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Studies on *Parmulariaceae* I. A phylogeny based on available sequence data; introducing *Parmulariales* ord. nov., and *Hemigraphaceae*, *Melaspileellaceae* and *Stictographaceae* fam. nov.

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Abstract

The family *Parmulariaceae* comprises three polyphyletic genera, but with very little data in GenBank and is presently placed in the order *Asterinales*. In this study, we re-analyze the available sequence data for taxa of the family and re-examine the type species of *Hemigrapha*, *Inocyclus* and *Parmularia*. The phylogenetic tree generated from maximum likelihood and Bayesian analyses of combined LSU-SSU sequence data demonstrate the relationships among *Hemigrapha*, *Inocyclus* and *Parmularia* species, and the relations of *Buelliella*, *Karschia*, *Labrocarpon*, *Lembosia*, *Melaspileella*, *Melaspileopsis* and *Stictographa*. We introduce *Parmulariales* ord. nov. to accommodate *Parmulariaceae* and the order *Asterinales* accommodates *Asterinaceae*, *Asterotexaceae*, *Hemigraphaceae* fam. nov., *Melaspileellaceae* fam. nov. and *Stictographaceae* fam. nov. Notes for each new order and families are provided. We confirm that *Asterinaceae sensu lato* is distant from *Asterinaceae sensu stricto* in the phylogenic analysis. The classification presented here is provisional, as more species are needed to re-collected and sequenced. We expect further support for our ordinal and familial lineages, as well as further novel lineages.

Keywords: 4 new taxa, taxonomy, phylogeny, biotrophs, lichenicolous fungi

Introduction

The family *Parmulariaceae* comprises more than 30 genera with extremely varied characters and is polyphyletic (Guatimosim *et al.* 2015). Genera such as *Aldona*, *Aldonata*, *Dictyocyclus* and *Hemigrapha* are not typical of the family when compared with the type (Inácio & Cannon 2008, Tian *et al.* 2015). There are few studies relating to the phylogeny of *Parmulariaceae* (Guatimosim *et al.* 2015, Hyde *et al.* 2016, Liu *et al.* 2017), as genera of this family are unculturable, therefore it is hard to obtain their DNA sequence data. However, sequence data is available for *Hemigrapha*, *Inocyclus* and *Parmularia* with DNA extracted from fresh specimens. The family is presently included in *Asterinales* (Hyde *et al.* 2013, Guatimosim *et al.* 2015), although this order affiliation needs verification.

Asterinales is an important order, commonly known as black mildews, forming dark, superficial colonies on living leaves (Hosagoudar *et al.* 2013a). This order is characterized by orbicular, dark, flattened thyriothecia with central dehiscent X- or Y- shaped openings. The thyriothecia of *Asterinales* usually contain few asci which are globose to oval, or clavate. Ascospores are commonly ellipsoid, conglobate and initially hyaline, becoming brown to dark brown when mature (Hosagoudar *et al.* 2013b, Hongsanan *et al.* 2014).

The order *Asterinales* previously included three families, *viz. Asterinaceae*, *Aulographaceae* and *Parmulariaceae* (Hyde *et al.* 2013). However, although somewhat similar, characters of these families provide few clues as to the natural placement of the genera within these families. Based on both phylogeny and morphology, Hongsanan *et al.* (2014) revised the order and accepted only a single family *Asterinaceae*. Subsequently, Ertz & Diederich (2015) sequenced several lichenicolous genera (*Buelliella*, *Hemigrapha*, *Karschia*, *Labrocarpon* and *Stictographa*), as well as two saprobic genera (*Melaspileella* and *Melaspileopsis*) and placed these genera in *Asterinales* genera *incertae sedis* on the

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basis of their phylogenic relationships close to taxa of *Asterinales*. Guatimosim *et al.* (2015) re-collected *Parmularia styracis* Lév., the type species of *Parmulariaceae* and designated it as an epitype. The authors placed *Parmulariaceae* in *Asterinales*, as the phylogenetic tree generated from Bayesian analysis of LSU sequence data, showed the *Parmularia* strains clustered with several new collections of *Asterinales* sequenced by Guatimosim *et al.* (2015), *viz. Asterina melastomatis* Lév., *A. crysophylli*, *Prillieuxina baccharidincola* (Rehm) Petr. and *Lembosia abaxialis* Firmino & R.W. Barreto. These taxa, however were treated as *Asterinaceae sensu lato* by Liu *et al.* (2017), as they separated from a separate set of *Asterina* species provided by different research groups. *Asterotexis* was previously placed in *Asterinaceae* (Inácio & Cannon 2008, Guerrero *et al.* 2011) and Guatimosim *et al.* (2015) had introduced a new family *Asterotexaceae* and a new order *Asterotexales* to accommodate this genus. Subsequently, Liu *et al.* (2017) synonymized *Asterotexales* under *Asterinales*, as strains of *Asterotexaceae* clustered with species of *Asterinaceae sensu stricto*.

Members of *Asterinales* are presently confused, with several genera *incertae sedis* groups. In this study, the morphology of *Hemigrapha*, *Inocyclus* and *Parmularia* are re-examined based on herbarium specimens. The family placements of the lichenicolous genera *Buelliella*, *Hemigrapha*, *Karschia*, *Labrocarpon* and *Stictographa*, as well as the saprobic genera *Melaspileella* and *Melaspileopsis* are partially resolved based on phylogenetic analysis. The placements of these genera are discussed and *Parmulariales* ord. nov., *Hemigraphaceae*, *Melaspileellaceae* and *Stictographaceae* families nov. are introduced.

Material and methods

Examination of specimens

Specimens (including types) of type species of *Hemigrapha*, *Inocyclus* and *Parmularia* were obtained from G, FH and S (abbreviations according to Index Herbariorum 2018). The study methods followed are those reported in Dai *et al.* (2014, 2017). Fruiting bodies were observed and photographed by Zeiss Stereo Discovery V8 with AxioCam ERc 5 s. Hand sections of the ascomata/ascostromata were mounted in distilled water on glass slides for microscopic studies and photomicrography. The morphologic characters were observed by differential interference contrast (DIC) using a Nikon ECLIPSE 80*i* compound microscope and photographed by Canon 600D digital camera fitted to the microscope (Dai *et al.* 2017). Ascomata, asci and ascospores etc. were measured using a Tarosoft (R) Image Frame Work program. Images used for figures were processed with Adobe Photoshop CS3 (Adobe Systems Inc., The United States). Index Fungorum (2018) numbers are provided for newly taxa and Facesoffungi numbers (Jayasiri *et al.* 2015) are provided for each taxa as well.

Phylogenetic analysis

DNA sequence data (LSU and SSU) of relevant taxa determined in Hyde *et al.* (2013), Guatimosim *et al.* (2015), Ertz & Diederich (2015) and Liu *et al.* (2017) were download from GenBank. Selected taxa belonging to *Asterinales*, *Botryosphaeriales*, *Capnodiales*, *Cladoriellales*, *Hysteriales*, *Jahnulales*, *Lichenoconiales*, *Lichenotheliales*, *Microthyriales*, *Myriangiales*, *Mytilinidiales*, *Natipusillales*, *Patellariales*, *Phaeotrichales*, *Tubeufiales*, *Valsariales* and *Venturiales* were included in the phylogenetic analysis to show the relationships among *Buelliella*, *Hemigrapha, Inocyclus*, *Karschia*, *Labrocarpon*, *Melaspileella*, *Melaspileopsis*, *Parmularia* and *Stictographa*. Sequences were aligned in MAFFT v. 7.215 (Katoh & Standley 2013) and manually edited in BioEdit (Hall 1999).

Maximum-likelihood (ML) analysis was performed in raxmlGUI v.1.0. (Stamatakis 2006, Silvestro & Michalak 2011) with 1000 bootstrap replicates. The PHYLIP format of combined alignment was exchanged and loaded from website http://sing.ei.uvigo.es/ALTER/. The best-fit nucleotide substitution model (GTR+G) for sequence data was selected by the online tool Findmodel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html).

Bayesian analyses were running using MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003). The best-fit model of evolution was obtained by using MrModeltest v. 2.3 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4. Six simultaneous Markov chains were run for 7,000,000 generations and trees were sampled every 100th generation (Dai *et al.* 2017). The burn-in was set to 0.25, and the run was automatically stopped when the average standard deviation of split frequencies reached below 0.01 (Maharachchikumbura *et al.* 2015).

