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Standard Paper

Two new species of *Placomaronea* (*Candelariaceae*: lichenized *Ascomycota*) in Peru, with a revision of secondary chemistry and cortical anatomy of *Placomaronea*, *Candelina* and *Candelariella*

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Abstract

Two new species, *Placomaronea fruticosa* and *P. placoidea*, are described. They were originally discovered in the southern Peruvian Andes at altitudes between 3000 and 4650 m. One specimen of *P. fruticosa* was subsequently also found among herbarium material collected in Argentina. *Placomaronea fruticosa* is terricolous in high altitude grasslands with rocky cliffs. It is characterized by its fruticose to subfruticose thallus, which is up to 8 mm tall and partially immersed in the substrate, its branches are bright to deep yellow, flattened and on the substrate surface their elongated apices resemble placodioid lobes of crustose species in the genus *Candelina*, whereas the cylindrical basal parts are pale beige to deep violet and mostly grow immersed in their substrate. The species has asci with over 20 ascospores in a 60–80 µm tall hymenium. *Placomaronea placoidea* is a saxicolous species, growing in rocky exposed areas. It is characterized by its tightly adnate, foliose, placodioid thallus with a bright to deep yellow upper surface. No fertile specimens were found. Both species newly described here are morphologically very similar to species of *Candelina* but are clearly distinguished by a cortex anatomy characteristic of *Placomaronea*. Cortex anatomy can thus be immensely useful to distinguish crustose and subfoliose genera in *Candelariaceae*, whereas secondary chemistry is shown to be quite uniform, with some chemotype variation of little taxonomic relevance. An updated ITS-only phylogeny of *Candelariaceae* is presented and compared with earlier phylogenies of the family. Several well-supported clades are identified, including *Candelina*, *Placomaronea* and *Protocandelariella*, but much of *Candelaria* and *Candelariella* s. lat. remain unresolved, and the relationships between the supported clades are not yet known. The limitations of currently available molecular data, primarily only ITS, are discussed, particularly in relation to the lack of support at species level, such as the two newly described species of *Placomaronea*. An updated key to currently accepted genera in *Candelariaceae* and all species of *Placomaronea* now known is provided.

A Spanish version of this abstract is provided in Supplementary Material File S1 (available online).

Keywords: Andes; biodiversity; high elevation; paraphyletic taxa

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Introduction

Candelariaceae Hakul. is a family of lichenized fungi that comprises four (4) genera based on morphological and anatomical features: *Candelaria* A. Massal., *Candelariella* Müll. Arg., *Candelina* Poelt, and *Placomaronea* Räsänen, *sensu* Poelt (1974), Westberg *et al.* (2007, 2011) and Lücking *et al.* (2016, 2017). Recently Kondratyuk *et al.* (2020), based on an analysis of the ITS, 28S nrLSU and 12S mtSSU regions downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), proposed three new genera, *Candelinella* S. Y. Kondr., *Opeltiella* S. Y. Kondr. and *Protocandelariella* Poelt ex D. Liu *et al.*, to accommodate groups of species currently placed in *Candelaria* and *Candelariella*. This proposal requires further revision before it can be accepted, because the phylogenetic trees presented in both of the most recent works on the subject (Westberg *et al.* 2007; Kondratyuk *et al.* 2020) have low support in their basal and only moderate resolution in some of the terminal branches. The available molecular data for *Candelariaceae* are still insufficient, mainly focusing on North America and Europe and largely absent from regions with conspicuous diversity, such as South America and Asia. Proposing substantial changes in the taxonomy of the family at this point seems premature and could lead to nomenclatural instability, especially if generic delimitations are based only on DNA, not clearly aligned with morphological or anatomical characteristics.

Until now, the study of the taxonomic diversity in the family has focused mainly on North America (Westberg & Nash 2002*a*, *b*, 2007; Westberg 2004, 2007*a*, *b*, *c*; Westberg & Arup 2011;

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Yakovchenko *et al.* 2017), with some additional work in Europe, Asia and Australia (Filson 1992; Westberg & Clerc 2012; Westberg & Sohrabi 2012; Dong & Hur 2018; Dong *et al.* 2019; Liu *et al.* 2019). Research in South America includes descriptions of a small number of new taxa (Räsänen 1939, 1941; Westberg & Frödén 2007; Etayo *et al.* 2021), range extensions (Osorio 1974), broad geographical treatments of the family with some incidental records from South America (Hakulinen 1954; Poelt 1974), and a monograph of *Placomaronea* (Westberg *et al.* 2009). As mentioned by Westberg & Frödén (2007), the family is presumably characterized by a great diversity in the Andean region, but there is still much work to do.

In Peru, six species of *Candelariaceae* have been reported (Ramos 2014): *Candelaria concolor* (Dicks.) Arnold, *C. fibrosoides* M. Westb. & Frödén, *Candelariella andicola* (Zahlbr.) Zahlbr., *Placomaronea candelarioides* Räsänen, *P. lambii* (Hakul.) R. Sant., and *P. mendozae* (Räsänen) M. Westb. This number is growing, with several recent new species in preparation (D. Ramos, unpublished data), principally in *Candelariella* which we believe has greater diversity in the Andean region than is currently known, requiring a more extensive revision.

Prior to our revision, six species of *Placomaronea* were known: *P. candelarioides*, *P. fuegiana* M. Westb. & Frödén, *P. kaernefeltii* M. Westb. *et al.*, *P. lambii*, *P. mendozae* and *P. minima* M. Westb. & Frödén. These are mainly distributed in the central and southern Andean region of South America, where they have been reported from Ecuador, Peru, Bolivia, Chile and Argentina. *Placomaronea mendozae* has also been reported from the United States, and *P. minima* from Lesotho, Africa. The genus is characterized by its fructicose, foliose, squamulose to crustose thallus, with pigments in the upper cortex and epihymenium arranged as 'hoods' on the tips of the outer hyphae, multi-spored asci (> 20), and hyaline, simple ascospores, commonly with two oil droplets but sometimes with one or multiple droplets (Westberg *et al.* 2009).

The present article describes two new species, *Placomaronea fruticosa* and *P. placoidea*, both collected in the southern Andes of Peru. Both morphologically much resemble species of *Candelina*, but molecular data place them into *Placomaronea*. Westberg *et al.* (2009) emphasized that among crustose to subfoliose genera of *Candelariaceae*, *Placomaronea* may best be characterized by its distinct cortex structure, and, according to Poelt (1974) secondary chemistry is deemed highly variable throughout the family. For comparison, we therefore also decided to study the cortex anatomy and secondary chemistry of *Candelariella*, *Candelina* and some of the previously described species of *Placomaronea*. An updated key to the genera and all species of *Placomaronea* currently known is provided.

Materials and Methods

The present study is primarily based on material collected by the first author during a field trip in 2018, in the southern Andes of Peru; these collections are deposited in the Herbario Sur Peruano (HSP). For comparison, particularly of the secondary chemistry and cortex anatomy, we also examined material of *Candelina*, *Candelariella* and *Placomaronea* from the following herbaria: ASU, COLO, LSU, MSC, NY and WIS. Detailed specimen information for all material examined is available from the *Consortium of Lichen Herbaria* (https://lichenportal.org/).

Morphological features were observed with a EDUblue1402-S and Wild M2Z dissecting microscope. Anatomical characteristics were studied with a Labortech-2005 and Zeiss Axio Lab A1 compound microscope. Hand-cut sections of the thallus and apothecia were mounted in distilled water. Standardized reagents of 10% aqueous solution of potassium hydroxide (K), chlorine household bleach (C), para-phenylendiamine crystals dissolved in ethanol (P) and Lugol's solution (I) were used for spot testing, and the reaction to UV light was checked. Anatomical measurements were taken using 'Scale Bar Tools for Microscopes' from ImageJ v. 1.52 software, previously calibrated to the microscope. A minimum of 10 measurements for each structure and 45 measurements of ascospores were made. Microphotographs were taken with a Canon EOS 12.2 MP camera mounted on the Labortech-2005 microscope and a Nikon D7000 mounted on the Zeiss Axio Lab A1. Macrophotographs were taken in a light box using a Nikon D800E and/or D810 camera with a 60 mm AF-D Micro-Nikkor lens mounted on a Novoflex macro stand.

Secondary metabolites were examined from a selection of specimens using standardized thin-layer chromatography, routinely using solvent C (Orange *et al.* 2001, 2010). Instead of the conventional upright TLC tanks, a horizontal HPTLC developing chamber was used (Arup *et al.* 1993). A protocol first suggested by Egan (2001) to document and conserve TLC results was modified here as follows: TLC plates were photographed with a Nikon D300 digital camera. Photographs were taken immediately after running the solvent, in long-wave (λ365 nm) and short-wave (λ254 nm) UV light, before applying 10% H_2SO_4. After H_2SO_4 treatment and charring in a laboratory oven for *c.* 8 min at 110 °C, a second set of photographs in visible light and long-wave UV (λ365 nm) were taken. Standard spot tests with reagents P, K and C were routinely carried out using methods described in Bungartz (2002). UV-fluorescence of thalli was studied under long-wave UV light (λ365 nm). Lugol's iodine was used to study asci following a routine protocol outlined in Bungartz (2002). Plates were subsequently analyzed in Mytabolites 1.0.0.0 (Lafferty *et al.* 2024), a software package that shares many functionalities with its popular predecessor Wintabolites, originally developed at the University of Essen (Mietzsch *et al.* 1992, 1993).

Specimens of the two new species examined are cited below (see descriptions). For comparison, we also studied thallus morphology, cortex anatomy and secondary chemistry of specimens for the following species:

Candelariella kansuensis H. Magn. **USA:** *Arizona*: *C. M. Wetmore* 55470 (608915, ASUL011864; MIN1408597/892474), 54366 (MIN1408598/783925), 55277 (MIN1408596/892475), 54907 (MIN1408599/783453).

Candelariella rosulans (Müll. Arg.) Zahlbr. **Mexico:** *Baja California*: *T. H. Nash* 4369 (579860, ASUL010372), 26322 (ASUL020311), 26332 (ASUL020306).—**USA:** *Arizona*: *W. C. Davis* 451 (577841, ASUL011839).

Candelariella vitellina (Hoffm.) Müll. Arg. **Mexico:** *Baja California*: *T. H. Nash* 38248 (514395, ASUL010373).—**USA:** *Arizona*: *W. C. Davis* 617 (578223, ASUL011791).

Candelina mexicana (B. de Lesd.) Poelt. **Mexico:** *Baja California Sur*: *T. H. Nash* 39918 (514805, ASUL034850). *Michoacán*: *R. S. Egan* 10704 (580307, ASUL034847). *Sonora*: *J. Marsh* 4776 (608870, ASUL010375).—**Venezuela:** *Mérida*: *K. Kalb* 24021 (WIS-L-0115751), *T. H. Nash* 29010 (514728, ASUL034863), 29040 (ASUL034864). *Lara*: *K. Kalb* 25843 (WIS-L-0141004), *T. H. Nash* 28949 (ASUL034865).—**USA:** *Arizona*: *T. H. Nash* 41651 (514893, ASUL010374). *Texas*: *C. Fox* T120 (580243, ASUL034888).

Candelina submexicana (B. de Lesd.) Poelt. **Mexico:** *Chihuahua*: *T. H. Nash* 13514 (580008, ASUL034785), 36550 (514668, ASUL034815), 36104 (ASUL034797).—**Peru:** *Ica*: *W. A. Weber* s. n. (L-66445, 314320, COLO-L-0063762), *W. A. Weber* s. n. (L66445 pkt 2, 414653, COLO).—**USA:** *Arizona*: *R. Kulich* 16A (ASUL003433).

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New Mexico: *B. D. Ryan* 22143-a (514803, ASUL034889), *R. D. Worthington* 31956 (537897, ASUL024890).

Placomaronea candelarioides Räsänen. **Argentina:** *Tucumán*: *T. H. Nash* 28034 (604432, ASUL010349), *R. C. Harris* 4254461 (NY 4254461). *Catamarca*: *I. M. Lamb* 5596 (MSC0135846).—**Bolivia:** *La Paz*: *D. Ugent* s. n. (MIN 1298531). *Potosí*: *D. Ugent* s. n. (MIN 1298532).—**Peru:** *Puno*: *D. Ugent* s. n. (MIN 1298534).

Placomaronea lambii (Hakul.) R. Sant. **Argentina:** *Tucumán*: *I. M. Lamb* 5413 (MSC0112896—isotype).

Placomaronea mendozae (Räsänen) M. Westb. **USA:** *Arizona*: *T. H. Nash* 25430 (580255, ASUL010350).

