

## An accurate circumscription of the poorly known *Punctelia negata* (Nyl.) Krog

Luciana S. Canêz<sup>1</sup>, Adriano A. Spielmann<sup>1</sup>, Marcelo P. Marcelli<sup>2</sup> & John A. Elix<sup>3</sup>

<sup>1</sup>Universidade Federal de Mato Grosso do Sul, Centro de Ciências Biológicas e da Saúde, Laboratório de Botânica-Liquenologia, Caixa Postal 549, CEP 79070-900, Campo Grande, Mato Grosso do Sul, Brazil. canez.luciana@gmail.com

<sup>2</sup>Instituto de Botânica, Núcleo de Pesquisa em Micologia, Av. Miguel Stéfano 3687, CEP 04301-902, São Paulo, Brazil

<sup>3</sup>Australian National University, Research School of Chemistry, Building 137, Canberra, A.C.T. 2601, Australia

Recebido em 03.III.2015

Aceito em 28.X.2016.

**ABSTRACT** – The holotype of *Punctelia negata* and its isotypes were studied and compared to similar species. The type study confirmed that *P. negata* has a pale lower surface and lobules on the upper surface rather than a black lower surface and no vegetative propagules, as reported previously. Brazilian collections identified as *P. negata* present a black lower surface; therefore should be referred to *P. borrerina* (Nyl.) Krog. At present, *P. negata* is only known from the type locality in Colombia.

**Keywords:** Brazil, Colombia, *Punctelia borrerina*

**RESUMO** – Uma circunscrição precisa para a espécie pouco conhecida *Punctelia negata* (Nyl.) Krog. O holótipo de *Punctelia negata* e seus isótipos foram estudados e comparados com espécies relacionadas. O estudo dos tipos mostrou que a espécie tem superfície inferior castanha clara e produz lóbulos na superfície superior ao invés de superfície inferior negra e sem propágulos como reportado previamente na literatura. As coleções brasileiras identificadas como *P. negata* apresentam superfície inferior negra e devem ser referidas como *P. borrerina* (Nyl.) Krog. Até o presente momento, *P. negata* é conhecida apenas para a localidade tipo na Colômbia.

**Palavras-chave:** Brasil, Colômbia, *Punctelia borrerina*

### INTRODUCTION

*Punctelia* (Parmeliaceae) was first proposed by Krog (1982) to include 18 species but currently represents 46 taxa (Egan & Aptroot 2004, Kalb 2007, Canêz & Marcelli 2007, 2010a, b, Marcelli et al. 2009, 2011, Lendemer & Hodkinson 2010, Hodkinson & Lendemer 2011), which are characterized by the presence of pseudocyphellae in the upper cortex, filiform or unciform conidia, and the presence of cortical atranorin and lecanoric, gyrophoric or fatty acids in the medulla.

The color of the lower surface has been used as a key species character since the genus was first proposed (Hale 1965, Krog & Swinscow 1977) and some current molecular data corroborate this approach (Crespo et al. 2004, Lendemer & Hodkinson 2010). Of all the *Punctelia* species, seventeen have a black lower surface but only six lack vegetative propagules (Canêz 2009, Canêz & Marcelli 2010b).

*Punctelia* [*Parmelia*] *negata* was described by Nylander (1872) in a footnote with a very brief diagnosis and the Lindig's specimen as a collection. He differentiated the new species from *Parmelia* [*Punctelia*] *rudecta* Ach. and *Parmelia* [*Punctelia*] *borreri* Smith ex Turner, by longer ascospores, which are 18–21 × 9–11 μm in *P. negata*, while *P. borreri* has the ascospores 11–15 × 8–11 μm and *P. rudecta* 14–18 × 9–12 μm. Nylander also added as

character the presence of more rugose amphithecia and longer conidia in *P. negata*, without giving the size of the conidia. The color of the lower surface was not used by Nylander (1872) for distinguishing these three taxa, but it was treated as having a black lower surface by Krog (1982).

Without examining the type, Lyngby (1914) distinguished *Punctelia negata* from other, mainly Brazilian species, *P. riograndensis* (Lyngby) Krog and *P. microsticta* (Müll. Arg.) Krog, by its supposed isidiate thallus. Later on, Krog (1982) included *P. negata* in her key as having a black lower surface but not producing vegetative propagules.

