

## A phylogenetic framework for reassessing generic concepts and species delimitation in the lichenized family *Trypetheliaceae* (Ascomycota: Dothideomycetes)

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**Abstract:** We provide an expanded and updated, 2-locus phylogeny (mtSSU, nuLSU) of the lichenized fungal family *Trypetheliaceae*, with a total of 196 ingroup OTUs, in order to further refine generic delimitations and species concepts in this family. As a result, the following 15 clades are recognized as separate genera, including five newly established genera: *Aptrootia*, *Architypethelium*, *Astrothelium* (including the bulk of corticate species with astrothelioid ascospores; synonyms: *Campylothelium*, *Cryptothelium*, *Laurera*), *Bathelium* s. str. (excluding *B. degenerans* and relatives which fall into *Astrothelium*), the reinstated *Bogoriella* (for tropical, lichenized species previously placed in *Mycomicrothelia*), *Constrictolumina* gen. nov. (for tropical, lichenized species of *Arthopyrenia*), *Dictyomeridium* gen. nov. (for a subgroup of species with muriform ascospores previously placed in *Polymeridium*), *Julella* (provisionally, as the type species remains unsequenced), *Marcelaria* (*Laurera purpurina* complex), *Nigrovothelium* gen. nov. (for the *Trypethelium tropicum* group), *Novomicrothelia* gen. nov. (for an additional species previously placed in *Mycomicrothelia*),

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*Polymeridium* s. str., *Pseudopyrenula*, *Trypethelium* s. str. (*T. eluteriae* group), and *Viridothelium* gen. nov. (for the *Trypethelium virens* group). All recognized genera are phenotypically characterized and a discussion on the evolution of phenotypic features in the family is given. Based on the obtained phylogeny, species delimitations are revised and the importance of characters such as thallus morphology, hymenial inspersion, and secondary chemistry for taxonomic purposes is discussed, resulting in a refined species concept.

**Key words:** evolution, genus concepts, lichenized fungi, phenotypic characters, species concepts

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## Introduction

*Trypetheliaceae* is one of the dominant elements of lichen communities in tropical rainforests, dry forests, and savannas (Komposch & Hafellner 2000, 2003; Komposch *et al.* 2002; Aptroot *et al.* 2008, 2013; Aptroot 2009*a, b*). The family was traditionally considered to belong in a suite of crustose, lichen-forming fungi with pyrenocarpous ascomata, and bitunicate (fissitunicate) asci, and was alternatively placed in the orders *Pyrenulales* or *Melanommatales* (Barr 1979, 1987; Harris 1984, 1991; Kirk *et al.* 2001; Eriksson *et al.* 2004; Cannon & Kirk 2007). Eriksson (1981) elaborated a detailed scheme about the putative evolution of this group, postulating that a trypethelioid precursor, the so-called  $\alpha$ -*Trypetheliaceae*, gave rise to both the *Pyrenulaceae* and *Trypetheliaceae*. *Pyrenulales* are now classified near *Verrucariales*

within the Eurotiomycetes, whereas *Melanommatales* have been synonymized with *Pleosporales* in the Dothideomycetes (Berbee 1996; Liew *et al.* 2000; Lumbsch & Lindemuth 2001; Weerakoon *et al.* 2012), which generated ambiguity as to the correct placement of *Trypetheliaceae*. Inclusion of a single taxon in the first AFTOL study (Lutzoni *et al.* 2004) suggested placement of the family within Dothideomycetes, which was subsequently confirmed by Del Prado *et al.* (2006) with a more target-oriented taxon sampling, and by Nelsen *et al.* (2009) and Schoch *et al.* (2009) with phylogenetic studies focusing on Dothideomycetes. However, the family consistently clustered outside the *Pleosporales* and a separate order, *Trypetheliales*, was established for it (Aptroot *et al.* 2008). While Nelsen *et al.* (2009) demonstrated the close relationship between tropical *Mycromicrothelia* species and *Trypetheliaceae*,

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a subsequent phylogenetic study suggested inclusion of these species within the family, together with further species in the collective genera *Arthopyrenia* and *Julella* (Nelsen et al. 2011). A second family, *Polycoccaceae*, which chiefly includes lichenicolous fungi, has been recently established and included in *Trypetheliales* (Ertz et al. 2015).

Genera in *Trypetheliaceae* were traditionally separated by thallus structure, ascoma disposition, and ascospore type (Letrouit-Galinou 1957, 1958; Makhija & Patwardhan 1988, 1993; Harris 1989a, b, 1990, 1991, 1995; Aptroot 1991a, b, 2009a, b; Aptroot et al. 2008; Lumbsch & Huhndorf 2010). Thus, the core group with corticate thalli and typically astrothelioid ascospores (with diamond-shaped lumina) was divided into five genera according to ascoma disposition and ascospore septation: *Trypethelium* (solitary to aggregate ascomata with apical, separate ostioles, ascospores transversely septate), *Laurera* (solitary to aggregate ascomata with apical, separate ostioles, ascospores muriform), *Astrothelium* (solitary to aggregate ascomata with lateral, separate or fused ostioles, ascospores transversely septate), *Cryptothelium* (aggregate ascomata with lateral, fused ostioles, ascospores muriform), and *Campylothelium* (solitary ascomata with lateral, separate ostioles, ascospores muriform). In addition, the genus *Pseudopyrenula* was distinguished by its white, ecorticate thallus, combined with astrothelioid ascospores, whereas *Polymeridium* was defined as having an ecorticate thallus and thin-walled ascospores. This classification was not only considered artificial (Harris 1989a, 1995; Del Prado et al. 2006; Aptroot et al. 2008), but was also flawed logically, since species with separate or fused ostioles were united under a single genus (*Astrothelium*) if ascospores were transversely septate but separated into two genera (*Campylothelium*, *Cryptothelium*) if ascospores were muriform. Also, species in the *Trypethelium eluteriae* group have ascospores more similar to those of *Polymeridium*, lacking diamond-shaped lumina.

Harris (1989b, 1991, 1995) was the first to try and establish more natural genera, such as *Bathelium*, characterized by a suite of morpho-anatomical and chemical characters. The

genera *Aptrootia* and *Architrypethelium* were introduced more recently for species with unique ascospore types (Aptroot 1991b; Lücking et al. 2007). Molecular phylogenetic studies then revealed the inclusion of some species of *Arthopyrenia*, *Julella*, and *Mycomicrothelia* in a basal position in the family (Nelsen et al. 2009, 2011), all with morphologies similar to *Polymeridium* and *Pseudopyrenula* (with ecorticate thalli and mostly exposed ascomata).

Generic delimitation within *Trypetheliaceae* was evaluated in an expanded study (Nelsen et al. 2014), illustrating that a number of genera recognized within *Trypetheliaceae* were well defined, while the boundaries of several others required substantial adjustments. Thus, the bulk of the species with a corticate thallus and astrothelioid ascospores was suggested to be included in a single clade, *Astrothelium*, regardless of ascomatal disposition or ascospore septation; with such a modified concept, *Cryptothelium* and *Laurera* would become synonyms of *Astrothelium*, although no formal changes were proposed. In contrast, *Trypethelium* was to be retained for species in the *T. eluteriae* group, with aggregate, sessile pseudostromata and a unique ascospore type. The genera *Aptrootia* and *Architrypethelium*, and presumably also *Campylothelium*, were confirmed as monophyletic, whereas *Bathelium* s. str. with muriform ascospores formed a separate clade outside *Astrothelium*, with some species with transversely-septate ascospores previously assigned to this genus now included in *Astrothelium*. In addition, the genus *Marcelaria* was established for the enigmatic species *Laurera purpurina* and its relatives (Aptroot et al. 2013). Differences in ascospore type and ontogeny were found to reflect this modern understanding of phylogenetic relationships within the family (Sweetwood et al. 2012).

Species circumscriptions within *Trypetheliaceae* were also rather schematically based on ascospore size and septation, and the formation of anthraquinone and perylenequinone pigments, recognizing c. 200 taxa (Harris 1984; Aptroot 1991b; Del Prado et al. 2006; Aptroot et al. 2008). Characters such as hymenial impersation, lichexanthone synthesis (Harris

1991, 1995, 1998), and particularly thallus morphology were rarely, if at all, considered to be taxonomically important. The pigments, on the other hand, were subject to numerous rather detailed studies on their chemical structures and possible functions (Stensiö & Wachtmeister 1969; Culberson & Culberson 1970; Mathey & Hoder 1978; Harris 1984; Mathey *et al.* 1987, 1994; Aptroot 1991*b*; Mathey & Lukins 2001; Manojlovic *et al.* 2010).

Here we present a further, much expanded phylogenetic study based on two loci, the mitochondrial small subunit (mtSSU) and the nuclear large subunit (nuLSU) of the rDNA cistron, including a total of 196 operational taxonomic units (OTU's) spanning the entire diversity of the family. While providing a much-refined framework for revised generic delimitations, this study also focuses on the problem of species delimitation and the potential importance of phenotypic characters that have hitherto been neglected for taxonomic purposes in this family.

## Material and Methods

We included all available mtSSU and nuLSU data on *Trypetheliaceae* published in previous studies (Lutzoni *et al.* 2004; Del Prado *et al.* 2006; Nelsen *et al.* 2009, 2011, 2014) and added a total of 155 new sequences (100 mtSSU, 55 nuLSU) for 117 OTUs (Table 1). The only genera putatively placed within the family for which sequence data could not be obtained were *Exiliseptum* (Harris 1984) and *Melanophloea* (Aptroot & Schumm 2012). *Cladospodium cladosporioides* was used as outgroup following Nelsen *et al.* (2014).

For newly generated sequences, the Sigma-Aldrich REDEExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, USA) was used to isolate DNA, following the manufacturer's instructions, except only 10–30 µl of extraction buffer and 10–30 µl dilution buffer were used, and a 20× DNA dilution was then used in subsequent PCR reactions. A portion of the fungal mitochondrial small subunit (mtSSU) was amplified and sequenced using combinations of the following primers: mrSSU1, mrSSU2, mrSSU2R, mrSSU3R (Zoller *et al.* 1999), MSU7 (Zhou & Stanosz 2001), mrSSU-1/2-5'-mpn and mrSSU-2/3-3'-mpn (Nelsen *et al.* 2011). Additionally, a portion of the fungal nuclear large subunit (nuLSU) was amplified and sequenced using combinations of the primers f-nu-LSU-0116-5'/ITS4A-5' (Nelsen *et al.* 2011, 2012), AL2R (Mangold *et al.* 2008), f-nu-LSU-0287-5'-mpn (Nelsen *et al.* 2011), LR3 (Vilgalys & Hester 1990), LR3R (reverse

complement of LR3), LR4 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>), LR5 and LR6 (Vilgalys & Hester 1990).