We generated ITS sequences for three specimens, including the holotype of both new species: *Ramos* 2899 (GenBank Accession number PQ807198) and 2908a (holotype: GenBank Accession number PQ807199) for *P. placoidea*, and *Ramos* 2946 (holotype: GenBank Accession number PQ807200) for *P. fruticosa*. Sequences have been submitted to GenBank (https://www.ncbi.nlm.nih.gov). DNA was extracted using the Soltis Lab CTAB DNA protocol (Doyle & Doyle 1987; Cullings 1992). ITS1 and ITS4 primers (White *et al.* 1990) were used to select the ITS region. Standard amplification procedures were followed (McCune & Curtis 2012). Forward and reverse sequences were inspected and combined by hand using Geneious Prime v. 2023.0.3 (https://www.geneious.com). All sequences were checked against the NCBI database (https://www.ncbi.nlm.nih.gov) for contamination. Additional ITS sequences were obtained from GenBank, comprising all sequences available for *Placomaronea*, including those used by Westberg *et al.* (2009) and, where available, one or two representatives for every species in *Candelariaceae*. Unfortunately, it was not possible to include any ITS sequences cited in Kondratyuk *et al.* (2020) since it appears that none of these have been submitted to GenBank. Reviewing the recent publication by Kondratyuk *et al.* (2020), we initially planned to add mtSSU and nuLSU sequences to our analysis, but only very few sequences for these loci exist and not a single one for *Placomaronea* or *Candelina*. Any attempt to construct a multilocus phylogeny of *Candelariaceae* therefore seems very premature.

Following Westberg *et al.* (2007, 2009), we chose *Pleopsidium chlorophanum*, *P. flavum* and *Pycnora xanthococca* as outgroups for our analysis. The sequences were initially aligned using MAFFT v. 7.490 (Katoh & Standley 2013), then manually trimmed and adjusted, removing regions that we considered problematic because of introns. IQ-TREE v. 2.1.3 (Minh *et al.* 2020) was then used to create maximum likelihood trees, using the default settings for an ultrafast bootstrap analysis, with 1000 bootstrap repetitions and automatic selection of the substitution model. We also ran a Bayesian analysis using the Markov chain Monte Carlo (MCMC) method (Larget & Shimon 1999) as implemented in MrBayes v. 3.2.7 (Ronquist *et al.* 2012), with substitution model nset=6 rates=invgamma. Two independent runs continued until the standard deviation between split frequencies dropped below 0.01. The final tree was rendered with FigTree v. 1.4.4 (Rambaut & Drummond 2012). Alignment and log files are available on FigShare (https://doi.org/10.6084/m9.figshare.28098074).

Results

Secondary chemistry

Table 1 summarizes the results of specimens analyzed by thin-layer chromatography and their cortical spot test reactions with K. The following chemotypes can be distinguished (Figs 1 & 2):

- Chemotype A: pulvinic acid (major), 4-hydroxypulvinic acid (minor), pulvinic dilacetone (minor or trace), calycin (minor or trace); with a series of unidentified terpenoids.
- Chemotype B: same chemistry as A, but no terpenoids.
- Chemotype C: same chemistry as chemotype A, but no pulvinic dilacetone; with the same unidentified terpenoids as chemotype A.
- Chemotype D: characterized only by pulvinic acid (major) and 4-hydroxypulvinic acid (minor); no other secondary metabolites present.

Among the material examined, all *Candelariella rosulans* and *C. vitellina* belong to chemotype B, reacting distinctly K+ red. *Candelariella kansuensis* is represented only by chemotype D, specimens reacting K− or K± weakly reddish. Most specimens of *Candelina* react distinctly K+ red, few K+ weakly reddish, and a small number are K−. Material of *C. mexicana* belongs to chemotypes A or C, and specimens of *C. submexicana* to chemotypes A or B; no specimens of *Candelina* were found that belong to chemotype D. Most specimens of *Placomaronea* belong to chemotype D (mostly reacting K± weakly reddish or K−, few K+ distinctly red); one specimen of *P. candelarioides* and one of *P. mendozae* were found to belong to chemotype B (both K+ distinctly red).

Anatomy

Most of the differences in cortex anatomy correspond with the three genera as currently recognized (Table 2): *Placomaronea* (thin colourless coating shedding off; peppered pigment hoods; thick cortex of cortical paraplectenchymatous hyphae), *Candelina* (no colourless coating; no hoods, but with a thick layer of closely cemented pigment granules; only the uppermost hyphae paraplectenchymatous), and *Candelariella* (no colourless coating; no hoods; thin layer of loose pigment granules; cortical hyphae mostly parallel, proso- to indistinctly paraplectenchymatous). Only in '*Candelariella*' *kansuensis* can an almost identical cortical anatomy to *Placomaronea* be observed; the only difference is the extremely thick outermost colourless coating of *C. kansuensis*, causing a smooth, waxy, almost shiny thallus surface.

Phylogeny

Our phylogenetic analyses strongly support the inclusion of the new species in *Placomaronea* (100% bootstrap; Fig. 3). Within *Placomaronea*, the two new species form a distinct, well-supported clade within *P. mendozae* (100% BS); together all three (*P. fruticosa*, *P. placoidea* and *P. mendozae*) form a well-supported clade (99% BS) that is sister to *P. candelarioides* and *P. fuegiana*.

The relationships between *P. fruticosa*, *P. placoidea* and *P. mendozae* remain poorly resolved. *Placomaronea fruticosa* is included in the same clade as *P. placoidea*, which in turn is part of the clade that forms *P. mendozae*. Both *P. placoidea* and *P. mendozae* are thus paraphyletic.

Our analysis recovers polysporous species of *Candelariella* into two separate but strongly supported, monophyletic clades: one with *C. borealis* M. Westb. and *C. placodizans* (Nyl.) H. Magn. (100% BS), the other one corresponding to *Candelariella* s. str., with *C. coralliza* (Nyl.) H. Magn., *C. efflorescens* R. C. Harris & W. R. Buck, *C. faginea* Nimis *et al.*, *C. granuliformis* M. Westb., *C. lutella* (Vain.) Räsänen, *C. vitellina* and *C. xanthostigma* (Ach.) Lettau (98% BS). Polysporous species of *Candelaria* (*C. asiatica* D. Liu & J.S. Hur, *C. concolor*, *C. fibrosa* (Fr.) Müll. Arg. and *C. murrayi*

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Table 1. Secondary chemistry and K spot tests in selected specimens of *Candelariaceae.* Testing the thallus surface, K+ indicates a strong red colour reaction to 10% potassium hydroxide; K± indicates a weak reddish reaction (barely visible after several minutes); K− indicates no reaction.

Taxa	Chemotype A pulvinic acid & 4-hydroxypulvinic acid, pulvinic dilacetone, calycin, terpenoids	Chemotype B same as A, no terpenoids	Chemotype C same as A, no pulvinic dilacetone	Chemotype D only pulvinic acid and 4-hydroxypulvinic acid
Candelariella kansuensis		**USA:** *C. M. Wetmore* 54366 (MIN1408598/783925); K+		**USA:** *C. M. Wetmore* 55470 (ASUL011864; MIN1408597/892474); K− *C. M. Wetmore* 55277 (MIN1408596/892475); K± *C. M. Wetmore* 54907 (MIN1408599/783453); K−
C. rosulans		**Mexico:** *T. H. Nash* 4369 (ASUL010372); K+ *T. H. Nash* 26322 (ASUL020311); K+ *T. H. Nash* 26332 (ASUL020306); K+ **USA:** *W. C. Davis* 451 (ASUL011839); K+		
C. vitellina		**Mexico:** *T. H. Nash* 38248 (ASUL010373); K+ **USA:** *W. C. Davis* 617 (ASUL011791); K+		
Candelina mexicana	**Mexico:** *T. H. Nash* 39918 (ASUL034850); K+ *J. Marsh* 4776 (ASUL010375); K+ **Venezuela:** *K. Kalb* 25843 (WIS-L–0141004); K+ *T. H. Nash* 29040 (ASUL034864); K± *T. H. Nash* 28949 (ASUL034865); K±		**Mexico:** *R. S. Egan* 10704 (ASUL034847); K+ **USA:** *T. H. Nash* 41651 (ASUL010374); K− *C. Fox* T120 (ASUL034888); K− **Venezuela:** *K. Kalb* 24021 (WIS-L–0115751); K− *T. H. Nash* 29010 (ASUL034863); K−	
C. submexicana	**Mexico:** *T. H. Nash* 13514 (ASUL034785); K+ *T. H. Nash* 36550 (ASUL034815); K+ **Peru:** *R. D. Worthington* 31956 (ASUL024890); K+ **USA:** *R. Kulich* 016A (ASUL003433); K+ *B. D. Ryan* 22143-a (ASUL034889); K+	**Mexico:** *T. H. Nash* 36104 (ASUL034797); K+ **Peru:** *W. A. Weber* s. n. (L–66445; COLO-L–0063762); K± *W. A. Weber* s. n. (L–66445 (pkt 2)); K±		
Placomaronea candelarioides		**Peru:** *D. Ugent* s. n. (MIN 1298534); K+		**Argentina:** *T. H. Nash* 28034 (ASUL010349); K− *R. C. Harris* 34601 (NY 04254461); K− *I. M. Lamb* 5596 (MSC0135846); K+ **Bolivia:** *D. Ugent* s. n. (MIN 1298531); K+ *D. Ugent* s. n. (MIN 1298532); K−
P. fruticosa				**Argentina:** *T. H. Nash* 27947 (ASUL010376); K± **Peru:** *D. Ramos* 2946 (HSP—holotype); K±
P. lambii				**Argentina:** *I. M. Lamb* 5413 (MSC0112896—isotype); K+
P. mendozae		**USA:** *T. H. Nash* 25430 (ASUL010350); K+		
P. placoidea				**Peru:** *D. Ramos* 2908a (HSP—holotype); K±

