



Hondaria, a new genus of Collemataceae (Ascomycota lichenized) from South America

Marcos Junji Kitaura^{1*} , Mayara Camila Scur¹ , Josiane Vogel Cortina Theodoro¹ , Ana Cláudia Piovezan-Borges²  and Aline Pedroso Lorenz¹ 

Received: March 12, 2020

Accepted: June 26, 2020

ABSTRACT

Collema leptosporum was originally included in Collemataceae as part of the *Collema fasciculare* group, an informal group that also included *C. fasciculare*, *C. papuanorum*, and *C. uviforme*. However, molecular data from *C. fasciculare* showed that this species belongs to Arctomiaceae, and all species in this informal group were relocated to *Arctomia*, although no molecular data were generated and analyzed for *C. leptosporum*, *C. papuanorum* and *C. uviforme*. To investigate the phylogenetic relationships of *Collema leptosporum*, currently *Arctomia leptospora*, we analyzed three DNA loci and examined morphological and anatomical features of specimens collected near the type locality. Genetic data suggest that this species is not included in Arctomiaceae and should be treated as a new genus in Collemataceae. *Hondaria* gen. nov. is characterized by having the longest transversely-septate ascospores in the family ((100–)120–175(–200) × 2–4(–5) μm). This study also suggests that the structures characterizing the *C. fasciculare* group are a result of convergent evolution, since this group includes species from different distantly related species.

Keywords: *Arctomia leptospora*, *Collema leptosporum*, *C. fasciculare* group, jelly lichens, South America biodiversity

Introduction

The landscape of the west-central region of Brazil, especially near the border with Bolivia and Paraguay, is composed of a mosaic of vegetation formations that include the Pantanal wetlands, the Brazilian savanna (Cerrado) and the Chaco (Pott & Pott 1994). The first paper on the diversity of lichenized fungi describe the species from this region based on an analysis of material obtained during the First Regnellian Expedition 1892–1894 (Malme 1897). More than 20 new species were described by the Swedish botanist Gustaf Malme (Spielmann & Canêz 2012), including the jelly lichen *Collema leptosporum* (Collemataceae, Peltigerales;

Malme 1924). This species was originally reported from Corumbá then subsequently from the Campo Grande municipality in Brazil (Oliva *et al.* 1992; Prado *et al.* 1999), and from the Chaco region in Paraguay (Degelius 1974).

The ascospores of *Collema leptosporum* are acicular and transversely septate, like *C. fasciculare*, *C. papuanorum*, and *C. uviforme*. So, these species were included in an informal subgeneric group, the *C. fasciculare* group, due to the combination of a “crustose” thallus with corticolous habit and the very long ascospores (Degelius 1974). Later, all four species were excluded from the Collemataceae (Peltigerales) and transferred to *Arctomia* (Arctomiaceae, Arctomiales), due to the phylogenetic placement of

¹ Laboratório de Ecologia e Biologia Evolutiva, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, Mato Grosso do Sul, Brazil

² Laboratório de Ecologia, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, Mato Grosso do Sul, Brazil

* Corresponding author: junjimjk@gmail.com

C. fasciculare that was revealed by DNA sequences (Otálora & Wedin 2013). No molecular data were generated and analysed for *C. leptosporum*, *C. papuanorum* and *C. uviforme*. Ultimately, considering molecular data and apothecium structure (including ontogeny), Jørgensen (2014) suggested that *C. fasciculare* should not be included in *Arctomia*, but rather in *Gabura*, another genus of Arctomiaceae. This was confirmed by Magain *et al.* (2020). The other three species of the *C. fasciculare* group remained allocated to *Arctomia*, including *Arctomia leptospora*.

This present research aimed at investigating the phylogenetic affinities of *Arctomia leptospora*, in combination with a reanalysis of its morphological features.

Materials and methods

We analyzed 15 specimens of *Arctomia leptospora*: three new specimens collected in the field, and 12 borrowed from the Universidade Federal de Mato Grosso do Sul herbarium (CGMS), Campo Grande, Brazil. The 15 specimens were all collected in Mato Grosso do Sul state in the west-central region of Brazil. We used only freshly collected material for molecular analyses (Tab. 1).

The specimens were analyzed with an Olympus SZX7 stereomicroscope and an Olympus CX22LED microscope, and images were captured with a Canon EOS Rebel T3i digital camera.

Preliminary identification was based on descriptions from the literature (Malme 1924; Degelius 1974). To characterize the genus, we used the characters utilized by Otálora *et al.* (2014) in the descriptions of genera of Collemataceae. The nomenclature of the pseudo-tissues observed in apothecia was adopted by Degelius (1954, 1974) and Kitaura & Marcelli (2013).

DNA extraction was performed with small fragments of the thalli following the methods used by Kitaura *et al.* (2018). We used the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) to amplify the nuITS region, the mrSSU1 and mrSSU3R primers (Zoller *et al.* 1999) for the mrSSU region, and the MCM7-709 and MCM7-1348 primers (Schmitt *et al.* 2009) for the MCM7 region. The 25 µL PCR reactions contained 1× PCR Buffer (Promega), 0.2 µM of each primer, 0.2 µM of dNTPs, 2 µM of MgCl₂, 1 unit of DNA polymerase (Promega) and 5–20 ng of genomic DNA. PCR reactions were carried out in the Veriti Thermal Cycler (Applied Biosystems) following the conditions described by Kitaura *et al.* (2018) for the mrSSU and nuITS regions and by Otálora & Wedin (2013) for MCM7. Sequencing was done by Macrogen Inc. (South Korea) and the sequences obtained were deposited in GenBank after assembly (Tab. 1).