The 10 µl PCR reactions consisted of 5 µM of each PCR primer, 3 mM of each dNTP, 2 µl of 10 mg/ml 100X BSA (New England BioLabs, Ipswich, Massachusetts, USA), 1.5 µl 10× PCR buffer (Roche Applied Science, Indianapolis, Indiana, USA), 0.5 µl Taq, approximately 2 µl diluted DNA, and 2 µl water or 2.5–5 µl REDEExtract-n-Amp PCR Ready Mix (Sigma-Aldrich, St. Louis, Missouri, USA), 5 µM of each PCR primer, 2 µl diluted DNA and 2–4.5 µl water. The PCR cycling conditions were as follows: 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 53 °C (mtSSU), 55 °C (nuLSU: AL2R/LR3) or 60 °C (nuLSU: f-nu-LSU-0116-5'/ITS4A-5') with LR3 or LR6) for 1 min, and 72 °C for 1 min, followed by a single 72 °C final extension for 7 min. Samples were visualized on an ethidium bromide-stained 1% agarose gel under UV light and bands were gel extracted, heated at 70 °C for 5 min, cooled to 45 °C for 10 min, treated with 1 µl GELase (Epicentre Biotechnologies, Madison, Wisconsin, USA) and incubated at 45 °C for at least 24 h. The 10 µl cycle sequencing reactions consisted of 1–1.5 µl of Big Dye version 3.1 (Applied Biosystems, Foster City, California, USA), 2.5–3 µl of Big Dye buffer, 1–6 µM primer (primers listed above), 0.75–2 µl GELase-treated PCR product and water. Cycle sequencing was performed using one of the following conditions: 96 °C for 1 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min or instead 96 °C for 1 min, followed by 40 cycles of 96 °C for 10 s, 45 °C for 5 s and 60 °C for 4 min. Samples were precipitated and sequenced in an Applied Biosystems 3730 DNA Analyzer (Foster City, California, USA), and sequences assembled in Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA). DNA analyses were performed at the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum.

Sequences were automatically aligned using MAFFT 6.935 with sorting function (Katoh *et al.* 2009). The unaligned sequences were also subjected to assessment of alignment ambiguity through the Guidance web server (Penn *et al.* 2010*a, b*). No substantial ambiguity was detected for the nuLSU locus, whereas three large, ambiguously aligned regions were found in the mtSSU locus. These were removed from the alignment and separately recoded using PICS-Ord 1.0 (Lücking *et al.* 2011). After testing for potential conflict between the trimmed mtSSU and the nuLSU loci using Compat.py 3.0 (Kauff & Lutzoni 2002, 2003), with no conflicts detected, the two loci were merged together with the PICS-Ord codes (see Supplementary Material A, available online) and a combined, partitioned maximum likelihood analysis (by locus and codes) was performed in RAXML 7.2.8 (Stamatakis 2006), employing a GTRGAMMA model for the nucleotide partitions and GTR for the code partition. Support was estimated by performing 1000 fast bootstrap pseudoreplicates (Stamatakis *et al.* 2008).

For selected portions of the resulting topology, we employed the Shimodeira-Hasegawa (SH) test as implemented in RAXML 8.0.2 to test whether alternative topologies could be rejected.

TABLE 1. *GenBank Accession numbers and voucher information for taxa and samples used in this study. INB → CR indicates that the collections previously held at INB (National Biodiversity Institute, Costa Rica) are currently in the process of being transferred to CR (National Herbarium, Costa Rica).*

Species	Extract	Country	Collector	Number	Repository	GenBank Accession numbers	
						mtSSU	nuLSU
<i>Cladosporium cladosporioides</i>	(outgroup)					FJ190628	DQ678057
<i>Aptrootia elatior</i>	MPN560B	New Zealand	<i>Knight</i>	O61815	(OTA)	KM453821	KM453754
<i>A. robusta</i>	MPN235B	Australia	<i>Lumbsch</i>	20012	(F)	KM453822	KM453755
<i>A. terricola</i>	DNA1501	Costa Rica	<i>Lücking</i>	17211	(F, INB → CR)	DQ328995	KM453756
<i>Architrypethelium lauropaluanum</i>	MPN48	Peru	<i>Nelsen</i>	Cit1P	(F)	KX215566	KX215605
<i>A. nitens</i>	MPN257	Panama	<i>Lücking</i>	27038	(F, PMA)	KM453823	KM453757
<i>A. uberinum</i>	MPN489	Brazil	<i>Nelsen</i>	s. n.	(F, SP)	–	KM453758
<i>Astrothelium aeneum</i>	MPN302	Panama	<i>Lücking</i>	27056	(F, PMA)	–	KX215606
<i>A. aff. crassum</i>	MPN335	Brazil	<i>Cáceres</i>	6011	(F, ISE)	KM453827	KM453761
<i>A. aff. megaspermum</i>	MPN190	Philippines	<i>Rivas Plata</i>	2093	(F, Saint Louis University)	KM453852	KM453787
<i>A. aff. norisianum</i>	MPN23B	Peru	<i>Nelsen</i>	Cit1B	(F)	KX215578	KX215607
<i>A. aff. obscurum</i>	MPN194	Philippines	<i>Rivas Plata</i>	2175	(F, Saint Louis University)	–	KX215608
<i>A. aff. sepultum</i>	MPN229	Costa Rica	<i>Lücking</i>	21027	(F)	–	KX215609
<i>A. aff. sepultum</i>	MPN63C	Peru	<i>Nelsen</i>	4001a	(F)	GU327690	GU327714
<i>A. bicolor</i>	MPN139	USA	<i>Nelsen</i>	4002a	(F)	GU327706	GU327728
<i>A. carassense</i>	MPN438	Brazil	<i>Lücking</i>	31004	(F, SP)	KM453849	KM453784
<i>A. cecidiogenum</i>	N/A	Costa Rica	<i>Lücking</i>	s. n.	(F, INB → CR)	DQ328991	–
<i>A. cinereorosellum</i>	MPN191	Philippines	<i>Rivas Plata</i>	2110	(F, Saint Louis University)	KM453873	KM453809
<i>A. cinereorosellum</i>	MPN199C	Philippines	<i>Rivas Plata</i>	2106	(F, Saint Louis University)	–	KX215610
<i>A. cinnamomeum</i>	AFTOL110	Costa Rica	<i>Lücking</i>	15322b	(DUKE, F, INB → CR, USJ)	AY584632	AY584652
<i>A. crassum</i>	MPN98	Peru	<i>Nelsen</i>	s. n.	(F)	GU327685	GU327710
<i>A. croceum</i>	MPN55	Peru	<i>Nelsen</i>	211D	(F)	KX215567	KX215611
<i>A. degenerans</i>	DNA1496	Costa Rica	<i>Lücking</i>	17502b	(CR, F, INB → CR, USJ)	DQ328987	–
<i>A. degenerans</i>	DNA1497	Costa Rica	<i>Lücking</i>	16657	(F)	DQ328988	KX215612
<i>A. degenerans</i>	MPN266	Panama	<i>Lücking</i>	27131x	(F, PMA)	–	KX215613
<i>A. degenerans</i>	MPN267	Panama	<i>Lücking</i>	27109	(F, PMA)	KM453835	KM453770
<i>A. degenerans</i>	MPN397	Galapagos	<i>Rivas Plata</i>	4065	(CDS, F)	KM453838	KM453773
<i>A. diplocarpum</i>	MPN134	USA	<i>Nelsen</i>	s. n.	(F)	KX215568	–
<i>A. diplocarpum</i>	MPN210	Nicaragua	<i>Lücking</i>	28529	(F, HNMN)	KM453846	KM453781
<i>A. endochryseum</i>	MPN436	Brazil	<i>Lücking</i>	31088	(F, SP)	KM453837	KM453772
<i>A. erubescens</i>	MPN96	Peru	<i>Nelsen</i>	AnaG	(F)	KX215569	KX215614
<i>A. euthelium</i>	MPN226	Thailand	<i>Lücking</i>	24075	(F, RAMK)	–	KX215615
<i>A. euthelium</i>	MPN22B	Philippines	<i>Rivas Plata</i>	1194B	(F)	–	KX215616
<i>A. floridanum</i>	DNA1503	Costa Rica	<i>Lücking</i>	17090b	(F, USJ)	DQ329007	–
<i>A. floridanum</i>	MPN132	USA	<i>Nelsen</i>	4008	(F)	GU327705	GU327727
<i>A. floridanum</i>	MPN214	Nicaragua	<i>Lücking</i>	28558	(F, HNMN)	–	KX215617



TABLE 1 (continued).