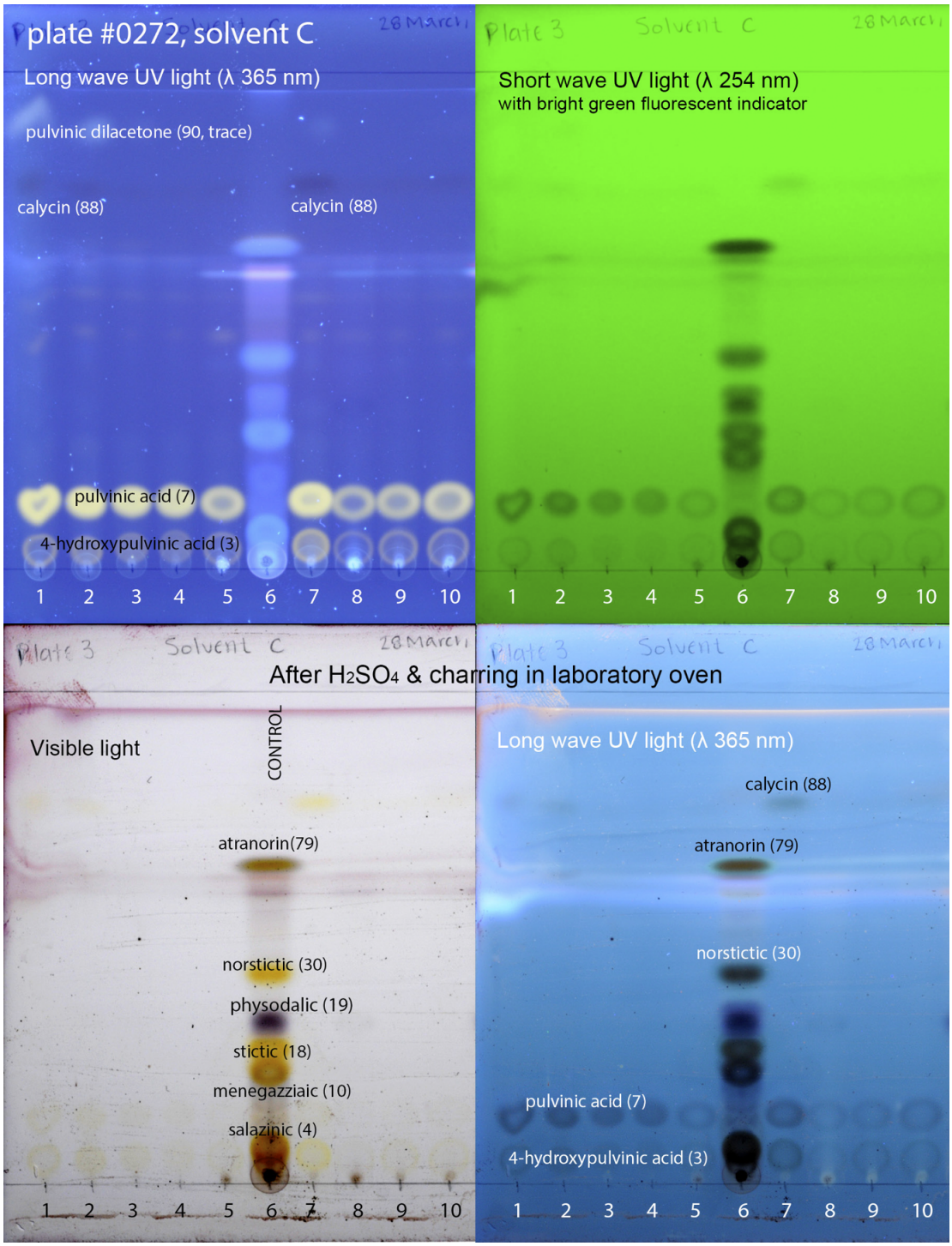

Figure 1. Thin-layer chromatography plate of selected specimens of *Candelariaceae*; numbers correspond to the following specimens: 1) *Candelina submexicana* (*W. A. Weber* 314320 (L-66445; COLO-L-0063762)), 2) *Candelina submexicana* (*W. A. Weber* 314320 (L-66445; pkt 2)), 3) *Placomaronea placoidea* (*D. Ramos* 2908a (HSP—holotype)), 4) *Placomaronea fruticosa* (*D. Ramos* 2946 (HSP—holotype)), 5) *Placomaronea candelarioides* (*T. H. Nash* 28034 (ASUL010349)), 6) Control (mixture of specimens with known secondary metabolites: *Hypogymnia physodes* (L.) Nyl., *Hypotrachyna microblasta* (Vain.) Hale, *Parmelia sulcata* Taylor, *Parmotrema crinitum* (Ach.) M. Choisy, *Physcia adscendens* H. Olivier), 7) *Placomaronea mendozae* (*T. H. Nash* 25439 (ASUL010350)), 8) *Placomaronea lambii* (*I. M. Lamb* 5413 (MSC0112896—isotype)), 9) *Placomaronea candelarioides* (*R. C. Harris* 34601 (NY 04254461)), 10) *Placomaronea candelarioides* (*I. M. Lamb* 5596 (MSC0135846)). Numbers 1, 2 & 7 = chemotype B (pulvinic dilacetone, calycin, pulvinic acid, 4-hydroxypulvinic acid, no terpenoids); 3, 4, 5, 8, 9 & 10 = chemotype D (only pulvinic acid with 4-hydroxypulvinic acid). In colour online.

Poelt) also form a well-supported, monophyletic clade (95% BS). In addition, our analysis confirmed two monophyletic clades of octosporous species of *Candelariella*: one that includes *Candelariella aurella* (Hoffm.) Zahlbr., *C. medians* (Nyl.) A.L. Sm., *C. plumbea* Poelt & Vězda and *C. ruzgarii* Halıcı *et al.* (100% BS), the other including *Candelariella aggregata* M. Westb., *C. antennaria* Räsänen and *C. viae-lacteae* G. Thor & V. Wirth (99% BS).

Morphology

Placomaronea fruticosa and *P. placoidea* are not easily categorized as crustose, foliose, or fruticose (Figs 4A–D & 5A–C). Both resemble crustose lichens closely, but their lobes radiating on the substrate surface have a distinctly corticate upper and lower side (Figs 4C & D, 5B). At least anatomically, these thalli are foliose.

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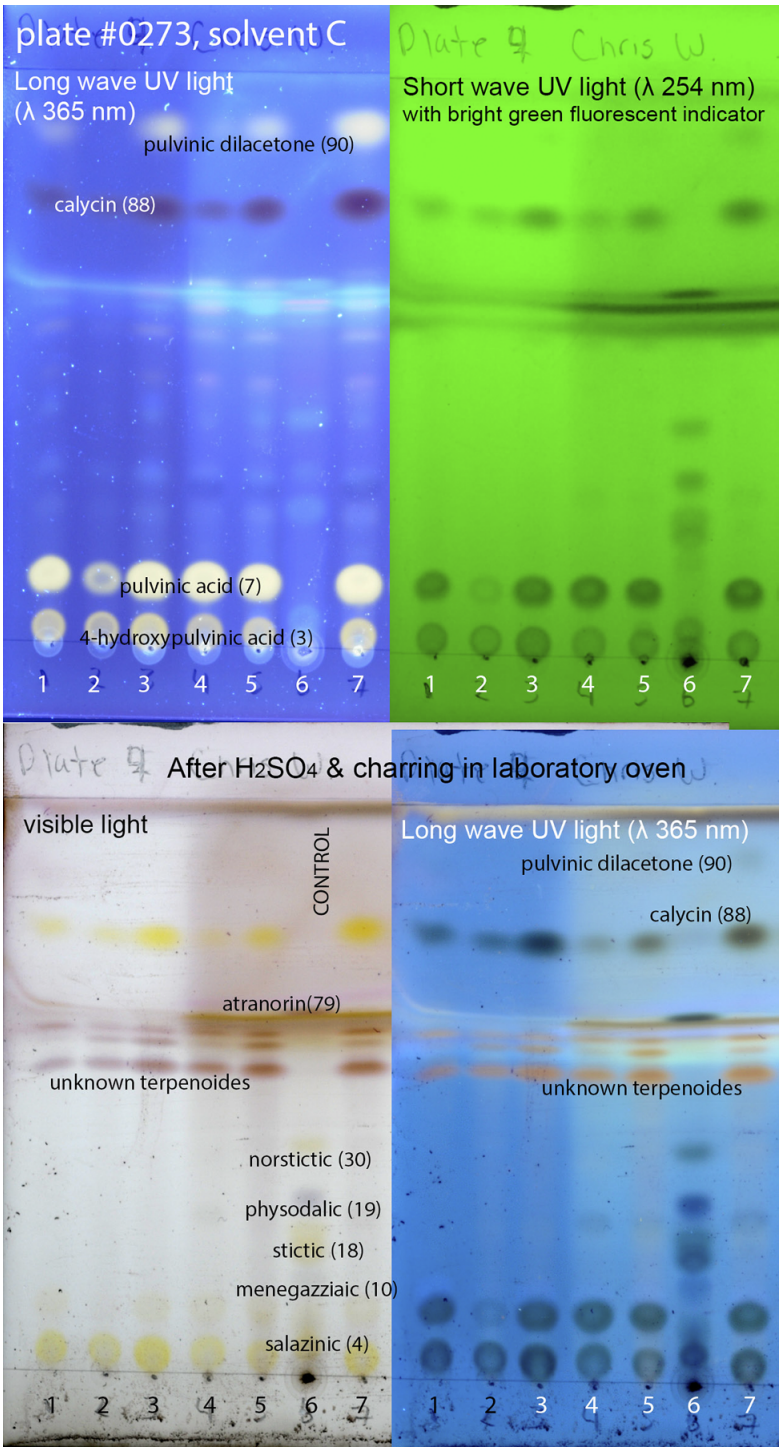

Figure 2. Thin-layer chromatography plate of selected specimens of *Candelariaceae*; numbers correspond to the following specimens: 1) *Candelina submexicana* (*R. D. Worthington* 31956 (ASUL024890)), 2) *Candelina mexicana* (*C. Fox* T120 (ASUL034888)), 3) *Candelina submexicana* (*T. H. Nash* 36550 (ASUL034815)), 4) *Candelina mexicana* (*K. Kalb* 24021 (WIS-L-0115751)), 5) *Candelina mexicana* (*T. H. Nash* 39918 (ASUL034850)), 6) Control (mixture of specimens with known secondary metabolites: *Hypogymnia physodes*, *Hypotrachyna microblasta*, *Parmelia sulcata*, *Parmotrema crinitum*, *Physcia adscendens*), 7) *Candelina mexicana* (*K. Kalb* 25843 (WIS-L-0141004)). Numbers 1, 3, 5 & 7 = chemotype A (pulvinic dilacetone, calycin, pulvinic acid, 4-hydroxypulvinic acid, unknown terpenoids); 2 & 4 = chemotype C (calycin, pulvinic acid, 4-hydroxypulvinic acid, unknown terpenoids). In colour online.

Table 2. Cortex anatomy in *Candelariaceae*.

	Candelariella	*Candelina*	*Placomaronea*	'*Candelariella*' *kansuensis*
Hyaline layer	/	/	thin (cell residue?)	very thick (coating?)
Granular pigments	thin, loose	thick, cemented	'peppered' hoods	'peppered' hoods
Cortex thickness	thin	thin	thick	thick
Cortex structure	mostly *proso*plectenchymatous	few cell rows *para*plectenchymatous	distinctly *para*plectenchymatous	distinctly *para*plectenchymatous

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Figure 3. Phylogeny of the family *Candelariaceae* based on maximum likelihood analysis (ML) of ITS. Support is shown as ML bootstrap values (left)/Bayesian MCMC posterior probabilities (right); branches with strong support (bootstrap ≥ 95% and MCMC ≥ 0.99) are indicated with thick lines. Polysporous clades are indicated with an asterisk (*) and shaded red (colour version). Octosporous clades are shaded pale blue (colour version). GenBank Accession numbers or voucher information are provided after the taxon names. The new species are in bold at the bottom of the tree. In colour online.

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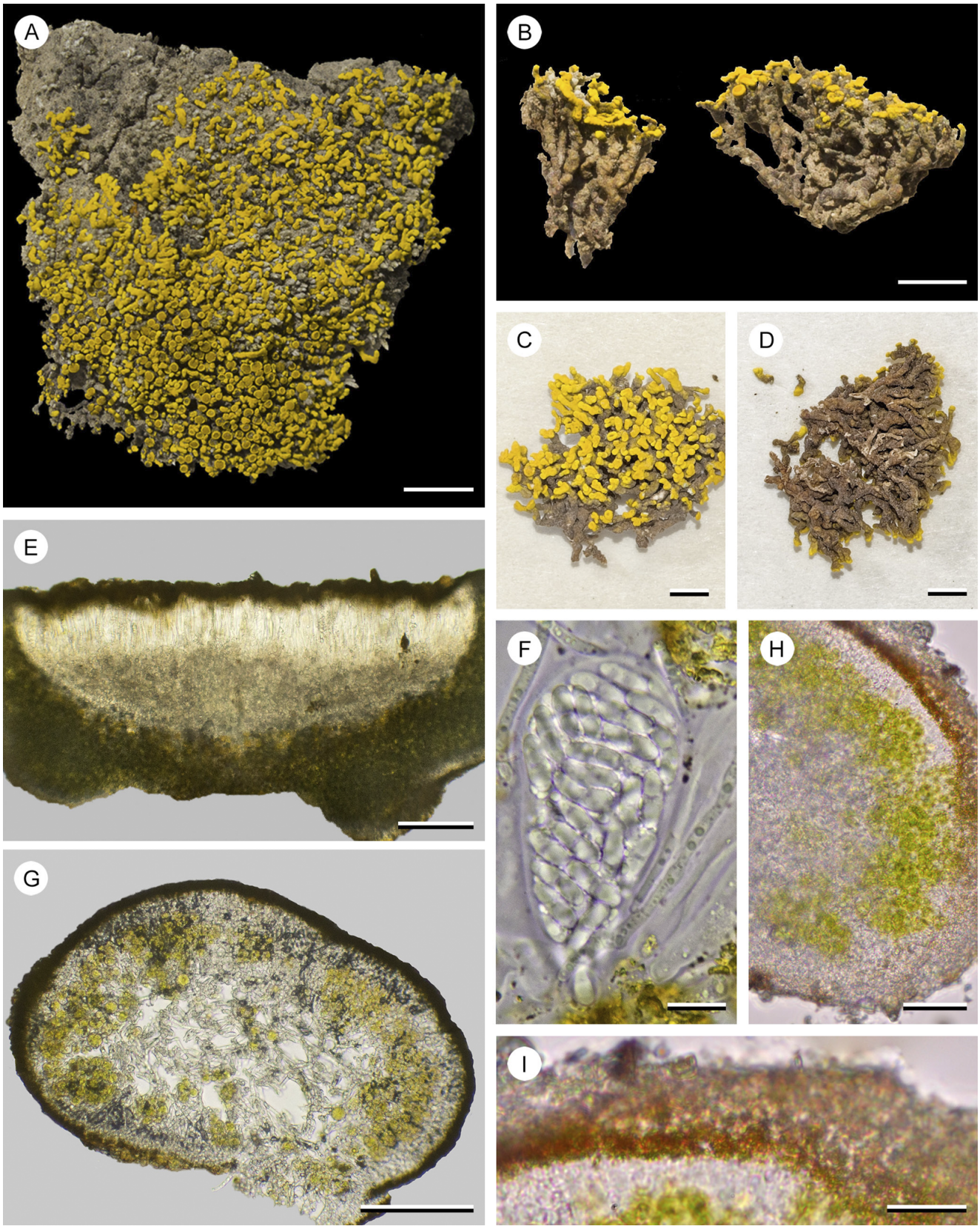

Figure 4. *Placomaronea fruticosa* (*Ramos* 2946, HSP—holotype). A, surface view of the thallus, placodioid lobes spreading irregularly across the soil substrate, fruticose parts of thallus embedded in the soil. B–D, samples prepared by removing soil substrate to illustrate fruticose growth below horizontally spreading surface lobes. B, lateral view. C, view of the upper side. D, view of the lower side. E, section of an apothecium. F, polysporous ascus. G, hand-cut section of fruticose part of the thallus with medullary hyphae throughout the centre, surrounded by a photobiont layer and cortex. H, hand-cut section of the lateral part of a thallus lobe, showing the medulla, photobiont and cortical layers. I, close-up of the cortex, with a distinct layer of paraplectenchymatous cells, apically capped by cells with pigment hoods, peppered in pigment granules, covered in a thin, barely distinct coating of hyaline residue. Scales: A–D = 5 mm; E & G = 100 μm; F & I = 10 μm; H = 25 μm. In colour online.

