



Original Paper

Small Parmeliaceae (lichenized Ascomycota) of Ilhabela State Park and nearby areas, São Paulo state, Brazil

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Abstract

A survey of lichens at Ilhabela State Park and nearby areas in São Sebastião Island revealed the occurrence of 12 taxa belonging to six genera of small foliose Parmeliaceae, which are characterized by usually small thalli (hardly > 10 cm in diameter) with narrow lobes or laciniae less than 1 cm wide. Comments are provided for the species registered.

Key words: Atlantic rainforest, lichens, mycobiota, restinga wood, São Sebastião Island.

Resumo

O levantamento de líquens no Parque Estadual de Ilhabela e seus arredores na Ilha de São Sebastião revelou a ocorrência de 10 táxons pertencentes a seis gêneros de pequenas Parmeliaceae foliosas, caracterizadas pelos talos geralmente pequenos (dificilmente > 10 centímetros de diâmetro), com lobos ou lacineas estreitas de largura inferior a 1 cm. São apresentados comentários para as espécies registradas.

Palavras-chave: Mata Atlântica, líquens, micobiota, restinga, Ilha de São Sebastião.

Introduction

With the 16th longest coastline in the world, the Brazilian Atlantic coastline extends over nearly 7.5 thousand kilometers, comprising many geographical features, including beaches, bays, reefs, and islands (CIA 2017). The Atlantic Rainforest represents the major vegetation type found in many coastal areas, including the islands. The diversity of lichens still remains poorly investigated in most coastal formations, such as restinga woods, mangroves, and rocky shores.

Parmeliaceae is the most representative family in Brazilian biomes with regard to the diversity of genera and species (Marcelli 1998). During the past two decades, it has been the focus of Brazilian researchers, who showed the occurrence of hundreds of species, including new records and new species. Several recent surveys have significantly contributed to the

diversity and distribution of small Parmeliaceae genera like *Bulbothrix* Hale, *Canoparmelia* Elix & Hale, *Crespoa* Lendemer & Hodgkinson, *Hypotrachyna* (Vain.) Hale, *Parmelinopsis* Elix & Hale, *Parmelinella* Elix & Hale, and *Relicina* (Hale & Kurok.) Hale (e.g., Benatti 2011, 2012a-h, 2013a-c, 2014a-c; Benatti & Elix 2012; Benatti & Lendemer 2014; Benatti & Marcelli 2007; Benatti *et al.* 2008; Canêz *et al.* 2009; Gerlach & Eliasaro 2012, 2014; Jungbluth *et al.* 2008; Marcelli & Canêz 2008; Marcelli *et al.* 2007, 2011; Spielmann & Marcelli 2008a,b). The small Parmeliaceae comprises species easily recognizable by their small thalli (usually < 10 cm diameter) with narrow lobes or laciniae (often < 1 cm wide).

Recently, a survey of foliose lichens in Cananéia, Cardoso, Comprida, and Ilhabela Islands (23°45'40"S, 45°24'44"W), São Paulo state, reported 16 species of *Leptogium* (Kitaura

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et al. 2017), however, the lichen diversity at this region remains largely unexplored. In the present study, we aimed at identifying the species of small Parmeliaceae that occur at Ilhabela Island, state of São Paulo, Brazil.

Material and Methods

Specimens of small foliose Parmeliaceae were collected at Ilhabela (23°45'36"S, 45°24'36"W) State Park, São Paulo state, Brazil, during several field expeditions from 2012 to 2013. Vegetational types include mostly Atlantic Rainforest, with Restinga Wood along the Eastern Coast. Accordingly to the climate classification of Köppen-Geiger, the island is classified as Tropical Aw. The altitude ranges from sea level up to almost 1400 m. The methodology used for the collection and study of lichenized fungi is described in Fink (1905), Hale (1979, 1987), Malcolm & Galloway (1997) and Benatti & Marcelli (2007). Collected specimens were observed under an Eikonol EK3ST stereomicroscope and Motic TYPE 102M microscope and the macroscopic and microscopic characteristics of somatic and reproductive structures of the thalli were analyzed. Identification was performed by comparison of published descriptions of specimens from different locations. Chemical tests consisted in spot tests, UV light exposure (360 nm), microcrystal tests and Thin Layer Chromatography (TLC) in the following eluents: (I) toluene: ethyl acetate: acetic acid 6:4:1 v/v/v and (II) toluene: acetic acid 85:15 v/v, following the methodology described in Asahina & Shibata (1954), Walker & James (1980), White & James (1985), Huneck & Yoshimura (1996) and Orange *et al.* (2001). All collected specimens were deposited at SP herbarium.