One hundred twenty-six years after Nylander described *P. negata*, Ribeiro (1998) cited specimens of this species from the Minas Gerais and São Paulo states in Brazil. Subsequently, Mazzitelli et al. (1999) mentioned *P. negata* specimens from Rio Grande do Sul state and Eliasaro (2001) from Paraná state in Brazil. All of these authors considered the species to lack vegetative propagules and to have a black lower surface as presented according to Krog (1982).

Recent information on the type of *Parmelia negata* has not been published and there is no detailed description of this species in the literature for comparing samples. Here we provide such a description as well as taxonomic and geographic notes on this poorly known South American species.

## MATERIAL AND METHODS

We studied type material of *Punctelia negata* from H, M and FH-TUCK as well as the types of morphologically related species. Furthermore, we were able to study material from Rio Grande do Sul and Paraná States, identified as *P. negata*, from HAS and UPCB.

The specimens were described according to the set of morphological characters and protocol used by Canêz & Marcelli (2006) and Canêz (2009), ascospores and conidia were measured on specimens mounted in water under a light microscope.

All specimens were examined under UV light for fluorescence and spot tests were made. The reagents potassium hydroxide (K), sodium hypochlorite (C) and para-phenylenediamine (P) were used. Thin Layer Chromatography (TLC) was carried out with solvent C (toluene and acetic acid – 170: 30) and microcrystallization with G.E. (glycerin and glacial acetic acid – 1:3) and G.A.W. (glycerin, alcohol and water – 1:1:1), following Huneck & Yoshimura (1996). Whenever necessary, the chemistry of the type materials was determined by High Performance Liquid Chromatography (HPLC) according Elix *et al.* (2003).

## RESULTS AND DISCUSSION

### The type specimens

In comments under *Punctelia subpraesignis* (Stirton) Krog, Krog & Swinscow (1977) considered the holotype of *P. negata* to be present in H-NYL with an isotype in M, possibly influenced by Hale, who labeled the respective specimens as holotype (in 1959) and lectotype (in 1961). However, Hale never published this information and Nylander (1872) mentioned just the exsiccate Lindig 735 in the protologue, with no reference to any herbarium, but there are exsiccates housed in several herbaria (see comments below).

The holotype (Fig. 1A) is a very minute specimen c. 1.5 cm broad that has a half an apothecium and is glued onto a card. The card has Nylander's handwriting on chemistry and diagrams of ascospores and conidia. The isotype (Fig. 1B) in M, identified initially as *Parmelia borrieri* var. *rudecta* (Ach.) Tuck., is more representative than the holotype and has the original label from its collector, Lindig. The specimen is composed of seven fragments, including apothecia. By carefully studying this material, it became obvious that the H-NYL fragment is a small part of this larger collection.

Another specimen from the same collection and labeled as "Ex-herbarium Lindig n° 735" was found in the Tuckerman herbarium (FH-TUCK) identified as *Parmelia borrieri* var. *rudecta* (= *P. rudecta*) (Fig. 1C). In 1967, Hale labeled it as an "isotype of *Punctelia negata*" and, in fact, the specimen has the same features as the holotype and as recorded in the protologue, therefore it is an isotype that has never been published before. Prior to publishing the

new species, this specimen Lindig 735 was cited under *Parmelia borrieri* var. *rudecta* by Nylander (1863) along with a second specimen, Lindig 2547, both from Colombia. Unfortunately, we were unable to locate the latter specimen.

### The misunderstood history of *Punctelia negata*

Although Nylander's (1872) diagnosis is in accordance with the International Code of Nomenclature of Algae, Fungi and Plants, it is not currently helpful in differentiating *P. negata* from other *Punctelia* species. He used two quite distinctive species for comparison with *P. negata*: *P. rudecta* (holotype in H!), an isidiate species with lecanoric acid and *P. borrieri* (holotype in BM!), a sorediate species with gyrophoric acid.

After studying the holotype and isotypes we realized that *Punctelia negata* has lobules on the upper surface and a pale brown lower surface, together with long-filiform conidia, ascospores longer than 20 µm and a C- medulla, producing neither soredia nor isidia. However, over the years, the poor diagnosis coupled with the absence of type studies and the lack of any other specimens contributed to mistakes in the species concept.

