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New species and records of lichens from Bolivia

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Abstract

Fuscidea multispora Flakus, Kukwa & Rodr. Flakus and *Malmidea attenboroughii* Kukwa, Guzow-Krzemińska, Kosecka, Jabłońska & Flakus are described as new to science based on morphological, chemical and molecular characters. *Lepra subventosa* var. *hypothamnolica* is genetically and chemically distinct from *L. subventosa* var. *subventosa* and a new name, *Lepra pseudosubventosa* Kukwa & Guzow-Krzemińska, is proposed due to the existence of *Lepra hypothamnolica* (Dibben) Lendemer & R.C. Harris. *Pertusaria muricata*, recently transferred to *Lepra*, is kept in the genus *Pertusaria* due to the highest similarity of ITS sequence with members of *Pertusaria*. The occurrence of *Micarea hedlundii* in the Southern Hemisphere is confirmed based on molecular evidence from Bolivian population. *Lepra pseudosubventosa* and *Pertusaria muricata* are reported as new to South America, and 20 taxa as new to Bolivia. *Lepraria stephaniana*, previously known only from the type locality, is reported from two more sites. An ascosporic state is reported for the first time for *Lepra amaroides*, as are new chemotypes. Molecular markers were used to place some sterile, sorediate crustose lichens in the family Graphidaceae. The phylogenetic positions of some sterile *Malmidea* specimens within Malmidaceae are also discussed.

Key words: sterile lichens, molecular systematics, biodiversity, Ascomycota

Introduction

Bolivia may have one of the richest lichen biota in South American countries (e.g., Flakus & Lücking 2008; Kukwa & Flakus 2009; Flakus *et al.* 2011, 2012, 2016; Oset & Kukwa 2012; Kukwa *et al.* 2012, 2013, 2014; Ertz *et al.* 2015; Rodriguez *et al.* 2016), but the diversity and distribution of lichens still remain insufficiently explored. This paper improves our knowledge of these deficiencies, and includes many records of sterile sorediate and isidiate lichens with crustose thalli, including two species described as new to science and one redesignated name.

Material and Methods

Taxon sampling

The studied material is deposited in the following herbaria: KRAM, LPB, UGDA and UPS. Lichen substances were identified with thin layer chromatography (TLC) (Culberson & Kristinsson 1970; Orange *et al.* 2001). In some cases, the colour reaction with C (commercial bleach), K (water solution of potassium hydroxide) and Pd (alcohol solution of paraphenylenediamine) were made (Orange *et al.* 2001), as well as the colour of the thallus checked in the ultraviolet light (UV). All measurements of apothecial and thallus structures were made in water. Species reported as new to Bolivia are marked with an asterisk (*) and those new to South America with two asterisks (**).

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DNA extraction, PCR amplification and DNA sequencing

DNA was extracted directly from either pieces of thalli or ascomata using a modified CTAB method (Guzow-Krzemińska & Węgrzyn 2000), GeneMATRIX Plant & Fungi DNA Purification Kit (Eurx, Poland) or QIAamp DNA Investigator Kit (QIAGEN) following the manufacturers' protocols. Genomic DNA was used for PCR amplifications of the internal transcribed spacer region (nucITS) using ITS1F (Gardes & Bruns 1993) and ITS4 primers (White *et al*. 1990), a part of the mitochondrial small subunit (mrSSU) of the ribosomal RNA using mrSSU1 and mrSSU3R primers (Zoller *et al.* 1999) and the nuclear ribosomal large subunit (nucLSU) using ITS4A-5' (Nelsen *et al.* 2011), LROR (Rehner & Samuels 1994), LR5 and LR7 primers (Vilgalys & Hester 1990). The same primers were used for sequencing. In most cases StartWarm HS-PCR Mix (A&A Biotechnology) was used for amplification. PCR amplifications of nucITS and mrSSU were performed in a Mastercycler (Eppendorf) using programs previously described (Guzow-Krzemińska *et al.* 2016; Guzow-Krzemińska *et al.* 2018) or in case of ITS4A-5'/LR5 the following PCR parameters were employed: initial denaturation at 95ºC for 5 min followed by 35 cycles at 95ºC for 1 min, 60ºC for 1 min and 72ºC for 1 min, and a final elongation step at 72ºC for 7 min. For *Fuscidea* and *Micarea* spp., the final PCR reaction volume was 25 µl and contained either 2 or 5 µl of genomic DNA, master mix AmpliTaq® 360 DNA Polymerase (10X PCR buffer and 25 mM $MgCl_2$) following the manufacturer's protocol, 0.2 µl of Bovine Serum Albumin (NEB, USA) and 1 µl of each primer (Flakus *et al.* 2019). Thermal cycling parameters were performed according to Rodriguez-Flakus & Printzen (2014). PCR products were visualized on agarose gels in order to determine DNA fragment lengths. Subsequently, PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing was performed in Macrogen (the Netherlands/South Korea). The newly obtained sequences were deposited in NCBI's database with specific accession numbers (Table 1).

Sequence alignment and phylogenetic analysis

The newly generated nucITS rDNA and mrSSU rDNA and nucLSU sequences were compared to the sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) using Megablast search (Altschul *et al*. 1990). The alignments and further phylogenetic analyses were performed separately for each group of species following different parameters. The mrSSU *Malmidea* alignment was generated using Seaview software (Galtier *et al*. 1996; Gouy *et al.* 2010) employing muscle option followed with Gblocks selection of poorly aligned sites (Castresana 2000). In the case of the concatenated *Fuscidea* and mrSSU *Micarea* phylogenetic analyses, the assembling of strains, manual edition and consensus sequences were performed in Geneious Pro version 5.0.4. Multiple-sequence alignment for each gene was performed using MAFFT algorithm (Katoh *et al.* 2005) on the GUIDANCE server (http://guidance.tau.ac.il/). The GUIDANCE tool assigns a confidence score (from 0 to 1) for each sequence position and base pair aligned. The default cutoff score of 0.93 was used for the removal of unreliably aligned positions. The selection of the best partition for our data and substitution model for each partition was performed in Partition Finder 2 (Lanfear *et al.* 2016) implemented in CIPRES Scientific gateway portal (http://www.phylo.org/portal2/) (Miller *et al.* 2010). Five partitions for *Fuscidea* concatenated alignment were found (ITS1: SYM+G, 5.8S: SYM+G+I, ITS2: SYM+G, mrSSU: GTR+G and nucLSU: SYM+G+I), and a single partition for *Micarea* (mrSSU: GTR+G) under greedy search algorithm and Bayesian Information Criterion (BIC) (Lanfear *et al.* 2012). Phylogenetic analyses were carried out using a heuristic search for the maximum likelihood (ML) implemented in RaxMLGUI version 0.9 beta 2 (Stamatakis 2006, Silvestro & Michalak 2012) with 1000 replicates and GTRGAMMA or GTRGAMMAI model respectively.

All data were analysed using Markov Chain Monte Carlo (MCMC) to infer the Bayesian approach as implemented in MrBayes 3.2.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) at CIPRES Science Gateway (Miller *et al.* 2010) or MrBayes 3.2.6 (Ronquist *et al.* 2012). The *Malmidea* dataset was analysed using GTR model with 10 M generations, two independent runs, each with four chains, and the output of MrBayes for *Malmidea* studies was analyzed with the program Tracer v.1.5 (Rambaud & Drummond 2007) and the initial 25% of trees were discarded as burn-in and the majority-rule consensus tree was calculated to obtain posterior probabilities (PP). In the case of *Fuscidea* and *Micarea*, the posterior probability analyses were carried out with the following parameters, i.e. 20 M generations, three independent runs, each with four chains that were incrementally heated using a factor of 0.15, and 50% of the sampled trees were discarded as burn-in. The running stopped when the standard deviation dropped below 0.01. The phylogenetic trees were drawn using FigTree 1.4.2 (Rambaud 2009). ML bootstrap support (BS) and PP values are given above the branches on each phylogenetic tree.

TABLE 1. GenBank Accession numbers for newly generated (in bold) and downloaded from GenBank sequences used in this study. Specimens are vouchered in UGDA, LPB or KRAM (for details see the text).

Species	Voucher/reference	nucITS rDNA	mrSSU	nucLSU
Caloplaca erythrantha	Kukwa 16782	**MK543176**		
Caloplaca erythrantha	Kukwa 16939a	**MK543175**		
Candelariella vitellina	Wedin *et al.* (2005)		AY853315	AY853363
Candelariella vitellina	Favero-Longo *et al.* (upubl.)	AJ640085		
Chapsa thallotrema	Kukwa 19753		**MK542859**	**MK542898**
Fuscidea arboricola	Mark *et al.* (2016)	KX132962		
Fuscidea austera	Miadlikowska *et al.* (2014)		KJ766395	KJ766562
Fuscidea austera	Zahradníková *et al.* (2017)	KY874026	KY874033	KY874045
Fuscidea australis	Zahradníková *et al.* (2017)	KY874022	KY874034	KY874044
Fuscidea cyathoides var. *corticola*	Zahradníková *et al.* (2017)	KY874015	KY874027	KY874037
Fuscidea cyathoides var. *corticola*	Zahradníková *et al.* (2017)	KY874016		KY874039
Fuscidea cyathoides var. *corticola*	Zahradníková *et al.* (2017)	KY874028	KY874028	
Fuscidea cyathoides var. *cyathoides*	Miadlikowska *et al.* (2014)		KJ766396	KJ766563
Fuscidea cyathoides var. *cyathoides*	Zahradníková *et al.* (2017)	KY874018	KY874030	KY874038
Fuscidea cyathoides var. *cyathoides*	Bylin *et al.* (2007)		EF659763	
Fuscidea cyathoides var. *cyathoides*	Bylin *et al.* (2007)		EF659761	
Fuscidea cyathoides var. *sorediata*	Zahradníková *et al.* (2017)	KY874013	KY874029	KY874046
Fuscidea elixii	Zahradníková *et al.* (2017)	KY874020	KY874035	KY874043
Fuscidea gothoburgensis	Zahradníková *et al.* (2017)	KY874024	KY874036	KY874042
Fuscidea intercincta	Zahradníková *et al.* (2018)	MG669003	MG669084	MG669077
Fuscidea kochiana	Zahradníková *et al.* (2017)	KY874023	KY874031	KY874041
Fuscidea lightfootii	Zahradníková *et al.* (2018)	MG669015	MG669088	MG669079
Fuscidea mollis	Wedin *et al.* (2005)		AY853321	AY853369
Fuscidea multispora	Flakus 25608.1	**MK561630**	**MK561613**	**MK561631**
Fuscidea pusilla	Zahradníková *et al.* (2017)	KY874025	KY874032	KY874040
Fuscidea pusilla	Bylin *et al.* (2007)		EF659767	
Fuscidea pusilla	Bylin *et al.* (2007)		EF659765	
Fuscidea recensa	Bylin *et al.* (2007)		EF659766	
Lepra amaroides	Kukwa 16157	**MK543177**		
Lepra amaroides	Kukwa 16892	**MK543178**		
Lepra amaroides	Kukwa 16901	**MK543179**		
Lepra pseudosubventosa	Kukwa 16672	**MK543180**		
Lepra subventosa	Kukwa 14699	**MK543181**		
Malmidea attenboroughii	Kukwa 19645		**MK542860**	
Malmidea aff. *flavopustulosa* 1	Kukwa 19309		**MK542861**	
Malmidea aff. *flavopustulosa* 1	Kukwa 19313		**MK542862**	
Malmidea aff. *flavopustulosa* 1	Kukwa 19764		**MK542863**	
Malmidea aff. *flavopustulosa* 1	Kukwa 19765		**MK542864**	
Malmidea aff. *flavopustulosa* 1	Kukwa 19785		**MK542865**	
Malmidea aff. *flavopustulosa* 2	Kukwa 18999		**MK542866**	
Malmidea aff. *flavopustulosa* 2	Kukwa 19006		**MK542867**	
Malmidea cf. *perisidiata*	Kukwa 19612		**MK542868**	
Malmidea cf. *polycampia*	Kukwa 19002		**MK542869**	
Malmidea sp.	Kukwa 18858		**MK542870**	
Micarea hedlundii	Flakus 25384		**MK561614**	
Ocellularia erodens	Kukwa 19258	**MK543182**	**MK542871**	**MK542899**
Ocellularia erodens	Kukwa 19754	**MK543183**	**MK542872**	**MK542900**
Ocellularia erodens	Kukwa 19732	**MK543184**	**MK542873**	**MK542901**
Ocellularia erodens	Kukwa 19568	**MK543185**	**MK542874**	**MK542902**
Ocellularia erodens	Kukwa 19668		**MK542875**	**MK542903**
Ocellularia microsorediata	Kukwa 19658		**MK542876**	**MK542904**
Pertusaria muricata	Kukwa 16954	**MK543186**	**MK542877**	**MK542905**
Umbilicaria crustulosa	Hestmark *et al.* (2011)	HM161499		HM161591
Umbilicaria crustulosa	Lumbsch *et al.* (2004)		AY300919	
Umbilicaria proboscidea	Kelly *et al.* (2011)	FR799304		
Umbilicaria proboscidea	Lumbsch *et al.* (2004)		AY300920	AY300870

