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ORIGINAL ARTICLE

Additions to neotropical stereoid fungi (Polyporales, Basidiomycota): one new species of *Lopharia* and one new combination in *Phlebiopsis*

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Abstract
Stereoid fungi are an artificial group with mostly effused-reflexed to stipitate basidiomata, smooth hymenophore, and hyaline spores. From recent surveys in the Brazilian Atlantic Forest, Caatinga, and Cerrado, six specimens of this group had their identity tested with the nrITS and nrLSU sequences. Two of them were nested within the *Lopharia s.s.* clade and represent a new species *L. erubescens*, characterized by the dextrinoid reaction of the cystidia, and small basidia and spores. The other four were initially identified as *Hjortstamia amethystea*, but nested in the *Phlebiopsis* clade. Thus, we proposed the new combination, *Phlebiopsis amethystea*. We also provide keys to neotropical *Lopharia s.l.* and *Phlebiopsis s.l.* and allied species.

Keywords Brazil · *Hjortstamia* · *Porostereum* · Taxonomy

Introduction

Stereoid fungi are an artificial group of Basidiomycota characterized by resupinate, effused-reflexed to stipitate basidiomata, a smooth hymenial surface, and hyaline, smooth spores (Welden 2010). Also considered to be among the corticioid fungi (Bernicchia and Gorjón 2010), this group was traditionally assembled in a single genus, *Stereum* Hill (Burt 1920). The genus was subsequently divided based on morphological features, and with the addition of molecular techniques, the stereoid fungi were known to be distributed among several lineages of Agaricomycetes (Hibbett et al. 2014), with *Stereum s.s.* being in the Russulales (Miller et al. 2006).

In Welden's (2010) monograph on neotropical stereoid fungi, 13 genera were recognized, including *Lopharia s.l.* (Polyporales) which comprises resupinate and effused-reflexed species with dimitic hyphal system, simple septate or clamped hyphae, and metuloid cystidia. Among the species included in the genus, there is *L. amethystea* (Hjortstam & Ryvarden) A.L. Welden, which is a rather common species in Brazil; *L. amethystea* is also placed in *Hjortstamia* Boidin & Gilles due to the lack of clamp connections and a brownish cystidia wall (Boidin and Gilles 2002; Ryvarden 2010). Recently, the type species of *Hjortstamia* (*H. friesii* (Lév.) Boidin & Gilles) was combined with *Phlebiopsis* Jülich (Phanerochaetaceae, Polyporales; Miettinen et al. 2016) based on morphology, consistent with the placement of *H. crassa*, a similar species, in the genus *Phlebiopsis* as a result of multigene phylogenetic analyses (Floudas and Hibbett 2015). However, morphological boundaries between *Hjortstamia*, *Phlebiopsis* (which also includes poroid species; Chen et al. 2018) and the recently described *Phaeophlebiopsis* Floudas & Hibbet are unclear, and their separation is possible only at the molecular level (Floudas and Hibbett 2015). Several neotropical species of *Hjortstamia* have not yet been sequenced, including *H. amethystea*, *H. mexicana* (A.L. Welden) Boidin & Gilles, and *H. novae-granata* (A.L. Welden) Hjortstam & Ryvarden, and their phylogenetic affinities are still unknown.

Lopharia s.s. (Polyporaceae, Polyporales) was recently emended by Liu et al. (2018), based on a three-gene

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phylogeny and morphological analyses. The genus now includes resupinate to effused-reflexed species, with monodimitic hyphal system, clamp connections, metuloid cystidia, and large basidia. It is a widespread white-rot genus, with a limited number of species. Several *Lopharia s.l.* listed in Welden's monograph (2010) were combined in *Porostereum* Pilát (Hjortstam and Ryvarden 1990; Ryvarden 2010), but the morphological differentiation between the two genera is vague.

In the present paper, we describe one new neotropical species of *Lopharia s.s.* based on morphology and nrITS and nrLSU sequences phylogeny. Furthemore, we report the sequencing of *H. amethystea* specimens for the first time and describe their phylogenetic position as determined using the abovementioned genes.

Material and methods

Study area

Specimens of stereoid fungi were collected in the Amazonia (Maiandeua Island, state of Pará), Atlantic Rainforest (Biological Reserve of Pedra Talhada, state of Alagoas, and Mata do Pau-Ferro Ecological Reserve, state of Paraiba), Caatinga (Missão Velha, state of Ceará, Serra das Confusões National Park, state of Piauí, and Sítio do Carro Quebrado, state of Pernambuco), and Cerrado (RPPN/UFMS Campus, state of Mato Grosso do Sul). They were deposited at the Herbarium Padre Camille Torrend (URM) at the Universidade Federal de Pernambuco (UFPE), with duplicates in the Natural History Museum herbarium, University of Oslo (O). Exsiccatae previously deposited at URM were also studied.

Morphological studies

Macroscopic analyses were performed on fresh specimens in field condition and after drying. On dried specimens, a drop of 3% potassium hydroxide solution (3% KOH) was poured over the basidioma to observe possible color changes of the basidioma. Slides were prepared with 3% KOH for measurements. For spore size, additional information is provided: L, means spore length; W, means spore width; Q, variation in the ratios of spore length/width. Slides were prepared with Melzer's reagent to observe amyloid or dextrinoid reactions of any structures. The absence of reaction with Melzer's reagent is indicated with "IKI-" (Ryvarden 2004). The presence of cyanophilic reaction was observed on preparations with Lactophenol cotton blue (CB). Color descriptions are based on the Methuen handbook (Kornerup and Wanscher 1978).

