

The rediscovery of *Xanthoria* (*Teloschistaceae*) in Brazil

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Abstract: After the first collection by Malme in 1892, a specimen of the genus *Xanthoria* was rediscovered growing on the public library wall in a coastal city from southern Brazil. The specimen is morphologically, chemically, and genetically similar to *X. parietina*.

Introduction

Brazil is a megadiverse country in terms of lichen diversity, with about 4.000 described species (Aptroot *et al.*, in prep.), although some well-known genera, like *Xanthoria* (Fr.) Th. Fr., 1860, are rarely found. Belonging to *Teloschistaceae* Zahlbr, one of the largest families of lichenized fungi (Arup *et al.* 2013, Lücking *et al.* 2017), *Xanthoria parietina* (L.) Th. Fr. is a widely studied species, especially in Europe and North America, where it is locally abundant (Brodo *et al.* 2001, Lindblom 1997, Smith *et al.* 2009).

In Brazil the genus *Xanthoria* was reported once (Malme 1926) as *Xanthoria parietina* f. *albicans* (Müll. Arg.) Hillm., to Pelotas, Rio Grande do Sul State. This form was recognized by Hillmann (1920), Malme (1926), and Grassi (1953), having as differential features the thalli whitish in the center, becoming gradually yellow towards the margins (Grassi 1953).

This study reports the rediscovery of the only known foliose *Teloschistaceae* that occur in Brazil. Since it is eye-catching because of its yellow thallus, it is quite surprising that it was found again only now, more than 100 years after Malme's first collection.

Material and Methods

The specimen (A.A. Spielmann 11001) was collected on the walls of a library in Rio Grande municipality, Rio Grande do Sul State, about 60 km from where Malme found and reported the species for the first time. The thalli were collected with a knife and later scanned at 1200 dpi. The macroscopy and microscopy methods followed the standards in Lichenology. The chemical analyses by TLC and micro-crystallization were performed according to Huneck & Yoshimura (1996) and Orange *et al.* (2010).

A small portion of the lichen thallus was sampled, and the DNA was isolated with Wizard Genomic DNA Purification Kit (Promega). The internal transcribed spacer region (nuITS), considered the universal DNA barcode for fungi (Schoch *et al.* 2012; Leavitt *et al.* 2013), was amplified using the ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) primers. The 25 µL PCR reaction contained: 1X Taq buffer (Promega), 0.2mM of dNTP set, 0.2µM of each primer, 25 mM of MgCl₂, 1U of DNA Polymerase (Promega), and ca. 20–50 ng of template DNA. The

PCR conditions were: 2 min at 95°C for initial denaturation, followed by 30 cycles of 30 s at 95°C, 1 min at 58.6°C, 1 min at 72°C, and a 10 min final elongation at 72°C. PCR product was purified with ammonium acetate and sequenced in an ABI 3730 XL DNA Analyzer (Applied Biosystems). Initial analyses using BLAST showed that the most similar hits for the nuITS sequence obtained in this study were from *Xanthoria parietina* specimens. We selected 71 sequences from *X. parietina* with known geographical origin available on GenBank (Supplementary Information). Additional species of the genus were chosen according to Arup *et al.* (2013): *X. aureola* (Ach.) Erichsen; *X. calcicola* Oxner; *X. ectaneoides* (Nyl.) Zahlbr.; *X. mediterranea* Giralt, Nimis & Poelt; *Xanthoria* cf. *stiligera*_KC179409 *X. monofoliola* S.Y. Kondr. & Kärnefelt; and *X. resendei* Poelt & Tav. *Rusavskia elegans* (Link) S.Y. Kondr. & Kärnefelt was used as outgroup (Table 1). Sequences were aligned in Geneious 9.1.2 program (Kearse *et al.* 2012), using the MAFFT plugin v7.308 (Katoh *et al.* 2002) adjusted with the G-INS-i algorithm, scoring matrix 1PAM / k = 2, and remaining parameters set as default as standard. A Maximum-likelihood analysis was implemented in the RAxML 8.2.12 program using the GTRGAMMA model and 1000 replicates of bootstrap (Stamatakis 2014). The resulting tree was visualized using FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results and Discussion

Xanthoria parietina (L.) Th. Fr., Lichenes Arctoi: 69, 1860. Fig. 1A.