Haplotype network

Sequences of nucITS rDNA marker from specimens of *Lepra amaroides, L. pseudosubventosa* and *L. subventosa* s.str. were aligned using Seaview software (Galtier *et al.* 1996; Gouy *et al.* 2010). TCS network (Clement *et al.* 2002) was created as implemented in PopART software (http://popart.otago.ac.nz).

The species

****Caloplaca erythrantha*** (Tuck.) Zahlbr.

This corticolous lichen is known from tropical to subtropical areas; to date it has been reported from Bermuda, Brazil, Colombia, Costa Rica, Cuba, Guadeloupe, Jamaica, Mexico, USA, and Trinidad and Tobago (Wetmore 2007).

ITS sequences were obtained from both collections (Tab. 1) and BLASTn search showed 97 or 98% of identity to the sequence of *C. erythrantha* deposited in GenBank (accession no FJ349101). The phylogeny including both sequences will be published elsewhere.

Material examined. BOLIVIA. Dept. Tarija, **Prov. Aniceto Arce**, close to la Mamora between Tarija and Bermejo, 22º09'51"S, 64º40'03"W, elev. 1320 m, disturbed Tucumano-Boliviano forest with *Tillandsia*, corticolous, 27 July 2015, *M. Kukwa 16782* (LPB, UGDA); **Prov. Burnet O'Connor**, close to Soledad, old road between Entre Ríos and Chuquisaca, 21º39'45"S, 64º07'22"W, 1750 m, Boliviano-Tucumano forest with shrubs and *Alnus acuminata*, on *Alnus acuminata*, 31 July 2015, *M. Kukwa 16939a* (LPB, UGDA).

****Chapsa thallotrema*** Lücking & N. Salazar

So far reported from Brazil, Costa Rica, Panama and Venezuela (Lumbsch *et al.* 2011; Rivas Plata *et al.* 2013; Sipman *et al.* 2012; Lima *et al.* 2016).

Our material of *Ch. thallotrema* is sterile with well-delimited soralia. The mrSSU and nucLSU sequences were obtained from our specimen (Tab. 1) and show 98 and 99% of identity respectively to the sequences of *Ch. thallotrema* deposited in GenBank (mrSSU: accession no JX421013; nucLSU: accession nos JX465319, JX467681, JX465306).

Chapsa thallotrema is morphologically and chemically very similar to the esorediate *Ch. sublilacina* (Ellis & Everh.) M. E. S. Cáceres & Lücking. It differs only in the production of soredia and according to Sipman *et al.* (2012) the latter can represent a non-sorediate counterpart of *Ch. thallotrema*. Newly generated nucLSU sequence of *Ch. thallotrema* is very similar (98% of identity) to that of *Ch. sublilacina* deposited in GenBank (accession no HQ639600) which suggests they may represent the same species, but more variable molecular markers need to be studied.

Material examined. BOLIVIA. Dept. La Paz: **Prov. Abel Iturralde**, between Santa Rosa de Maravillas and Tumupasa, 13°58'43"S, 67°58'14"W, elev. 300 m, natural Preandean Amazon forest, corticolous, 25 May 2017, *M. Kukwa 19753* (LPB, UGDA).

Fuscidea multispora Flakus, Kukwa & Rodr. Flakus *sp. nov.* Mycobank MB 830057. Figs 1 & 2

Diagnosis: Differs from *Fuscidea lightfootii* in having smaller ascospores, 16-spored asci, lack of soredia, different substrate preferences (foliicolous) and secondary chemistry (sekikaic acid).

Type:—BOLIVIA. Dept. Cochabamba: **Prov. Carrasco**, Parque Nacional Carrasco, Meruvia close to Monte Punku, 17º35'06"S, 65º14'54"W, elev. 3283 m, *Podocarpus* and *Polylepis* forest, foliicolous on *Podocarpus* sp., 26 Nov. 2014, *A. Flakus 25608.1* (holotype KRAM!; isotype LPB!).

Thallus foliicolous, greyish-green to brownish, verruculose, not continuous to almost continuous, verrucae developing on dark prothallus or on thin thallus layer, thallus 3–6 mm in diam., verrucae irregular in shape, 100–200 mm in diam, 20–100 µm tall, covered by thin dark brown cortical layer, c. 5 µm thick, inside with small crystals (K+ dissolving); *soralia* lacking; *prothallus* usually evident, dark brown to black; *photobiont* chlorococcoid, cells 5–12 µm in. diam; *ascomata* apothecia, dark brown to black, scattered, sessile, sometimes constricted at the base, marginate, rounded in shape, 0.2–0.6 mm in diam; *disc* dark brown to black, matte, epruinose, flat to slightly concave; *excipulum* evident from the beginning, concolorous with the disc, 40–80 µm wide, colourless inside, not inspersed by crystals or oil droplets, I–, composed of radiating hyphae with lumina 1–3 µm wide, outer part strongly gelatinized and containing brown pigment; *epihymenium* dark brown pigmented, K+ olive (greenish grey), N+ first grey than orange-brown, 5–10 µm thick; *hymenium* colourless to brown in the upper part (with less amount of the same pigment as in epithecium), not inspersed by oil droplets, 60–70(–80) µm high, strongly agglutinated, I+ blue, K/I+ blue, strongly lax after treatment by K; *paraphyses* colourless, rather branched at the top, composed of hyphae with lumina 1–1.5 µm wide, strongly thickened (2–4 µm wide) apically, with pigmented caps; *subhymenium* colourless, c. 10–15 µm high; *hypothecium* colourless to brownish in lower part, inspersed by clusters of crystals (size 3–15 µm in diam.), 30–50 µm high; *asci* 16-spored, clavate, K/I+ blue at the top, *Fuscidea*-type (according to Hafellner 1984), 40–60 × 10–15 µm; *ascospores* colourless, simple, ellipsoid to bacillar, usually constricted in the middle or slightly curved, with 2 large guttules, with rather thick walls (c. 0.5 µm thick), without epispore, frequently with indistinct pseudosepta, (6–)7–8.5(–9) × 3.5–4.5 µm (n=30); *conidiomata* not seen.

Chemistry: Sekikaic acid by TLC.

Etymology: The name refers to the multispored asci.

Distribution and habitat: The species is known only from the type locality in the Andean forest dominated by *Podocarpus* and *Polylepis* trees.