Exploratory analyses using the megablast tool (Altschul *et al.* 1990) were performed to compare the new sequences with the reference sequences in GenBank. The blast results revealed that all sequences generated in the present study

were closest to the Collemataceae species. Therefore, the sequences used in the Collemataceae phylogeny studies were selected for this study for the mrSSU and MCM7 regions (Wedin *et al.* 2009; Otálora *et al.* 2010a; Otálora *et al.* 2013; Bjelland *et al.* 2017; Kitaura *et al.* 2018; Košuthová *et al.* 2019), and the available related nuITS sequences (Otálora *et al.* 2008; Otálora *et al.* 2010b; Jayalal *et al.* 2014; Magain & Sérusiaux 2014; Kitaura *et al.* 2018; Marthinsen *et al.* 2019). Due to the *C. fasciculare* group's circumscription history, the sequences of *Gabura borbonica* and *Gabura fascicularis* (Arctomiaceae) were also added to the study dataset (Otálora & Wedin 2013; Magain *et al.* 2020).

The sequences were aligned separately for each marker using the MAFFT v7.308 (Katoh *et al.* 2002) plugin in Geneious v9.1.2 (Kearse *et al.* 2012) with the auto option. After manual adjustments, the Gblocks webserver was used for the mrSSU alignment to exclude unreliably aligned sites, employing all less stringent options (http://molevol.cmima.csic.es/castresana/Gblocks_server.html).

Substitution models were defined for each region according to jModelTest2 (Guindon & Gascuel 2003; Durrin *et al.* 2012), selecting the Akaike information criteria (AIC) that suggested TPM3fu+I+G (mrSSU) and GTR+I+G (MCM7 and nuITS) as the best fitting models. Phylogenetic trees were estimated using Bayesian (BA) and Maximum Likelihood (ML) approaches with the nuITS, mrSSU and MCM7 regions and mrSSU and MCM7 concatenated. The analyses were performed in the CIPRES Science Gateway portal (Miller *et al.* 2010). The BA tree was estimated using the Metropolis-coupled Bayesian Markov chain Monte Carlo algorithm implemented in MrBayes 3.2.2 (Ronquist *et al.* 2012). Two runs of 10 million generations employing four simultaneous chains were executed. Trees were saved every 10,000 generations. The first 25% of the generated trees were discarded as burn-in, and convergence of the chains was assessed using Tracer v1.7.1 (Rambaut *et al.* 2018). The ML tree was built in RaxML 8 (Stamatakis 2014) and implemented in Geneious using the GTRGAMMA model, 1000 bootstrap pseudoreplicates, and the remaining parameters set as default. *Staurolemma omphalarioides* (Anzi) P.M. Jørg. & Henssen and *Pannaria rubiginosa* (Thunb.) Delise were used as outgroups, following Otálora *et al.* (2013) and Košuthová *et al.* (2019). The program FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used to edit the trees.

Results

Phylogenetic analyses

We generated DNA sequences of the three freshly-collected specimens of *Arctomia leptospora* for the nuITS, mrSSU and MCM7 regions (Tab. 1).

For the phylogenetic analyses, the alignments of the mrSSU region (731 base pairs) and MCM7 (565 base pairs) were analyzed separately and concatenated, resulting in a



**Hondaria, a new genus of Collemataceae (Ascomycota lichenized)
from South America**

Table 1. Information about DNA sequences used in this study. New sequences are indicated in bold. Herb. = Herbarium's acronym.