Species	Extract	Country	Collector	Number	Repository	GenBank Accession numbers	
						mtSSU	nuLSU
<i>Astrothelium floridanum</i>	MPN216	Nicaragua	Lücking	28564	(F, HNMN)	–	KX215618
<i>A. floridanum</i>	MPN304	Panama	Lücking	27131a	(F, PMA)	KM453876	KM453811
<i>A. gigantosporum</i>	MPN590	Panama	Lücking	33037	(F, PMA)	KM453851	KM453786
<i>A. grossum</i>	MPN259	Panama	Lücking	27045	(F, PMA)	KM453834	KM453769
<i>A. grossum</i>	MPN262	Panama	Lücking	27083	(F, PMA)	–	KX215619
<i>A. grossum</i>	MPN263	Panama	Lücking	27131v	(F, PMA)	–	KX215620
<i>A. grossum</i>	MPN264	Panama	Lücking	27084	(F, PMA)	–	KX215621
<i>A. grossum</i>	MPN47	Peru	Nelsen	4000a	(F)	GU327689	GU327713
<i>A. grossum</i>	MPN657	Panama	Nelsen	s. n.	(F, PMA)	KX215570	KX215622
<i>A. grossum</i>	MPN703	Brazil	Cáceres	11137	(F, ISE)	–	KM453765
<i>A. grossum</i>	MPN751	Puerto Rico	Mercado	590	(F, UPR)	–	KX215623
<i>A. grossum</i>	MPN770	Fiji	Lumbsch	20556h	(F)	KM453831	KM453766
<i>A. inspersoaneum</i>	MPN45	Peru	Nelsen	Cit1K	(F)	KX215571	–
<i>A. kunzei</i>	MPN201B	El Salvador	Lücking	28120	(F, ITIC)	–	KX215624
<i>A. kunzei</i>	MPN203B	El Salvador	Lücking	28137	(F, ITIC)	–	KX215625
<i>A. laevigatum</i>	MPN430	Brazil	Lücking	31010	(F, SP)	KX215572	–
<i>A. laevithallinum</i>	MPN442	Brazil	Lücking	31061	(F, SP)	KM453836	KM453771
<i>A. leucoconicum</i>	MPN42	Peru	Nelsen	4000c	(F)	KM453830	KM453764
<i>A. leucosessile</i>	MPN258	Panama	Lücking	27059	(F, PMA)	KM453828	KM453762
<i>A. leucosessile</i>	MPN265	Panama	Lücking	27093	(F, PMA)	–	KX215626
<i>A. leucosessile</i>	MPN58C	Peru	Nelsen	AnaE	(F)	–	KX215627
<i>A. leucosessile</i>	MPN713	Brazil	Cáceres	11201	(F, ISE)	KM453869	KM453805
<i>A. macrocarpum</i>	MPN131	USA	Nelsen	s. n.	(F)	KX215573	KX215628
<i>A. macrocarpum</i>	MPN260	Panama	Lücking	27077	(F, PMA)	KM453829	KM453763
<i>A. macrocarpum</i>	MPN261	Panama	Lücking	27052	(F, PMA)	–	KX215629
<i>A. macrocarpum</i>	MPN339	Brazil	Cáceres	6014	(F, ISE)	–	KX215630
<i>A. macrocarpum</i>	MPN57C	Peru	Nelsen	AnaA	(F)	–	KX215631
<i>A. megaspermum</i>	AFTOL2094	Gabon	Ertz	9725	(BR)	GU561847	FJ267702
<i>A. megaspermum</i>	MPN138	USA	Nelsen	s. n.	(F)	KX215574	KX215632
<i>A. megaspermum</i>	MPN234	Costa Rica	Lücking	s. n.	(F)	KX215575	KX215633
<i>A. megaspermum</i>	MPN32B	Thailand	Nelsen	s. n.	(F, RAMK)	KX215576	–
<i>A. meristosporum</i>	MPN189	Philippines	Rivas Plata	2108	(F, Saint Louis University)	KM453850	KM453785
<i>A. meristosporum</i>	MPN198	Philippines	Rivas Plata	2128	(F, Saint Louis University)	–	KX215634
<i>A. neogalbineum</i>	MPN51	Peru	Nelsen	Cit1T	(F)	KX215577	KX215635
<i>A. neogalbineum</i>	MPN711	Brazil	Cáceres	11100	(F, ISE)	KM453877	KM453812
<i>A. neoinspersum</i>	MPN61C	Peru	Nelsen	AnaJ	(F)	–	KX215636
<i>A. neoinspersum</i>	MPN62	Peru	Nelsen	s. n.	(F)	KM453866	KM453802

TABLE 1 (continued).

Species	Extract	Country	Collector	Number	Repository	GenBank Accession numbers	
						mtSSU	nuLSU
<i>Astrothelium nicaraguense</i>	MPN205	Nicaragua	Lücking	28503	(F, HNMN)	–	KX215637
<i>A. nicaraguense</i>	MPN211	Nicaragua	Lücking	28546	(F, HNMN)	–	KX215638
<i>A. nicaraguense</i>	MPN213	Nicaragua	Lücking	28551	(F, HNMN)	–	KX215639
<i>A. nitidiusculum</i>	MPN704	Brazil	Cáceres	11297	(F, ISE)	KM453868	KM453804
<i>A. nitidiusculum</i>	MPN768	Fiji	Lumbsch	20547i	(F)	–	KX215640
<i>A. norisianum</i>	MPN52C	Peru	Nelsen	4000d	(F)	KM453848	KM453783
<i>A. obtectum</i>	MPN422	Brazil	Lücking	31242	(F, SP)	KM453832	KM453767
<i>A. perspersum</i>	AFTOL2099	Gabon	Ertz	9716	(BR)	GU561848	FJ267701
<i>A. phlyctaena</i>	MPN373	USA	Nelsen	4167	(F)	–	KX215641
<i>A. phlyctaena</i>	MPN376	USA	Nelsen	4166	(F)	–	KX215642
<i>A. phlyctaena</i>	MPN381	USA	Nelsen	4173	(F)	–	KX215643
<i>A. phlyctaena</i>	MPN386	USA	Nelsen	4149	(F)	–	KX215644
<i>A. pulcherrimum</i>	MPN313	Panama	Lücking	27046	(F, PMA)	KM453879	KM453814
<i>A. pupula</i>	MPN224	Colombia	Lücking	26305	(F, UDBC)	KM453880	KM453815
<i>A. purpurascens</i>	MPN53C	Peru	Nelsen	s. n.	(F)	KM453847	KM453782
<i>A. robustum</i>	MPN209	Nicaragua	Lücking	28519	(F, HNMN)	–	KX215645
<i>A. robustum</i>	MPN212	Nicaragua	Lücking	28547	(F, HNMN)	–	KX215646
<i>A. robustum</i>	MPN221	Guatemala	Lücking	25558	(BIGU, F)	–	KX215647
<i>A. robustum</i>	MPN222	Guatemala	Lücking	25559	(BIGU, F)	–	KX215648
<i>A. robustum</i>	MPN223	Guatemala	Lücking	25534	(BIGU, F)	–	KX215649
<i>A. robustum</i>	MPN754	Costa Rica	Mercado	586	(F)	KM453826	KM453760
<i>A. rufescens</i>	MPN143	Brazil	Nelsen	B1	(F, SP)	–	KX215650
<i>A. rufescens</i>	MPN345	Argentina	Lücking	30510	(CTES, F)	–	KX215651
<i>A. rufescens</i>	MPN346	Argentina	Lücking	30511	(CTES, F)	–	KX215652
<i>A. sanguinarium</i>	MPN765	Brazil	Cañez	3133	(CGMS, F)	KM453853	KM453788
<i>A. sanguinarium</i>	MPN766	Brazil	Cañez	3135	(CGMS, F)	KX215579	KX215653
<i>A. sanguinarium</i>	MPN767	Brazil	Cañez	3137a	(CGMS, F)	KX215580	KX215654
<i>A. scoria</i>	MPN310	Panama	Lücking	27181	(F, PMA)	–	KX215655
<i>A. scorizum</i>	MPN336	Brazil	Lücking	29814	(F, SP)	KM453872	KM453808
<i>Astrothelium</i> sp. (pycnidia)	MPN133	USA	Nelsen	s. n.	(F)	KX215581	KX215656
<i>Astrothelium</i> sp. (pycnidia)	MPN162	Brazil	Nelsen	B20	(F, SP)	KX215582	KX215657
<i>Astrothelium</i> sp. (pycnidia)	MPN390	USA	Nelsen	4082A	(F)	–	KX215658
<i>A. subcatervarium</i>	MPN97	Peru	Nelsen	4009a	(F)	GU327707	GU327729
<i>A. subendochriseum</i>	MPN202B	El Salvador	Lücking	28121	(F, ITIC)	–	KX215659
<i>A. subinterjectum</i>	MPN157	Brazil	Nelsen	B15	(F, SP)	KX215583	KX215660
<i>A. subscoria</i>	MPN217	Nicaragua	Lücking	28640	(F, HNMN)	KM453878	KM453813
<i>A. subscoria</i>	MPN325	Bolivia	Lücking	29010	(F)	KX215584	KX215661

TABLE 1 (continued).

Species	Extract	Country	Collector	Number	Repository	GenBank Accession numbers	
						mtSSU	nuLSU
<i>Astrothelium tuberculosum</i>	DNA1504	Costa Rica	Lücking	16306a	(F, INB → CR)	DQ329008	–
<i>A. variolosum</i>	MPN41	Peru	Nelsen	Cit1F	(F)	KX215585	KX215662
<i>A. variolosum</i>	MPN43	Peru	Nelsen	s. n.	(F)	KM453833	KM453768
<i>Bathelium lineare</i>	MPN741	Vietnam	Gueidan	2078	(F)	KM453839	KM453774
<i>B. madreporiforme</i>	MPN354	Brazil	Lücking	23290	(F, SP)	KM453840	KM453775
<i>B. madreporiforme</i>	MPN355	Brazil	Lücking	23271	(F, SP)	–	KX215663
<i>B. madreporiforme</i>	MPN356	Brazil	Lücking	23248	(F, SP)	–	KX215664
<i>B. porinosporum</i>	MPN743	Vietnam	Gueidan	3040	(F)	KM453841	KM453776
<i>B. porinosporum</i>	MPN744	Vietnam	Gueidan	3055	(F)	KX215586	KX215665
<i>B. porinosporum</i>	MPN745	Vietnam	Gueidan	3041	(F)	–	KX215666
<i>B. porinosporum</i>	MPN747	Vietnam	Gueidan	3053	(F)	KX215587	KX215667
<i>B. tuberculosum</i>	MPN112	India	Lumbsch	19733j	(F)	–	KX215668
<i>B. tuberculosum</i>	MPN113	India	Lumbsch	19739z	(F)	–	KX215669
<i>B. tuberculosum</i>	MPN81	India	Lumbsch	19739z	(F)	KM453842	KM453777
<i>Bogoriella hemisphaerica</i>	MPN102	Nicaragua	Lücking	28641	(F, HNMN)	GU327695	GU327719
<i>B. miculiformis</i>	MPN101B	Nicaragua	Lücking	28637	(F, HNMN)	GU327696	GU327720
<i>B. minutula</i>	MPN567	Thailand	Nelsen	s. n.	(F, RAMK)	KM453856	–
<i>Constrictolumina bifera</i>	MPN574	Thailand	Nelsen	s. n.	(F, RAMK)	KM453824	–
<i>C. cinchonae</i>	MPN333	Brazil	Lücking	29583	(F, SP)	JN872349	JN872351
<i>C. cinchonae</i>	MPN417	Brazil	Lücking	s. n.	(F, SP)	KM453825	KM453759
<i>C. planorbis</i>	MPN330	Brazil	Lücking	29532	(F, SP)	–	KX215670
<i>C. planorbis</i>	MPN331	Brazil	Lücking	29587	(F, SP)	–	KX215671
<i>C. planorbis</i>	MPN332	Brazil	Lücking	29585	(F, SP)	–	KX215672
<i>C. planorbis</i>	MPN334	Brazil	Lücking	29584	(F, SP)	JN872350	JN872352
<i>C. planorbis</i>	MPN352	Brazil	Lücking	23289	(F, SP)	–	JN872353
<i>C. planorbis</i>	MPN353	Brazil	Lücking	23228	(F, SP)	–	JN872354
<i>Constrictolumina</i> sp.	MPN575	Thailand	Nelsen	s. n.	(F, RAMK)	KX215588	–
<i>Dictyomeridium propomense</i>	MPN359	Venezuela	Lücking	26103	(F, VEN)	KM453860	JN887403
<i>Julella fallaciososa</i>	MPN141	USA	Nelsen	s. n.	(F)	JN887411	JN887399
<i>J. fallaciososa</i>	MPN547	USA	Nelsen	s. n.	(F)	JN887412	JN887400
<i>Marcelaria cumingii</i>	MPN552	Thailand	Parmmen	s. n.	(F)	KM453854	KM453789
<i>M. purpurina</i>	MPN323A	Brazil	Cáceres	2009	(F, ISE)	KM453855	KM453790
<i>Nigrovothelium bullatum</i>	MPN114	India	Lumbsch	19701g	(F)	KX215589	KX215673
<i>N. bullatum</i>	MPN579	Thailand	Parmmen	32	(F)	KX215590	KX215674
<i>N. bullatum</i>	MPN82	India	Lumbsch	19701g	(F)	KX215591	KX215675
<i>N. tropicum</i>	MPN130	USA	Nelsen	s. n.	(F)	KM453883	KM453819
<i>N. tropicum</i>	MPN195	Philippines	Rivas Plata	2155	(F, Saint Louis University)	–	KX215676



