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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 *Rhizodermea 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*.

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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 Boudier (1907), considered *Pseudotryblidium* as belonging to *Patellariaceae*, while in later classifications it was included in *Helotiales incertae sedis* (*e.g.* Nannfeldt 1932, Jaklitsch *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

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	KR859090
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 molliuscula		MH868355	MH856839
Dermea molliuscula		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 frangulae		GU973600	KR859208
Pezicula heterochroma		KR859002	KR859210
Pezicula neocinnamomea		KR859007	KR859215
Pezicula radicicola		KR859028	KR859236
Pezicula rubi		KR859039	KR859247
Phlyctema vagabunda		KR859069	KR859275
Polyphilus sieberi		MG719706	na
Polyphilus sieberi		MG719704	na
Polyphilus sieberi		MG719703	na
Pseudofabraea citricarpa		KR859075	na
Pseudotryblidium neesii	TU86401 / HE299	**MK894285**	**MK894292**
Pseudotryblidium neesii	TU86402 / HE300	**MK894286**	**MK894293**

Table 1. (Continued).

Species name	Voucher / Lab code	LSU acc. no.	ITS acc. no
Pseudotryblidium neesii	TU86400 / HE301	**MK894284**	na
Pseudotryblidium neesii	Zimmermann M274 / PS337	na	**MK894295**
Pseudotryblidium neesii	Zimmermann M271 / PS338	**MK894287**	**MK894298**
Pseudotryblidium neesii	Zimmermann M273 / PS339	na	**MK894297**
Pseudotryblidium neesii	Zimmermann M272 / PS340	na	**MK894296**
Rhizodermea veluwiensis		KR859076	HM002555

LA-W (Tedersoo *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 tsugae* (*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 *diagnfreq* 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:S25257).

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 repositiory 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).