Species	GenBank Accession no.			Origin	Voucher	Herb.	Reference
	nulTS	mtSSU	MCM7				
<i>Blennothallia crispa</i>	-	MK445278	MK451920	Spain	Westberg S-F315217	S	Košuthová <i>et al.</i> (2019)
<i>Callome multipartita</i> 1	-	EU982557	-	Spain	Etayo 20255	S	Otálora <i>et al.</i> (2013)
<i>Callome multipartita</i> 2	-	GQ259019	-	Norway	Haugan 7015	O	Wedin <i>et al.</i> (2009)
<i>Callome multipartita</i> 3	MK811803	-	-	Norway	S. Reiso & T. Høitomt	O	Marthinsen <i>et al.</i> (2019)
<i>Collema furfuraceum</i> 1	-	EU982567	JX992982	Norway	Otálora 819	A	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2013)
<i>Collema furfuraceum</i> 2	GQ396263	-	-	Spain	MA-16260	MA	Otálora <i>et al.</i> (2010b)
<i>Collema leptaleum</i>	-	JX992928	JX992986	Argentina	Wedin 8822	S	Otálora <i>et al.</i> (2013)
<i>Collema nigrescens</i>	-	EU982563	JX992989	Spain	Aragón 80/04	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2013)
<i>Collema undulatum</i>	DQ466044	-	-	Spain	MA-16036	MA	Otálora <i>et al.</i> (2008)
<i>Enchylium tenax</i> 1	-	EU982556	JX992998	Spain	Etayo 20214	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2013)
<i>Enchylium tenax</i> 2	-	EU982580	JX992999	Spain	Otálora 010707	S	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2013)
<i>Gabura borbonica</i>	MK571781	JX030032	-	Reunion Island	Magain & Sérusiaux N952	LG	Magain <i>et al.</i> (2020)
<i>Gabura fascicularis</i>	-	KC118988	KC118995	Spain	Aragon & Martinez 3417	MA	Otálora & Wedin (2013)
<i>Gabura fascicularis</i>	-	KC118987	KC118997	Sweden	Karström 562	UPS	Otálora & Wedin (2013)
<i>Gabura insignis</i> 1	MK571780	MK571787	-	Ireland	Sérusiaux N3786	LG	Magain <i>et al.</i> (2020)
<i>Gabura insignis</i> 2	MK571777	MK571789	-	USA	DiMeglio 322 P6281	OSC	Magain <i>et al.</i> (2020)
Hondaria leptospora 1	MN653001	MN653004	MT415829	Brazil	J.B.Paula 02	CGMS	This study
Hondaria leptospora 2	MN653002	MN653005	MT415830	Brazil	J.B.Paula 03	CGMS	This study
Hondaria leptospora 3	MN653003	MN653006	MT415831	Brazil	J.B.Paula 04	CGMS	This study
<i>Lathagrium auriforme</i>	-	JX992913	JX992973	Norway	Nordin 4621	UPS	Otálora <i>et al.</i> (2013)
<i>Lathagrium fuscovirens</i>	-	JX992923	JX992983	Sweden	Tibell 23588	UPS	Otálora <i>et al.</i> (2013)
<i>Leptogium antarcticum</i>	KY171869	KY171880	-	Antarctica	Koch 5528	CGMS	Kitaura <i>et al.</i> (2018)
<i>Leptogium azureum</i>	KJ409609	-	-	South Korea	-	-	Jayalal <i>et al.</i> (2014)
<i>Leptogium burnetiae</i>	KJ409601	-	-	South Korea	-	-	Jayalal <i>et al.</i> (2014)
<i>Leptogium byssinum</i>	-	KT240180	KT240183	Norway	Westberg S-F264803	S	Košuthová <i>et al.</i> (2019)
<i>Leptogium crispatellum</i>	-	JX992945	JX993009	New Zealand	Wedin 9206	S	Otálora <i>et al.</i> (2013)
<i>Leptogium cyanescens</i>	-	EU982561	JX993010	Panamá	Etayo 18743	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2013)
<i>Leptogium denticulatum</i>	-	JX992948	JX993013	Argentina	Wedin 8698	S	Otálora <i>et al.</i> (2013)
<i>Leptogium denticulatum</i>	KJ409597	-	-	South Korea	-	-	Jayalal <i>et al.</i> (2014)
<i>Leptogium furfuraceum</i> 1	EU982634	EU982553	JX993017	Spain	Aragon 175/97	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2010b)
<i>Leptogium furfuraceum</i> 2	EU982655	-	-	Spain	MA-09431	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2010b)
<i>Leptogium hibernicum</i>	-	JX992952	JX993020	New Zealand	Wedin 8751	S	Otálora <i>et al.</i> (2013)
<i>Leptogium krogiae</i>	-	KX013744	KX013728	Kenya	Krog s.n. (O-L-188661)	O	Bjelland <i>et al.</i> (2017)
<i>Leptogium hibernicum</i>	-	JX992952	JX993020	New Zealand	Wedin 8751	S	Otálora <i>et al.</i> (2013)
<i>Leptogium pedicelatum</i>	KJ409611	-	-	South Korea	-	-	Jayalal <i>et al.</i> (2014)
<i>Leptogium puberulum</i>	KY171876	KY171887	-	Antarctica	Bernardo 441	CGMS	Kitaura <i>et al.</i> (2018)
<i>Leptogium pseudofurfuraceum</i>	EU982649	-	-	USA	ASU-N38938	ASU	Otálora <i>et al.</i> (2010b)
<i>Leptogium saturninum</i>	DQ466043	EU982569	JX993034	France	Argüello 2000	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2010b)
<i>Paracollema italicum</i> 1	-	JX992925	JX992984	Croatia	Nordin 2708	UPS	Otálora <i>et al.</i> (2013)
<i>Paracollema italicum</i> 2	-	JX992926	JX992985	Croatia	Nordin 2763	UPS	Otálora <i>et al.</i> (2013)
<i>Parmeliella brisbanensis</i>	KF704278	-	-	Reunion Island	R1019	LG	Magain & Sérusiaux (2014)
<i>Pseudoleptogium diffractum</i>	-	JX992949	JX993015	Sweden	Nordin 2529	UPS	Otálora <i>et al.</i> (2013)
<i>Rostania ceranisca</i>	-	MK445267	MK451922	Sweden	Westberg PL433	UPS	Košuthová <i>et al.</i> (2019)
<i>Rostania multipunctata</i>	-	JX992930	JX992988	Greece	Nordin 3160	UPS	Otálora <i>et al.</i> (2013)
<i>Rostania occultata v. occultata</i>	-	MK445266	MK451924	Sweden	Westberg PL467	UPS	Košuthová <i>et al.</i> (2019)
<i>Rostania occultata v. populina</i>	-	JX992932	JX992990	Greece	Llop 56060303	S	Otálora <i>et al.</i> (2013)
<i>Scytinium biatorinum</i>	-	JX992940	JX993003	Sweden	Jonsson 5500	UPS	Otálora <i>et al.</i> (2013)
<i>Scytinium callopismum</i>	-	JX992915	JX992975	Spain	Etayo 19783	MA	Otálora <i>et al.</i> (2013)
<i>Scytinium imbricatum</i>	-	MK445264	MK451929	Sweden	Hermansson 18777	UPS	Košuthová <i>et al.</i> (2019)
<i>Scytinium intermedium</i>	-	MK445263	MK451930	Sweden	Nordin 7385	UPS	Košuthová <i>et al.</i> (2019)
<i>Scytinium magnussonii</i>	-	EU982565	JX993022	Spain	Otálora 20104	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2013)



Table 1. Cont.