Trees were viewed in TreeView (Page 1996), and additionally layouts were made with Adobe Illustrator CS v. 5. Maximum-likelihood bootstrap values (MLBP) greater than 50 % and Bayesian posterior probabilities (BYPP) greater than 0.80 are given. The sequences used in this study are listed in table 1. The combined alignment and phylogenetic tree were submitted at TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S22032).

TABLE 1. Sequences data with GenBank accession numbers used in this study are provided.

Organism	Strain	GenBank accession numbers	
		LSU	**SSU**
Aliquandostipite khaoyaiensis	SS3321	EF175650	EF175628
Apiosporina collinsii	CBS 118973	GU301798	GU296135
Asterina cestricola	TH 591	GU586215	GU586209
Asterina crysophylli	VIC 42823	KP143738	
Asterina cynometrae	MFLU 13-0373	KX845436	
Asterina fuchsiae	TH 590	GU586216	GU586210
Asterina melastomatis	VIC 42822	KP143739	
Asterina phenacis	TH 589	GU586217	GU586211
Asterina siphocampyli	ppMP 1324	HQ701140	
Asterina weinmanniae	TH 592	GU586218	GU586212
Asterina zanthoxyli	TH 561	GU586219	GU586213
Asterina cynometrae	MFLU 13-0373	KX845436	
Asterotexis cucurbitacearum	PMA M-0141224	HQ610510	
Asterotexis cucurbitacearum	VIC 42814	KP143734	
Aulographum hederae	CPC 21373	KM386981	
Aulographum hederae	MFLUCC 13-0001	KM386980	
Bambusaria bambusae	MFLUCC 12-0851	KP687812	KP687962
Batistinula gallesiae	VIC 42514	KP143736	
Botryosphaeria fusispora	MFLUCC 10-0098	NG_042722	JX646823
Buelliella physciicola	Ertz 19173	KP456148	
Buelliella physciicola	Ertz 18113	KP456147	
Buelliella poetschii	Ertz 18115 (BR)	KP456149	
Buelliella poetschii	Ertz 18116 (BR)	KP456150	
Caliciopsis pinea	AFTOL-ID 1869	DQ678097	DQ678043
Capnodium coffeae	CBS 147.52	NG_027576	DQ247808
Cladoriella eucalypti	CPC 10953	DQ195790	DQ195801
Cladoriella rubrigena	CBS 124760	GQ303304	
Dissoconium aciculare	CBS 204.89	GU214419	GU214523
Dothiorella thailandica	MFLUCC 11-0438	JX646813	JX646829
Glonium circumserpens	CBS 123342	FJ161208	FJ161168
Glonium circumserpens	CBS 123343	FJ161200	FJ161160
Hemigrapha atlantica	Ertz 14014 (BR)	KP456151	
Hysterium angustatum	MFLU 16-1179	KX611364	KX611365
Inocyclus angularis	VIC 39747	KP143731	
Inocyclus angularis	VIC 39748	KP143732	
Inocyclus angularis	VIC 39749	KP143733	
Jahnula aquatica	R68.1	EF175655	EF175633
Jahnula bipileata	F49.1	EF175657	EF175635
Karschia cezannei	Ertz 19186	KP456154	
Karschia talcophila	Ertz 16749	KP456155	
Labrocarpon canariense	Ertz 16907	KP456158	
Labrocarpon canariense	Ertz 16308	KP456157	

.....continued on the next page

TABLE 1 (Continued**)**

Organism	Strain	GenBank accession numbers	
		LSU	**SSU**
Lembosia albersii	MFLU 13-0377	KM386982	
Lembosia xyliae	MFLU14-0004	KT283685	
Lempholemma polyanthes	11294-L1(2/2)	AF356691	
Leptoxyphium fumago	CBS 123.26	GU214430	GU214535
Lichenoconium aeruginosum	JL359-09	HQ174269	
Lichenoconium lecanorae	JL382-10	HQ174263	
Lichenothelia convexa	L1608	KC015070	KC015085
Lichenothelia convexa	L1609	KC015071	KC015086
Melaspileella proximella	G.M. 2015-04-29	KY654708	
Melaspileella proximella	G.M. 2014-05-25	KY654747	
Melaspileopsis cf. *diplasiospora*	Ertz 16247	KP456164	
Melaspileopsis cf. *diplasiospora*	Ertz 16624	KP456165	
Mendogia macrostroma	MFLU 13-0642	KU863104	KU872109
Microthyrium microscopicum	CBS 115976	GU301846	GU296175
Microthyrium propagulensis	IFRD 9037	KU948989	
Myriangium duriaei	CBS260.36	NG_027579	NG_013129
Myriangium hispanicum	CBS 247.33	KX887067	GU371744
Myrmaecium rubrum	CBS 109505	GU456324	GU456303
Natipusilla decorospora	L_A236_1A	HM196369	HM196376
Natipusilla naponensis	L_AF217_1B	HM196372	HM196379
Neodeightonia microspora	MFLUCC 11-0483	KU863099	KU872105
Parmularia styracis	VIC 42447	KP143728	
Parmularia styracis	VIC 42450	KP143729	
Parmularia styracis	VIC 42587	KP143730	
Patellaria cf. *atrata*	BCC 28876	GU371828	GU371836
Patellaria cf. *atrata*	BCC 28877	GU371829	GU371837
Peltula auriculata	AFTOL-ID 892	DQ832330	
Phaeotrichum benjaminii	CBS 541.72	AY004340	AY538349
Prillieuxina baccharidincola	VIC 42817	KP143735	
Psiloglonium simulans	CBS 206.34	FJ161178	FJ161139
Rhytidhysteron rufulum	MFLUCC 14-0577	KU377565	KU377570
Stictographa lentiginosa	Ertz 17570	KP456170	
Stictographa lentiginosa	Ertz 17447	KP456169	
Teratosphaeria fibrillosa	CBS 121707	KF902075	GU296199
Trichodelitschia bisporula	CBS 262.69	GU348996	GU296202
Tubeufia filiformis	MFLUCC 16-1135	KY092411	
Tubeufia javanica	MFLUCC 12–0545	KJ880036	KJ880035
Valsaria insitiva	CBS 123098	GU460204	GU456310
Venturia inaequalis	ATCC 60070	EF114712	EF114737
Venturia populina	CBS 256.38	GU323212	GU296206

Results and discussion

Phylogeny

A data set of combined LSU-SSU sequence data of 82 strains, including the outgroup taxon *Caliciopsis pinea* (AFTOL-ID 1869), was used to determine the relationship among *Hemigrapha*, *Inocyclus* and *Parmularia*. The lichenicolous *Buelliella*, *Karschia*, *Labrocarpon* and *Stictographa*, as well as saprobic *Melaspileella* and *Melaspileopsis* genera are placed in *Asterinales*. The phylogenetic tree generated from maximum likelihood (ML) and Bayesian analyses of LSU-SSU sequence data demonstrate that *Parmulariaceae* forms a sister clade with *Asterinaceae sensu lato* with high bootstrap support (MLBS/BYPP 100/1.00) and forms a separate lineage from *Asterinales* (Fig. 1). The phylogenetic analysis (Fig. 1) based on the representative sequences of the major orders in Dothideomycetes support *Parmulariaceae* as an individual entity. A new order *Parmulariales* is therefore established to accommodate this family.

Guatimosim *et al.* (2015) mentioned that *Parmulariaceae* is polyphyletic, perhaps because the authors considered the case that *Inocyclus* was phylogenetically far from *Parmulariaceae*. However, on the basis of re-examination of isotype of the type species *Inocyclus psychotriae* (Syd. & P. Syd.) Theiss. & Syd., *Inocyclus* is not morphologically and representatively similar with *Parmulariaceae*.

