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A new *Bunodophoron* species (*Sphaerophoraceae*, *Lecanorales*) from the Neotropics

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Abstract: This is the first part of an ongoing taxonomic treatment of *Bunodophoron* (*Sphaerophoraceae*, *Lecanorales*) in the Neotropics, based on the molecular phylogenetic analysis of three markers together with studies of morphology and chemistry, and using the general mixed Yule coalescence (GMYC) method to delimit species boundaries. In the Neotropics, species in this genus grow on the ground or on shrubs in the páramos, and as epiphytes in the montane rainforests. We describe here a new species from the páramos of Colombia, *Bunodophoron crespoae* Soto, M. Prieto & Wedin sp. nov., and discuss its distinction from another large and common páramo species *Bunodophoron flabellatum* (Hue) Soto, M. Prieto & Wedin comb. nov. Both species are primarily terrestrial in the páramos, although *B. flabellatum* may occasionally also grow as an epiphyte. *Bunodophoron crespoae* is characterized by the white, *c.* 10–13 cm long, subterete to narrowly flattened, main branches. It differs from the otherwise similar *B. flabellatum* by being distinctly subterete, more abundantly branched, and by having smaller ascospores. Both are distinguished from the primarily epiphytic *B. melanocarpum* by the considerably larger thallus size, with the main branches of *B. melanocarpum* rarely exceeding 3·5 cm in length and 2 mm in width.

Key words: lichenized fungi, nomenclature, phylogeny, species boundaries, systematics, taxonomy

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Introduction

Sphaerophoraceae (*Lecanorales*, Lecanoromycetes) contains large, often eye-catching and frequently collected mazaediate macrolichens. The genus *Bunodophoron* A. Massal. was resurrected following a hypothesis on the phylogeny of the family, based on morphological and chemical characters (Wedin 1993). It includes the majority of the species previously classified in *Sphaerophorus* Pers., and differs from *Sphaerophorus* s. str. in ascospore shape and ontogeny. *Bunodophoron* has globose spores with an irregular ornamentation consisting of an amorphous material adhering to the spore wall following release from the asci, whereas *Sphaerophorus* has broadly ellipsoidal spores where a thick secondary spore wall is developed when the spores are still inside the asci (Tibell 1981, 1984; Wedin 1990, 1991, 1992, 1993; Wedin & Tibell 1991; Kantvilas & Wedin 1992). *Bunodophoron* is further characterized (and differs from *Sphaerophorus* and the closely related *Leifidium* Wedin) by having a more or less dorsiventrally flattened thallus, subapically to ventrally exposed mazaedia and rod-shaped conidia. Species of *Bunodophoron* usually contain stictic or protocetraric acid (frequently together with related substances) in the medulla, whereas species of *Sphaerophorus* usually contain thamnolic or squamatic acid. A detailed

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discussion of taxonomic concepts and relevant characters can be found in Wedin (1993) and Wedin (1995*a*). The position of the mazaediate *Sphaerophoraceae* was subsequently analyzed in a series of molecular phylogenies by Wedin and co-workers. *Sphaerophoraceae* was confirmed to be lecanoralean (Wedin *et al.* 1998) and to include the small non-mazaediate genera *Neophyllis* (F. Wilson) F. Wilson and *Austropeltum* Henssen *et al.* (Wedin & Döring 1999; Wedin *et al.* 2000). This relationship was further supported by all these genera sharing an anatomically and ontogenetically similar boundary tissue between ascomatal and thalline tissues, which is presumably a synapomorphy for the family (Henssen *et al.* 1992; Döring *et al.* 1999; Döring & Wedin 2000). The closest relative to *Sphaerophoraceae* is likely to be the mazaediate *Calycidiaceae* (Wedin 2002; Prieto *et al.* 2013).

Bunodophoron is widespread in humid and temperate regions, particularly of the Southern Hemisphere, for which Wedin (1995*a*) revised the whole family. Currently *c.* 20 species are accepted in *Bunodophoron* worldwide (Wedin 1993, 1995*a*; Lumbsch *et al.* 2011) but there is no modern treatment for any tropical region (however, see Tibell (1982, 1987), Wedin (1992, 1995*a*) and Lumbsch *et al.* (2011) for discussions on single species) and many additional species can be expected to occur in moist high-altitude areas. Most species are epiphytic but several can occur on rocks and soil. In the neotropical region, *Bunodophoron* species are often important and conspicuous components in the páramos (Fig. 1A). They belong to a species group within *Bunodophoron* with sphaerophorin, stictic and constictic acids, and with comparatively small, greyish to brownish grey spores (Wedin 1993, 1995*a*). Only two species are currently accepted in the Neotropics: *B. formosanum* (Zahlbr.) Wedin and *B. melanocarpum* (Sw.) Wedin (Tibell 1982). These taxa are not well understood and the names are probably applied only by tradition to samples with or without isidioid outgrowths along the main branches, respectively (Wedin 1995*a*). The application of the name *Lichen melanocarpum* Sw. described from Jamaica, one of the oldest names available, will be important to clarify in a future paper. The present study is an introduction to *Bunodophoron* in the Neotropics and deals with two large and morphologically distinct *Bunodophoron* species which occur predominantly in the páramo areas, one of which is described as new. The páramo is a very diverse and species-rich neotropical ecosystem above the continuous timberline, dominated by shrubby grasslands and boggy areas. The lichen vegetation is comparatively poorly studied but baseline studies are summarized by Sipman (1999, 2002). Examples of recent ecological and systematic studies in páramo areas include González *et al.* (2017) and Lücking *et al.* (2014).