Results and Discussion

Thirty specimens of small Parmeliaceae were studied. We identified ten species belonging to *Bulbothrix* Hale (one species), *Canoparmelia* Elix & Hale (two), *Crespoa* Lendemmer & Hodgkinson (one), *Parmelinella* Elix & Hale (two), *Parmelinopsis* Elix & Hale (three) and *Remototrachyna* Divakar & Crespo (one), which are commented below.

Due to the presence of a constantly dense ombrophilous forest, it was difficult to find thalli that were mature or not damaged by chlorosis or necrosis. Even with a high search effort, almost all thalli in the study area were found in small open

areas such as clearings, posts, lookouts, roadsides, and near water courses or waterfalls. We were not able to examine and identify some of the specimens collected due to the fragmented and depleted condition of the badly damaged thalli.

Species registered

1. *Bulbothrix ventricosa* (Hale & Kurok.) Hale. *Phytologia* 28(5): 481. 1974. MB 341620.

Parmelia isidiza var. *domingensis* Vain., *Annales Academiae Scientiarum Fennicae* 6A(7): 17. 1915.

Species description: Hale (1976b); Benatti (2012d).

Specimens examined: Ilhabela, Parque Estadual de Ilhabela, Ilha de São Sebastião, Cachoeira da Toca old farm, 23°47'S, 45°21'W, 6.XII.2012, leg., M.N. Benatti *et al.* 3412, 3413, 3418; Cocaia track, entrance to Costabela, 15.V.2013, leg., M.N. Benatti *et al.* 3533.

This is one of the most common species of *Bulbothrix* in several Brazilian vegetation types (Benatti 2012d). It is mainly recognized by its larger lobed aspect (with rounded and broad lobes, often between 1 and 5 mm wide) compared to other *Bulbothrix* species, its distinct bulbate cilia with simple apices, the varied (often mottled) coloration of the lower cortex and the presence of medullary norstictic acid (the latter two characteristics also common to the non-isidiate *B. viatica* Spielmann & Marcelli). Our specimens were poorly developed with 2 to 3 cm in diameter (with the exception of a single larger, 6 cm-wide thallus), and the presence of laciniae of often small width (only a few > 3 mm wide). The laminal ciliar bulbs were scarce to common, and most specimens showed darkly mottled lower cortices, with a predominance of dark brown and black shades. All specimens have plenty of isidia, although no apotheciate material was found. The presence of atranorin and norstictic acids were confirmed by TLC.

2. *Canoparmelia caroliniana* (Nyl.) Elix & Hale. *Mycotaxon* 27: 278. 1986. MB 128704.

Parmelia caroliniana Nylander, *Flora* 68: 614. 1885.

Species description: Benatti (2014c).

Specimens examined: Ilhabela, Ilhabela State Park, Ilha de São Sebastião, Cocaia track, entrance to Costabela, 23°47'S, 45°21'W, 15.V.2013, leg., M.N. Benatti, L.B. Moro & C. Mayumi 3532, 3538, 3555, 3557.

The isidiate *C. caroliniana* together with the sorediate *C. texana* (Tuck.) Elix & Hale are among the most common *Canoparmelia* species

found in Brazil, even in urbanized or other types of anthropized areas. Interestingly, the specimen *M.N. Benatti et al.* 3357 presented a more accentuate medullary reaction to spot tests (almost as reddish as expected for *C. sanguinea* Marcelli, Benatti & Elix) but TLC confirmed traces of perlatolic acid derivatives. Although confirmed by TLC, the perlatolic acid was not clearly evidenced due to the low concentration found. This might be due to the contact with other species that are growing on the same substrate and have similar compounds which could have been leached by rainfall. The atranorin and chloroatranorin acids might have occurred at low concentrations or even been absent in our material, as they were not detected on the plates.

3. *Canoparmelia cryptochlorophaea* (Hale) Elix & Hale. Mycotaxon 27: 278. 1986. MB 128708.

Parmelia cryptochlorophaea Hale, The Bryologist 62: 18. 1959.

Species description: Hale (1976a, as *Pseudoparmelia cryptochlorophaea*).