Asterinales forms a strongly supported clade (MLBS/BYPP 98/1.00) in the data matrix. Two major lineages can be defined within *Asterinales*. One that contains taxa of *Stictographaceae*, with the genera *Karschia*, *Labrocarpon* and *Melaspileopsis* as well as *Buelliella physciicol*a Poelt & Hafellner. Currently, *Buelliella* is not included in *Stictographaceae*, until sequences from the type species are available. The relationships among these genera are poorly supported, probably because of lack of protein genes, and this result is similar to that of Ertz & Diederich (2015). Other groups of *Asterinales* are *Asterinaceae sensu stricto*, *Asterotexaceae*, *Hemigraphaceae* and *Melaspileellaceae*, together with several genera *incertae sedis*. The clade of *Asterinaceae sensu stricto* is stable and strongly supported (MLBS/BYPP 99/1.00, Fig. 1). Strains of type species of *Melaspileellaceae* form a branch at the base of *Asterinales*. *Hemigrapha* was previously placed in *Parmulariaceae* and then removed to *Asterinales incertae sedis* by Ertz & Diederich (2015). It forms a single branch within *Asterinales* and therefore *Hemigraphaceae* is introduced here. *Inocyclus*, a genus also previously assigned to *Parmulariaceae* forms a sister clade with two *Lembosia* species (*L. xyliae* X. Y. Zeng *et al.* and *L. albersii* Henn.). The type species of *Inocyclus* has not been sequenced, therefore *Inocyclus* is retained in *Asterinales incertae sedis*. *Lembosia* was previously included in *Asterinaceae* (Hongsanan *et al.* 2014) based on the sequences obtained from a fresh specimen *L. albersii*, however few strains were included in the phylogenic tree in Hongsanan *et al.* (2014). According to our study, two *Lembosia* species are phylogenetically separate from *Asterinaceae* (Fig. 1). *Lembosia* needs further collections with molecular data of type species to establish its relationship with *Asterinaceae*.

Taxonomy

Asterinales M.E. Barr ex D. Hawksw. & O.E. Erikss., Syst. Ascom. 5(1): 177 (1986)
Synonyms: *Asterotexiales* Firmino, O.L. Pereira & Crous [as 'Asterotexiales'], Persoonia 35: 238 (2015)

Hemigraphaceae D.Q. Dai & K.D. Hyde, fam. nov.
Index Fungorum number: IF554062; Facesoffungi number: FoF 03910

Biotrophic on thallus of lichens. **Sexual morph**: *Ascostromata* solitary to gregarious, in groups, superficial, black to dark brown, coriaceous, stellate, irregularly opening from the centre to margin, conical in section. *Peridium* composed of two parts; outer part, a black layer, composed of thick-walled cells of *textura angularis*; inner part thin, composed of light brown cells of *textura angularis*. *Hamathecium* composed of a few brown, unbranched, filamentous, septate, pseudoparaphyses around asci. *Asci* 8-spored, bitunicate, clavate to cylindric-clavate, subglobose, with an ocular chamber and a short pedicel. *Ascospores* 3-seriate to irregularly arranged, brown, ellipsoid, 1-septate, with larger upper cell and narrower lower cell, smooth-walled. **Asexual morph**: Undetermined.

Type genus: *Hemigrapha* (Müll. Arg.) R. Sant. ex D. Hawksw., Kew Bull. 30(1): 9 (1975)

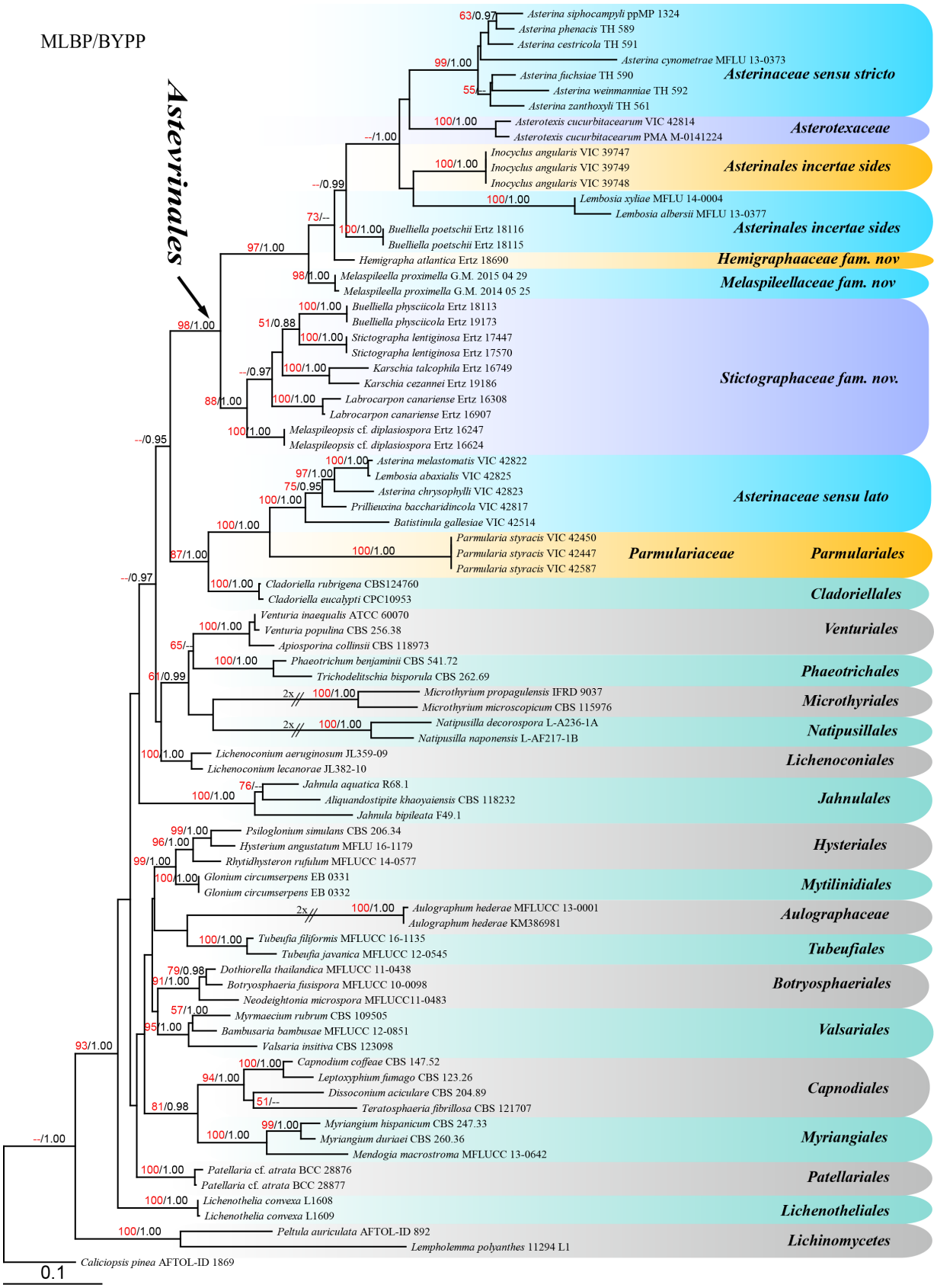

FIGURE 1. Maximum likelihood phylogenetic tree generated by RAxML (GTR+G model) based on analysis of combined LSU and SSU sequence data. ML values (>50 %) resulting from 1000 bootstrap replicates and Bayesian posterior probabilities greater than 0.80 are given at the nodes. The strain numbers are noted after the species names. The tree is rooted to *Dendrographa decolorans* (DUKE 0047570).

Notes:—The genus *Hemigrapha*, typified by *H. asteriscus* (Müll. Arg.) R. Sant. ex D. Hawksw (Basionym: *Melanographa asteriscus* Müll. Arg.), was introduced by Hawksworth (1975). This genus is a communalistic taxon having a biotrophic life-style (Diederich & Wedin 2000, Ertz & Diederich 2015). Inácio & Cannon (2008) revised the genera of *Parmulariaceae* but did not include *Hemigrapha* in the family. However, Lumbsch & Huhndorf (2010), Hyde *et al.* (2013) and Wijayawardene *et al.* (2014) included this genus in *Parmulariaceae*. The lichenicolous fungi, *Hemigrapha* is similar to *Viegasella* (Tian *et al.* 2015) in having star-shaped ascomata, but differs in its smooth-walled ascospores (*Viegasella* has verrucose ascospores). *Hemigrapha* is characterized by dark, superficial, star-shaped ascomata forming on a thallus of lichens, clavate to subglobose asci and brown, ellipsoid, 2-celled ascospores (Diederich & Wedin 2000). Based on the morphology of the re-examined type in this paper, *Hemigrapha* is not similar with the family type of *Parmulariaceae*. Ertz & Diederich (2015) included *H. atlantica* in their phylogenetic analysis and indicated that this genus belongs to order *Asterinales* based on LSU sequence data, without assigning the family placement. In this study, *Hemigrapha atlantica* Diederich & Wedin forms a single clade clustering within the order *Asterinales* (Fig. 1). *Hemigrapha atlantica* phylogenetically forms as a sister species with the generic type *H. asteriscus*, seen in Ertz & Diederich (2015). Therein a new family *Hemigraphaceae* is introduced to accommodate this single genus in *Asterinales*.