Materials and Methods

Specimens and morphology

The specimens studied were collected in areas of montane forest and páramo in Colombia and Ecuador. Additional specimens were sourced from the Universidad Distrital Francisco José Caldas (UDBC), Universidad del Valle (CUVC), Universidad de Caldas (FAUC), Swedish Museum of Natural History (S), Museum of Evolution at Uppsala University (UPS) and Herbarium des Botanischen Gartens und Botanischen Museums Berlin-Dahlem (B).

The specimens were studied using light microscopy, and a selection of samples was investigated with high performance thin-layer chromatography (HPTLC; Arup *et al.* 1993; Orange *et al.* 2001) using solvent systems B and C. Morphological terms and measurements follow Wedin (1995*a*).

For the phylogenetic analyses, we used a subset of neotropical samples (Table 1) with an additional number of *Bunodophoron* species belonging to the species group with stictic acid and small grey spores, to serve as functional outgroups based on the phylogeny in Wedin (1993). The resulting tree was rooted using one of these, *B. ramuliferum*.

Molecular techniques

DNA was extracted from 23 samples (Table 1) using DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Three protein coding genes were amplified: β-tubulin, RNA polymerase II largest subunit (*RPB1*) and DNA replication licensing factor (*Mcm7*). PCR amplifications were performed using Illustra™ Hot Start Mix RTG PCR beads (GE Healthcare, UK) in a 25 µl volume, containing 5 µl of DNA, 0·5–1·2 µM of each primer and distilled water.

FIG. 1. *Bunodophoron* in the Neotropics. A, typical Colombian páramo, an important habitat for *Bunodophoron* in the Neotropics; B, *B. crespoae* growing among *Cladonia* and bryophytes in Colombian páramo; C, *B. crespoae* (holotype), thallus, note apothecia in the uppermost branch. Scale: C = 1 cm.

TABLE 1. *Specimens used for the phylogenetic study of* Bunodophoron, *with GenBank Accession numbers for newly produced sequences.*

Species	Sample identity	Extraction code	GenBank Accession numbers β-tubulin	*RPB1*	*Mcm7*	Country
B. crespoae	*Moncada* 45 (UDBC)	ES1	MF954554	MF954577	MF954535	Colombia
B. crespoae	*Moncada* 16 (UDBC)	ES27	MF954555	MF954578	MF954539	Colombia
B. crespoae	*Moncada* 24 (UDBC)	ES28	MF954556	MF954579	MF954544	Colombia
B. crespoae	*Arango* 46 (CUVC)	ES52	MF954557	MF954580	MF954543	Colombia
B. crespoae	*Soto & Barrera* 20I (S)	ES53	MF954558	MF954581	MF954540	Colombia
B. crespoae	*Soto & Barrera* 21I (CUVC)	ES54	MF954559	MF954582	MF954546	Columbia
B. flabellatum	*Hurtado* s. n. (CUVC)	ES13	MF954564	MF954587	MF954537	Colombia
B. flabellatum	*Moncada et al.* 69 (UDBC)	ES26	MF954565	MF954588	MF954541	Colombia
B. flabellatum	*Benitez, González & Prieto* 4000 (S)	MW128	MF954562	MF954585	MF954548	Ecuador
B. flabellatum	*Benitez & Prieto* 4007 (S)	MW129	MF954563	MF954586	MF954542	Ecuador
B. melanocarpum	*Benitez, González & Prieto* 4006 (S)	ES62	MF954572	MF954595	MF954538	Ecuador
B. melanocarpum	*Benitez & Prieto* 4008 (S)	ES65	MF954573	MF954596	MF954549	Ecuador
B. melanocarpum	*Malaver* 97 (UDBC)	ES3	MF954567	MF954590	MF954547	Colombia
B. melanocarpum	*Coca* 1703 (FAUC)	ES6	MF954568	MF954591	MF954536	Colombia
B. melanocarpum	*Coca* 1806 (FAUC)	ES8	MF954569	MF954592	MF954550	Colombia
B. melanocarpum	*Soto* 26 (CUVC)	ES16	MF954570	MF954593	MF954551	Colombia
B. melanocarpum	*Home* s. n. (CUVC)	ES21	MF954571	MF954594	MF954545	Colombia
B. australe	*Wedin* 8046 (S)	CO334	MF954553	MF954576	MF954530	New Zealand
B. diplotypum	*Rivas Plata & Lücking* 1195 (F)	MWE107	MF954560	MF954583	MF954532	Philippines
B. dodgei	*Wedin* 8630 (S)	MWE101	MF954561	MF954584	MF954531	Argentina
B. formosanum	*Wedin* 3529 (UPS)	MWE152	MF954566	MF954589	MF954552	Australia
B. notatum	*Wedin* 9188 (S)	MWE134	MF954574	MF954597	MF954533	New Zealand
B. ramuliferum	*Wedin* 8652 (S)	MWE102	MF954575	MF954598	MF954534	Argentina