DNA extraction, PCR amplification and sequencing

Fragments from the basidiomata (30–50 mg) were removed and placed in 1.5 ml tubes and stored at − 20 °C until DNA extraction was performed. The fragments were grinded with liquid nitrogen or homogenized in 2 ml tubes containing 0.17 g of glass beads (425–600 μm) and one 6.35 mm ceramic sphere using the FastPrep-24™ 5G Instrument (MP Biomedicals). DNA was extracted using a modified method described in Goés-Neto et al. (2005) and Rodrigues et al. (2009). The reaction mix and parameters for PCR amplification of the ITS and LSU regions were as described by Lima-Júnior et al. (2014), using the primer pairs ITS1-ITS4 or ITS4-ITS5 and LR0R-LR5, respectively (White et al. 1990; Lima-Júnior et al. 2014). Negative controls containing all components of the reaction mix, but exchanging DNA by water, were used in each procedure to detect possible contamination. The PCR products were purified either with ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA) or E.Z.N.A.® Cycle-Pure Kit (Omega Bio-tek®) following the manufacturer's recommendations. Samples were Sanger sequenced at the Plataforma Tecnológica de Genômica e Expressão Gênica do Centro de Biociências, UFPE, Brazil, or sent to Stab Vida Lda (Madan Parque, Caparica, Portugal). Cycle sequencing was carried out with the same primers as amplification reactions (Moncalvo et al. 2000). All obtained sequences were deposited in NCBI GenBank (National Center for Biotechnology Information, Bethesda, MD, USA).

Phylogenetic analyses

The electropherograms were analyzed and edited using the 2.0 Staden Package software (Staden et al. 1998). Ready sequences were subjected to a BLASTn search in the NCBI GenBank (National Center for Biotechnology http://www.ncbi.nlm.nih.gov) to recover similar sequences. Reference sequences for datasets were chosen based on studies of Justo and Hibbett (2011), Floudas and Hibbett (2015), and Liu et al. (2018) in addition to those recovered from Genbank through BLASTn. These sequences were used in the dataset to study the phylogenetic relationships (Table 1). Each gene region was aligned with the MAFFT v.7 online interface (Katoh et al. 2017) using default settings (http://mafft.cbrc.jp/alignment/server), and then improved manually using MEGA 7 (Kumar et al. 2018), and combined to the form of a concatenated dataset.

The phylogenetic analyses and tree construction were performed using the maximum likelihood method (ML) and

Table 1 Specimens used in this study. The sequences in bold were generated in this study

Species	Voucher	Country	Gen Bank accession Number	
			ITS	LSU
Dentocorticium bicolor	FP-150666	Belize	KY948710	KY948878
Dentocorticium bicolor	He 2772	China	MF626354	MF626378
Dentocorticium bicolor	He 2757	China	MF626355	MF626379
Dentocorticium portoricence	He 2161	USA	MF626356	MF626380
Dentocorticium portoricence	He 2202	USA	MF626357	MF626381
Dentocorticium sulphurellum	T609	Canada	JN165015	JN164815
Dentocorticium taiwanianum	Wu 9907-1 (holotype)	China	MF626363	MF626387
Dentocorticium taiwanianum	He 3383	China	MF626361	MF626388
Dentocorticium ussuricum	He 3322	China	MF626360	MF626384
Dentocorticium ussuricum	He 3294	China	MF626359	MF626383
Lopharia ayresii	He 2778	China	MF626353	MF626376
Lopharia ayresii	He 20120724	China	MF626352	MF626375
Lopharia* aff. *cinerascens	**URM 93328**	**Brazil**	**MK993643**	**MK993637**
Lopharia cinerascens	CBS 485.62	USA	MH858220	MH869821
Lopharia cinerascens	He2188	USA	MF626350	MF626373
Lopharia cinerascens	FP105043sp	USA	JN165019	JN164813
Lopharia erubescens	**URM 93246**	**Brazil**	**MK993641**	**MK993636**
Lopharia erubescens	**URM 93247 (type)**	**Brazil**	**MK993642**	**-**
Lopharia mirabilis	Dai 5147	China	MF626342	MF626365
Lopharia mirabilis	Yuan 2532	China	MF626343	MF626366
Lopharia sinensis	He 2428 (holotype)	China	MF626347	MF626370
Lopharia sinensis	He 2424	China	MF626349	MF626372
Phlebiopsis amethystea	**URM 93248**	**Brazil**	**MK993644**	**MK993638**
Phlebiopsis amethystea	**URM 84741**	**Brazil**	**MK993645**	**MK993639**
Phlebiopsis amethystea	**URM 92985**	**Brazil**	**MK993646**	**MK993640**
Phlebiopsis amethystea	**URM 87790**	**Brazil**	**MK993647**	**MK995634**
Phlebiopsis flavidoalba	FD-263	USA	KP135402	KP135271
Phlebiopsis flavidoalba	OM 17896	USA	KX752607	KX752607
Phlebiopsis flavidoalba	103F9C-AM	Brazil	MG751231	-
Phlebiopsis flavidoalba	**URM 87826**	**Brazil**	**MK993648**	**MK995635**
Phlebiopsis gigantea	FP-70857-Sp	USA	KP135390	KP135272
Phlebiopsis gigantea	CBS 935.70	Germany	MH860011	MH871798
Phlebiopsis yunnanensis	CLZhao 3990	China	MH744141	MH744143
Phlebiopsis yunnanensis	CLZhao 3958	China	MH744140	MH744142
Phlebiopsis crassa	SWFC001804	China	MK811352	-
Phlebiopsiscrassa	JRH101909-1	USA	MF773600	-
Phlebiopsis pilatii	no voucher	China	KY971603	-
Phlebiopsis ravenelii	CBS 411.50	France	MH856691	MH868208
Porostereum fulvum	LY:18496	France	MG649453	MG649455
Porostereum fulvum	LY:18491	France	MG649452	MG649454
Porostereum spadiceum	CBS 476.48	France	MH856440	MH867984
Porostereum spadiceum	KUC20080728-31	Korea	JX463660	JX463654
Stereum hirsutum	AFTOL-ID 492	not mentioned	AY854063	MH874407

