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Standard Paper

Hypotrachyna neohorrescens, a new species in the subgenus *Parmelinopsis* (*Parmeliaceae*) from Brazil

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Abstract

This study describes a new species of *Hypotrachyna* subgenus *Parmelinopsis* from the south-eastern Cerrado (Brazilian savannah), a biodiversity hotspot. The species is especially common in open vegetation, including urban environments. *Hypotrachyna neohorrescens* sp. nov. is morphologically and chemically similar to *H. horrescens*. Nevertheless, phylogenetic analyses of the nuITS and mtSSU regions revealed that *H. neohorrescens* is a distinct species and closely related to the North American *H. mcmulliniana*, differing by the size of the laciniae and ascospores.

Key words: Cerrado, lichenized fungi, phylogeny, taxonomy

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Introduction

With more than 260 described species, the genus *Hypotrachyna* (Vain.) Hale belongs to *Parmeliaceae*, a hyperdiverse family of lichenized fungi (Lücking *et al.* 2017). Phylogenetic reconstructions indicate a probable generic origin in the neotropical region, where the split of the major *Hypotrachyna* clades occurred between the Eocene and Oligocene, with South America being the main centre of diversification, represented by *c.* 120 species (Sipman *et al.* 2009; Cubas *et al.* 2018). The *Hypotrachyna* clade includes a *sensu stricto* group and several well-supported clades recognized as the subgenera *Cetrariastrum* (Sipman) Divakar *et al.*, *Everniastrum* (Hale ex Sipman) Divakar *et al.*, *Longilobae* Divakar *et al.*, *Parmelinopsis* (Elix & Hale) Divakar *et al.* and *Sinuosae* Divakar *et al.* (Divakar *et al.* 2013).

Currently considered a subgenus of *Hypotrachyna*, *Parmelinopsis* is represented by 28 species, 10 of which occur in Brazil: *Hypotrachyna cryptochlora* (Vain.) D. Hawksw. & A. Crespo, *H. damaziana* (Zahlbr.) Krog & Swinscow, *H. heteroloba* (Zahlbr.) Divakar *et al.*, *H. horrescens* (Taylor) Krog & Swinscow, *H. jamesii* (Hale) Divakar *et al.*, *H. minarum* (Vain.) Krog & Swinscow, *H. schindleri* (Hale) Divakar *et al.*, *H. spathulata* (Kurok.) Krog & Swinscow, *H. spumosa* (Asahina) Krog & Swinscow, and *H. subfatiscens* (Kurok.) Swinscow & Krog (Canêz 2005; Martins *et al.* 2008; Benatti 2012; Lendemer & Allen 2020). *Parmelinopsis* is a genus that was previously segregated from *Parmelina* Hale by Elix & Hale (1987), encompassing species with a white medulla, emaculated thallus, narrow and apically truncated laciniae, simple marginal cilia, simple to dichotomous rhizines, cylindrical to bifusiform conidia (*c.* 3–5 µm) and commonly orcinol tridepsides as the main medullary chemical group (Elix & Hale 1987; Benatti 2012). However, this circumscription of *Parmelinopsis* proved to be polyphyletic in molecular phylogenies, indicating multiple origins of these morphological and chemical characters in the *Hypotrachyna* clade (Divakar *et al.* 2006, 2013). Thus, the subgenus *Parmelinopsis* currently encompasses species with eciliate laciniae up to 6 mm wide, mostly dichotomous rhizines, and depsides derived from β-orcinol and (or) orcinol as main chemical compounds. These characteristics are not exclusive and can also be found in other subgenera (Divakar *et al.* 2013; Lendemer & Allen 2015, 2020).