TABLE 1 (continued).

Species	Extract	Country	Collector	Number	Repository	GenBank Accession numbers	
						mtSSU	nuLSU
<i>Nigrovothelium tropicum</i>	MPN197	Philippines	<i>Rivas Plata</i>	2173	(F, Saint Louis University)	–	KX215677
<i>N. tropicum</i>	MPN25	Thailand	<i>Nelsen</i>	4003	(F, RAMK)	GU327708	GU327730
<i>N. tropicum</i>	MPN318	Panama	<i>Lücking</i>	27092	(F, PMA)	–	KX215678
<i>N. tropicum</i>	MPN44	Peru	<i>Nelsen</i>	Cit1J	(F)	KX215592	KX215679
<i>N. tropicum</i>	MPN561	Thailand	<i>Nelsen</i>	s. n.	(F, RAMK)	KX215593	KX215680
<i>N. tropicum</i>	MPN658	Panama	<i>Nelsen</i>	s. n.	(F, PMA)	KX215594	–
<i>Novomicrothelia oleosa</i>	MPN700	Brazil	<i>Cáceres</i>	11821	(F, ISE)	KM453857	KM453794
<i>N. oleosa</i>	MPN95	Peru	<i>Nelsen</i>	4007a	(F)	GU327697	GU327721
<i>Polymeridium albocinereum</i>	MPN439	Brazil	<i>Lücking</i>	s. n.	(F, SP)	KM453858	KM453795
<i>P. catapastum</i>	MPN358	Venezuela	<i>Lücking</i>	26052	(F, VEN)	KM453859	JN887402
<i>P. subvirescens</i>	MPN702	Brazil	<i>Cáceres</i>	11110	(F, ISE)	–	KX215681
<i>Pseudopyrenula</i> aff. <i>subgregaria</i>	MPN288	Panama	<i>Lücking</i>	27124b	(F, PMA)	–	KX215682
<i>P. diluta</i>	MPN362	Venezuela	<i>Lücking</i>	26062	(F, VEN)	KM453861	KM453797
<i>P. diluta</i>	MPN697	Brazil	<i>Lücking</i>	31068	(F, SP)	KM453862	KM453798
<i>P. endoxanthoides</i>	MPN106	Thailand	<i>Lücking</i>	24079	(F, RAMK)	GU327699	GU327724
<i>P. endoxanthoides</i>	MPN573	Thailand	<i>Nelsen</i>	s. n.	(F, RAMK)	KX215595	–
<i>P. subgregaria</i>	DNA1502	Costa Rica	<i>Lücking</i>	17619	CR, F, INB → CR)	DQ328997	–
<i>P. subgregaria</i>	MPN292	Panama	<i>Lücking</i>	27053	(F, PMA)	KM453864	KM453800
<i>P. subgregaria</i>	MPN297	Panama	<i>Lücking</i>	27014	(F, PMA)	–	KX215683
<i>P. subgregaria</i>	MPN391	USA	<i>Nelsen</i>	4082b	(F)	KM453863	KM453799
<i>P. subgregaria</i>	MPN565	Thailand	<i>Nelsen</i>	s. n.	(F, RAMK)	KX215596	–
<i>P. subgregaria</i>	MPN568	Thailand	<i>Nelsen</i>	s. n.	(F, RAMK)	KX215597	KX215684
<i>P. subnudata</i>	MPN293	Panama	<i>Lücking</i>	27014r1	(F, PMA)	KM453865	KM453801
<i>Trypethelium eluteriae</i>	MPN111	India	<i>Lumbsch</i>	19701a	(F)	KM453874	GU327726
<i>T. eluteriae</i>	MPN562	Thailand	<i>Nelsen</i>	s. n.	(F, RAMK)	KX215598	KX215685
<i>T. eluteriae</i>	MPN563	Thailand	<i>Nelsen</i>	s. n.	(F, RAMK)	KX215599	KX215686
<i>T. eluteriae</i>	MPN576	Thailand	<i>Nelsen</i>	s. n.	(F, RAMK)	KX215600	–
<i>T. eluteriae</i>	MPN580	Thailand	<i>Parmen</i>	32	(F)	KX215601	KX215687
<i>T. foveolatum</i>	MPN351	Argentina	<i>Lücking</i>	30515	(CTES, F)	KM453881	KM453816
<i>T. inamoenum</i>	MPN228	Thailand	<i>Lücking</i>	24125	(F, RAMK)	KM453875	KM453810
<i>T. platyleucostomum</i>	MPN349	Argentina	<i>Lücking</i>	30512	(CTES, F)	KM453870	KM453806
<i>T. platyleucostomum</i>	MPN350	Argentina	<i>Lücking</i>	30512	(CTES, F)	KX215602	KX215688
<i>T. rubroplatystomum</i>	MPN54	Peru	<i>Nelsen</i>	s. n.	(F)	KM453871	KM453807
<i>T. rubroplatystomum</i>	MPN64	Peru	<i>Nelsen</i>	s. n.	(F)	–	KX215689
<i>T. rubroplatystomum</i>	MPN65C	Peru	<i>Nelsen</i>	s. n.	(F)	KX215603	KX215690
<i>T. sprengelii</i>	MPN200B	El Salvador	<i>Lücking</i>	28013	(F, ITIC)	–	KX215691
<i>T. sprengelii</i>	MPN382	USA	<i>Nelsen</i>	4169	(F)	KM453867	KM453803

TABLE 1 (continued).

Species	Extract	Country	Collector	Number	Repository	GenBank Accession numbers	
						mtSSU	nuLSU
<i>Trypethelium subeluteriae</i>	DNA1144	Australia	Lumbsch	19113k	(F)	DQ328989	—
<i>T. subeluteriae</i>	DNA1218	Australia	Lumbsch	19112g	(F)	DQ328990	—
<i>T. subeluteriae</i>	DNA1505	Costa Rica	Lücking	17611	(F, INB → CR, USJ)	DQ329009	—
<i>T. subeluteriae</i>	MPN144	Brazil	Nelsen	B2	(F, SP)	—	KX215692
<i>T. subeluteriae</i>	MPN49C	Peru	Nelsen	s. n.	(F)	KM453882	KM453818
<i>T. subeluteriae</i>	MPN748	Vietnam	Gueidan	3043	(F)	KX215604	KX215693
<i>T. subeluteriae</i>	MPN749	Vietnam	Gueidan	3052	(F)	—	KX215694
<i>Viridothelium tricolor</i>	MPN268	Panama	Lücking	27125	(F, PMA)	KM453843	KM453778
<i>V. tricolor</i>	MPN271	Panama	Lücking	27057	(F, PMA)	—	KX215695
<i>V. tricolor</i>	MPN399	Venezuela	Lücking	32241	(F, VEN)	KM453844	KM453779
<i>V. virens</i>	MPN646	Panama	Nelsen	s. n.	(F, PMA)	KM453845	KM453780
<i>V. vonkonratii</i>	MPN497	USA	Nelsen	s. n.	(F)	KM453884	KM453820
	MPN764	Fiji	Lumbsch	20551a	(F)	—	KM453817

## Results and Discussion

The final dataset consisted of 196 ingroup OTUs and 1272 characters (mtSSU: 690; nuLSU: 491; mtSSU PICS-Ord codes: 91). The basal portion of the best-scoring maximum likelihood tree (Figs 1 & 2) included several clades representing the artificial genera *Arthopyrenia*, *Julella*, and *Mycomicrothelia*, all with ecorticate thalli and more or less euseptate ascospores. Species formerly placed in *Arthopyrenia* s. lat. and *Mycomicrothelia* s. lat. did not form monophyletic clades and the backbone in this part of the tree was poorly supported. Therefore, while the name *Bogoriella* was separately reinstated for the bulk of tropical, lichenized *Mycomicrothelia* species (Aptroot & Lücking 2016), here we established new genera (see below) for the well-supported core clade of tropical, lichenized *Arthopyrenia* species (*Constrictolumina*) and for *Mycomicrothelia oleosa* (*Novomicrothelia*), for which monophyly with the other three *Mycomicrothelia* species (*Bogoriella*) was rejected (SH test,  $P < 0.01$ ). No formal change was proposed for *Julella fallaciosa* since the available data do not allow us to conclude whether this taxon is conspecific with the type of the genus. Two further lineages representing tropical, lichenized species previously assigned to *Arthopyrenia* were provisionally placed in *Constrictolumina*, since monophyly of these lineages with the latter could not be rejected (SH test,  $P > 0.05$ ).