Species	GenBank Accession no.			Origin	Voucher	Herb.	Reference
	nuITS	mtSSU	MCM7				
<i>Scytinium palmatum</i>	-	JX992959	JX993025	Sweden	Nordin 5369	UPS	Otálora <i>et al.</i> (2013)
<i>Scytinium parvum</i>	-	JX992933	JX992992	Sweden	Thor 4300	UPS	Otálora <i>et al.</i> (2013)
<i>Scytinium plicatile</i>	-	GQ259033	JX993030	Sweden	Nordin 5566	UPS	Wedin <i>et al.</i> (2009)
<i>Scytinium pulvinatum</i>	-	MK445262	MK451931	Russia	Pystina 17352	UPS	Košuthová <i>et al.</i> (2019)
<i>Scytinium subtile</i>	-	JX992970	-	Sweden	Nordin 5861	UPS	Otálora <i>et al.</i> (2013)
<i>Scytinium tenuissimum</i>	-	JX992971	-	Spain	Aragón 1682/97	MA	Otálora <i>et al.</i> (2013)
<i>Scytinium turgidum</i>	-	EU982592	JX993040	Spain	Aragón 1671/98	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2013)
<i>Pannaria rubiginosa</i>	-	AY340513	JX993042	Portugal	Purvis, James & Smith 27/4/95	BM	Wiklund & Wedin (2003); Otálora <i>et al.</i> (2013)
<i>Pannaria rubiginosa</i>	AF429280	-	-	Norway	Anonby 870	BG	Ekman and Jørgensen (2002)
<i>Staurolemma omphalioides</i>	-	EU982560	JX993043	Spain	Aragón 83/04	MA	Otálora <i>et al.</i> (2010a); Otálora <i>et al.</i> (2013)
<i>Staurolemma omphalioides</i>	KJ533503	-	-	Norway	G. Gaarder 5553	TRH	Bendiksby <i>et al.</i> (2014)

final dataset with a total of 35 species of Collemataceae, and 3 species of Arctomiaceae, covering all the primary clades of Collemataceae. The nuITS alignment (424 base pairs) was composed of 13 species of Collemataceae and 3 of Arctomiaceae (Tab. 1).

The trees of the concatenated dataset of the MCM7 and mrSSU regions (Fig. 1), nuITS (Fig. 2) and, mrSSU and MCM7 single region analyses (Supplementary material 1) showed that the *Gabura* species (Arctomiaceae) is a separate group; and *Arctomia leptospora* and the remaining Collemataceae form a well-supported group.

Arctomia leptospora is not included in any of the known genera of Collemataceae; and, therefore, it is assigned here to the new genus *Hondaria*, with the single species *Hondaria leptospora*.

Hondaria M.J. Kitaura & A.P. Lorenz, gen. nov.

Mycobank number: MB 835521

Type species: *Hondaria leptospora* (Malme) M.J. Kitaura, M.C. Scur & A.P. Lorenz

Etymology – The generic name is a tribute to Dr. Neli Kika Honda, who has dedicated her scientific career to study the chemistry of the lichens from Mato Grosso do Sul since 1992 (Oliva *et al.* 1992).

Thallus foliose, medium-sized (3–5 cm across), homoiomerous, black to dark olive brown when dry (Fig. 3A, B, C); lobes irregular in outline, irregularly branched, 1.5–3.0 mm wide, plane; lobe surface plane and with longitudinal ridges when dry, not swollen; cortex with amorphous layer without euparaplectenchymatous cells (Fig. 3D); isidia granular, laminal, marginal, and on the margin of apothecia, simple to grouped; tomentum not observed. Apothecia usually present, frequent, laminal, pedicellate, ornamented by isidia (Fig. 3B); disc plane to slightly concave, reddish brown; proper exciple euparaplectenchymatous (Degelius 1954, Fig. 3C and E). Asci 150–170 × 12.5–17.5 µm. Ascospores (100–)120–175(–200) × 2–4(–5) µm, acicular, straight to curved, transversally 5–8-septate. Pycnidia not observed.

Hondaria leptospora (Malme) M.J. Kitaura, M.C. Scur & A.P. Lorenz, comb. nov.

Mycobank number: MB 835522

≡ *Collema leptosporum* Malme, Ark. Bot. 19(8): 6 1924. Type – Brazil, Mato Grosso do Sul state, Corumbá municipality, Malme s/n (LD, S, UPS - syntypes).

≡ *Arctomia leptospora* (Malme) Otálora & Wedin, Lichenologist 45(3): 302 2013.

Etymology – The epithet *leptospora* refers to thin ascospores of the species, 3–4(–5) µm thick.

Description – see description of the genus and more details in Malme (1924) and Degelius (1974).

Known distribution – On cortex in west-central Brazil near the border region with Bolivia, and Paraguay.

Material examined – Brazil, Mato Grosso do Sul state, Aquidauana municipality, Vila Palmeiras, 17 Nov 1993, N.K. Honda & Devincenzi 061H, 095DH (CGMS); IDEM, Campo Grande municipality, campus of Universidade Federal de Mato Grosso do Sul (UFMS), 24 Jul 2017, J.B. Paula & M.J. Kitaura 02, 03, 04 (CGMS); IDEM, campus of UFMS, 19 Jan 2011, A.L. Simal & P.H.R. Medeiros 22 (CGMS 42162); IDEM, campus of UFMS, 29 Feb 1989, I. Riquelme 061 (CGMS); IDEM, Vila da Base Aérea, on cortex, 25 Feb 1989, I. Riquelme 094, 273, 288 (CGMS); IDEM, Jardim Itatiaia, 13 Mar 1991, I. Riquelme 282, 283 (CGMS); IDEM, Jaraguari municipality, furnas do Dionísio, 13 Nov 2015, C.M. Bernardo 813 (CGMS); IDEM, 20°08'54.3" S, 54°34'14.9" W 435 m. alt., 12 Sep 2015, C.M. Bernardo, A.A. Spielmann, M.J. Kitaura *et al.* 762 (CGMS); IDEM, 20°08'54.1" S, 54°34'15.1" W, 425 m alt., 22 Nov 2011, A.L. Simal, L.S. Canêz & A.A. Spielmann 92 (CGMS).

Notes – The species is characterized by the presence of granular isidia (Fig. 3A, B), a euparaplectenchymatous proper exciple (Fig. 3C and E), and transversely septate ascospores with (100–)120–175(–200) × 2–4(–5) µm (Degelius 1974).



