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Two new species of the *Parmotrema subrugatum* group from the coast of São Paulo State, southeastern Brazil

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Abstract — During a survey of the *Parmeliaceae* in natural ecosystems and urbanized coastal areas of southeastern Brazil, two new *Parmotrema* species containing alectoronic acid were discovered: *P. hyperlaciniatulum* and *P. restingense*. These species are described and compared to *P. subrugatum*.

Key words — *Parmotrema lacinulatulum*, *Parmotrema maraense, Parmotrema wainioi*

Introduction

The genus *Parmotrema* A. Massal. is characterized by lobes with broad rotund apices and naked lower margins, the absence of pseudocyphellae, the frequent occurrence of marginal cilia, simple rhizines, and thick-walled, ellipsoid ascospores (Brodo et al. 2001, Nash & Elix 2002). More than 300 species are known worldwide (Nash & Elix 2002), and about one third of them occur in Brazil.

Two new species containing alectoronic acid are described in the present paper. These species were discovered by the authors during research on the broad-lobed species of *Parmeliaceae* at the coast in São Paulo State, Brazil (Benatti 2005), primarily situated between the municipalities of Ubatuba (23°02'S, 45°04'W) and Itanhaém (24°11'S, 46°47'W). This region includes urbanized areas and rocky shores, as well as mangrove and restinga forests as the predominant vegetation types.

The most common species of *Parmotrema* producing alectoronic acid in Brazil can be separated into two characteristic subgroups: (1) the *P. wainioi* group, with ascospores ca. 15–25 µm, filiform conidia over 6 µm long and abundant long cilia and (2), the *P. subrugatum* group, with larger ascospores 25–40 µm long, unciform conidia up to 6 µm long and shorter, less abundant cilia.

Both of the new species lack vegetative propagules, are corticolous in coastal mangrove or restinga forests, and belong to the *P. subrugatum* subgroup. Although we have included substantive information about the new species, more detailed morphological and chemical comparisons with other somewhat similar species can be found in Benatti (2005).

Material and methods

Specimens were distinguished by morphological characters using standard stereoscopic and light microscopes. Anatomical sections, including those of apothecia and pycnidia, were made with a razor blade by hand. The chemical constituents were checked by spot tests with potassium hydroxide (K), sodium hypochlorite (C) and *para*-phenylenediamine (P), and also examined under UV light (360 nm). Chemical constituents were identified by thin-layer chromatography (TLC) using solvent C (Bungartz 2001), high performance liquid chromatography (HPLC) (Elix et al. 2003) and comparison with authentic samples.

Since we had encountered problems dealing with the many morphological terms present in the literature, we specify here that lacinules represent adventitious, ribbon-like secondary outgrowths from the primary lobe margins. Lobules are similar, but short and rounded.

The diagnosis for each taxon refers exclusively to holotype characters and the English descriptions and comments to all the material studied.

The species

Parmotrema hyperlaciniatulum Benatti, Marcelli & Elix, **sp. nov.** Fig. 1

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Species cum thallo simili Parmotrematis lacinulatuli sed magis robusto et crasso, lobis angustis laciniatis demum lacinulatis, cortex superior continuus et emaculatus, ciliis parvis, conidiis minoribus et unciformibus differt. Atranorinam, chloroatranorinam, acidum alectoronicum, acidum α-collatolicum, acidum β-alectoronicum, acidum β-collatolicum, acidum dehydrocollatolicum, acidum dehydroalectoronicum, methyl pseudoalectoronatum, et methyl pseudo-α-collatolatum continens.

Holotype: Brazil, São Paulo State, Municipality of Itanhaém, Padre Manoel da Nóbrega Highway (SP-55) Km 108, at the crossing point with the Itanhaém River, mangroves by the side of the highway at the right margin of the river, 24°10'48.7"S, 46°48'07.1"W, 1 m alt., on trunk of *Rhizophora mangle* L., leg. M.P. Marcelli & L.R. Fontes 1670, 01-X-1979 (SP).