The strongly supported remainder of the tree contained lineages with astrothelioid ascospores and/or corticate thalli. This large clade was rather well supported in most parts, suggesting up to 15 lineages which we mostly interpret as distinct genera (Figs 1–4). *Pseudopyrenula* formed a supported, monophyletic sister group to all remaining genera which were split into two larger clades (Figs 1 & 2). The first of these two clades included *Polymeridium* s. str., the *Trypethelium tropicum* clade (*Nigrovothelium*), the *Polymeridium proponens* clade (*Dictyomeridium*), for which monophyly with *Polymeridium* s. str. was rejected (SH test,  $P < 0.01$ ), the *Trypethelium virens* clade (*Viridothelium*), *Marcelaria*, and *Trypethelium* s. str. (Fig. 2). The *Trypethelium virens* clade

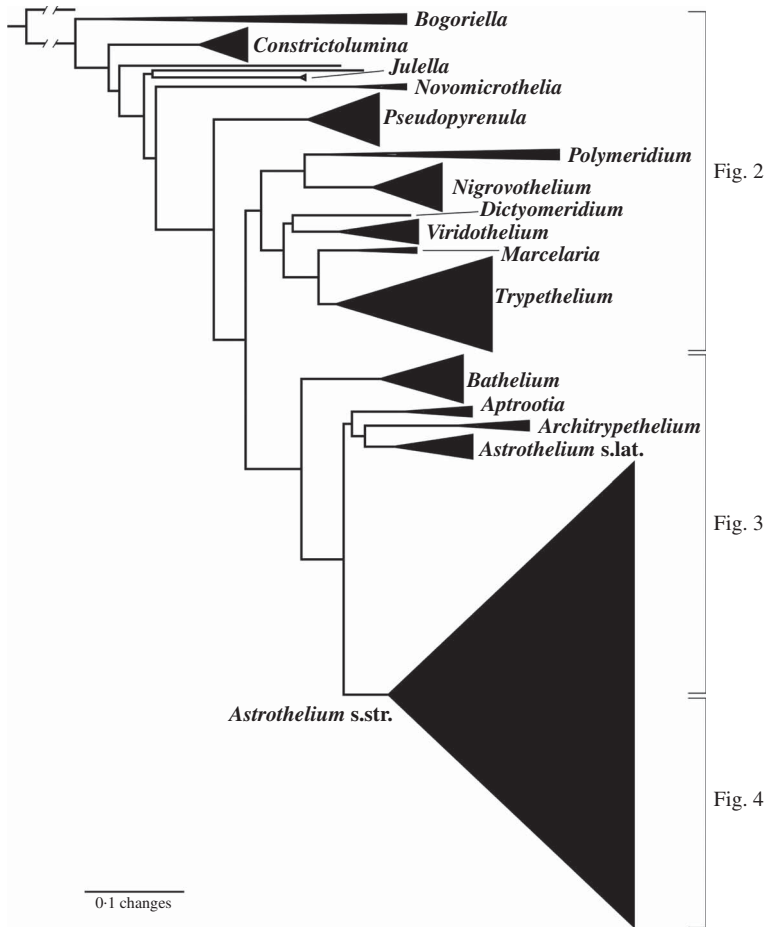
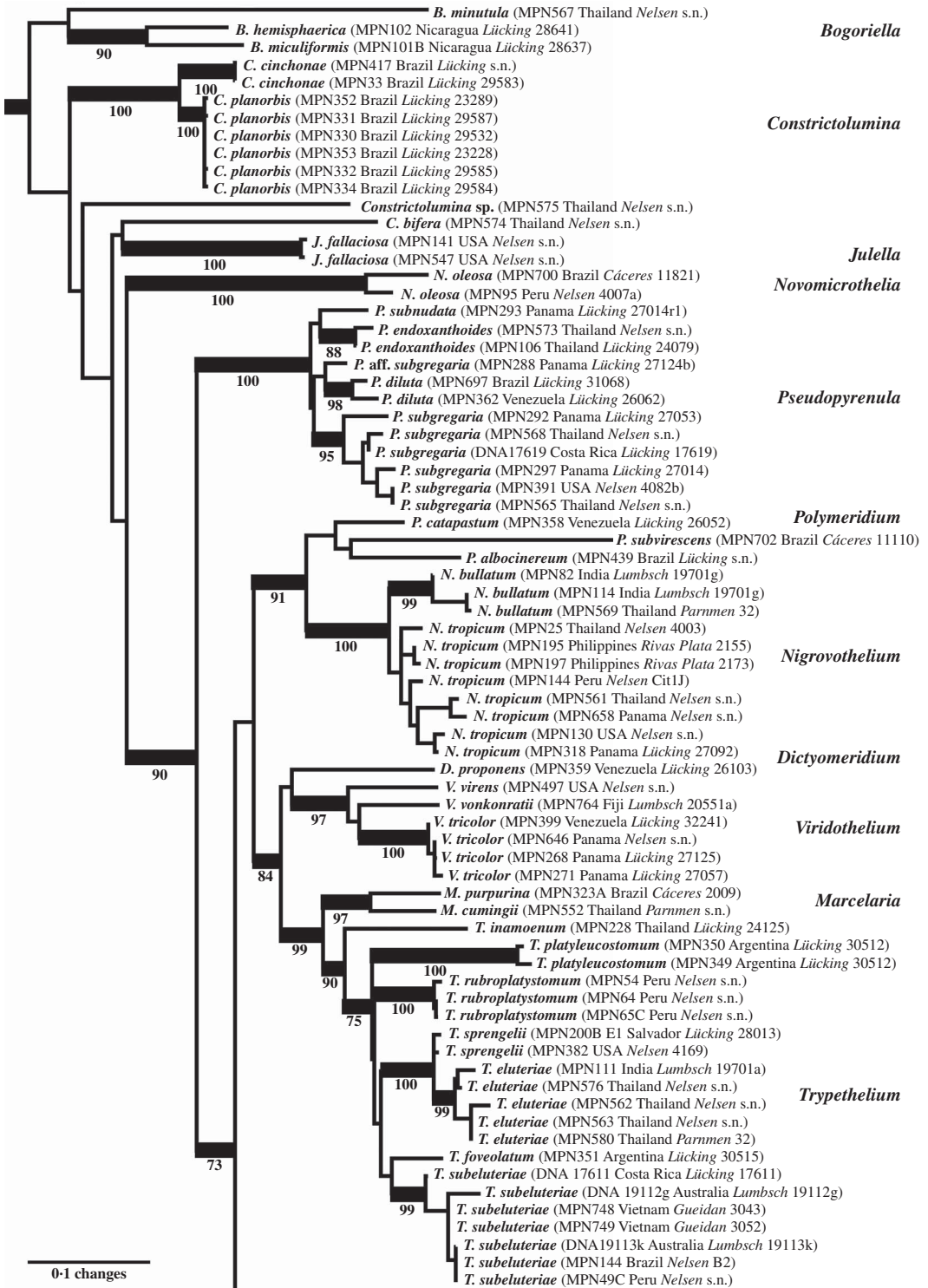


FIG. 1. Combined mtSSU-nuLSU-PICS-Ord cartoon tree of *Trypetheliaceae* based on maximum likelihood analysis. Accepted genera are labelled. Separate detailed figures (which also include bootstrap support values) are indicated.

had previously been identified with the name *Campylothelium* (Nelsen *et al.* 2014), but this was based on a misidentification of one of the species contained in this clade as *Campylothelium puiggarii* which turned out to represent an undescribed species unrelated to *C. puiggarii*; we now assume that *Campylothelium* falls into the *Astrothelium* clade although the type, *Campylothelium puiggarii*, has not yet been sequenced. The sister group relationship of *Polymeridium* s. str. with the *Trypethelium tropicum* clade was supported, as well as the sister group relationship of *Marcelaria* and *Trypethelium* s.

str. and the position of the *Polymeridium proponens* (*Dictyomeridium*) and the *Trypethelium virens* (*Viridothelium*) clades close to *Marcelaria* and *Trypethelium* (Fig. 2).

The second clade included the genera *Bathelium* s. str., *Aptrootia* and *Architrypethelium* as well as a small and a large clade comprising species traditionally classified in the genera *Astrothelium*, *Bathelium*, *Cryptothelium*, *Laurera*, and *Trypethelium*, with *Astrothelium* as the oldest available name (Figs 1, 3 & 4). *Bathelium* s. str. formed a strongly supported sister group to the remaining clades in this portion of the tree (Fig. 3), but the relationships between



the genera *Aptrootia*, *Architrypethelium*, and *Astrothelium* were not supported and not fully resolved, due to the presence of a small clade of species that would morphologically be referable to *Astrothelium* but clustered, without support, with *Aptrootia* and *Architrypethelium* (Fig. 3). The separate mtSSU and nuLSU trees had similar topologies, with a small *Astrothelium* clade separated from the large *Astrothelium* clade; however, in the mtSSU tree, *Architrypethelium* was nested within the small clade and *Aptrootia* within the large clade (see Supplementary Materials B & C, available online); for both loci, the alternative hypothesis that the two *Astrothelium* clades form a monophyletic group could not be rejected (SH test,  $P > 0.05$ ). Therefore, we adopted a conservative solution, retaining *Aptrootia* and *Architrypethelium* as separate genera, due to their distinctive features, and treating all other species in this large, terminal clade in a single genus, *Astrothelium*.

All genus-level lineages as delimited here are characterized by a combination of phenotypic features and we formally recognize 15 genera at this time within *Trypetheliaceae* (Aptroot & Lücking 2016), excluding *Fulella* (since the phylogenetic position of the type species is unknown) but including *Distothelia*, which has not yet been sequenced. The lineages at the base of the tree are for the time being recognized in three genera, all with ecorticate thalli, exposed, black ascumata and ascospores of variable types but not astrothelioid (i.e. lacking the endospore causing the diamond-shaped lumina characteristic of most other genera). These are *Bogoriella* (reinstated for tropical, lichenized species previously placed in *Mycocomicrothelia*; ascospores euseptate, brown), *Constrictolumina* (for tropical, lichenized species previously placed in *Arthopyrenia*; ascospores subdistoseptate, often with incomplete septal invaginations, hyaline), and *Novomicrothelia* (for an additional species previously placed in *Mycocomicrothelia*).