In Brazil, species of *Hypotrachyna* subgenus *Parmelinopsis* are common in the Cerrado ecoregion or province, including open areas of savannah and seasonal forests, as well as urban environments (Marcelli 1993). The Cerrado is a biodiversity hotspot characterized by different landscapes, ranging from tropical grasslands to seasonal forests, with numerous species adapted to the frequent fires that occur during the six-month dry season (Batalha 2011; Strassburg *et al.* 2017; Oliveira *et al.* 2021). However, studies of widespread lichenized fungi from this highly seasonal region are still scarce (Jungbluth 2006).

This study introduces *Hypotrachyna neohorrescens* Jungbluth, Marcelli & Lorenz, a new species from the south-eastern Cerrado, provides a detailed description and infers its phylogenetic position.

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Material and Methods

Morphological data: study location, sampling and identification

Specimens of *Hypotrachyna neohorrescens* sp. nov. were collected on trees of forest fragments in southern Cerrado (Fig. 1). Furthermore, specimens of *Hypotrachyna minarum*, a species that morphologically resembles *H. horrescens*, were collected in restingas (Atlantic forests adapted to coastal plains) in southern Brazil. Morphological descriptions are based on all specimens examined, using a Nikon SMZ645 stereomicroscope (Nikon Corporation, Japan) and an Olympus CX22LED optical microscope (Olympus Corporation, Japan). Chemical compounds were identified by spot tests (K, C, KC and P) and thin-layer chromatography (TLC), using solvents A and C (Orange *et al.* 2010).

The specimens of *H. neohorrescens* examined were deposited in the herbaria of the Universidade Federal de Santa Maria (PALM) and the Instituto de Botânica (SP), and the specimens of *H. minarum* were deposited in the herbarium of the Universidade Federal de Mato Grosso do Sul (CGMS). In addition, we undertook morphological studies of the lectotype of *Parmelia* [*Hypotrachyna*] *horrescens* (Taylor) Krog & Swinscow (FH-TAYL), kindly loaned to us by the curator of herbarium FH (Farlow Herbarium, Harvard University).

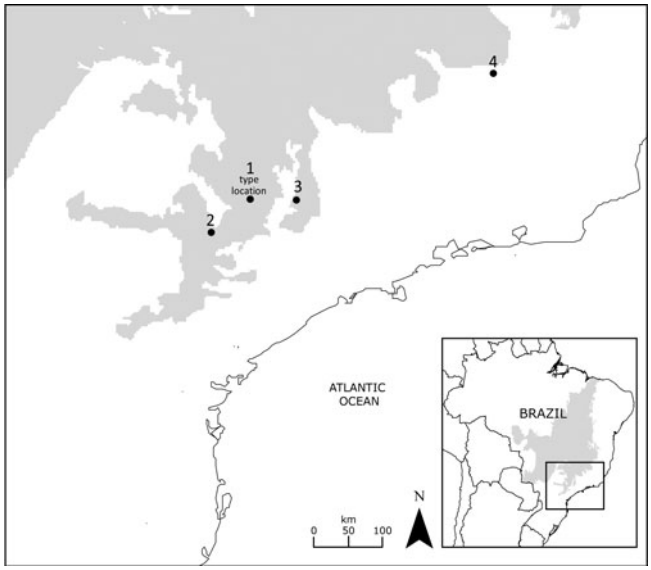

Fig. 1. Sampling points of *Hypotrachyna neohorrescens* in the south-eastern Cerrado ecoregion of Brazil. 1, *P. Jungbluth* 3160, 3317 (type), 3161, 3264 and 3266. 2, *P. Jungbluth* 2690 and 2709. 3, *P. Jungbluth* 2642 and 2647. 4, *P. Jungbluth* 2294. The Cerrado distribution is shown in grey.

