The Lichenologist **49**(6): 597–605 (2017) doi:10.1017/S0024282917000512

Ocean view: a first assessment of the littoral, crustose lichen biota of south Brazil

André APTROOT, Emerson Luiz GUMBOSKI and Marcela Eugenia da Silva CÁCERES

Abstract: The crustose lichen biota of coastal rocks in South Brazil was investigated. Several distinct lichen zones were found: a littoral black zone, a supralittoral yellow zone and a grey zone with species restricted to either exposed granite, dry overhangs, damp overhangs or places subjected to run-off. *Dendrographa austrosorediata* is newly described, with a crustose, flat to partly curling up or blister-like thallus which is much dissected, whitish grey, 0·1–0·2 mm thick; surface minutely densely cracked, rimose, with cracks intersecting at each *c.* 0·1 mm; soredia whitish to bluish grey but asymmetrically blackened in the direction facing the light, originating on the thallus surface, in initially discrete convex soralia. The phylogenetic position of this new species was traced by molecular methods. *Stigmidium marinum*, generally regarded as a lichenicolous fungus, was found as a free-living lichen, thousands of kilometres away from the nearest known occurrence of any purported host.

Key words: *Dendrographa*, maritime, saxicolous, *Stigmidium*

Accepted for publication 9 *May* 2017

Introduction

The lichen biota of Brazil is still incompletely known. However, with over 4750 species recorded to date, it is already a country containing a relatively high number of lichen species. As every year around 200 species are added to this list, about half of which are newly described, it will soon become the country with the highest number of lichen species. Yet there are still whole states and habitats that have not been sampled at all.

The coast of Brazil is mostly known for its wide and long sandy beaches, only interrupted by river estuaries with mangrove forest. In the southern states of Paraná, Santa Catarina and Rio Grande do Sul, these beaches are interspersed with coastal granite rocks that often rise as steep cliffs directly from the sea. The targeted study of the lichen mycobiota on these coastal cliffs began in 1890 (Vainio 1890), to be taken up again in 1984 with the report of some macrolichens (Osorio & Fleig 1984*a*, *b*), and then subsequently discontinued for many years but taken up again recently, mainly with the study of *Parmeliaceae* and *Cladoniaceae* (Benatti *et al.* 2008; Benatti & Marcelli 2009*a*, *b*; Gumboski & Eliasaro 2011, 2012*a*, *b*; Gerlach & Eliasaro 2012, 2014*a*, *b*).

All over the Northern Hemisphere, as well as in Australia and New Zealand, specialized lichen biota of coastal rocks have been reported, consisting partly of littoral species that are restricted to those coastal rocks (e.g. Arup 1995; Gilbert 2000). Usually, different zones with characteristic lichens can be recognized: a littoral black zone, a supralittoral yellow zone and a grey zone, the latter with some microhabitats such as dry and wet overhangs, exposed rocks and places with run-off. Species from the lower zones and the dry overhangs are generally restricted to maritime habitats. The lichen biota of coastal rocks have rarely been reported from tropical regions.

A. Aptroot: ABL Herbarium, G.v.d.Veenstraat 107, NL-3762 XK Soest, The Netherlands. Email: andreaptroot@gmail.com
E. L. Gumboski: Departamento de Ciências Biológicas, Universidade da Região de Joinville, CEP: 89219-710, Joinville, Santa Catarina, Brazil.
M. E. S. Cáceres: Departamento de Biociências, Universidade Federal de Sergipe, CEP: 49500-000, Itabaiana, Sergipe, Brazil.

In 2015, the second author organized an excursion of the Reunião Brasileira de Estudos Liquenológicos in Santa Catarina which allowed the authors to study the lichens on coastal cliffs in the area.

Material and Methods

Identification and descriptive work was carried out in Soest using an Olympus SZX7 stereomicroscope and an Olympus BX50 compound microscope with interference contrast, connected to a Nikon Coolpix digital camera. Sections were mounted in tap water, in which all measurements were also taken. The specimens from this study are preserved in JOI and ABL. The chemistry of selected specimens was investigated by thin-layer chromatography (TLC) using solvent A (Orange *et al.* 2001).