Bayesian algorithm (BA). The best-fist models of evolution were identified for the concatenated dataset from jModelTest (Posada 2008). The ML analyses were performed in MEGA 7 software and including 5000 bootstrap replicates. BA analyses were run in the TOPALi v2.5 (Milne et al. 2004) with 5×10^6

generation. Trees were visualized with MEGA 7 and the layouts were edited in the Microsoft PowerPoint.

Results

Phylogenetic analyses

Eight specimens were sequenced, generating both ITS and LSU sequences for each one. The combined dataset (ITS + LSU) included 43 and 40 sequences, respectively, with *Stereum hirsutum* (Willd.) Pers. as outgroup, and comprised 1572 characters including gaps, of which 678 belonged to ITS (1–678) and 894 to LSU (679–1572). The best models were TRN+G+I for ML analyses and SYM+G for BA.

The results of the phylogenetic analyses generated from ML and BA showed quite similar tree topologies and small or no significant differences in the statistical support values. The ML tree topology with bootstrap support values (BS) and posterior probabilities (PP) from Bayesian inference of phylogeny (BI) is presented in Fig. 1. Two specimens clustered in a well-supported clade (BS = 100%; PP = 1.0) within the equally well-supported *Lopharia s.s.* clade (BS = 92%; PP = 1.0), and confirm that those sequences belong to a new species, are described below. The specimens of *Hjortstamia amethystea* clustered in a strongly supported clade (BS = 98%; PP = 0.98) in the *Phlebiopsis* clade (BS = 100%; PP = 1.0), thus the new combination *Phlebiopsis amethystea*, are proposed.

Taxonomy

Lopharia erubescens Xavier de Lima, sp. nov.

MycoBank: MB831392

Type: Brazil, State of Alagoas, Municipality of Quebrangulo, Biologial Reserve of Pedra Talhada, moist submontane broadleaf forest 9°15'00.7"S, 36°25'38.3"W, on dead hardwood, leg. V. Xavier de Lima, 12 Oct 2018, VXL620 (holotype URM 93247).

Etymology: e.ru.bes.cens. N.L. neut. adj. erubescens means becoming red, in reference to the dextrinoid reaction of cystidia.

Basidioma resupinate, thin, tightly adnate, ceraceous when fresh and dry (Fig. 2a, b). Hymenophore smooth, white to pale yellow, in some parts may crack revealing the white subiculum or substrate. Margin thinning out, irregular, fibrillose, white. No reaction of any part of basidioma with 3% KOH.

Hyphal system monomitic, CB-. Hyphae with clamp connections, thin-walled. Subiculum thin to absent, composed mostly of interwoven and richly branched thin-walled hyphae, 1.5–4 μm in diameter, with numerous small rhomboid crystals; thicker, straight hyphae with clamps at regular intervals often found, from which the subhymenium originates. Hyphae from the margin similar to the subiculum. Subhymenium composed of vertically oriented hyphae, compact, up to 150 μm thick, hyphae agglutinated and difficult to discern in older parts, thinning out towards the margin where basidia and cystidia are loose or scattered, hyphae 2.5–3.5 μm in diameter.

Cystidia as metuloids, numerous, arising from the subiculum and subhymenium, immersed or projecting above the hymenium, subulate to ventricose, hyaline, thick-walled, upper-half to two-thirds densely encrusted with hyaline crystals (Fig. 2d), rarely naked, 35–53 × 8–12 μm including crystals, CB+, dextrinoid in Melzer's reagent in the lower portion (Fig. 2e).

Basidia clavate, 20–27 × 3.5–6 μm, with a basal clamp, tetrasterigmate, sterigma up to 2.5 μm; basidioles cylindrical to distinctly clavate.

Basidiospores cylindrical, some slightly curved, hyaline, thin-walled, smooth, with visible guttulae, 5–6 × 1.5–2.5 μm, Q = 2.5–3.3, L = 5.3 μm, W = 1.9 μm, IKI-, CB- (Fig. 2h).

Remarks: The dextrinoid reaction in the metuloid bases is a distinctive feature that differentiates *L. erubescens* from the other species of the genus, as well as the smaller dimensions of basidia, cystidia and spore size and shape. The thin to almost absent subiculum and agglutinated subhymenium is also observed in *L. ayresii*, *L. mirabilis*, and *L. resupinata*; the latter species has numerous crystals in the subiculum (Liu et al. 2018). *Lopharia erubescens* subhymenium can be extremely compact in older parts, composed of agglutinated and collapsed cells, difficult to discern under the microscope, and towards the margin; the subhymenium becomes thinner and much less compact.

Ecology: Both specimens were found on 1.8–2.5 cm diameter logs, in advanced stage of decay.

Additional material examined: Brazil, the same locality as holotype, on dead hardwood, leg. V. Xavier de Lima, 12 Oct 2018, VXL619 (URM 93246).