Phylogenetic analyses

Prior to DNA extraction, thallus fragments were immersed in acetone for 20 min to remove the secondary compounds. The fragments were then dried in the open air until the acetone evaporated completely. DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. The nuITS region was amplified using the universal primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). To amplify the mtSSU and nuLSU regions, we used the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999), and LR1R (Döring *et al.* 2000) and LR6 (Vilgalys & Hester 1990), respectively. The 25 µl PCR reactions contained the following: 1× buffer, 0.2 mM dNTPs, 0.2 µM of each primer, 3.0 mM $MgCl_2$, 1U Taq DNA polymerase (Promega) and *c.* 20 ng of DNA template. The PCR conditions for the amplification of the nuITS region were as follows: initial denaturation for 2 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing between 54–56 °C for 30 s, extension at 72 °C for 1 min 10 s and a final extension at 72 °C for 5 min. For the mtSSU and nuLSU regions, we applied the following: initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 1 min 30 s and a final extension at 72 °C for 10 min. The PCR reactions were performed in an Eppendorf Mastercycler® Gradient thermal cycler. The amplification products were visualized in 1% agarose gel stained with GelRed® (Biotium). Macrogen Korea performed DNA purification and sequencing.

For assembly and quality evaluation of the DNA sequences generated, we used Geneious® 9.1.6 (Kearse *et al.* 2012). Sequences from GenBank were selected to encompass the main clades of the *Hypotrachyna* clade (details in Table 1). *Hypotrachyna cirrhata* (Fr.) Divakar *et al.* (subgenus *Everniastrum*) was chosen as outgroup due to its close phylogenetic relationship with *Hypotrachyna* subgenus *Parmelinopsis* (Divakar *et al.* 2013). Multiple sequence alignments for each locus were performed using MAFFT v.7 (Katoh & Standley 2013), with the auto option and subsequent manual inspection. We removed the ambiguous sites from the alignments with Gblocks 0.97b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), selecting all less stringent options (Talavera & Castresana 2007).

Exploratory analyses using BLAST tools revealed that the nuLSU sequences obtained were up to 85% identical to mtDNA of the green algal genus *Trebouxia* Puymaly, the associated photobiont. Therefore, the nuLSU sequences were excluded from subsequent analyses. Two datasets were used, one with the sequences of the nuITS and mtSSU regions concatenated and one with only the nuITS region, the universal DNA barcode for fungi (Schoch *et al.* 2012). Phylogenetic analyses were based on maximum likelihood (ML) and Bayesian (B/MCMC) approaches, performed on the Cipres Science Gateway webserver (https://www.phylo.org/). The ML analysis was performed using RAxML v.8 (Stamatakis 2014), with the GTRGAMMA model and 1000 bootstrap pseudoreplicates. We used jModelTest 2.1.6 (Darriba *et al.* 2012) to verify the best nucleotide substitution model, using the Akaike information criterion (Akaike 1974). The model GTR + G was selected for the nuITS region, and HKY + I + G for mtSSU. For the tree reconstruction based on Bayesian inference, the program MrBayes 3.2.7 (Ronquist *et al.* 2012) was used with two parallel Markov chain Monte Carlo (MCMC) chains with 10 million generations, saving every 1000th tree. The first 25% of the sampled trees was discarded as burn-in. The convergence of the Bayesian analysis chains was verified using Tracer v.1.7 (Rambaut *et al.* 2018), with the effective sample size (ESS) ≥ 200 considered as indicative. Branches with bootstrap values ≥ 70% for ML, and posterior probabilities ≥ 0.95 for Bayesian inference were considered to be supported. These values were also used to compare maximum likelihood and Bayesian analyses to check for conflicts in the resulting topologies.

Additionally, pairwise genetic distances were calculated for the nuITS region (using the alignment with the ambiguous sites removed) with PAUP v.4.0b10 (Swofford & Sullivan 2003), using ML as a distance measure, while pairwise distances between different sequences are given as the number of nucleotide

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Table 1. Sequences of *Hypotrachyna* used in the phylogenetic analyses with voucher information and GenBank Accession numbers. New sequences are shown in bold.