DNA extraction, amplification and sequencing were carried out in Santander (ALVALAB): total DNA was extracted from dry specimens using a modified protocol based on Murray & Thompson (1980). A portion of each sample was blended with the aid of a micropestle in 600 µl CTAB buffer (CTAB 2%, NaCl 1·4 M, EDTA (pH 8·0) 20 mM, Tris-HCl (pH 8·0) 100 mM). The resulting mixture was incubated for 15 min at 65 ºC. A similar volume of chloroform:isoamilalcohol (24:1) was added and carefully mixed with the samples until an emulsion formed. It was then centrifuged for 10 min at 13 000 **g**, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µl of ddH_2O. PCR amplification was performed with the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999) for the mtSSU region, and LR0R and LR5 (Vilgalys & Hester 1990; Cubeta *et al.* 1991) for 28S nrDNA. PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (for 45, 30 and 45 s respectively) and a final 72 °C step for 10 min. PCR products were checked in 1% agarose gels and positive reactions were sequenced with one of the PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

Phylogenetic analyses

BLAST (Altschul *et al.* 1990) was used to check the most closely related sequences of the family *Roccellaceae* in INSD public databases and a combined 28S rDNA- *RPB2* dataset of this family, similar to that in Ertz *et al.* (2015), was built. Species of *Opegraphaceae* were used as outgroups. Sequences were first aligned in MEGA 5.0 (Tamura *et al.* 2011) software with its ClustalW application and then corrected manually. The final alignment included 405/826 (28S rDNA) and 484/844 (*RPB2*) variable sites. The aligned loci were loaded in PAUP* 4.0b10 (Swofford 2001) and subjected to MrModeltest v.2.3 (Nylander 2004). The model GTR+Γ+I was implemented in MrBayes v.3.1 (Ronquist & Huelsenbeck 2003) where a Bayesian analysis was performed (two simultaneous runs, six chains, temperature set to 0·2, sampling every 100th generation) until convergence parameters were met after *c.* 7 000 000 generations (after which the Bayesian analyses were carried out), the standard deviation having fallen

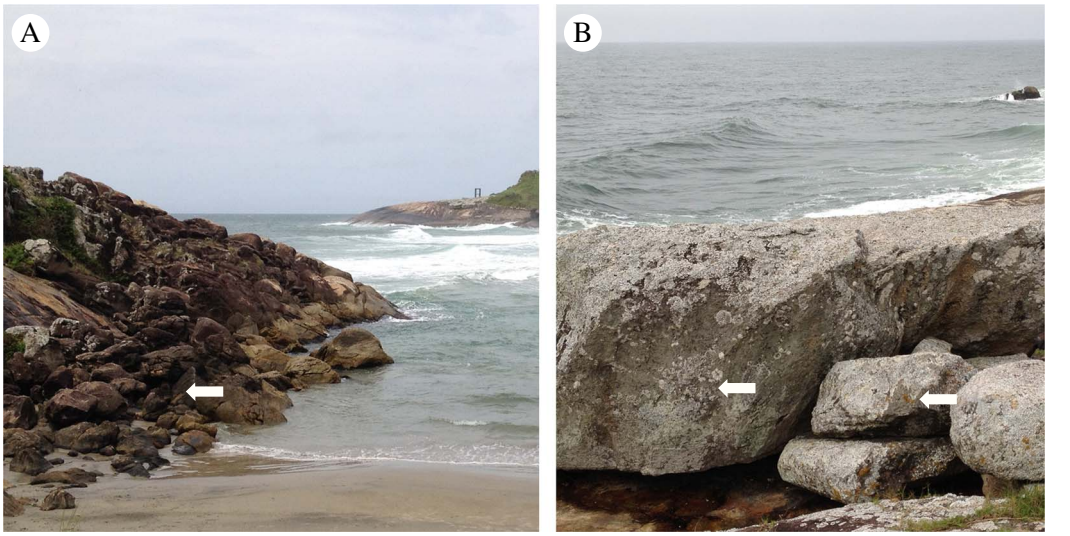

FIG. 1. Coastal rocks in Santa Catarina, Brazil. A, north of Prainha, showing a lower supralittoral zone with algae where the undescribed *Teloschistaceae* are growing (arrow); B, south of Prainha, showing grey zone, including overhanging parts (left arrow) and some yellow supralittoral *Teloschistaceae* (right arrow). In colour online.

https://doi.org/10.1017/S0024282917000512 Published online by Cambridge University Press

below 0·01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis 2006) using the standard search algorithm (data partitioned, 2000 bootstrap replications). Significance threshold was set above 0·95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Results

Crustose lichens were found to be abundantly present on the rock faces investigated. Three distinct lichen zones were found: a littoral black zone, a supralittoral yellow zone and a grey zone with species restricted to either exposed granite, dry overhangs, damp overhangs or places subjected to run-off. Therefore the whole range of variation in microhabitat was found to be present.

In the littoral black zone, only one species was found, viz. *Stigmidium marinum* (Deakin) Swinscow.