Specimens examined: Ilhabela, Ilhabela State Park, track close to the meteorological station near the lookout, 23°47'S, 45°21'W, 4.IX.2012, leg., *M.N. Benatti et al.* 3370; municipal plant nursery Cachoeira da Água Branca, 23°47'S, 45°21'W, 6.XII.2012, leg., *M.N. Benatti et al.* 3425; SABESP area, southern part of the island, Bairro Bixiga, 23°47'S, 45°21'W, 06.XII.2012, leg., *M.N. Benatti et al.* 3456 (p.min.p.); Bairro da Feiticeira, 23°47'S, 45°21'W, 6.XII.2012, leg., *M.N. Benatti et al.* 3461.

This species was cited for the first time for the state of São Paulo by Jungbluth (2006), who collected specimens at several spots of Cerrado vegetation in the interior of the state. This is the first record for coastal areas. The presence of capitate and raised marginal soralia and the medullary reaction to spot tests (K+ faintly rose and KC+ purple rose) are characteristic of this species. The KC reaction for the specimen *M.N. Benatti* 3370 gave a more purple-reddish tone. The presence of cryptochlorophaeic acid was confirmed, although atranorin might have occurred at low concentrations as it was not detected in the TLC plate. We examined several specimens of different species from the study area that presented low concentrations of atranorin, which has a solar light filter function, and interestingly, all these specimens were collected from relatively shaded places and reacted to K spot tests, showing the presence, albeit weak, of the substance. Like

the specimens found by Jungbluth (2006), our specimens did not have ascomata or pycnidia.

4. *Crespoa cf. scrobicularis* (Kremp.) Benatti & Lendemer, Brittonia 66: 288. 2014. MB 802265.

Parmelia scrobicularis Kremp., Videnskabelige Meddelelser Naturhistorik Forening Kjöbenhavn 25: 10. 1873.

Species description: Benatti & Lendemer (2014).

Specimens examined: Ilhabela, Ilhabela State Park, southern part of the island, 27.VI.2012, 23°47'S, 45°21'W, leg., *M.N. Benatti, L. Moro & M. Boro* 3335.

We collected a single specimen that was growing together with a specimen of *Heterodermia* sp. This species is characterized by the scrobiculate thallus. Atranorin, stictic and constictic acids were confirmed by TLC and menegaziac acid was not present. An unidentified substance with Rf close to 43 was found solely in eluent I, but it could not be determined. As the specimen of *C. scrobicularis* was growing together with a species of *Heterodermia*, the substance might have leached from the later to our specimen. The thallus did not produce apothecia, pycnidia, soredia or isidia, as in the case of several other specimens found in the study area and as the propagule-producing species *C. crozalsiana* (B. de Lesd. ex Harm.) Lendemer & Hodk. and *C. carneopruinata* (Zahlbr.) Lendemer & Hodk. Therefore, it could only be identified as *Crespoa cf. scrobicularis*, which has been previously reported for other areas in the state of São Paulo (Jungbluth 2006; Benatti 2014b).

5. *Parmelinella cinerascens* (Lyngé) Benatti & Marcelli. Opuscula Philolichenum 11: 27. 2012. MB 563647.

Parmelia cinerascens Lyngé, Arkiv för Botanik 13 (13): 104. 1914.

Species description: Benatti (2012h).

Specimens examined: Ilhabela, Ilhabela State Park, Cocaia track, entrance to Costabela, 15.V.2013, 23°47'S, 45°21'W, leg., *M.N. Benatti, I.H. Schoenlein-Crusius & C. Mayumi* 3548.

We found only a single specimen at the study area. This species is easy recognizable due to the presence of small lobes, axillary or marginally sparse cilia that are very short and simple, a black lower cortex and medullary salazinic acid. It is similar to *Parmelinella salacinifera*, from which it differs by the paler and brown lower cortex, and the scarce to absent simple cilia. This material was one of the few well-developed thalli of small

Parmeliaceae found (approximately 8 cm wide), very isidiate and with a single, relatively mature apothecium. The hymenia, however, did not present asci with mature ascospores.

6. *Parmelinella salacinifera* (Hale) Marcelli & Benatti. *Mycosphere* 5: 780. 2014. MB 810474.

Parmelia salacinifera Hale. *Contributions from the U.S. National Herbarium* 36: 157. 1964.

Species description: Benatti (2014a).

Specimens examined: Ilhabela, Ilhabela State Park, Cocaia track, entrance to Costabela, 15.V.2013, 23°47'S, 45°21'W, leg., M.N. Benatti, I.H. Schoenlein-Crusius & C. Mayumi 3540.