Key to known neotropical *Lopharia s.s.* and *Porostereum*

1. Cystidia hyaline……..……2
1. Cystidia pale yellow to brown……...……3
2. Cystidia with dextrinoid reaction; spores cylindrical, 5–6 μm long…….....…….....….*L. erubescens*
2. Cystidia non-dextrinoid; spores ellipsoid, 10–16 μm long.……..…….....…….....*L. cinerascens*
3. Hyphal system monomitic; spores 13–15 μm ….....…..…....…..…….*Porostereum pilosiusculum*
3. Hyphal system dimitic; spore 5–8 μm.…….....4
4. Hymenophore lilaceous; most cystidia encrusted…. ……...……..….....*P. lilacinum*
4. Hymenophore buff, ochraceous, brown; cystidia smooth or encrusted……...……....…5

5. Cystidia smooth, large, up to 470 µm long… ..……...……..……..….*P. sharpianum*
5. Cystidia encrusted, 35–60 µm long…….. ……..…….*P. spadiceum*

Phlebiopsis amethystea (Hjortstam & Ryvarden) Chikowski & C.R.S. Lira, comb. nov.

MycoBank: MB831393

Basionym: *Porostereum amethysteum* Hjortstam & Ryvarden, Synopsis Fungorum 4: 27, 1990.

Type specimen described in Hjortstam and Ryvarden (1990), Ryvarden (2010) and Welden (2010).

Remarks: *Phlebiopsis amethystea* is easily recognized by the effused-reflexed basidioma, purplish colors of the smooth hymenophore, and brownish hyphae. It differs from the other species of the genus by the dark brown cystidia. It is morphologically very similar to *Ph. crassa*, but can be distinguished by several characteristics: (1) context is darker than hymenial surface in *Ph. amethystea* (Fig. 3d), whereas in *Ph. crassa* the context is paler (Fig. 4b) (Hjortstam and Ryvarden 1990); (2) hymenophore color may vary, but it has consistently purple hues in the dried specimens of *Ph. amethystea* analyzed (Fig. 3a, b, c d), while *Ph. crassa* presents brownish and reddish tints (Fig. 4a, b); (3) *Ph. amethystea* has three types of cystidia, of hymenial, and contextual origin, whereas only one type of cystidia originating from the context is present in *Ph. crassa*; (4) contextual metuloids in *Ph.*

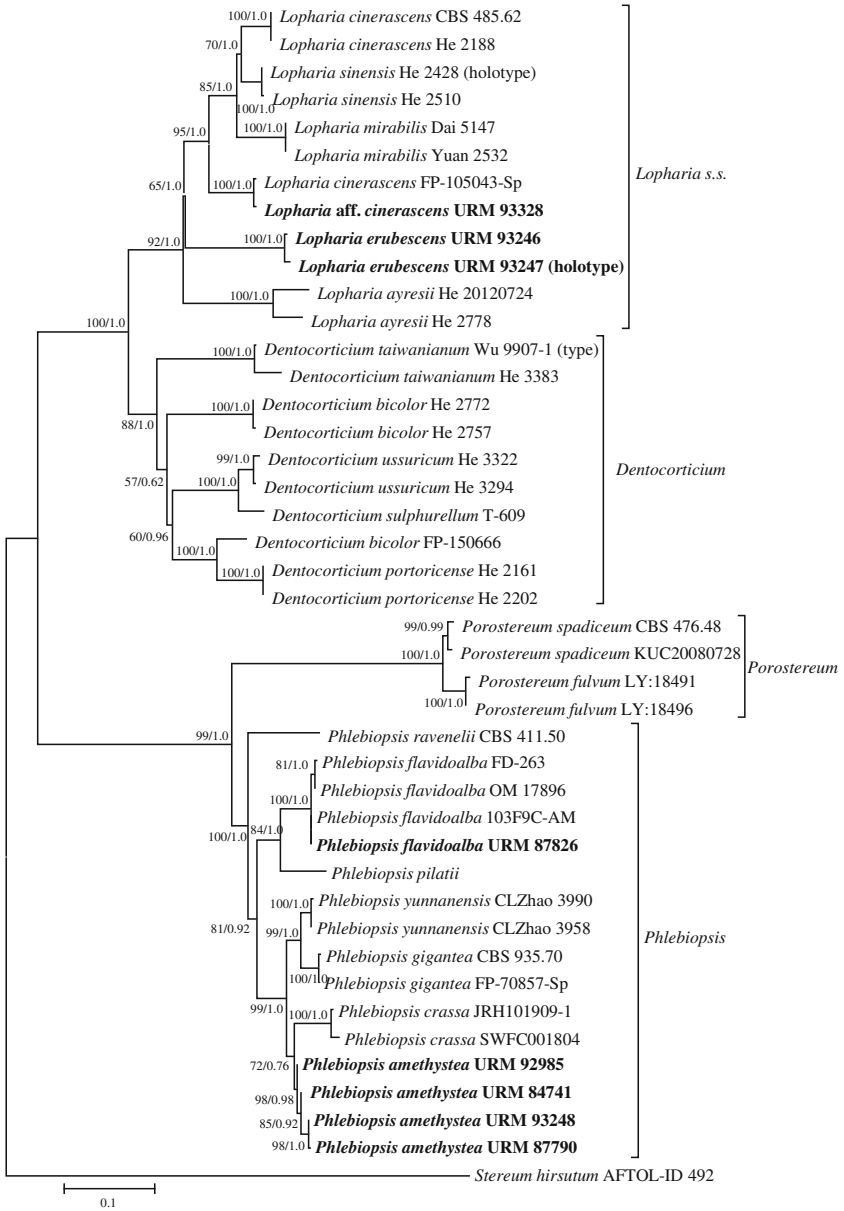

Fig. 1 Phylogenetic reconstruction for *Dentocorticium*, *Lopharia*, *Phlebiopsis*, and *Porostereum* inferred from a combined dataset of ITS and nLSU. Parsimony bootstrap generated by ML (higher than 50%) and BA posterior probabilities (higher than 0.70) are shown along the branches