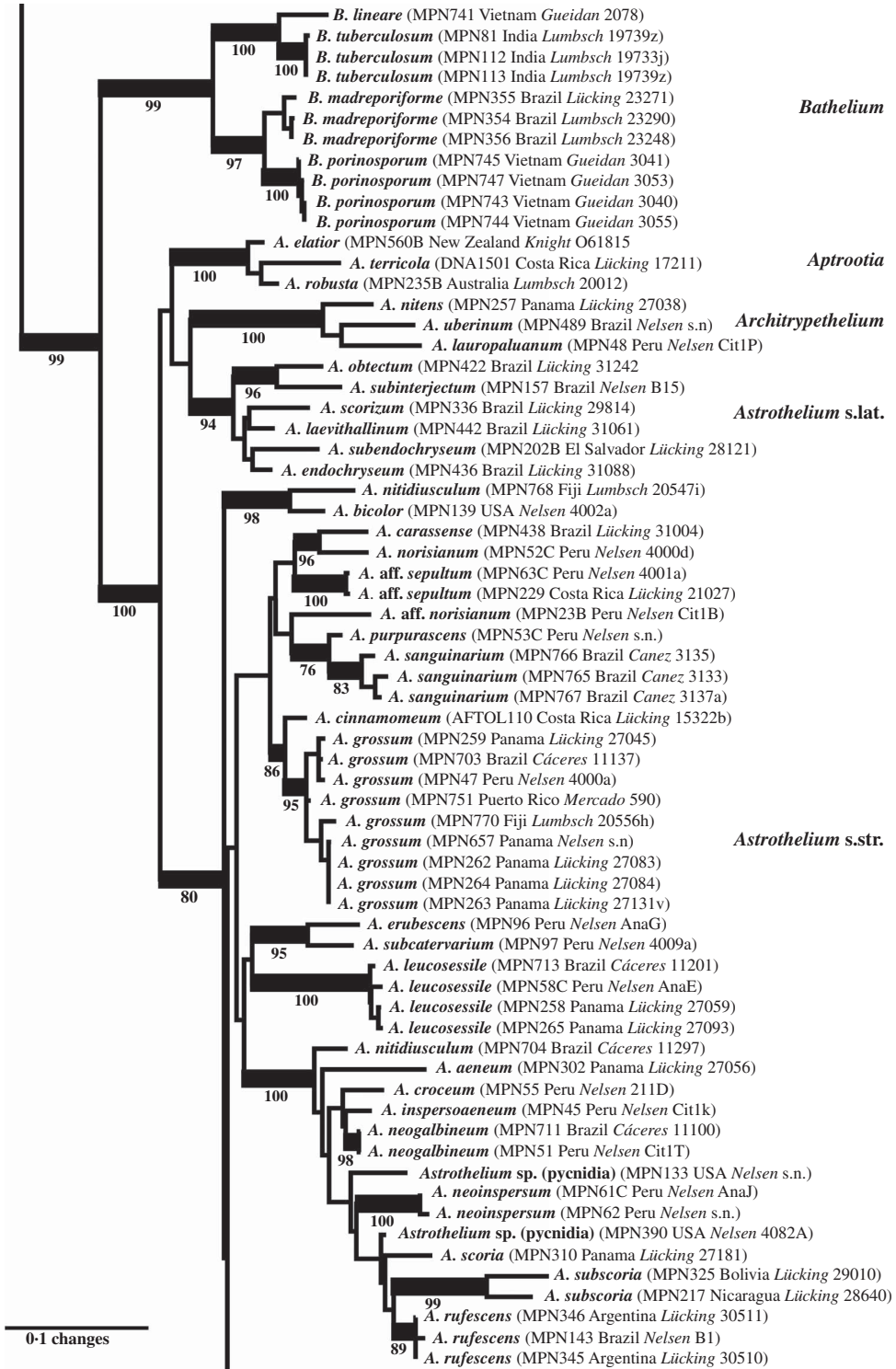
The genus *Fulella*, previously assigned to *Arthopyreniaceae* or *Thelenellaceae* (Mayrhofer 1987; Harris 1995), has recently been placed in *Halolellaceae* and *Didymosphaeriaceae*, respectively (Hyde et al. 2013; Ariyawansa et al. 2014) but no molecular data are yet available for the non-lichenized, European type species, *F. buxi* Fabre. The sequenced material falling within *Trypetheliaceae* fits the North American temperate species *F. fallaciosa*, which might or might not be congeneric with *F. buxi*, so further data are required to resolve this issue; for the time being, we do not formally accept *Fulella* as a genus within *Trypetheliaceae*. The genus as currently circumscribed might well turn out to be polyphyletic, similar to *Arthopyrenia* s. lat. and *Mycocomicrothelia* s. lat. A potentially available genus name for the lineage falling within *Trypetheliaceae*, should *F. fallaciosa* prove to be unrelated to *F. buxi*, is *Polyblastiopsis* Zahlbr. based on *P. sericea* (A. Massal.) Zahlbr., which appears to be related to, or conspecific with, *F. fallaciosa* (Purvis et al. 1992; Aptroot & van den Boom 1995; Harris 1995).

*Pseudopyrenula* is characterized by a morphology similar to *Bogoriella* and *Constrictolumina*, but with astrothelioid ascospores with diamond-shaped lumina. In both morphology and position, *Pseudopyrenula* provides a true transitional genus between the base of the tree and the more derived taxa, combining a primitive morphology with a derived ascospore type.

The genera *Polymeridium* and *Dictyomeridium* share the plesiomorphic morphology of ecorticate thalli and largely exposed, black ascumata with the aforementioned genera and their ascospores are also euseptate. Given their supported, nested position within a clade largely characterized by astrothelioid ascospores, the euseptate ascospores of *Polymeridium* and *Dictyomeridium* could be a secondary reversal (loss of endospore) due to the ecology of these species occurring mostly in dry forest biomes (Harris 1984,

FIG. 2. Combined mtSSU-nuLSU-PICS-Ord tree of *Trypetheliaceae* (basal and central portion of the tree: *Bogoriella* to *Trypethelium*) based on maximum likelihood analysis. Thick lines indicate bootstrap support  $\geq 70\%$  and exact bootstrap support values are given below branches. Accepted genera are in larger font and in bold.





1991, 1995; Cáceres 2007; Aptroot *et al.* 2008, 2013). However, this needs to be tested with a further expanded dataset, although the thin-walled ascospores of *Trypethelium* s. str. (see below) would support this notion. *Polymeridium* was initially described as a section of *Arthopyrenia* (Müller 1883), while Harris (1975) eventually raised the section to a separate genus within *Trypetheliaceae* (Tucker & Harris 1980). The morphological distinction between *Polymeridium* s. str. and *Dictyomeridium* is not yet fully understood, but the latter appears to include species with lateral ostioles and muriform ascospores. The separate placement of this group was anticipated in earlier works (Tucker & Harris 1980; Harris 1991), and *Polymeridium proponens* had at some point been assigned to *Campylothelium* (Tucker & Harris 1980).

The pantropical *Trypethelium tropicum* complex is supported sister to *Polymeridium* s. str. and could be included in the latter based on topology alone, but since it differs in the corticate thallus and astrothelioid ascospores, we prefer to recognize it as a new genus, *Nigrovothelium*. *Nigrovothelium* is morphologically distinguished from *Astrothelium* by the completely exposed, sessile ascomata (at least partly covered by thallus or pseudostromatic in *Astrothelium*) and from *Bathelium* by the absence of pseudostromata and the black colour of the ascomata. *Marcelaria* has been characterized already in a separate paper (Aptroot *et al.* 2013) as comprising species with exposed, strongly pigmented pseudostromata, which are somewhat similar to those of *Trypethelium* s. str. but are not distinctly pseudostromatic and produce muriform ascospores.

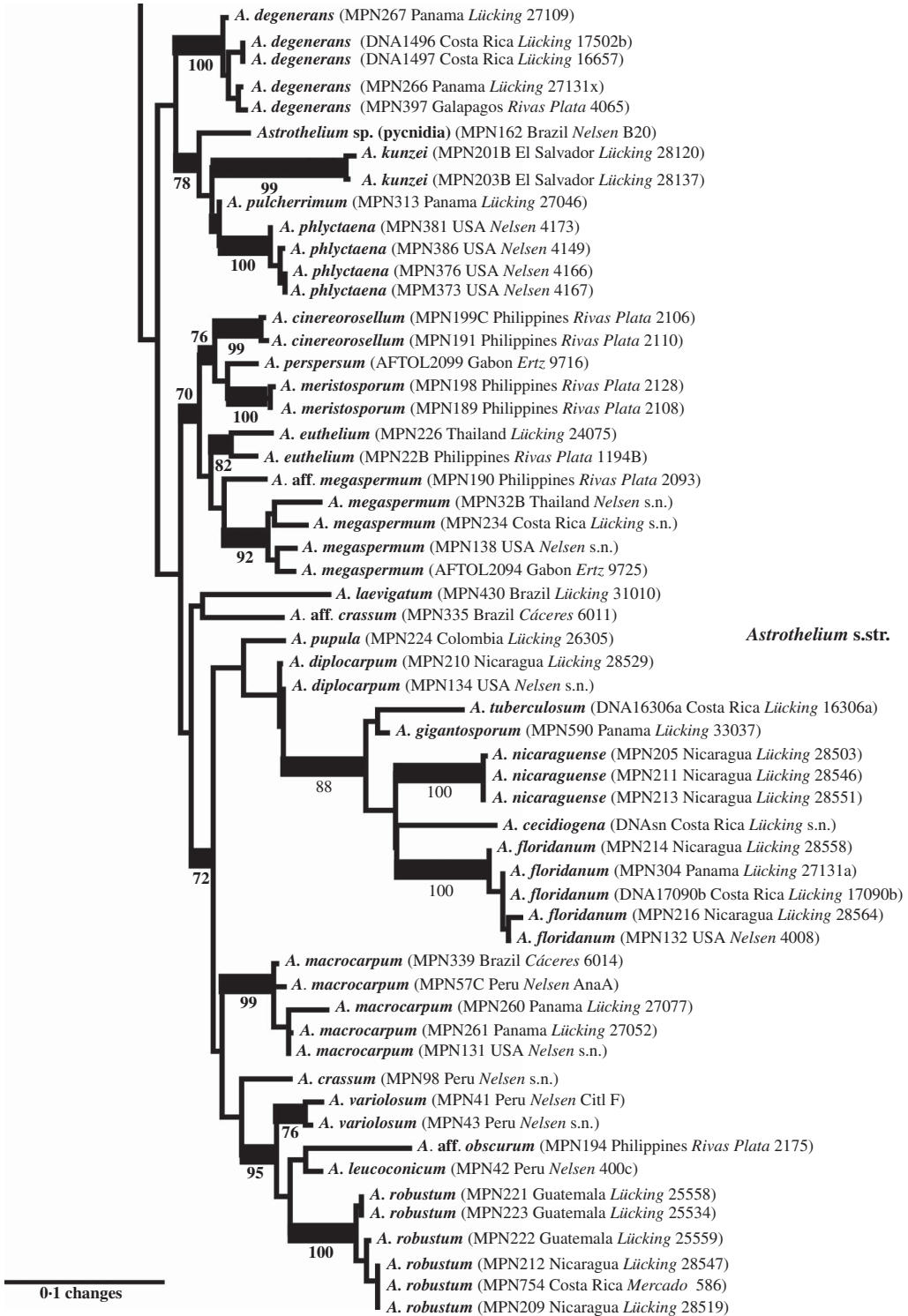
*Trypethelium* s. str. is well delimited by its prominent to sessile pseudostromata with apical ostioles and the subdistoseptate, transversely-septate ascospores with more or less rectangular lumina, different to the astrothelioid ascospores in *Astrothelium* and

