

Parmotrema hydrium, a new species of Parmeliaceae in southeastern Brazil

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Received: 9 May, 2013. Accepted: 14 August, 2013

ABSTRACT

Parmotrema hydrium is a new lichen species discovered during a survey of Parmeliaceae in the Cantareira mountain range of southeastern Brazil. In its medullary chemistry, the species contains several fatty acids and other substances, including small amounts of atranorin, typically restricted to the soralia and young lobes.

Key words: cilia, Parmeliaceae, *Parmotrema hababianum*, *Parmotrema grayanum*, soredia

Introduction

The parmelioid lichen genus *Parmotrema* A. Massal. (Parmeliaceae) is characterized by broad, rotund lobe apices, the absence of pseudocyphellae, the frequent occurrence of marginal cilia, a naked lower surface at the margins, simple rhizines, and thick-walled, ellipsoid ascospores (Brodo *et al.* 2001, Nash & Elix 2002). More than 300 species are known worldwide (Nash & Elix 2002), with over 90 cited in the earliest list for Brazil (Marcelli 2004), which was recently expanded to include over 120 species (Benatti & Marcelli 2008, 2009a, 2009b, 2010, 2011; Benatti *et al.* 2008, 2010; Donha & Eliasaro 2006; Eliasaro & Donha 2003; Gerlach & Eliasaro 2012; Marcelli & Benatti 2008, 2010a, 2010b, 2011; Marcelli *et al.* 2007, 2008, 2011; Spielmann & Marcelli 2009). The medullary chemistry is highly variable, representing many chemosyndromes and including a number of unknown substances.

Here, we describe a new species containing undetermined several fatty acids (without most of the common acids found as medullary substances, except for what is probably atranorin in soralia and young lobes). This species was discovered during a survey of the Parmeliaceae species occurring in the Cantareira mountain range, located north of the city of São Paulo, within the state of São Paulo, in southeastern Brazil. The new species has rather rounded lobes, is marginally sorediate and has simple cilia. Only corticolous specimens were found.

Material and methods

Morphological characters were studied using standard stereoscopic and compound light microscopes. Anatomical

sections were cut by hand with a razor blade. All five specimens examined lacked apothecia and pycnidia. The chemical constituents were initially checked by spot tests with potassium hydroxide (K), sodium hypochlorite (C) and para-phenylenediamine (P), as well as being examined under UV light (360 nm). Subsequently, chemical constituents were identified by thin-layer chromatography (TLC) using solvent C (Bungartz 2001), following the standard methods described in Elix & Ernst-Russell (1993) and Orange *et al.* (2001). Samples were also examined by toluene, ethyl acetate, acetic acid (6:4:1 v/v). The samples were submitted to TLC together with all substances commonly found in species with similar morphology (e.g., alectoronic acid, gyrophoric acid and psoromic acid).

For high-performance liquid chromatography (HPLC), pieces of the thallus (ca. 5 × 5 mm) were extracted with 200 µl of methanol at room temperature for 1 h. Extracts were filtered through 0.2 µm polytetrafluoroethylene filter plates (Acroprep Advance; Pall Corporation, East Hills, NY, USA) and diluted 10-fold with methanol. Samples were analyzed in a liquid chromatograph (1260; Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, an incorporated degasser and diode array detection. Substances were separated on a Poroshell 120 EC-C18 column (2.7 µm, 3.0 × 50.0 mm; Agilent Technologies) regulated to 30°C, at a flow rate of 1.4 ml/min. Two solvent systems were employed. Solvent A was aqua bidest (LiChrosolv[®]; Merck, Darmstadt, Germany) containing 30% methanol and 0.0625% trifluoroacetic acid; and solvent B was 100% methanol. The methanol and trifluoroacetic acid were obtained from VWR International (HiPerSolv CHROMA-NORM[®]; BDH Prolabo, Lutterworth, UK). The HPLC system was equilibrated to solvent A for 2 min. After washing

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the needle automatically in 100% methanol, we injected 10 µl of extract. The run started with 100% solvent A and continued isocratically for 0.18 min at a flow rate of 1.4 ml/min. After 0.18 min, solvent B was increased to 58% within 5 min, then up to 100% in 5 min, and then isocratically in 100% solvent B for an additional 0.82 min. At the end of the run, solvent A was increased to 100% within 0.5 min and the column was flushed with 100% solvent A for 2 min before a new run was started.