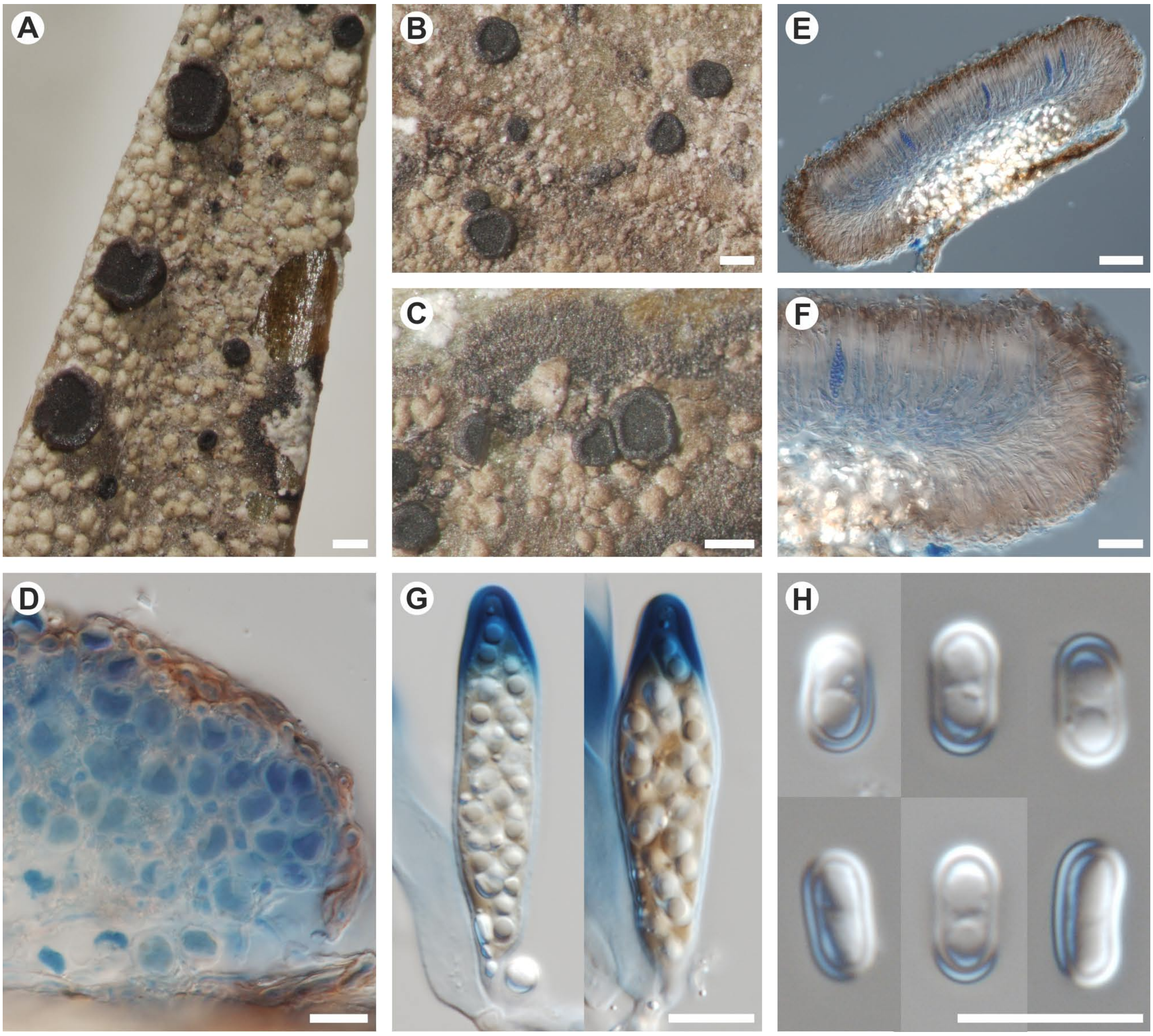

FIGURE 1. *Fuscidea multispora* (holotype). A–C, habit showing black marginate apothecia, verruculose thalli and black prothallus (C); D, cross section of thallus verrucae showing photobiont cells and brown cortical layer (mounted in LPCB), E, cross section of apothecium showing brown pigment in epithecium and outer part of exciple and hypothecium inspersed by crystals (mounted in LPCB); F, cross section of apothecium showing exciple composed of radiately arranged hyphae and part of hymenium with capitate paraphyses (mounted in LPCB); G, *Fuscidea*-type asci with 16-ascospores (mounted in Lugol soluton); H, ascospores. Scales: A–C—250 µm; D—10 µm; E—50 µm; F—25 µm; G–H—10 µm.

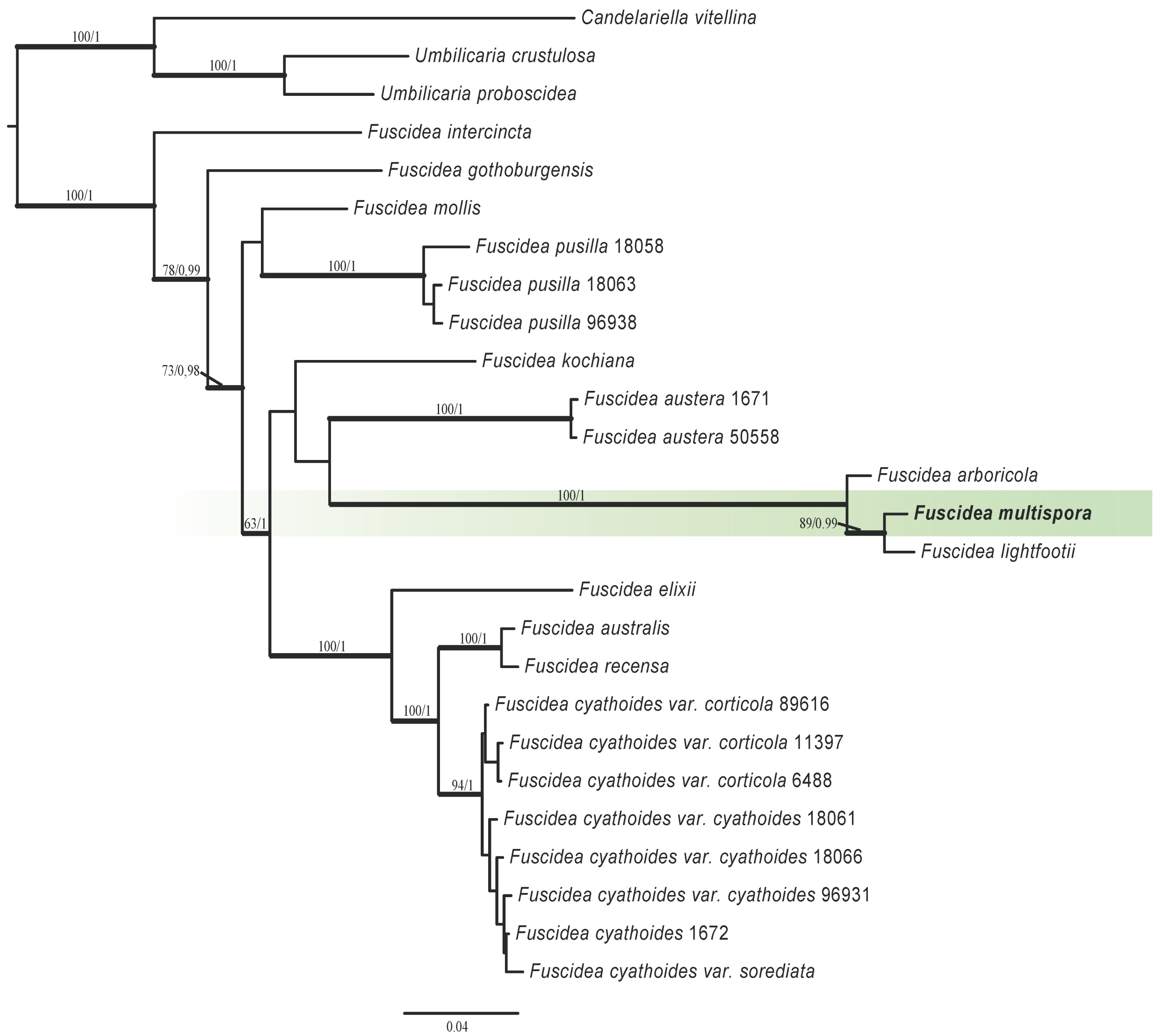

FIGURE 2. Maximum likelihood phylogenetic tree of *Fuscidea* inferred from concatenated three loci data set (nucLSU, nucITS and mrSSU) within Fuscideaceae. High nodal support values are indicated by bold branches, including ML bootstrap values ≥ 75% and MCMC posterior probability ≥ 0.9. The newly described *F. multispora* is in bold and highlighted. *Candellariella vitellina*, *Umbilicaria crustulosa* and *U. proboscidea* were used as outgroup.

Notes: The new species is characterized by dark brown to black lecideoid apothecia, greenish-grey to brownish and verrucose thallus, distinct dark prothallus, 16-spored asci, non-septate, usually constricted in the middle, hyaline ascospores and the presence of sekikaic acid. The genus *Fuscidea* V. Wirth & Vězda consists mainly of saxicolous and corticolous lichens (Wirth & Vězda 1972; Kantvilas 2001; Fryday 2008; Zahradníková *et al.* 2017), but so far only *F. fulva* (Malme) Kalb was known as foliicolous; this is a rare lichen known only from the Atlantic rain forest in southern Brazil, but clearly differs from *F. multispora* by larger ascospores (10–15 × 5–7 μm) and 8-spored asci (Lücking 2008).

The new species, due to its lecideoid apothecia and 16-spored asci, does not fully fit any genus in the Fuscideaceae Hafellner (Umbilicariales J. C. Wei & Q. M. Zhou) (Hafellner 1984; Kantvilas 2001; Miadlikowska *et al.* 2006; Bylin *et al.* 2007; Zahradníková *et al.* 2017), and without molecular data it would be difficult to assign it properly at the genus level. As currently circumscribed, *Fuscidea* taxa develop lecideoid apothecia (very rarely incomplete thin thalline margin can be developed in some species) and has 8-spored asci. The 16-spored asci of *F. multispora* make the new species similar to *Maronea* A. Massal., which is characterized by lecanoroid apothecia and multispored asci (Magnusson 1925, 1934; Wirth & Vězda 1972; Kantvilas 2001; Fryday 2008). Similar taxonomic problem concerns *M. afroalpina* Brusse, described form South Africa (Drakensberg), which has lecideoid apothecia and multispored asci

(Brusse 1989). This species can be easily separated from *Fuscidea multispora* by its ecology (saxicolous substrate preference), white pruinose apothecia, and the asci producing more ascospores (± 100) (Brusse 1989).

Despite morphological disparities, *F. multispora* is clearly placed in our phylogenetic analyses in the *Fuscidea* clade (Fig. 2). In Maximum likelihood and Bayesian analyses the topologies of the trees were very similar. A total of 23 sequences of *Fuscidea* were included in a final concatenated alignment of 2220 bp length (Fig. 2). The phylogeny shows that *F. multispora* is placed in a highly-supported clade (BS 100%, PP 1) together with *F. arboricola* Coppins & Tønsberg and *F. lightfootii* (Sm.) Coppins & P. James. Those species can easily be separated from *F. multispora* by the different substrate preference (corticolous), the production of soredia, 8-spored asci, and the production of different lichen metabolites (divaricatic acid in *F. lightfootii* and fumarprotocetraric acid in *F. arboricola*) (Tønsberg 1992; Gilbert *et al.* 2009; Zahradníková *et al.* 2018).

The genus *Fuscidea* is poorly studied in the Neotropics. Recently a new corticolous species, *F. tropica* van den Boom & Kalb was described from mountain ranges in Brazil, Guatemala and Venezuela (Boom *et al.* 2014). This species also contains sekikaic acid, but clearly differs from *F. multispora* in the thicker, rimose-areolate and warted thallus, 8-spored asci, larger (up to 1.4 mm in diam.) apothecia and longer ascospores [8.5–11(–12) μm long] (Boom *et al.* 2014).

Calvelo and Liberatore (2002) reported *F. cyathoides* (Ach.) V. Wirth & Vězda and *F. impolita* (Müll. Arg.) Hertel from Argentina, and Aptroot (2002) *F. kochiana* (Hepp) V. Wirth & Vězda and *F. lightfootii* from Brazil. All those species differ from *F. multispora* in 8-spored asci. Additionally, *F. cyathoides* has larger apothecia (up to 1.4 mm in diam.), bean-shaped ascospores, produces fumarprotocetraric acid, and usually grows on rocks (rarely on tree bark), *F. impolita* is a saxicolous species with larger ascospores and contains divaricatic acid, and *F. kochiana* grows on rocks, develops emarginate apothecia immersed in a thick thallus and contains divaricatic acid (Fryday 2000, 2008; Gilbert *et al.* 2009; Zahradníková *et al.* 2017); for differences from *F. lightfootii* see above.

****Haematomma persoonii*** (Fée) A.Massal.

This species is widely distributed, being reported from Africa (e.g., Ethiopia, Mozambique, Kenya, Tanzania, Uganda, Zimbabwe), Asia (Japan, Philippines, Sri Lanka, Thailand), Australia and Oceania (New Caledonia), and North and South America (Argentina, Brazil, Chile, Costa Rica, Ecuador, Jamaica, Mexico, Paraguay, Puerto Rico, USA, Venezuela) (Staiger & Kalb 1991).

