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Melaspilea demissa (Tuck.) Zahlbr. (lichenized Ascomycota) in eastern North America with a key to North American species of *Melaspilea* s. lat.

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Abstract: Recently collected specimens of the crustose lichen *Melaspilea demissa* from south-eastern USA have been compared with those of *Melaspilea* spp. previously determined from North America. A review of both the historical and contemporary treatments of this species is provided. A lectotype was selected from the type collection of *M. demissa* in FH and is here proposed as it best matches incomplete citations in the original treatment. We also discuss the nomenclatural and taxonomic status of the name *Opegrapha cymbiformis* var. *deformis* (considered a synonym of *M. gibberulosa*). North American specimens of *M. gibberulosa* were found to be misidentifications, as were specimens attributed to *M. lentiginosula*, *M. mesophlebia* and *M. octomera*. We therefore recommend that these species be removed from the North American lichen checklist. We also present a key to North American *Melaspilea*.

Key words: lichens, *Melaspileaceae*, morphology, *Opegrapha*, taxonomy

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Introduction

The lichen genus *Melaspilea* Nyl. (Ascomycota) in its traditional sense contains species with arthonioid (round to irregular-shaped) to opegraphoid (ellipsoid to lirellate) ascomata producing 1-septate spores with thin walls and septa, often becoming brown when mature (Ryan & Nimis 2004; Sanderson *et al.* 2009). Species in this genus are either lichenized or lichenicolous, with the lichen species often barely lichenized and some possibly non-lichenized on bark, a situation comparable to *Arthopyrenia* s. lat. (Harris 1995). The genus is highly heterogeneous (Ryan & Nimis 2004; Sanderson *et al.* 2009), including several distinct morphotypes. The type species, *M. arthonioides* (Fée) Nyl., forms immersed, arthonioid ascomata with exposed discs and reduced excipula (Fink 1935; Redinger 1938; Thomson 2003). A similar morphology is found in other species, such as *M. maculosa* (Fr.) Müll. Arg. In contrast, many species have sessile, opegraphoid ascomata with well-developed excipula and often-concealed discs. Whereas the latter are easily confused with *Opegrapha* s. lat. or, more rarely, *Graphis*, the arthonioid morphotype resembles *Arthonia* s. lat., *Arthothelium* and the recently described genus *Eremithallus* (Lücking *et al.* 2008; Lumbsch *et al.* 2011). Another genus similar to the opegraphoid taxa of *Melaspilea* is *Hemigrapha* (Müll. Arg.) R. Sant. ex D. Hawksw., a small genus of chiefly lichenicolous species which shares with *Melaspilea* the opegraphoid ascomata

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and 1-septate, thin-walled, hyaline to brown spores, but differs in the thin, plate-like excipulum (Hawksworth 1975; Matzer 1996; Diederich & Wedin 2000).

The phylogenetic relationships and exact circumscription of *Melaspilea* were unclear until recently. The genus has been variously connected with *Arthoniales*, *Caliciales*, and *Ostropales* (Wirth & Hale 1963; Poelt 1969; Eriksson 1982; Ryan & Nimis 2004; Sanderson *et al.* 2009; Lumbsch & Huhndorf 2010), but also with the Dothideomycetes (Von Arx & Müller 1975). In a recent molecular phylogenetic study, Ertz & Diederich (2015) showed that *Melaspilea* s. lat. consists of several unrelated lineages, all situated within Dothideomycetes: *Melaspilea* s. str. was found closely related to or conspecific with *Eremithallus* in *Eremithallales* (which, however, differs from the type of *Melaspilea arthonioides* in the distinctly zeorine, lobulate margins), whereas other species form several genera within *Asterinales*. Apparently, many more species require molecular study to be accurately placed. Due to their usually small size and nondescript nature, these taxa are often overlooked or misidentified, which can have an impact on the accuracy of site biodiversity assessments.

During the first author's exploration of lichen diversity in parts of the south-eastern United States (North Carolina, South Carolina and Virginia), a small, thin, corticolous, crustose lichen was encountered with a shiny tan thallus and minute, scattered lirellae. Superficially this microlichen resembled *Opegrapha* Ach. (*Opegraphaceae*), but the brownish, 2-celled spores, together with the multilayered excipulum, place it in *Melaspilea* Nyl. (*Melaspileaceae*). While the determination of these specimens to *Melaspilea* s. lat. was straightforward, species identification proved to be a challenge due to the lack of a current treatment containing keys and species descriptions for this genus in North America. Recent literature includes regional lichen floras that cover only few species [three species in Wisconsin (Thomson 2003); one in the Sonoran Desert (Ryan & Nimis 2004); and one in the Ozark Highlands (Harris & Ladd 2005)]. The only seemingly comprehensive revision of the genus in North America was in Bruce Fink's posthumous work, *The Lichen Flora of the United States* (1935), which includes a key and descriptions of 12 species. However, the validity of some of the species described therein is uncertain. Therefore, the specimens were reported as *Melaspilea* sp. in the resulting checklists (Perlmutter 2008; Hodkinson *et al.* 2009; Hodkinson 2010; Perlmutter & Beeching 2011).

In an effort to identify the aforementioned material, detailed descriptions were made and compared with loaned specimens from other herbaria, as well as recent and historical species treatments in the literature. Similar analyses were made of six species from SCT's Louisiana collections. The undetermined specimens keyed to both *Melaspilea demissa* and *M. deformis* in Fink (1935). Consequently, European material named *M. deformis* was also examined.

Materials and Methods

Our material consisted of 17 collections of unidentified *Melaspilea* from North Carolina, South Carolina and Virginia (NCU), plus six specimens from Louisiana (LSU, SBBG). We also examined Louisiana collections of *M. amota* Nyl., *M. arthonioides* (Nyl.) Fée, *M. deformis* (Schaer.) Nyl., *M. maculosa* (Fr.) Müll. Arg., and *M. tribuloides* (Tuck.) Müll. Arg. (all from LSU, SBBG). Material on loan from F, MICH, MSC, WIS, and US, was also studied for many of these same species, along with *M. constrictella* (Stirton) A. L. Sm., *M. cupularis* Müll. Arg., *M. demissa* (Tuck.) Zahlbr., *M. octomera* Müll. Arg., and *M. proximella* Nyl. For *M. cinerascens* (Willey) Fink and *M. lentiginosula* (Nyl.) A. L. Sm., two additional species listed by Fink (1935), adequate material could not be located. Type material of several species traditionally assigned to *Melaspilea*, including *M. arthonioides*, *M. demissa, M. deformis* and *M. maculosa*, was loaned from FH, G and UPS and examined for comparison with the south-eastern USA material.

Material was examined under an AO Spencer binocular dissecting microscope (by GBP) or a Wild dissecting microscope (by SCT) with an emphasis on ascomatal size, as this was a character used in Fink (1935) for species determination. Spot tests (K, C, KC and P) as well as UV tests were conducted on thalli. Ascomatal sections were prepared in water mounts and examined under light microscopy using a Zeiss Standard 14 compound microscope (by GBP) or a Leitz Wetzlar compound microscope (by SCT). Tissue characters (excipular carbonization, hymenial height and colour, thickness of paraphyses, and ascus abundance) and

spore characters (colour, number per ascus, width and length measurements) were recorded. K and KI tests were performed on section mounts based on notes in Ryan & Nimis (2004). We paid particular attention to the presence of heaped ascomata, a feature that characterized *M. constrictella* and *M. lentiginosula*, but is absent from other North American species. The ascomata are not only clustered but growing over one another in groups of ~8–12.

Chemistry was tested by RL using thin-layer chromatography (Orange *et al.* 2010) on three specimens of the material in question. Due to the endoperidermal nature of these taxa, we ran comparative TLC on material scraped directly off the bark surface, containing ascomata and endoperidermal thallus, and bark tissue from underneath the uppermost periderm layers. TLC was performed in solvent C.

Descriptions of the ascomatal and spore features of each collection of the unknown *Melaspilea* species were made and compared with those derived from loaned material of known *Melaspilea* species. Comparisons were also made with published species descriptions in the literature. A distribution map of all specimens determined as *M. demissa* was created with the aid of Google maps. In addition, a key to the examined *Melaspilea* s. lat. species in North America was generated.

Results and Discussion

A total of 31 specimens of the undetermined south-eastern species was examined (17 from NCU, five from LSU, and nine from MICH). All match Fink's description of the genus *Melaspilea*, which agrees with later treatments (Thomson 2003; Ryan & Nimis, 2004; Sanderson *et al.* 2009). More specifically, the undetermined specimens all share the following characteristics: a pale tan matt or shiny, thin to endoperidermal thallus, in some specimens bearing dark contact lines; minute lirellae with closed to open, blackish brown discs; brownish hymenial tissues that react KI+ pale bluish; and abundant, clavate, 8-spored asci bearing 2-celled, hyaline to brownish spores. In Fink (1935), the unknown species keyed to either *M. demissa* or *M. deformis*. The Fink key relied strongly on ascomatal size and shape; however, we learned that these characters are somewhat variable.