The compounds were detected at 210, 254, 280 and 310 nm and the spectra of each peak ($\lambda = 190\text{--}650$ nm, in 2-nm steps) were recorded automatically. Spectra and retention time were computer-matched against a library of spectra from authentic metabolites derived under identical conditions using OpenLAB CDS ChemStation software (Agilent Technologies).

The new species

Parmotrema hydrium Benatti, Gernert & Schmitt, sp. nov.
Figs 1-3

MycoBank: MB 803849

Diagnosis: Similar to *Parmotrema hababianum* but with soralia labriform to mostly irregularly linear, rather than capitate to labriform, and containing several fatty acids in the medulla, with atranorin restricted to soralia and some young lobes.

Holotype: Brazil, city and state of São Paulo, Cantareira mountain range, Cantareira State Park, on tree alongside the Pé-de-Galinha road to Mairiporã, leg. M.P. Marcelli, O. Yano, A. Rezende & F.M.M. Coppola 11580, 25-VI-1991 (SP).

- Thallus: up to 7.0 cm wide, subcoriaceous, corticolous, greenish gray to dusky gray in the herbarium, lobate
- Lobes: irregularly branched, 1.5-6.5 mm wide (very rarely, ~ 11.5 mm wide), contiguous to overlapping laterally, adnate to slightly raised, loosely attached
- Apices: \pm plane, \pm rounded
- Margin: flat, sinuous to crenate or subirregular, entire to slightly incised, occasionally sublacinulate, ciliate, axils oval
- Upper surface: smooth and continuous, occasionally with irregular transverse cracks on older parts.
- Maculae: absent
- Adventitious marginal lacinules or lobules: absent to scarce, short, simple, flat, $0.1\text{--}0.5 \times 0.5\text{--}0.8$ mm, truncate to subtruncate, occasionally ciliate, underside concolorous with the lower marginal zone
- Soredia: farinose to subgranular, often darkening and becoming blackish, soralia labriform to sublinear, marginal or occasionally formed on the apices of the lacinulae, occasionally laminal and capitate on younger lobes
- Pustules and isidia: absent
- Cilia: black, with simple apices, $0.3\text{--}1.5 \times \text{ca } 0.03$ mm, frequent along the margins appearing on the crenae, dispersed and intercalate with the soralia

- Medulla: white, without K+ purple pigmented spots
- Lower surface: black, shiny, smooth to rugose or slightly veined, moderately rhizinate
- Marginal zone: shiny, pale brown and attenuate, 0.5-2.0 mm wide, usually ivory or variegated and distinct on sorediate lobes, smooth to subrugose or slightly veined, weakly papillate, becoming rhizinate in the transition zone towards the center
- Rhizines: black, simple, $0.1\text{--}1.2 \times \text{ca } 0.05$ mm, irregularly grouped, scarce to frequent or abundant
- Apothecia and pycnidia: not seen

Spot reactions: upper cortex K+ yellow, UV-; medulla K- to variably K+ yellow, C-, KC-, P- to variably P+ sulfur yellow/yellow-orange at soralia or occasionally and randomly on younger lobes, UV-. The K+ and P+ reactions might be due to concentrations of atranorin in different parts, particularly the young lobes and soralia.

TLC: cortical atranorin; medullary unidentified substances, possible traces of terpenoids and sterols.