Amplifications were performed using the following programs: for β-tubulin, initial denaturation at 95 °C for 5 min followed by 4 cycles of 95 °C for 30 s, 54 °C for 40 s and 72 °C for 1 min, 4 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, 35 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 8 min; for *RPB1* and *Mcm7*, initial denaturation at 95 °C for 5 min followed by 4 cycles of 95 °C for 30 s, 57 °C for 40 s and 72 °C for 1 min, 4 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, 35 cycles of 95 °C for 30 s, 53 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 8 min.

β-tubulin was amplified using the newly designed primers bTub-75F-Buno and bTub-803R-Buno or bTub-75F-Buno (Table 2) and Bt10-LM (Myllys *et al.* 2001), or in some cases in two steps using the newly designed primer pairs bTub-75F-Buno and bTub-486R-Buno, and bTub-462F-Buno and bTub-803R-Buno (Table 2). The *RPB1* was amplified with the primers RPB1-Af (Stiller & Hall 1997) and RPB1-773R-Sphaero (Table 2), or in some cases in two steps using the newly designed primer pairs RPB1-46F-Buno and RPB1-404R-Sphaero, and RPB1-367F-Buno and RPB1-773R-Sphaero (Table 2). For *Mcm7* we used the newly designed primers MCM7-SphaeF and MCM7-SphaeR, or in some cases in two steps using the primer pairs MCM7-SphaeF and MCM7-SphaeIntR, and MCM7-SphaeIntF and MCM7-SphaeR (Table 2).

PCR products were subsequently purified using the enzymatic method Exo-sap-IT (USB Corporation, Santa Clara, California, USA) or when multiple bands were amplified, products were size-fractionated on a 1% agarose gel run in TBE buffer, stained with GelRed™ (Biotium Inc.), visualized over a UV transilluminator, excised and purified using QIAquick spin columns (Qiagen). The purified PCR products were sequenced with the BigDye Terminator Kit v.3.1 (ABI Prism, USA), using the PCR primers.

Sequences were assembled and edited using Sequencher v.4.10.1 (Genes Codes Corporation, Ann Arbor) and deposited in GenBank (Table 1). Subsequently, sequences were aligned manually using MacClade 4.01 (Maddison & Maddison 2001) utilizing the amino acid translation. Introns were delimited manually by comparing with sequences already annotated in GenBank and excluded from phylogenetic analyses.

TABLE 2. *Sequences of newly designed primers used in the phylogenetic study of* Bunodophoron.

Protein coding gene	Primer	Sequence
β -tubulin		
	bTub-75F-Buno	5'-CTAGGCATCAAGCAACAAATATGTTCC-3'
	bTub-486R-Buno	5'-ACCTCGTTGTCGATACAGAAAGTC-3'
	bTub-462F-Buno	5'-CCTTACAACGCAACTCTRTCTGTTC-3'
	bTub-803R-Buno	5'-TCGTACATCTGCTGGGTCAATTCTG-3'
RPB1		
	RPB1-46F-Buno	5'-GCCCCTGTCTTCCATGTTGGTATG-3'
	RPB1-404R-Sphaero	5'-ACGGATTTCGGGTTGGATRTTTCC-3'
	RPB1-367F-Buno	5'-CGTCGACGAAGATGCACCGCTKG-3'
	RPB1-773R-Sphaero	5'-AGCTGCTCAAATTCATTCACGACATG-3'
Mcm7		
	MCM7-SphaeF	5'-AAGCCCGCAGTCCAGGTCAATGCGT-3'
	MCM7-SphaeIntF	5'-CAATCCCGGAGATGTTGTTGATAT-3'
	MCM7-SphaeIntR	5'-TCAGGAGCCCAGCTCTAATTGCCT-3'
	MCM7-SphaeR	5'-CGGGGTCTCCCATCAAGCAGATGTT-3'

Phylogenetic analyses

Each individual gene region was analyzed using maximum likelihood-based inference (ML) as implemented in RAxML v.8.1.11 (Stamatakis 2014) with a GTRGAMMA model for tree inference. Bootstrapping was performed with a GTRCAT model and 1000 replicates. In order to check for gene-tree incongruence, we compared maximum likelihood bootstrap values (ML-BS) between the individual gene trees, considering a conflict among clades when a supported clade (bootstrap support >70%) for one marker was contradicted with significant support by another. Since no supported nodes were in conflict, the data were combined into a single concatenated data matrix. The combined maximum likelihood (ML) analyses were run with six distinct partitions (first and second codon positions of the *Mcm7*, β-tubulin and *RPB1* and the third codon position of the *Mcm7*, β-tubulin and *RPB1*), using a GTRGAMMA model of molecular evolution and rate heterogeneity with unlinked parameters and 1000 bootstrap replicates.