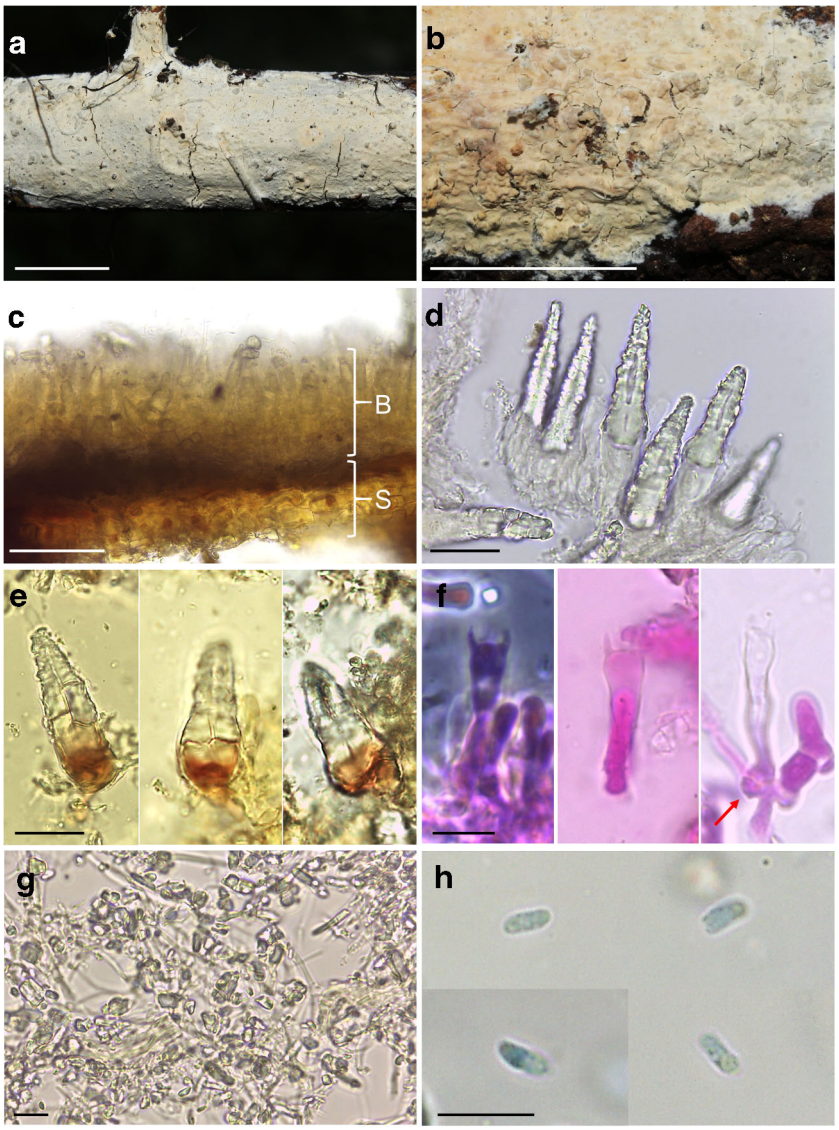

Fig. 2 *Lopharia erubescens* sp. nov. **a**, **b** Macromorphological aspect in field. **a** Voucher URM 93247 (holotype). **b** Voucher URM 93246. **c** Vertical section showing the basidioma (B) and the substrate (S). **d** Cystidia of subhymenial origin, in KOH 3%. **e** Dextrinoid reaction at the cystidial base, in Melzer's reagent. **f** Basidia. Red arrow pointing to the basal clamp. **g** Subiculum. **h** Spores in Cotton Blue Lactophenol. Images **c**, **d**, **e**, **f**, **g**, and **h** are from the holotype. Bars: **a**, **b** = 1 cm; **c** = 100 μm; **d**, **e** = 20 μm; **f**, **g**, **h** = 10 μm

amethystea are brown and dark brown, bear persistent coarse crystals and are scattered in the basidioma (Fig. 3g), whereas in *Ph. crassa* metuloids are very abundant, yellowish, and pale brown, and bear much finer crystals that easily dissolve and detaches in microscopic preparations (Fig. 4c, d, e).

Material examined: *Phlebiopsis amethystea*: Brazil: Alagoas, Quebrangulo, Biological Reserve of Pedra Talhada, 9°15'01" S, 36°25'38" W, 758 m asl., leg. V. Xavier de Lima, 9 Sep. 2018, VXL555 (URM 93252); leg. V.R.T. Oliveira, 19 Jul 2018 (URM 92583); Ceará, Missão Velha, 7°14'58" S, 39°08'35" W, leg. T.B. Gibertoni, 28 Mar 2011, TBG125 (URM 83375); Taianguá, 3°43'48"S, 40°59'33"O leg. C.R.S. Lira, 2012 (URM 84741); Mato Grosso do Sul, Campo Grande, 20°30'28" S, 54°37'02" W, leg. D.C. dos Santos (URM 92985), Pará, Maracanã, Maiandeua Island, 0°35'42" S, 47°34'54" W, leg. E. L. Campos, Jul 1998 (URM 76945), leg. E.L. Campos, May 1999 (URM 76945), Paraíba, Areia, Mata do Pau Ferro, 6°57'47" S, 35°41'30" W, leg. C.R.S. Lira 9, Nov. 2010 (URM 83070), leg. C.R.S. Lira, 24 Aug 2012, CL307 (URM 87790), Pernambuco, Recife, Dois Irmãos Zoo Botanical Park, 8°00'59" S, 35°35'09" W, leg. M. Rajchenberg, 5 Aug 2009, MJ-D1 (URM 83789), Triunfo, Carro Quebrado Farm, 7°51'17' S, 38°06'06" W, 700–1100 m asl., leg. C.R.S. Lira, 12 Jul 2012, CL161 (URM 93248), Piaui, Caracol, Serra das Confusões, 9°16'42" S, 43°19'48" W, A. Gomes-Silva, 28 Mar 2011, AC184 (URM 83376). *Phlebiopsis crassa*: USA, NC, Dare County, Roanoke Island, leg. unknown, 17 Aug 1957, (URM 13206); MD, Anne Arundel County, Herald Harbor, on *Ilex opaca*, leg. C.R. Benjamin, 30 Jul 1962 (URM 29832).