other genera. The aforementioned notion that the euseptate ascospores in *Polymeridium* and *Dictyomeridium* could represent a secondary reversal from astrothelioid forms is in part supported by ascospore ontogeny in *Trypethelium* s. str., where the finally subdistoseptate ascospores pass through an astrothelioid stage early in their ontogeny (Sweetwood *et al.* 2012). Aptroot *et al.* (2008) suggested that the *T. eluteriae* group might be closely related to *Bathelium*, a hypothesis that is not supported here. Makhija & Patwardhan (1992, 1993) suggested that *Trypethelium* s. str. includes subgroups with slightly different pseudostromatal anatomy: those of *T. eluteriae* and *T. sphaerocephalum* are separated from the thallus by the absence of cortical, algal and medullary layers, and instead a cortical layer is produced beneath the pseudostroma, and ascomata are surrounded by a single layer, either hyaline or filled with yellow to orange crystals. In contrast, pseudostromata in *T. subeluteriae* contain a cortical layer both beneath and above them.

The only conceivable morphological difference between *Viridothelium* and *Astrothelium* are the subdistoseptate versus astrothelioid ascospores. The ascospores of *Viridothelium* closely resemble those of *Trypethelium* s. str., but the two genera differ in ascoma morphology: solitary to diffusely pseudostromatic in *Viridothelium* and distinctly pseudostromatic in *Trypethelium* s. str. Possible inclusion of *Viridothelium* within *Trypethelium* s. str. is rejected by the topology (SH test,  $P < 0.01$ ).

The genus *Bathelium* is partially confirmed here as a monophyletic entity, but several species placed in this genus by Harris (1995) fall into *Astrothelium*, notably those with small, 3-septate ascospores and with rather low pseudostromata, such as *A. degenerans*. *Bathelium* s. str. instead produces strongly prominent to sessile, very conspicuous pseudostromata. This concept coincides in part with that of Trevisan (1853) and

FIG. 3. Combined mtSSU-nuLSU-PICS-Ord tree of *Trypetheliaceae* (central portion of the tree: *Bathelium* to *Astrothelium* p.p.) based on maximum likelihood analysis. Thick lines indicate bootstrap support  $\geq 70\%$  and exact bootstrap support values are given below branches. Accepted genera are in larger font and in bold.



Massalongo (1860). An additional difference is that the ascospores are subdistoseptate in *Bathelium* and astrothelioid in *Astrothelium*, for example *A. degenerans*, a feature best observed in species with transversely septate ascospores.

The two genera *Aptrootia* and *Architrypethelium*, originally defined based on their peculiar ascospores (dark brown with a hard outer shell in *Aptrootia* and large, 3-septate and often dark brown in *Architrypethelium*), are confirmed as monophyletic and retained here. The ascospores of both genera pass through an astrothelioid stage before producing their characteristic mature structure (Sweetwood *et al.* 2012) which supports the close relationship between these genera and *Astrothelium*.

Finally, *Astrothelium* as defined here includes the bulk of species in the family, characterized by rather thick, corticate thalli and astrothelioid ascospores. The genera *Campylothelium*, *Cryptothelium*, and *Laurera* are now considered synonyms of this genus and most species previously classified as *Trypethelium* are also placed here (Aptroot & Lücking 2016). Harris (1995) had already argued that the delimitation of these genera was artificial, and predicted that many species from these genera would eventually be placed in a single genus, although he suggested *Laurera* (Reichenbach 1841) as a potential name, which is, however, younger than *Astrothelium* (Eschweiler 1824). While most of the species in this considerably emended genus have rather thick, corticate, often olive-green thalli with the ascomata usually immersed or covered by a thallus layer, much variation is found in ascoma disposition, being either solitary or aggregate or fused with either apical or lateral ostioles; also, the ascospores range from transversely septate to muriform but are without exception hyaline. While muriform ascospores usually do not display diamond-shaped lumina, their ontogeny passes through an astrothelioid

stage (Sweetwood *et al.* 2012), explaining the observed close relationship between species with differently septate ascospores, for instance the well-known *A. megaspermum* with large, muriform ascospores, falling in a small, supported clade together with taxa with small, transversely septate ascospores, *A. cinereorosellum* and *A. perspersum* (Fig. 4).

The results presented here also provide a refined understanding of species delimitation in *Trypetheliaceae*. Characters such as thallus and ascoma morphology, hymenium inspersation, and the presence of lichexanthone, as used in Harris (1984), have been successively neglected when delimiting species, so that more recently, species were almost exclusively defined by pigments and ascospore septation and size (e.g. Harris 1991, 1995); even ascospore size was at some point considered of limited value, such as in *Pseudopyrenula* (Harris 1998). On the other hand, taxa such as *Trypethelium eluteriae* and *T. subeluteriae* have been distinguished by successive workers based on very subtle morphological and chemical differences (Makhija & Patwardhan 1992; Harris 1995; Aptroot *et al.* 2008). Without independent evidence, arguments for either splitting or lumping might be valid yet remain subjective, but fortunately can be tested using a phylogenetic framework. As an example, the separation of *Trypethelium eluteriae* and *T. subeluteriae* was supported by our results (Fig. 2).

More importantly, we repeatedly found that species originally identified with names using available keys were located in different places in the tree, especially in the newly defined genus *Astrothelium*, with the commonly applied names *A. (Trypethelium) aeneum*, *A. nitidiusculum*, and *A. ochroleucum* (Figs 3 & 4). These morphotaxa apparently comprise many, often unrelated species, such as *A. leucosessile*, *A. kunzei*, and *A. inspersaeneum* (Aptroot & Lücking 2016). Similar results were found in *Pseudopyrenula* and *Nigrovothelium* (Fig. 2), including the reinstated *Pseudopyrenula endoxanthoides* and the newly recognized

FIG. 4. Combined mtSSU-nuLSU-PICS-Ord tree of *Trypetheliaceae* (distal portion of the tree: *Astrothelium* p.p.) based on maximum likelihood analysis. Thick lines indicate bootstrap support  $\geq 70\%$  and exact bootstrap support values are given below branches. Accepted genera are in larger font and in bold.

*Nigrovothelium bullatum* (Aptroot & Lücking 2016; Lücking *et al.* 2016). In these cases, examination of the sequenced material revealed that clades were distinguished by characters such as hymenium inspersion and presence of lichexanthone, but especially by the morphology of thallus and ascomata. Thus, features such as a bullate thallus or the degree of dispersion and aggregation or emergence of the ascomata and pseudostromata appear to be species-specific. This suggests that a much more refined species concept has to be applied within the family, similar to the situation found in *Graphidaceae*, especially the megadiverse genera *Graphis* and *Ocellularia*, where gross morphology had been similarly neglected but was found to be diagnostic (Lücking 2009, 2014, 2015; Lücking *et al.* 2009). Based on these findings, the refined species concept laid out in the monographic synopsis of the family (Aptroot & Lücking 2016) increases the number of recognized species in *Trypetheliaceae* based on names reinstated from prior synonymy by *c.* 70, in addition to well over 100 new species (Aptroot & Cáceres 2016; Aptroot *et al.* 2016a, b; Flakus *et al.* 2016; Lücking *et al.* 2016).

### Taxonomic Novelties

#### **Constrictolumina Lücking, M. P. Nelsen & Aptroot gen. nov.**

Mycobank No.: MB 816872

Differing from *Arthopyrenia* s. str. in the lichenized thallus and the ascospores with thicker walls, and usually forming secondary endospore thickenings resembling incomplete septa.

Type: *Constrictolumina cinchonae* (Ach.) Lücking, M. P. Nelsen & Aptroot (holotype).

*Thallus* not corticate.

*Ascomata* single, roughly conical, erumpent to prominent and more or less exposed, not in distinct pseudostromata but sometimes fused sideways. *Ostiole* apical. *Hamathecium* hyaline, clear, filaments thick at the base, thinner above, not anastomosing. *Asci* clavate. *Ascospores* 1–3-septate, rarely submuriform, with irregular endospore formation, sometimes with pseudosepta, often one or two cells with secondary endospore

invaginations resembling incomplete septa, smooth or ornamented, hyaline, very rarely becoming brownish, often becoming granular ornamented.

*Pycnidia* sometimes present.

*Notes.* This aggregate of tropical, lichenized species is separated here for the first time into a formally described genus, following the realization that this group forms part of the family *Trypetheliaceae*, unrelated to the non-lichenized representatives of *Arthopyrenia* s. str. (Nelsen *et al.* 2009, 2011; Hyde *et al.* 2013). Details of the species were given primarily by Harris (1975, 1995), as part of a variously defined genus *Arthopyrenia* s. lat. Including the two species sequenced and recombined here, the new genus unites nine tropical taxa treated elsewhere in this issue (Aptroot & Lücking 2016). *Constrictolumina* exhibits a unique hamathecium structure different from the remainder of the *Trypetheliaceae*, one of the reasons why this group was not previously suspected to be related to the latter.