To select models of nucleotide substitution, we used jModelTest 2.0 (Guindon & Gascuel 2003; Darriba *et al.* 2012) using the Akaike Information Criterion (AIC) for model selection. The GTR model (Rodríguez *et al.* 1990) with an estimated proportion of invariable sites was selected for all partitions of the β-tubulin and *RPB1*, while HKY (Hasegawa *et al.* 1985) with an estimated proportion of invariable sites was selected for all partitions of the *Mcm7*. Bayesian inference was carried out through Markov chain Monte Carlo (MCMC) sampling, as implemented in MrBayes 3.2.3 (Ronquist *et al.* 2011). The analyses consisted of two parallel searches, each with four chains run for 10 M generations and initiated with random starting trees. The chains were sampled every 100 generations from the posterior distribution. A burn-in sample of 25 000 trees was discarded for each run. The remaining 150 000 trees (pooled from both independent runs) were used to assemble a majority-rule consensus tree and to estimate branch lengths and posterior probabilities (PPs). To determine if the chains had converged, verify if mixing was appropriate and choose a suitable burn-in, we plotted the log-likelihood values against the time generation with Tracer v.1.5.0 (Rambaut & Drummond 2007). We assumed stationarity of the chains when log-likelihood values reached the same stable equilibrium value for each independent run (Huelsenbeck & Ronquist 2001) and when the average standard deviation of split frequencies across runs dropped below 0·01. We also tested convergence with the AWTY program (Wilgenbusch *et al.* 2004; Nylander *et al.* 2008). Maximum likelihood, Bayesian analysis and the selection of models were run on the CIPRES Science Gateway v.3.3 (Miller *et al.* 2010).

Species delimitation

We used the general mixed Yule coalescence (GMYC) single-threshold method to delimit species boundaries (Pons *et al.* 2006; Monaghan *et al.* 2009). This method is based on the differential branching patterns generated by speciation (Yule process) and intraspecific events (coalescent events) observed in an ultrametric tree. The null model assumes that all individuals belong to a single species and branching patterns indicate a coalescent process. The ultrametric tree used in this analysis was built in BEAST v.1.8 (Drummond *et al.* 2012) with the combined alignment, using BEAUti v.1.8 (Drummond *et al.* 2012) to prepare the input with partitions and nucleotide substitution models as used in the previous Bayesian analysis. We implemented an uncorrelated relaxed lognormal clock (Drummond *et al.* 2006) and selected a Yule tree prior, with default values for remaining priors. BEAST analyses were run for

40 million generations, logging parameters and trees every 1000 generations. Convergence, mixing and effective sample sizes (ESS) of parameters were checked using Tracer v.1.5.0 (Rambaut & Drummond 2007). A burn-in of 1000 trees was removed from each analysis. The remaining trees were used to generate a maximum clade credibility tree with TreeAnnotator v.1.8.2 (Drummond *et al*. 2012). The GMYC single-threshold model was implemented in the splits package (Ezard *et al*. 2009; Fujisawa & Barraclough 2013) for R v.3.0.3 (R Core Team 2013) using the functions of the ape package (Paradis *et al*. 2004).

Results and Discussion

PCR and sequencing resulted in 69 sequences (Table 1). The combined data set consisted of 2025 unambiguously aligned sites, 726 for β-tubulin, 573 for *Mcm7* and 726 for *RPB1*. The best maximum likelihood tree with bootstrap support and posterior probabilities is depicted in Fig. 2. The samples sourced from the Neotropics form three monophyletic groups. The likelihood of the GMYC model (183·8607) was significantly superior to the likelihood of the null model (178·1938), with a likelihood ratio test P value <0·05. Four putative species were supported using this method. The number of entities (represented by single sequences) was higher (4) than the number of clusters represented by at least two sequences (3). Groups corresponded to morphotype species (Fig. 2; entities A–C) except for the specimen ES65 which was listed as a different species (Fig. 2; entity D). The first two clades obtained in the phylogenetic analysis and supported by the GMYC single-threshold method are interpreted here as currently unrecognized species. For one of these we have found an existing name but the other is described here as new. The third group is here provisionally called *Bunodophoron melanocarpum*, based on morphological similarities with the lectotype (Wedin 1995*b*). The GYMC analysis suggests that this third clade represents two species, which supports that what we currently include within *B. melanocarpum* is still an unresolved species complex where additional sampling will be required to fully address species boundaries.