HPLC: Each sample analyzed contained atranorin, chloroatranorin and lichesterinic acid. For some of the samples, additional substances were detected: SP264958 and SP264968 (protolichesterinic acid); SP416110 (unknown substance, possibly a depside); thuringione; protolichesterinic acid; unknown substance, SP265140 (thuringione and protolichesterinic acid)

Additional specimens examined: Brazil, city and state of São Paulo, Cantareira mountain range, Sítio da Cachoeira, on tree exposed to direct sun, leg. M.P. Marcelli, O. Yano, A. Rezende & F.R.C. Lourenço 9771, 9772, 20-VII-1990 (SP); idem, on shaded tree next to a spring, leg. M.P. Marcelli, O. Yano, A. Rezende & F.R.C. Lourenço 9791, 20-VII-1990 (SP); idem, Cantareira State Park, on tree trunk in the woods, leg. M. N. Benatti 1066, VI-2000 (SP).

Comments: This species is morphologically somewhat similar to *Parmotrema hababianum* (Gyel.) Hale. The development of the soralia is somewhat similar, initially labriform and evolving to sublinear, unlike those of *P. perlatum*, which typically have a "pearl necklace" aspect. The medullary chemistry of the new species is also distinct.

Results and discussion

Parmotrema hydrium was shown to have a distinctive medullary chemistry, containing several fatty acids as well as traces of medullary terpenoids and sterols, without the more common acids often found in *Parmotrema* species with similar morphological characteristics, e.g. alectoronic acid (*P. rampoddense*), psoromic acid (*P. direagens*), stictic acid (*P. perlatum*), gyrophoric acid (*P. sancti-angelii*) (Benatti & Marcelli 2009a, 2010; Brodo *et al.* 2001; Divakar & Upreti 2005; Elix 1994; Hale 1965; Nash & Elix 2002; Spielmann & Marcelli 2009; Swinscow & Krog 1988). Although it was not the main substance found in all specimens, some samples contained protolichesterinic acid, like the morphologically similar *P. hababianum*



Figure 1-3. Holotype of *Parmotrema hydrium*. 1. Habitus. 2. Detail of the marginal soralia and cilia. 3. Detail of spot tests on the medulla and soralia (arrows). Scale bars = 1 cm.

(norlobaridone and protolichesterinic acid) or *P. grayanum* (protolichesterinic acid only), which might indicate that the new species is associated with this group. The substances were tested together with the samples used in different solvents of TLC, and not even traces of other commonly found substances were detected, as confirmed by HPLC.

Positive K and P spot tests were irregular in *Parmotrema hydrium*, usually being positive only at the soralia and in random areas of younger lobes, and even variable on a single thallus. Even the yellowish reactions of these reagents differed from those normally observed in speci-

mens of other *Parmotrema* species with atranorin. In *P. hydrium*, the yellow color of the K and P reactions were close to sulfur yellow, darkening to yellow-orange after some time. The reactions were often negative or weak in the older parts of the thallus, or even at some of the soralia. The possibility of atranorin concentrations at soralia and young lobes was discussed with colleagues, who also judged that this could be the only explanation for the positive K and P reactions, because the TLC and HPLC had revealed no other substances that could account for the reaction. Atranorin is an UV-negative substance that runs

highest on the TLC plate and, because of this distance, can become quite diluted.

Because of the absence of clearly recognizable secondary metabolites in the TLC, the specimens were initially impossible to identify. They were in good condition, so the possibility that they were lacking substances due to deterioration could be ruled out. It was not possible to determine whether lichesterinic and protolichesterinic acid represented trace, minor or major substances, because of the limited quantity of material, which had already been sampled extensively in our attempts to elucidate the secondary metabolite composition. The following species are most similar:

- *Parmotrema praesorediosum*: The overall labriform aspect of the soralia is initially similar to those observed in *P. praesorediosum*, but soon becomes more linear. In addition, *P. praesorediosum* is eciliate and contains different medullary fatty acids, namely praesorediosic and protopraesorediosic acids (Divarkar & Upreti 2005; Hale 1965; Marcelli & Benatti 2010b; Nash & Elix 2002; Spielmann & Marcelli 2009; Swinscow & Krog 1988).
- *Parmotrema hababianum*: In *P. hababianum*, the marginal zone is mostly white at the lower cortex, whereas a white margin is seen in *P. hydrium* only at the lobes bearing soralia, as is common in most sorediate *Parmotrema* species. The medullary chemistry is distinct, *P. hababianum* containing medullary protolichesterinic acid, as well as norlobaridone (KC+, rose), and having no parts that react to K or P tests (Hale 1965).
- *Parmotrema grayanum*: Although *Parmotrema hydrium* is somewhat similar to *P. grayanum*, the latter is a saxicolous species that also contains only protolichesterinic acid in the medullary chemistry and does not present K or P test positivity in any of its parts (Hale 1965; Divakar & Upreti 2005; Elix 1994; Swinscow & Krog 1988). The soralia of *P. hydrium* are closer to those of *P. praesorediosum* (more labriform than linear), whereas *P. grayanum* is ciliate, with coarse thick cilia.

Overall, the new species is morphologically closer to *Parmotrema hababianum*, although somewhat chemically closer to *P. grayanum*. However, there are some issues regarding the specimens found: they all are corticolous, whereas all citations of *P. grayanum* mention only saxicolous specimens; K+ and P+ yellow-orange reactions were found for soralia and young lobes, such reactions being unknown for *P. grayanum* and atranorin being the only substance detected by both TLC and HPLC that could explain those reactions; and (according to the literature) *P. grayanum* contains only protolichesterinic acid as a medullary substance.

As described by Hale (1965), *Parmotrema grayanum* is a whitish gray, commonly pruinose, coriaceous lichen, with coarse, thick marginal cilia. The specimens of the new species found do not share these features. The type specimen of *P. grayanum* is purportedly in an herbarium in Paris, France (PC). Unfortunately, requests sent to the herbarium went

unanswered. There are no known isotypes or paratypes. The type locality of *P. grayanum* is Neilgherries, India.

Among other similar species, *Parmotrema ciliiferum* differs from *P. hydrium* by its more irregular, linear marginal soralia, the medullary chemistry (*P. ciliiferum* containing constipatic acid and not atranorin, lichesterinic acid or protolichesterinic acid) and a total absence of positive spot test reactions in any of its parts.

Parmotrema mordenii is apparently the only other species that has medullary atranorin (the reactions are even more uniform throughout the medulla) but is an eciliate, saxicolous species (Marcelli & Benatti 2010b).

The soralia spot test results for *Parmotrema hydrium* were very similar to those reported for *P. direagens*, another marginally sorediate and ciliate species, which differs in that it has much broader lobes (6.0-12.0 to 10.0-20.0 mm wide) and contains psoromic acid in its medullary chemistry (Divarkar & Upreti 2005; Elix 1994; Hale 1965; Swinscow & Krog 1988).

All *Parmotrema hydrium* specimens were corticolous. At present, this species is known only from the type region, a mountainous area of Atlantic Forest in somewhat open areas mostly at 1500-2200 m in elevation. The species is named after the Cantareira mountain range (*Serra da Cantareira* in Portuguese, literally translated as "Pitcher mountain range"), where the specimens analyzed were originally collected.

Acknowledgments

The authors wish to thank Neli Kika Honda, of the *Universidade Federal de Mato Grosso do Sul* (UFMS, Federal University of Mato Grosso do Sul), for the TLC data; John Alan Elix and Marcelo Pinto Marcelli, for their comments and suggestions; the peer reviewers, for their critical review of the manuscript; and John Alan Elix, for the revision of the English. This study received financial support from the German state of Hessen, via the Integrative Fungal Research Program of the *Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz* (LOEWE, State Offensive for the Development of Scientific and Economic Excellence), funded by the Hessian State Ministry of Higher Education, Research and the Arts. The first author also thanks FAPESP (Fundação para o Amparo à Pesquisa do Estado de São Paulo) for the grant nº 00/01009-1.

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