Just above the black zone (Fig. 1A), in the lower supralittoral, there is a zone containing a number of *Teloschistaceae* with thallus colours predominantly yellow to orange, hence the name "yellow zone". Several different additional species were found that are yet to be determined. The yellow *Teloschistaceae* that occur slightly higher up in the intermediate supralittoral belt could all be identified as species which are known from other areas in Brazil.

In the grey zone (Fig. 1B), the higher supralittoral belt, several microhabitats can be distinguished such as exposed granite, dry overhangs, damp overhangs or places subjected to run-off. Dry overhangs of maritime rocks in at least warm-temperate regions usually abound with *Arthoniales*, especially *Roccellaceae*. The overhangs in southern Brazil are no exception to this, with species of *Dendrographa*, *Enterographa*, *Lecanographa*, *Opegrapha* s. lat. and *Roccellographa*. Fruticose *Roccellaceae*, which can be abundant in such places, were absent but *Roccellographa circumscripta* (Leight.) Erzt & Tehler (reported by Gumboski & Eliasaro 2012*b*) has a partly lobate thallus. Several species from *Opegrapha* s. lat. were present, of which only *O. lithyrga* Ach. could be identified with some certainty. The taxonomy of the group is still too poorly known to warrant the description of the other species. The species of *Enterographa* has only pycnidia present on the thalli and cannot be identified with certainty. The *Dendrographa* is different from all other species

TABLE 1. *Crustose and microfoliose maritime lichens on the Santa Catarina coast, Brazil.*

Microhabitat	Lichen species
Upper grey zone	
exposed	*Diploschistes euganeus* (A. Massal.) Zahlbr.
	Dirinaria aegialita (Afzel. ex Ach.) B. J. Moore
	Hyperphyscia adglutinata (Flörke) H. Mayrh. & Poelt
	Lecanora sulfurescens Fée
	L. wilsonii Müll. Arg.
	Lecidella asema (Nyl.) Knoph & Hertel
	L. carpathica Körb.
	L. chodatii (Samp.) Knoph & Leuckert
	Physcia convexa Müll. Arg.
	P. erumpens Moberg
	Pyxine albovirens (G. Mey.) Aptroot
	P. cocoes (Sw.) Nyl.
	Rinodina oleae Bagl.
	R. oxydata (A. Massal.) A. Massal.
	Sarcogyne privigna (Ach.) A. Massal.
	Scoliciosporum camptosporum (Vain.) Aptroot
	S. umbrinum (Ach.) Arnold
	Tephromela atra Fée
	Trapelia coarctata (Turner) M. Choisy
damp overhanging	*Agonimia opuntiella* (Buschardt & Poelt) Vězda
	Endocarpon pallidulum (Nyl.) Nyl.
	Flakea papillata O.E. Erikss.
	Physcia atrostriata Moberg
	Porina chlorotica (Ach.) Müll. Arg.
	Rinodina oleae Bagl.
	R. oxydata (A. Massal.) A. Massal.
influenced by run-off	*Peltula bolanderi* (Tuck.) Wetmore
	P. clavata (Kremp.) Wetmore
	Leptogium isidiosellum (Riddle) Sierk
dry overhanging	*Dendrographa austrosorediata* Aptroot & Gumboski
	Lecanographa farinulenta (Müll. Arg.) Egea & Torrente
	Opegrapha lithyrga Ach.
Intermediate upper yellow supralittoral zone	*Caloplaca brouardii* (B. de Lesd.) Zahlbr.
	C. isidiosa (Vain.) Zahlbr.
	C. subvitellina (Müll. Arg.) Zahlbr.
	Roccellographa circumscripta (Leight.) Ertz & Tehler
Lower littoral zone	*Stigmidium marinum* (Deakin) Swinscow

https://doi.org/10.1017/S0024282917000512 Published online by Cambridge University Press