Hondaria, a new genus of Collemataceae (Ascomycota lichenized)
from South America

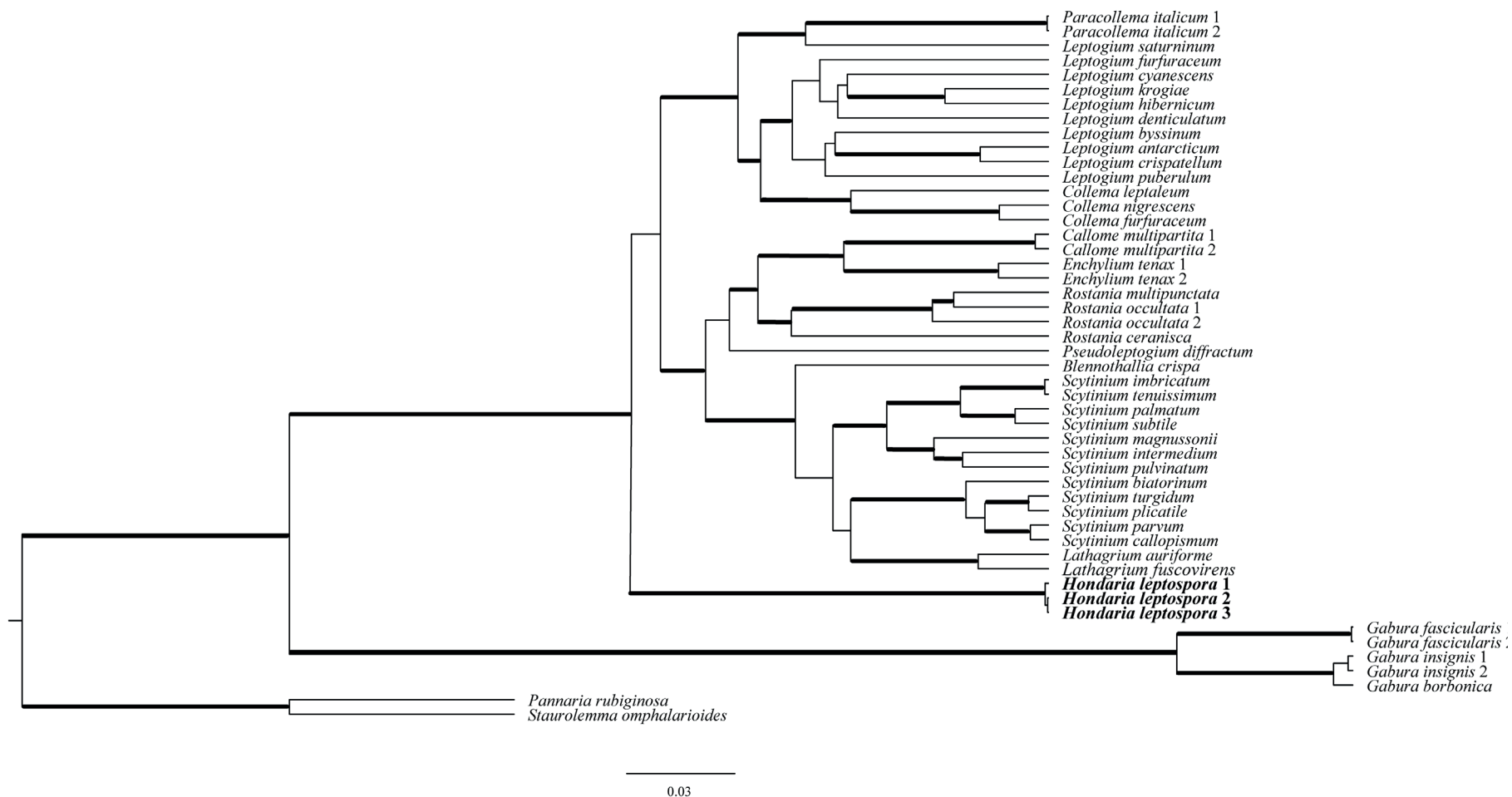
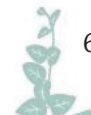


Figure 1. Phylogenetic relationships of *Hondaria*, other Collemataceae and *Gabura* (Arctomiaceae) based on Bayesian analysis of a 2-locus data set (mrSSU and MCM7). Thickened branches indicate support branches (posterior probabilities ≥ 0.90 and bootstrap $\geq 70\%$).



Discussion

Our phylogenetic analysis showed that *Arctomia leptospora* is not a member of *Arctomia*, *Gabura* (Arctomiales) or *Collema*. Instead, the three sequenced specimens belong to a novel clade of the Collemataceae, and is here recognized as a new genus, *Hondaria*. Consequently, the previously recognized *Collema fasciculare* group is composed of species from two different families and orders of Ascomycota that show some level of phenotypic convergence. Such notable convergence has already been reported for Arctomiaceae vs. Collemataceae (Otálora & Wedin 2013), and Arctomiaceae vs. Massalongiaceae (Ertz *et al.* 2017, Magain *et al.* 2020).

In addition to phylogeny, our study revealed that certain anatomical characteristics can also be used to separate *Hondaria leptospora* from *Arctomia* and *Gabura*. The apothecia of *Gabura fasciculare* have a thin proper exciple composed

by euthyplectenchymatous cells and a thin thalline exciple (Degelius 1954; Otálora & Wedin 2013), whereas *H. leptospora* has a thick paraplectenchymatous proper exciple and a thin cortex of thalline exciple (Figs. 3C and E).

Anatomically, *Arctomia papuanorum* and *A. uviforme* are more similar to *H. leptospora* than to *G. fasciculare* since they also have a thick paraplectenchymatous proper exciple. These two species might therefore also belong in the genus *Hondaria*; however, formal combinations are not proposed here, since DNA sequences are not yet available for these taxa. *Hondaria leptospora* produces the longest and thinnest ascospores of the previously defined *C. fasciculare* group (Degelius 1974; Otálora & Wedin 2013; see Tab. 2).

These results highlight that much remains to be investigated about lichenized fungi in South America and elsewhere. Furthermore, for jelly lichens it appears essential to integrate molecular, morphological, and anatomical data to accurately assess their phylogenetic relationships and classification.