Thallus up to 14 cm wide, subcoriaceous to coriaceous, corticolous, grayish green but becoming dark gray in the herbarium, primarily lobed to sublobed, ultimately developing dense secondary laciniae; lobes irregularly branched, 1.5–4.0(–5.0) mm wide, primary lobes contiguous to ± imbricate, adnate to loosely adnate, secondary lacinules ascending, unattached, eventually twisted and subcanaliculate; apices ± plane to subconcave, subrotund; margin smooth to irregularly dissected, plane to ± ascending, weakly undulate in part, entire to incised, ciliate. Upper surface continuous to weakly and irregularly cracked, smooth to subrugose, sometimes with verrucae becoming papillose; maculae weak to distinct, linear, laminal, more obvious at the distal parts, sometimes developing fissures; lacinules linear and long, regularly spreading from margins, abundant at the thallus center, simple then dichotomously or irregularly branched, subcanaliculate to canaliculate, 0.2–15.0(–30.0) × 0.2–0.9(–1.1) mm, truncate, crowded, often covering parts of the upper surface, sometimes with papillose verrucae, underside cream or black. Soralia, pustules and isidia absent. Cilia black, simple or rarely furcate, 0.1–1.7(–2.4) × ca. 0.05 mm, frequent along the margins of the lobes and lacinules. Medulla white, with orange pigmented spots often present in the lower portion. Lower surface black, shiny, smooth to rugose, unevenly papillate; marginal zone shiny to opaque, usually pale brown but soon turning cream colored at the start of lacinules growth, smooth to rugose, unevenly papillate 0.5–4.5(–6.0) mm wide, naked; rhizines black, simple, sometimes agglutinated, 0.20–0.70 (–1.3) × 0.05–0.15 mm, sparse or frequent, grouped. Apothecia submarginal to subterminal, common, often originating on the lacinules, concave, 0.3–9.2 mm wide, substipitate, margins smooth to crenate or dentate-lacinulate, usually eciliate or rarely with scarce cilia, amphithecia and stipe smooth but becoming rugose with age; disc brown, epruinose, imperforate; ascospores ellipsoid, (22.5–)24.5–38.0(–40.0) × 14.0–21.5 μm, epispore (2.5–)3.0–3.5 μm wide. Pycnidia submarginal, common, abundant on the lacinules, with brown or black ostioles; conidia short unciform, 4.0–5.0 × ca. 1.0 μm.

Color reactions: upper cortex K+ yellow, UV–; medulla K–, C–, KC+ rose, P–, UV+ bluish green, and a K+ dark reddish pigment in the lower portions.

TLC/HPLC: cortical atranorin (minor) and chloroatranorin (minor); medullary alectoronic acid (major), α-collatolic acid (major), β-alectoronic acid (minor), β-collatolic acid (minor), dehydrocollatolic acid (minor), dehydroalectoronic acid (trace), methyl pseudoalectoronate (trace) and methyl pseudo-α-collatolate (trace).

Paratypes: Brazil, São Paulo State, Municipality of Itanhaém, Padre Manoel da Nóbrega Highway (SP-55) Km 108, at the crossing point with the Itanhaém River, mangrove by the highway´s side at the river´s right margin, 24°10'48.7"S, 46°48'07.1"W, 1 m alt., on trunk of *Rhizophora mangle*, leg. M.P. Marcelli & L.R. Fontes 1669, 10-I-1979 (SP); idem,

on tree trunk, leg. M.P. Marcelli & A. Mathey 1672, 05-VIII-1981 (SP); idem, on trunk of *Laguncularia racemosa* C.F. Gaertn., leg. M.P. Marcelli & L.R. Fontes 2386, 01-IV-1988 (B); idem, on tree trunk, leg. M.P. Marcelli, B. Marbach & C.H. Ribeiro 29380, 21-VIII-1995 (G).

Comments: This species is characterized by the absence of vegetative propagules, the narrow lobes which become laciniate and subcanaliculate and develop dense lacinules at the apices and margins, the substipitate apothecia with dentate-lacinulate, eciliate or sparsely ciliate margins, and pale brown lower margins which turn cream at the beginning of lacinule formation. An orange K+ dark red pigment is often present at the lower portions of the medulla, but this was not detected with HPLC.

The verrucae (or papillae) on the upper surface of the lobes and the lacinules resemble stout isidia, but lack a constricted base present in true isidia. They often support pycnidia.

Parmotrema hyperlaciniatulum differs from *P. subrugatum* and other species of this complex by the short, weakly inflated apothecia stipes (longer and markedly inflated in *P. subrugatum*) although the stipes do appear larger when developing on subcanaliculate lobe apices. Although most of the apothecia are eciliate, we noted that some apothecia in each specimen examined had a few poorly developed cilia.