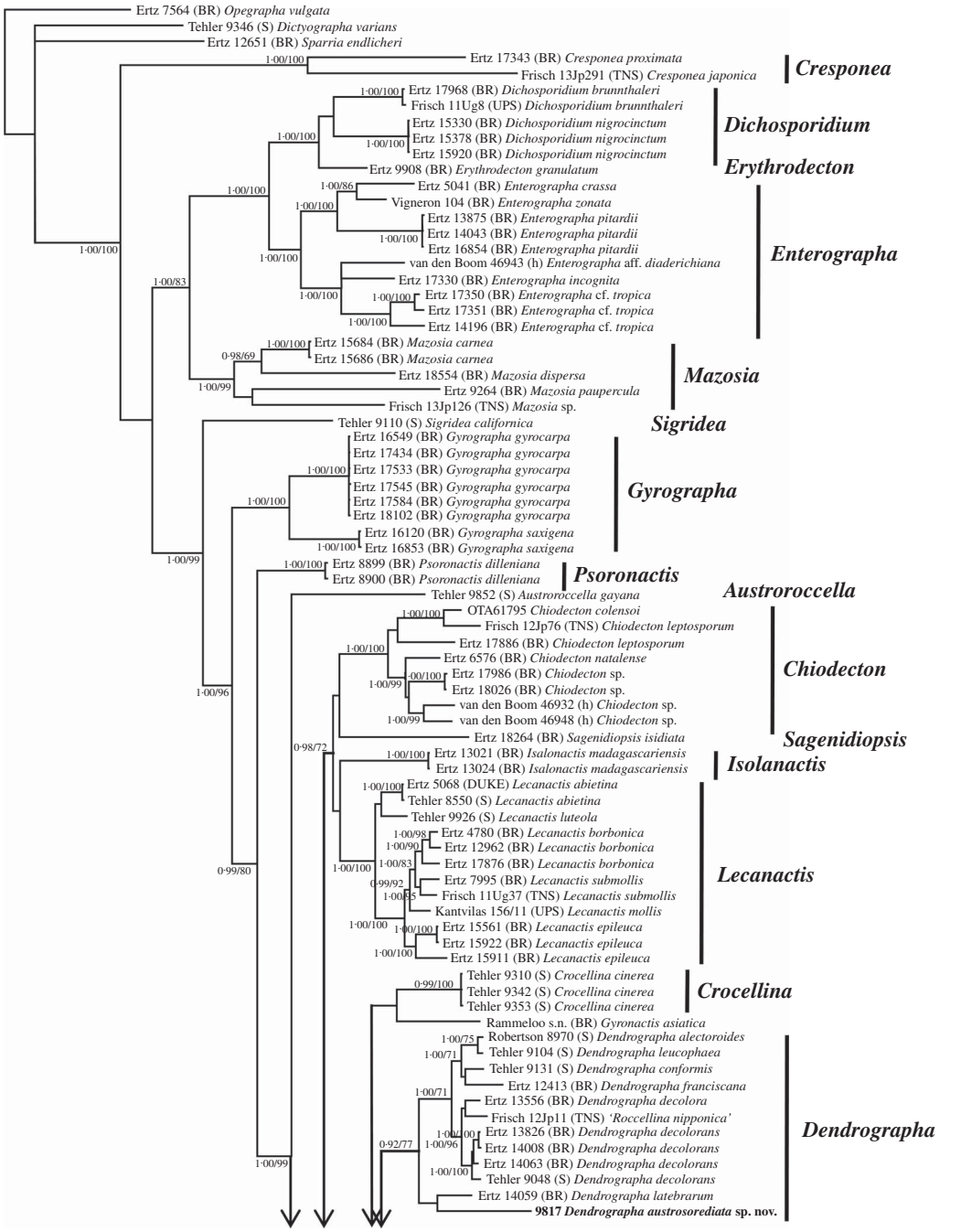

FIG. 2. Phylogenetic position of *Dendrographa austrosorediata* (shown in bold). Consensus phylogram obtained in MrBayes after the analysis of a combined 28S rDNA and *RPB2* dataset. Bayesian PP (set above 0.95) and ML BP (set at 70%) are given adjacent to nodes. Only nodes supported by both analyses are shown.
Continued on following page.

https://doi.org/10.1017/S0024282917000512 Published online by Cambridge University Press