Comparisons with other North American *Melaspilea*

Several species we examined have ascomata that are arthonioid (round to irregular). *Melaspilea proximella* [now referred to the reinstated genus *Melaspileella* (Ertz & Diederich 2015)] has minute discs [0·1–0·2(–0·3) mm diam.], and occurs in the British Isles (Sanderson *et al.* 2009) but is quite rare in USA collections [five of *M. proximella* were recorded from 64 institutions as posted on CNALH; US specimens examined by GBP were from Vermont and New Hampshire (one each)]. Four other North American species examined with arthonioid ascomata are *M. amota*, *M. arthonioides*, *M. constrictella* and *M. maculosa*. *Melaspilea arthonioides* and *M. maculosa* are relatively common species with large ascomata: 0·8–1·5 mm diam. in *M. arthonioides* and 1·0–1·5 mm diam. in *M. maculosa*. The type specimen of *M. maculosa*, loaned from UPS, was examined by ERP and found to contain ascomata that were heavily carbonized with indistinguishable internal tissues. The spores of determined *M. maculosa* specimens are comparable (18–24 × 8–11 μm) to those of the undetermined material. *Melaspilea maculosa* appears to be a Gulf Coast species, frequent in southern Louisiana (Müller 1895; Fink 1935; Tucker 2010), particularly on bark of *Fraxinus*; *M. arthonioides* appears to be more widespread (Fink 1935; Thomson 2003; Harris & Ladd 2005). According to published concepts, *M. amota* has smaller ascomata (0·2–0·8 mm diam.) than *M. arthonioides* and *M. maculosa*; however, the type material of *M. arthonioides* in G has very small ascomata, up to 0·5 mm diam., and hence the taxonomy and correct nomenclature of these species needs to be further studied. We were unable to locate satisfactory material of *M. constrictella*; several collections were examined, although some were fragments and others appeared to be other species. Fink (1935) described the ascomata of *M. constrictella* as elongate, but Redinger (1938) described them as round, elliptical or 2–3-lobed.

We also examined species that had opegraphoid (ovoid to lirellate) ascomata. According to Fink (1935), *M. demissa* grows on bark and has the smallest lirellae (0·2–0·4 × 0·1–0·2 mm) while *M. cinerascens* has larger lirellae (0·4–0·8 × 0·10–0·25 mm) and grows on old wood. However, no

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satisfactory material of *M. cinerascens* could be found, as the only collection we examined, from Louisiana (*Langlois* s. n., MICH 113010), keyed to *M. constrictella.* The descriptions for *M. cinerascens* and *M. constrictella* in Fink (1935) are nearly identical, except that the ascomata in *M. constrictella* are sometimes clustered in heaps. *Melaspilea tribuloides* [now referred to the genus *Melanographa* (Ertz & Diederich 2015)] is distinct among the lirellate *Melaspilea* in being lichenicolous and commonly found on the brilliant red crustose thalli of *Pyrenula cruenta*, as well as other *Pyrenula* species and *Trypethelium*. Two very rare species, *M. cupularis* and *M. epigena*, are also lichenicolous (on *Pyrenula* spp. and *Reimnitzia santensis*, respectively).

We are disregarding *Melaspilea octomera* Müll. Arg., mentioned by Fink (1935); it was described as having 5–7-septate spores and examination of the type material revealed it belonging to *Opegrapha astraea*; also, several examined specimens named *M. octomera* were determined by SCT and GBP as *O. astraea* Tuck. Similarly, *M. mesophlebia* Müll. Arg. (Esslinger 2011) should be transferred to *Opegrapha* or related taxa. Further discussion regarding *O. astraea* will be presented in a forthcoming report.

A key to the above species represent ing *Melaspilea* s. lat. is presented below. Detailed descriptions and further discussion of these *Melaspilea* taxa will be presented in forthcoming reports.

Nomenclatural and taxonomic status of *Melaspilea deformis*

We borrowed three putative specimens of *Melaspilea deformis* for study: two from MICH (collected in New Hampshire) and one from MSC (collected in Michigan). All keyed to *M. demissa* using Fink (1935), and matched characters with the undetermined material; however, one was later determined by SCT to be *M. constrictella*. For further comparison we examined what we presumed to be type material of *M. deformis* loaned from ASU and G (by GBP), and at G (by PC). These specimens are from the *L. E. Schaerer Exsiccati*, No. 283, collected in Bern, Switzerland (as *Opegrapha cymbiformis* var. *deformis*). A review of the literature revealed *M. deformis* to be synonymized with *M. gibberulosa* (Ach.) Zwackh [now referred to the genus *Hazslinszkya* (Ertz & Diederich 2015)], first by Zwackh (1862), who cited one of Schaerer's *Exsiccati* no. 283 in his treatment. According to Redinger (1938), the earliest reference to synonyms of *M. gibberulosa*, established as *Arthonia gibberulosa* Ach. (Acharius 1810), is *M. deformis* (Schaer.) Nyl., originally described as *Opegrapha cymbiformis* var. *deformis* Schaer. (Schaerer 1836). Ertz & Diederich (2015) interpreted the name *Opegrapha cymbiformis* var. *deformis* as illegitimate, since Schaerer (1836) listed *Arthonia gibberulosa* as a synonym, and hence the name would automatically be typified by the type of the latter. However, ICN Art. 52 only applies to names that should have been adopted at the same rank and hence *Opegrapha cymbiformis* var. *deformis* cannot be automatically typified by the type of *Arthonia gibberulosa*. Instead, one of the synonyms listed at the same rank of variety, viz. *Opegrapha verrucarioides* var. *marmorata* Ach. and *O. verrucarioides* var. *megalyna* Ach., would automatically typify *O. cymbiformis* var. *deformis*. Fortunately, at least one of them also represent *M. gibberulosa*, as judged from digital images of the types and their annotations in JSTOR: the holotype of *O. verrucarioides* var. *marmorata* features the annotation *Melanoth. deformis* (apparently by Nylander), which refers to an overlooked combination proposed by Nylander (1856) before recombining the species again as *Melaspilea deformis* (Nylander 1858). In the latter, Nylander (1858) also lists *O. verrucarioides* var. *marmorata* as synonym of *M. deformis*. We therefore agree with Ertz & Diederich (2015) that the name *O. cymbiformis* var. *deformis* is illegitimate and synonymous with *Melaspilea* (*Hazslinszkya*) *gibberulosa*, but that it should be automatically typified with the type of *O. verrucarioides* var. *marmorata*.

Melaspilea (*Hazslinszkya*) *gibberulosa* is a widely distributed European species (e.g. Redinger 1938; Nimis 1993; Llimona & Hladun 2001; Bielczyk *et al.* 2004; Santesson *et al.* 2004; Wirth *et al.* 2013) that is also reported from the USA (Thomson 2003). However, the material referred to in Thomson (2003) was determined by SCT to be *M. constrictella*. *Melaspilea gibberulosa* is distinct from the undetermined material by having ascomata that are arthonioid with flat, sunken, black discs bordered by thin, black rims, and smaller spores (Redinger 1938; Thomson 2003). An additional specimen from the Little Carpathians of present-day Slovakia (*Zahlbruckner* s. n., s. d., US) was also examined.

Specimens examined from Schaerer's *Exsiccati* no. 283 (Fig. 1) revealed spore heterogeneity among specimens, containing 2-celled spores and 4–6-celled transversely septate spores, suggesting the exsiccati to be a mixture of *Melaspilea* and *Opegrapha* material. However, the specimens that do bear the 2-celled trait also match other morphological and anatomical characters described by Redinger (1938) for *M. gibberulosa*, and Lindsay (1872) for *M. deformis*, including: thin, pale thallus; short, round-elongate ($0{\cdot}2–1{\cdot}2 \times 0{\cdot}2–0{\cdot}4$ mm) ascomata with open, blackish brown discs; a lateral, carbonized exciple; and brownish ascomatal tissues as well as the brownish spore colour. Two additional specimens from the exsiccate in G were examined by GBP and found to have the following distinctions from the undetermined material: broadly irregular ascomata ($0{\cdot}5–1{\cdot}0 \times 0{\cdot}2–0{\cdot}5$ mm) that are immersed to erumpent, compared to prominent to sessile lirellae; distinct paraphyses compared to sometimes indistinct ones in a hymenial gel; smaller asci ($34–53 \times 10–14$ μm compared to $42–85 \times 10–25$ μm); and smaller, 2-celled, hyaline spores ($12–17 \times 5–7$ μm, compared to $14–22 \times 6–10$ μm). Based on these differences, we conclude that the undetermined species is not *M. gibberulosa*. Fink's description of *M. deformis* as lirellate appears inconsistent with other treatments of this species, namely Redinger (1938) and Thomson (2003), as well as our own observations.

