipulum and hypothecium brown to dark brown, of textura globulosa to textura prismatica type, KOH + deep red (pigment dissolving). Paraphyses hyaline, filiform, simple to bifurcate, 1.5–2(–2.5) μm diam, septate, tips slightly swollen and glued together. Asci inamyloid (no apical ring structure visible in iodine solutions), cylindric-clavate, with a short stalk, 8-spored. Ascospores (1–)2–4-celled, ellipsoid to oblong to ovoid, hyaline, yellowish to brown when overmature, biseriate. Asexual morph not observed.

Hosts: Abies alba and A. grandis.
Distribution: Austria (Maurer et al. 1983, Hafellner 2001), France (Boudier 1907, van den Boom & Breuss 2002), Germany (Nimis et al. 2018), Italy (Crivelli et al. 1981), Poland (lectotype), Slovenia (Nimis et al. 2018), Switzerland (Zimmermann 2011), USA: Idaho, Montana, Oregon and Washington (this paper).

Fig. 2. *Pseudotryblidium neesii*. A–E. American population on *Abies grandis*, with apothecia growing through the corticolous thallus of the lichen *Ochrolechia montana* (best seen in section B), simulating a lichenicolous growth (A: apothecium of *P. neesii* beside apothecium of *O. montana*) (Haldeman 2078). F, G. European population on *Abies alba* (Zimmermann M272). Scale bar (in A: same for all photos) = 200 µm.

Fig. 3. *Pseudotryblidium neesii* (Haldeman 2078). A. Section through apothecium, in water. B. *Idem*, showing excipular structure, in water. C. *Idem*, showing reaction with K (inner parts not yet reacting). D–G. Paraphyses, asci and ascospores, in K/I, using DIC optics. Scale bars: A–C = 50 µm, D–G = 10 µm.
Specimens examined (with collection numbers and GenBank accession codes in parentheses): Switzerland (all on A. alba, all in G): Kanton Bern: Rüti bei Büren, Rütiwald, Leibach, 2018, Zimmermann M271 (MK894287, MK894298), M272 (MK894296); Röthenbach, Vordere Naterswald, 2018, Zimmermann M283; Schüpfen, Bütschwil, Unterholz, 2018, Zimmermann M285, M286. Kanton Jura: Sauley Nirveux, 2018, Zimmermann M273 (MK894297); La Joux, Envers des Combles, 2018, Zimmermann M274 (MK894295), Feusi & Zimmermann M291. USA (all on Abies grandis): Idaho: Benewah Co., above St Joe City, 2017, Haldeman 2078 (TU86401, hb. Diederich; MK894285, MK894292); Clearwater Co., 12 km E of Elk River, 2017, Haldeman 2102 (TU86400, MK894284); id., hillside above Orogrande Creek, 2016, Haldeman 1479 (TU87199); id., west side of Dworshak Reservoir, 2015, Haldeman 804B (TU87200); id., 2 km NE of Deer Creek Reservoir (SW of Headquartters), 2017, Haldeman 2135 (hb. Diederich); Kootenai Co.: 2 mi N of Fernan Saddle, 2015, Haldeman 690 (TU86402; MK894286, MK894293); id., near Twin Lakes, 2015, Haldeman 647 (TU87201); Latah Co., SE of Deary, Thuya plicata forest, 2015, Haldeman 1060 (hb. Haldeman); Shoshone Co., Idaho Panhandle National Forest NE of Clarkia, 2015, Haldeman 1053 (hb. Diederich); id., Hammond Creek, off North Fork of the St Joe River, 2017, Haldeman 2649 (hb. Diederich, hb. Haldeman). Montana: Mineral Co., Two Mile Creek just W of St. Regis, 2017, Haldeman 2309 (TU87202, hb. Diederich). Oregon: Hood River Co., along Hwy 35, 0–1 km N of Pollalie Creek Bridge/Copper Spur Rd jct., 2001, Tønsberg 29121 (BG L-71948). Washington: Chelan Co., Little Chumstick Creek, 2018, Haldeman 2917 (hb. Haldeman); Ferry Co., Lynx Creek west of Inchelium, 2019, Haldeman 3312 (OSC); Whatcom Co., Baker Lake Trail, 2019, Haldeman 3347 (hb. Haldeman).

Exsiccate examined: Peziza neesii Flot. Poland ['Silesia']: Buchwald, Grünbusch, parasitic on Loxospora elatina ['parasitans in crusta Zeoria elatinae'], undated, J. von Flotow. Rabenh., Klotzschii Herb. Viv. Mycol. no. 1419 (HAL).

Pseudographis rufonigra (Saut.) Diederich & Baral, comb. nov. MycoBank MB833089. Fig. 4. Basionym: Peziza rufonigra Saut., Flora, Regensburg 24: 314. 1841. Type: Austria, ad abietes, Obersee, 31. Aug. 1841. (LINN ÖNB). [Later lectotypified by Jaklitsch et al. (2010)]

DISCUSSION

The species of Dermateaceae are often associated with gymno- and angiosperms, being endophytes fruiting on bark (Jaklitsch et al. 2016). With a few exceptions, the species of Dermateaceae are host-specific (Abeln et al. 2000, Jaklitsch et al. 2016), and the host-specificity also holds for Pseudotryblidium neesii, being known on white fir (Abies alba) in Europe (Rehm 1890, Zimmermann 2011), and on grand fir (A. grandis) in North America. Pseudotryblidium was not found on A. lasiocarpa, another common Abies species in Idaho (Patterson et al. 1985) that has frequently been inspected by one of us (MH). European records of P. neesii on other tree species rather than Abies are likely to be misidentifications, e.g. for Phacographa zwackii (Rehm 1890, Nannfeldt 1932, Hafellner 2009), a lichenicolous fungus inhabiting Phytisca argenta and having positive iodine reactions of hymenial structures (Hafellner 2009), in contrast to P. neesii having negative iodine reactions.