Parmotrema lacinulatulum Krog from East Africa is superficially similar and we initially thought that the present material might represent this species. However *P. lacinulatulum* has a thinner and more fragile thallus, much broader lobes (5.0–8.0 mm), longer cilia (3.0–4.0 mm), a more continuous, emaculate upper cortex, longer sublageniform conidia (7.0–7.5 μm) and lacks a K+ orange pigment in the medulla (Krog 1991).

The lacinules of *P. hyperlaciniatulum* often cover large portions of the upper surface and extend to several centimeters long. With the aging of the thallus, the older, primary lobes die and disintegrate, but the subcanaliculate lacinules continue to grow and resemble somewhat small specimens of *Everniastrum*.

The mature thalli of *P. hyperlaciniatulum* must be collected and handled with care; otherwise, they may crumble since the older parts that keep the terminal parts together are often no longer present. This species is named after its habit, where the lobes gradually change their form, becoming laciniate and ultimately densely lacinulate.

Parmotrema restingense Marcelli, Benatti & Elix**, sp. nov.** Fig. 2

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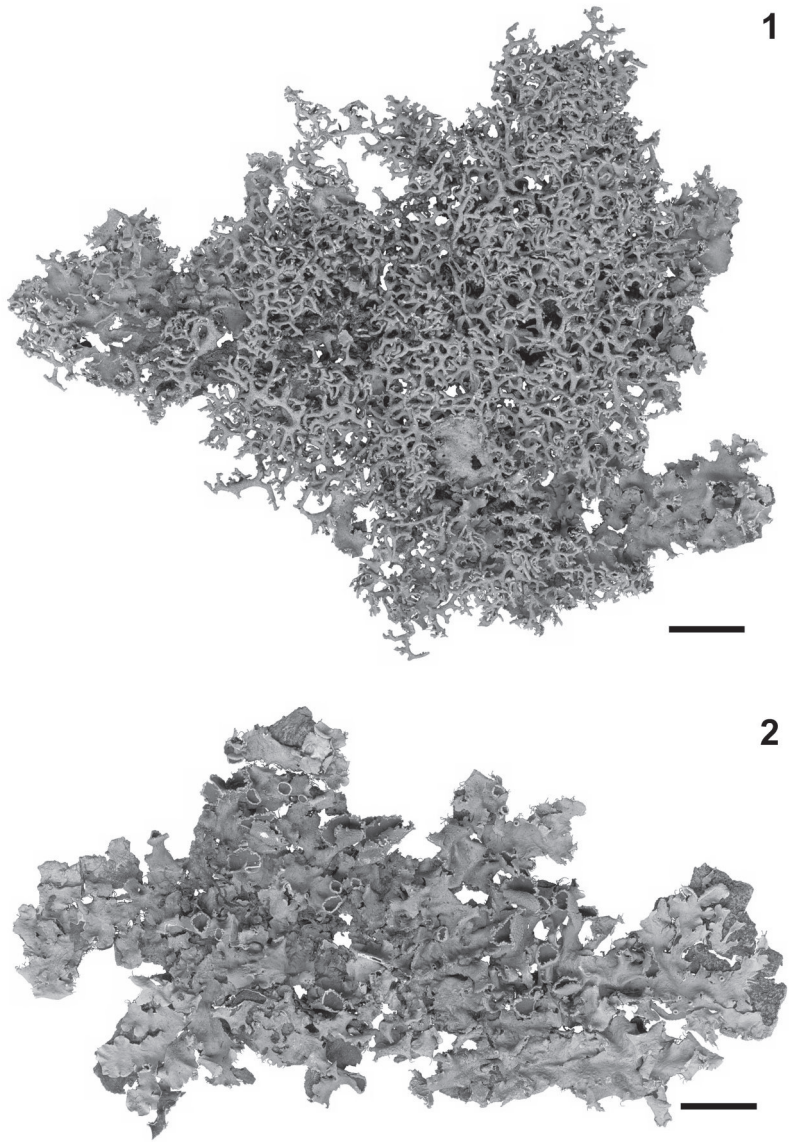
Species cum thallo simili Parmotrematis subrugati sed lobis angustatis, margine irregulariter sublacinulatis, margine inferior non continuus albida et apothecia laevigata vel ex parte lacinulata denticulata, eciliata differt. Atranorinam, chloroatranorinam, acidum alectoronicum, acidum α-collatolicum, acidum β-alectoronicum, acidum β-collatolicum, methyl pseudoalectoronatum, et methyl pseudo-α-collatolatum continens.