Original description of *Melaspilea demissa*

Melaspilea demissa (as *Opegrapha demissa*) was originally described by Edward Tuckerman (1872) in a discussion of North American *Opegrapha* species:

> "*O. demissa*—a description of which is for the present reserved, is marked by larger, rather sunken fruit, scattered over an indistinct pale spot on the bark of Holly, Witch Hazel, and Poison Dogwood in southern Massachusetts (Mr. Willey) and yet larger spores… Thalline features of *O. demissa* as yet very obscure; but the plant is not parasitic. Ascomata commonly 1 mm in length, scattered and simple, white within. Spores in eights (in clavate thekes) bilocular, and constricted at the middle, brown, 0,016–23 mm. long, 0,006–9 mm. wide. Paraphyses not always indistinct. With iodine, in some specimens, only the tips of gravid thekes shew a slight bluish tinge; but in others, the blue reaction is more marked."

We interpret "Mr. Willey" as Henry Willey, a student of Tuckerman's who contributed substantially to the understanding of the north-eastern US lichen biota, in particular that of the *Arthoniales* and of Massachusetts. In his posthumous work, *A Synopsis of the North American Lichens* (Tuckerman 1888, edited by Willey), Tuckerman provides a more correct treatment of *M. demissa*. This treatment in full is as follows:

> '7. *O. demissa*, Tuckerm.; "thallus thin, whitish or obsolete; ascomata minute, elliptical, simple, white within, the disk at length somewhat dilated. Spores 8 in clavate thekes, 2-locular, finally brown, constricted in the middle, 16–23 by 6–9 mic. Paraphyses now distinct." On *Rhus venenata* and other barks. New Bedford, Mass., *Willey*. [Maryland and Virginia, *Dr. Eckfeldt.*] *Genera, p. 199.*'

These descriptions appeared to match the south-eastern material well, including the I+ reaction which was also found in all specimens tested. To further explore the potential determination of the south-eastern specimens as *M. demissa*, we examined specimens of *M. demissa* from FH, MICH and US, including type material collected by Willey in New Bedford, Massachusetts. Willey made excellent notes with measurements and drawings of spores, including one drawing of an ascomatal section. Two sets of specimens are considered syntypes: one from FH and labelled "TYPE" includes

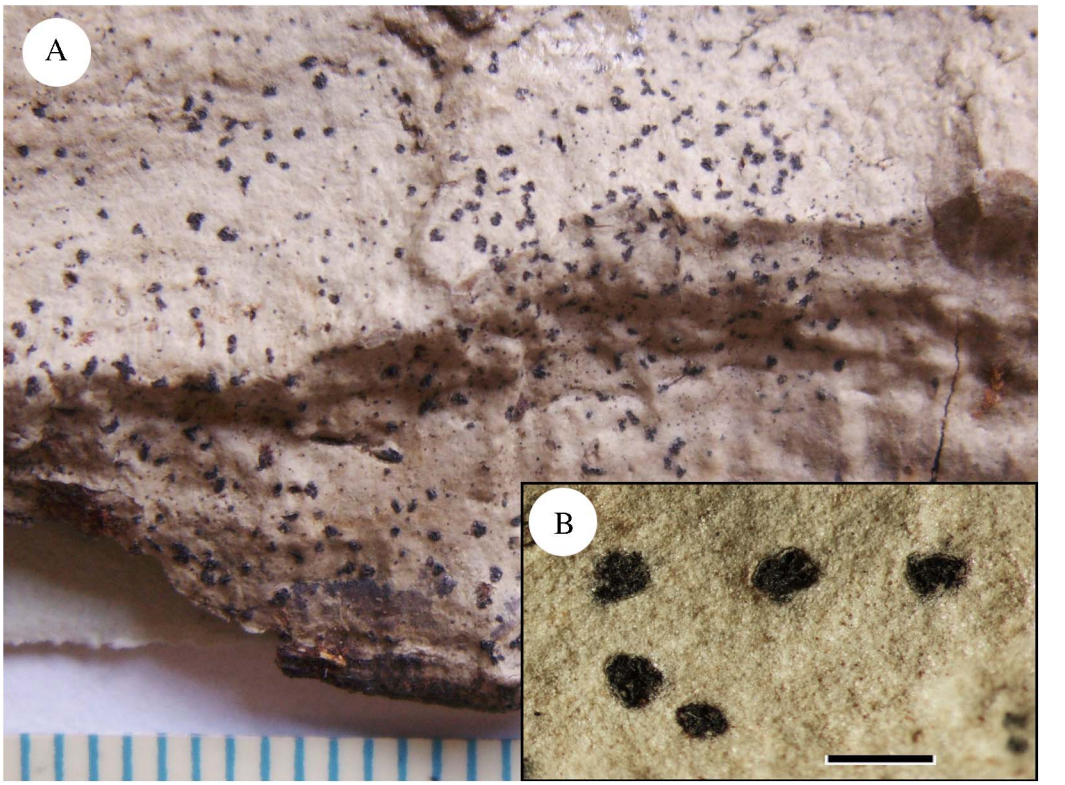

FIG. 1. *Melaspilea gibberulosa* specimen (*Schaerer Exsiccati* 283, G barcode G00294512), the original material of *Opegrapha cymbiformis* var. *deformis* which is, however, automatically typified by the type of *O. verrucarioides* var. *marmorata*. A, habit. scale increments = 1 mm; B, detail with apothecia, scale bar = 1 mm. In colour online.

three specimens; another set of three from US is here considered isolectotypes. From the type material, we selected *Willey* s. n., 1870 [FH] as the lectotype because it contains the most informative notes and drawings (Fig. 2) and it corresponds to the phorophyte (*Rhus venenata*) first mentioned in Tuckerman's citation. The historical specimens matched the south-eastern material as well as Tuckerman's description, both morphologically (thin thallus with scattered, short lirellae) and anatomically [drawings depicted an ascoma with lateral exciple, brownish tissues, an endophloedal thallus and 2-celled slipper-shaped spores that were hyaline to brownish and measured (16–)18–23(–25) × 7–10(–13) μm] (Fig. 2). Additional specimens of *M. demissa* from Pennsylvania and Tennessee (US) were also examined and found to match both the type and south-eastern material.

To further compare the south-eastern USA material with the historical material, a multivariate analysis was conducted using PC-ORD (McCune & Mefford 2011). A matrix of 59 specimens and 11 characters as well as ecoregion was created from material examined at NCU and then underwent a cluster analysis to explore the data for groupings (see Appendix). Cluster analysis involved the Sorensen (Bray Curtis) distance measure and the Flexible Beta group linkage method with beta set to −0·25, as recommended in McCune & Grace (2002). Clustering resulted in the south-eastern material grouping with the *M. demissa* type specimens and others identified as this species in the US collection, further

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FIG. 2. *Melaspilea demissa*, lectotype (*Willey* s. n., 1870, FH). A, thallus, raised areas are lenticels of the phorophyte; B, spores in ascus; C, specimen proposed as lectotype with notes and drawings (*Willey* s. n., 1870, FH barcode 00377321). Scales: A=5 mm; B=20 μm. In colour online.

supporting the south-eastern material being *M. demissa* (Fig. 3).

As mentioned before, many species of *Melaspilea* superficially resemble *Opegrapha* as both taxa bear thin thalli and small, usually prominent to sessile, lirellate ascomata with well-developed excipula. This is evident as several *Melaspilea* species were originally placed in *Opegrapha*. During our comparisons we discovered that specimens

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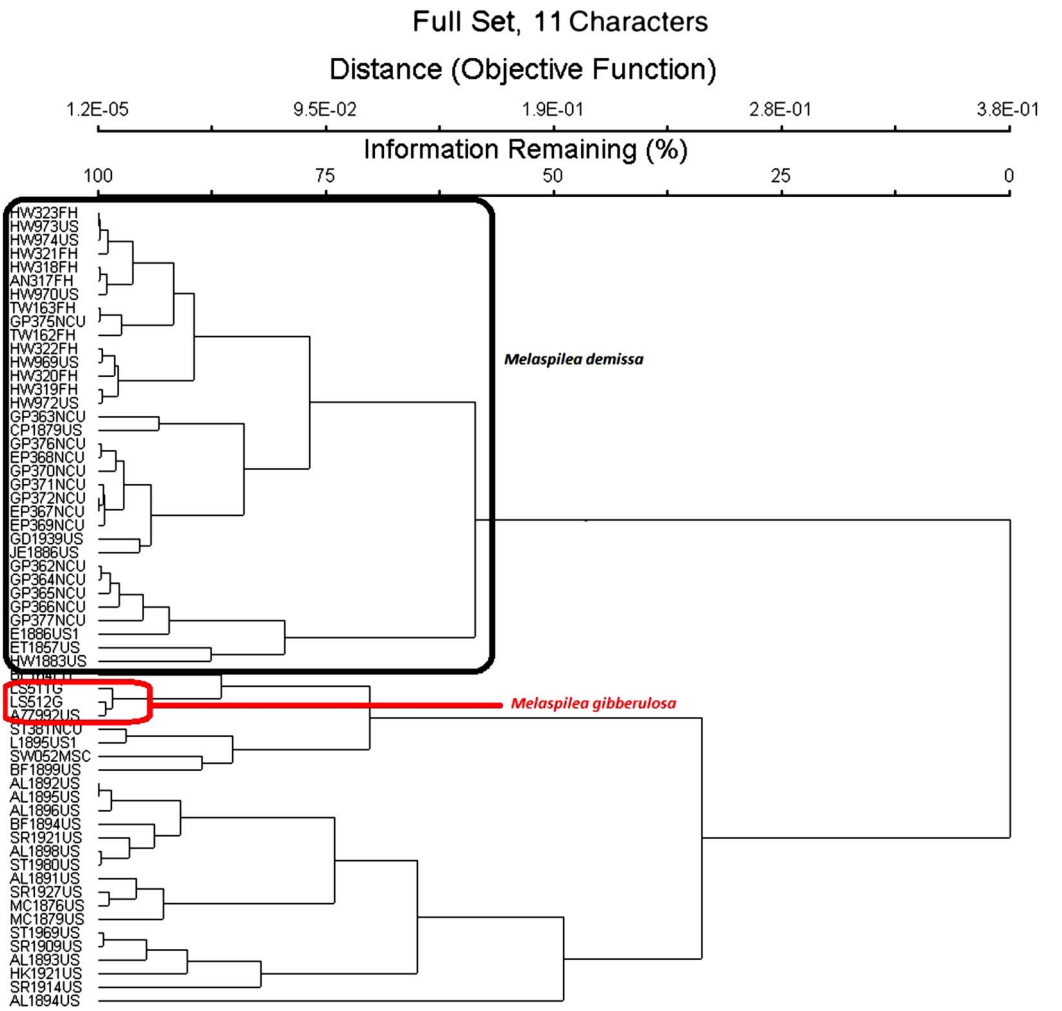