Type species: *Hemigrapha asteriscus*, Figure 2.

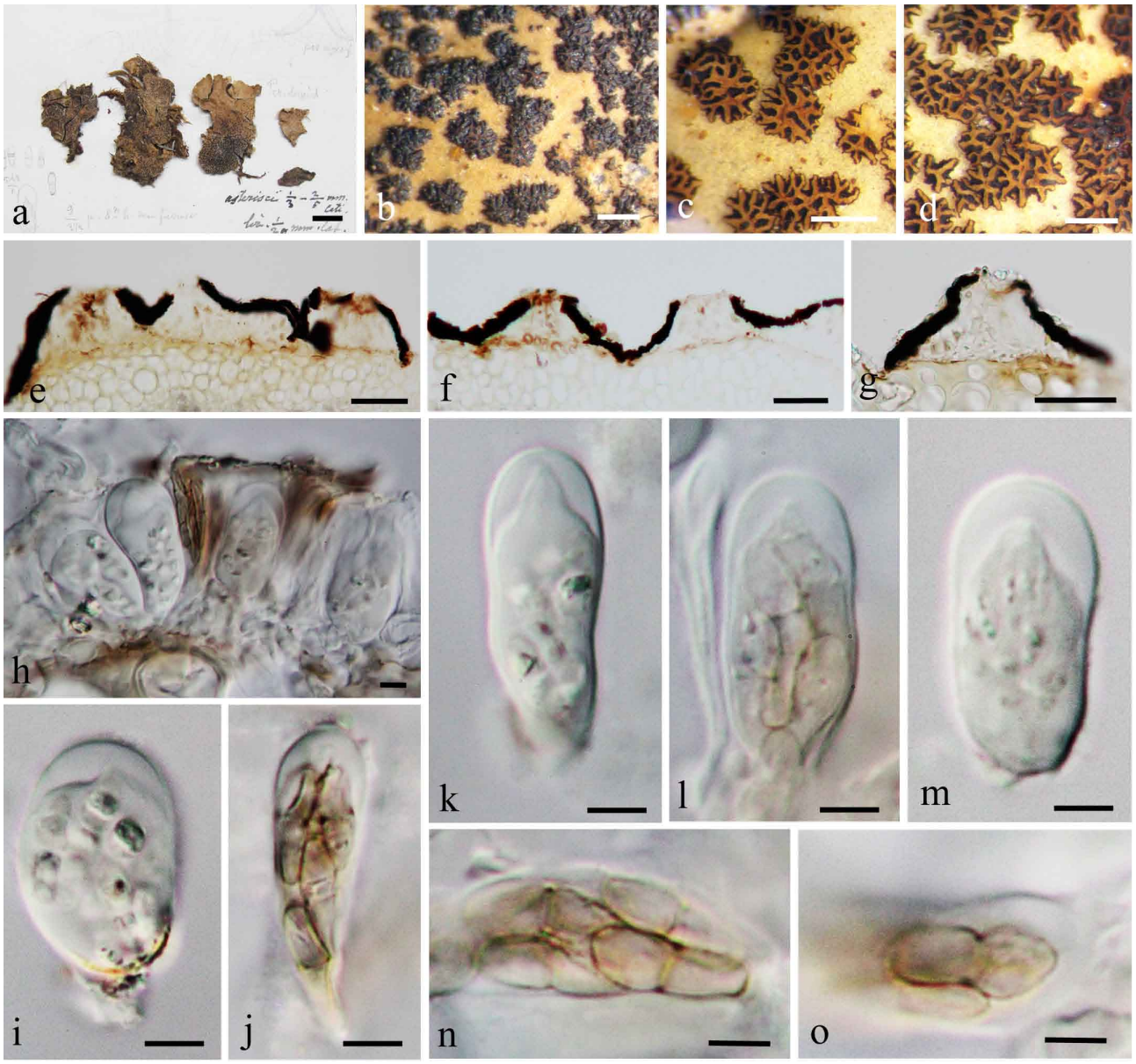

FIGURE 2. *Hemigrapha asteriscus* (G 00292584, syntype). a. Herbarium material. b–d. Appearance of ascostromata on host surface. c, d. Ascostromata in the water. e–j. Section of ascostroma. h. Asci with hamathecial tissues. i–m. Asci. n, o. Ascospores. Scale bars: a = 20 mm, b–d = 500 µm, e–g = 50 µm, h–o = 5 µm.

Facesoffungi number: FoF 02312

Basionym: *Melanographa asteriscus* Müll. Arg., Flora, Regensburg 65(33): 519 (1882)

Syntype:—G 00292584

Biotrophic on lichens. **Sexual morph**: *Ascostromata* 1–1.5 mm diam., solitary to gregarious, in groups, superficial, black to dark brown, coriaceous, stellate, irregularly opening from the centre to margin, conical in section, 50–115 µm diam., 30–55 µm high. *Peridium* 7–10 µm thick, composed of two layers; outer part, black, composed of thick-walled cells of *textura angularis*; inner part thin, composed of light brown cells of *textura angularis*. *Hamathecium* composed of few brown, unbranched, 1–1.5 µm wide, filamentous, septate, pseudoparaphyses around asci. *Asci* 19–30 × 8–15 µm ($\overline{x}$ = 24.4 × 10.9 µm, n = 20), 8-spored, bitunicate, clavate to cylindric-clavate, subglobose, with an ocular chamber and a short pedicel. *Ascospores* 8.5–12.5 × 3–4 µm ($\overline{x}$ = 10.1 × 3.7 µm, n = 20), 3-seriate to irregularly arranged, brown, ellipsoid, 1-septate, with large upper cell and narrower lower cell, smooth-walled. **Asexual morph**: Undetermined.

Material examined:—AUSTRALIA, New South Wales, Mount Kosciuszko, on thallus of *Peltigera dolichorrhiza* (Lichen). Müller J. 1882 (G 00292584, syntype).

Melaspileellaceae D.Q. Dai & K.D. Hyde, fam. nov.

Index Fungorum number: IF554063; Facesoffungi number: FoF 03911

Saprobic on trees and shrubs. **Sexual morph**: *Ascostromata* solitary, superficial, dark to black, coriaceous, rounded. *Peridium* outer layers composed of black, thick-walled cells; inner layers composed of light brown to hyaline cells of *textura angularis*. *Hamathecium* of dense, hyaline, unbranched, filamentous, septate pseudoparaphyses around asci, brown at the apices. *Asci* 8-spored, bitunicate, wide clavate to subglobose, with a round apex and a short pedicel. *Ascospores* 2-seriate to irregularly arranged, hyaline, ellipsoid, 1-septate, with slightly larger upper cell, with slightly narrower lower cell, smooth-walled, bearing 2–3 appendages which disappear when dry. **Asexual morph**: Undetermined.

Type genus: *Melaspileella* (P. Karst.) Vain.

Notes:—*Melaspileella* was introduced by Vainio (1921) and was placed in *Melaspileaceae* within the order *Arthoniales* (Index Fungorum 2018). Ertz & Diederich (2015) lectotypified the genus with *Melaspileella proximella* (Nyl.) and provided SSU sequence data. LSU sequence data was earlier submitted in GenBank for its synonym *Banhegyia setispora* L. Zeller & Tóth. Based on the phylogenetic analysis, Ertz & Diederich (2015) transferred *Melaspileella* to *Asterinales* without assigning it to a family. In our study, the generic type *Melaspileella proximella* forms a single clade close to *Hemigrapha* in *Asterinales* with high bootstrap support (MLBS/BYPP 97/1.00) (Fig. 1). *Melaspileella* is morphologically similar to *Karschia* in having rounded and dark ascomata, but is distinct in the phylogenic analysis (Fig. 1). Herein, *Melaspileellaceae* is introduced to accommodate a single genus *Melaspileella*.

Type species: *Melaspileella proximella* (Nyl.) Ertz & Diederich

Stictographaceae D.Q. Dai & K.D. Hyde, fam. nov.