Material examined. BOLIVIA. Dept. Tarija: **Prov. Aniceto Arce**, close to la Mamora between Tarija and Bermejo, 22º09'51"S, 64º40'03"W, elev. 1320 m, disturbed Tucumano-Boliviano forest with *Tillandsia*, corticolous, 27 July 2015, *M. Kukwa 16787* (LPB, UGDA); **Prov. Burnet O'Connor**, old road between Entre Ríos and Tarija, 21º29'13"S, 64º11'42"W, 1535 m, Boliviano-Tucumano forest, corticolous, 31 July 2015, *M. Kukwa 16955* (LPB, UGDA).

****Heterocyphelium triseptatum*** Aptroot & M.Cáceres

This recently described species was previously known only from Brazil and Tanzania (Aptroot *et al.* 2017). It was previously reported from Bolivia as *Heterocyphelium* aff. *leucampyx* (Tuck.) Vain. (Flakus *et al.* 2013).

Material examined. BOLIVIA. Dept. Beni: **Prov. Itenez**, Chaco lejos area near Bella Vista village, 13°16'24"S, 63°42'26"W, 140 m, lowland Amazon forest, corticolous, 17 Aug. 2008, *A. Flakus 12419, P. Rodriguez* (LPB, KRAM); Dept. Santa Cruz: **Prov. José Miguel de Velasco**, Sendero de goma near Florida village, 14°37'48"S, 61°12'02"W, 170 m, lowland Amazon secondary forest, on bark, 16 Dec. 2009, *A. Flakus 16082.1 & P. Rodriguez* (LPB).

****Hypocenomyce scalaris*** (Ach.) M. Choisy

This species has been reported from Asia, Australia, Europe, North and South America (Timdal 1984; Sipman *et al.* 2006); in South America it was previously known only from Colombia (Sipman *et al.* 2006).

Material examined. BOLIVIA. Dept. Chuquisaca: **Prov. Zudañez**, Área Natural de Manejo Integrado El Palmar, La Cascada below El Palmar, 18º41'23"S, 64º54'26"W, elev. 2740 m, Boliviano-Tucumano forest with *Podocarpus*, Lauraceae and palms, on palm, 15 July 2015, *M. Kukwa 16184* (LPB, UGDA); sendero El Palmar, 18º41'28"S, 64º54'32"W, elev. 2600–2876 m, forest with palms and shrubs, on palm, 15 July 2015, *M. Kukwa 16913e* (LPB, UGDA, as admixture in specimen of *Hypotrachyna*).

*__Lepra amaroides__ (H. Magn.) I. Schmitt, Hodkinson & Lumbsch

Type:—URUGUAY. **Rivera**, Camino de Rivera a Marco Lopez. Sur pierres dans lieu sombre. 24 Feb. 1947, *H.S Osorio 1070* (holotype UPS L-22975—picture seen). Analysed by Archer & Elix by HPLC: lichexanthone (major) and hypothamnolic acid (major).

Chemistry: Four chemotypes found in Bolivian material: I with lichexanthone and hypothamnolic acid, II with lichexanthone and squamatic acid, III with lichexanthone and thamnolic acid, and IV with lichexanthone, and barbatic and hypothamnolic acids.

Distribution: Previously known only from Uruguay in South America (Magnusson 1950).

Notes: Two samples (*Kukwa 16901 & 16172*) found in the Bolivian material contained lichexanthone and hypothamnolic acid and thus represented *Lepra amaroides* s.str. (Archer 1993). Additional specimens morphologically very similar to *L. amaroides* were also studied, but they contained thamnolic acid (*Kukwa 16157*) or squamatic acid (*Kukwa 16633*) instead of hypothamnolic acid or additionally barbatic acid (*Kukwa 16627, 16640, 16642, 16892, A. Flakus 23898.1*); they were all morphologically similar, and also very similar to *L. subventosa* and *L. pseudosubventosa*. Specimens from which nucITS sequences were obtained represented most of the chemical variation and haplotype network analyses showed that *L. subventosa* and *L. pseudosubventosa*, both containing picrolichenic acid, are different from samples lacking that substance (Fig. 3). As the genetic differences between samples representing *Lepra amaroides* and those containing barbatic and hypothamnolic acids or thamnolic acid are very low, it was concluded that they represent one species (Fig. 3). No sequence of the squamatic acid chemotype was obtained, but this substance is biogenetically related to barbatic, hypothamnolic and thamnolic acids (Elix 2014), therefore this chemotype was included in *L. amaroides.*

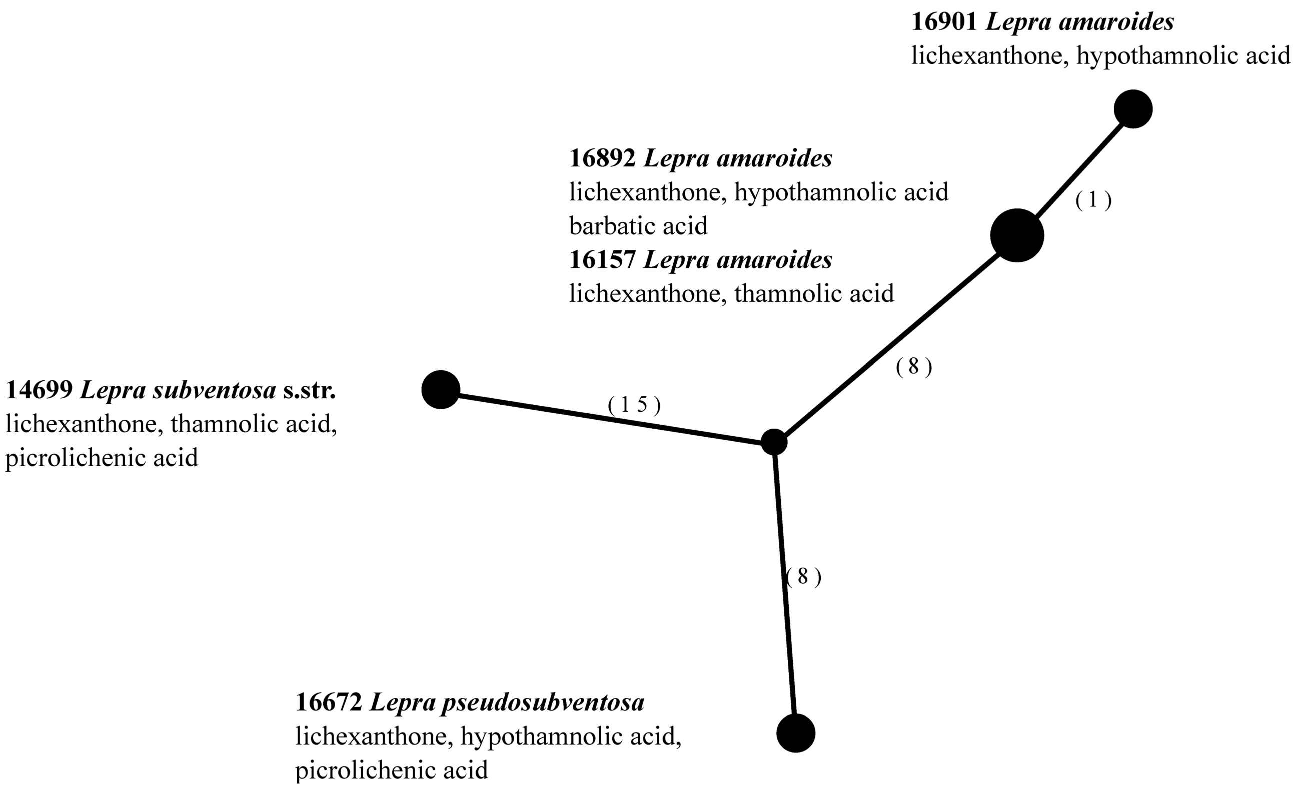

FIGURE 3. Haplotype network showing relationships among ITS haplotypes between *Lepra amaroides, L. pseudosubventosa* and *L. subventosa* s.str. Sizes of circles are proportional to the number of specimens per haplotype. Chemotypes are described below specimen's data. Numbers in brackets near lines between haplotypes represent number of mutational steps.

The records of an undetermined *Pertusaria* species, which was reported as a host of *Melaspilea tucumana* Flakus, Etayo & Kukwa (Flakus *et al.* 2014), belong to *Lepra amaroides* and represent chemotype IV.

According to Archer (1993) *L. amaroides* was known only in a sterile sorediate stage. Two Bolivian samples (*Kukwa 16172 & 16901*) are fertile and produce disciform apothecia, which are developing in soralia. Discs are dark brown, white pruinose being exposed in soralia in groups of 1–3. Asci are 1-spored with large ascospores (105–195 × 25–70 µm).

Material examined. BOLIVIA. Dept. Chuquisaca: **Prov. Zudañez**, Área Natural de Manejo Integrado El Palmar, Muy Orquo, on road from El Palmar to Loman, 18º47'46"S, 64º51'31"W, elev. 2879 m, open area, saxicolous, 14 July 2015, *M. Kukwa 16901* (LPB, UGDA; TLC: lichexanthone and hypothamnolic acid); ibidem; 14 July 2015, *M. Kukwa 16892* (LPB, UGDA; TLC: lichexanthone, barbatic and hypothamnolic acids); La Cascada below de El Palmar, 18º41'23"S, 64º54'26"W, elev. 2740 m, Boliviano-Tucumano forest with *Podocarpus*, *Lauraceae* and palms, saxicolous, 15 July 2015, *M. Kukwa 16172* (LPB, UGDA; TLC: lichexanthone and hypothamnolic acid); ibidem, *M. Kukwa 16157* (LPB, UGDA; TLC: lichexanthone, thamnolic acid); Dept. Tarija: **Prov. Aniceto Arce**, Papachacra, near Papachacra valley, 21°41'14"S, 64°30'19"W, elev. 2050 m, open vegetation with shrubs, 7 Aug. 2012, *A. Flakus 23898.1*, *M. Kukwa 10967a* (KRAM, LPB, UGDA; TLC: lichexanthone, barbatic and hypothamnolic acids); Reserva Nacional de Flora y Fauna Tariquía, close to la Cumbre between Padcaya and campamento los Alisos, 21º59'49"S, 64º36'11"W, elev. 3297 m, open area with *Puya* and rocks, saxicolous, 24 July 2015, *M. Kukwa 16633* (LPB, UGDA; TLC: lichexanthone, squamatic acid); ibidem, 22º00'00"S, 64º36'29"W, elev. 3158 m, saxicolous, 24 July 2015, *M. Kukwa 16627* (LPB, UGDA; TLC: lichexanthone, and barbatic and hypothamnolic acids); ibidem, 21º59'15"S, 64º36'12"W, elev. 3295 m, open area with rocks, saxicolous, 24 July 2015, *M. Kukwa 16640, 16642* (LPB, UGDA; TLC: lichexanthone, and barbatic and hypothamnolic acids).

Lepra erythrella (Müll. Arg.) I. Schmitt, B.G. Hodk. & Lumbsch

The species was previously known only from Australia and the Galapagos Islands (Archer 1991, 1997; Schmitt & Lumbsch 2004; Bungartz *et al.* 2015).