FIG. 3. Cluster diagram of *Melaspilea* specimens examined. See Table in Appendix and text for specimen codes.

of *M. octomera*, a species mostly collected by A. B. Langlois in Louisiana, were actually *Opegrapha astraea* Tuck., a more widespread species that is characterized by a black prothallus and white pruinose lirellae with black excipular margins and multi-septate spores (Tuckerman 1888; Fink 1935). We therefore propose that *M. octomera* be synonymized with *O. astraea*. Further discussion will be presented in a future paper.

A comparison of descriptions of *Melaspilea* and *Opegrapha* in Redinger (1938) and Ryan & Nimis (2004) show some overlap in thallus and ascomatal characters, with a wider variation in *Opegrapha*. Redinger (1938) separated the two genera on the basis of spore and paraphysal characters. He reports *Melaspilea* to have ellipsoid and 1-septate spores and simple paraphyses, whereas in *Opegrapha* spores are multi-septate, fusiform to needle-shaped, and paraphyses are branched. Paraphyses are usually difficult to see in *Melaspilea* species, but spore traits of shape, septation and wall thickness (thin in *Melaspilea*, somewhat thickened in *Opegrapha*) are more reliable and provide an easier means of

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separating the two genera. *Opegrapha* has been shown to be polyphyletic (Ertz *et al*. 2009; Ertz & Tehler 2011), and *Melaspilea* is well known to be heterogenous (Ryan & Nimis 2004; Sanderson *et al*. 2009). Further study of *Melaspilea* specimens could better resolve the taxonomy of this intriguing genus.

We conclude that the recently collected *Melaspilea* specimens from south-eastern North America bearing a pale tan, matt or shiny thallus and short lirellae are *M. demissa*, and our observations agree with a specimen collected by Gunnar Degelius in the Great Smoky Mountains National Park of eastern Tennessee (*Degelius* s. n., 18 ix 1939, US). The range of this species, from comparison with other material, seems to be widespread in eastern North America.

Here we present a revised treatment of *Melaspilea demissa*, followed by a revised key to North American *Melaspilea* s. lat. from material examined in this study. It is possible that specimens currently identified as *Melaspilea* sp. can now be determined to species with greater confidence using our treatment and key below. Detailed descriptions of the species examined will be presented in a forthcoming paper.

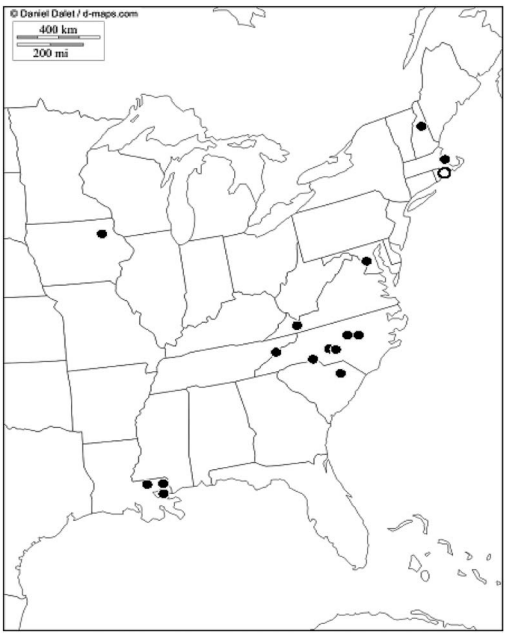

FIG. 4. Distribution map for *Melaspilea demissa* based on specimens examined. Open circle represents type locality of New Bedford, Massachussetts.

The Species

Melaspilea demissa (Tuck.) Zahlbr.

MycoBank No.: MB395311

Opegrapha demissa Tuck, *Gen. Lich.*: 199 (1872); type: USA, Massachusetts, Bristol Co., New Bedford, 1870, *Willey* 488 (FH!—selected here as lectotype).

(Fig. 2)

Life form. Crustose, lichenized fungus.

Thallus thin to endoperidermal, as pale to tan, or ± shiny stain, continuous, sometimes with a dark prothallus and/or contact lines. *Photobiont Trentepohlia*, endoperidermal; clusters of associated chlorococcoid alga often observed lying on the thallus surface.

Ascomata opegraphoid, scattered to clustered, elliptical to elongate, mostly short black lirellae, 0·2–1·3 × 0·2–0·3 mm, prominent to sessile, straight to curved, usually unbranched but some may have short branches; *disc* closed to open, opening in water, blackish brown, epruinose. *Exciple* carbonized, lateral and extending into the substratum, thickened basally, lined apically and proximally with melanized cells in some specimens. *Epihymenium* brown to dark brown or absent; *hymenium* hyaline to brownish, 58–68(−100) μm, gel-like, the gel and brownish coloration dissolving in K, KI+ pale blue; *hypothecium* pale tan to orangish brown; *paraphyses* unbranched, thickened with darkened, swollen tips. *Asci* clavate, abundant, 8-spored. *Spores* 2-celled, septum slightly constricted or not, one cell often larger, hyaline to light brownish when mature, wall smooth or warty, (10−)14–22(−26)×(−5)6−10(−13) μm.

Chemistry. Spot tests: thallus K–, C–, PD–, UV+ yellowish. TLC: comparative TLC showed no substances for *Perlmutter* 1284 (including the deeper bark layers) and unknown terpenoids for *Perlmutter* 2073; in

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the case of the latter, two distinct and one faint spot were found in the uppermost layers containing the lichen thallus and two distinct spots in the deeper bark layer, suggesting that these spots represent bark substances. If occurring in the uppermost bark layers only (upper of the three spots), it cannot be said with certainty whether the substance originates from the lichen or the bark, but the complete absence of substances in 1284 suggests that these lichens do not contain secondary substances.

Distribution. Appears to be widespread in eastern USA, found in DC, IA, LA, MA, MD, NH, NC, SC, PA, TN, VA, WI (Fig. 4).

Ecology. Corticolous on smooth bark of understorey stems and branches in shaded forests.

Key to North American *Melaspilea* species

This key was developed from a comparison of specimens attributed to *Melaspilea* s. lat. in several North American herbaria. The nomenclature of several species has been adjusted according to the recent study by Ertz & Diederich (2015). The following taxa were found to be misidentifications and are excluded from this key: *M. gibberulosa, M. lentiginosula, M. mesophlebia* and *M. octomera. Melaspilea interjecta* was recently reported from a collection in Oregon (Villella *et al.* 2013) and is herein included. Detailed descriptions of taxa in this key other than *M. demissa* will be presented in a subsequent publication.

1 Lichenicolous; hypothecium colourless 2
Not lichenicolous; hypothecium colourless or brown 4

2(1) Apothecia arthonioid (round to irregular; erumpent), 0·2–0·4 mm diam.; on *Pyrenula* . . . . . **M. cupularis** Müll. Arg.
Apothecia opegraphoid (lirellate; sessile); on *Pyrenula* or other crustose lichens 3

3(2) Apothecia 0·3–0·9 × 0·1–0·2 mm; disc closed, with lirella tips blunt or short-acute; spores 15–21 × 6–9 μm; on *Pyrenula, Thelotrema* or *Trypethelium* . . . . . **Melanographa tribuloides** (Tuck.) Müll. Arg. (*Melaspilea tribuloides* (Tuck.) Müll. Arg., *Opegrapha tribuloides* Tuck.)
Apothecia to 1·0 × 0·2 mm; disc open, with lirella ends pointed; spores 10·0–12·6 × 5·5–7·0 μm; on *Reimnitzia santensis* (syn. *Leptrotrema santense*) . . . . . **M. epigena** Müll. Arg.