≡ *Lichen parietinus* L., Species Plantarum 2: 1143, 1753. Type: Tab. XXIV, fig. 76A in Dillenius, Historia Muscorum, 1742. Lectotype selected by Jørgensen *et al.* 1994: 379, epitype: the corresponding specimen in herb. Dillenius (OXF), according to Lindblom (1997).

Description of the rediscovered specimen. Thallus yellowish, especially in the lobe tips, becoming faint yellowish gray to whitish towards the center, forming rosettes up to 5 cm in diameter; lobes broad, 1-5 mm wide, flat or usually concave near the lobe tips. Soredia or isidia absent. Lower surface whitish, attached to the substrate by sparse, short rhizines. Apothecia abundant, 0.7–2.0 mm in diameter, slightly flat to more frequently concave, with dark yellow discs and thallus-colored margins. Ascospores colorless, polarilocular, 8 per ascus, 11–14 × 5–7 μm. Pycnidia and conidia not seen.

Chemistry. Thallus K+ wine reddish, parietin (major), other anthraquinones (minor) and sterols.

Specimens examined. BRAZIL, RIO GRANDE DO SUL, Rio Grande, Biblioteca Riograndense, 32°01'48.2"S, 52°05'50.5"W, 05 m alt., in the wall of the library, more or less humid site, 06.IX.2012, A.A. Spielmann 11001 (CGMS).

Known distribution. Cosmopolitan. Africa, Asia, Australia, Europe, North America (e.g. Lindblom 1997, Aptroot 2008) and South America, where it was recorded to Argentina (Grassi 1953, Calvelo & Liberatore 2002), Brazil (Malme 1926), Chile (Galloway & Quilhot 1998) and Uruguay (Osorio 1979, 1981, 2001).

Xanthoria parietina is one of the best-known and studied lichens in the world, especially in Europe. Its morphological plasticity is famous, and several varieties and forms were described (e.g. Hillmann 1920). Here we found what would be named *X. parietina* f. *albicans*; however, due to the reasonable amount of morphological variation already known for *X. parietina* and related species (Lindblom 1997; Scherrer and Honegger 2003; Eichenberger 2007), the specimen examined was not assigned to any infraspecific rank.

After the exploratory analyses (Supplementary Information), a subset of 22 sequences of *X. parietina* was chosen, representing the main lineages found and covering a wide geographical range. Phylogenetic analysis revealed that most nuITS sequences belong to a cosmopolitan clade of *X. parietina*, with surprising low divergence among geographically distant specimens (maximum 1.5%). *Xanthoria coomae* S.Y. Kondr. & Kärnefelt and *X. polessica* S. Y. Kondr. & A. P. Yatsyna, recently synonymized as *X. parietina*, are included in this clade (Tsurykau *et al.* 2020).



Figure 1. A – *Xanthoria parietina* (Spielmann 11001). Scale in millimeters. B – Bibliotheca Rio-grandense, the specimens were collected in the front walls.

Nested to this clade are the sequences of *Xanthoria* cf. *stiligera* (KC179409) and *X. monofoliola* (EU681293). Furthermore, as had already been described by Scherrer and Honegger (2003), some specimens identified as *X. parietina* were positioned in a sister clade, here named as Mediterranean-Atlantic Clade (Fig. 2). This clade includes sequences from South Africa, Spain (Mallorca), Italy (Sicily), and the Canary Islands. The Brazilian specimen was positioned in this clade, whose sequences were identical or only 0.4% divergent. These two clades of *X. parietina* were also detected with other loci (hydrophobin gene, beta-tubulin gene, and mtSSU; Eichenberger 2007), reinforcing the hypothesis of more than one species. Eichenberger (2007) highlighted that large lobes and a papery appearance characterize the Mediterranean-Atlantic Clade samples (referred to as “Clade B” samples). Despite the morphological and phylogenetic evidence, this probably new species has never been formally described.