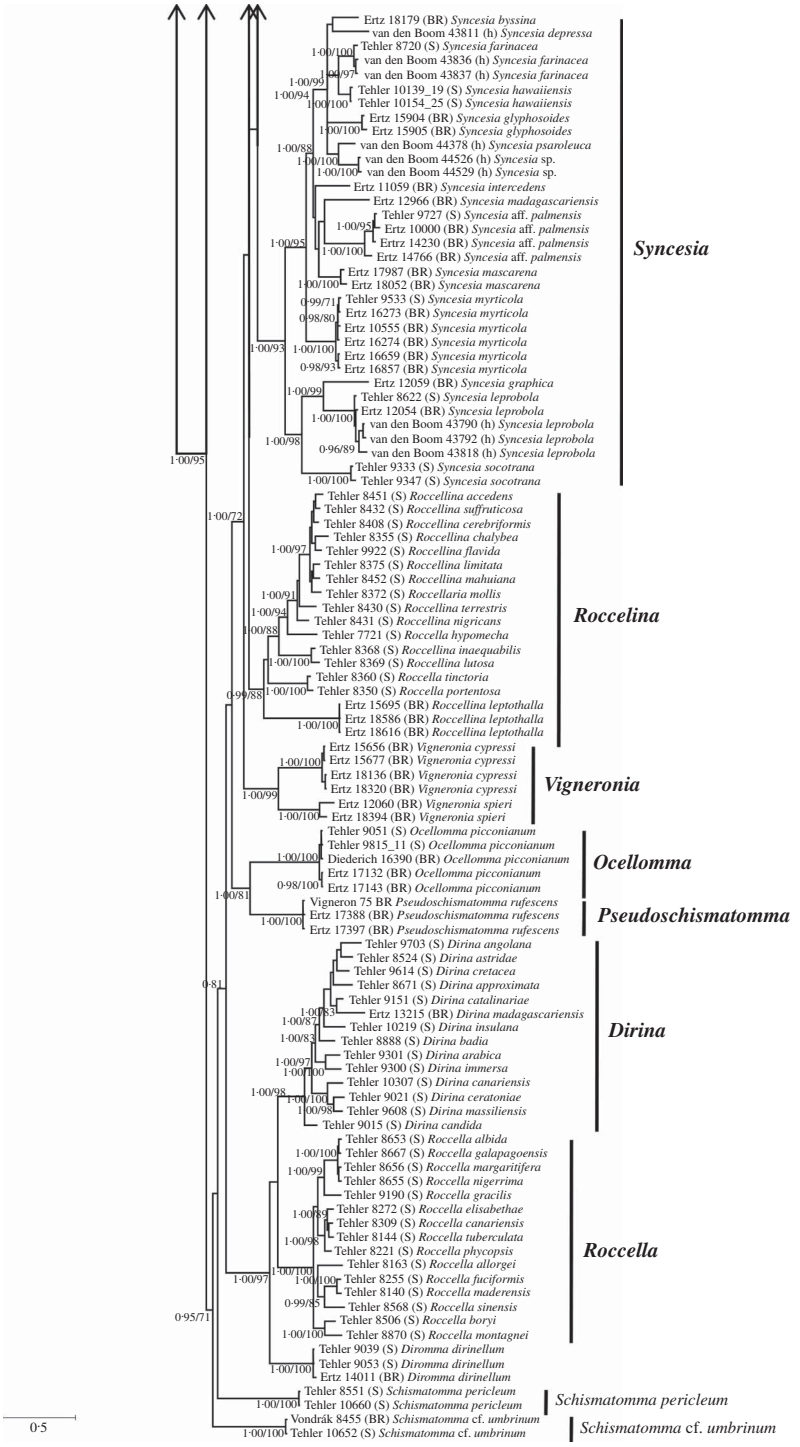

FIG. 2. *Continued from facing page.*

https://doi.org/10.1017/S0024282917000512 Published online by Cambridge University Press

accepted in the genus and is therefore described here as new. It is one of the few crustose species in the genus.

Places that can be classified as wet overhangs are generally shaded and damp. Lichens growing here are usually only partly maritime. In southern Brazil, mostly ubiquitous cosmopolitan species such as *Agonimia opuntiella* (Poelt & Buschardt) Vězda, *Endocarpon pallidulum* (Nyl.) Nyl., *Flakea papillata* O. E. Erikss., *Physcia atrostriata* Moberg, *Porina chlorotica* (Ach.) Müll. Arg., *Rinodina oleae* Bagl. and *R. oxydata* (A. Massal.) A. Massal. were found in this microhabitat.

In places with frequent run-off, lichens with cyanobacteria were dominant. The pantropical *Peltula bolanderi* (Tuck.) Wetm., *P. clavata* (Kremp.) Wetm. and *Leptogium isidiosellum* (Riddle) Sierk were present, as well as several so far unidentified *Lichinaceae* (i.e. probably one or more *Pterygiopsis* species).

The exposed granitic rocks of the grey zone are the richest in lichen species, just as elsewhere in the world. Here the majority of the macrolichens of the coastal rock also occur. The simple explanation is that the environment is less extreme and harbours many species that can also grow inland. Most of the crustose species identified so far seem to be cosmopolitan, while a considerable number of the reported macrolichens had a more restricted distribution. One *Ramalina* was present, viz. *R. gracilis* (Pers.) Nyl. *Ramalina* is often the dominant lichen on coastal rocks around the world and the species are often partly endemic, just as here, which is rather unusual among lichens. The crustose and microfoliose species found by us are listed in Table 1. The list is far from complete as only a small number of coastal cliffs have been systematically sampled and not all species could be identified.