4(1) Saxicolous, western USA. . . . . **M. interjecta** (Leight.) A. L. Sm.
Corticolous, central and eastern USA 5

5(4) Apothecia arthonioid (round to irregular, erumpent to sessile) 6
Apothecia opegraphoid (lirellate, sessile) 9

6(5) Apothecia large, (0·4–)0·8–1·0 mm diam. 7
Apothecia small, up to 0·4(–0·8) mm diam 8

7(6) Throughout eastern USA; apothecia not erumpent, 0·4–1·2 mm diam.; spores 12–17 × 6·0–8·5 μm. . . . . **M. arthonioides** (Fée) Nyl. (*Lecidea arthonioides* Fée)
In southern USA only; apothecia erumpent, 0·9–1·9 mm diam.; spores 18–24 × 8–11 μm. . . . . **M. maculosa** (Fr.) Müll. Arg. (*Glyphis maculosa* Fr., *Melaspilea angulosa* Nyl.)

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8(6) Apothecia 0·2–0·8 mm diam., erumpent; hypothecium brown; spores 14–18 × 7·5–12·5 μm . . . . . **M. amota** Nyl.
Apothecia 0·1–0·2(–0·3) mm diam., sessile; hypothecium colourless or brown; spores 18–22 × 7–9 μm. . . . . **Melaspileella proximella** (Nyl.) Ertz & Diederich (*Arthonia proximella* Nyl., *Melaspilea proximella* (Nyl.) Nyl. ex Norrlin)

9(5) Apothecia sometimes heaped; hypothecium colourless; spores 12–17 × 4·5–6·5 μm . . . . . **M. constrictella** (Stirt.) A. L. Sm. (*Opegrapha constrictella* Stirt.)
Apothecia not heaped; hypothecium brown or colourless; spores 12–24 × 6·0–12·5 μm 10

10(9) Exciple thin . . . . . **M. cinerascens** (Willey) Fink (*Opegrapha cinerascens* Willey)
Exciple thick, at least basally . . . . . **M. demissa** (Tuck.) Zahlbr. (*Opegrapha demissa* Tuck.)

Conclusions

This study revealed that the south-eastern USA material collected in recent surveys is *Melaspilea demissa*. We have reviewed the type material, from which a lectotype was selected and is presented here. From a wider study of additional species in comparison with the recent south-eastern collections, we have amassed sufficient morphological and anatomical information to develop a key to North American *Melaspilea* species, primarily sorted by habit (lichenized or lichenicolous), substratum (saxicolous or corticolous), ascomatal features (mainly shape as either arthonioid or opegraphoid, as well as size), spore dimensions, and geography. We present this key to aid the identification of *Melaspilea* specimens in current and future North American lichen surveys.

However, it should be re-emphasized that *Melaspilea* is a heterogenous taxon in strong need of revision. The ascomatal morphology of the arthonioid taxa and opegraphoid taxa appears so distinct as to suggest separate or multiple lineages, even within these morphological groups. To best resolve the accuracy of *Melaspilea* taxonomy, we recommend molecular study of this problematic taxon. Until then, we hope that the arthonioid and opegraphoid crustose lichens studied here can be appropriately identified and included in biodiversity assessments.

Specimens Examined

Specimen information is presented here in abridged form. Full specimen records are available online on websites of the Smithsonian Institution's National Herbarium (US) (http://botany.si.edu/), Uppsala's Natural History Museum (UPS) (http://130.238.83.220:81/home.php), Conservatoire et Jardin botaniques de la Ville de Genève (G) (http://www.ville-ge.ch/cjb/), and on the Consortium of North American Lichen Herbaria web portal (for all other listed holdings) (http://lichenportal.org/).

Melaspilea amota Nyl. **USA:** *Florida*: Lake Co., N of Eustis, *Rapp* 716 (MICH); Alachua Co., Gainesville, *Rapp* s. n. (FLAS). *Louisiana*: Natchitoches Parish, Bayou Millieu, *Langlois* s. n. (US); Point Coupee Parish, Chenal, *Langlois* 775 (US); St. Martinville/Lafayette Parish border, Bayou Tortue, *Langlois* s. n. (LSU, MICH, NY, US); Iberville Parish, *Tucker* 13394 (LSU, SBBG).

Melaspilea arthonioides (Fee) Nyl. Locality unknown, *Anonymous* s n., s. d. (G) (as *Lecidea arthonioides*, type). **USA:** *Florida*: Lake Co., north of Eustis on tree trunk, 12 vii 1928, *Rapp* s. n. (MICH); Monroe Co., Pumpkin Key, iv 1921, *Kelly* s. n. (US); Seminole Co., Sanford, *Rapp*; *Lichenes Exs*. 238 (FLAS, NEB, VT, US), ix 1921, *Rapp* s. n. (US). *Iowa*: Fayette Co., Fayette, vii 1894, *Fink* s. n. (US). *Louisiana*: East Baton Rouge Parish, Baton Rouge, *Tucker* 7297 (US), *Tucker* 21224 (SBBG); Iberville Parish, *Tucker* 13345 (LSU, SBBG). *Massachusetts*: Hampshire County, Hadley, 17 vii 1899, *Tuckerman* s. n. (US). *Minnesota*: Yellow Medicine Co., Granite Falls, *Fink* 32A (MICH). *Missouri*: Franklin Co., Meramec State Park, *Buck* 49642 (NY); Taney Co., Mark Twain National Forest, *Harris* 47747 (NY);

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Washington Co., Pea Ridge Conservation Area, *Harris* 47910-A (NY).

Melaspilea cinerascens (Willey) Fink. **USA:** *Louisiana*: *Langlois* s. n., s. d. (MICH).

Melaspilea constrictella (Stirt.) A. L. Sm. **USA:** *Louisiana*: *Langlois* 1766 (US). *Michigan*: Alpena Co., 8 miles SW of Alpena on *Populus*, 1974, *Wang* 964 (MSC). *Wisconsin*: Sawyer Co., Flambeau River State Forest on *Pinus strobus*, *Hale* 2081 (WIS).

Melaspilea cupularis Müll. Arg. **USA:** *Louisiana*: St. Martinville Parish, St. Martinville, *Langlois* s. n. (MICH).

Melaspilea demissa (Tuck.) Zahlbr. **USA:** *District of Columbia*: Takoma Park on *Rhus*, *Williams*, Decades of American Lichens 284 (DUKE, FH, ISC, MICH, US). *Iowa*: Clayton County, 1894, *Fink* s. n. (MICH); Fayette County, 1894, *Fink* s. n. (MICH). *Louisiana*: St. Tammany Parish, Abita, 26 xi 1891, *Langlois* s. n. (US); Washington Parish, *Tucker* 28427C (LSU). *Massachusetts*: Bristol County, New Bedford, *Willey* s. n., 1870 "On *Rhus venenata*", "On Ash", *Willey* s. n., 1862–1898, "On Holly", *Willey* s. n., 1862–1898 "On *Rhus venenata*", *Willey* s. n., 1862–1898, "On Witch-hazel" (syntype collection, FH and US), *Willey* s. n., 1881 "On Cherry", *Willey* s. n., 1884 "On *Rhus copallina*", *Willey* s. n., 1862–1898 (US), *Willey* 239, New Bedford, Bristol Co. (MIN). *New Hampshire*: Coos Co., Jefferson, *Willey* s. n., s. d. (MICH); White Mountains, *Willey* s. n., s. d. (MICH). *North Carolina*: Gaston County, Crowder's Mountain, *Green* s. n., s. d. (MICH); Orange County, Mason Farm Biological Reserve, *Perlmutter* 827 (on *Viburnum*), 912 (on *Viburnum*), 929 (on *Prunus*), 1456 (on *Cornus*), 1470 (on hardwood sapling) (all NCU); Randoph Co., Purgatory Mt. on hardwood trunk, 25 ix 2011, *Rivas Plata* s. n., (NCU); Ridges Mt. on *Prunus*, 24 ix 2011, *Rivas Plata* s. n. (2 specimens, NCU); Wake Co., Turnipseed Nature Preserve, *Perlmutter* 2073 (on *Ilex*), 2325 (on *Ilex*), 2344 (on *Prunus*), 2345 (on *Ilex*), 2604 (on *Prunus*). *Pennsylvania*: 1886, *Eckfeldt* s. n. (US); *Lehnert*, s. n., s. d. (MICH). *South Carolina*: 1886, *Eckfeldt* s. n. (US); Darlington Co., Black Creek watershed on *Aralia*, *Perlmutter* 1495 (NCU); Florence Co., Back Swamp on *Ilex*, *Perlmutter* 1571 (NCU). *Tennessee*: Sevier County, Great Smoky Mountains National Park, 18 ix 1939, *Degelius* s. n. (US). *Virginia*: Grayson Co., Jefferson National Forest on hardwood trunk, *Perlmutter* 1284 (NCU).