Material examined. BOLIVIA. Dept. Cochabamba: **Prov. Carrasco**, Parque Nacional Carrasco, between Meruvia and Monte Punku, 17º34'43"S, 65º15'25"W, elev. 3082 m, *Podocarpus* forest, epixylic, 26 Nov. 2014, *M. Kukwa 15055* (LPB, UGDA); Dept. La Paz: **Prov. Nor Yungas**, Chuspipata, old road Coroico-La Paz, 16°18'31"S, 67°48'51"W, elev. 3000 m, semi-natural Yungas forest, corticolous, 23 Nov. 2016, *M. Kukwa 19158, 19161 & 19170* (LPB, UGDA).

****Lepra pseudosubventosa*** Kukwa & Guzow-Krzemińska *nom. et stat. nov.* Mycobank MB 830059

Basionym *Pertusaria subventosa* var. *hypothamnolica* A.W. Archer & Elix (1993: 147); syn. *Lepra subventosa* (Malme) I. Schmitt, B.G. Hodk. & Lumbsch var. *hypothamnolica* (A.W. Archer & Elix) A.W. Archer & Elix

Etymology: The name refers to the similarity to *Lepra subventosa*.

Chemistry: Lichexanthone, and picrolichenic and thamnolic acids by TLC.

Distribution: Lepra pseudosubventosa was previously known only from Australia and Papua New Guinea (Archer & Elix 1993).

Notes: Lepra subventosa s.l. appears to be morphologically uniform, but variable in the secondary chemistry (Archer & Elix 1993; Wei *et al.* 2017) which apparently reflects the genetic differences as several species can be hidden under that name (Wei *et al.* 2017). Archer & Elix (1993) recognized three varieties within *L. subventosa*, including *L. subventosa* var. *hypothamnolica*, which is morphologically very similar to *L. subventosa* var. *subventosa*, but differs in the presence of hypothamnolic acid (thamnolic acid present in *L. subventosa* var. *subventosa*) (Archer & Elix 1993). Based on newly obtained ITS sequences of *L. subventosa* var. *hypothamnolica* and *L. subventosa* var. *subventosa* we found high variation between those taxa (Fig. 3) which suggest that *L. subventosa* var. *hypothamnolica* should be treated at the species level. Due to the existence of *L. hypothamnolica* (Dibben) Lendemer & R.C. Harris (Lendemer & Harris 2017), the new name *L. pseudosubventosa* is proposed here.

Material examined. BOLIVIA. Dept. Tarija: **Prov. Aniceto Arce**, Reserva Nacional de Flora y Fauna Tariquía, la Cumbre between Padcaya and campamento los Alisos, 21º59'02"S, 64º36'22"W, 3280 m, open area with rocks, saxicolous, 25 July 2015, *M. Kukwa 16672* (LPB, UGDA); **Prov. Burnet O'Connor**, la Cumbre close to antenas del gasoducto, old road between Entre Ríos and Tarija, 21º26'11"S, 64º24'42"W, 2769 m, open area with shrubs and sandstone rocks, saxicolous, 1 Aug. 2015, *A. Flakus* 25713 (KRAM, LPB).

Material of L. subventosa var. subventosa examined. BOLIVIA. Dept. La Paz: **Prov. Bautista Saavedra**, Área Natural de Manejo Integrado Nacional APOLOBAMBA, between la Curva and Charazani, 15º08'09"S, 69º02'03"W, elev. 3780 m, open area with shrubs, on saxicolous bryophytes, 15 Nov. 2014, *M. Kukwa 14699* (LPB, UGDA). AUSTRALIA. New South Wales: **Lord Howe Island**, along ridge to Malabar Hill, 31°31'16"S, 159°03'50"E, elev. 80 m, on basalt, 23 June 1992, *J. A. Elix 32952*, *Elix, Lich. Australas. Exs. 291* (HAL).

Lepra variolosa (Kremp.) I. Schmitt, A.W. Archer & Lumbsch

Previously reported from South America (Brazil) (Krempelhuber 1876; Archer & Elix 2017).

Material examined. BOLIVIA. Dept. Cochabamba: **Prov. Carrasco**, Parque Nacional Carrasco, near Río López Mendoza, 17º30'25"S, 65º16'51"W, elev. 2248 m, lower montane Yungas cloud forest, Andino montano (Montano), corticolous, 27 Nov. 2014, *M. Kukwa 15079a* (LPB, UGDA).

Lepraria stephaniana Elix, Flakus & Kukwa

This species has been recently described from Bolivia and known only from the type locality (Flakus *et al.* 2011).

Material examined. BOLIVIA. Dept. La Paz: **Prov. Abel Iturralde**, between Santa Rosa de Maravillas and Tumupasa, 13°58'43"S, 67°58'14"W, elev. 300 m, natural Preandean Amazon forest, corticolous, 25 May 2017, *M. Kukwa 19740* (LPB, UGDA); Dept. Santa Cruz: **Prov. Ichilo,** Parque Nacional y Área Natural de Manejo Integrado Amboró, Sendero a la Cascada, near Villa Amboró, 17°44'02"S, 63°35'05"W, elev. 470 m, transition Chaceño-Amazon forest, in the valley, corticolous, 11 May 2017, *M. Kukwa 19267* (LPB, UGDA).

****Leucodecton glaucescens*** (Nyl.) Frisch

This species has been reported from Australia, Bahamas, Belize, Brazil, Cambodia, Costa Rica, Dominican Republic, El Salvador, Jamaica, Mexico, Trinidad and Tobago, and USA (Sipman 2008; Rivas Plata *et al.* 2013; Sipman *et al.* 2012; Moon *et al.* 2013, Lücking 2015).

Material examined. BOLIVIA. Dept. Santa Cruz: **Prov. Ichilo**, Parque Nacional y Área Natural de Manejo Integrado Amboró, near La Chonta, Sendero de Mirador, 17°39'31"S, 63°42'21"W, elev. 450 m, primeval Amazon forest, corticolous, 13 May 2017, *M. Kukwa 19363* (LPB, UGDA).

Malmidea attenboroughii Kukwa, Guzow-Krzemińska, Kosecka, Jabłońska & Flakus *sp. nov.* MycoBank MB 830058. Figs 4 & 5

Diagnosis: Differs from other *Malmidea* species in having minutely verrucose to granulose-isidiate thallus, verrucose margin containing internal medullary chambers, K+ orange yellow pigment present in thalline and excipular medulla, and ascospores measuring 12–16 × 7.5–9 µm.

Type:—BOLIVIA. Dept. La Paz: **Prov. Abel Iturralde**, SE of Tumupasa, Jardin Botánico UMSA, 14°09'46"S, 67°52'02"W, alt. 400 m, semi-natural Preandean Amazon forest, roadside, corticolous, 24 May 2017, *M. Kukwa 19645* (holotype UGDA!; isotype LPB).

Thallus crustose, corticolous, continuous or cracked, minutely verrucose to granulose-isidiate (best seen in the transversal section), greenish-grey, dull, up to 200 µm thick, granules when discrete 50–125 µm diam.; *medulla* in thallus and excipular granules pale yellow, K+ orange yellow; *prothallus* thin, white, fibrous; *hypothallus* visible in some cracked areas of the thallus, white; *apothecia* sessile, rounded to irregular, up to 1.0 mm diam.; *disc* plane, flesh-coloured to brown; *excipulum* thin, up to 55 µm broad, distinct, not prominent, smooth to verrucose due to the presence of internal medullary chambers (*granifera* type), cream-coloured to dark grey-brown, in some apothecia darker around the discs, yellow in parts where the outer margin layer is abraded above medullary chambers containing yellow pigment, externally paraplectenchymatous with small cells, hyaline, but close to hymenium dark brown, with internal medullary chambers filled with more or less loosely arranged hyphae and pigment; *hypothecium* up to 50 µm high, brown, K–; *epithecium* pale orange-brown; *hymenium* up to 90 µm high, colourless; *asci* 8-spored; *ascospores* simple, broadly ellipsoidal, with evenly thickened walls, 12–16 × 7.5–9 µm. *Photobiont* chlorococcoid.

Chemistry: Traces of two unknown substances by TLC in Rf classes C3 and C5. Yellow pigment reacting K+ yellow-orange in groups of crystals present in medulla of thallus and granules, and in medullary internal chambers of apothecial margin.

Etymology: The species is named after Sir David F. Attenborough, an English broadcaster and naturalist, for his major contributions to the popularization of knowledge about biodiversity and nature protection.

Distribution and habitat: Known only from the type locality in Preandean Amazon forest in Bolivia.

Notes: Due to its granulose-isidiate thallus this species is morphologically similar to *Malmidea cineracea* Breuss & Lücking, but the latter differs in its smooth and entire margin with inner part densely encrusted with yellowish-brown granules (K+ greenish-yellow), and yellow pigments in its thallus medulla (K–) (Breuss & Lücking 2015). Other species with similar isidiate thalli differ in having a white medulla (e.g. *M. furfurosa* (Tuck. ex Nyl.) Kalb &

Lücking), granular to coralloid isidia (e.g., *M. perisidiata* (Malme) Kalb & Lücking) or different ascospores size (e.g., *M. corallophora* Aptroot & Schumm) (Kalb *et al.* 2011; Schumm & Aptroot 2012; Breuss & Lücking 2015).

Based on mrSSU dataset, *M. attenboroughii* belongs the *Malmidea* genus, being placed in a well-supported clade together with *M. bakeri* (Vain.) Kalb, Rivas Plata, *M. chrysostigma* (Vain.) Kalb, Rivas Plata & Lumbsch & Lumbsch and *M. variabilis* Kalb (Fig. 5); however, the relationships within the group are poorly supported, probably due to very short mtSSU sequences available in GenBank (in most cases of 324 bp). All three species develop thalli which are never granulose-isidiate, but in addition they differ in other characters. *Malmidea variabilis* has a thallus with distinct and large verrucae, smaller ascospores (9–12 × 6–8 μm) and contains atranorin (absent in *M. attenboroughii*). This species was known only from Asia (Thailand) (Kalb *et al.* 2011, Breuss & Lücking 2015). *Malmidea bakeri* has a densely verrucose thallus, dark brown apothecial discs and contains atranorin as a major secondary metabolite; similarly to *M. variabilis*, it is known from Asia (Thailand) (Kalb *et al.* 2011, Breuss & Lücking 2015). *Malmidea chrysostigma* has a densely verrucose thallus with a golden orange medulla that reacts K+ blood red to red–violet, and its ascospores are longer (Kalb *et al.* 2011, Breuss & Lücking 2015).

Two other species which have morphologically similar apothecia, *M. granifera* (Ach.) Kalb, Rivas Plata & Lumbsch and *M. leucogranifera* M. Cáceres & Lücking, have densely verrucose thalli (both species), dark brown to blackish apothecial disc (*M. granifera*) or orange-brown hypothecium and apothecia (*M. leucogranifera*) (Cáceres 2007; Cáceres *et al.* 2012; Breuss & Lücking 2015). Another species with similar apothecial morphology and ascospore size is *M. piperis* (Spreng.) Kalb, Rivas Plata & Lumbsch, but that species has a compact excipulum of conglutinated, radiating hyphae (*piperis* type) (Cáceres 2007; Kalb *et al.* 2011, Breuss & Lücking 2015).