Taxonomy

Bunodophoron crespoae Soto, M. Prieto & Wedin sp. nov.

MycoBank No.: MB 821588

A large and eye-catching terrestrial species characterized by the white, *c*. 10–13 cm long, subterete to narrowly flattened, main branches. It differs from the otherwise similar *Bunodophoron flabellatum* by being distinctly subterete, more abundantly branched, and by having smaller ascospores. From *B. melanocarpum* it differs by being much larger, white and by growing on the ground.

Type: Colombia, Cauca, Páramo Gabriel López, 59 km along the road from Popayán to Huila, alt. *c*. 3200 m, on the ground among *Sphagnum*, 13 March 2016, *E. Soto & Á. Barrera* 20I (CUVC—holotype; S—isotype). GenBank Accession numbers: MF954558 (β-tubulin), MF954581 (*RPB1*), MF954540 (*Mcm7*).

(Fig. 1B & C)

Thallus elongated, forming extensive colonies on the ground. *Fertile branches* subterete to narrowly flattened, sparingly branched, *c*. (9·5–)10·5–11·6–12·7(–13·0) cm long ($n = 8$), (2·3–)2·5–3·2–3·9(–4·3) mm wide ($n = 7$); secondary branchlets laminal, elongate, abundantly branched, dichotomous, subterete to narrowly flattened. *Upper surface* creamy white, smooth to slightly wrinkled. *Lower surface* white, smooth to slightly wrinkled.

Ascomata sparse, terminal, 0·8–2·2 mm wide ($n = 3$), usually on poorly differentiated supporting branches. *Mazaedia* subapically exposed; thalline receptacle rupturing early and soon disappearing. *Ascospores* (4·0–)4·5–5·4–6·3(–8·0) µm diam. ($n = 40$), pale greyish to dark grey.

Pycnidia common, in the apices of terminal branchlets. *Conidia* not seen.

Secondary chemistry. Sphaerophorin (major), stictic and constictic acids (frequently as trace or in low concentrations).

Etymology. We proudly name this very distinct species after our friend and mentor Ana Crespo.

Ecology. This species is found on the ground associated with mosses (frequently *Sphagnum*) and species of *Cladonia* in the páramo, in moderately exposed sites. It is

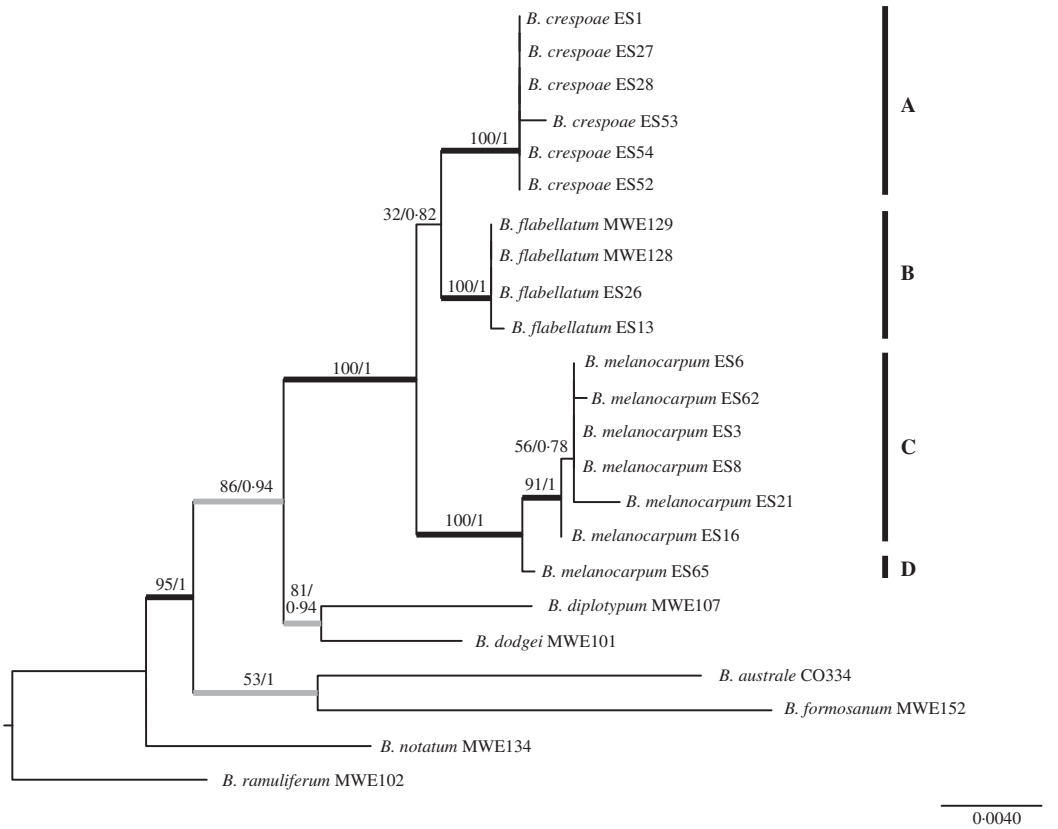

FIG. 2. Best phylogenetic tree for *Bunodophoron* from RAxML with bootstrap support (ML-BS) and posterior probabilities (PP) obtained in the Bayesian analysis. Branch support values are ordered as ML-BS/PP. Clades supported by both analyses (ML-BS ≥70, PP ≥0·95) are marked with thicker black branches and with thicker grey branches when the node is only supported by one of the two analyses. The GMYC entities recovered are depicted in the right side of the figure (entities A–D).

currently known only from páramo areas in the south-east of Colombia.