Nylander's protologue does not clarify whether *P. negata* is an isidiate species nor the color of the lower surface, but interestingly Lynge concluded that *P. negata* was an isidiate species with black lower surface, probably following Nylander's comparison with *P. borrieri* and *P. rudecta*. According to Krog (1982) *P. negata* has black lower surface, filiform conidia and no vegetative propagules. After Nylander's publication, Lynge and Krog were the only authors to mention *P. negata*; both just presented keys, without any detailed description or comments on the species.

One specimen from Peru (Matucana, on rocky slope, leg. G.S. Bryan 67, March 14<sup>th</sup>-18<sup>th</sup>, 1929) housed in FH was determined by Hale in 1971 (in a label) as *Punctelia* cf. *negata*. Unfortunately, this material is now just a powder comprising a mixture of C+ rose and C- fragments, so it was not possible to determine this specimen.

Unfortunately, the specimens recorded from Minas Gerais and São Paulo State by Ribeiro (1998) were not found in SP herbarium. However, the specimens cited by Mazzitelli *et al.* (1999) from Rio Grande do Sul and Eliasaro (2001) from Paraná were studied. These authors identified the specimens according to Krog (1982). The specimens have a black lower surface and lack propagules, so they do not correspond to *P. negata* s. st. Such Brazilian specimens are better identified as *P. borrierina* (Nylander) Krog, an elobulate species with a black lower surface. Furthermore, they have long-filiform conidia, ascospores shorter than 20 µm and fatty acids in the medulla.

Thus, according to our studies, the three specimens in H-NYL (holotype), M and FH-TUCK (isotypes) are the only representatives of this species, currently known just from the type locality in Colombia. We have tried, unsuccessfully, to obtain more Colombian material from different herbaria, but none was located.

*Punctelia negata* (Nyl.) Krog, Nordic Journal of Botany 2: 291. 1982. *Parmelia negata* Nyl., Flora 55: 547. 1872. Type: Colombia, Bogotá, Nova Granata, 2600 m alt., 1860, leg. A. Lindig 735 [H-NYL! 35038 – holotype, M!, FH-TUCK! – isotypes].

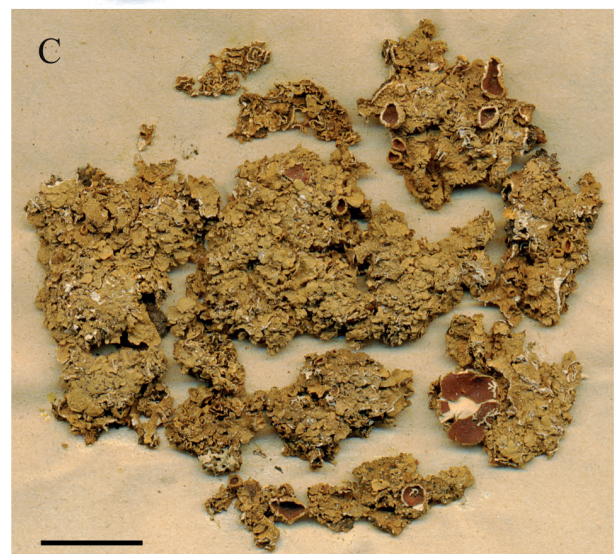
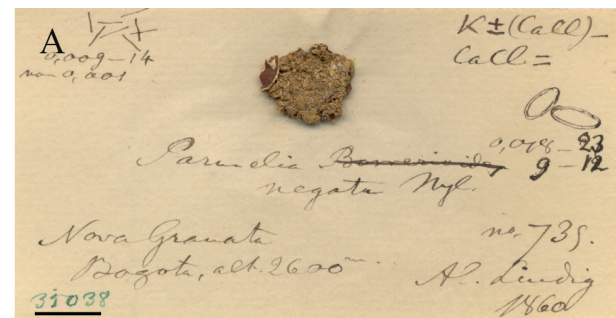
(Figs. 1A - C)