Melaspilea epigena Müll. Arg. **USA:** *Louisiana*: East Baton Rouge Parish, Centurion Place subdivision, lichenicolous on *Reimnitzia santensis*, *Tucker* 15903 (LSU).

Melaspilea gibberulosa (Ach.) Zwackh. [now *Hazslinszkya gibberulosa* (Ach.) Körb.] **Switzerland:** *Bern*: on St. Peter's island on *Juglans, Schaerer* s. n., s. d. 507734 (ASU), G00294511 and G00294512 (G), NEB00032057 (NEB) (as *Opegrapha cymbiformis* f. *deformis*, syntype collection).—**Slovakia:** *Little Carpathians*: *Zahlbruckner* s. n., s. d., (US).

Melaspilea maculosa (Fr.) Mull. Arg. Locality unknown. Ref.: K. Vetensk. *Acad. Handl.* **1820:** 44 (1820), *Anonymous* s. n., s. d., L-104319, 182909 (as *Glyphis maculosa*, holotype, UPS). **USA:** *Florida*: Seminole Co., Sanford, *Rapp* 238 (MICH); Escambia Co., Big Lagoon State Recreation Area, *Platt* 52 (LSU, NCU). *Louisiana*: Bois Levert, 26 xii 1898, *Langlois* s. n. (US); St. Martinville Parish, St. Martinville, *Langlois* 776 (US), on *Celits*, *Langlois* 857 (MICH), *Langlois* 1033 (MICH, SBBG); East Baton Rouge Parish, Baton Rouge, on *Fraxinus*, *Tucker* 11951 (ASU, BRY, DUKE, FLAS, MIN, MSC, OMA, SBBG, TENN, WIS); Burden Research Plantation, on *Fraxinus*, *Tucker* 17252 (NCU); Ben Hur Farm, on *Fraxinus*, *Tucker* 21332 (US); West Feliciana Parish, *S. Tucker* 12821 (LSU).

Melaspilea proximella (Nyl.) Nyl. ex Norrlin. [now *Melaspileella proximella* (Nyl.) Ertz & Diederich] **USA:** *New Hampshire*:Coos Co., Jefferson, 1883, *Willey* s. n. (US). *Vermont*: 1879, *Pringle* s. n. (US).

Melaspilea tribuloides (Tuck.) Müll. Arg. [now *Melanographa tribuloides* (Tuck.) Müll. Arg.] **USA:** *Alabama*: Mobile Co., Mobile, 1876, *Curtis* s. n. (US). *Florida*: Duval Co., Ft. George, *Calkins* s. n. (MICH); Seminole Co., Sanford, *Rapp* 2751 (US). *Louisiana*: Catahoula Parish, Sicily Island Wildlife Management Area, on *Pyrenula* (as *Melanotheca*) *cruenta*, *Tucker* 27467B (LSU); East Baton Rouge Parish, Forest Park, on *Pyrenula cruenta*, *Tucker* 9997 (NCU, SBBG); East Feliciana Parish, Idlewild Research Plantation, on *Pyrenula* (as *Melanotheca*), *Tucker* 18437B (MICH, MIN, MSC, OMA, SBBG); St. Tammany Parish, Fontainebleau State Park, on *Pyrenula* (as *Melanotheca*) *cruenta*, *S. Tucker* 18374 (SBBG). *North Carolina*: 1879, *Curtis* s. n. (US).

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References

Acharius, E. (1810) *Lichenographia Universalis*. Gottingen: I. F. Danckwerts.

Bielczyk, U., Lackovicova, A., Farkas, E. E., Lokos, L., Liska, J., Breuss, O. & Kondratyuk, S. Y. (2004) *Checklist of Lichens of the Western Carpathians. Biodiversity of the Carpathians Vol. 1*. Krakow: W. Szafer Institute of Botany, Polish Academy of Sciences.

Diederich, P. & Wedin, M. (2000) The species of *Hemigrapha* (lichenicolous Ascomycetes, *Dothideales*) on *Peltigerales*. *Nordic Journal of Botany* **20:** 203–214.

Eriksson, O. (1982) The families of bitunicate Ascomycetes. *Opera Botanica* **60:** 1–209.

Ertz, D. & Diederich, P. (2015) Dismantling *Melaspileaceae*: a first phylogenetic study of *Buelliella*, *Hemigrapha*, *Karschia*, *Labrocarpon* and *Melaspilea*. *Fungal Diversity*: DOI 10.1007/s13225-015-0321-1

Ertz, D. & Tehler, A. (2011) The phylogeny of *Arthoniales* (Pezizomycotina) inferred from nucLSU and *RPB2* sequences. *Fungal Diversity* **49:** 47–71.

Ertz, D., Miądlikowska, J., Lutzoni, F., Dessein, S., Raspé, O., Vigneron, N. & Diederich, P. (2009) Towards a new classification of the *Arthoniales* (Ascomycota) based on a three-gene phylogeny focusing on the genus *Opegrapha*. *Mycological Research* **113:** 141–152.

Esslinger, T. L. (2011) *A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada*. Fargo: North Dakota State University. Available from: http://www.ndsu.nodak.edu/instruct/esslinge/chcklst/chcklst7.htm [First Posted 1 December 1997, most recent version (#17) 16 May 2011].

Fink, B. (1935) *The Lichen Flora of the United States*. Ann Arbor, Michigan: University of Michigan Press.

Harris, R. C. (1995) *More Florida Lichens, Including the 10 ¢ Tour of the Pyrenolichens*. Bronx, New York: Published by the Author.

Harris, R. C. & Ladd, D. G. (2005) *Preliminary Draft: Ozark Lichens: Enumerating the lichens of the Ozark Highlands of Arkansas, Illinois, Kansas, Missouri, and Oklahoma*. Prepared for the 14th Tuckerman Lichen Workshop. Eureka Springs, Arkansas: Published by the authors.

Hawksworth, D. L. (1975) Notes on British lichenicolous fungi, I. *Kew Bulletin* **30:** 183–203.

Hodkinson, B. P. (2010) A first assessment of lichen diversity for one of North America's 'biodiversity hotspots' in the southern Appalachians of Virginia. *Castanea* **75(1):** 126–133.

Hodkinson, B. P., Harris, R. C. & Case, M.A. (2009) A checklist of Virginia lichens. *Evansia* **26:** 64–88.

Lindsay, W. L. (1872) Memoir on the spermagones and pycnides of crustaceous lichens. *Transactions of the Linnean Society of London* **28:** 189–318.

Llimona, X. & Hladun, N. L. (2001) Checklist of the lichens and lichenicolous fungi of the Iberian Peninsula and Balearic Islands. *Bocconea* **14:** 1–581.

Lücking, R., Lumbsch, H. T., Di Stefano, J. F., Lizano, D., Carranza, J., Bernecker, A., Chaves, J. L. & Umaña, L. (2008) *Eremithallus costaricensis* (Ascomycota: Lichinomycetes: *Eremithallales*), a new fungal lineage with a novel lichen symbiotic lifestyle discovered in an urban relict forest in Costa Rica. *Symbiosis* **46:** 161–170.

Lumbsch, H. T. & Huhndorf, S. M. (2010) Myconet Volume 14. Part One. Outline of Ascomycota – 2009. Part Two. Notes on Ascomycete Systematics. Nos. 4751–5113. *Fieldiana Life and Earth Sciences* **1:** 1–64.

Lumbsch, H. T., Ahti, T., Altermann, S., Amo De Paz, G., Aptroot, A., Arup, U., Bárcenas Peña, A., Bawingan, P. A., Benatti, M. N., Betancourt, L. *et al.* (2011) One hundred new species of lichenized fungi: a signature of undiscovered global diversity. *Phytotaxa* **18:** 1–127.

Matzer, M. (1996) *Lichenicolous Ascomycetes with Fissitunicate Asci on Foliicolous Lichens. (Mycological Papers 171)*. Wallingford: CAB International.

McCune, B. & Grace, J. B. (2002) *Analysis of Ecological Communities*. Gleneden Beach, Oregon: MjM Software Design.

McCune, B. & Mefford, M. J. (2011) *PC-ORD. Multivariate Analysis of Ecological Data. Version 6.0*. Gleneden Beach, Oregon: MjM Software.

Müller, J. (1895) Graphideae Eckfeltianae in Louisiana et Florida: lecte, additis observationibus in Graphideas Calkinsianas eiusdem regionis. *Bulletin de L'Herbier Boissier* **3(2):** 41–50. Available online through the Biodiversity Heritage Library: www.biodiversitylibrary.org (Accessed 2 April 2012).

Nimis, P. L. (1993) *The Lichens of Italy: An Annotated Catalogue*. Monografie XII. Torino: Museo Regionale di Scienze Naturali.

Nylander, W. (1856) Synopsis du genre *Arthonia*. *Mémoires de la Société Impériale des Sciences Naturelles de Cherbourg* **4:** 85–104.