In a traditional sense, Dermateaceae included species with a pigmented exciple of textura angularis or textura globulosa type (Nannfeldt 1932, Nauta & Spooner 2000). Abeln et al. (2000) and Verkley (1999) suggested restricting the family to three genera, Dermea, Neofabrea and Pezicula. These three genera differ from each other mainly by ascomatal characters: the typical ascomata of Dermea are dark brown to black and hard or leathery (Groves 1946, Mehrebi et al. 2018), while they are brighter in colour, softer, fleshy or waxy in Neofabrea and Pezicula (Verkley 1999). The ascomata of Pseudotryblidium are horny, dark brown to almost black (Fig. 2), and thus quite similar to those of Dermea. Many species of Dermateaceae have a pigmented excipulum and hypothecium that turn intensively deep red to violet in KOH (Jaklitsch et al. 2016). The structure of the excipulum and hypothecium (textura globulosa to prismatic type) with a brown pigment (Fig. 3) turning deep red in KOH supports an inclusion of Pseudotryblidium in Dermateaceae.

Dermea neesii was shown by Groves (1946) who divided it into four morphological groups based on characters of the asexual morph of the fungus (shape of conidia). The paraphyly of the genus was suggested by Abeln et al. (2000) and Verkley et al. (2003), and our study supports this hypothesis. Groves (1946) showed that D. piceina and D. acerina deviate from the core group of Dermea in several aspects, especially by the oblong-ellipsoid, hyaline conidia that are straight to slightly curved and therefore more similar to those of Pezicula. The presence of an asexual morph in P. neesii has neither been described in the literature nor observed by us. Our attempts to obtain pure cultures of P. neesii from freshly collected specimens from Switzerland unfortunately failed. The monotypic Rhizoderma, isolated mainly from ericaceous hosts (Lin et al. 2010, Verkley et al. 2010), is known to produce only chlamydospore-like structures in culture.

We do not propose any new taxonomical combinations for Dermea acerina and D. piceina or for Rhizoderma. There are two reasons to postpone this taxonomic act: 1) low support to internal relationships within Dermateaceae indicating the need for including more taxa and more genes into the analysis, and 2) lack of clear morphological support (esp. asexual morph) separating Pseudotryblidium and related Dermea and Rhizoderma species from the core group of Dermea.

Additional materials examined: Dermea acerina. Ukraine, Kharkiv Oblast, local protected area Forest Park (Sokolniki-Pomery) (50.66° N, 36.24° E), on bark of a fallen trunk of Acer tataricum, May 2018, Akulov (TU104990; MK894288, MK894299); on dead twigs of A. tataricum, Oct. 2016, Akulov (TU104992). Dermea cerasi. Ukraine. Kharkiv Oblast, local protected area Lesopark (Sokolniki-Pomery) (50.66° N, 36.24° E), thin dead attached twigs of Prunus cerasus, May 2018, Akulov (TU104987; MK894291, MK894302); Ivano-Frankivsk Oblast, the vicinity of Sheshory village, by Korvyak stow (48.35° N, 24.99° E), thin dead attached branches of P. cerasus, Aug. 2017, Akulov (TU104988;
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MK894290, MK894301). *Dermea piceina* (asexual morph). **Ukraine**, Zakarpattia Oblast, the vicinity of High-altitude experimental station, the base of Pozhizhevska mountain (48.14° N, 24.52° E), on *Picea abies* bark of a thin dead attached branch, Aug. 2017, Akulov (TU104989; Fig. 4. Lectotype of *Peziza rufonigra* (Sauter, W Krypto 1917-0001238). A. Apothecia growing through the thallus of the lichen *Phlyctis argena*, simulating a lichenicolous growth. B. Section through apothecium, in water. C, D. Ascospores reacting violet brown in Lugol. E, F. Hymenium with asci, ascospores and paraphyses, in water, using DIC optics. Scale bars: A = 0.5 mm, B = 100 µm, C–F = 10 µm.
ACKNOWLEDGEMENTS

Rasmus Puusepp (Tartu) is thanked for performing lab work. We warmly thank the curators of BG, HAL, H and W, and A. Akulov (Kharkiv) for the loan of herbarium specimens, the curator of CWU for preparing and sending us macro- and microphotographs of Dactylopsora neesii, and the curator of B for searching for the original material of Peziza lecanorae. Hans-Otto Baral (Tübingen) helped us with the study of the type of Peziza rufonigra; he and Kadri Pärtel (Tartu) are thanked for helpful discussions about helotialean fungi. We are grateful to James Lendemer (New York) and Linda in Arcadia (Arkadias, Greece) for nomenclatural advice. We also thank Sara Goeking of the Rocky Mountain Research Station for a review of this document. Both reviewers are thanked for their constructive criticism that improved the original version of the manuscript. The financial support of AS was provided by IUT 20-30 and by the European Regional Development Fund (Centre of Excellence EcolChange). The financial support for the North American field work done by MH was provided by the US Forest Service’s Forest Inventory and Analysis Units from both the Rocky Mountain and the Pacific Northwest Research Stations.

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