Characterization. This species is characterized by the large, subterete, comparatively richly branched, white thallus. *Bunodophoron flabellatum* has a more distinctly flattened thallus and larger spores, and *B. melanocarpum* is generally much smaller and more slender, and is predominantly epiphytic. It is currently difficult to state anything about its closest relatives as we lack a larger phylogeny of *Bunodophoron*, but it is morphologically distinct from all other currently accepted species (Wedin 1993, 1995*a*).

Additional specimens examined. **Colombia:** *Cauca*: Páramo de Gabriel López, km 59 de la vía que lleva de Popayán a Huila, alt. *c.* 3200 m, sobre suelo entre *Sphagnum*, 2016, *E. Soto & Á. Barrera* 21I (CUVC); Páramo de Gabriel López, alt. *c.* 3200 m, 2012, *B. Moncada* 16, 24, 45 (UDBC); Páramo de Puracé, Laguna de San Gabriel, *c.* 3300 m, sobre suelo entre pajonales, 2016, *K. Arango* 46 (CUVC). *Valle*: Parque Nacional Natural Farallones de Cali, alt. *c.* 3650 m, 2017, *E. Soto* 25PP (CUVC).

Bunodophoron flabellatum (Hue) Soto, M. Prieto & Wedin comb. nov.

MycoBank No.: MB 821589

Dufourea flabellata Hue, *Nouv. Arch. Mus. Hist. Nat.* **4:** 61 (1899); type: Colombia ("Bolivia"), Tolima *Goudot* (PC—holotype).