This isidiate species is similar to *P. cinerascens*, but is promptly identified by its pale brown lower cortex and the very scarce to absent marginal or axillary simple short cilia. It also presented medullary salazinic acid, which is common for *Parmelinella* spp. We were not able to elucidate if all specimens identified under this name belong to a single species, although they are overall nearly identical in morphology, anatomy and chemistry to all specimens described in Benatti (2014a). The only difference found was regarding the frequency of the tiny marginal cilia, with specimens completely eciliate, with a few cilia, to some with cilia in a few parts of their marginal area (Benatti 2014a). As in the case of most species registered in the present study, we only found one specimen.

7. *Parmelinopsis damaziana* (Zahlb.) Elix & Hale. *Mycotaxon* 29: 242. 1987. MB 130583.

Parmelia damaziana Zahlbr. *Bulletin de l'Herbier Boissier* 5: 541. 1905.

Species description: Hale (1976c)

Specimens examined: Ilhabela, Ilhabela State Park, track close to the meteorological station near the lookout, 04.IX.2012, 23°47'S, 45°21'W, leg., M.N. Benatti, L. Moro, M. Boro & C. Mayumi 3365.

The only specimen collected was fertile with plenty of mature apothecia. This species commonly present hyaline, simple, ellipsoid to subglobose ascospores that measure (8–)10–15(–16) × 8–12 µm, and does not produce neither soredia nor isidia. Its morphological and chemical varieties are discussed in detail by Jungbluth (2006), but overall the single specimen examined herein was similar to those described by this author for Cerrado areas of São Paulo state. The chromatographic analysis showed the presence of atranorin and also of an unidentified substance (with a well-marked spot)

with Rf 43 in the eluent 2. According to literature and simultaneous experiments, this substance might be 2,4-di-O-metilgíroforic acid, also found by Jungbluth (2006) for *P. damaziana* growing on Cerrado vegetation. Besides the perceptible but evanescent medullary reaction KC⁺ reddish-rose, other components, even possibly gíroforic acid, were at too low concentrations in the extract to be detected.

8. *Parmelinopsis cf. minarum* (Vain.) Elix & Hale. *Mycotaxon* 29: 243. 1987. MB 130590.

Parmelia minarum Vain., *Acta Societatis pro Fauna et Flora Fennica* 7: 48. 1890.

Species description: Benatti (2012e).

Specimens examined: Ilhabela, Parque Estadual de Ilhabela, track close to the meteorological station near the lookout, 4.IX.2012, 23°47'S, 45°21'W, leg., M.N. Benatti, L. Moro, M. Boro & C. Mayumi 3358, 3368, 3371; Municipal plant nursery Cachoeira da Água Branca, 6.XII.2012, 23°47'S, 45°21'W, leg., M.N. Benatti, L. Moro & C. Mayumi 3447; eastern part of the island, around Praia de Castelhanos and restinga with houses, 14.V.2013, 23°47'S, 45°21'W, leg., M.N. Benatti, H. Schoenlein-Crusius, L. Moro & C. Mayumi 3500, 3509; Cocaia track, road to Costabela, 15.V.2013, 23°47'S, 45°21'W, leg., M.N. Benatti, I.H. Schoenlein-Crusius & C. Mayumi 3556.

This species, together with *P. spumosa*, are one of the most common small Parmeliaceae found at the study area. It is easily recognizable in the field due its narrow and short laciniate thallus and the isidiate upper cortex. Most specimens collected, however, consisted of very small thalli of less than 4 cm wide, except for a single one of approximately 7 cm wide. The chromatographic analysis showed a few differences between specimens. Atranorin was overall present but some specimens presented three medullary compounds while others had two substances of the gyrophoric tridepsides group. Analysis by microcrystalization did not show the presence of gyrophoric acid. We were not able to compare the chromatographic behaviour due to the absence of data in the literature, but it appears to be related to the *P. horrescens* complex. In this study, we found only one specimen (M.N. Benatti et al. 3368) that had mature apothecia but unfortunately, the hymenium was damaged. The specimens M.N. Benatti et al. 3500 and 3368 both showed a similar chromatographical profile. The collection of more specimens and the use of other analytical techniques such as Liquid Chromatography-Mass Spectrometry (LC-MS) are needed for a conclusive characterization of these substances.

9. *Parmelinopsis spumosa* (Asahina) Elix & Hale. Mycotaxon 29: 243. 1987. MB 130596.

Parmelia spumosa Asahina, Journal of Japanese Botany 26: 259. 1951.

Species description: Benatti (2012e).