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Morphologically, these thalli cannot be distinguished reliably from species of *Candelina*, where Poelt (1974) also described placodioid growth in combination with a lower cortex. *Placomaronea placoidea* is a close lookalike of *Candelina submexicana* and is easily confused. The lobes of *P. placoidea* are generally smaller, and more flattened and hollow inside; both are most reliably distinguished by their different cortex anatomy.

In the field, on the surface of the soil, thalli of *P. fruticosa* look almost identical to those of their saxicolous counterpart, *P. placoidea*, both having radiating placodioid lobes with an upper and lower cortex. This appearance is, however, deceiving. Below the surface, thalli of *P. fruticosa* are distinctly fruticose, extending upwards from deep within their substrate (Fig. 4B). Emerging from the soil, the coralloid branches spread laterally to mimic placodioid growth. Lobes of *P. placoidea* are hollow (Fig. 5D), whereas the centre of the lobes and coralloid branches of *P. fruticosa* contain hyphae, forming a lax medulla (Fig. 4G). No fertile material of *P. placoidea* has been found; only specimens of *P. fruticosa* were collected with apothecia (Fig. 4A).

Taxonomic Section

Placomaronea fruticosa Ramos, Hollinger & Bungartz sp. nov.

MycoBank No.: MB 857051

Differs from other species in the genus by a fruticose thallus part that is up to 8 mm deep and immersed in its soil substrate.

Type: Perú, Cusco, Condoroma, Carretera Condoroma-Oscollo, Roquedal rodeado por césped de puna, en suelo [along the road from Condoroma to Oscollo, rocky area surrounded by puna grass, within the soil], 15.2887°S, 71.1388°W, 4650 m alt., 6 May 2018, *D. Ramos* 2946 (HSP-17855—holotype!, GenBank Accession no. PQ807200; ASUL010486—isotype!).

(Fig. 4)

Thallus dimorphic, with a spread-out placodioid thallus on the substrate surface, and a subterranean fruticose part buried up to 8 mm within the soil, the placodioid part on the surface generally ±ellipsoid in outline, 1–3 cm diam., irregularly branched, towards the centre increasingly immersed in its substrate; the apical branches emerging from the soil horizontally spreading outwards, flattened, elongated, lobate, resembling placodioid crustose thalli, but the lobes corticate below, branch tips linear or spathulate, 2.6–5.6 mm long and 0.4–1.0 mm wide; basal, immersed thallus vertical, ±terete to distinctly cylindrical, solid, beige to violet (in parts), to 8 mm tall. *Surface* lacking pruina, the flattened placodioid part with a bright yellow to deep yellow upper side, the lower side whitish to beige; surface of the immersed cylindrical thallus beige to brown all around, in parts often violet. *Cortex* 14–30 µm thick, on the upper surface of the lobes differentiated into a hyaline, paraplectenchymatous layer 5–8 cells thick, capped by ±inflated terminal cells, their walls forming pigment hoods, 'peppered' on the outside with pigment granules, coated by a thin hyaline residue flaking off; lower cortex along the lobe edges a continuation of the upper one, becoming thinner, with less pigmentation on the lower site of the lobes and along the immersed, fruticose part of the thallus. *Photobiont* trebouxioid. *Medulla* white, lax.

Apothecia lecanorine, laminal or at the emerging apices of the cylindrical, immersed branches, 0.3–0.8 mm diam., thalline margin entire, thin, crenate, proper margin indistinct; *disc* plane, of the same colour or slightly darker than the thallus, without pruina. *Hymenium* 60–80 µm tall, inspersed with small oil droplets; *paraphyses* to 2 µm wide in the centre, straight, capitate, simple or subapically sparsely branched; *epihymenium* orange-brown to golden; *subhymenium* and *hypothecium* hyaline, also inspersed; *proper exciple* to 12 µm wide. *Asci* clavate 47–59 × 19–30 µm. *Ascospores* ellipsoid, (8–)11–13.5(–15) × 3–4.5 µm (n = 73), more than 20 per ascus, hyaline, simple or rarely with a central septum, most with two oil drops (bigutulate).

Pycnidia not observed.

Chemistry. Pulvinic acid (major) and 4-hydroxypulvinic acid (minor), no other secondary metabolites detected (chemotype D). Cortex K± very faintly reddish, C−, KC−, P−, UV−; medulla, all reactions negative; hymenium IKI+ blue.

Etymology. The name refers to its main characteristic, the subterranean fruticose growth.

Distribution. Terricolous, at elevations between 3800 and 4650 m, in exposed habitats among Andean grassland in the southern Andes of Peru (Cusco and Tacna), to Argentina.

Remarks. Although some species in *Placomaronea* show tendencies to form thalli ±elevated from their substrate (e.g. the 'umbilicate' lobes of *P. candelarioides*), *P. fruticosa* is the only species currently known where the major part of its thallus is fruticose (even though this fruticose part remains entirely immersed and only the placodioid lobes extend across the surface). *Placomaronea fruticosa* is also the only species known to grow on soil; all other species in the genus are saxicolous.

Additional specimens examined. **Perú:** *Cusco*: Condoroma, Carretera Condoroma-Oscollo, Roquedal rodeado por césped de puna, 15.2887°S, 71.1388°W, 4650 m alt., 2018, *D. Ramos* 2949 (HSP). *Tacna*: Candarave, Zona rocosa camino al poblado Yucamani, 17.2666°S, 70.2349°W, 3250 m alt., 2018, *D. Ramos* 2767 (HSP). *Ticaco*: Roquedal al lado de la carretera de Candarave a Ticaco, 17.2486°S, 70.0792°W, 3676 m alt., 2018, *D. Ramos* 2835 (HSP).— **Argentina:** *Jujuy*: 43 km E of La Quiaca, lower part of Sierra de Santa Victoria, along route 5 (dirt road to Santa Victoria), 22°08′S, 65°18′W, 3807 alt., alpine area, on soil, 1989, *T. H. Nash* 27947 (512253, ASUL010376).

Placomaronea placoidea Ramos, Hollinger & Bungartz sp. nov.

MycoBank No.: MB 857052

Differs from other species of *Placomaronea* by a tightly adnate thallus of placodioid radiating lobes with a distinct lower cortex, resembling species of *Candelina* but distinguished by a hollow medulla and a *Placomaronea*-type cortex (i.e. a paraplectenchymatous hyaline layer capped by pigment hoods, 'peppered' with pigment granules, coated in a thin hyaline residue flaking off).

Type: Perú, Arequipa, Cayarani, Roquedal al lado del camino de entrada al poblado de Cayarani, en rocas [rocky area next to the entrance road to the town of Cayarani, on rock], 14°40′10.8″S, 72°01′25.5″W, 3955 m alt., 5 May 2018, *D. Ramos* 2908a (HSP-17815—holotype!, GenBank Accession no. PQ807199; ASUL010485—isotype!).

(Fig. 5)

Thallus resembling placodioid crustose lichens, but the tightly adnate lobes with a lower cortex and thus foliose, irregularly branching lobes forming small rosettes, irregular to ±ellipsoid in outline, up to 6 cm diam.; lobes elongate, irregularly spreading from the thallus centre, simple to scarcely and irregularly branched,

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closely adjoining to ±imbricate, linear to apically barely spathulate, 2.0–4.9 mm long and 0.7–1.4 mm wide. *Upper surface* plane to undulate, mostly dull, but with some shiny areas, epruinose, bright yellow especially towards the lobe apices, often discoloured and darker yellow or even brownish in the thallus centre, lower surface beige, becoming paler towards the thallus centre, difficult to remove intact from its substrate. *Cortex* 10–30 µm thick, on the upper surface of the lobes differentiated into a hyaline, paraplectenchymatous layer 3–8 cells thick, capped by ±inflated terminal cells, their walls forming pigment hoods, 'peppered' on the outside with pigment granules, coated by a thin hyaline residue flaking off; lower cortex continuous with the upper cortex and of similar thickness along the lobe edges, but becoming thinner and almost devoid of pigments towards the thallus centre; lobes initially compact, but soon becoming hollow, the medulla at least in part not filling the interior of the thallus. *Photobiont* trebouxioid. *Medulla* white, composed of lax, occasionally anastomosing hyphae that are eventually confined to the upper and lower parts of the lobes, hollowed out in the centre.

Apothecia not observed.

Pycnidia scarce to abundant, inconspicuous, forming small depressions on the upper surface, ostioles concolorous with the surface; *conidia* ellipsoid, hyaline, (3.0–)3.5–4.5 × 1.2–1.9 µm.

Chemistry. Pulvinic acid (major) and 4-hydroxypulvinic acid (minor), no other secondary metabolites detected (chemotype D). Cortex K± very faintly reddish, C−, KC−, P−, UV−; medulla, all reactions negative.

Etymology. The name refers to the placodioid growth.

Distribution and ecology. Saxicolous, in exposed and sunny habitats, at elevations between 3000–4000 m, so far recorded only in the southern Andes of Peru (Cusco and Arequipa), but presumably with a wider distribution.

Remarks. The species is distinguished by its adnate, foliose-placodioid thallus resembling species of *Candelina* but distinctly different by its cortical anatomy and hollow medulla (see Discussion).

Additional specimens examined. **Perú:** *Cusco*: Santo Tomás, Roquedal en rodal de *Puya raimondii*, Carretera al borde del río Cayarani, 14.7771°S, 72.0412°W, 4082 m alt., 2018, *D. Ramos* 2899 (HSP, GenBank Accession no. PQ807198). *Ocoruro*: Carretera Ocoruro-Espinar, 14.9377°S, 71.1915°W, 3990 m alt., 2018, *D. Ramos* 2931 (HSP). *Arequipa*: Huaynacotas Rodal de Puya alrededores de la comunidad de Puyca, 14.9340°S, 72.7108°W, 4108 m alt., 2017, *D. Ramos* 2524 (HSP). *Chuquibamba*: Matorral en ladera, pasando el pueblo de Chuquibamba, 15.8295°S, 72.6615°W, 3004 m alt., 2017, *D. Ramos* 2502 (HSP).

Key to the genera of *Candelariaceae*

1 Thallus surface grey, cortex lacking yellow pigment granules; only the apothecia and pycnidial ostioles yellow; apothecia biatorine ..... '**Protocandelariella**' [*Candelariella subdeflexa* & *C. blastidiata*]
Thallus surface bright to egg-yolk yellow, with a distinct layer of pigment granules; apothecia lecanorine 2

2(1) Upper cortex with a few layers of hyaline, proso- to indistinctly paraplectenchymatous cells; apically inspersed by a thin to moderately thickened layer of pigment granules ..... **Candelariella** s. lat.
Upper cortex with few to several layers of hyaline, distinctly paraplectenchymatous cells; apically either with a thick and dense layer of closely aggregated pigment granules, or with inflated cortical cells capped by pigment hoods, 'peppered' on their outside with pigment granules 3

3(2) Upper cortex of hyaline, distinctly paraplectenchymatous cells covered by a thick layer of densely aggregated pigment granules (even in squash preparations these granules do not easily dissociate); the cortex on the outside lacking any hyaline residue or coating 4
Upper cortex of hyaline, distinctly paraplectenchymatous cells bottomped by a distinct layer of inflated cells that are capped by yellow pigment 'hoods', these 'peppered' in abundant pigment granules (observed only in thin sections or squash preparations); the hooded cells on the outside covered either by a thin, ±indistinct hyaline residue, or by a thick, ±layered, hyaline coating 5

4(3) Thalli distinctly foliose, divided into minute, ±flattened lobes that are loosely spreading outwards and/or ±upwards; lobes often crowded, irregularly overlapping or ±tiled, occasionally appearing almost fruticose; attached either by short, multi-cellular hapters and/or by long bundles of hyphae forming true rhizines ..... **Candelaria**
Thalli placodioid, lobes convex, closely appressed and therefore appearing crustose, although with a distinct lower cortex; not attached by multi-cellular hapters, but in part directly and broadly adhered, occasionally attached also by individual hyphae (German: '*Suchhyphen*'); these, however, not aggregating to form true rhizines ..... **Candelina**