Thallus lobate, 1.3 × 1.2 cm wide; lobes irregularly branched, lobes (1–) 2–3 mm wide, adnate, crowded, adpressed, apices rounded, margins with an inconspicuous black line, smooth, crenate. Upper surface continuous, emaculate, smooth or becoming rugose towards the center, lobulate; pseudocyphellae inconspicuous, abundant, punctiform, plane, 0.03–0.06 mm diam., laminal or rarely marginal. Lobules densely crowded over the thallus, the larger ones approaching the dimensions of some lobes, (0.15–) 0.3–0.45 (–0.75) × 0.15–0.42 (–0.6) mm, plane, apices rounded, laminal and a few marginal pseudocyphellae present, lower cortex present, pale brown to dark brown. Soredia and isidia absent. Medulla white, K+ purple pigment absent. Lower surface pale brown or dark brown in some areas (especially at the apices of the lobules), opaque, densely papillate to smooth in some areas; marginal zone the center, ±becoming dark brown, opaque, smooth, naked to rarely papillate; rhizines sparse, concolorous with the lower surface, rarely darker, simple, 0.15–0.3 × 0.03–0.06 mm. Apothecia plane or slightly concave, 1.5–3.8 (–6) mm diam., sessile, submarginal, margin smooth, amphithecia pseudocyphellate and irregularly rugose, when immature smooth and lacking pseudocyphellae, disc dark brown, epruinose; epihymenium 20–25 µm thick, hymenium 82–87 µm high, subhymenium 62–67 µm thick; ascospores ellipsoid, 19–23 × 9–10 µm. Pycnidia laminal, ostiole black; conidia long-filiform, 10–13 × 1 µm. Anatomy thallus 82–95 µm thick, upper cortex 15–20 µm thick, algal layer 17–25 µm thick, medulla 37 µm thick, lower cortex 12 µm thick.

**Spot test:** Upper cortex K+ yellow, UV–; medulla K–, C–, KC–, P–, UV–. Atranorin (Hale's label in 1959); atranorin and fatty acids as in *P. reddenda* (Stirt.) Krog (Krog's label in 1978).

HPLC (isotype in M): constipatic acid [major], protoconstipatic acid [major], dehydroconstipatic acid [minor], protodehydroconstipatic acid [minor], atranorin [minor], chloroatranorin [minor].

**Remarks:** *Punctelia negata* is characterized by the presence of lobules on the upper surface, a pale brown lower surface, long-filiform conidia, ascospores 19–23 µm long, and a C– medulla. *Punctelia negata* is similar to *P. bolliana* (Müll. Arg.) Krog (lectotype in G!), which also has a C– medulla but is readily distinguished by its unciform conidia, shorter ascospores (11–16 µm long) and the presence of marginal lacinules (longer than they are wide) rather than laminal lobules (wider than long). Furthermore, the pseudocyphellae in *P. bolliana* are



Figs. 1 A-C. A. Holotype of *Punctelia negata* in H-NYL; B. Isotype in M; C. Isotype in FH-TUCK. Bars = 10 mm.

restricted to the amphithecia and apices of the lacinules, while the pseudocyphellae in *P. negata* are abundant all over the upper surface, including the lobules. *Punctelia purpurascens* Marcelli & Canêz is also similar but differs in having unciform conidia and in containing a pale yellow, K+ purple medullar pigment in the distal areas of the thallus. *Punctelia nebulata* Elix & J. Johnston (holotype in CANB!) has a pale lower surface, C– medulla and filiform conidia, but differs in having very sparse pseudocyphellae,

a strongly plicate-rugose upper surface and no lobules. Both *P. hypoleucites* (Nyl.) Krog (lectotype in H-NYL!) and *P. graminicola* (B. de Lesd.) Egan (lectotype in ASU!) also have pale lower surface. However, both species are distinguished by producing lecanoric acid as the major medullar substance (C+ rose).

### ACKNOWLEDGEMENTS

We would like to thank the curators of the following herbaria for the loan of critical collections: BM, CANB, FH-TUCK, F, H, H-NYL, M, HAS, SP, and UPCB. The first author wishes to thank the Fundação de Amparo a Pesquisa do Estado de São Paulo for the Doctoral scholarship, the International Association for Plant Taxonomy, as well as the American Bryological and Lichenological Society for their financial support. The third author has been supported by a research grant of Conselho Nacional de Desenvolvimento Científico e Tecnológico. The authors are also grateful to Dr. Teuvo Ahti (Finland) for his valuable assistance and also to Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul.