Index Fungorum number: IF554064; Facesoffungi number: FoF 03912

Lichenicolous on the thallus of lichen. **Sexual morph**: *Ascomata* solitary, scattered to clustered, superficial, cymbiform to lirelliform, or rounded, a slit-like disc, black to dark brown, with a centre ostiole opening by a slit or star-shaped fissure. *Peridium* thick, outer layers composed of black to dark brown, thick-walled cells of *textura angularis*; inner layers composed of light brown to hyaline cells of *textura angularis*. *Hamathecium* of dense, hyaline, filamentous, septate pseudoparaphyses, unbranched or occasionally branched at upper part around the asci. *Asci* 8-spored, bitunicate, wide clavate to subglobose, with an ocular chamber, apically rounded and short pedicellate. *Ascospores* 2-seriate to irregularly arranged, hyaline, becoming light brown to dark brown, ellipsoid, 1-septate, with slightly larger upper cell, with slightly narrower lower cell, smooth-walled. **Asexual morph**: Undetermined.

Type genus: *Stictographa* Mudd, Man. Brit. Lich.: 226 (1861)

Notes:—*Stictographaceae* is introduced here to accommodate several lichenicolous genera or with saprobic life-cycles, and is characterized by cymbiform to lirelliform, or a slit-like disc, black to dark brown ascomata, wide clavate to subglobose asci and brown, ellipsoid ascospores with a single septum. *Stictographa* was collected on the exclusive host *Phaeographis dendritica* Muell in western Europe. It is morphologically similar to *Labrocarpon* Etayo & Pérez-Ortega in having cymbiform ascomata opening by a long slit. However, they can be distinguished in the phylogenetic analysis (Fig. 1). The monotypic genus *Labrocarpon* was introduced by Pérez-Ortega & Etayo (2010) and is typified

by *L. canariense* (D. Hawksw.). In the phylogenetic tree (Fig 1), the type species of *Labrocarpon* clusters within *Stictographaceae*. *Karschia* Körb. was described by Körber (1865) and is typified by *K. talcophila* (Ach.) Körb. Barr (1979) placed *Karschia* in *Patellariaceae* Corda. Then it was transferred to *Lichenotheliaceae*, *Lichenotheliales* by Thambugala *et al.* (2014a) based on the morphology observed from herbarium material. Ertz & Diederich (2015) re-examined the type specimen and sequenced the type species. According to the phylogenetic analysis of Ertz & Diederich (2015), *Karschia* forms a single clade close to *Labrocarpon*, *Melaspileopsis* and *Stictographa*. Our phylogenetic tree (Fig. 1) shows the same result and *Karschia* is phylogenetically distant from *Lichenotheliales*. Thus, *Karschia* is placed in *Stictographaceae*. *Melaspileopsis* was introduced by Ertz & Diederich (2015) and is typified by *M. diplasiospora* (Nyl.) Ertz & Diederich. Index Fungorum (2018) lists the current name of the type species is *Melaspilea diplasiospora* (Nyl.) Müll. Arg. However, the phylogenic analysis shows it does not belong to *Melaspileaceae* Walt. Watson, but clusters close to the clade of *Stictographa* (Fig. 1). *Melaspileopsis* is therefore placed in *Stictographaceae*.
Type species: *Stictographa lentiginosa* (Lyell ex Leight.) Mudd, Man. Brit. Lich.: 226 (1861)

Other genera included:
Karschia Körb., Parerga lichenol. (Breslau) 5: 459 (1865)
Type species: *Karschia talcophila* (Ach.) Körb., Parerga lichenol. (Breslau) 5: 460 (1865)

Labrocarpon Etayo & Pérez-Ortega, in Pérez-Ortega & Etayo, Lichenologist 42(3): 271 (2010)
Type species: *Labrocarpon canariense* (D. Hawksw.) Etayo & Pérez-Ortega [as 'canariensis'], in Pérez-Ortega & Etayo, Lichenologist 42(3): 272 (2010)

Melaspileopsis (Müll. Arg.) Ertz & Diederich, Fungal Diversity 71: 161 (2015)
Type species: *Melaspileopsis diplasiospora* (Nyl.) Ertz & Diederich, Fungal Diversity 71: 161 (2015)

Asterinales* genera *incertae sedis
Inocyclus Theiss. & Syd., Annls mycol. 13(3/4): 211 (1915)
Facesoffungi number: FoF 02315

Parasitic on upper surface of living leaves. **Sexual morph**: *Ascostromata* solitary, scattered or gregarious, superficial, circular or ellipsoidal, flat, black, opening by radiating fissures to irregular slits, internal part of stromata thick and containing a single locule, with a thin light brown tissue under the loculate layer, reaching leaf surface, composed of dark brown cells of *textura angularis*; upper wall of ascostromata comprising dark brown tissue of cells of *textura prismatica*. *Locules* immersed under the upper wall of stromata. *Hamathecium* composed of hyaline, septate, filamentous pseudoparaphyses, extending above the asci. *Asci* 8-spored, bitunicate, subglobose, obovoid, clavate-cylindrical, with a short pedicellate, thick-walled, with rounded apex, smooth, with a large apical chamber. *Ascospores* irregularly arranged, initially hyaline and becoming pale brown to dark brown, ellipsoidal, apiospores, 1-septate, with larger upper cell and shorter lower cell, thin-walled, smooth-walled. **Asexual morph**: See notes.

Notes:—*Inocyclus* was introduced by Theissen & Sydow (1915) for taxa forming black ascostromata opening by radiating fissures to irregular slits on living leaves. It includes eight accepted species, of which two species (*I. discoideusis* (Racib.) Arx and *I. angularis* Guatim. & R.W. Barreto) were reported on ferns (*Polypodiaceae* J. Presl & C. Presl) (Guatimosim *et al.* 2014a). *Inocyclus angularis* has conidiomata intermixed with the ascomata, occupying the same stromata located in the central region of the colonies and hyaline, aseptate, fusiform to clavate conidia reported by Guatimosim *et al.* (2014a). However, the asexual morph of generic type is undetermined. Guatimosim *et al.* (2015) included *I. angularis* in the phylogenetic analysis based on LSU sequences data. It is indicated that *I. angularis* is phylogenetically separate from *Parmulariaceae* but close to species of *Asterotexis*, thus Guatimosim *et al.* (2015) placed *Inocyclus* in *Asterotexales incertae sedis*. However, *Asterotexales* was synonymized under *Asterinales* by Liu *et al.* (2017). Based on the phylogenic analysis in this study (Fig. 1), *Inocyclus* clusters within *Asterinales* and is close to several *Lembosia* species. However, the type species of *Inocyclus* has not been sequenced yet, so we place *Inocyclus* in *Asterinales* genera *incertae sedis*.

Type species: *Inocyclus psychotriae* (Syd. & P. Syd.) Theiss. & Syd., Annls mycol. 13(3/4): 211 (1915), Figure 3, 4