5(4) Upper cortex covered in a thick, layered, hyaline coating (gelatinous?) which disintegrates on the outside into minute granules ..... **Candelariella kansuensis**
Upper cortex with a thin hyaline residue (disintegrating cells?), easily overlooked, not forming a thick coating .... **Placomaronea**

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Key to the species of *Placomaronea*

1 Thallus crustose, i.e. most of the lower surface broadly attached and lacking a lower cortex, the upper cortex extending towards the edge and along the side, and only slightly below where the areoles become subsquamulose to squamulose (species superficially resembling *Candelariella* s. lat., but generally with a smooth, epruinose surface due to their distinctly different cortex anatomy; see Key to the genera) 2

Thallus closely appressed or even immersed in the substrate and thus appearing crustose, but with a distinct lower cortex throughout 4

2(1) Apothecia, if present, with epruinose, shiny discs; hymenium typically < 90 µm tall ((70–)80–90(–95) µm); paraphyses apically forked, the uppermost cells inconspicuously swollen (submoniliform), with distinctly swollen end cells; thallus areoles subsquamulose to almost squamulose, mostly broadly attached (gomphate), but some almost stalked (peltate) ..... ***P. mendozae**

[*thalli of *P. mendozae* closely resemble *Candelariella vitellina*, which has a distinctly different cortex anatomy and occasionally forms a more granular thallus; the two species can further be distinguished by their ascospores, with spores of *P. mendozae* being generally < 3.5 µm wide with a length/width ratio ≥ 3.0 (n = 80), whereas spores of *C. vitellina* are typically wider than 3.5 µm with a length/width ratio ≤ 2.9 (n = 150)]

Apothecia, if present, with faintly to distinctly pruinose discs; hymenium typically > 95 µm tall ((85–)90–100(–120) µm); paraphyses simple to rarely branched, uppermost cells straight, the end cells barely inflated; thallus areoles strongly convex (bullate) or ±flattened, becoming subsquamulose (sterile material might be impossible to distinguish from *P. mendozae*) 3

3(2) Thallus areoles flattened, their edges notched, ±crenate and slightly raised (sub-squamulose); apothecial discs plane; hymenium 85–95(–120) µm tall; known from central and southern Andes, Africa ..... **P. minima**

Thallus areoles convex, swollen, their edges turned downwards (bullate); apothecial discs convex; hymenium 90–100 µm; known from southern South America (Tierra del Fuego (Argentina and Chile)) ..... **P. fuegiana**

4(1) Thallus resembling umbilicate foliose lichens, but not composed of few or even a single broad lobe, instead of multiple, narrow, well-defined and closely adjoining lobes; individual lobes elongate, distinctly branched, radiating from the centre; there attached by a single, broad holdfast, or occasionally with few additional, secondary attachment points ..... **P. candelarioides**

Thallus placodioid, i.e. with relatively short lobes in the centre, radiating outwards and forming distinct rosettes, but not branching from a central holdfast 5

5(4) Thallus thick, lobes broad and convex, particularly in the thallus centre often crowded and strongly convoluted, in part overlapping, almost tiled 6

Thallus thin, lobes narrow, flattened, spread out and discrete, even in the centre of the thallus not or barely overlapping (in overall appearance closely resembling species of *Candelina*, but with more narrow, less convex and distinctly flattened lobes) 7

6(5) Thallus forming thick cushions; the central lobes much inflated, crowded, ±upright, almost spathulate; marginal lobes ±elongate, typically at least twice as long as wide (length/width ratio > 2); internally with a stratified anatomy, differentiated into upper cortex, algal layer, medulla and lower cortex; apothecia, if present, with a disc darker than the margin; paraphyses capitate, subapically branched; hymenium 70–90 µm tall; specimens growing saxicolous or muscicolous (over rocks) .... **P. lambii**

Thallus forming small rosettes; central lobes short, stout, imbricate; marginal lobes short and broad, no more than twice as long as wide (length/width ratio ≤ 2); internally not stratified (i.e. with an undifferentiated accumulation of algae packed between upper and lower cortex); apothecia, if present, with a disc ±concolorous with its margin; paraphyses barely capitate, simple or barely branched; hymenium 90–135 µm tall; specimens found on rock (saxicolous) ..... **P. kaernefeltii**

7(5) Thallus dimorphic, with fruticose, cylindrical segments buried up to 8 mm deep inside compacted soil; on the substrate surface forming flattened, spread-out, discrete lobes closely resembling regular placodioid thalli (immersed parts not visible unless specimens are removed from the soil) ..... **P. fruticosa**

Thallus not dimorphic, placodioid, closely appressed to the rock substrate, forming flattened, spread-out, discrete lobes ..... **P. placoidea**

Discussion

Secondary chemistry

Poelt (1974, p. 190) suggested that among *Candelariaceae* '…Their chemistry is mostly uniform…' ('…Ihr Chemismus ist weitgehend einheitlich…') and that the presence of 'stictaurin' (i.e. pulvinic dilacetone and calycin occurring together; Zopf 1907) was generally characteristic. In their revision of *Placomaronea*, Westberg *et al.* (2009) list only secondary metabolites generally characteristic for the genus, not for individual species, reporting calycin, pulvinic dilacetone (= pulvic acid lactone), vulpinic acid and pulvinic acid. This agrees largely with our own analyses, although vulpinic acid could not be confirmed and, for the first time, 4-hydroxypulvinic acid is reported, present in virtually all specimens examined.

Overall, chemical variation in the material examined is low. Based on quantitative analyses using high performance liquid chromatography (HPLC), Westberg (2005) distinguished only two chemosyndromes, ChA (with calycin as the dominant metabolite; characteristic for most species of *Candelariella*, *Placomaronea*, *Candelina*, and the 8-spored species of *Candelaria*) and ChB

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(with the secondary metabolites in approximately equal quantities; documented for polyspored species of *Candelaria*, *Candelariella subdeflexa*, and possibly *Candelariella lutella*).

We did not have access to HPLC and the chemotypes distinguished here are based on secondary metabolites detected by TLC. Here we document variation in whether terpenoids are present or not and if pulvinic dilacetone and/or calycin are absent, occur in minor concentration, or as a trace (Table 1). Whereas a much larger number of specimens and taxa needs to be analyzed to assess if any of this variation correlates with particular genera or species. This may seem surprising. In many groups of lichenized fungi, secondary chemistry is taxonomically relevant and chemotype patterns are often species-specific. Only recently, however, Díaz-Escandón *et al.* (2022) suggested including *Candelariaceae* within *Lichinomycetes*, based on their much smaller genomes, arguing that these fungi are characterized by 'reduced arsenals of carbohydrate-degrading enzymes and secondary metabolite gene clusters' (Díaz-Escandón *et al.* 2022, p. 5209).

Most surprising in the material analyzed is the inconsistency of reddish spot test reactions in K. Zopf (1895) reported that pure calycin reacts K+ red, but in the presence of pulvinic dilacetone (= pulvinic acid lactone) forms a complex called 'stictaurin', which no longer reacts with K. This observation cannot be confirmed. Virtually all specimens that contain both calycin and pulvinic dilacetone react distinctly K+ red, or at least K± weakly reddish (chemotypes A & B; Table 1), and specimens that contain only calycin and lack pulvinic dilacetone instead mostly react K− (chemotype C; Table 1). Curiously, even some specimens that contain neither pulvinic dilacetone nor calycin react distinctly K+ red, or K± weakly reddish.

Poelt (1974) suggested, based on observations by Santesson, that polyporic acid might be responsible for a K+ weakly reddish spot test reaction in *Candelina mexicana*. For the material that we examined, this cannot be confirmed. Polyporic acid is a deep red pigment, whereas the medulla of *C. mexicana* is bright yellow, no different in colour than the cortex of that species (Fig. 7B). It seems unlikely that terphenylquinones would be present in a family of lichens generally characterized by pulvinic acid derivatives. Instead, in much of the material that we examined, we discovered a substance that most likely refers to 4-hydroxypulvinic acid, a pulvinic acid derivative (in solvent C it occurs at R_f 3 as a distinctly pale yellow spot in λ 365 nm UV light before H_2SO_4 treatment; the same spot is pale yellow in visible light after H_2SO_4 treatment, and it turns dull greenish yellow in λ 365 nm UV light after H_2SO_4 treatment; Figs 1 & 2). This secondary metabolite was present in all chemotypes and for all chemotypes at least some specimens displayed a positive spot test K reaction. If 4-hydroxypulvinic acid is indeed responsible for this reaction, the strength of the reaction may be correlated with its concentration in the cortex. Comparing whether secondary metabolites show up on a thin-layer chromatography plate as saturated, pale or barely visible (major, minor or trace) depends on many factors and it is, at least to some extent, subject to experimental variation. More sensitive, quantitative analytical methods are therefore necessary to assess whether differences in concentration of 4-hydroxypulvinic acid might explain the variation in K reactions documented here.

Anatomy

In his revision of *Candelariaceae*, describing the genus *Candelina*, Poelt (1974) was the first to recognize anatomical differences in the cortical structure of the family. He emphasized that *Candelina*, though appearing crustose-placodioid, is characterized by a well-defined lower cortex of few rows of closely aggregated cells, attached to its substrate with what he called 'searching hyphae', or 'Suchhyphen', whereas the lower cortex of *Placomaronea*, illustrating *P. candelarioides* as an example, has a lower cortex of several closely packed, dense cell layers (Poelt 1974, p. 190, fig. 1). Westberg *et al.* (2009), distinguishing *Placomaronea* from all other genera of *Candelariaceae*, also emphasized cortex anatomy, which they described as 'well-developed paraplectenchymatous… the pigments form[ing] characteristic hoods…usually also by the presence of a thin, gelatinous epicortex' (Westberg *et al.* 2009, p. 526).

Closely comparing the cortical anatomy of *Candelariella*, *Candelina* and *Placomaronea* (Figs 4–9), the distinction of the three genera at first seems less obvious than the assessment by Westberg *et al.* (2009) suggests. All three genera show at least the tendency to form a paraplectenchymatous cortex. In all three, the yellow pigments are present as minute granules in the outer part of the cortex. A cursory comparison of, for example, the crustose-subsquamulose *P. mendozae* with *Candelariella rosulans* may not, at first, suggest conspicuous differences: both have a thin layer of unpigmented, somewhat paraplectenchymatous cells, apparently topped by yellow pigment granules. Only upon closer examination are the differences revealed. The outer cell layer of *P. mendozae* is distinctly paraplectenchymatous, and its cells have pigmented cell walls, the yellow granules adhering to these pigment caps. When these cells die off, they leave behind a residue of unpigmented organic material shedding off, a layer that Westberg *et al.* (2009) referred to as 'gelatinous epicortex' (Fig. 6B & C). This is not the case for *Candelariella rosulans*; the species lacks this residue of organic material and the uppermost cells of the cortex are not distinctly paraplectenchymatous (Fig. 8C).

Originally we assumed that the organic material was a residue of dead cortical cells, perhaps better called an epinecral layer, not an epicortex. However, the thick, hyaline, layered material covering the cortex of '*Candelariella*' *kansuensis* shows the same 'shedding' at its surface. Thus, the cortex of *Placomaronea* and '*Candelariella*' *kansuensis* appears structurally identical (compare Figs 4–6 with Fig. 9). Since we do not understand its origin, we prefer to call it a 'coating of layered organic material'.

Macroscopically, the genera also differ: the densely packed pigment granules on the surface of *Candelariella rosulans* cause a finely pruinose surface, whereas the surface of *P. mendozae* appears waxy, the coating of hyaline organic material smoothing the surface, covering pigment granules below. In *Candelina* the thallus surface appears dull, not pruinose but not smooth either, possibly a result of the much more densely packed pigment granules. The newly described *Placomaronea fruticosa* and *P. placoidea* not only appear similar macroscopically because of their placodioid growth form, but also from the outside their surface does not look different from the species of *Candelina*. Some material of *P. fruticosa*, a specimen at ASU from Argentina (*T. H. Nash* 27947, ASUL010376), was previously even annotated by Westberg as *Candelina* cf. *submexicana*. Entirely embedded in the substrate, the basal, fruticose growth of this specimen is easily overlooked. In addition, although the material has smaller, slightly narrower lobes, only careful examination of the cortex anatomy will reveal the distinct diagnostic differences of *Placomaronea*: the paraplectenchymatous cortex, apically with 'peppered pigment hoods', covered by a thin colourless coating sloughing off.