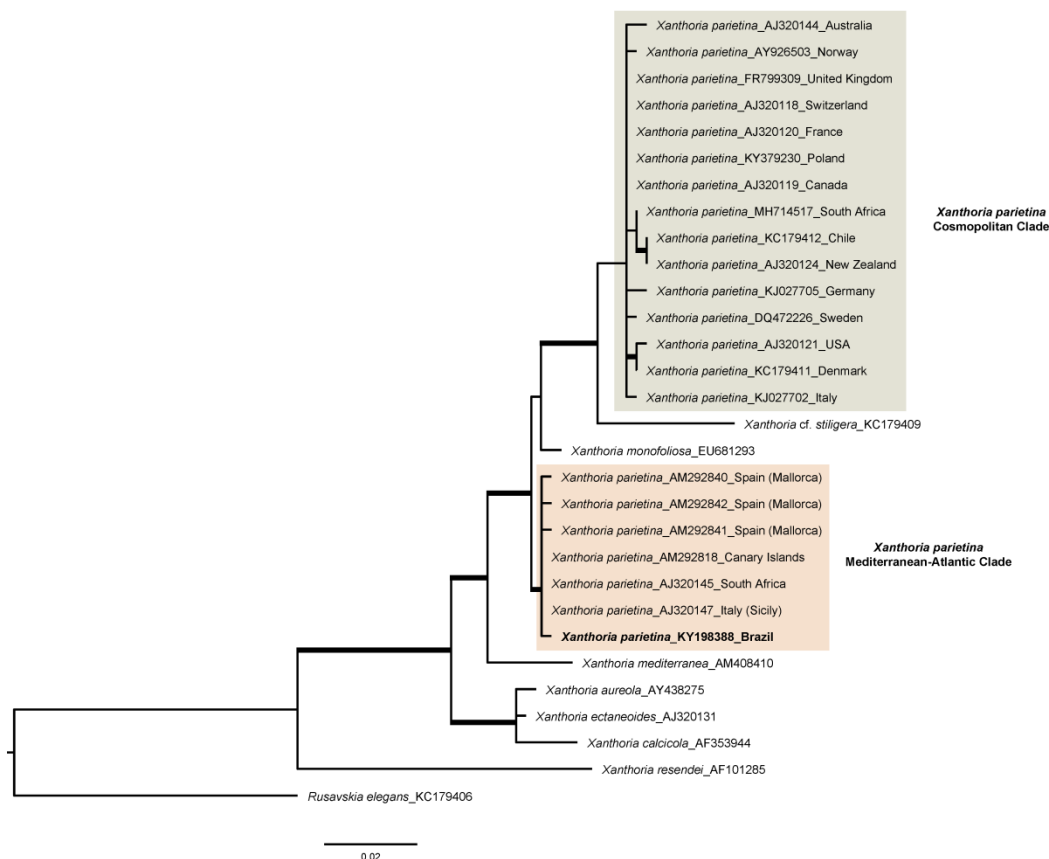


Figure 2. Maximum likelihood tree based on nuITS sequences of *Xanthoria* species. Thicker lines represent branches with bootstrap values ≥ 70 .

How to explain the growth of this species in the south of Brazil? Lindblom (1997) pointed out that in North America, especially in the northern part of the distribution area, *X. parietina* frequently grows on anthropogenic substrates. Despite belonging to a different clade, this is the case of the specimen studied here. Rio Grande was in the past the main entry into the Rio Grande do Sul State, due to the presence of a port in the city. Even Malme, in his First Regnellian Expedition, probably used this way (Malme 1897). Right next to the port of Rio Grande is the “Bibliotheca Rio-grandense” (established in 1846, Wikipédia). The specimens seen here were collected just on the walls of the library (Fig. 1B). Considering that *X. parietina* is an easy species to recognize due

to its yellow color, it is probably rare in southern Brazil. Therefore, as a member of a coastal clade, its distribution pattern may reflect, in addition to their ecological preferences, the role of dispersion influenced by human occupation. However, one has to be in mind that the same species can behave differently in different regions, as pointed out by Aptroot (2004).