Some of the lichens encountered, including all species in the lower yellow (lower supralittoral) zone, could not be identified as any described species. One undescribed species was found in overhangs. It was sequenced as it was sterile. ITS (KY986702), 28S rDNA (KY986703) and mtSSU (KY986704) sequences were obtained, but we failed to obtain an *RPB2* sequence. In the analysis only 28S rDNA was used for our specimen, but *RPB2* was used for other taxa from which it was available. Two analyses were carried out: a Bayesian simulation in MrBayes and a ML analysis in RAxML. The tree depicted (Fig. 2) is the Bayesian tree, with nodes annotated with PP (Bayesian) and BP (ML) values.

The new species clustered with one of the few other known crustose *Dendrographa* species, *D. latebrarum* (Ach.) Ertz & Tehler (Fig. 2), and is described here in that genus.

Dendrographa austrosorediata Aptroot & Gumboski sp. nov.

MycoBank No.: MB 820870

Saxicolous *Dendrographa* from overhanging coastal rock, with a crustose, flat to partly curling or blister-like thallus which is much dissected, whitish grey, 0·1–0·2 mm thick; surface minutely densely cracked, rimose, with cracks intersecting every *c.* 0·1 mm; soredia whitish to bluish grey but asymmetrically blackened in the direction facing the light, originating on the thallus surface, in initially discrete convex soralia.

Type: Brazil, Santa Catarina State, municipality of São Francisco do Sul, S of Prainha, 26°14'06"S, 48°30'05"W, on coastal granite, *c.* 10 m alt., 8 October 2015, *M. E. S. Cáceres & A. Aptroot* 27936 (JOI—holotype; ABL—isotype).

(Fig. 3)

Thallus crustose, individual thalli not distinguishable, colonies indeterminate and covering large areas, flat to partly curling up or blister-like, much dissected, epruinose or slightly pruinose, whitish grey, 0·1–0·2 mm thick; surface minutely densely cracked, rimose with cracks intersecting at each *c.* 0·1 mm; cortex 20–40 µm thick; *medulla* not differentiated, sometimes surrounded by a thin, black prothalline line; alga trentepohlioid, *c.* 6–11 µm diam. *Soredia* present, whitish to bluish grey, but asymmetrically blackened in the direction facing the light, originating on the thallus surface in initially discrete convex soralia of *c.* 0·2–0·4 mm diam. and up to 0·3 mm high, later covering most of the thallus; soredia round to ellipsoid, *c.* 18–32 µm diam., surface hyphae partly blackened.

Apothecia unknown.

Pycnidia not observed.

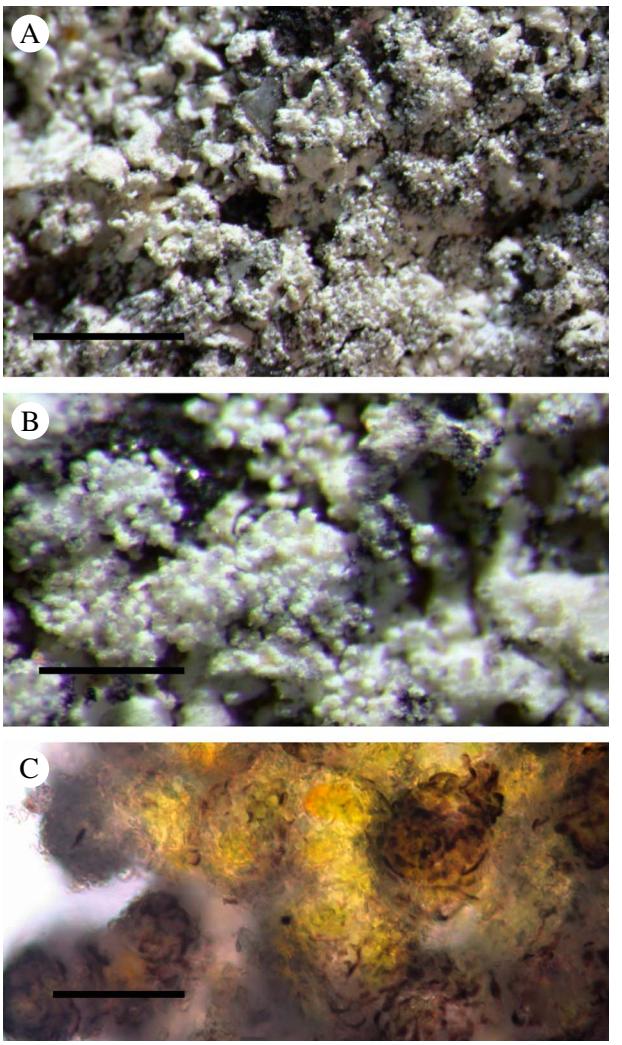

FIG. 3. *Dendrographa austrosorediata* (holotype). A, thallus; B, detail of thallus; C, soredia. Scales: A = 1 mm; B = 0·5 mm; C = 10 µm. In colour online.