Facesoffungi number: FoF 02316

Lectotype:—K 180637

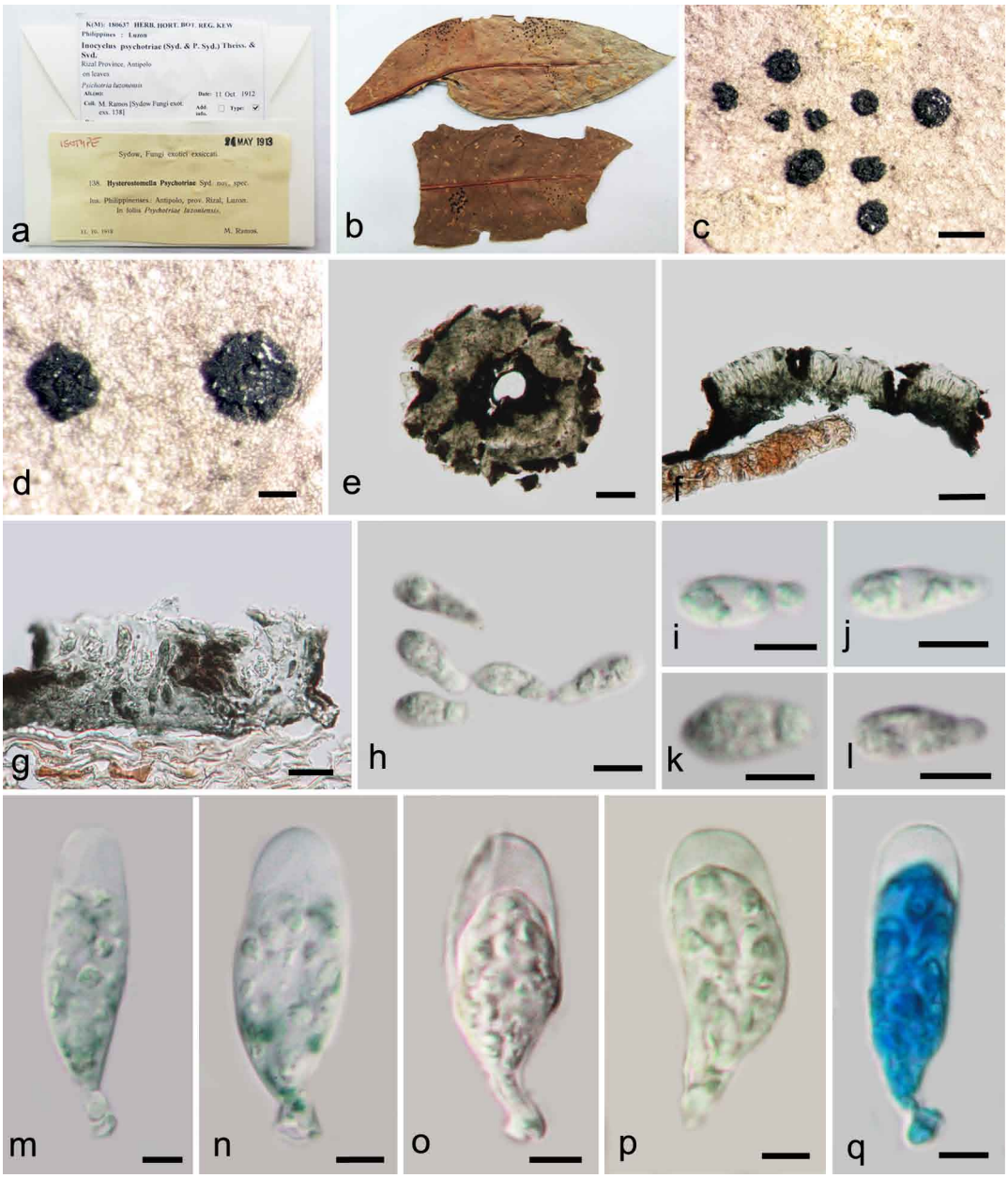

FIGURE 3. *Inocyclus psychotriae* (K 180637, lectotype). a, b. Herbarium material. c, d. Ascostromata on leaves. E. Squash mount of dark brown to black ascostromata. f, g. Hand section of ascostroma (Note: the peridium without apical cells). h–l. Ascospores immersed in KOH. m–q. Asci with ascospores immersed in KOH. Scale bars: c =500 µm, d = 200 µm, e = 100 µm, f = 50 µm, g = 20 µm, h–l = 5 µm, m–q = 10 µm.

Parasitic on upper surface of living leaves. **Sexual morph**: *Ascostromata* 0.3–0.7 mm diam., 100–150 µm high, solitary, scattered or gregarious, superficial, circular or ellipsoidal, flat, black, opening by radiating fissures to irregular slits, internal part of stromata thick and containing a single locule, with a thin light brown tissue under the loculate layer, reaching leaf surface, composed of dark brown cells of *textura angularis*; upper wall of ascostromata comprising 15–20 µm thick, dark brown tissue of cells of *textura prismatica*. *Locules* 140–160 diam., 100–125 µm high, immersed under the upper wall of stromata. *Hamathecium* composed of hyaline, septate, 2–3 µm wide, filamentous pseudoparaphyses, extending above the asci. *Asci* 23–38 × 8–10.5 µm ($\overline{x}$ = 29.7 × 9.8 µm, n = 20), 8-spored, bitunicate, subglobose, obovoid, clavate-cylindrical, with rounded apex, with a short pedicellate, thick and smooth-walled, with a large apical chamber. *Ascospores* 8–11.5 × 3–4 ($\overline{x}$ = 9.3 × 3.8 µm, n = 20), irregularly arranged, initially hyaline and becoming pale brown to dark brown, ellipsoidal, apiosporous, 1-septate, with larger upper cell and shorter lower cell, thin-walled, smooth-walled. **Asexual morph**: Undetermined.

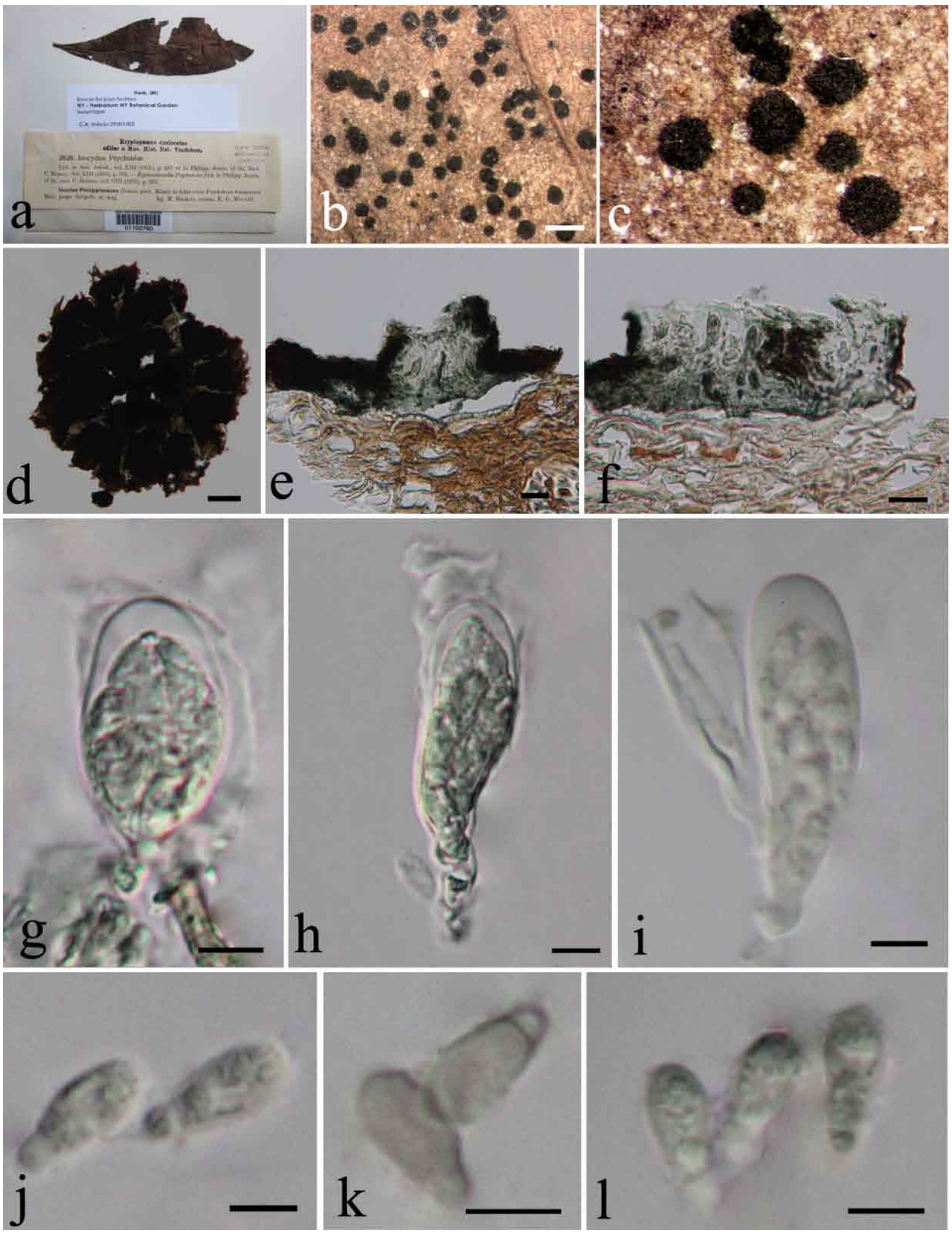

FIGURE 4. *Inocyclus psychotriae* (NY 01102760, isolectotype). a. Herbarium material. b, c. Ascostromata on leaves. D. Squash mount of dark brown to black ascostroma. e, f. Hand section of ascostroma (Note: the peridium without apical cells). g–h. Asci with ascospores immersed in water. i. Asci with ascospores immersed in KOH. j–l. ascospores. Scale bars: b = 1 mm, c = 100 µm, d–f = 20 µm, g–l = 5 µm.