Figures 1–2. 1. The holotype of *P. hyperlaciniatulum*.
2. The holotype of *P. restingense*.
Bar = 1 cm.

Holotype: *Brazil, São Paulo State, Municipality of Cananéia, near the continental raft port to Cananéia, mangrove at the roadside, 24°59'10.2"S, 47°57'06.1"W, 1 m alt., on tree trunk, leg. M.P. Marcelli & J. Vieira Filho 1593, 23-XII-1979 (SP).*

Thallus up to 16.0 cm wide, submembranaceous to subcoriaceous, ramulicolous or corticolous, pale greenish gray becoming darker in the herbarium, lobate to sublobate. Lobes (1.5–)2.5–6.0(–9.0) mm wide, irregularly branched, contiguous to crowded, adnate, ascending when bearing apothecia, loosely attached; apices ± plane to subconvex and involute, subrotund to irregular; margin smooth near the apices, turning subcrenate or irregular, ± flat to ascending or subundulate, involute or revolute, entire to irregularly incised, partially dentate-sublacinulate, ciliate. Upper surface continuous but becoming irregularly cracked with age, smooth to subrugose; maculae weak to distinct, punctiform or sometimes aggregate and linear, laminal but more frequently appearing on the amphithecia and apothecial stipes. Adventitious lacinules generally sparse, very short, irregularly distributed along the lobe margins but occasionally intermixed with some small irregular lobules, simple or irregular, flat, 0.3–1.4(–2.5) × 0.2–0.7 mm, truncate or acute, underside concolorous with the lower margin or cream on lobes with apothecia. Cilia black, simple to furcate or rarely irregular, 0.2–2.8 × ca. 0.05 mm, frequent to abundant along the margins but scarce or absent at the apices of young lobes. Medulla white, rarely with spots of an orange pigment in the older parts. Soredia, pustulae and isidia absent. Lower surface black, shiny, smooth to rugose, weakly papillate or veined; marginal zone shiny, brown, smooth to subrugose, 1.5–5.5(–9.0) mm wide, naked, turning cream, white, or variegated under lobes with apothecia; rhizines black, simple, sometimes furcate or irregular, 0.10–1.60(–2.30) × 0.05(–0.15) mm, few to frequent but more abundant in some parts, occasionally becoming agglutinated, grouped. Apothecia submarginal or subterminal, originating in part from subcanaliculate lobes apices, common, ± concave to urceolate, becoming fissured and distorted with age, up to 9.5 mm wide, stipes inflated, margins smooth when young, then subcrenate and short dentate-lacinulate, eciliate, amphithecia and stipe smooth when young, becoming rugose, veined or vertically folded with age, sometimes with papillose wrinkles; discs brown, epruinose, imperforate; ascospores ellipsoid, (19.0–)25.0–36.0(–40.0) × (12.0–)14.0–18.0(–24.0) µm, epispore 2.5–4.0(–5.0) µm thick; pycnidia submarginal, frequent to abundant, with black ostioles; conidia unciform, (3.0–)4.0–5.0(–6.0) × ca. 1.0 µm.

Color reactions: upper cortex K+ yellow, UV–; medulla K–, C–, KC+ rose, P–, UV+ bluish green, with a K+ dark reddish pigment frequent only in old or necrotic areas of some thalli.

TLC/HPLC: cortical atranorin (minor) and chloroatranorin (minor); medullary alectoronic acid (major), α-collatolic acid (major), β-alectoronic acid (trace),

β-collatolic acid (trace), methyl pseudoalectoronate (trace) and methyl pseudo-α-collatolate (trace).