Chemistry. Thallus and soredia C+ red. TLC: erythrin & lecanoric acid.

Ecology and distribution. On maritime granite, on overhanging rockfaces. Known only from Brazil.

Discussion

In the new phylogenetic classification of the *Roccellaceae* (Ertz *et al*. 2015), some crustose species are classified in the genus *Dendrographa* Darb. which traditionally contained only a few fruticose species. The available material of the new species is sterile but characteristic enough to be described and recognized, even in the field. Its placement in *Dendrographa* is based on a phylogenetic analysis (Fig. 2) using two genes. We present here an analysis of a larger group because ITS produced no matches, with the genera *Roccellina* and *Dendrographa* as top results, while LSU was only 90% similar to both

https://doi.org/10.1017/S0024282917000512 Published online by Cambridge University Press

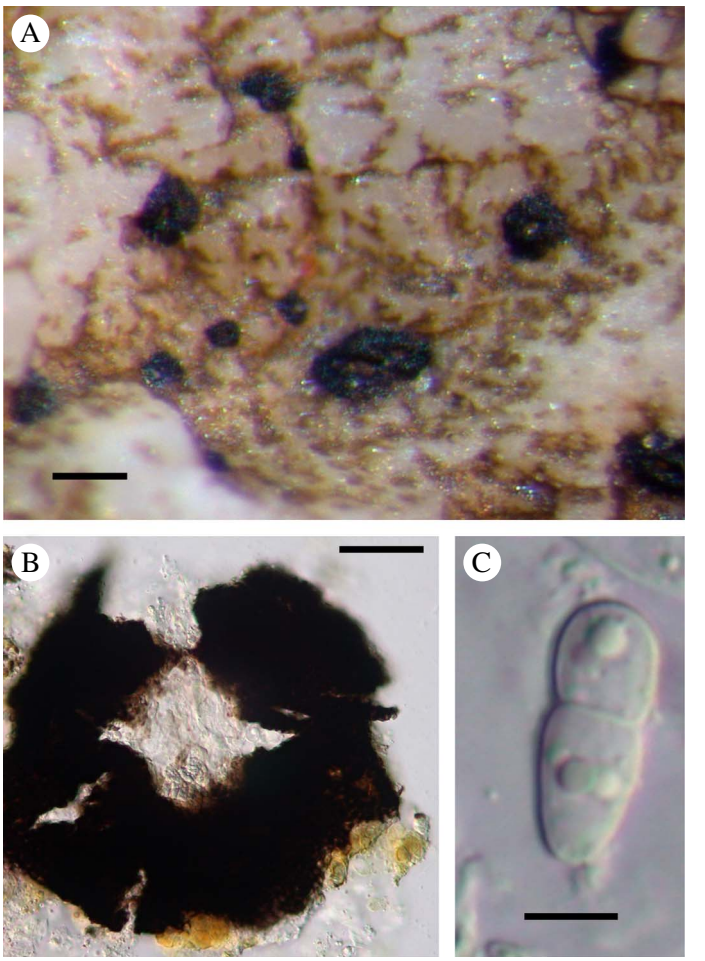

FIG. 4. *Stigmidium marinum* (*Cáceres & Aptroot* 27943). A, thallus; B, flattened perithecium in surface view revealing *Dilabofilum* algal cells around the margin; C, ascospore. Scales: A = 0·1 mm; B = 25 µm; C = 5 µm. In colour online.

genera. Without a broader analysis, it was not possible to decide whether it was a *Roccelina* or a *Dendrographa* after the BLAST results (or whether it even belonged to another genus). Moreover, the noise in the LSU sequence could alter BLAST results significantly, making it too risky to decide if it was a *Dendrographa* without conducting an analysis.

Stigmidium marinum (Fig. 4) is a somewhat surprising find, especially as it is generally regarded as a lichenicolous fungus. It is usually reported as an obligate parasite on some littoral *Verrucaria* species. However, it has been repeatedly observed occurring as a free-living lichen and is treated as such by van Herk & Aptroot (2004). Its occurrence in the littoral zone of southern Brazil, thousands of kilometres away from the nearest known occurrence of any purported host, proves once more that this is a lichenized fungus. It is lichenized with *Dilabofilum*.

MESC thanks CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for a research grant (Processo 311706/2012-6); AA thanks the Stichting Hugo de Vries-fonds for a travel grant; ELG thanks the Universidade da Região de Joinville – UNIVILLE for support provided for the event. We are grateful to Pablo Alvarado (ALVALAB, Santander) for carrying out the sequencing and phylogenetic analysis.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215:** 403–410.