Species	Sample ID	Voucher	Locality	nuITS	mtSSU
Subgenus *Parmelinopsis*					
Hypotrachyna afrorevoluta	01	MAF-Lich-10409	Spain: Canary Islands	DQ279529	DQ287839
	02	NY-2328235	USA: North Carolina	MT482207	MT482225
	03	NY-2328105	USA: North Carolina	MT482206	MT482226
H. appalachensis	01	NY-2606541	USA: North Carolina	MT482160	MT482254
	02	NY-2606549	USA: North Carolina	MT482161	-
H. britannica	01	MAF-Lich-15415	Ireland: Kerry	GQ919273	GQ919221
	02	NY-2795290	USA: North Carolina	MT482155	MT482261
H. cryptochlora	01	MAF-Lich-10398	China: Yunnan	DQ279535	DQ287845
	02	NY-2327559	USA: South Carolina	MT482203	MT482228
	03	NY-1885857	USA: North Carolina	MT482215	-
H. exsecta	01	MAF-Lich-10381	China: Yunnan	DQ279497	DQ287806
	02	MAF-Lich-10380	China: Yunnan	DQ279498	DQ287807
H. horrescens	01	MAF-Lich-9913	Spain: La Coruña	AY581085	AY582321
	02	NY-2357898	USA: North Carolina	MT482181	MT482237
	03	NY-2329051	USA: North Carolina	MT482195	MT482236
	04	NY-2329248	USA: North Carolina	MT482178	MT482240
	05	MAF-10399	Spain: Canary Islands	DQ279536	DQ287846
	06	MAF-10400	Spain: Ponevedra	DQ279537	DQ287847
	07	NY-3722141	USA: Tennessee	MT482187	-
	08	NY-3722347	USA: Tennessee	MT482188	-
H. kauffmaniana	01	NY-2356582	USA: North Carolina	MT482200	MT482231
	02	NY-2794602	USA: North Carolina	MT482157	MT482257
H. mcmulliniana	01	NY-2356553	USA: North Carolina	MT482197	MT482234
	02	NY-2356567	USA: North Carolina	MT482174	MT482245
	03	NY-2328946	USA: North Carolina	MT482171	MT482248
	04	NY-2328867	USA: North Carolina	MT482196	MT482235
	05	NY-2329035	USA: North Carolina	MT482180	MT482238
	06	NY-2329184	USA: North Carolina	MT482175	MT482244
	07	NY-2328963	USA: North Carolina	MT482172	MT482247
	08	NY-3721310	USA: Tennessee	MT482186	-
	09	NY-3722066	USA: Tennessee	MT482193	-
H. minarum	01	MAF-Lich-7639	Spain: Cádiz	AY581086	AY582322
	02	NY-2356512	USA: North Carolina	MT482176	MT482243
	03	NY-2329265	USA: North Carolina	MT482179	MT482239
	04	MAF-10401	Spain: Canary Islands	DQ279538	DQ287848
	05	MAF-13968	Australia: Queensland	DQ279539	DQ287849
	06	MAF-10220	China: Yunnan	AY611110	AY611168
	07	NY-3721830	USA: Tennessee	MT482182	-
	09	***A.S. Rodrigues* 594**	**Brazil: Rio Grande do Sul**	**MZ919271**	**MZ919145**
	10	***A.S. Rodrigues* 614**	**Brazil: Rio Grande do Sul**	**MZ919272**	**MZ919146**
H. neodamaziana	01	MAF-Lich-10182	Australia: Queensland	AY611107	AY611166

(*Continued*)

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Table 1. (*Continued*)