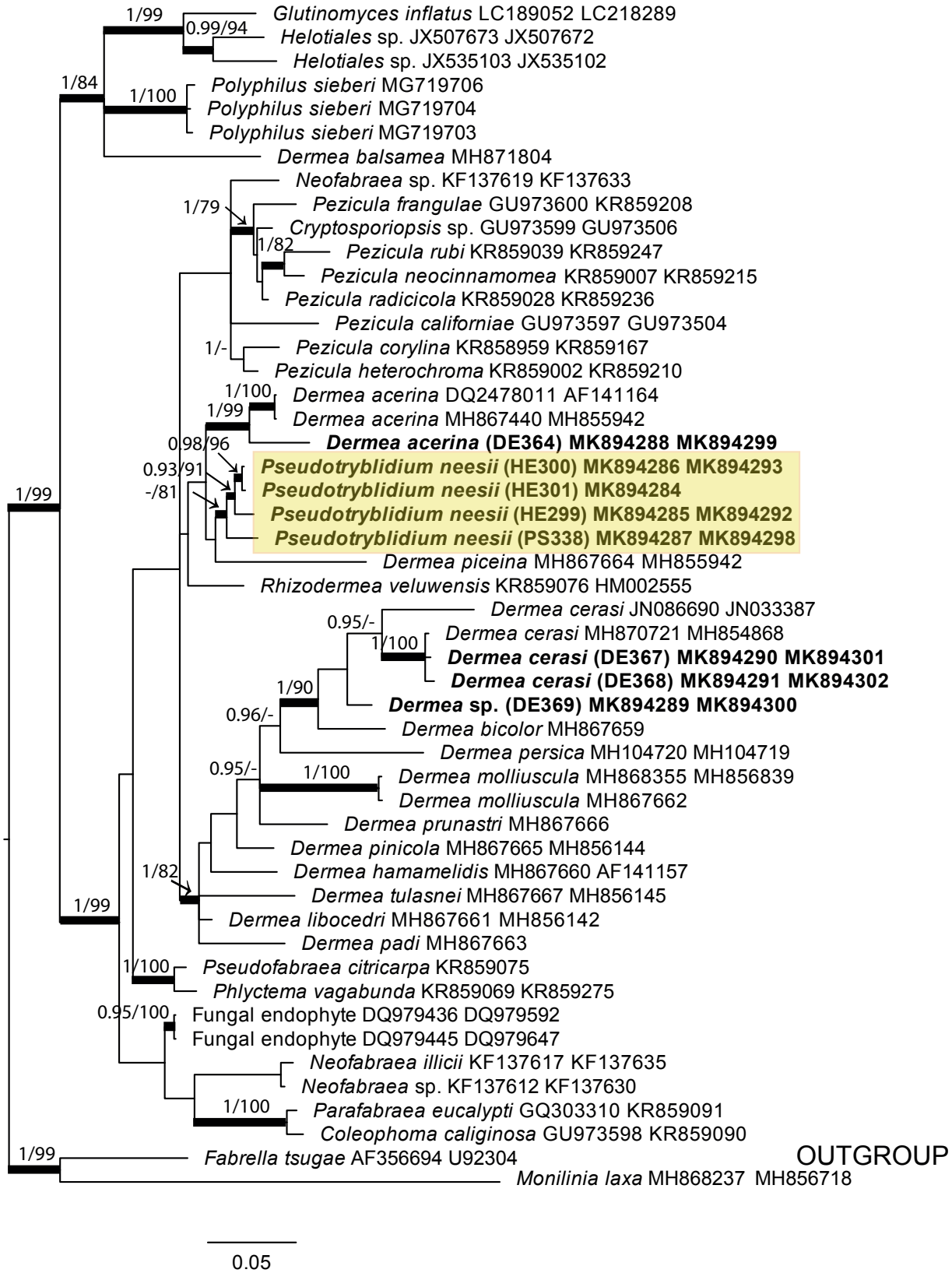


Fig. 1. The rDNA LSU + ITS based Bayesian phylogeny showing the position of *Pseudotryblidium neesii* (yellow box) within *Dermateaceae*. The branches with posterior probabilities (PP) ≥ 0.95 and bootstrap values (BS) ≥ 75 % are considered as supported. GenBank accession numbers are at the tips of the tree.

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. cerasi* formed a well-supported clade (PP = 1, BS = 82 %; Fig. 1), while *Pseudotryblidium neesii* together with *Dermea piceina*, *D. acerina* and *Rhizodermea 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 *Polyphilus sieberi* (*Hyaloscyphaceae*, *Helotiales*).

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 (*l.c.*) 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 (*l.c.*) referred to several previously published descriptions and cited several examined exsiccatae, 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 Tauern) 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 exciple 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*., isolectotypes).

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)* **5**: 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. *Epihymenium* 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 brownish when overmature, biseriate. *Asexual morph* not observed.

Hosts: *Abies alba* and *A. grandis*.