Material examined:—PHILIPPINES, Luzon, Antipolo, Rizal, on living leaves of *Psychotria luzoniensis* (Cham. & Schltdl.) Fern.-Vill., 11 October 1912, M. Ramos, (K 180637, lectotype).

Other specimen examined:—PHILIPPINES, Luzon, Antipolo, Rizal, on living leaves of *Psychotria luzoniensis* (Cham. & Schltdl.) Fern.-Vill., M. Ramos and E.D. Merrill (NY 01102760, isolectotype).

Parmulariales D.Q. Dai & K.D. Hyde, ord. nov.

Index Fungorum number: IF554065; Facesoffungi number: FoF 03913

Epiphytic or *parasitic* on living leaves of plants and ferns or *lichenicolous*. *Colonies* superficial, dark brown to black, rounded to irregular. *Hyphae* usually spreading out from ascomata border. **Sexual morph:** *Ascostromata* solitary, scattered, gregarious, or in groups, superficial to immersed, shield-like, elliptical, dark brown to black, carbonaceous to membranaceous, strongly flattened. *Locules* immersed in ascostromata or in longitudinal ridges radiating from the centre. *Peridium* composed of dark to hyaline, thick to thin cells of *textura prismatica* to *textura angularis*.

Hamathecium of hyaline, septate pseudoparaphyses with brown apices, or pseudoparaphyses lacking. *Asci* 8-spored, bitunicate, broad cylindrical to subglobose, with an ocular chamber. *Ascospores* 2-seriate to irregular arranged, hyaline to dark brown, oblong to ellipsoid, 1-septate, smooth- to verrucose-walled. **Asexual morph**: Undetermined.

Notes:—*Parmulariaceae* has had a varied history in terms of its higher-level placement (Inácio & Cannon 2008), with placements in the orders *Dothiorales*, *Microthyriales*, *Hemisphaeriales*, *Dothideales* and *Hysteriales* (Müller & von Arx 1962, Ainsworth 1971, Luttrell 1973, von Arx & Müller 1975, Barr 1979, Hawksworth *et al.* 1983, 1995). The family has also been placed in Dothideomycetes family *incertae sedis*, as DNA sequence data was not available (Eriksson 1999, Kirk *et al.* 2001). Kirk *et al.* (2008) followed this placement. Inácio & Cannon (2008) reviewed the family with a detailed study, however they did not assign the family *Parmtoulariaceae* to any order. Lumbsch & Huhndorf (2010) maintained *Parmulariaceae* in Dothideomycetes family *incertae sedis*. Hyde *et al.* (2013) re-examined the types of bitunicate families and placed *Parmulariaceae* in the order *Asterinales* based on morphological similarities with *Asterinaceae*. Guatimosim *et al.* (2015) sequenced the type species of type genus of *Parmulariaceae* and placed this family in order *Asterinales*, as *Parmulariaceae* was phylogenetically close to several *Asterina* species. However, Liu *et al.* (2017) placed above mentioned *Asterina* taxa in *Asterinaceae sensu lato* and their lineages are separate from *Asterinales sensu stricto*. Thus, the ordinal placement of *Parmulariaceae* is undetermined. The family *Parmulariaceae* is morphologically and phylogenetically distinct from families of *Asterinales* and justifies a separate order in *Dothideomycetes* (Fig. 1). Thus, *Parmulariales* is recommended here to accommodate a single family *Parmulariaceae*.

Type family: *Parmulariaceae* E. Müll. & Arx ex M.E. Barr, Mycologia 71(5): 944 (1979)

Facesoffungi number: FoF 02296

Epiphytic or *parasitic* on living leaves of plants and ferns or thallus of lichens. *Colonies* superficial, dark brown to black, rounded to irregular. *Hyphae* spreading out from ascomata, brown, septate, branching, sinuous, usually lacking appressoria. **Sexual morph**: *Ascostromata* solitary, scattered, or gregarious, superficial to immersed, shield-like, elliptical to boat-shaped, dark brown to black, carbonaceous to membranaceous, strongly flattened, or longitudinal slits slightly protuberant. *Locules* immersed in ascostromata or in longitudinal ridges radiating from the centre. *Stromata wall* thin to thick, composed of black, large cells of *textura prismatica*. *Wall of locules* thin to thick, composed of dark brown to hyaline smaller cells of *textura angularis*. *Hamathecium* of dark brown to hyaline, septate, pseudoparaphyses or pseudoparaphyses lacking. *Asci* 8-spored, bitunicate, long ovate, broad cylindrical, broad-clavate, or obclavate, globose to subglobose, short pedicellate, with a distinct ocular chamber, thick-walled. *Ascospores* 2–multi-seriate, hyaline to dark brown, oblong to ellipsoid, 1-septate, smooth- to verrucose-walled. **Asexual morph**: Undetermined.

Notes:—The family *Parmulariaceae* was invalidly introduced by Müller & von Arx (1962) to accommodate species with colonies forming on the surface of living plant tissues, mostly leaves, and was later validated by Barr (1979). *Parmulariaceae* contains a range of ascomata types, asci and ascospores forms (Inácio & Cannon 2008), as numerous genera were placed in this family. Lumbsch *et al.* (2007) included 34 genera in *Parmulariaceae* and Inácio & Cannon (2008) included 35 genera. Kirk *et al.* (2008) included 34 genera with 24 synonyms as well as 119 species. Inácio *et al.* (2012) introduced a new genus *Antoniomyces* Inácio, according to its similarity of morphology with *Parmulariaceae*. Hyde *et al.* (2013) accepted 31 genera and excluded four genera *viz. Coccodothis* Theiss. & Syd., *Dothidasteroma* Höhn., *Englerodothis* Theiss. & Syd. and *Perischizon* Syd. & P. Syd. from this family. Guatimosim *et al.* (2014b) introduced a new genus *Rhagadolobiopsis* Guatim. & R.W. Barreto based on morphology. Thambugala *et al.* (2014b) re-examined the herbarium of type species of *Placostromella* Petr. and assign it in *Parmulariaceae*.

The family *Parmulariaceae* can be compared with *Asterinaceae*, however, it differs in having ascostromata produce multi-locules which forming longitudinal ridges radiating from the centre surrounded by thick wall, and formed a developed base, pseudoparaphyses, ellipsoidal to obclavate asci, and usually lack appressoria (Inácio & Cannon 2008, Inácio 2012, Hyde *et al.* 2013). Species of *Asterinaceae* have superficial hyphae mostly with appressoria, small thyriothecia with a thin upper wall layer and poorly developed base, subglobose asci, lack pseudoparaphyses, (Pirozynski & Shoemaker 1970, Inácio 2012, Hongsanan *et al.* 2014). *Parmulariaceae* can also be compared with *Englerulaceae* whose species are usually parasitic on living leaves. *Englerulaceae* however it differs in having small, superficial, globose and deliquescent ascomata on mycelium (Dai *et al.* 2014).

Type genus: *Parmularia* Lév., Annls Sci. Nat., Bot., sér. 3 5: 286 (1846)

Facesoffungi number: FoF 02297

Epiphytic on upper surface of leaves. *Colonies* foliicolous, superficial, dark brown to black, circular. *Hyphae* growing at base of ascomata, brown, septate, branching, sinuous, lacking appressoria. **Sexual morph**: *Ascostromata* large, solitary to groups, gregarious, superficial, shield-like, dark brown to black, carbonaceous, flattened, with ridges radiating from the centre to the outer rim; ridges containing elongated locules, which open by a longitudinal slit and

containing numerous asci; cells of ascostromata wall thick-walled and elongated and composed of black, amorphous tissues in the stromatic crust. *Locules in vertical section* subglobose, immersed within ascostroma. *Upper wall of ascostromata* thick, comprised of several black layers, composed of dark brown cells of *textura prismatica*. *Cells between locules* composed of brown to hyaline cells of *texture angularis*. *Hamathecium* of hyaline, septate, filamentous pseudoparaphyses, swollen, brown and verrucose at their apices. *Asci* 8-spored, thick-walled, bitunicate, cylindrical, short-pedicellate, with a distinct ocular chamber. *Ascospores* 2–3-seriate, hyaline and becoming pale brown when mature, ellipsoidal, 1-septate, with large and round upper cells, with narrow and long lower cells, constricted at the septum, smooth-walled. **Asexual morph**: Undetermined.