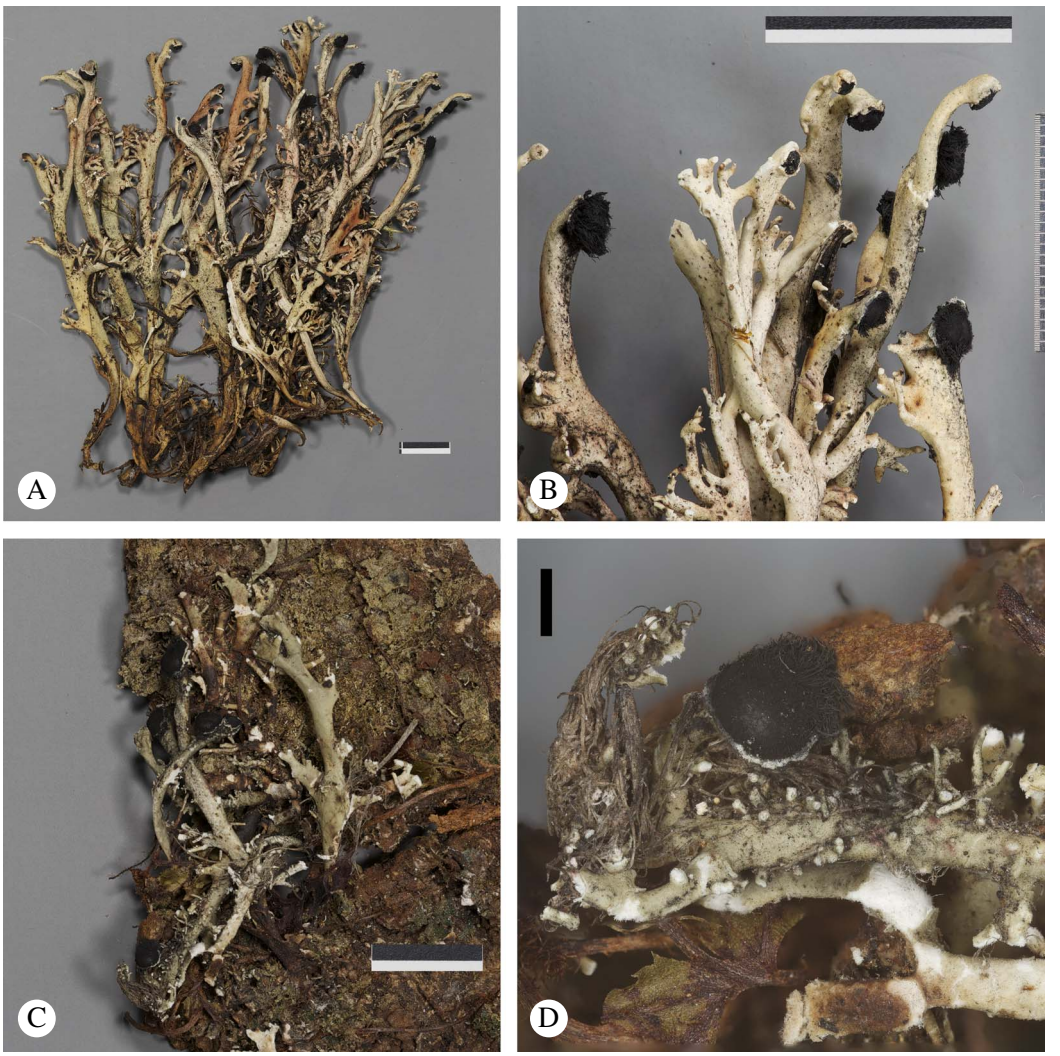

FIG. 3. *Bunodophoron* in the Neotropics. A, *B. flabellatum*, thallus, overview (epitype); B, *B. flabellatum*, close-up of apothecia (epitype); C, *B. melanocarpum*, thallus, overview (*Coca* 1703, FAUC); D, *B. melanocarpum*, close-up of apothecium (*Coca* 1703, FAUC). Scales: A–C = 1 cm; D = 1 mm.

Epitype: Ecuador, Loja, Podocarpus National Park, Cajanuma, 4°06'59"S, 79°09'41"W, 3337 m, páramo, on soil, 29.6.2010, *A. Benítez*, *Y. González & M. Prieto* 4000 (S—epitype, designated here). GenBank Accession numbers: MF954562 (β-tubulin), MF954585 (*RPB1*), MF954548 (*Mcm7*).

(Fig. 3A & B)

Thallus erect, elongated, forming extensive, compact to loose colonies to small patches over tree trunks and frequently on the ground in páramos. *Fertile branches* narrowly to broadly flattened, sparingly branched, *c.* (3·0–)3·4–5·3–7·2(–9·5) cm long ($n = 30$), (1·9–)2·5–3·2–3·9(–4·5) mm wide ($n = 24$); secondary branches laminal, sparsely branched, flattened. *Upper surface* pale greyish to white, smooth to slightly wrinkled. *Lower surface* white, smooth to slightly wrinkled.

Ascomata sparse, terminal, (1·2–)1·7–2·4–3·1(–4·0) mm wide ($n = 34$), usually on

poorly differentiated supporting branches that are frequently bent and ± involuted. *Mazaedia* subapically to ventrally exposed; thalline receptacle rupturing early and soon disappearing. *Ascospores* (5·0–)5·9–6·6–7·3 (–9·0) µm diam. ($n = 125$), pale greyish to dark grey.

Pycnidia common, in the apices of terminal branchlets. *Conidia* bacilliform, (5·0–)5·1–5·8–6·5(–7·0) × 2 µm.

Secondary chemistry. Sphaerophorin (major), stictic acid and constictic acid (frequently in low concentrations).

Ecology. This species is found growing on soils in páramos or on trees in montane forests.

Characterization. Bunodophoron flabellatum is a large, flattened and sparingly branched species which frequently grows on the ground in páramos but also occurs as an epiphyte in the forests of the same areas. In the páramos, it is usually erect and the ultimate parts of the fertile branches are often then characteristically involuted. This feature is interpreted as a response to keeping the mazaedia horizontal. It differs from the otherwise similar *B. crespoae* by being distinctly flattened, more sparingly branched, and by having larger ascospores. From *B. melanocarpum* it differs by being much larger, having larger spores, and by predominantly growing on the ground. *Bunodophoron flabellatum* was included in the phylogeny of Wedin (1993) as *Bunodophoron* species "A", where it was the sister to a group formed by *B. ohlssonii*, *B. macrocarpum*, *B. notatum* and *B. scrobiculatum*. This phylogeny was, however, based on morphology and chemistry only, and these relationships have not yet been tested by molecular approaches. Only one of these species, *B. notatum*, was included in the current phylogeny (Fig. 2) and is clearly not closely related.

Nomenclatural note. *Dufourea flabellata* Hue was described based on material from Tolima, a volcano (and province) in what is today Colombia. The type is sterile, as this species often is, but the flattened, large terrestrial thallus is distinctive. We epitypify the name on sequenced material to achieve nomenclatural stability. The epitype, *Prieto* 4000, is a typical, but richly fertile, terrestrial sample.