Specimens examined: Ilhabela, Ilhabela State Park, track close to the meteorological station near the lookout, 4.IX.2012, 23°47'S, 45°21'W, leg., *M.N. Benatti, L. Moro, M. Boro & C. Mayumi 3354, 3355, 3359, 3360, 3372*.

This species is easily recognizable by its eciliate thallus with coarse sorediate bursting pustules, differing from similar species such as *P. subfatiszens*, which is also eciliate but has non sorediate pustules. The chromatographical analysis showed some differences between specimens. All specimens examined contained atranorin but most had at least three other medullary compounds (some specimens contained two compounds). The low production of some substances and the leeching of other substances from other lichens present on the same substrate could justify these discrepancies. Using microcrystalization, only one thalli clearly showed presence of gyrophoric acid. Other compounds could not be determined, as documented by Jungbluth (2006) for specimens from Cerrado. The specimens *M.N. Benatti et al. 3360* and *3289* were partially different chromatographically. The specimen *M.N. Benatti et al. 3360* showed presence of three tridepsides together with atranorin, whereas the specimen *M.N. Benatti et al. 3289* showed only two tridepsides with atranorin. Besides this difference, we were not able to make further identifications of substances using microcrystalization.

The substances present in the specimens of both *P. minarum* and *P. spumosa* were tridepsides derived from orcinol (e.g., Hüneck & Yoshimura 1996). However, the chromatographical profiles and microcrystalization tests did not show the presence of gyrophoric acid (although they reacted C+ and KC+ rose- reddish, as expected for this substance in spot tests), even with the eluents tested (I and II) and microcrystal preparations. Again, most specimens were quite small with a few centimetres wide and therefore, they could not be used for further tests without destroying the whole specimens.

10. *Remototrachyna cf. costaricensis* (Nyl.) Divakar & Crespo. Taxon 97: 586. 2010. MB 546535.

Parmelia costaricensis Nyl. Journal of Botany British and Foreign 13: 225.1877.

Species description: Spielmann & Marcelli (2008b).

Specimens examined: Ilhabela, Ilhabela State Park, southern part of the island, 27.VI.2012, 23°53'S, 45°25'W, leg., *M.N. Benatti, L. Moro & M. Boro 3336*; Cachoeira da Toca old farm, 6.XII.2012, 23°47'S, 45°21'W, leg., *M.N. Benatti, L. Moro & C. Mayumi 3408*; Cocaia rack, road to Costabela, 15.V.2013, 23°47'S, 45°21'W, leg., *M.N. Benatti, I.H. Schoenlein-Crusius & C. Mayumi 3531*.

This species was the only Parmeliaceae with dichotomously branched rhizines (previously in *Hypotrachyna* before recent studies confirmed that a group of species comprised another genus) found at the study area. It presented relatively large lacinae (up to 5 mm wide, i.e. broader and more rotund than in other species of *Parmelinopsis*) and abundant isidia. Atranorin and chloroatranorin were not detected by chromatography, possibly due to their low concentrations in the specimens (as the K+ yellow reaction occurred in the spot tests). The chromatography showed also three spots (Rf around 35, 40 and 45 in eluent I) that we were not able to identify. The chemistry variability is yet not fully comprehended (e.g., Nash *et al.* 2002; Louwhoff & Elix 2002; Jungbluth 2006) but this is currently the only known isidiate species with dichotomously branched rhizines that show no medullary reaction to any spot tests (K, C, KC, and P).

The specimens *M.N. Benatti et al. 3531* and *3336* were slightly different chromatographically, but both contained atranorin. The spots revealed with the *p*-anisaldehyde: sulfuric acid indicated that the substances might belong to the class of terpenes, since fatty acids are not revealed with this substance. Microcrystalization also did not show the presence of fatty acids, including protoliqueterinic and caperatic acids. Constipatic acid is very difficult to detect with this technique. Flakus *et al.* (2011) reported the presence of atranorin, constipatic acid complex (\pm , only very rarely absent) and an unidentified pigment (\pm , only in one specimen). Our results are in agreement with the substances reported by Flakus *et al.* (2011) in Bolivian specimens. It is possible that some of the specimens collected in South America and identified as *R. costaricensis* could represent chemotypes, since it is still premature to confirm that they belong to different species.

Aside from our work on the genus *Leptogium* (Kitaura *et al.* 2017) there are no other inventories of lichens in the study area. Therefore, this study adds new and further information to the diversity of

the local mycobiota. In addition, it represents one of the few surveys (e.g., Benatti 2012b,d) performed hitherto in areas of the Atlantic Rainforest.

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