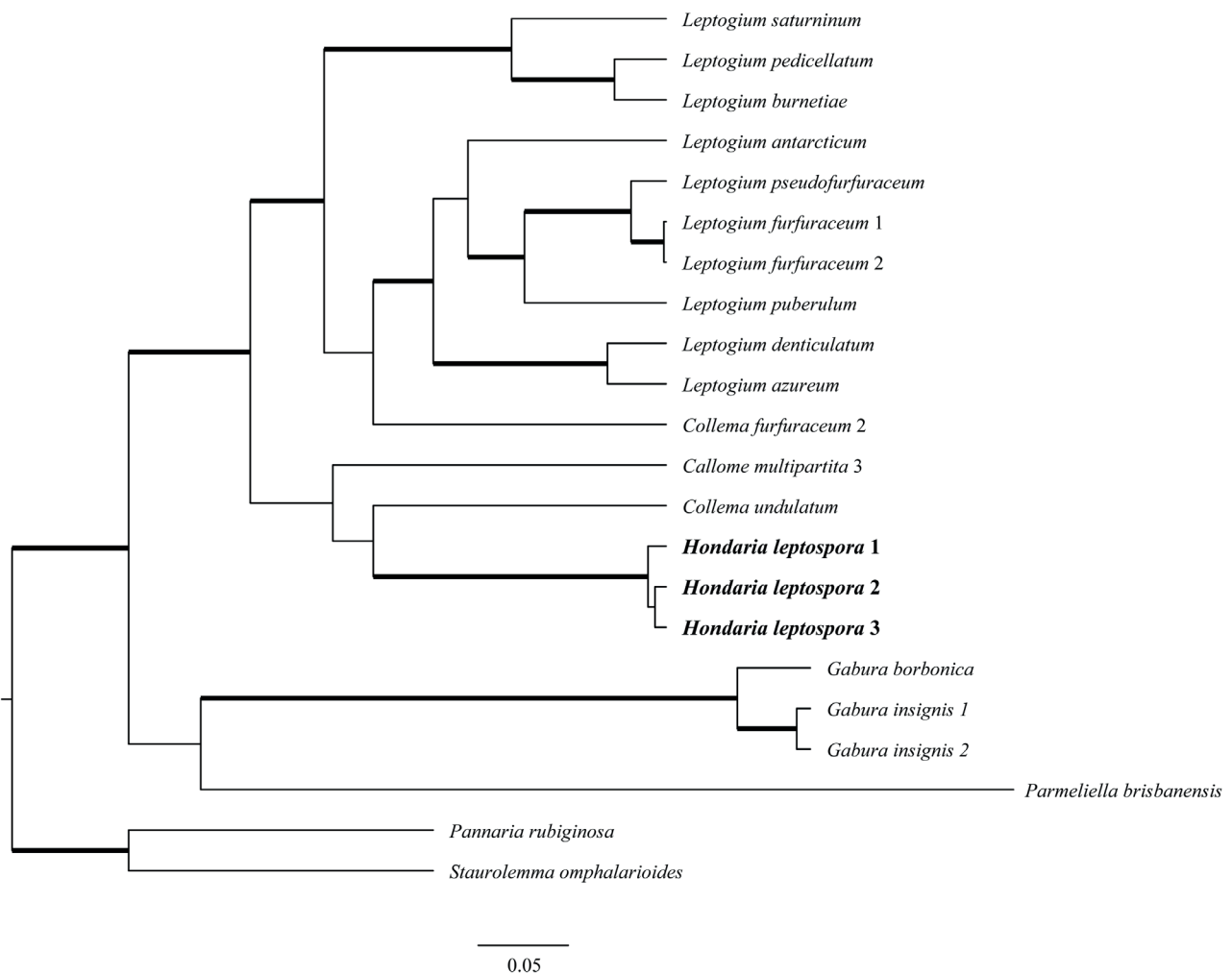


Figure 2. Phylogenetic relationships of *Hondaria*; other Collemataceae and *Gabura* (Arctomiaceae) based on Bayesian analysis of the nuITS region. Thickened branches indicate support branches (posterior probabilities ≥ 0.90 and bootstrap $\geq 70\%$).

***Hondaria*, a new genus of Collemataceae (Ascomycota lichenized)
from South America**

Table 2. Comparison among ascospore characteristics of the *Collema fasciculare* group.

Species	Ascospores per ascus	Forms	Number of cells	Measure of ascospores
<i>Arctomia papuanorum</i> (Degel.) Otálora & Wedin	6 or 8 per ascus	Usually straight in asci forming a fascicle or sometimes twisted or in part ± strongly curved	Many celled (up to c. 15, short or somewhat extended cells)	80–110 × 6.5 μm
<i>Arctomia uviforme</i> (Hue) Otálora & Wedin	8 per ascus	± twisted, when free straight or often strong curved or twisted	Usually 8 celled (4–10 celled)	(34–)40–60(–88) × 2.5–4.5 μ
<i>Gabura fascicularis</i> (L.) P. M. Jørg.	6 or 8 per ascus	Vermiform and plastic, in water straight or curved in various ways	10–17 celled	52–95 × 4.5–5.0(–6.5) μm
<i>Collema fasciculare</i> var. <i>microcarpum</i> (Müll. Arg.) Degel.	Not informed	Acicular to rarely subbacillar	(8–) 12–20 celled	Not mentioned
<i>Collema fasciculare</i> var. <i>colensoi</i> C. Bab.	Not informed	Acicular	12–16 celled	56–86(–110) × 2.5–4.5 μ
<i>Hondaria leptospora</i>	Usually 8 per ascus	Straight or twisted or curved in asci, when free straight or somewhat curved (seldom strongly)	Usually 8 celled	(100–)120–175(–200) × 2–4(–5) μm