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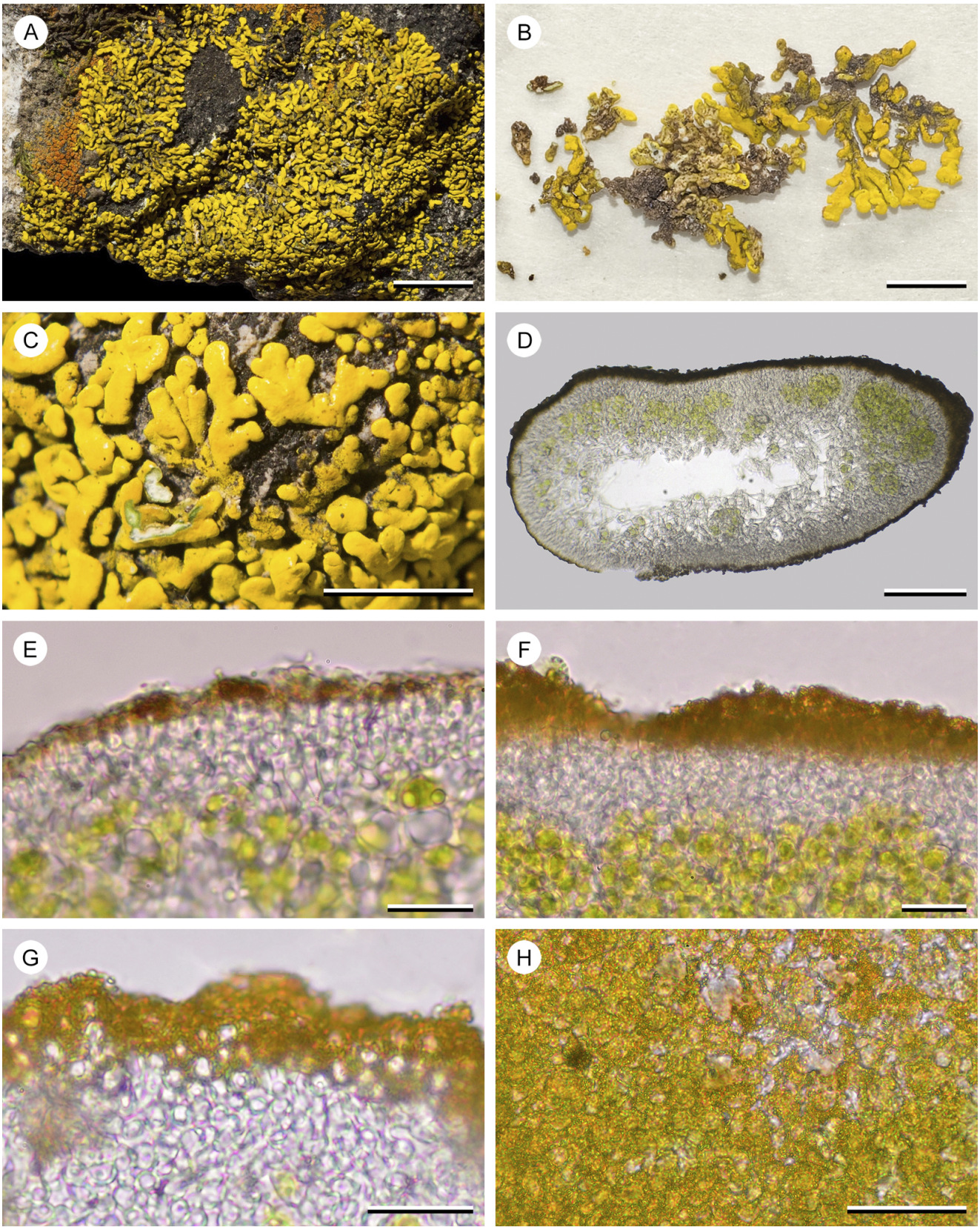

Figure 5. *Placomaronea placoidea* (*Ramos* 2908a, HSP—holotype). A, overview of the placodioid thallus covering a rock. B, thallus lobes removed from their substrate showing the upper and lower sides; upper cortex yellow, lower cortex brown, beige to ivory. C, close-up of lobes adhering to the rock substrate, cortex in part damaged showing the photobiont layer and white medulla. D, hand-cut section of hollow thallus lobe. E–G, hand-cut sections of photobiont layer and upper cortex. E, thin section, showing distinctly paraplectenchymatous hyaline cells, apically with a pigmented layer and a thin, barely distinct coating of hyaline residue. F, thick section with photobiont layer, hyaline and pigmented part of the cortex. G, thin section, showing distinctly paraplectenchymatous hyaline cells, apically capped by cells with pigment hoods peppered in pigment granules, covered in a thin, barely distinct coating of hyaline residue. H, squash preparation of inflated apical cortex cells with pigment hoods covered in pigment granules. Scales: A = 1 cm; B = 3 mm; C = 5 mm; D = 100 μm; E & F = 20 μm; G = 15 μm; H = 25 μm. In colour online.

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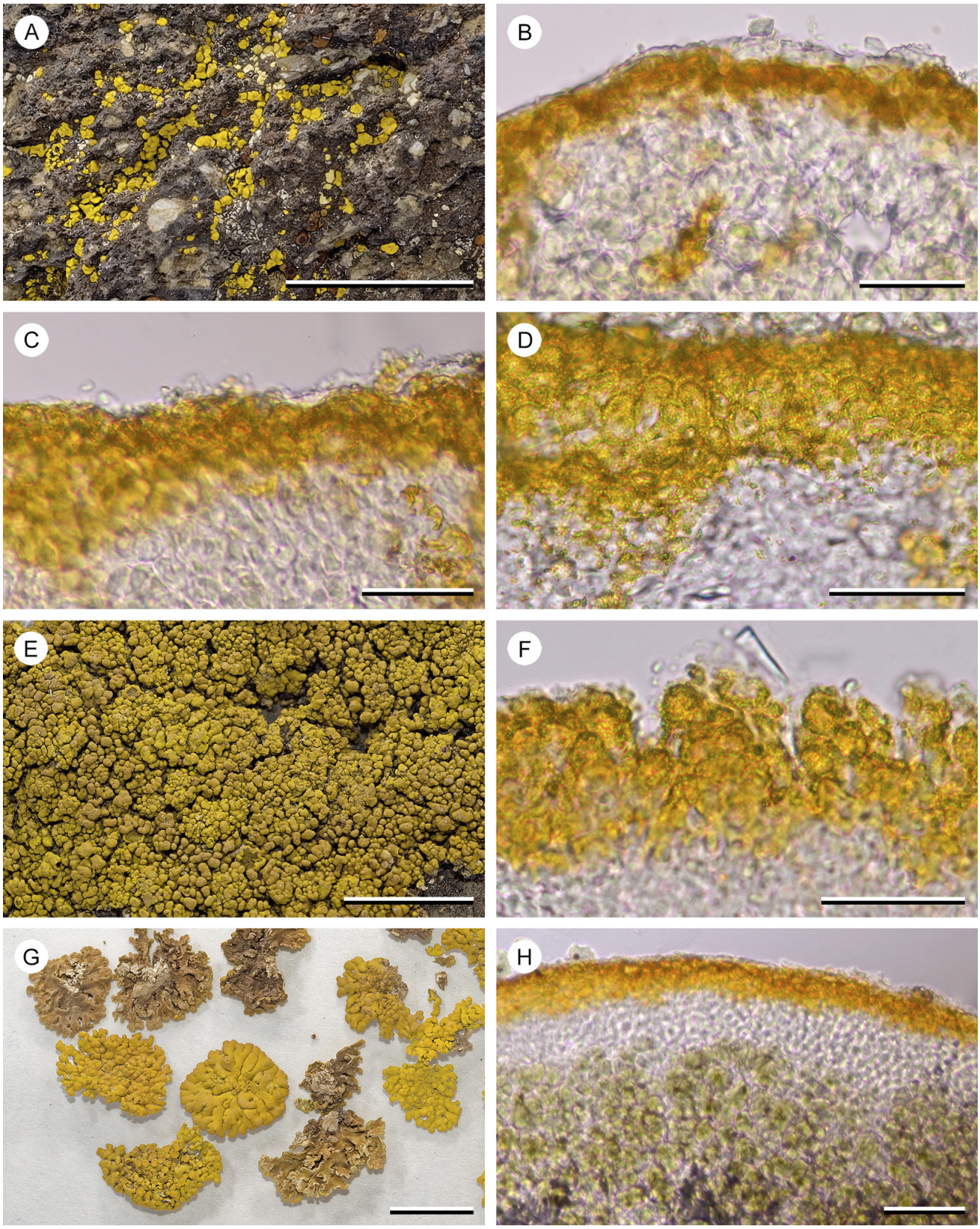

Figure 6. A–D, *Placomaronea mendozae* (*T. H. Nash* 25430 (ASUL010350)). A, squamulose thallus. B & C, paraplectenchymatous cortex with apically pigmented cells, covered by a hyaline coating, possibly the residue of dead cells. D, hooded pigment cells coated with pigment granules. E & F, *Placomaronea lambii* (*I. M. Lamb* 5413 (MSC0112896—isotype)). E, inflated erect thallus areoles. F, uppermost cortex with hooded pigment cells coated with pigment granules, covered by hyaline coating, possibly the residue of dead cells. G & H, *Placomaronea candelarioides* (*T. H. Nash* 28034 (ASUL010349)). G, upper and lower view of umbilicate thalli (some indistinctly attached by broadened multiple holdfasts). H, paraplectenchymatous cortex with apically pigmented cells, covered by a hyaline coating, possibly the residue of dead cells. Scales: A, E & G = 1 cm; B, C, D, F & H = 25 μm. In colour online.

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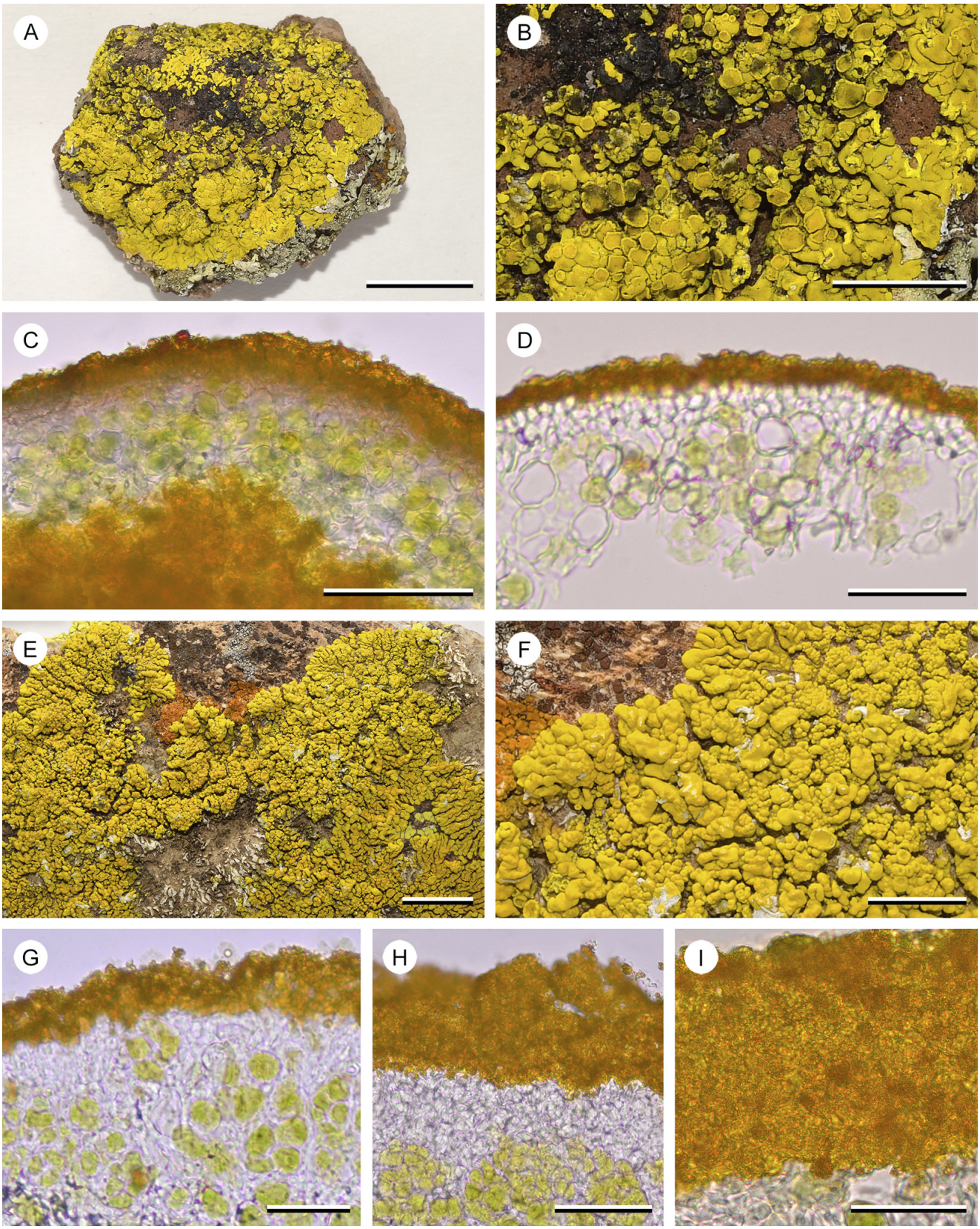

Figure 7. A–D, *Candelina mexicana* (*T. H. Nash* 39918 (ASUL034850)). A, overview of placodioid thallus. B, central part of the thallus, parts of the cortex damaged, the bright yellow medulla exposed. C, hand-cut section of the thallus, with a thin paraplectenchymatous cortex capped by a dense layer of pigment granules, green photobiont layer and yellow medulla below. D, thin section with two layers of paraplectencymatous cortical cells, capped by a dense layer of pigment granules. E–I, *Candelina submexicana* (*R. D. Worthington* 31956 (ASUL024890)). E, overview of placodioid thallus. F, detail of thallus lobes with sessile, adnate apothecia, parts of the cortex damaged, the white medulla exposed. G & H, hand-cut sections of the thallus showing the cortex; photobiont layer transitioning into a narrow layer of hyaline paraplectenchymatous cells, capped by a dense layer of pigment granules, hyaline coating absent. I, close-up of the dense layer of yellow pigment granules (squash preparation). Scales: A & E = 1 cm; B & F = 5 mm; C & H = 50 μm; D, G & I = 25 μm. In colour online.