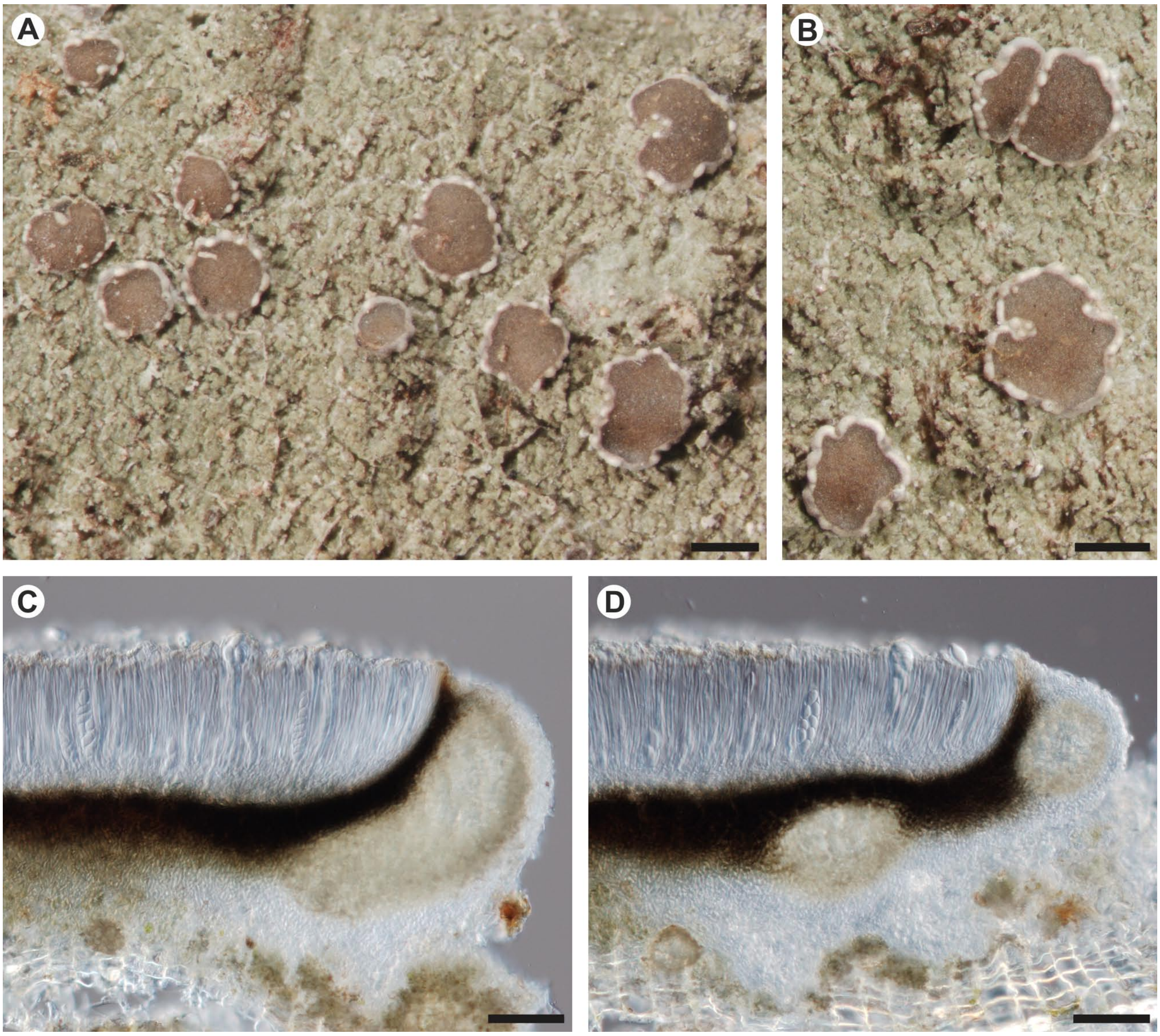

FIGURE 4. *Malmidea attenboroughii* (holotype). A–B, habit showing apothecia with distinct verrucose margin and granulose-isidiate thalli; C–D, cross section of apothecium showing paraplectenchymatous exiple with one (C) and two (D) internal medullary chambers (creamy coloured and opaque areas). Scales: A–B—500 μm; C–D—50 μm.

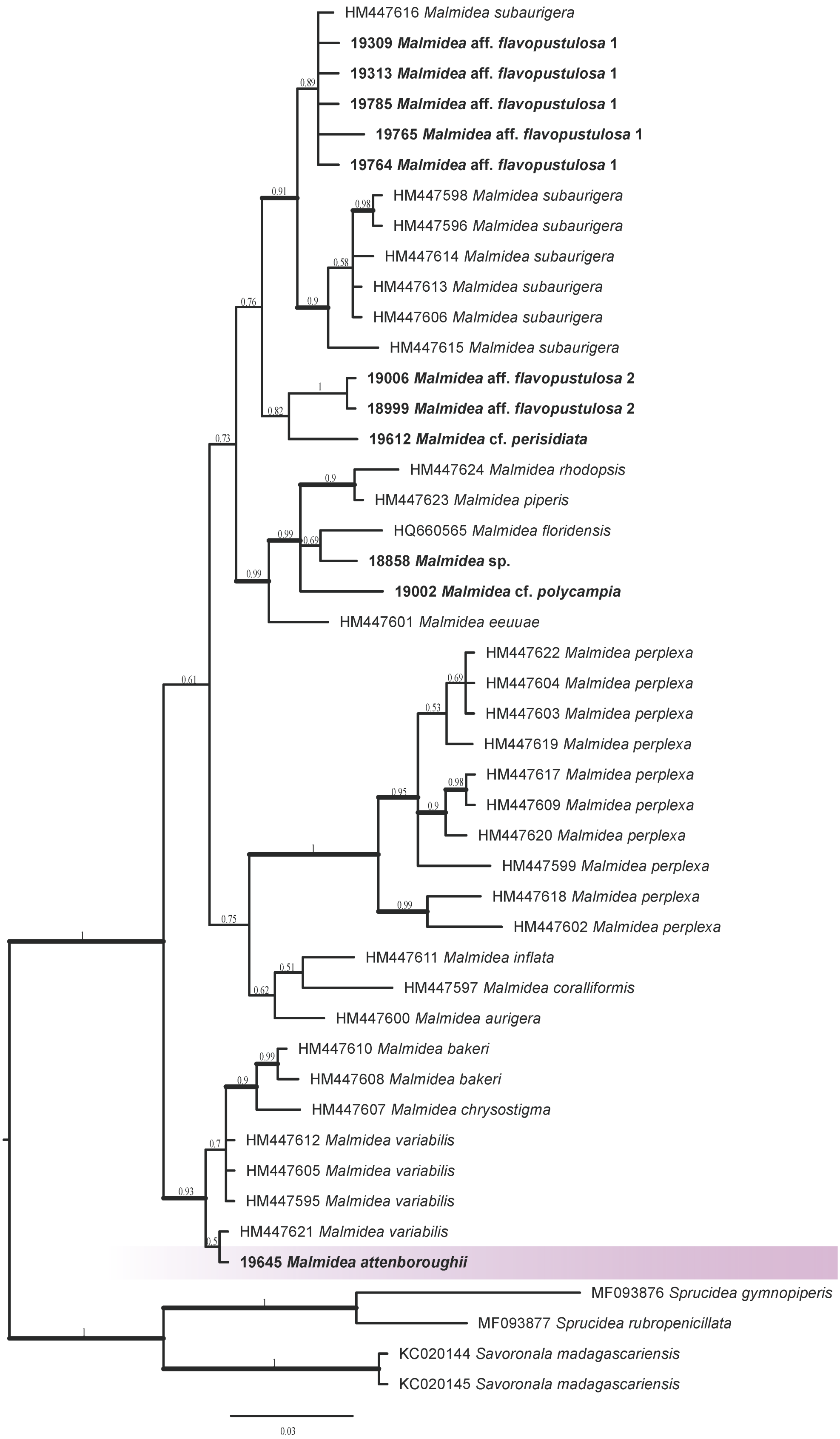

FIGURE 5. Bayesian phylogenetic tree of *Malmidea* based on mrSSU data set. Posterior probabilities are shown above branches. Internal branches, considered strongly supported, are represented by thicker lines. The newly sequenced specimens are marked in bold and collecting numbers precede the species names. In case of sequences downloaded from GenBank accession numbers precede the species names. The newly described *M. attenboroughii* is highlighted. *Savoronala madagascariensis* and two species of *Sprucidea* were used as outgroup.

Several sorediate and isidiate, but sterile, specimens of *Malmidea* (Fig. 5) were also sequenced. Those with a thallus producing pustules with a yellow medulla and punctiform to confluent soralia, and therefore similar to *M. flavopustulosa* (Cáceres & Lücking) Cáceres & Kalb, form two distinct clades (*M.* aff. *flavopustulosa* 1 and 2). They are morphologically similar and cannot be separated from each other based on thallus characters, therefore, without apothecia it is impossible to refer the name *M. flavopustulosa* to one of these clades; furthermore, all those specimens contain atranorin, which was not reported by Cáceres (2007).

A sample named *M.* cf. *polycampia* is morphologically also similar to *M. flavopustulosa* but lacks a yellow pigment and is thus similar to *M. polycampia* (Tuck.) Kalb & Lücking, but without apothecia its identity, as also in the case of isidiate material tentatively referred to as *M. perisidiata* (Malme) Kalb & Lücking, remains unclear.

Additional material of Malmidea examined. Malmidea* aff. *flavopustulosa* 1**. BOLIVIA. Dept. La Paz: **Prov. Abel Iturralde**, N of Tumupasa, 14°07’42”S, 67°53’37”W, alt. 395 m, Preandean Amazon forest, roadside, corticolous, 22 May 2017, *M. Kukwa 19764, 19765 & 19785* (LPB, UGA); Dept. Santa Cruz: *Prov. Ichilo*, Parque Nacional y Área Natural de Manejo Integrado Amboró, Macuñucu, 17°43’38”S, 63°35’38”W, elev. 465 m, Chaceño-Amazon forest, corticolous, 12 May 2017, *M. Kukwa 19309 & 19313* (LPB, UGA). ***Malmidea* aff. *flavopustulosa* 2**. BOLIVIA. Dept. La Paz: **Prov. Franz Tamayo**, between Mapiri and Apollo, 14º52’22”S, 68º21’10”W, elev. 1750 m, remnants of forest near pasture, corticolous, 19 Nov. 2016, *M. Kukwa 18999 & 19006* (LPB, UGA). ***Malmidea* cf. *perisidiata. BOLIVIA. Dept. La Paz: **Prov. Abel Iturralde**, between Ixiamas and Tumupasa, Orilla de Cuñaca, 13°56’44”S, 68°02’07”W, elev. 330 m, natural Preandean Amazon forest, corticolous, 23 May 2017, *M. Kukwa 19612* (LPB, UGA). ***Malmidea* cf. *polycampia***. BOLIVIA. Dept. La Paz: **Prov. Franz Tamayo**, between Mapiri and Apollo, 14º52’22”S, 68º21’10”W, elev. 1750 m, remnants of forest near pasture, corticolous, 19 Nov. 2016, *M. Kukwa 19002* (LPB, UGA). ***Malmidea* sp.** BOLIVIA. Dept. La Paz, **Prov. Franz Tamayo**, between Mapiri and Apollo, 14º38’51”S, 68º24’44”W, elev. 1520 m, remnants of forest on savanna, corticolous, 18 Nov. 2016, *M. Kukwa 18858* (LPB, UGA).