Species	Sample ID	Voucher	Locality	nuITS	mtSSU
H. neodissecta	01	MAF-Lich-13986	South Africa: Western Cape	DQ279510	DQ287820
	02	F, MAF-Lich-19622	Kenya: Western Province	JN943836	KR995336
H. neohorrescens	**01**	***P. Jungbluth* 3160**	**Brazil: São Paulo**	**MW969682**	**MW980901**
	02	***P. Jungbluth* 2690**	**Brazil: São Paulo**	**MW969683**	**MW980902**
	03	***P. Jungbluth* 2642**	**Brazil: São Paulo**	**MW969684**	**MW980903**
	04	***P. Jungbluth* 2709**	**Brazil: São Paulo**	**MW969685**	**MW980904**
	05	***P. Jungbluth* 2647**	**Brazil: São Paulo**	**MW969686**	**MW980905**
	06	***P. Jungbluth* 3266**	**Brazil: São Paulo**	**MW969687**	**MW980906**
	07	***P. Jungbluth* 3264**	**Brazil: São Paulo**	**MW969688**	**MW980907**
	08	***P. Jungbluth* 3161**	**Brazil: São Paulo**	**MW969689**	**MW980908**
	09	***P. Jungbluth* 3317–TYPE**	**Brazil: São Paulo**	**MZ919273**	**MZ919147**
H. pluriformis	01	KRAM-L	Bolivia: Aniceto Arce	KF380912	KF380995
H. revoluta	01	MAF-Lich-6047	Spain: Puerto Urkiola	AY611075	AF351166
	02	NY-2356534	USA: North Carolina	MT482202	MT482229
	03	MAF-13989	South Africa: Western Cape	DQ279523	DQ287833
H. showmanii	01	MAF-Lich-15618	USA: Pennsylvania	GQ919287	GQ919234
	02	NY-1080325	USA: Pennsylvania	KF380916	KF380999
H. spumosa	01	NY-1885632	USA: North Carolina	MT482220	-
	02	NY-1886244	USA: North Carolina	MT482216	-
H. subfatiscens	01	MAF-Lich-6878	Australia: Queensland	AY611108	AF351174
Subgenus *Everniastrum*					
Hypotrachyna cirrhata	01	MAF-13976	Peru	DQ279487	DQ287795
	02	MAF-10374	China: Yunnan	DQ279486	DQ287794

substitutions per site (s/s). We used the barcode gap values (a threshold close to 0.015–0.017 s/s) proposed for *Parmeliaceae* (Del-Prado *et al.* 2010) to distinguish *H. neohorrescens* from the phylogenetically closest species.

Results

Novel sequences from nuITS and mtSSU regions were obtained for nine specimens of *H. neohorrescens* (collected from sampling points 1, 2 and 3 in Fig. 1), and two specimens of *H. minarum* (Table 1). The final data matrix contained 63 concatenated sequences of the markers nuITS and mtSSU with a length of 1271 characters (see Supplementary Material File S1, available online). There were no conflicts between the phylogenies based on maximum likelihood and Bayesian inference of the concatenated matrix, so only the Bayesian tree is shown in Fig. 2. *Hypotrachyna neohorrescens* sp. nov. formed a new clade, closely related to the North American *H. mcmulliniana* Lendemer & J. L. Allen. The single marker matrix containing 63 nuITS sequences with 472 characters resulted in trees with a similar topology, but they did not recover complete reciprocal monophyly of *H. neohorrescens* and *H. mcmulliniana* (Supplementary Material Fig. S1, available online). This lack of resolution reflects the low divergence among the nuITS sequences. The mean genetic distance between *H. neohorrescens* and *H. mcmulliniana* was 0.0185 s/s, ranging from 0.0142 to 0.0205 s/s.

In addition, we also verified the phylogenetic position of *H. minarum*, a widely distributed species with its type specimen from Brazil. The *H. minarum* clade included sequences from Australia, Brazil, China, the United States and Spain (Fig. 2). Interestingly, nuITS distances up to 0.017 s/s indicated that more than one species might be in this clade. The Brazilian specimens analyzed in this study were characterized by laminal isidia without cilia (as described for North American populations in Lendemer & Allen (2020)), marginal lobes with rare cilia, and the presence of atranorin and gyrophoric acid, together with an unidentified compound (migrated above gyrophoric acid in solvent C in the TLC profile).