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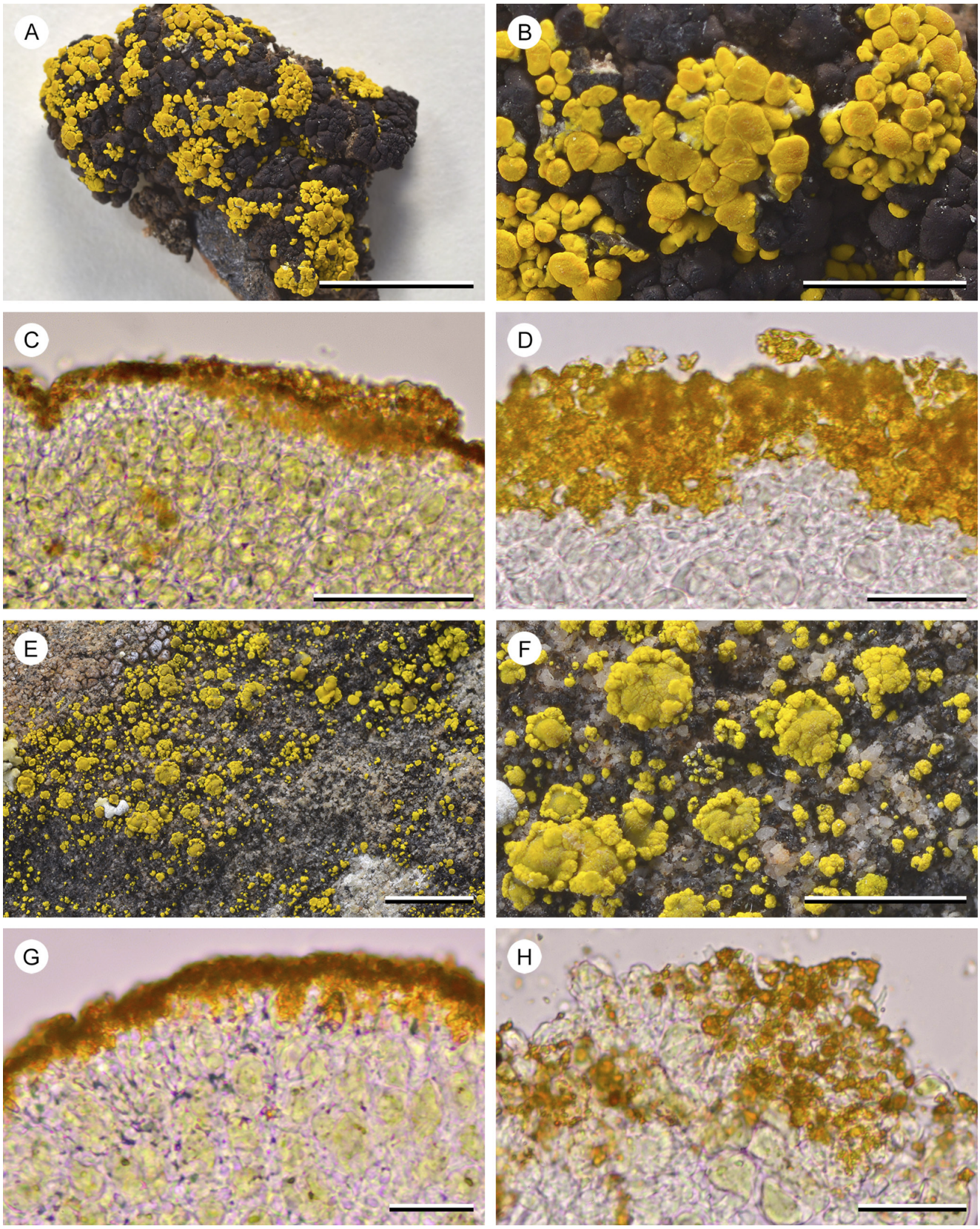

Figure 8. A–D, *Candelariella rosulans* (*W. C. Davis* 451 (ASUL011839)). A, overview of subsquamulose areoles with abundant apothecia. B, detail of the thallus with sessile, adnate apothecia. C, hand-cut section of the thallus, showing a thin layer of proso- to indistinctly paraplectenchymatous hyaline cells above the photobiont layer, apically covered by an accumulation of pigment granules. D, close-up of hyaline cortex cells and aggregation of pigment granules. E–H, *Candelariella vitellina* (*W. C. Davis* 617 (ASUL011791)). E, overview of thallus granules with scattered apothecia. F, detail of the thallus granules with scattered apothecia. G, hand-cut and somewhat squashed section of the thallus, showing a thin layer of proso- to indistinctly paraplectenchymatous hyaline cells above the photobiont layer, apically covered by an accumulation of pigment granules. H, close-up of the hyaline cortex cells and aggregation of pigment granules (squash preparation). Scales: A = 1 cm; B & F = 2 mm; E = 5 mm; C = 50 μm; D, G & H = 25 μm. In colour online.

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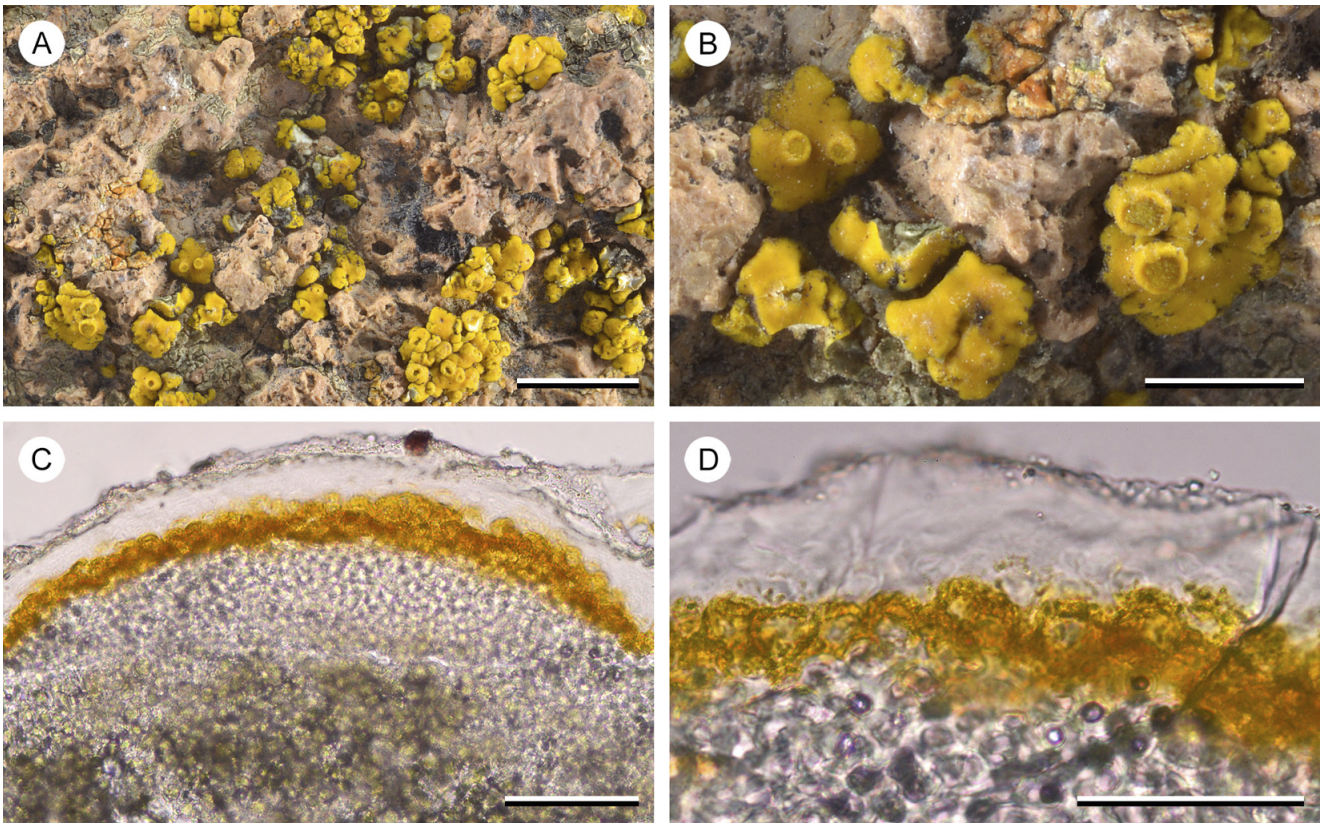

Figure 9. *Candelariella kansuensis* (*C. M. Wetmore* 55470 (ASUL011864)). A, overview of inflated, waxy thallus squamules. B, detail of squamules with few sessile, adnate apothecia. C, hand-cut section of the thallus, showing thick paraplectenchymatous layer of cortical cells, capped by swollen, hooded cells 'peppered' by pigment granules, covered by a thick hyaline, distinctly layered coating that apically erodes into minute, hyaline granules. D, close-up of the swollen, apical cells capped by yellow pigment hoods 'peppered' by pigment granules, covered in hyaline coating. Scales: A = 5 mm; B = 2 mm; C & D = 50 μm. In colour online.

Phylogeny

Our trees are generally similar to those of *Candelariaceae* previously published (e.g. Westberg *et al.* 2007, 2009; Kondratyuk *et al.* 2020; Halıcı *et al.* 2023). All of the studies agree in several broad aspects: *Candelariella blastidiata* Yakovch. and *C. subdeflexa* (Nyl.) Lettau form a well-supported, monophyletic clade basal to the rest of the family (100% BS). Kondratyuk *et al.* (2020) named this clade *Protocandelariella.* Also, *Candelina* and *Placomaronea* each form well-supported, monophyletic clades (99% and 100% BS, respectively). Polyspory must have evolved independently multiple times. Our phylogeny suggests that this occurred at least four times: three times in *Candelaria* and *Candelariella* s. lat., once when *Placomaronea* emerged.

Our phylogeny also agrees with Westberg *et al.* (2009) in so far as the uniquely characteristic aspects of the cortex anatomy in *Placomaronea* must have evolved independently in *Candelariella kansuensis*: both are characterized by a distinctly paraplectenchymatous cortex with an outer layer of cells with granular-pigmented hoods. It remains unclear, however, whether the thick hyaline coating observed in *C. kansuensis* is analogous to what appears to be a residue of cells in *Placomaronea.*

Although several clades in our analysis received strong bootstrap support, the relationships between clades (and within clades) remains unresolved. Most clades formed a massive polytomy in the middle of the tree. The relationships between the clades in this polytomy were sensitive to the alignment and which particular ITS sequences we chose to include. For example, we were unable to determine with any support which clade might be sister to *Placomaronea.* Westberg *et al.* (2009) placed it sister to *Candelariella complanata*, and Westberg *et al.* (2011) placed it in a polytomy similar to ours but with a few small changes: they included *Candelariella aurella* in the clade with *Candelaria* s. str., *Candelariella* s. str. and *C. antennaria* + *C. aggregata* (we found the position of the *C. aurella* + *C. plumbea* group to be unstable and left it in the polytomy), and they separated out a clade with *C. corviniscalensis* C.A. Morse & M. Westb., *C. kansuensis* and *C. rosulans* in a position basal to the polytomy (some versions of our tree had weak support for a similar basal clade which also included *C. clarkii* and *C. spraguei* (Tuck.) Zahlbr., but other versions did not, and so the conservative tree we present here leaves these three species unresolved).