Additional specimens examined. **Colombia:** *Arauca*: Sierra Nevada del Cocuy, Quebrada el Playon, Plan de San José, on soil, 3680 m, 1973, *A. M. Cleff* 10095 (B); 2 km ENE of Boqueron de Cusirí, Cabeceras de la Quebrada El Playón, Sierra Nevada del Cocuy, 4250 m, *A. M. Cleff* 9069 (B). *Departamento de Cundinamarca*: Parque Nacional Natural Sumapaz, 3500–3700 m, 2004, *B. Moncada* 2296, 2335 (UDBC). *Cauca*: Inzá, 2014, *B. Moncada et al.* 69 (UDBC). *Meta*: Páramo de Sumapaz, Cerro Nevado del Palacio, on soil, 3715 m, 1973, *A. M. Cleff* 8215 (B). *Nariño*: Municipio Pasto, Cordillera Centro Oriental, microcuenca las Tiendas, inicio del páramo del Bordoncillo, 1°12'N, 77°08'W, 3450–3510 m, on soil, 1997, *B. R. Ramírez* 10617 (B). *Risaralda*: Santa Rosa de Cabal, Vereda La Linda Cortaderal, *c.* 3100m, 2014, *A. Hurtado* s. n. (CUVC); Municipio Santa Rosa, alrededores de la Finca la Sierra, 3725 m, epiphyte, 1982, *J. Aguirre & S. R. Gradstein* 1389 (B); Parque Nacional Natural Los Farallones de Cali, on soil, 3140 m, *Cardenas* s. n. (CUVC).—**Ecuador:** *Loja*: Madrigal Private Reserve, secondary montane forest, epiphyte, 2700 m, 4°3'9"S, 79°9'55"W, 2010, *A. Benítez & M. Prieto* 4007 (S).

Bunodophoron melanocarpum (Sw.) Wedin

MycoBank No.: MB 412621

Lichen melanocarpum Sw., *Nov. Gen. et Spec. Plant.*: 147 (1788); type: Jamaica, *Swartz* (SBT—lectotype, designated by Wedin 1995*b*; UPS—Thunberg, isolectotype).—*Sphaerophorus melanocarpus* (Sw.) DC. in Lam. & DC., *Flora Franç.*, ed. 3, vol. 6: 178 (1815).

(Fig. 3C & D)

Thallus erect to prostrate, with rather scattered branches. *Fertile branches* sparsely branched, subterete to narrowly flattened, (2·0–)2·4–2·9–3·4(–3·5) cm long ($n = 11$), (1·2–)1·2–1·5–1·8(–2·0) mm wide ($n = 10$). *Upper surface* greenish grey, smooth to wrinkled at the base of the receptacle. *Lower surface* white, scrobiculate to foveolate.

Ascomata sparse to abundant, terminal, *c.* 1·5–2·5 mm wide. *Mazaedia* subapically exposed; thalline receptacle sometimes remaining around the base of the

mazaedium, wrinkled. *Ascospores* globose, (4·0–)5·0–5·7–6·4(–8·0) µm diam. ($n = 102$), pale greyish to dark grey.

Pycnidia common, along the sides and in the apices of terminal branchlets. *Conidia* not seen.

Secondary chemistry. Sphaerophorin (major), stictic acid and constictic acid (frequently in low concentrations).

Ecology. This species is found on trees in montane forests in moderately exposed sites, and on the ground in páramos.

Notes. *Bunodophoron melanocarpum* differs from the other two species treated here in the considerably smaller size (shorter and more slender thallus) and by being predominantly epiphytic. It further differs from *B. flabellatum* by the smaller spores. There has been some confusion around the name *Lichen melanocarpum* Sw. since it was described from Jamaica by Swartz in 1788 (Wedin 1993, 1995*b*). For a long time, it was generally utilized for mazaediate macrolichens (*Sphaerophorus* s. lat.) with a flattened thallus and subapically oriented mazaedia. The description given here is very preliminary and we suspect that several morphologically distinct epiphytic species are included under this name. This is supported by the results obtained in the species delimitation analyses.

Specimens examined. **Colombia:** *Boyacá*: Villa de Leyva, Santuario de Fauna y Flora de Iguaque, 2800 m, epiphyte, 2002, *B. Moncada & R. Dávila* 1777 (UDBC). *Cundinamarca*: Choachí, El Verjón, 3080 m, 2007, *K. Maláver et al.* 97 (UDBC). *Caldas*: Río Sucio, 2800 m, epiphyte, *J. Home* s. n. (CUVC). *Nariño*: La Cruz, Tajumbima, Complejo Volcánico Doña Juana Cascabel, 2014, *E. Soto-Medina* 26n (CUVC). *Risaralda*: Santuario, Parque Nacional Natural Tatamá, Valle de Las Mirlas, 3564 m, epiphyte, 2011, *L. Coca* 1806 (FAUC); Santuario, Parque Nacional Natural Tatamá, El Mirador, 3337 m, epiphyte, 2011, *L. Coca* 1703 (FAUC).—**Ecuador:** *Loja*: Saraguro, Loma del Oro, 3°40'52"S, 79°14'24"W, 3300 m, páramo, on soil, 2010, *A. Benítez, Y. González & M. Prieto* 4006 (S); Madrigal Private Reserve, secondary montane forest, on dead tree, 2600 m, 4°2'36"S, 79°10'20"W, 2010, *A. Benítez & M. Prieto* 4008 (S).

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