Thus, based on its morphology, phylogenetic relationships (inferred with concatenated markers) and geographical distribution, we consider *H. neohorrescens* to be a new species and highlight its main diagnostic characteristics below and in Table 2.

Taxonomy

Hypotrachyna neohorrescens Jungbluth, Marcelli & Lorenz sp. nov.

MycoBank No.: MB 841994

Morphologically similar to *Hypotrachyna horrescens* but differs by its phylogenetic position (based on concatenated nuITS and

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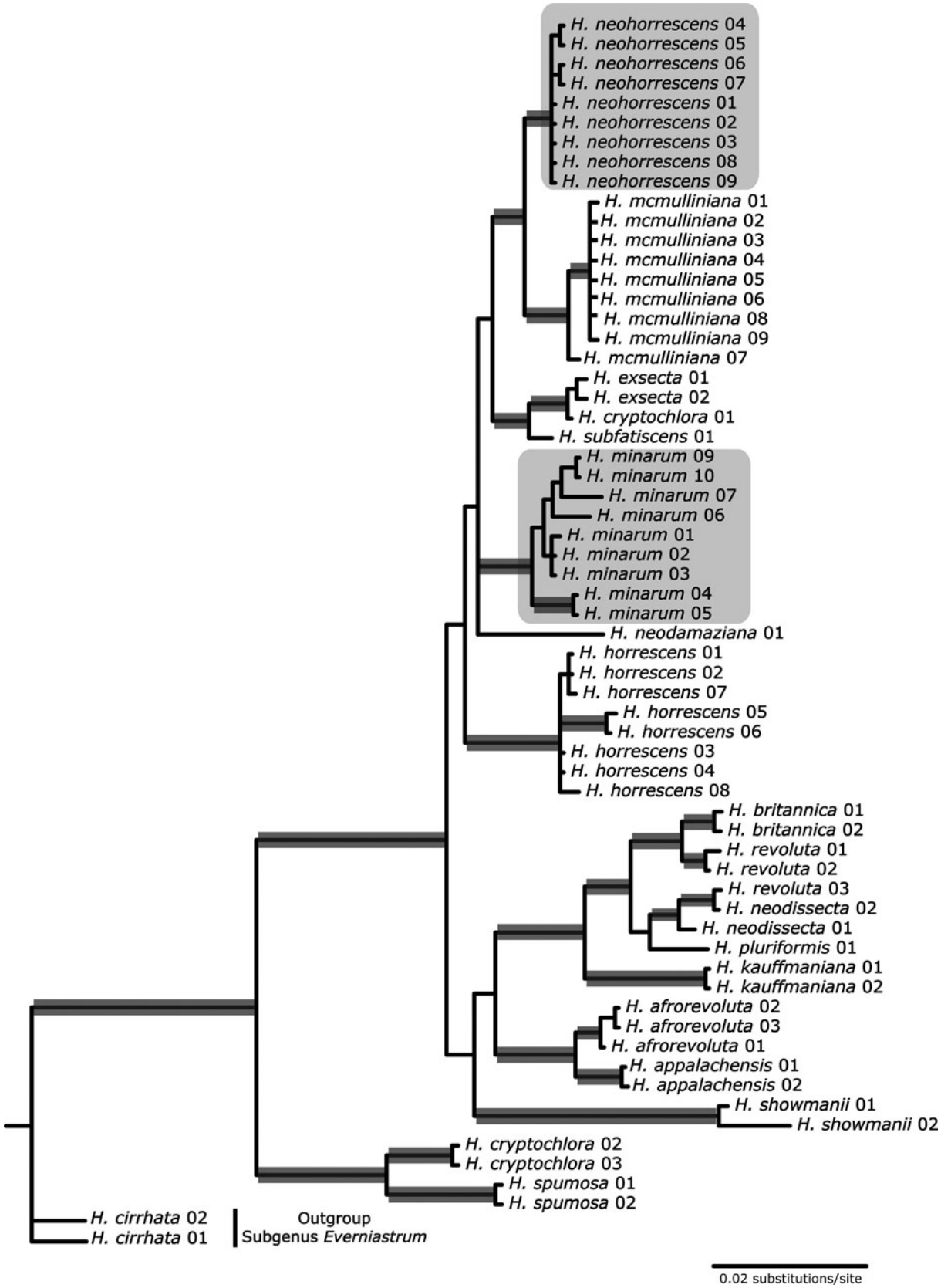