Candelariella thus remains paraphyletic, and relationships between various clades of *Candelaria*, *Candelariella*, *Candelina* and *Placomaronea* remain for the most part unresolved. Notably, we were unable to confirm support for two of the three genera proposed by Kondratyuk *et al.* (2020), namely *Candelinella* and *Opeltiella.* For example, one of the sequences available for *Opeltiella fruticans* (Poelt & Oberw.) S.Y. Kondr. (EF535207) appears to be nested within the same clade as *Candelinella deppeanae* (M. Westb.) S.Y. Kondr. and *C. makarevichiae* (S.Y. Kondr. *et al.*) S.Y. Kondr.

The phylogenies published so far (Westberg *et al.* 2007, 2009; Kondratyuk *et al.* 2020; Halıcı *et al.* 2023), although similar overall, nevertheless do not exactly correspond in their topologies, even though individual clades are supported by high bootstrap values. It

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is difficult to assess what causes these discrepancies. We had hoped that improving clade resolution and overall tree topology might be possible by adding additional loci. Unfortunately, all eight sequences newly generated by Kondratyuk *et al.* (2020) remain unavailable. More importantly, of the sequences available in GenBank for different loci (303 ITS, 24 mtSSU and 21 nuLSU), not a single specimen had all three loci sampled. Constructing a multilocus 'consensus' tree based on such sparse and poorly overlapping data does not seem warranted, especially since some of the sequences appear to be of low quality. For example, a BLAST search with the mtSSU sequence of *Candelariella terrigena* (DQ986884) retrieves *Porina*. In addition, the mtSSU sequence for *Candelaria concolor* (MN508267) is missing numerous base pairs in nucleotide regions which are otherwise well conserved for other mtSSU sequences of *Candelariaceae*. This suggests that attempts to accurately resolve the overall phylogeny for the family remain premature without adding additional sequences from a broader array of loci. Whether any of the new genera proposed by Kondratyuk *et al.* (2020) deserve recognition cannot be assessed without access to the specimens, sequences and alignments used in that study.

With regard to the species newly described here, our phylogenetic analysis suggests that they form a well-supported, monophyletic clade within *Placomaronea*. However, distinguishing the two from *P. mendozae* or recognizing *P. fruticosa* and *P. placoidea* as separate species is, unfortunately, not supported by the current topology. Not only are the two species part of a clade that includes *P. mendozae*, but *P. fruticosa* is also nested inside a clade that includes both *P. placoidea* and *P. mendozae*. Based on the molecular data, the hypothesis that the three taxa might be conspecific cannot therefore be rejected. Our phylogeny instead suggests that the conspicuously different growths forms could be interpreted as environmental modifications of a single, morphologically extremely plastic species: *P. mendozae*.

However, evolutionary processes such as introgression or incomplete lineage sorting could also explain this discrepancy, particularly in rapidly evolving lineages (Tremble *et al.* 2019). These processes are known to result in polyphyly within trees that are based on the analysis of a single gene only, even after speciation (Maddison & Knowles 2006). Using a small number of, or even single, loci to construct a phylogeny can result in a topology where the gene tree is incongruent with the overall species tree (Górniak *et al.* 2021). Such phenomena ultimately can be resolved only by expanding the scope of the molecular analysis to include additional loci and additional specimens, yielding a consensus tree which more accurately reflects the overall phylogeny of the species (Maddison & Knowles 2006). Within a single gene tree, polymorphisms from an ancestor may persist in a new lineage for some time, even after species divergence (Maddison 1997). Relying on a single gene to construct a phylogeny, the parent population may then appear to be paraphyletic with respect to its descendants (Carstens & Knowles 2007). Monophyly of the single gene tree may eventually be restored via lineage sorting and extinction, but typically only after sufficient time has passed. It has been argued that this depends on generation time and effective population size of the species involved (Knowles & Carstens 2007). In such a situation, accurately inferring the species tree by molecular phylogenetic reconstruction requires increased sampling, adding both individuals and additional loci (Maddison & Knowles 2006). It is not inconceivable that some of these processes are also responsible for the phylogeny inferred here.

Our analysis, where a phylogeny is based solely on ITS but is also conspicuously incongruent with distinct anatomical and morphological characters, suggests that ITS may not offer sufficient information to accurately resolve the correct phylogeny of these species. Even though nrITS is now widely accepted as the universal barcoding region for fungi (Nagy *et al.* 2012; Schoch *et al.* 2012; Xu 2016), its effectiveness in reliably distinguishing some fungal lineages is increasingly being questioned (Badotti *et al.* 2017; Parks *et al.* 2019). Some authors suggest that this insufficient resolution can indeed be a result of incomplete lineage sorting or hybridization (Pino-Bodas *et al.* 2013; Tremble *et al.* 2019).

Lack of sufficient ITS resolution has been documented for a variety of fungal lineages (O'Donnell & Cigelnik 1997; Nilsson *et al.* 2008; Thiery *et al.* 2016; Hughes *et al.* 2018; Kruse *et al.* 2018*a*, *b*; Tremble *et al.* 2019; Stadler *et al.* 2020), including some that are lichenized (Pino-Bodas *et al.* 2013; Moncada *et al.* 2014; Steinová *et al.* 2022; Di Meglio & Goward 2023). It has been suggested that, even within groups of lichenized fungi where ITS generally offers good resolution (e.g. Magain & Sérusiaux 2015; Moncada *et al.* 2020), augmenting the data with secondary barcodes will sometimes be necessary, particularly when dealing with rapidly evolving lineages (Lücking *et al.* 2020).

Morphology

Despite inconclusive molecular data, accepting *Placomaronea mendozae* as one single, highly polymorphic species seems untenable. Both *P. fruticosa* and *P. placoidea* are characterized by a thallus morphology conspicuously different from *P. mendozae*.

It could be argued, of course, that the two new species are simply environmentally induced morphotypes. Countless examples of dimorphic lichens are known where a basal crustose or squamulose primary thallus gives rise to a secondary fruticose thallus. However, these transitions are easily recognized when studying specimens and no such transitional morphotypes could be observed here: *Placomaronea mendozae* is composed of minute, scattered subsquamulose to barely squamulose, broadly attached, gomphate areoles. Even exuberant specimens are barely squamulose, not closely aggregated, the squamules broadly attached to their substrate. The thalli are not hollow like the placodioid lobes of *P. placoidea*, and unlike *P. fruticosa*, subsquamulose areoles of *P. mendozae* do not show any tendency to become fruticose.

Crustose, placodioid lichens transitioning into coralloid fruticose lichens have been described from distantly related species in *Caloplaca* s. lat. Along the Pacific coast of Baja California and Baja California Sur in Mexico, *Polycauliona thamnodes* (Poelt) Arup *et al.* forms placodioid thalli in less exposed microhabitats, but transitions into distinctly coralloid fruticose outgrowths at more exposed sites. In the Atacama of southern Peru and northern Chile, *Follmannia orthoclada* (Zahlbr.) Frödén *et al.* also transitions from distinctly placodioid into entirely fruticose specimens. The crustose thalli are found at the most sheltered sites, and the fruticose ones at the most exposed habitat sites. Obviously, the morphology of these two not closely related species is highly plastic; it can be interpreted as fog-induced and is clearly an adaptation of the morphology to microhabitat variation at the sites where these species grow.

Interpreting the different morphologies of *P. fruticosa* and *P. placoidea* as also being environmentally induced, however, ignores how these thalli presumably arose. Both species have been found in ecologically similar areas; *Placomaronea placoidea* grows on the surface of rocks embedded in soil, and these surrounding soils are inhabited by thalli of *P. fruticosa*, which develop as an adaptation to the soil in which the species grows. At these sites the loamy soil is hardened and compact and if the lichen thalli were not

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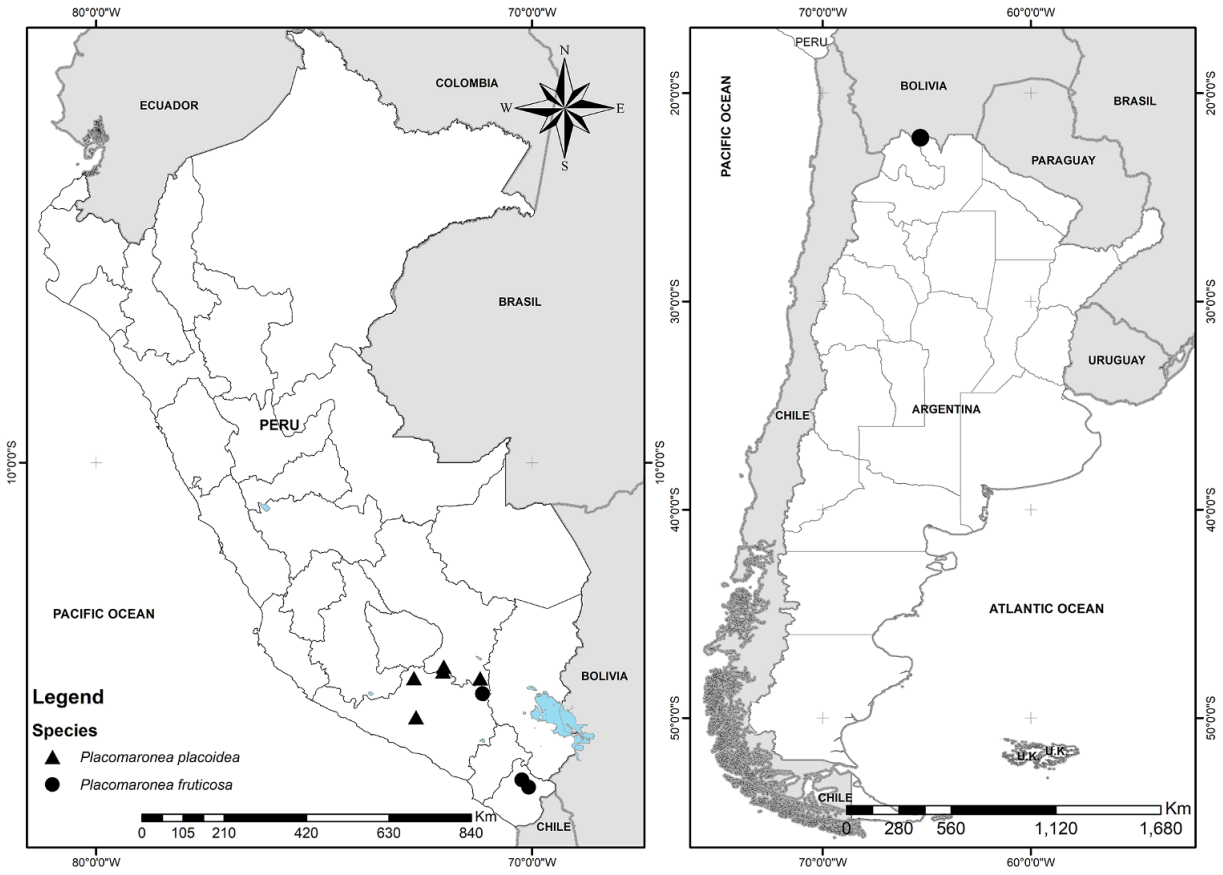

Figure 10. Known distribution of *Placomaronea fruticosa* (circles) and *P. placoidea* (triangles) in Peru and Argentina. In colour online.

'rooted' deeply inside their substrate these lichens could not compete with grasses. Thalli of *P. placoidea* growing nearby obviously cannot penetrate the rock they grow on and any tendencies to develop a fruticose growth form would necessarily result in erect thalli emerging from the rock surface. Such thalli have, however, not been observed. Transitional forms, where lobes of *P. fruticosa* creep onto rocks from the surrounding soil, have also not been found. Both species thus maintain their distinct morphologies, even when growing side by side. Both also maintain distinct anatomical differences. The lobes of *P. placoidea* consistently have a hollow medulla, whereas the fruticose stalks of *P. fruticosa* as well as its placodioid lobes on the substrate surface are solid.

Conclusion

Our decision to describe *Placomaronea placoidea* and *P. fruticosa* at species level follows recent guidelines published by Lücking *et al.* (2021). Both form nested (paraphyletic residual) clades that are morphologically and anatomically distinct. The geographical distributions of the new species overlap with one another (Fig. 10) and with that of *P. mendozae*. At this stage, with only a small number of specimens known, it is possible that all three are either fully sympatric or, more likely, *P. placoidea* and *P. fruticosa* have a nested, more limited distribution within the broader range of one another and, by comparison, also within the range of the much more widely distributed *P. mendozae*. In any case, the three species are clearly not allo-, para- or peripatric. According to the categorization schema distinguishing main patterns of phylogenetic topologies in Lücking *et al.* (2021, p. 130, fig. 10), *Placomaronea placoidea* and *P. fruticosa* should therefore be recognized not as subspecies, but as species.

Supplementary Material. The Supplementary Material for this article can be found at http://doi.org/10.1017/S0024282925000040.

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