Paratypes: Brazil, São Paulo State, Municipality of Cananéia, Cardoso Island, restinga wood of Marujá Village, post-dune restinga vegetation at the southern part of the island, wood of bushes and small trees, 25°14'S, 48°01'W, 5 m alt., on small tree thin branch, leg. M.P. Marcelli 1747, 1751, 1752, 1753, 1754, 1755, 1756, 1761, 1762, 1763, 1764, 1766, 1767, 1768, 1769, 1770, 1771, 1772, 1775 (SP), 1759 (B) 20-X-1981. Municipality of Iguape, Barra do Ribeira, between Suamirim "River" and the ocean, low restinga forest near the mangrove, 24°38'S, 47°22'W, 2 m alt., on small tree trunk, leg. M.P. Marcelli & O. Yano 6375, 15-VII-1989 (G); idem, on thin small tree branch, leg. M.P. Marcelli & O. Yano 6872, 6873, 18-VII-1989 (SP); idem, sand dunes vegetation, 24°38'S, 47°22'W, 5 m alt., thin branch of small tree, leg. M.P. Marcelli & O. Yano 6808, 10-VII-1989 (SP); idem, urban zone, 24°39'S, 47°22'W, 5 m alt., tree trunk at the sidewalk, leg. M.P. Marcelli & O. Yano 7112, 7117, 7134, 22-VII-1989 (SP). Municipality of Ilha Comprida, Gambôa Nóbrega, 25°01'S, 47°54'W, 1 m alt., small tree trunk, leg. M.P. Marcelli 1594, 16-II-1982 (SP); idem, central area of the island, low restinga forest behind the propriety of the Kitaura family, 24°51'S, 44°42'W, 2 m alt., thin branch of small tree, leg. M.N. Benatti, A.A. Spielmann, L.S. Canêz, M.J. Kitaura & M.P. Marcelli 1730, 1748, 1749 (SP), 1747 (ASU), 02-IV-2004. Municipality of Peruíbe, margin of Guaraú River, mangrove at the edge of the river, 24°23'S, 47°02'W, 5 m alt., on trunk of *Rhizophora mangle*, leg. M.P. Marcelli & O. Yano 3907, 3909, 3927, 23-VII-1988 (SP).

Comments: *Parmotrema restingense* is characterized by the absence of vegetative propagules, the densely ciliate margins that are sparsely and irregularly sublacinulate, the apothecia with smooth or shortly denticulate, always eciliate margins, and a lower cortex which is brown at the margins becoming white or cream only under the apothecia.

This is the most common species of the alectoronic acid containing group along the coast of São Paulo State. Previously it may well have been mistaken for *P. subrugatum*, which has a shiny white margin and only becomes pale brown in a very narrow transition zone towards the black center. In *P. restingense* the marginal zone is always brown, becoming white to ivory colored only under lobes bearing apothecia.

The frequent, ramified, subcanaliculate lacinules of *P. subrugatum* are very different from the uneven, short and simple, dentate lacinules seen in *P. restingense*. While the lacinules in *P. restingense* rarely exceed 1.5 mm in length (usually resulting from the irregular incised margins), those in *P. subrugatum* are regular in shape and branching pattern and may exceed 1 cm in length.

Similarly, the apothecia of *P. restingense* invariably have a smooth, eciliate margin that only becomes dentate with age, while those of *P. subrugatum* sometimes have apical cilia and frequent small lacinules (see below). The epithet refers to the predilection of the species for restinga forest habitats at the southeastern Brazilian littoral.

Parmotrema subrugatum (Kremp.) Hale, Phytologia 28: 339. 1974. FIG. 3

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≡ *Parmelia subrugata* Kremp., Verh. Zool. Bot. Gesell. Wien 18: 320. 1868.

HOLOTYPE: Brazil, Rio de Janeiro State, Serra dos Órgãos (Organ Mountains), leg. Helmreichen s.n. (M!).