Fig. 2. Phylogenetic relationships of *Hypotrachyna* subgenus *Parmelinopsis* based on maximum likelihood (ML) and Bayesian inference analyses from nuITS and mtSSU sequences. The Bayesian tree is shown here. Thickened branches indicate ML bootstrap values ≥ 70% and posterior probabilities ≥ 0.95. Grey boxes indicate the species for which novel sequences have been generated in this study.

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Table 2. Comparison of the morphological characters of *Hypotrachyna neohorrescens*, *H. horrescens, H. mcmulliniana* and *H. minarum.* Information for *H. mcmulliniana* is based on Lendemer & Allen (2020) and for *H. horrescens* on the lectotype (FH-TAYL).

	H. neohorrescens	*H. horrescens*	*H. mcmulliniana*	*H. minarum*
Vegetative propagules	laminal isidia with frequent apical cilia	laminal isidia with sparse apical cilia	laminal isidia with sparse to occasional apical cilia	laminal isidia lacking apical cilia
Laciniae/lobe (mm)	sublinear, 0.5–1.7 wide at the base of the branches, 1.5–2.5 maximum width	sublinear, (0.7–)1.0–2.5 wide at the base of the branches, 1.5–2.5 maximum width	sublinear, 1.0–4.0 wide (probably maximum width)	sublinear, 1.1–3.5 wide
Marginal cilia	abundant	frequent	infrequent	infrequent
Rhizines	simple	simple to rarely dichotomously branched	simple to dichotomously branched	simple to irregularly branched
Ascospores (µm)	(12.5–)16.0(–19.0) × (8.8–)10.2(–11.3)	16–18 × 10–12 (according to Hale (1976))	9.9–13.8 × 5.1–8.1	not measured (no apothecia found)
Chemistry	atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid, 5-*O*-methylhiascic acid and gyrophoric acid	atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid, 5-*O*-methylhiascic acid and gyrophoric acid	atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid, 5-*O*-methylhiascic acid and gyrophoric acid.	atranorin, gyrophoric acid and unidentified compound (R_f above gyrophoric acid in solvent C).

mtSSU regions). Additionally, *H. neohorrescens* is phylogenetically close to *H. mcmulliniana,* differing in lacinia size (0.5–1.7 mm vs 1.0–4.0 mm wide), ascospore size (12.5–19.0 × 8.8–11.3 µm vs 9.9–13.8 × 5.1–8.1 µm) and geographical distribution (South America vs North America).

Type: Brazil, São Paulo State, municipality of Itirapina, Estação Experimental de Itirapina, Instituto Florestal, corticolous, tree in a well-preserved area of Cerrado seasonal forest, named Valério, *c.* 818 m alt., 22°13′10.0″S, 47°51′05.7″W, 10 December 2012, *P. Jungbluth, M. J. Kitaura* & *S. A. Adachi* 3317 (PALM—holotype). GenBank Accession nos: MZ919273 (nuITS) and MZ919147 (mtSSU).