Notes:—*Parmularia* is typified by *P. styracis* Lév., introduced by Léveillé (1846). *Parmularia* is similar to *Mintera* in having elongate locules radiating from a central point. However, *Mintera* has mycelium with appressoria, and each black ascostroma with a single locule which aggregate into star-shaped structures. In *Parmularia*, a single ascostroma contains many locules. *Parmularia* comprises numerous species with 53 names in Index Fungorum (2018) and Kirk *et al.* (2008) estimated there are 30 species. However, Inácio & Cannon (2008) compared only six *Parmularia* species, all from Brazil and only one species from New Zealand. *Parmularia* is characterized by large, black, shield-like, flattened ascostromata with ridges radiating from the centre to the outer rim, and the ridges comprising elongated, radiating locules, filamentous pseudoparaphyses with brown and verrucose apices and ellipsoid, 1-septate ascospores (Inácio & Cannon 2008, Hyde *et al.* 2013).

Type species: *Parmularia styracis* Lév., Annls Sci. Nat., Bot., sér. 3 5: 286 (1846). Figure 5

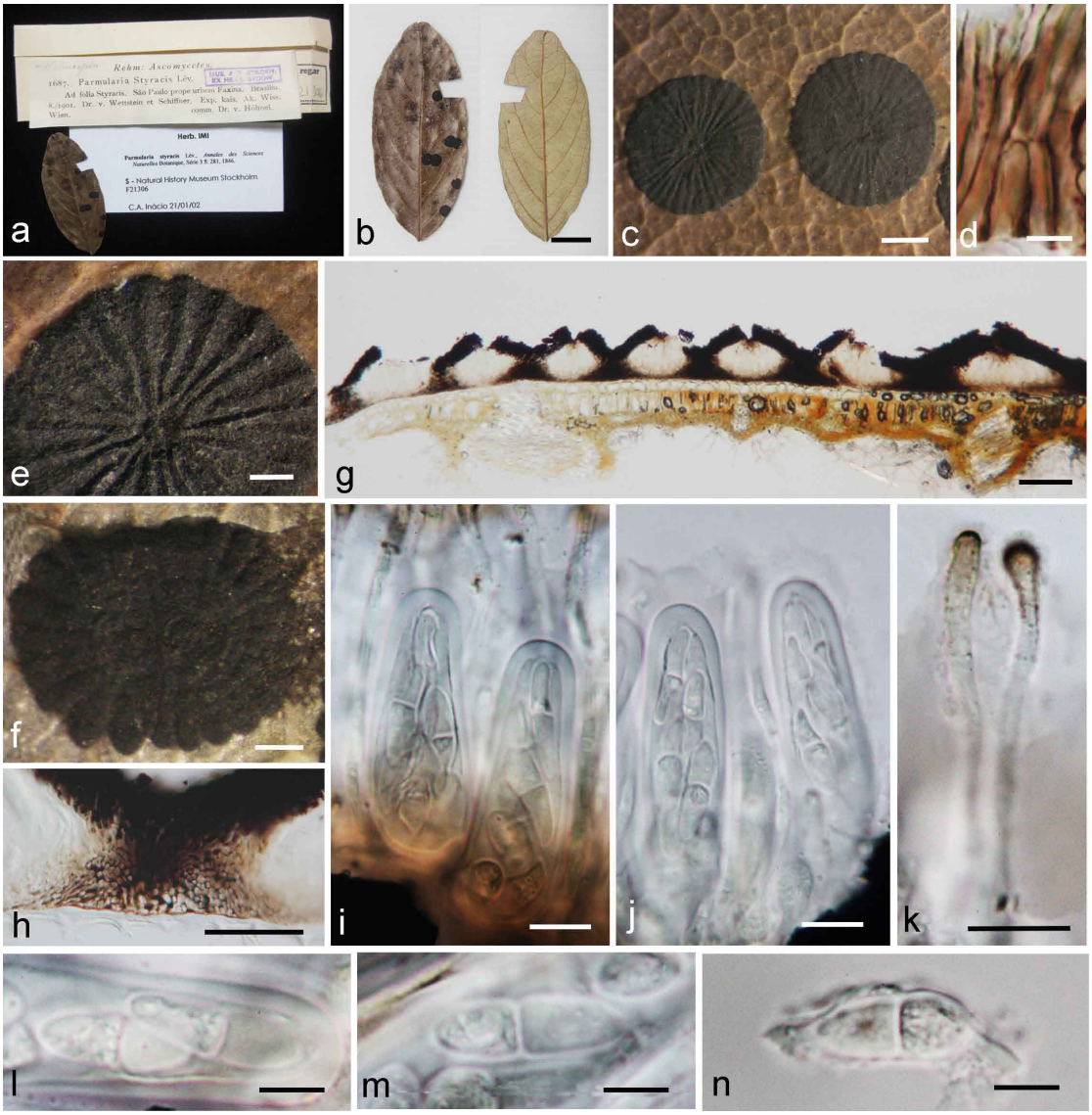

FIGURE 5. *Parmularia styracis* (S F21306). A. Herbarium material. B. Appearance of ascostromata on upper of leaf surface. c, e, f. Black shield-like ascostroma. d. Dark brown ascostroma wall. G. Sections of ascostroma showing multi-locules. H. Wall cells of ascostroma. i, j. Asci with ascospores. K. Pseudoparaphyses with brown swollen apices. l–n. Immature hyaline ascospores. Scale bars: b =10 mm, c =1 mm, d = 5 µm, e, f = 500 µm, g=100 µm, h = 50 µm, i–k = 10 µm, l–n = 10 µm.

Facesoffungi number: FoF 02298

Epiphytic on upper surface of leaves. *Colonies* foliicolous, superficial, dark brown to black, round. *Hyphae* growing out from ascomata, brown, septate, branching, sinuous, lacking appressoria. **Sexual morph**: *Ascostromata* 1–3 mm diam., solitary to gregarious, or in groups, superficial, shield-like, dark brown to black, carbonaceous, flattened, with ridges radiating from the centre to outer rim; ridges containing elongated locules, which open by a longitudinal slit and contain numerous asci, cells of ascostromata thick-walled and elongate and composed of black, amorphous tissues in the stromatic crust. *Locules* in vertical section: 150–250 µm diam., 80–120 µm high, subglobose, immersed in ascostromata. *Upper wall of ascostromata* 20–40 µm thick, comprising several black layers, composed of dark brown cells of *textura prismatica*. *Cells between locules* composed of brown to hyaline cells of *texture angularis*. *Hamathecium* of hyaline, septate, filamentous pseudoparaphyses, 50–80 × 2–3.5 µm, swollen, brown and verrucose at their apices. *Asci* 45–70 × 12–16 µm ($\overline{x}$ = 58.2 × 13.9 µm, n = 20), 8-spored, thick-walled, bitunicate, broadly cylindrical, short-pedicellate, with a distinct ocular chamber. *Ascospores* 13.5–16.5 × 4–6 µm ($\overline{x}$ = 15.6 × 5.1 µm, n = 20), 2–3-seriate, hyaline becoming pale brown when mature, ellipsoidal, 1-septate, with larger and rounder upper cells, with narrow and longer lower cells, constricted at the septum, smooth-walled. **Asexual morph**: Undetermined.

Material examined:—BRAZIL, Brasilia, São Paolo, near Urbem Faxina, on leaves of *Styracis* sp., August 1901, v. Wettstein & Schiffner (S F21306).

Notes:—*Parmularia styracis* described from leaves of *Styrax* L., collected in Brazil (Léveillé 1846). Guatimosim *et al.* (2015) recollected and epitypified this species and provided a phylogenetic analysis based on LSU sequence data. Inácio & Cannon (2008) recorded the ascostromata of *P. styracis* as 0.3–1.6 mm in diam.; however, Guatimosim *et al.* (2015) indicated the epitype having 1–3 mm diam. ascostromata. We also found the ascostromata to be 1–3 mm diam. on the material we examined. The holotype of this species was collected in Brazil on the leaves of *Styracis* sp. and was deposited in PC (Index Herbariorum 2018), however, a later specimen collected from same country on same host was examined in this study.

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