Nylander, W. (1858) Prodromus lichenographicae Galliae et Algeriae. *Actes de la Société Linnéenne de Bordeaux* **21:** 249–467.

Orange, A., James, P. W. & White, F. J. (2010) *Microchemical Methods for the Identification of Lichens*, 2nd edn. London: British Lichen Society.

Perlmutter, G. B. (2008) The lichen biota of Mason Farm Biological Reserve, North Carolina. *Journal of the North Carolina Academy of Sciences* **124:** 82–90.

Perlmutter, G. B. & Beeching, S. Q. (2011) A preliminary checklist of lichens and lichenicolous fungi from the Pee Dee Region, South Carolina, USA. *Evansia* **28:** 27–35.

Poelt, J. (1969) *Bestimmungsschlüssel europäischer Flechten*. Lehre: Verlag von J. Cramer.

Redinger, K. (1938) *Graphidaceae*, in *Arthoniaceae, Graphidaceae, Chiodectonaceae, Dirinaceae, Roccellaceae, Lecanactidaceae, Thelotremaceae, Diploschistaceae, Gyalectaceae & Coenogoniaceae*. In *Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. IX Band, 2 Abt., Part 1*: 181–404. Leipzig: E. Kummer.

Ryan, B. D. & Nimis, P. L. (2004) *Melaspilea*. In *Lichen Flora of the Greater Sonoran Desert Region, Vol. 2* (T. H. Nash III, B. D. Ryan, C. Gries & F. Bungartz, eds): 358–359. Tempe, Arizona: Lichens Unlimited, Arizona State University.

Sanderson, N. A., Hawksworth, D. L. & Aptroot, A. (2009) *Melaspilea* Nyl. (1857). In *The Lichens of Great Britain and Ireland* (C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James, P. A. Wolseley & A. Orange, eds): 576–579. London: British Lichen Society.

Santesson, R., Moberg, R., Nordin, A., Tonsberg, T. & Vitikainen, O. (2004) *Lichen-forming and Lichenicolous Fungi of Fennoscandia*. Uppsala: Museum of Evolution, Uppsala University.

Schaerer, L. E. (1836) *Lichenum Helveticorum Spicilegium. Part II*. Bern: Burgdorfer.

Thomson, J. W. (2003) *Lichens of Wisconsin*. Madison, Wisconsin: Wisconsin State Herbarium, Department of Botany, University of Wisconsin.

Tucker, S. C. (2010) Lichens of Burden Research Plantation, Baton Rouge, Louisiana. *Evansia* **27:** 121–140.

Tuckerman, E. (1872) *Genera Lichenum: an Arrangement of the North American Lichens*. Amherst, Massachusetts: Edwin Nelson. Available online as an e-book on Internet Archive: http://www.archive.org/ (Accessed 2 April 2012).

Tuckerman, E. (H. Willey, ed.) (1888) *A Synopsis of the North American Lichens: Part II, Comprising the Lecidecei and (In Part) the Graphidacei*. New Bedford, Massachusetts: E. Anthony & Sons. Available online as an e-book on Internet Archive: http://www.archive.org/ (Accessed 18 March 2012).

Villella, J., Loring, S. & McCune, B. (2013) Lichens of southwest Oregon's Illinois River Watershed. *Bulletin of the California Lichen Society* **20:** 33–38.

Von Arx, J. A. & Müller, E. (1975) A re-evaluation of the bitunicate Ascomycetes with keys to families and genera. *Studies in Mycology* **9:** 1–159. Available online: http://www.cbs.knaw.nl/publications/1009/content_files/content.htm (Accessed January 2014).

Wirth, M. & Hale, M. E. (1963) The lichen family *Graphidaceae* in Mexico. *Contributions from the United States National Herbarium* **36:** 63–119.

Wirth, V., Hauck, M. & Schultz, M. (2013) *Die Flechten Deutschlands*. Bände 1 & 2. Stuttgart: Ulmer.

Zwackh, W. (1862) Enumeratio Lichenum Florae Heidelbergensis, Fortsetzung. *Flora* **45:** 518.

Appendix. PC-ORD Matrix of North American *Melaspilea* specimens examined including type material of *M. demissa* and *M. gibberulosa* (the latter from Europe)

Specimens are coded with the following scheme: collector's initials followed by the last three letters of the specimen barcode or, if not available, the collection year followed by herbarium acronym. Traits are categorized as either C (category) or Q (quantitative). Measurements are expressed as sample mean ± SD with the sample size in parenthesis, but are analyzed using PC-ORD by the mean only.

	Characters*										Region†
Specimens	C HABIT	C CONTLN	C ASCTYP	Q ASCLEN	C ASCEMR	C HYMINS	C PARAPH	C SPOSEP	Q SPOLEN	Q SPOWID	C REGION
HW323FH	1	0	2	0·65 ± 0·26 (n = 8)	3	1	1	1	19·4 ± 1·5 (n = 5)	8·2 ± 0·8 (n = 5)	1
HW322FH	1	1	2	0·70 ± 0·17 (n = 8)	3	1	1	1	20·4 ± 1·7 (n = 5)	8·5 ± 1·2 (n = 5)	1
HW321FH	1	0	2	0·73 ± 0·13 (n = 8)	3	1	2	1	19·8 ± 2·3 (n = 7)	9·4 ± 1·0 (n = 7)	1
HW318FH	1	0	2	0·50 ± 0·12 (n = 8)	3	1	2	1	17·3 ± 2·6 (n = 8)	8·9 ± 0·9 (n = 8)	1
HW319FH	1	1	2	0·65 ± 0·16 (n = 8)	3	1	2	1	19·0 ± 1·9 (n = 5)	8·5 ± 1·2 (n = 5)	1
HW320FH	1	1	2	0·65 ± 0·20 (n = 8)	3	1	2	1	21·1 ± 1·5 (n = 5)	8·5 ± 1·2 (n = 5)	1
TW163FH	1	0	2	0·4 ± 0·13 (n = 8)	3	1	2	1	18·4 ± 1·4 (n = 5)	8·3 ± 0·4 (n = 5)	2
BF164FH	1	0	2	0·40 ± 0·08 (n = 8)	3	1	1	1	14·8 ± 0·8 (n = 5)	5·8 ± 0·9 (n = 5)	14
AN317FH	1	0	2	0·68 ± 0·15 (n = 8)	3	1	2	1	18·0 ± 1·5 (n = 5)	9·2 ± 0·9 (n = 5)	1
TW162FH	1	0	2	0·53 ± 0·16 (n = 8)	3	1	2	1	20·4 ± 1·7 (n = 5)	8·2 ± 0·8 (n = 5)	2
HW969US	1	1	2	0·74 ± 0·21 (n = 7)	3	0	1	1	19·8 ± 1·8 (n = 6)	8·5 ± 1·5 (n = 6)	1
HW972US	1	1	2	0·76 ± 0·19 (n = 7)	3	1	1	1	18·7 ± 1·7 (n = 5)	8·2 ± 0·8 (n = 5)	1
HW970US	1	0	2	0·70 ± 0·20 (n = 7)	3	1	1	1	17·8 ± 1·4 (n = 6)	8·5 ± 0·0 (n = 6)	1
HW974US	1	0	2	0·80 ± 0·32 (n = 7)	3	1	1	1	19·8 ± 0·9 (n = 6)	8·8 ± 0·7 (n = 6)	1
HW973US	1	0	2	0·58 ± 0·19 (n = 8)	3	1	1	1	19·6 ± 2·9 (n = 4)	8·1 ± 1·6 (n = 4)	1
GP362NCU	1	0	2	0·75 ± 0·21 (n = 6)	3	0	2	1	17·9 ± 2·9 (n = 5)	7·4 ± 0·8 (n = 5)	3
GP363NCU	1	1	2	0·50 ± 0·10 (n = 5)	3	0	1	1	21·0 ± 1·6 (n = 8)	10·2 ± 0·9 (n = 8)	3
GP364NCU	1	0	2	1·1 ± 0·5 (n = 7)	3	0	2	1	17·0 ± 2·1 (n = 9)	7·7 ± 0·9 (n = 9)	3
GP377NCU	1	0	2	0·46 ± 0·09 (n = 5)	3	1	2	1	17·2 ± 1·8 (n = 8)	8·5 ± 1·2 (n = 8)	4
GP365NCU	1	1	2	0·64 ± 0·31 (n = 7)	3	0	2	1	16·7 ± 2·0 (n = 6)	7·8 ± 0·8 (n = 6)	3
GP366NCU	1	0	2	0·73 ± 0·21 (n = 7)	3	1	2	1	16·3 ± 1·2 (n = 3)	7·3 ± 1·3 (n = 3)	3
GP375NCU	1	0	2	0·42 ± 0·12 (n = 6)	3	1	2	1	18·4 ± 1·6 (n = 10)	9·7 ± 1·3 (n = 10)	2
GP376NCU	1	0	2	0·40 (n = 1)	3	1	1	1	19·8 ± 1·7 (n = 10)	9·7 ± 1·4 (n = 10)	2
GP370NCU	1	0	2	0·35 ± 0·09 (n = 8)	3	1	1	1	18·5 ± 3·6 (n = 9)	8·9 ± 1·3 (n = 9)	3
GP371NCU	1	0	2	0·54 ± 0·26 (n = 7)	3	0	1	1	18·5 ± 3·0 (n = 9)	9·5 ± 1·3 (n = 9)	3
GP372NCU	1	0	2	0·45 ± 0·13 (n = 4)	3	0	1	1	19·4 ± 3·0 (n = 9)	9·3 ± 2·1 (n = 9)	3
EP368NCU	1	0	2	0·68 ± 0·19 (n = 6)	3	1	1	1	19·6 ± 1·8 (n = 6)	9·6 ± 1·6 (n = 6)	3
EP367NCU	1	0	2	0·53 ± 0·13 (n = 4)	3	0	1	1	19·8 ± 1·4 (n = 6)	8·8 ± 0·7 (n = 6)	3
EP369NCU	1	0	2	0·48 ± 0·15 (n = 5)	3	0	1	1	19·7 ± 2·6 (n = 5)	10·5 ± 0·8 (n = 5)	3
ST381NCU	2	0	2	0·33 ± 0·13 (n = 8)	3	0	1	1	13·1 ± 1·5 (n = 4)	7·0 ± 0·4 (n = 4)	9
SW052MSC	1	0	2	0·25 ± 0·07 (n = 2)	3	0	1	1	11·8 ± 1·2 (n = 4)	5·8 ± 0·8 (n = 4)	6