### REFERENCES

- Canêz, L.S. 2009. Estudos taxonômicos em *Punctelia* (Parmeliaceae, Ascomycetes liquenizados) Thesis 274 p. Instituto de Botânica, São Paulo.
- Canêz, L.S. & Marcelli, M.P. 2006. Gêneros de Parmeliaceae (Ascomycetes Liquenizados) na localidade de Fazenda da Estrela, Vacaria, Rio Grande do Sul, Brasil. Caderno de Pesquisas. Série Biologia 18:38-74.
- \_\_\_\_\_. 2007. Two new species of *Punctelia* (Parmeliaceae) from Southern Brazil. Mycotaxon 99:211-216.
- \_\_\_\_\_. 2010a. *Punctelia osorioi*, a new species of *Parmeliaceae*. Mycotaxon 111:45-49.
- \_\_\_\_\_. 2010b. The *Punctelia microsticta*-group (*Parmeliaceae*). The Bryologist 113:728-738.
- Crespo, A., Divakar, P.K., Argüello, A., Gasca, C., & Hawksworth, D.L. 2004. Molecular studies on *Punctelia* species of the Iberian Peninsula, with an emphasis on specimens newly colonizing Madrid. Lichenologist 36:299-308.
- Egan, R.S. & Aptroot, A. 2004. *Punctelia*. In Lichen Flora of the Greater Sonoran Desert Region (Nash III T. H., Ryan, B., D., Diederich, P., Gries, C. & Bungartz, F., eds). Lichens Unlimited, Arizona State University, Tempe, Arizona, v. 2, p.431-436.
- Eliasaro, S. 2001. Estudio taxonomico y floristico sobre las Parmeliaceae sensu stricto (Ascomycota Liquenizados) del Segundo Planalto del Estado de Paraná, Brasil. Thesis 97p., Universidad de Buenos Aires, Buenos Aires.
- Elix, J.A., Giralt, M. & Wardlaw, J.H. 2003. New chloro-depsides from the lichen *Dimelaena radiata*. Bibliotheca Lichenologica 86:1-7.
- Hale, M.E. Jr. 1965. Studies on the *Parmelia borrieri* group. Svensk Botanisk Tidskrift 59(1):37-48.
- Hodkinson, B.P. & Lendemer, J.C. 2011. *Punctelia eganii*, a new species in the *P. rudecta* group with a novel secondary compound for the genus. Opuscula Philolichenum 9:35-38.
- Huneck, S. & Yoshimura, I. 1996. Identification of lichen substances. Springer. Berlin. 493 p.
- Kalb, K. 2007. New or otherwise interesting lichens. Bibliotheca Lichenologica 95:297-316.
- Krog, H. 1982. *Punctelia*, a new lichen genus in the Parmeliaceae. Nordic Journal of Botany 2:287-292.
- Krog, H. & Swinscow, T.D.V. 1977. The *Parmelia borrieri* group in East Africa. Norwegian Journal of Botany 24:167-177.
- Lendemer, J.C. & Hodkinson, B.P. 2010. A new perspective on *Punctelia subrudecta* (*Parmeliaceae*) in North America: previously-rejected morphological characters corroborate molecular phylogenetic evidence and provide insight into an old problem. Lichenologist 42:1-17.
- Lyngby, B. 1914. Die Flechten der ersten Regnellschen Expedition. Die Gattungen Pseudoparmelia gen. nov. und Parmelia Ach. Arkiv för Botanik 13(1):1-172.
- Marcelli, M.P., Jungbluth, P., Elix, J.A. 2009. Four new species of *Punctelia* from São Paulo State, Brazil. Mycotaxon 109:49-61.
- Marcelli, M.P., Canêz, L.S., Benatti, M.N., Spielmann, A.A., Jungbluth, P. & Elix, J.A. 2011. Taxonomical novelties in Parmeliaceae. Bibliotheca Lichenologica 106:211-224.
- Mazzitelli, S.M.A.M., Käffer, M.I., Cardoso, N. 1999. Lichens corticícolas de Porto Alegre, Rio Grande do Sul, Brasil. Iheringia. Série Botânica 52:55-63.
- Nylander W. 1863. Lichenes. Annales des Sciences Naturelles 19-20:286-382.
- \_\_\_\_\_. 1872. Observata lichenologica in Pyrenaeis orientilibus. I. Forca-Réale; II. La Preste-Costabonne. Flora Regensburg 55:545-554.
- Ribeiro, C.H. 1998. A família Parmeliaceae (Ascomycota liquenizados) em regiões montanhosas dos Estados de Minas Gerais, Rio de Janeiro e São Paulo. Dissertation 194p., Universidade de São Paulo, São Paulo.