#### **Constrictolumina cinchonae (Ach.) Lücking, M. P. Nelsen & Aptroot comb. nov.**

Mycobank No.: MB 816878

*Verrucaria cinchonae* Ach., *Synops. Lich.*: 90 (1814).—*Arthopyrenia cinchonae* (Ach.) Müll. Arg., *Flora* 66: 287 (1883); type: “cort. Cinchonae officinalis” (H-ACH 781B!—holotype).

#### **Constrictolumina planorbis (Ach.) Lücking, M. P. Nelsen & Aptroot comb. nov.**

Mycobank No.: MB 816879

*Verrucaria planorbis* Ach., *Synops. Lich.*: 92 (1814).—*Arthopyrenia planorbis* (Ach.) Müll. Arg., *Mem. Soc. Phys. Genève* 30: 27 (1888); type: “cort. Crotonis Cascariillae” (H-ACH, holotype, not seen).

#### **Dictyomeridium Aptroot, M. P. Nelsen & Lücking gen. nov.**

Mycobank No.: MB 816873



Differing from *Polymeridium* s. str. in the ascomata with lateral ostiole in combination with muriform ascospores.

Type: *Dictyomeridium proponens* (Nyl.) Aptroot, M. P. Nelsen & Lücking (holotype).

*Thallus* not corticate.

*Ascomata* single or a few aggregate, roughly conical to pyriform, erumpent to prominent and more or less exposed, not in distinct pseudostromata. *Ostioles* eccentric. *Hamathecium* hyaline, not interspersed. *Asci* with 2–8 ascospores. *Ascospores* muriform, hyaline, smooth, often IKI+ violet.

*Pycnidia* sometimes present.

*Notes.* This species aggregate is united here as a segregate of *Polymeridium*, with seven muriform-spored species with lateral ostiole (Aptroot & Lücking 2016). While *Dictyomeridium* is phylogenetically separate from *Polymeridium*, the morphological differences are subtle, since the latter includes some species with muriform ascospores or lateral ostioles, but not in combination. The type of *Dictyomeridium*, *D. proponens*, representing the most common species of the genus, was for a long time recognized under different names in the genus *Campylothelium*.

***Dictyomeridium proponens* (Nyl.)  
Aptroot, M. P. Nelsen & Lücking  
comb. nov.**

Mycobank No.: MB 816880

*Verrucaria proponens* Nyl., *Bull. Soc. Linn. Normandie*, sér. 2: 130 (1868).—*Polyblastia proponens* (Nyl.) Müll. Arg. *Flora* 65: 402 (1882).—*Campylothelium proponens* (Nyl.) Müll. Arg., *Hedvigia* 31: 286 (1892).—*Polyblastiopsis proponens* (Nyl.) Zahlbr., *Catal. Lich. Univ.* 1: 351 (1922).—*Polymeridium proponens* (Nyl.) R. C. Harris, *Bol. Mus. Paraense Emílio Goeldi, Ser. Bot.*, 7: 637 ('1991') [1993]; type: New Caledonia, Lifu, Loyalty Islands, *Thiebaut*. (H-NYL—holotype, not seen).

***Nigrovothelium* Lücking, M. P. Nelsen  
& Aptroot gen. nov.**

Mycobank No.: MB 816875

Differing from *Polymeridium* in the corticate thallus and astrothelioid ascospores, from *Bathelium* in the mostly single, black ascomata and astrothelioid ascospores, and

from *Astrothelium* in the fully exposed, sessile, black ascomata.

Type: *Nigrovothelium tropicum* (Ach.) Lücking, M. P. Nelsen & Aptroot (holotype).

*Thallus* corticate.

*Ascomata* usually single but often crowded and irregularly confluent, sessile, ovoid, not in pseudostromata. *Ostiole* apical. *Hamathecium* hyaline, clear, filaments thin, anastomosing paraphysoids, often interspersed with oil. *Asci* clavate. *Ascospores* transversely 3-septate, with distinct endospore formation creating diamond-shaped lumina (astrothelioid), hyaline.

*Notes.* The genus is described here to accommodate *Trypethelium tropicum* and at least one additional, newly recognized species (Lücking et al. 2016). While this genus resembles other genera in certain characters, such as *Pseudopyrenula* and *Astrothelium* in the astrothelioid ascospores and *Bathelium* in the exposed, dark ascomata, its unique combination of characters and its phylogenetic position, sister to the morphologically distinct *Polymeridium*, merit its recognition as a separate taxon.

***Nigrovothelium tropicum* (Ach.)  
Lücking, M. P. Nelsen &  
Aptroot comb. nov.**

Mycobank No.: MB 816881

*Verrucaria tropica* Ach., *Lichenogr. Univ.*: 278 (1810).—*Sagedia tropica* (Ach.) A. Massal., *Ricerch. Auton. Lich.*: 161 (1852).—*Pyrenula tropica* (Ach.) Trevis., *Spighe e Paglie*: 17 (1853).—*Spermatodium tropicum* (Ach.) Trevis., *Conspect. Verruc.*: 11 (1860).—*Pseudopyrenula tropica* (Ach.) Müll. Arg., *Flora* 66: 248 (1883).—*Trypethelium tropicum* (Ach.) Müll. Arg., *Bot. Jahrb. Syst.* 6: 393 (1885); type: America, Swartz (H-ACH 707A)—lectotype, designated here; BM-ACH—isolectotype).

***Novomicrothelia* Aptroot, M. P. Nelsen  
& Lücking gen. nov.**

Mycobank No.: MB 816876

Similar to *Bogoriella* but forming a separate phylogenetic clade, with the following substitutions in the large subunit nuclear ribosomal DNA (nuLSU; relative positions following Supplementary Material A, available online): 46, 47, 308, 449, 458 (A replaces C); 117, 298, 379, 397, 447, 459 (A replaces G); 32, 68, 81, 109–111, 311, 316, 355

(A replaces T); 45, 70 (C replaces A); 98 (C replaces G); 41, 87, 106, 195, 196, 351, 419, 457, 461 (C replaces T); 36, 383 (G replaces A); 322 (G replaces C); 44 (G replaces T); 83, 266, 307, 321, 361 (T replaces A); 40, 97, 320, 385, 440, 474 (T replaces C); 352, 354 (T replaces G); also differing from most species of *Bogoriella* in the interspersed hamathecium and ascospore wall invaginations besides the (sub-)median septum.

Type: *Novomicrothelia oleosa* (Aptroot) Aptroot, M. P. Nelsen & Lücking (holotype).

*Thallus* not corticate.

*Ascomata* single, roughly conical, erumpent to prominent and more or less exposed, not in pseudostromata. *Ostiole* apical. *Hamathecium* hyaline, interspersed with oil droplets, filaments thin, anastomosing paraphysoids. *Asci* clavate. *Ascospores* transversely 1-septate, with irregular endospore formation, becoming ornamented, brown, rather elongated.

*Pycnidia* sometimes present.

**Discussion.** The genus is described here to accommodate a single species that was until now united with temperate, non-lichenized fungi in the genus *Mycomicrothelia*. *Novomicrothelia* is phylogenetically distinct from the morphologically similar, reinstated genus *Bogoriella* but the phenotypic distinction between the two genera is not very clear yet; more data are needed for these basal lineages within the family to fully understand their phylogeny and classification. According to Harris (1995), *N. oleosa* is unique based on its interspersed hamathecium and ascospore wall invaginations similar to those found in *Constrictolumina* but the small number of species of *Bogoriella* sequenced so far does not allow us to conclude whether these are consistent differences. Two species currently accepted in *Bogoriella* have an interspersed hamathecium (Aptroot & Lücking 2016) but these have not yet been sequenced.

Since the phenotypic differences between *Novomicrothelia* and *Bogoriella* are not yet clear, but both form distantly related clades for which monophyly as a single clade was rejected, we provided diagnostic molecular features as allowed by the *Code*. The *Code* specified in such a case that the differential characters (i.e. relative columns and substitutions) need to be spelled out, and we provide a possible model for this case. It is obvious that further data might change these

characters, in particular reduce the number of diagnostic columns, but since this applies analogously to any phenotypic characters when further data are added, it does not make the diagnosis invalid.

***Novomicrothelia oleosa* (Aptroot)  
Aptroot, M. P. Nelsen &  
Lücking comb. nov.**

MycoBank No.: MB 816882

*Mycomicrothelia oleosa* Aptroot, *Biblioth. Lichenol.* 44: 133 (1991); type: Trinidad, Caroni, north bank road, Britton *et al.* 869 (NY!—holotype).

***Viridothelium* Lücking, M. P. Nelsen &  
Aptroot gen. nov.**

MycoBank No.: MB 816877

Differing from *Astrothelium* in the subdistoseptate ascospores and from *Trypethelium* s. str. in the absence of well-defined pseudostromata.

Type: *Viridothelium virens* (Tuck. ex Michener) Lücking, M. P. Nelsen & Aptroot (holotype).

*Thallus* corticate, often warted.

*Ascomata* simple or aggregated in pseudostromata, which can be hardly to clearly raised and are usually not of a different structure and colour from the thallus. *Ostioles* apical or eccentric, simple or fused. *Wall* hyphal (textura intricata), usually carbonized. *Hamathecium* interspersed with oil droplets or not, filaments thin, anastomosing paraphysoids. *Ascospores* subdistoseptate, with thin walls and only slightly thickened septa, hyaline, I– or weakly I+ violet-blue, transversely septate.

*Pycnidia* occasionally present.

**Notes.** This new genus accommodates *Trypethelium virens*, a taxon that has long been considered unique due to its northern temperate distribution and the non-astrothelioid, I+ weakly amyloid ascospores (Aptroot & Lücking 2016; Aptroot *et al.* 2016a, b; Lücking *et al.* 2016); several further, tropical species are also included in this clade. *Viridothelium* is superficially similar to *Astrothelium* but appears in a distant phylogenetic position; the main difference lies in

the subdistoseptate ascospores resembling those of *Trypethelium* s. str.

**Viridothelium virens (Tuck. ex Michener) Lücking, M. P. Nelsen & Aptroot comb. nov.**

Mycobank No.: MB 816883

*Trypethelium virens* Tuck. ex Michener, *W. Dard. Fl. Cest. ed. 3*: 453 (1853).—*Trypethelium eluteriae* var. *virens* (Tuck. ex Michener) Trevis., *Flora* **44**: 20 (1861); type: USA, Arkansas, Dardanelle, Michener, 1853 (FH—holotype, not seen; M!—isotype).

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SUPPLEMENTARY MATERIAL

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