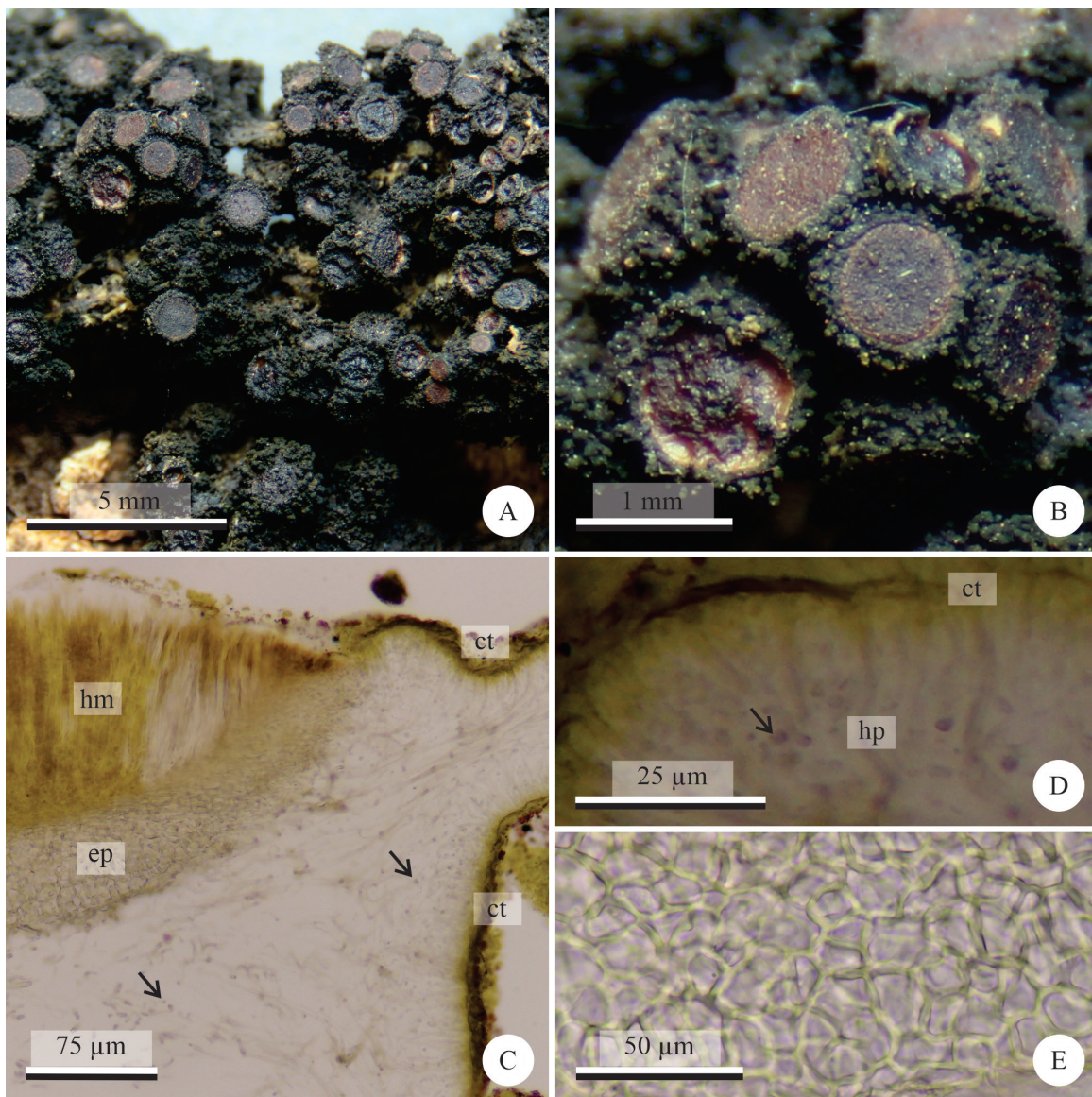


Figure 3. *Hondaria leptospora*. **A.** Specimen JBP 04. **B.** Detail of the ornamented apothecia with granular isidia on the margin. **C.** Diametral section of an apothecium. **D.** Detail of amorphous cortex. **E.** Detail of euparaplectenchymatous tissue. (hm = hymenium, ep = proper exciple, ct = cortex, hp = hyphae, arrow = cyanobacteria).