THALLUS up to 11.0 cm wide, subcoriaceous, corticolous, becoming dark greenish gray in the herbarium, lobate to sublobate. LOBES 2.5–7.0 mm wide, irregularly branched, crowded, not adnate, subascending and distorted, loosely attached; APICES plane to subconvex and revolute when lacinulate, subrotund to subirregular; MARGIN smooth near the apices, soon turning subirregular, ± flat to ascending and becoming subundulate, involute or revolute, normally giving the lobes a canaliculate aspect, entire to irregularly incised, commonly lacinulate, ciliate. UPPER SURFACE continuous but becoming irregularly cracked with age, subrugose to rugose; MACULAE usually distinct, punctiform and aggregated, appearing irregularly on the lamina or frequently forming on the amphithecia and stipes of the apothecia where they sometimes become linear. ADVENTITIOUS LACINULES very common, short to medium, regularly distributed along the apices and margins of the lobes, occasionally intermixed with some small irregular lobules, often agglomerated, simple at first but soon becoming irregularly dichotomously branched, flat to partially subcanaliculate, 1.2–8.3 × 0.3–1.2 mm, normally truncate, often ciliate, underside generally cream and concolorous with the lower margin. CILIA black, simple to sometimes furcate or irregularly ramified, 0.3–2.5 × ca. 0.05 mm, usually common along the margins but scarce or absent at the apices of young lobes. MEDULLA white, spots of orange pigments absent even in the older parts. SOREDIA, PUSTULAE and ISIDIA absent, but with some grouped papilloid, dactyliform, massive and ± ciliate structures resembling thick isidia without a constricted base, 0.4–1.5 × 0.2–0.5 mm, ramified, appearing on some parts of the cortex or sometimes on the stipes of the apothecia, sometimes difficult to distinguish from the young apothecia, partially developing into laminal lacinules similar to those on the margins. LOWER SURFACE black, shiny, smooth to subrugose, weakly papillate; MARGINAL ZONE naked, shiny to opaque, smooth to subrugose, 1.0–6.5 mm wide, normally cream or white in an almost continuous line in the distal portions; brown only in young, smaller lobes bearing no lacinules or apothecia; CENTER black; RHIZINES black, simple, rarely furcate or irregular, 0.20–1.40(–2.20) × 0.05(–0.15) mm, frequent to abundant at some parts, often becoming agglutinated. APOTHECIA submarginal or subterminal partially originating from the subcanaliculate lobes apices, common, ± concave to urceolate, normally fissuring and becoming distorted with age, up to 17.5 mm diam., stipes inflated, margins smooth when young, then denticulate and sometimes lacinulate, eciliate except at the apices of the lacinules, amphithecia