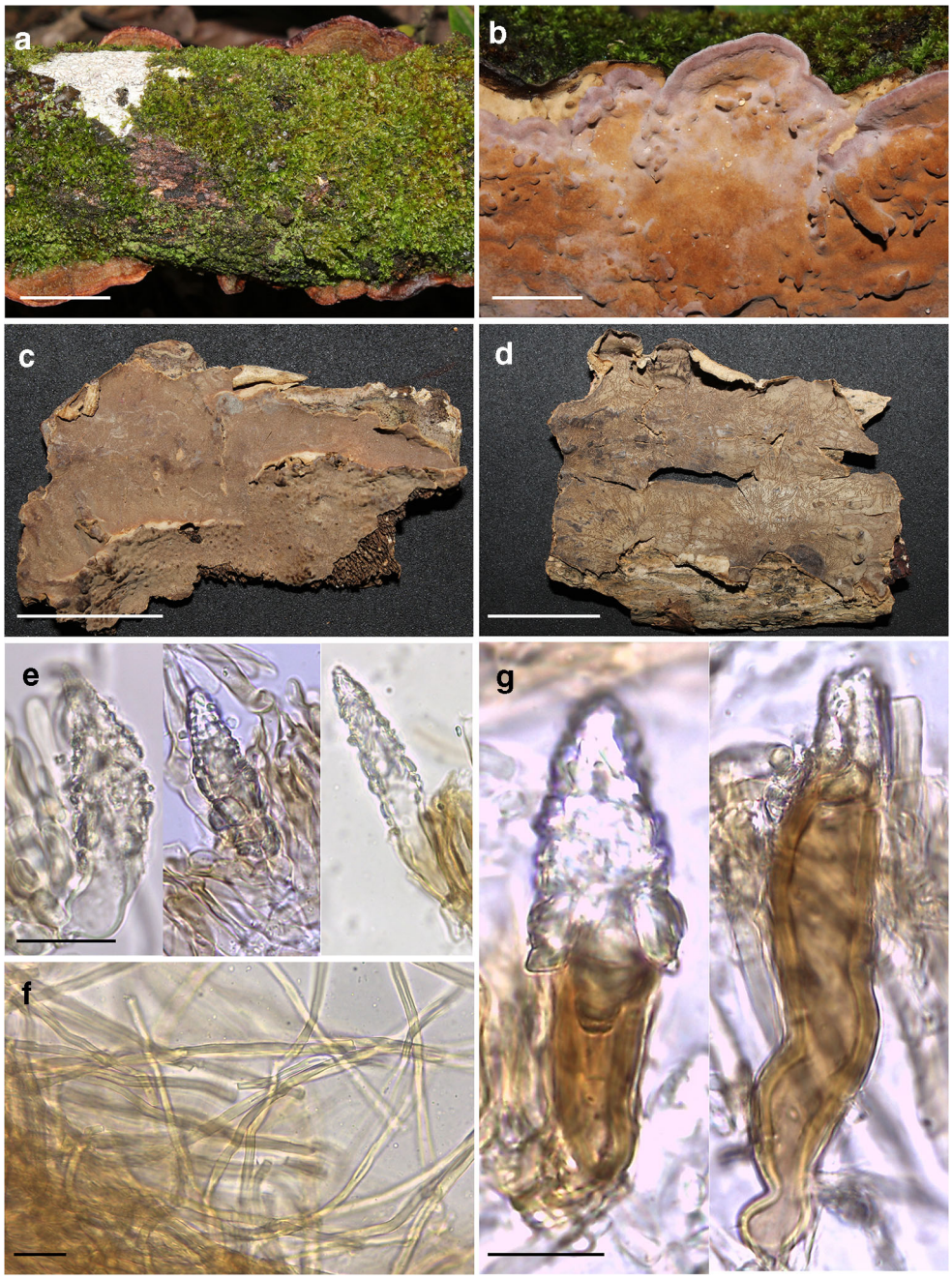

Fig. 3 *Phlebiopsis amethystea*. **a** Pilear surface and general aspect of the basidioma in field. Voucher URM 93252. **b** Hymenial surface in field. Voucher URM 93252. **c**, **d** Dried specimens URM 76944 and URM 87790. **e** Metuloid cystidia of hymenial origin (URM 76944). **f** Thick-walled hyphae of context and tomentum (URM 76944). **g** Metuloid cystidia of contextual origin (URM 76944). Bars: **a**, **b**, **c**, **d** = 2 cm; **e**, **f**, **g** = 20 μm