Arup, U. (1995) Littoral species of *Caloplaca* in North America: a summary and a key. *Bryologist* **98:** 129–140.

Benatti, M. N. & Marcelli, M. P. (2009*a*) Espécies de *Parmotrema* (*Parmeliaceae*, Ascomycota) do litoral centro-sul do Estado de São Paulo, Brasil. I. Grupos químicos girofórico e lecanórico. *Acta Botanica Brasilica* **23:** 1012–1026.

Benatti, M. N. & Marcelli, M. P. (2009*b*) Espécies de *Parmotrema* (*Parmeliaceae*, Ascomycetes liquenizados) com medula pigmentada do litoral centro-sul do Estado de São Paulo. *Hoehnea* **36:** 597–612.

Benatti, M. N., Marcelli, M. P. & Elix, J. A. (2008) Three new species of *Parmotrema* containing salazinic acid from the coast of São Paulo State, southeastern Brazil. *Mycotaxon* **103:** 41–52.

Cubeta, M. A., Echandi, E., Abernethy, T. & Vilgalys, R. (1991) Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology* **81:** 1395–1400.

Ertz, D., Tehler, A., Irestedt, M., Frisch, A., Thor, G. & van den Boom, P. P. G. (2015) A large-scale phylogenetic revision of *Roccellaceae* (*Arthoniales*) reveals eight new genera. *Fungal Diversity* **70:** 31–53.

Gerlach, A. C. L. & Eliasaro, S. (2012) Liquens parmelioides eciliados (*Parmeliaceae*, Ascomycota) em costões rochosos dos estados do Paraná e Santa Catarina, Brasil. *Acta Botanica Brasilica* **26:** 572–586.

Gerlach, A. C. L. & Eliasaro, S. (2014*a*) Espécies de *Parmeliaceae* (Ascomycota) ciliadas e sem máculas reticulares em costões rochosos dos Estados do Paraná e de Santa Catarina, Brasil. *Hoehnea* **41:** 321–336.

Gerlach, A. C. L. & Eliasaro, S. (2014*b*) *Parmotrema marcellii*, a new species of *Parmeliaceae* (lichenized Ascomycota) from Brazil. *Brazilian Journal of Botany* **37:** 597–600.

Gilbert, O. L. (2000) *Lichens*. London: HarperCollins.

Gumboski, E. L. & Eliasaro, S. (2011) *Cladonia litoralis* (*Cladoniaceae*), a new species from southern Brazil. *Bryologist* **114:** 665–667.

Gumboski, E. L. & Eliasaro, S. (2012*a*) Espécies de *Cladonia* P. Browne (*Cladoniaceae*, Ascomycota) do Supergrupo *Cladonia* em restingas e costões rochosos dos Estados do Paraná e de Santa Catarina, Brasil. *Hoehnea* **39:** 315–337.

Gumboski, E. L. & Eliasaro, S. (2012*b*) *Peterjamesia circumscripta* (Leight.) D. Hawksw. (*Roccellaceae*, Ascomycota): first record to continental South America. *Check List* (*São Paulo, Online*) **8:** 267–268.

Murray, M. G. & Thompson, W. F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* **8:** 4321–4325.

Nylander, J. A. A. (2004) *MrModeltest v2*. Evolutionary Biology Centre, Uppsala University.

Orange, A., James, P. W. & White, F. J. (2001) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Osorio, H. S. & Fleig, M. (1984*a*) Contribution to the lichen flora of Brazil. XIII. Maritime lichens from Torres, Rio Grande do Sul State. *International Journal of Mycology and Lichenology* **1:** 273–279.

Osorio, H. S. & Fleig, M. (1984*b*) Contribution to the lichen flora of Brazil. XV. Lichens from Torre Sul and Itapeva, Torres, Rio Grande do Sul State. *Comunicaciones Botánicas del Museo de Historia Natural de Montevideo* **4:** 1–7.

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Swofford, D. L. (2001) *PAUP*4.0b10: Phylogenetic Analysis Using Parsimony* (*and Other Methods*). Sunderland, Massachusetts: Sinauer Associates.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28:** 2731–2739.

Vainio, E. A. (1890) Étude sur la classification naturelle et la morphologie des lichens du Brésil. *Acta Societatis pro Fauna et Flora Fennica* **7** (1)**:** 1–246 & **7** (2)**:** 1–256.

van Herk, C. M. & Aptroot, A. (2004) *Veldgids Korstmossen*. Utrecht: KNNV Uitgeverij.

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172:** 4238–4246.

Zoller, S., Scheidegger, C. & Sperisen, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *Lichenologist* **31:** 511–516.