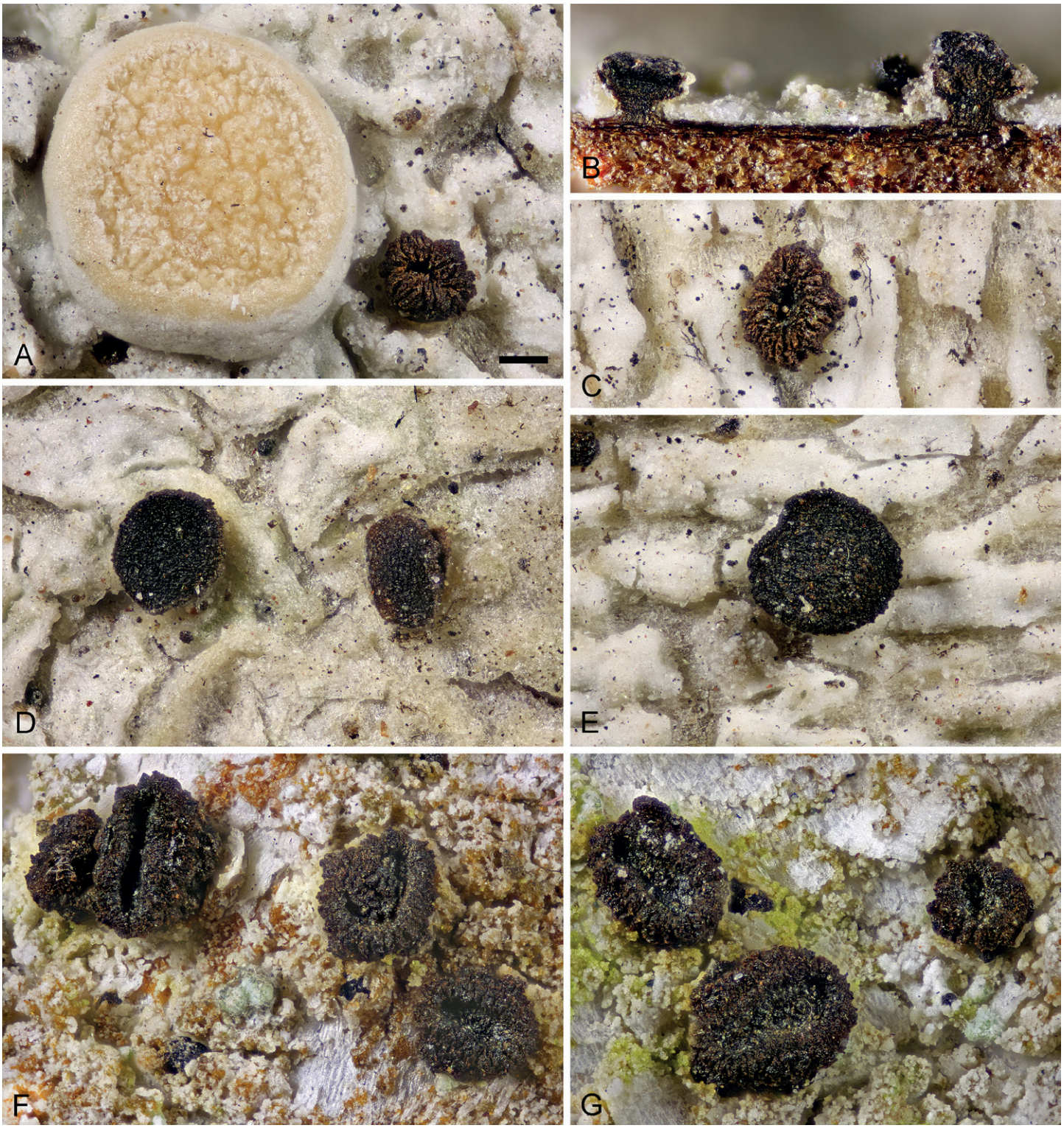
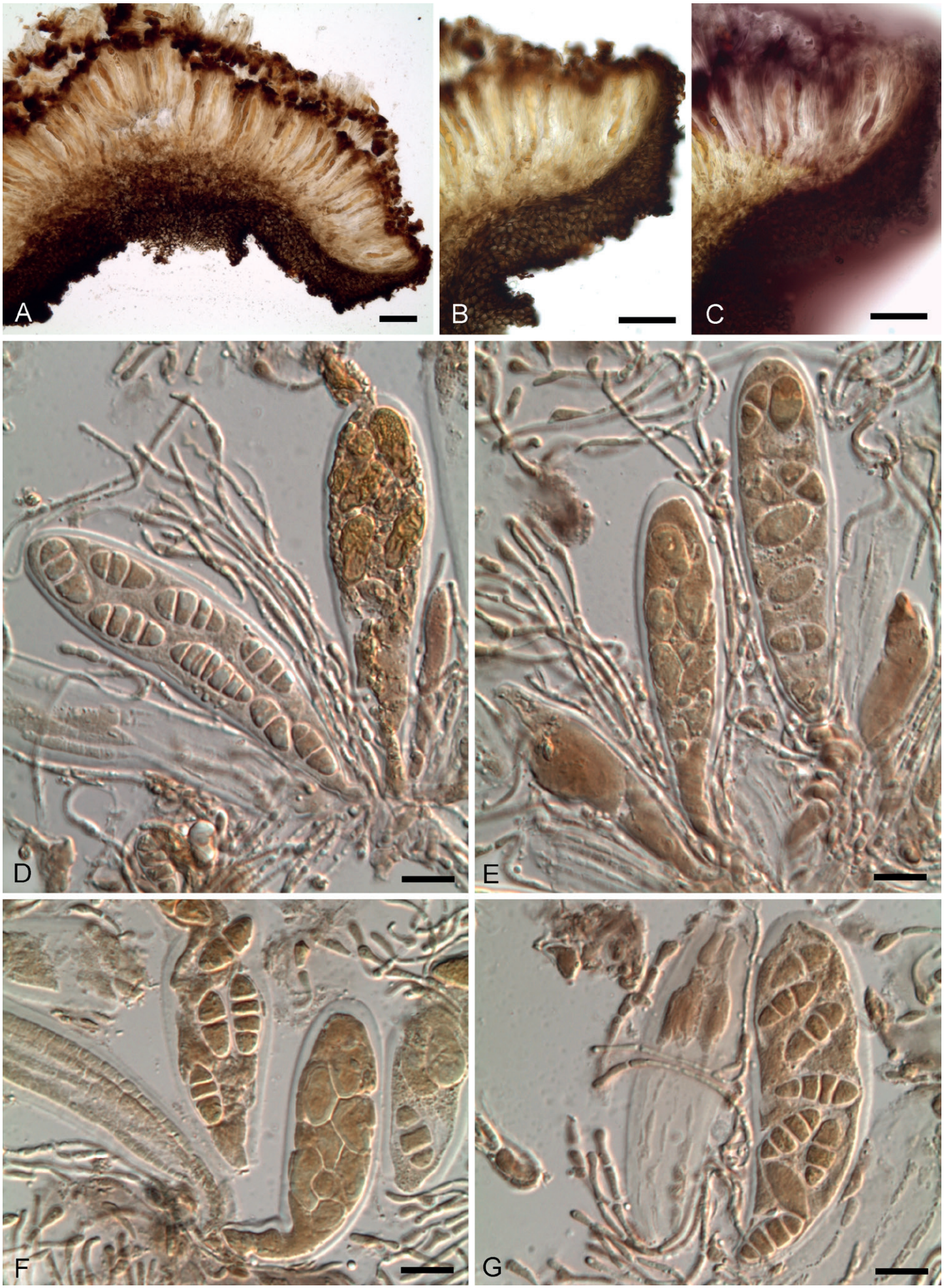