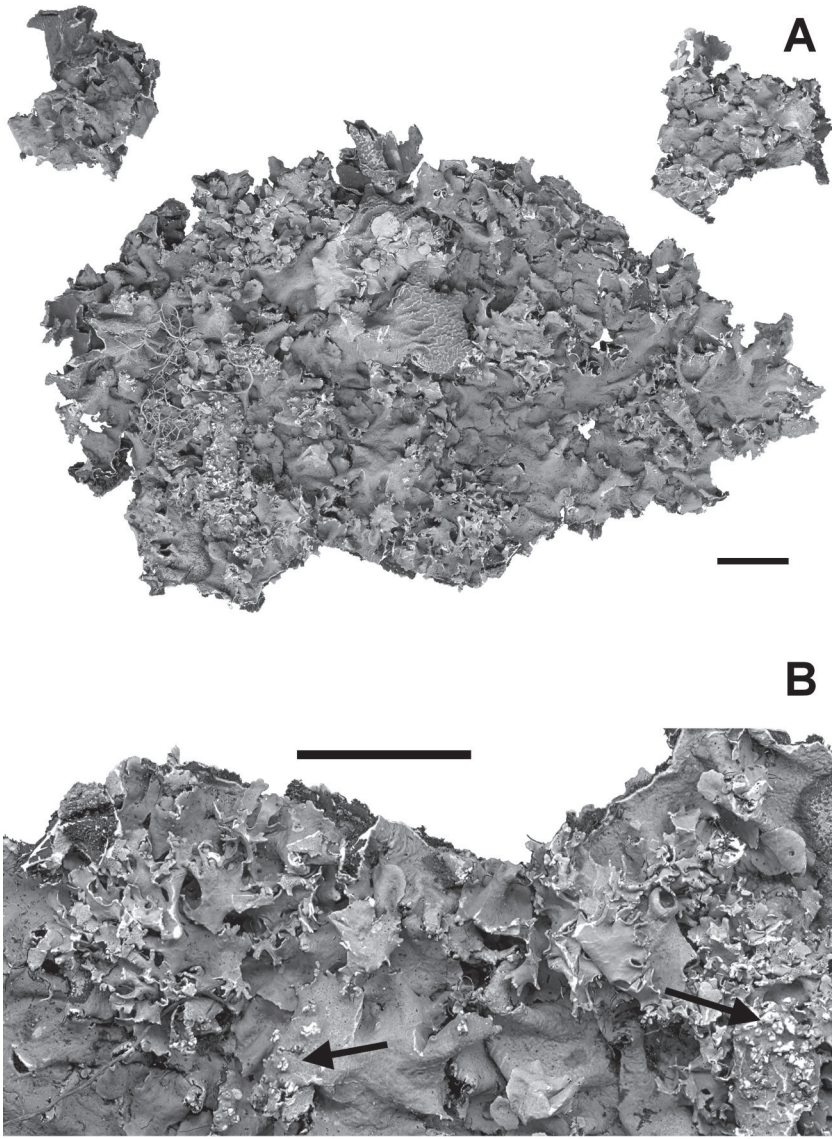

Figure 3. The holotype of *P. subrugatum*. A. The entire specimen.
B. Details of the marginal lacinules and the papilloid structures (arrows) that give rise to them.
Bars = 1 cm.

and stipe smooth when young, becoming strongly rugose and veined with age, sometimes with a few papilloid-isidioid structures as seen on the upper surface; disc dark brown, epruinose, imperforate or partially perforate when mature; ASCOSPORES ellipsoid to ovoid, (17.5–)26.5–35.0 × (12.0–)15.0–22.0 μm, epispore 2.5–4.0 μm thick; PYCNIDIA mainly submarginal and on the lacinules, frequent to abundant, with black ostioles; CONIDIA unciform, (4.0–5.0–6.0 × ca. 0.75 μm.

COLOR REACTIONS: upper cortex K+ yellow, UV–; medulla K–, C–, KC+ rose, P–, UV+ bluish green.

TLC: cortical atranorin; medullary alectoronic acid and α-collatolic acid, with or without rhodophyscin (fide Culberson 1969).

COMMENTS: *Parmotrema subrugatum* is characterized by the absence of vegetative propagules, the ciliate margins regularly developing dichotomously branched lacinules, the presence of laminal digitiform structures, the denticulate to lacinulate apothecia which are only ciliate at the apices of the lacinules, and by the black lower cortex with an usual white or cream marginal zone that is almost continuous along the distal parts of the thallus.

The name *P. subrugatum* has apparently been misapplied to several different species, some of which appear as synonyms in Hale's classic monograph on *Parmelia* subgen. *Amphigymnia* (Hale 1965).This species is apparently one of the most frequently confused species of those containing alectoronic acid, and its name has been misapplied for specimens which have a white (at least in part) lower marginal zone, eciliate apothecia, large ellipsoid ascospores (25–40 μm long) and short conidia (4–6 μm long).

Hale (1965) described *P. subrugatum* as having broad lobes (7–15 mm wide) with an ivory to brown or mottled lower margin. However, when comparing *P. maraense* Hale to *P. subrugatum* (Hale 1990), he refined his species concept, mentioning that *P. subrugatum* has a continuous white margin that turns dirty white with age.

The holotype of *P. subrugatum* (M!, FIGURE 3A) has an almost uniformly white marginal zone which distinguishes it from the other species of this group, where the marginal zone is initially brown and becomes pale only on aging. In this specimen, the margin is almost entirely shiny cream (probably white when freshly collected), with a few young lobes having a brown color.

This species normally forms abundant small, dichotomously branched lacinules along the margins throughout the thallus. In addition, they sometimes develop from the upper cortex, growing from scattered, isidioid-papillate structures (FIGURE 3B). These structures are quite different from anything we have seen in other species of the alectoronic chemical complex, and although

they resemble large, thick isidia without a constricted base, their function is not apparent. In some parts, they resemble poorly developed apothecial primordia, and can readily be confused with them. However, on further development, their shape diverges from that of primordial apothecia and eventually they may form dichotomously branched lacinules like those along the margins.

Poorly developed thalli of *P. subrugatum* and *P. restingense* may appear very similar. One should look for true lacinules along the margins and the overall color of the lower marginal zone for confirmation. The presence of the papillate structures on the upper surface is also important for distinguishing *P. subrugatum*.

Parmotrema subrugatum is a species described from southeast Brazil, from a place mostly covered by the Atlantic rainforest, perhaps little above 1000 m high (Serra dos Órgãos) where commonly the trees become shorter and the cloud forest begins to appear. The additional specimen studied came from a place with similar climate and vegetation but of higher latitude.

Additional specimen examined: Brazil, Rio Grande do Sul State, Municipality of Sobradinho, open place near the road, 29°24'20.2"S, 53°01'25.9"W, 375 m alt., corticolous, leg. A.A. Spielmann 360, 17-VII-2003 (SP).

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