Key to known neotropical *Phlebiopsis* and allied species

1. Dimitic with dendroid binding hyphae, cystidia absent or undifferentiated…..……..*Hjortstamia mexicana*
1. Monomitic or dimitic with typical skeletal hyphae, cystidia smooth or encrusted…….....……....2
2. Cystidia pale yellow to dark brown…….....3
2. Cystidia hyaline……..…7
3. Basidioma resupinate, hymenophore grayish, monomitic……...……..…...……4
3. Basidioma resupinate to effused-reflexed, hymenophore with purple, violet and reddish tints, dimitic, cystidia predominantly encrusted with crystals…….....……....6
4. Cystidia smooth and large, up to 120 μm long, subiculum distinct basidia 22–25 μm long…… …….....……*Hjortstamia novae-granata*
4. Cystidia encrusted, 22–51 μm long, subiculum very thin to indistinct, basidia 14–22 μm long ….....….5
5. Cystidia 31–51 μm long, subiculum difficult to discern from subhymeniu...……...*Phaeophlebiopsis ignerii*
5. Cystidia 22–38 μm long, subiculum very thin, brown……....…*Phaeophlebiopsis caribbeana*

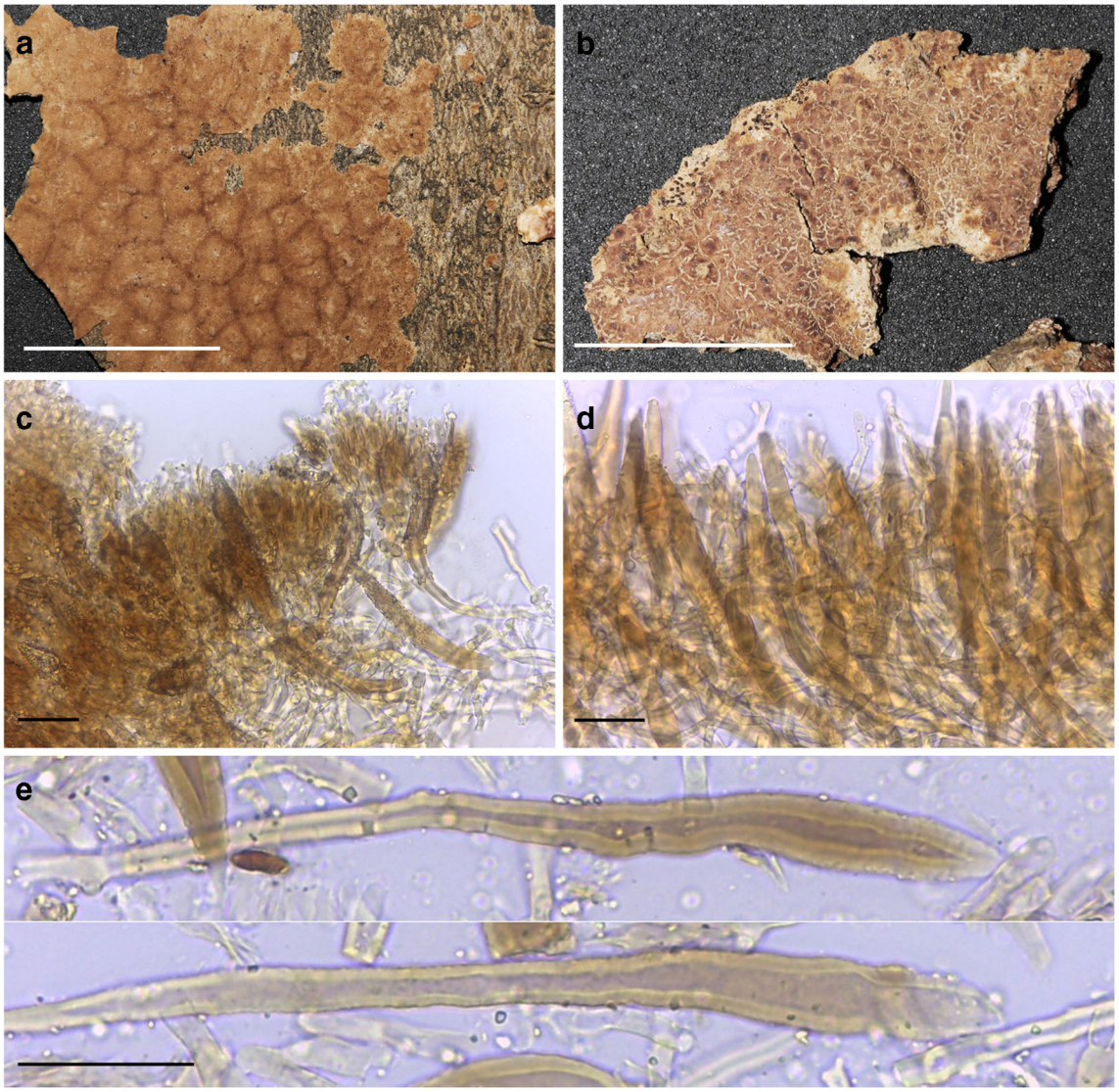

Fig. 4 *Phlebiopsis crassa*. **a**, **b** Dried specimens URM 13206 and URM 29832. **c**, **d** Vertical sections of the hymenophore, showing the contextual origin of cystidia (URM 13206). **e** Cystidia (URM 13206). Bars: **a**, **b** = 2 cm; **c**, **d**, **e** = 20 μm