Fig. 2. *Pseudotryblidium neesii*. **A–E.** American population on *Abies grandis*, with apothecia growing through the corticolous thallus of the lichen *Ochrolechia montana* (best seen in section B), simulating a lichenicolous growth (A: apothecium of *P. neesii* beside apothecium of *O. montana*) (*Haldeman*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

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. 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, Underholz, 2018, *Zimmermann* M285, M286. Kanton Jura: Sauley Nirveux, 2018, *Zimmermann* M273 (MK894297); La Joux, Envers des Combes, 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 Headquarters), 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, *Thuja 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).

Exsiccatae 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, ober Tauern, 20 Jun. 1837, on *Abies*, *Sauter* (W Krypto 1917-0001238, **lectotype** designated here, MycoBank: MBT389398); id. (W Krypto 1917-0001276, syntype).
Synonyms: *Hysterium pinicola* Nyl., *Obs. Pez. Fenn*.: 77. 1868. *Type*: "in Lapponia orientali prope Imandram lacum, Rasnavolok, N.I. Fellman, ad corticem abietis", type not located.
Pseudographis pinicola (Nyl.) Rehm, *Rabenhorst's Kryptogamen-Flora, Pilze – Ascomyceten* **1**(3): 99. 1888.

Karakehian *et al*. (2019) included this species and the generic type *Pseudographis elatina* (Ach.) Nyl. in a phylogenetic analysis and concluded that the genus belongs to the *Rhytismataceae* within the *Rhytismatales*. High quality macroscopic and microscopic photographs are presented by these authors.

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 zwackhii* (Rehm 1890, Nannfeldt 1932, Hafellner 2009), a lichenicolous fungus inhabiting *Phlyctis argena* 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 *Neofabraea* 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 *prismatica* type) with a brown pigment (Fig. 3) turning deep red in KOH supports an inclusion of *Pseudotryblidium* in *Dermateaceae*.

The heterogeneity of the genus *Dermea* 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 *Rhizodermea*, 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 *Rhizodermea*. 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 *Rhizodermea* species from the core group of *Dermea.*

Additional materials examined: *Dermea acerina.* **Ukraine**, Kharkiv Oblast, local protected area Forest Park (Sokolniki-Pomerki) (50.06° 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-Pomerki) (50.06° 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;

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.

MN061684). *Dermea* sp. **Ukraine**, Khmelnytskyi Oblast, between Humentsi and Kolubaivtsi, on fallen deciduous tree branches, Jul. 2106, *Akulov* (TU104991; MK894289, MK894300).

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