****Megalospora sulphurata*** Meyen **var. *nigricans*** (Müll. Arg.) Riddle

This variety has been reported from Argentina, Brazil, Jamaica, Mexico and Venezuela (Sipman 1983; Marcano *et al.* 1996).

Material examined. BOLIVIA. Dept. Tarija: **Prov. Burnet O’Connor**, 60 km from Tarija, new road between Tarija and Entre Ríos, 21º28’52”S, 64º17’41”W, 1837 m, Boliviano-Tucumano forest with *Podocarpus*, corticolous, 28 July 2015, *M. Kukwa 16929* (LPB, UGDA).

****Micarea hedlundii*** Coppins (Fig. 6)

Type:—NORWAY. **Oppland**, Ringebu, 5.5 km ENE of Ringebu, along Soraa, between Nyhamnsbekken and Ulveslabekken. Lat/long: 61:33N 10:13E. Alt.: 400 m, decorticated stump. 25 Aug. 1979, *L. Tibell 8657* (holotype UPS L-05554!).

This species has been described from Norway and is currently known from several localities in Europe (e.g., Austria, Germany, Norway, Lithuania, Poland, Sweden, Ukraine). It has also been reported from Africa (Rwanda), North America (Canada, USA) and South America (Chile) (Coppins 1983; Gowan & Brodo 1988; Motiejűnaitë 2005; Czarnota 2007).

The phylogenetic tree which includes sequences of *Micarea hedlundii* shows that the Bolivian specimen is placed with the European population in a highly supported monophyletic clade (BS 100%, PP 0.1, Fig. 7). Although it is not an aim of this study to resolve the position of the *Micarea* species within the genus, our molecular tree is very similar in topology to those proposed by Czarnota & Guzow-Krzemińska (2010) and Guzow-Krzemińska *et al.* (2016). Therefore, the occurrence of this species in the Southern Hemisphere is confirmed based on molecular evidence.

Material examined. BOLIVIA. Dept. La Paz: **Prov. Franz Tamayo**, Parque Nacional y Área Natural de Manejo Integrado Madidi, Chuñuna above Keara, 14º41’11”S, 69º05’30”W, 4053 m, *Polylepis pepei* forest, on lignum, 19 Nov. 2014, *A. Flakus 25384 & J. Quisbert* (KRAM, LPB).

****Myriotrema glauculum*** (Nyl.) Hale

This neotropical species is known only from Brazil, Cuba, Panama, Trinidad and Tobago (Hale 1978; Lücking 2015).

Material examined. BOLIVIA. Dept. La Paz: **Prov. Larecaja**, near Guanay, Aguada, 15°29’50”S, 67°55’50”W, elev. 1010 m, remnants of humid, natural forest near river, with large trees, corticolous, 21 Nov. 2016, *M. Kukwa 19095* (LPB, UGDA).

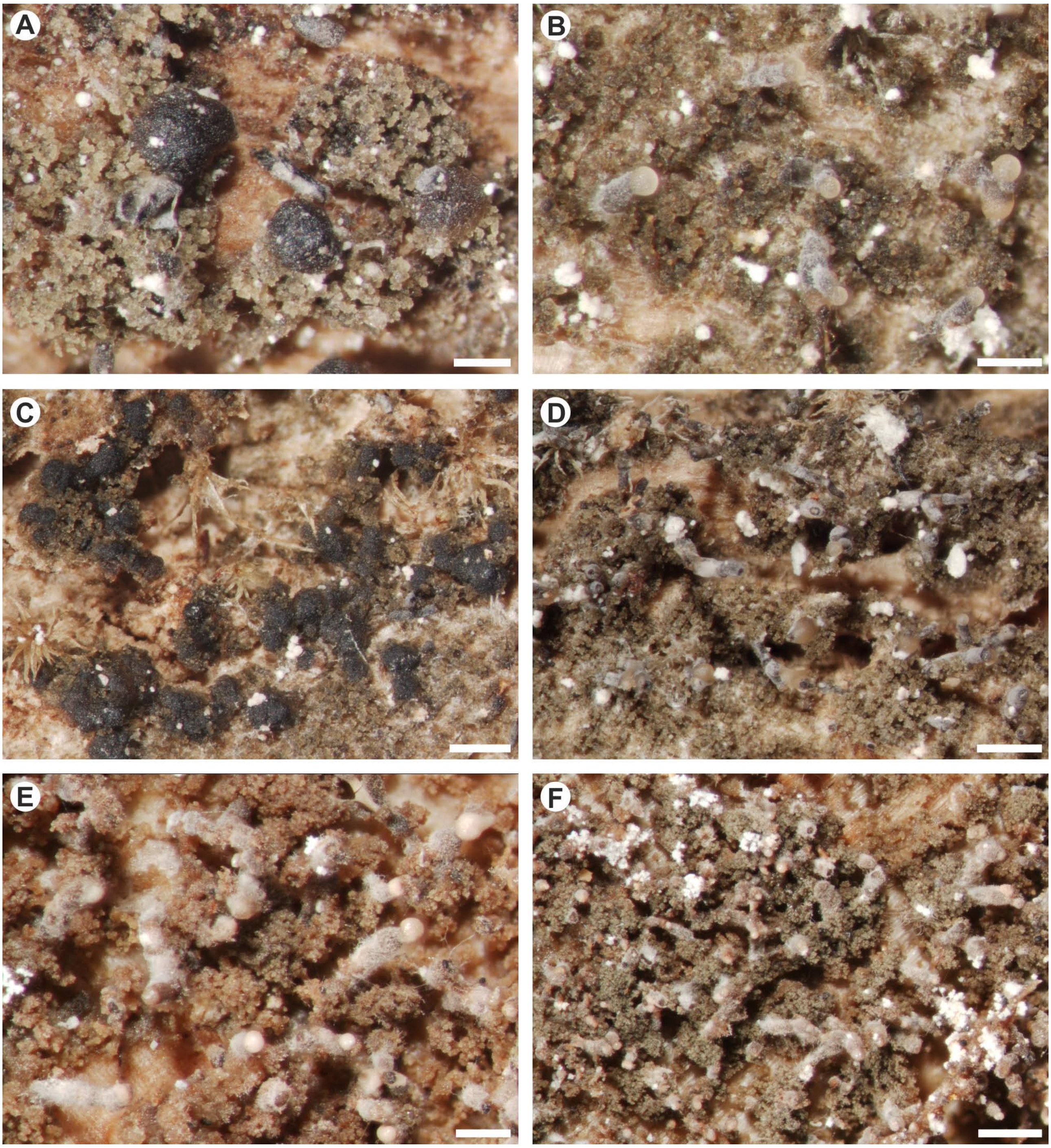

FIGURE 6. Habit of *Micarea hedlundii* showing thallus composed of goniocysts and ascomata or conidiomata. A–B, greyish black emarginate apothecia (Bolivia, *A. Flakus 25384*); C–D, stalked, moderately white tomentose pycnidia (Bolivia, *A. Flakus 25384*); E–F stalked, strongly white tomentose pycnidia (Norway, holotype). Scales: A, C, E—250 µm, B, D, F—500 µm.

***Ocellularia erodens** (R.C. Harris) Kraichak, Lücking & Lumbsch

This species has been reported from Cuba, Panama Peru, USA and Venezuela (Lücking *et al.* 2011; Rivas Plata & Lücking 2013; Rivas Plata *et al.* 2013; Kraichak *et al.* 2014).

All our specimens were sterile and sorediate. ITS, mrSSU and nucLSU markers were sequenced from five samples (*Kukwa 19258, 19754, 19732, 19568, 19668*; Tab. 1). ITS sequences for four of these (*Kukwa 19258, 19754, 19732, 19568*) show 93% of identity (using BLASTn search) to the sequence of *O. urceolaris* Ach. (GenBank accession no. AJ508680). The mrSSU sequences of all samples showed 98–99% of identity to two sequences of *O. erodens* (GenBank accession nos JX421526 and JX421523) and *O. auberianoides* (Nyl.) Müll. Arg. (GenBank accession nos

JX421549 and JX421548) and in the case of nucLSU 99–100% of identity to the sequence of *O. erodens* (GenBank accession no. JX421092) and 99% of identity to sequence *O. auberianoides* (GenBank accession nos JX421122 and JX421123).

The high similarity of molecular markers of *O. auberianoides*, *O. erodens* and *O. sorediigera* Kalb have already been reported by Lücking *et al.* (2011). They may represent one species with a variable size of ascospores and two reproductive modes, but this needs more molecular data and will be discussed in a forthcoming paper.

Material examined. BOLIVIA. Dept. La Paz: **Prov. Abel Iturralde**, between Ixiamas and Tumupasa, Orrilla de Cuñaca, 13°56’42”S, 68°02’00”W, elev. 335 m, natural Preandean Amazon forest, corticolous, 23 May 2017, *M. Kukwa 19568* (LPB, UGDA); between Santa Rosa de Maravillas and Tumupasa, 13°58’43”S, 67°58’14”W, elev. 300 m, natural Preandean Amazon forest, corticolous, 25 May 2017, *M. Kukwa 19732 & 19754* (LPB, UGDA); SE of Tumupasa, Jardin Botánico UMSA, 14°09’46”S, 67°52’02”W, elev. 400 m, semi-natural Preandean Amazon forest, by the road, partly cut, corticolous, 24 May 2017, *M. Kukwa 19668* (LPB, UGDA); Dept. Santa Cruz: **Prov. Ichilo**, Parque Nacional y Área Natural de Manejo Integrado Amboró, Sendero a la Cascada, near Villa Amboró, 17°44’02”S, 63°35’05”W, elev. 470 m, transition Chaqueño-Amazon forest, in a valley, corticolous, 11 May 2017, *M. Kukwa 19258* (LPB, UGDA).