Appendix. (Continued)

	Characters*										
Specimens	C HABIT	C CONTLN	C ASCTYP	Q ASCLEN	C ASCEMR	C HYMINS	C PARAPH	C SPOSEP	Q SPOLEN	Q SPOWID	Region† C REGION
LS511G	1	0	1	0·64 ± 0·21 ($n = 5$)	2	0	1	1	15·0 ± 1·7 ($n = 6$)	6·5 ± 0·7 ($n = 6$)	12
LS512G	1	0	1	0·58 ± -0·08 ($n = 5$)	2	0	1	1	12·8 ± 0·9 ($n = 6$)	6·5 ± 0·7 ($n = 6$)	12
AL1892US	1	1	1	0·55 ± 0·12 ($n = 8$)	2	1	1	1	16·8 ± 1·0 ($n = 6$)	8·5 ± 0·3 ($n = 6$)	9
AL1895US	1	1	1	0·58 ± 0·09 ($n = 8$)	2	1	1	1	16·1 ± 2·5 ($n = 6$)	8·5 ± 0·3 ($n = 6$)	9
AL1896US	1	1	1	0·58 ± 0·15 ($n = 8$)	2	1	1	1	18·7 ± 1·4 ($n = 4$)	8·9 ± 0·8 ($n = 4$)	9
ST1969US	1	0	1	0·88 ± 0·16 ($n = 8$)	2	0	1	1	23·0 ± 1·7 ($n = 4$)	11·5 ± 0·8 ($n = 4$)	9
SR1909US	1	0	1	1·18 ± 0·26 ($n = 8$)	2	0	1	1	22·5 ± 2·6 ($n = 4$)	11·1 ± 1·0 ($n = 4$)	10
BF1899US	1	0	1	0·54 ± 0·09 ($n = 8$)	3	1	1	1	11·9 ± 0·00 ($n = 3$)	6·8 ± 1·7 ($n = 3$)	8
ET1857US	1	0	1	0·6 ± 0·06 ($n = 6$)	2	1	1	1	16·2 ± 1·0 ($n = 4$)	7·7 ± 1·0 ($n = 4$)	1
BF1894US	1	0	1	0·56 ± 0·10 ($n = 7$)	2	0	1	1	17·8 ± 1·0 ($n = 4$)	8·5 ± 0·0 ($n = 4$)	7
HK1921US	1	1	1	2·08 ± 0·38 ($n = 6$)	2	0	1	1	22·1 ± 2·4 ($n = 4$)	9·8 ± 0·8 ($n = 4$)	11
SR1921US	1	0	1	1·08 ± -0·30 ($n = 8$)	2	0	1	1	17·4 ± 0·8 ($n = 4$)	8·9 ± 0·8 ($n = 4$)	10
L1895US1	1	1	2	0·74 ± 0·24 ($n = 8$)	3	0	1	1	13·2 ± 0·8 ($n = 4$)	6·0 ± 1·0 ($n = 4$)	9
GD1939US	1	0	2	0·45 ± 0·05 ($n = 6$)	3	0	1	1	19·1 ± 1·6 ($n = 4$)	8·1 ± 0·8 ($n = 4$)	4
AL1891US	1	0	2	0·42 ± 0·42 ($n = 5$)	3	0	1	1	19·0 ± 1·4 ($n = 2$)	7·5 ± 0·7(n – 2)	10
JE1886US	1	0	2	0·56 ± 0·14 ($n = 8$)	3	1	1	1	19·7 ± 2·6 ($n = 5$)	9·9 ± 0·8 ($n = 5$)	5
AZ7992US	1	0	1	0·51 ± 0·11 ($n = 8$)	3	0	1	1	13·6 ± 1·4 ($n = 4$)	6·8 ± 0·0 ($n = 4$)	12
E1886US1	1	0	1	0·57 ± 0·10 ($n = 7$)	3	0	1	1	16·9 ± 1·0 ($n = 5$)	7·7 ± 0·9 ($n = 5$)	3
SR1914US	1	0	1	1·19 ± 0·3 ($n = 7$)	1	0	1	2	22·1 ± 2·4 ($n = 4$)	5·5 ± 0·8 ($n = 4$)	10
AL1893US	1	0	2	1·05 ± 0·14 ($n = 8$)	2	0	1	1	20·0 ± 1·6 ($n = 4$)	10·7 ± 2·5 ($n = 4$)	9
AL1898US	1	1	1	1·34 ± 0·35 ($n = 8$)	2	0	1	1	18·7 ± 2·4 ($n = 4$)	8·1 ± 1·6 ($n = 4$)	9
ST1980US	1	0	1	1·15 ± 0·25 ($n = 8$)	2	0	1	1	19·0 ± 3·3 ($n = 5$)	9·9 ± 1·4 ($n = 5$)	9
AL1894US	1	0	1	0·88 ± 0·41 ($n = 6$)	3	1	1	2	32·3 ± 0·0 ($n = 2$)	6·0 ± 1·2 ($n = 2$)	9
CP1879US	1	0	1	0·34 ± 0·05 ($n = 7$)	3	0	1	1	20·5 ± 2·5 ($n = 3$)	10·5 ± 4·8 ($n = 3$)	1
HW1883US	1	0	1	0·25 ± 0·06 ($n = 4$)	3	0	1	1	14·6 ± 2·3 ($n = 6$)	6·4 ± 1·6 ($n = 6$)	1
SR1927US	2	0	2	0·60 ± 0·17 ($n = 7$)	3	0	1	1	16·2 ± 1·2 ($n = 2$)	8·5 ± 0·0 ($n = 2$)	10
MC1879US	2	0	2	0·50 ± 0·14 ($n = 6$)	3	0	1	1	17·5 ± 1·6 ($n = 6$)	8·2 ± 1·4 ($n = 6$)	13
MC1876US	2	0	2	0·47 ± 0·16 ($n = 6$)	3	0	1	1	17·1 ± 2·6 ($n = 8$)	6·8 ± 1·4 ($n = 8$)	10

*Characters are as follows: HABIT = lichenized (1) or lichenicolous fungus (2), CONTLN = contact lines absent (0) or present (1), ASCTYP = ascomatal type (1 = arthonioid, 2 = opegraphoid), ASCLEN = ascomatal length or diameter (mm), ASCEMR = ascoma emergence (1 = immersed, 2 = erumpent, 3 = sessile), HYMINS = hymenium inspersion (0 = not inspersed, 1 = inspersed), PARAPH = paraphyses (1 = not thickened, 2 = thickened), SPORSEP = spore septation (1 = one-septate, 2 = multiseptate), SPOLEN = spore length (μm), SPOWID = spore width (μm), REGION = EPA Level III ecoregion.;

†Ecoregions are numbered as follows: 1 = Northeastern Coastal Zone (Massachusetts), 2 = Southeastern Plains (South Carolina), 3 = Piedmont (North Carolina), 4 = Blue Ridge (Tennessee, Virginia), 5 = Ridge and Valley (Pennsylvania), 6 = Northern Lakes and Forests (Michigan), 7 = Driftless Area, 8 = Glaciated Plains (Minnesota), 9 = Mississippi Alluvial Plain (Louisiana), 10 = South Coastal Plain (Alabama, Florida), 11 = South Florida Coastal Plain (Florida), 12 = European Broadleaf Forest (Switzerland, Slovakia), 13 = Northeastern Highlands (New Hampshire, Vermont), 14 = Mid Atlantic Coastal Plain (North Carolina).