Xanthoria parietina is well known as a biomonitor model (Honegger 1996), with considerable morphological variation, generally interpreted as phenotypic plasticity resulting from the thalli growing in different substrate types (Lindblom 1997), for example. However, besides the recollection of this species in Brazil, this work also disclosed that specimens worldwide identified as *X. parietina* might belong to more than one evolutionary lineage, and its delimitation should be revisited.

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Literature

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Table 1. Information about the nuITS database used in this study.

Species	GenBank No.	Accession	Geographical origin	Reference
<i>Rusavskia elegans</i>	KC179406		Russia	Arup <i>et al.</i> 2013
<i>Xanthoria aureola</i>	AY438275		United Kingdom	Lindblom & Ekman 2005
<i>Xanthoria calcicola</i>	AF353944		Sweden	Arup & Grube 1999
<i>Xanthoria ectaneoides</i>	AJ320131		France	Scherrer & Honegger 2003
<i>Xanthoria mediterranea</i>	AM408410		Italy	Eichenberger 2007
<i>Xanthoria monofoliola</i>	EU681293		South Africa	Fedorenko <i>et al.</i> 2009
<i>Xanthoria parietina</i>	KY198388		Brazil	This study
<i>Xanthoria parietina</i>	KJ027702		Italy	Dal Grande <i>et al.</i> 2014
<i>Xanthoria parietina</i>	KJ027705		Germany	Dal Grande <i>et al.</i> 2014
<i>Xanthoria parietina</i>	KY379230		Poland	Felczykowska <i>et al.</i> 2017
<i>Xanthoria parietina</i>	FR799309		United Kingdom	Kelly <i>et al.</i> 2011
<i>Xanthoria parietina</i>	AY926503		Norway	Lindblom & Ekman 2006
<i>Xanthoria parietina</i>	DQ472226		Sweden	Lindblom & Ekman 2007
<i>Xanthoria parietina</i>	AJ320118		Switzerland	Scherrer & Honegger 2003
<i>Xanthoria parietina</i>	AJ320119		Canada	Scherrer & Honegger 2003
<i>Xanthoria parietina</i>	AJ320120		France	Scherrer & Honegger 2003
<i>Xanthoria parietina</i>	AJ320121		USA	Scherrer & Honegger 2003
<i>Xanthoria parietina</i>	AJ320124		New Zealand	Scherrer & Honegger 2003
<i>Xanthoria parietina</i>	AJ320144		Australia	Scherrer & Honegger 2003
<i>Xanthoria parietina</i>	AJ320145		South Africa	Scherrer & Honegger 2003
<i>Xanthoria parietina</i>	AJ320147		Italy (Sicily)	Scherrer & Honegger 2003
<i>Xanthoria parietina</i>	AM292818		Canary Islands	Eichenberger 2007
<i>Xanthoria parietina</i>	AM292840		Spain (Mallorca)	Eichenberger 2007
<i>Xanthoria parietina</i>	AM292841		Spain (Mallorca)	Eichenberger 2007
<i>Xanthoria parietina</i>	AM292842		Spain (Mallorca)	Eichenberger 2007
<i>Xanthoria parietina</i>	MH714517		South Africa	Wirth <i>et al.</i> 2018
<i>Xanthoria parietina</i>	KC179412		Chile	Arup <i>et al.</i> 2013
<i>Xanthoria parietina</i>	KC179411		Denmark	Arup <i>et al.</i> 2013
<i>Xanthoria resendei</i>	AF101285		-	Martin & Winka 2000
<i>Xanthoria cf. stiligera</i>	KC179409		Spain	Arup <i>et al.</i> 2013

Supplementary Information

Table S1. Information about the nuITS database used in this study.

Figure 1S. Maximum likelihood tree based on phylogenetic analysis of *Xanthoria* species using a dataset of nuITS sequences. Bootstrap values ≥ 70 are presented above the nodes.