6. Cystidia of up to three type, contextual metuloids brown to dark brown…….*Phlebiopsis amethystea*
6. Only contextual metuloids present, hyaline to pale brown……..…….....*Phlebiopsis crassa*
7. Hymenophore whitish, gray to buff, cystidia often with adventitious sept…….....……*Phlebiopsis gigantea*
7. Hymenophore cream to brown, cystidia non-septate…..……..……...……8
8. Basidioma effused-reflexed to distinctly pileate, dimitic…..……...……..*Phlebiopsis papyrina*
8. Basidioma resupinate, monomitic……...9
9. Subiculum thin to absent, spores 4–5 μm long…...…….....……*Phlebiopsis ravenelii*
9. Subiculum distinct, spores 5–7.5 μm long …….....10
10. Basidia 15–20 μm long, cystidia 7–10 μm wide…….....…….*Phlebiopsis galochroa*
10. Basidia 22–30 μm long, cystidia 10–18 μm wide……...……..*Phlebiopsis flavidoalba*

Discussion

The main morphological characteristics of *Lopharia erubescens* are in accordance with what is known for the genus, such as the clavate basidia, presence of metuloids, clamped hyphae, and IKI-, CB- smooth spores. However, several features of *L. erubescens* diverge from typical species of the genus, like the dextrinoid cystidia, size of basidia, and size and shape of

spores. Among the corticioid basidiomycetes, *Dextrinocystis* Gilb. & M. Blackw. and *Litschauerella* Oberw. (Trechisporales) also have dextrinoid cystidia, but they are thick-walled and multi-rooted, distinct from the typical metuloids observed in *Lopharia*. In the most recent emendation of *Lopharia*, Liu et al. (2018) indicated the basidia longer than 50 μm as a feature of the genus; however, *L. erubescens* has basidia around 25 μm long and spores narrower than all species of *Lopharia s.s.*

An additional specimen of *Lopharia* (URM 93328), which was found in northeast Brazil (Paraíba) in our phylogeny, was found to be of the same clade as *L. cinerascens* (Schwein.) G. Cunn. from the southern USA (MS, FP-105043). The holotype of *L. cinerascens* was found in the northern part of the USA (PA), as well as specimens He2188 (WI) and CBS 485.62 (NY). Liu et al. (2018) suggested that specimens from the north of THE USA belong to *L. cinerascens s.s.*, whereas the specimens found in the southern states of the USA may belong to a different species. Thus, it is likely that *L. cinerascens s.s.* is restricted to temperate climates, whereas morphologically similar specimens from subtropical and tropical climates represent a different species. Unfortunately, the specimen PB359 is sterile, and the dry specimen of the available FP-105043 culture is lost (Beatriz Ortiz-Santana, personal communication), making it such that the formal description of a new species is not advised.

Phlebiopsis amethystea, previously placed in *Hjortstamia*, is phylogenetically a member of *Phlebiopsis*, but the morphological affinities are vague. *Phlebiopsis* was introduced to accommodate *Thelephora gigantea* Fr. (Jülich 1978), but differs from *Ph. amethystea* and similar species, such as *Ph. crassa*, in several aspects, agglutination of hymenial elements, construction of subiculum, consistency of dried specimens and hyphae color. The morphological limits of *Phlebiopsis* are not clear (Eriksson et al. 1981), and recent phylogenetic studies (Floudas and Hibbett 2015; Miettinen et al. 2016) show that there is no obvious differentiation between lineages of *Phanerochaete s.l.* (*Phanerochaete s.s.*, *Phlebiopsis*, *Phaeophlebiopsis*, *Scopuloides* and others). *Phlebiopsis amethystea* is one of the most common stereoid fungi in Brazil, but seems to be rare elsewhere. Outside the type locality (Brazil), it was also recorded in Ecuador (Welden 2010) and Spain (Canary Islands; Beltrán-Tejera et al. 2013). The morphological similarities with *Ph. crassa*, a widespread species, may cause misidentification, and the geographical distribution of *P. amethystea* is apparently much wider. When the species was firstly described (Hjortstam and Ryvarden 1990), and even later (Ryvarden 2010; Welden 2010), only the type specimen and a few additional collections were studied.

Phlebiopsis amethystea was initially described as a species of *Porostereum* (Hjortstam and Ryvarden 1990), which was differentiated from *Lopharia* mostly by the smaller spores, and then further segregated into *Hjortstamia* because of the lack of clamp connections (Boidin and Gilles 2003). Of the 19 names of *Porostereum* listed in the *Index Fungorum* database, only four are now accepted in the genus. The type species of the genus, *P. phellodendri* Pilát, was not yet sequenced, making the concept of *Porostereum* somewhat unclear. However, Hjortstam and Ryvarden (1990) treated *P. phellodendri* Pilát as heterotypic synonym of *Thelephora spadicea* Pers., proposing a new combination *P. spadiceum* (Pers.) Hjortstam & Ryvarden, placed in the *Bjerkandera* clade (Phanerochaetaceae; Miettinen et al. 2016). Morphological limits between *Porostereum* and *Lopharia* are not clear, as both have clamped hyphae, mono-dimitic hyphal systems, and hyaline to brown thick-walled cystidia. With the exception of the simple septate species, which are now mostly combined in *Phlebiopsis*, we decided to keep the other *Lopharia s.l.* listed in Welden's monograph in the key. *Lopharia rimosissima* is not included in the key, as basidia and spore were not described nor found in the type specimen (Welden 2010). With this, we concluded that consistent identification is not possible.

In summary, there is no morphological feature for reliably differentiating the genera discussed here. Simple septate species are grouped in *Hjortstamia*, *Phaeophlebiopsis*, and *Phlebiopsis*. Besides generative hyphae with simple septa, this group is characterized by the mono-dimitic hyphal system, presence of metuloid cystidia, clavate basidia, and ellipsoid, CB-, IKI- spores. Species with clamped hyphae are in *Lopharia* and *Porostereum*, but they share all other features with the previous group. There are several species for which phylogenetically important DNA sequences are not available, especially those with tropical distribution, which in the future can improve our understanding of this group.

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