Acknowledgements

We thank the Universidade Federal de Mato Grosso do Sul, especially the curator of the CGMS herbarium for the loan of specimens, and we also thank the reviewers of the manuscript. M.J. Kitaura is grateful to CAPES for the post-doctoral support, and M.C. Scur and A.C. Piovezan-Borges are grateful to CAPES and FUNDECT for PhD scholarships. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-410.
- Bendiksby M, Mazzoni S, Jørgensen RH, Holien H. 2014. Combining genetic analyses of archived specimens with distribution modelling to explain the anomalous distribution of the rare lichen *Staurolemma omphalarioides*: long-distance dispersal or vicariance?. *Journal of Biogeography* 41: 2020-2031.
- Bjelland T, Bendiksby M, Frisch A. 2017. Geographically disjunct phylogenetic lineages in *Leptogium hibernicum* reveal *Leptogium krogiae* sp. nov. from East Africa. *The Lichenologist* 49: 239-251.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. doi: 10.1038/nmeth.2109
- Degelius G. 1954. The lichen genus *Collema* in Europa. *Acta Universitatis Upsaliensis, Symbolae Botanicae Upsalienses* 13: 1-499.
- Degelius G. 1974. The lichen genus *Collema* with special reference to the extra-European species. *Symbolae Botanicae Upsaliensis* 20: 1-215.
- Ekman S, Jørgensen PM. 2002. Towards a molecular phylogeny for the lichen family Pannariaceae (Lecanorales, Ascomycota). *Canadian Journal of Botany* 80: 625-634.
- Ertz D, Poulsen RS, Charrier M, Søchting U. 2017. Taxonomy and phylogeny of the genus *Steinera* (Arctomiales, Arctomiaceae) in the subantarctic islands of Crozet and Kerguelen. *Phytotaxa* 324: 201-238.
- Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118.
- Guindon S, Gascuel O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696-704.
- Jayalal U, Jang SH, Yu NH, Oh SO, HUR JS. 2014. Notes on the lichen genus *Leptogium* (Collemataceae, Ascomycota) in South Korea. *Mycobiology* 42: 120-131.
- Jørgensen PM. 2014. Taxonomy and nomenclature of *Collema fasciculare* (L.) G. H. Weber. *The Lichenologist* 46: 594. doi:10.1017/S0024282914000140
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059-3066.
- Kearse M, Moir R, Wilson A, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649.
- Kitaura MJ, Marcelli MP. 2013. A revision of *Leptogium* species with spherical-celled hairs (section *Mallotium* p.p.). *The Bryologist* 116: 15-27.
- Kitaura MJ, Scur MC, Spielmann AA, Lorenz-Lemke AL. 2018. A revision of *Leptogium* (Collemataceae, lichenized Ascomycota) from Antarctica with a key to species. *The Lichenologist* 50: 467-485.
- Košuthová A, Westberg M, Otálora MAG, Wedin M. 2019. *Rostania* revised: testing generic delimitations in Collemataceae (Peltigerales, Lecanoromycetes). *Mycologia* 47: 17-33.
- Magain N, Sérusiaux E. 2014. Do photobiont switch and *Cephalodia* emancipation act as evolutionary drivers in the lichen symbiosis. A case study in the Pannariaceae (Peltigerales). *PLOS ONE* 9: e89876. doi: 10.1371/journal.pone.0089876
- Magain N, Sprobble T, DiMeglio J, Nelson PR, Miadlikowska J, Sérusiaux E. 2020. Phylogenetic evidence for an expanded circumscription of *Gabura* (Arctomiaceae). *The Lichenologist* 52: 3-15.
- Malme GOA. 1897. Die Flechten der ersten Regnellischen Expedition. I. Einleitung. Die Gattung *Pyxine* (Fr.) Nyl. Bihang till Kongl. Svenska Vetenskaps-Akademiens Handlingar 23 III: 1-52.
- Malme GOA. 1924. Die Collematazeen des Regnellischen Herbars. *Arkiv för Botanik* 19: 1-29.
- Marthinsen G, Rui S, Timdal E. 2019. OLICH: A reference library of DNA barcodes for Nordic lichens. *Biodiversity Data Journal* 7: e36252. doi: 10.3897/BDJ.7.e36252
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. <http://www.phylo.org/>. 03 Mar. 2020.
- Oliva MLV, Mendes CR, Bueno NR, Honda NK, Sampaio MU, Sampaio CAM. 1992. Cysteine proteinase inhibitors in lichen (*Collema leptosporum* Malme). *Brazilian Journal of Medical and Biological Research* 25: 999-1002.
- Otálora MAG, Martínez I, Molina MC, Aragón G, Lutzoni F. 2008. Phylogenetic relationships and taxonomy of the *Leptogium* lichenoides group (Collemataceae, Ascomycota) in Europe. *Taxon* 57: 907-921.
- Otálora MAG, Aragón G, Molina MC, Martínez I, Lutzoni F. 2010a. Disentangling the *Collema/Leptogium* complex through a molecular phylogenetic study of the Collemataceae (Peltigerales, lichen-forming Ascomycota). *Mycologia* 102: 279-290.
- Otálora MAG, Martínez I, Aragón G, Molina MC. 2010b. Phylogeography and divergence date estimates of a lichen species complex with disjunct distribution pattern. *American Journal of Botany* 97: 216-223.
- Otálora MAG, Aragón G, Martínez I, Wedin M. 2013. Cardinal characters on a slippery slope - A re-evaluation of phylogeny, character evolution, and evolutionary rates in the jelly lichens (Collemataceae s. str). *Molecular and Phylogenetic Evolution* 68: 185-198.
- Otálora MAG, Wedin M. 2013. *Collema fasciculare* belongs in Arctomiaceae. *The Lichenologist* 5: 295-304.
- Otálora MAG, Jørgensen PM, Wedin M. 2014. A revised generic classification of the jelly lichens, Collemataceae. *Fungal Diversity* 64: 275-293.
- Pott A, Pott VJ. 1994. Plantas do Pantanal. Corumbá, EMBRAPA/CPAP.
- Prado SRT, Gorin PAJ, Stuelp PM, Honda NK, Iacomini M. 1999. An unusual juxtaposition of polysaccharide components of *Collema leptosporum*. *Carbohydrate Polymers* 40: 271-276.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 1-3. doi:10.1093/sysbio/syy032.
- Ronquist F, Teslenko M, Mark P, et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542.
- Schmitt I, Crespo A, Divakar PK, et al. 2009. New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* 23: 35-40.
- Spielmann AA, Cânez LS. 2012. Breve histórico sobre a taxonomia de líquens no Estado de Mato Grosso do Sul, Brasil. *Glalia* 4: 53-60.
- Stamatakis A. 2014. RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313.
- Wedin M, Wiklund E, Jørgensen PM, Ekman S. 2009. Slippery when wet: phylogeny and character evolution in the gelatinous cyanobacterial lichens (Peltigerales, Ascomycetes). *Molecular Phylogenetics and Evolution* 53: 862-871.
- White T, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ. (eds.) *PCR Protocols: A Guide to Methods and Applications*. New York, Academic Press Inc. p. 315-322.
- Wiklund E, Wedin M. 2003. The phylogenetic relationships of the cyanobacterial lichens in the Lecanorales suborder Peltigerales. *Cladistics* 19: 419-431.
- Zoller S, Scheidegger C, Sperisen C. 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming Ascomycetes. *The Lichenologist* 31: 511-516.