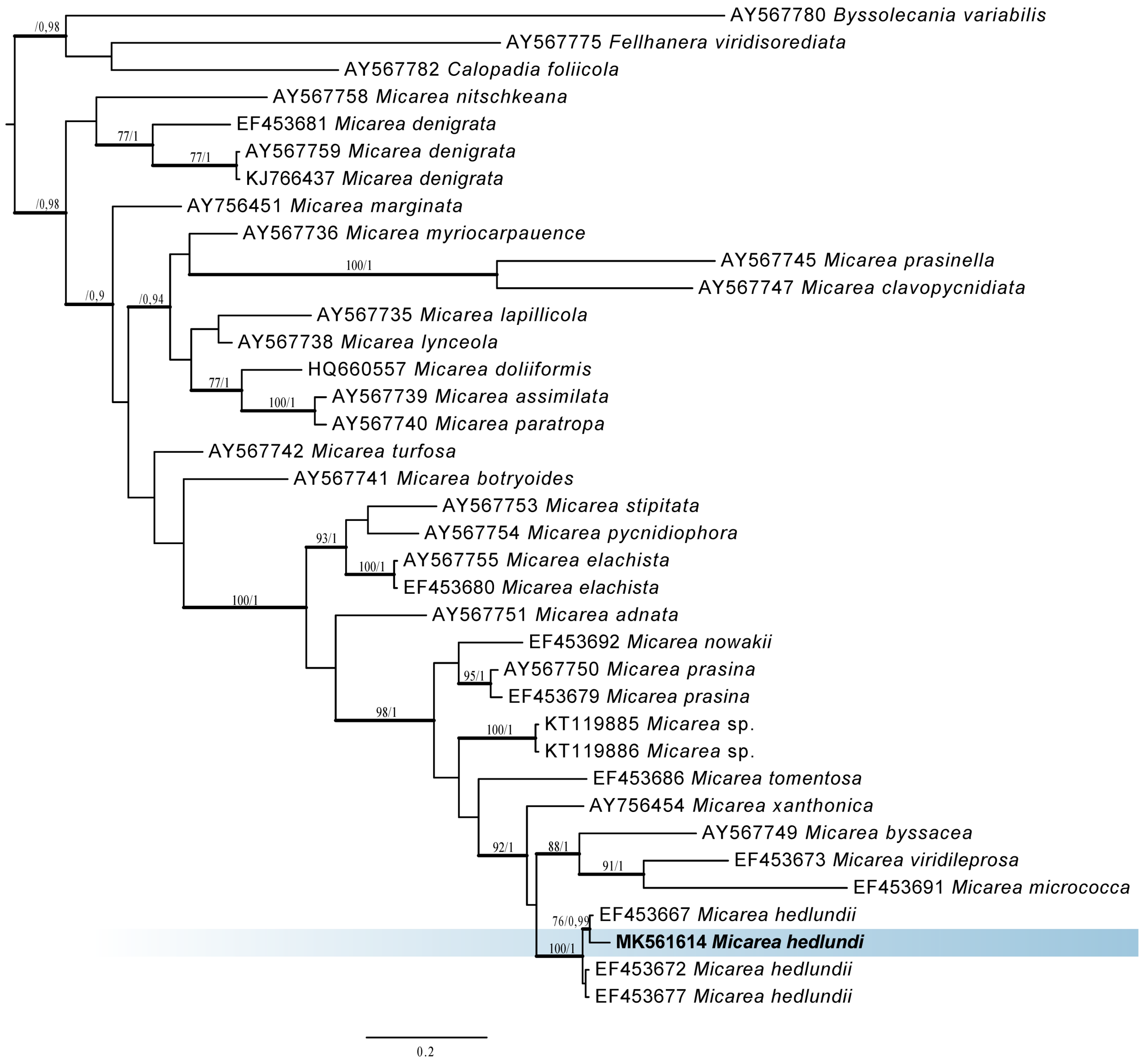

FIGURE 7. Maximum likelihood phylogenetic tree of *Micarea* based on mrSSU data set. High nodal support values are indicated by bold branches, including ML bootstrap values ≥ 75% and MCMC posterior probability ≥ 0.9. *Micarea hedlundii* is in bold and highlighted. GenBank, accession numbers precede the species names. *Byssolecania variabilis*, *Fellhanera viridisorediata* and *Calopadia foliicola* were used as outgroup.

***Ocellularia microsorediata** Rivas Plata & Lücking

Previously only reported from Peru (Rivas Plata & Lücking 2013; Rivas Plata *et al.* 2013; Ramos 2014).

All specimens are sterile. One specimen (*Kukwa 19658*, Tab. 1) has been sequenced and the nucLSU sequence shows 99% of identity (using BLASTn search) to the sequences of *Ocellularia microsorediata* deposited in GenBank (accession nos JX421573 and JX421572). The mrSSU sequence of the same sample also shows 99% of identity to the sequence of *O. microsorediata* (GenBank accession nos JX421172).

Material examined. BOLIVIA. Dept. La Paz: **Prov. Abel Iturralde**, between Ixiamas and Tumupasa, Orilla de Cuñaca, 13°56’44”S, 68°02’07”W, elev. 330 m, natural Preandean Amazon forest, corticolous, 23 May 2017, *M. Kukwa 19615* (LPB, UGDA); SE of Tumupasa, Jardin Botánico UMSA, 14°09’46”S, 67°52’02”W, elev. 400 m, semi-natural Preandean Amazon forest, by the road, corticolous, 24 May 2017, *M. Kukwa 19658* (LPB, UGDA); Tahua village, 13°51’54”S, 67°54’29”W, elev. 240 m, semi-natural Amazon forest, roadside, corticolous, 25 May 2017, *M. Kukwa 19712* (LPB, UGDA).

****Pertusaria muricata** J.C. David
syn. *Lepra muricata* (J.C.David) A.W.Archer & Elix

Previously reported from Australia and Mauritius (David & Hawskworth 1995; Archer & Elix 2017, 2018).

Pertusaria muricata, found only in a sterile state, has recently been transferred to the genus *Lepra* Scop. (Archer & Elix 2018), but the nucITS sequence (see Tab. 1) shows the highest similarity to *Pertusaria* spp.; for example, 87% of identity to sequence obtained from *P. alpina* Hepp ex Ahles (GenBank accession no. AF332128), while it is similar to *Lepra* spp. only in 5.8 S region. Moreover, the mrSSU sequence is most similar to *P. kalelae* Messuti (AY567989) with 98% of identity and the nucLSU is most similar to *P. pentelicii* J. Steiner (AF419327) and *P. tejocotensis* J. Steiner (AF279301) with 97% of identity. Those results suggest that currently this species may be better placed in *Pertusaria* DC, but its phylogenetic position needs further study.

Material examined. BOLIVIA. Dept. Tarija: **Prov. Burnet O’Connor**, old road between Entre Ríos and Tarija, 21º29’13”S, 64º11’42”W, elev. 1535 m, Boliviano-Tucumano forest, corticolous, 31 July 2015, *M. Kukwa 16954* (LPB, UGDA).

***Pertusaria patagonica** Müll. Arg.

Previously known only from Argentina and New Zealand (Messuti & Vobis 2002).

Material examined. BOLIVIA. Dept. Tarija: **Prov. Aniceto Arce**, Reserva Nacional de Flora y Fauna Tariquía, between la Cumbre and campamento los Alisos, 22º00’52”S, 64º36’24”W, elev. 2796 m, disturbed forest with *Polylepis*, saxicolous, 25 July 2015, *M. Kukwa 16658* (LPB, UGDA); Dept. Chuquisaca, **Prov. Zudañez**, Área Natural de Manejo Integrado El Palmar, La Cascada bajo de El Palmar, 18º41’23”S, 64º54’26”W, elev. 2740 m, Boliviano-Tucumano forest with *Podocarpus*, Lauraceae and palms, saxicolous, 15 July 2015, *M. Kukwa 16180, 16180a* (LPB, UGDA); El Palmar, 18º41’28”S, 64º54’32”W, 2600–2876 m, forest with palms and shrubs, saxicolous, 15 July 2015, *M. Kukwa 16908* (LPB, UGDA).

***Pertusaria tesselaria** Müll. Arg.

Previously known only from Brazil (Müller Argoviensis 1889; Wainio 1900; Archer & Elix 2017).

Material examined. BOLIVIA. Dept. Chuquisaca: **Prov. Zudañez**, Área Natural de Manejo Integrado El Palmar, Muy Orquo, on road from El Palmar to Loman, 18º47’46”S, 64º51’31”W, 2879 m, open area, table mountain of sandstone, on sandstone, 14 July 2015, *M. Kukwa 16892a* (LPB, UGDA).

***Ramboldia heterocarpa** (Fée) Kalb, Lumbsch & Elix

A tropical species previously known from Brazil, Costa Rica, Uruguay, Venezuela, Tanzania and Republic of South Africa (Kalb *et al.* 2008).

Material examined. BOLIVIA. Dept. Tarija: Prov. Aniceto Arce, Reserva Nacional de Flora y Fauna Tariquía, close to la Cumbre between Padcaya and campamento los Alisos, 22º00’00”S, 64º36’29”W, elev. 3158 m, open area with *Puya* and rocks, saxicolous, 24 July 2015, *M. Kukwa 16615* (LPB, UGDA).

***Sprucidea penicillata* (**Aptroot, M.Cáceres, Lücking & Sparrius) M.Cáceres, Aptroot & Lücking

This species with its peculiar greyish conidiomata was described from Brazil, Costa Rica, Papua New Guinea and Thailand (Aptroot *et al.* 2007; Cáceres *et al.* 2017).

Material examined. BOLIVIA. Dept. La Paz: **Abel Iturralde**, SE of Tumupasa, Jardin Botánico UMSA, 13°09'46"S, 67°52'02"W, elev. 400 m, semi-natural Preandean Amazon forest, roadside, corticolous, 24 May 2017, *A. Flakus 29308 & 29338, M. Kukwa 19650* (KRAM, LPB, UGDA).

Thrombium epigaeum (Pers.) Wallr.

A widely distributed species known from Africa, Europe, Asia, New Zealand, and North and South America (Purvis & Orange 2008).

Material examined. BOLIVIA. Dept. Cochabamba: **Prov. Tiraque**, Parque Nacional Carrasco, the crossroads below Cerro Juno, 17º19'50"S, 65º43'50"W, 4126 m, open high Andean vegetation, on soil, 29 Nov. 2014, *A. Flakus 25940.1* (KRAM, LPB).

Varicellaria velata (Turner) I. Schmitt & Lumbsch

This is a widespread species. It is known from Africa (e.g., Sierra Leone, Republic of South Africa), Asia (e.g., China, India, Indonesia, Japan, Sri Lanka), Australasia (e.g., Australia, New Guinea, New Caledonia, New Zealand), Europe (e.g., France, Germany, Great Britain, Italy, Sweden) North America (Canada, USA) and South America (Argentina, Brazil, Chile, Colombia, Ecuador, French Guiana, Paraguay, Uruguay) (Hekking & Sipman 1988; Archer & Messuti 1997; Messuti & Vobis 2002; Spielmann 2006; Nöske *et al.* 2007).

Material examined. BOLIVIA. Dept. Tarija: **Prov. Burnet O'Connor**, close to Soledad, old road between Entre Ríos and Chuquiaca, 21º39'45"S, 64º07'22"W, 1750 m, Boliviano-Tucumano forest with shrubs and *Alnus acuminata*, corticolous, 31 July 2015, *M. Kukwa 16942b* (LPB, UGDA).

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