(Fig. 3A & B)

Thallus corticolous, greenish grey, laciniate, 2–7 cm diam., adnate. *Proximal upper surface* continuous, densely isidiate. *Distal upper surface* continuous, smooth, becoming densely covered by young isidia, shiny, with a darker line near the tips, evidently thicker at the sinuses of the laciniae. *Laciniae* sublinear, mainly dichotomously branched, contiguous to slightly overlapping laterally, 0.5–1.7(–2.0) mm wide at the base of the branches, 1.5–2.5 mm maximum width; lateral margin smooth to crenate, slightly canaliculated; axils oval; apices subtruncate, flat with an involute tendency, procumbent. *Pruina* absent. *Maculae* absent. *Cilia* black, marginal, shiny, with acute ends, simple, abundant, mainly restricted in the axils and sinuses and in the isidia, up to 0.4 mm long. *Isidia* with pale to dark brown apices, mainly cylindrical, simple becoming very branched and coralloid in old thalli, erect, apices frequently ciliate, including the apices of the lateral branches; laminal, up to 0.5 mm. *Soralia* absent. *Medulla* white. *Distal lower surface* brown, shiny, smooth near the margins becoming papillate (small rhizines). *Proximal lower surface* black, shiny, slightly rugose, and veined. *Rhizines* black, simple, rarely irregularly branched, abundant, evenly distributed, up to 0.75 mm long.

Apothecia rare, concave, adnate, laminal, up to 2.5 mm diam.; margin smooth becoming isidiate; disc brown, shiny, without pruina. *Epithecium* 12.5–17.5 µm high; hymenium 50.0–62.5 µm high; subhymenium 50.0–62.5 µm high. *Ascospores* ellipsoid, (12.5–)16.0(–19.0) × (8.8–)10.2(–11.3) µm, episporium 1.0–2.0 µm (apothecia and ascospores found only in *P. Jungbluth* 2294).

Pycnidia rare, laminal to submarginal. *Conidia* bacilliform to bifusiform, (3.0–)4.0–6.0 µm.

Chemistry. Upper cortex K+ yellow, UV−; medulla K−, C−, KC+ pink, P−, UV−. Atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid ('horrescens complex'), 5-*O*-methylhiascic acid and gyrophoric acid.

Etymology. The specific epithet refers to the morphological similarity to *H. horrescens* and its known distribution in the neotropical region.

Distribution and habitat. The species is commonly found in south-eastern Cerrado, mainly in the states of São Paulo and Minas Gerais, Brazil.

Intraspecific morphological variation. There is a conspicuous presence of ciliate isidia in some specimens of this species, although there are exceptions. Populations of *H. neohorrescens* from Itirapina (collection point 1 in Fig. 1), for example, show abundantly ramified isidia with conspicuous apical cilia (Fig. 3B) whereas other specimens do not have ciliate isidia, instead resembling *Hypotrachyna minarum*, a species common in Brazil (Fig. 3C & D). This populational variation was also observed in *H. horrescens* by Hale (1971). However, *H. horrescens* and *H. neohorrescens* can be distinguished from *H. minarum* by the presence of the 3-methoxy-2,4-di-*O*-methylgyrophoric acid.

Additional specimens examined. **Brazil:** *Minas Gerais State*: Catas Altas, Parque Natural do Caraça (Sanctuary of Caraça), tree from the forest between Atlantic and Cerrado seasonal forests, trail to 'Piscina', corticolous, 1365 m, 20°06′08.2″S, 43°30′04.1″W, 2010, *P. Jungbluth* 2294 (PALM). *São Paulo State*: Itirapina, Estação Experimental de Itirapina, Instituto Florestal, corticolous, tree in a well-preserved area of Cerrado seasonal forest, named Valério, *c.* 818 m, 22°13′10.0″S, 47°51′05.7″W, 2012, *P. Jungbluth, M. J. Kitaura* & *S. A. Adachi* 3160, 3161 (PALM); *ibid*., on tree singed by fire in savannah (Cerrado s. str.), 757 m, 22°12′23″S, 47°54′26″W, 2012, *P. Jungbluth, M. J. Kitaura* & *S. A. Adachi* 3264, 3266 (PALM). *Mogi-Guaçu*: Martinho Prado District, Reserva Biológica de Mogi-Guaçu, Fazenda Campininha,

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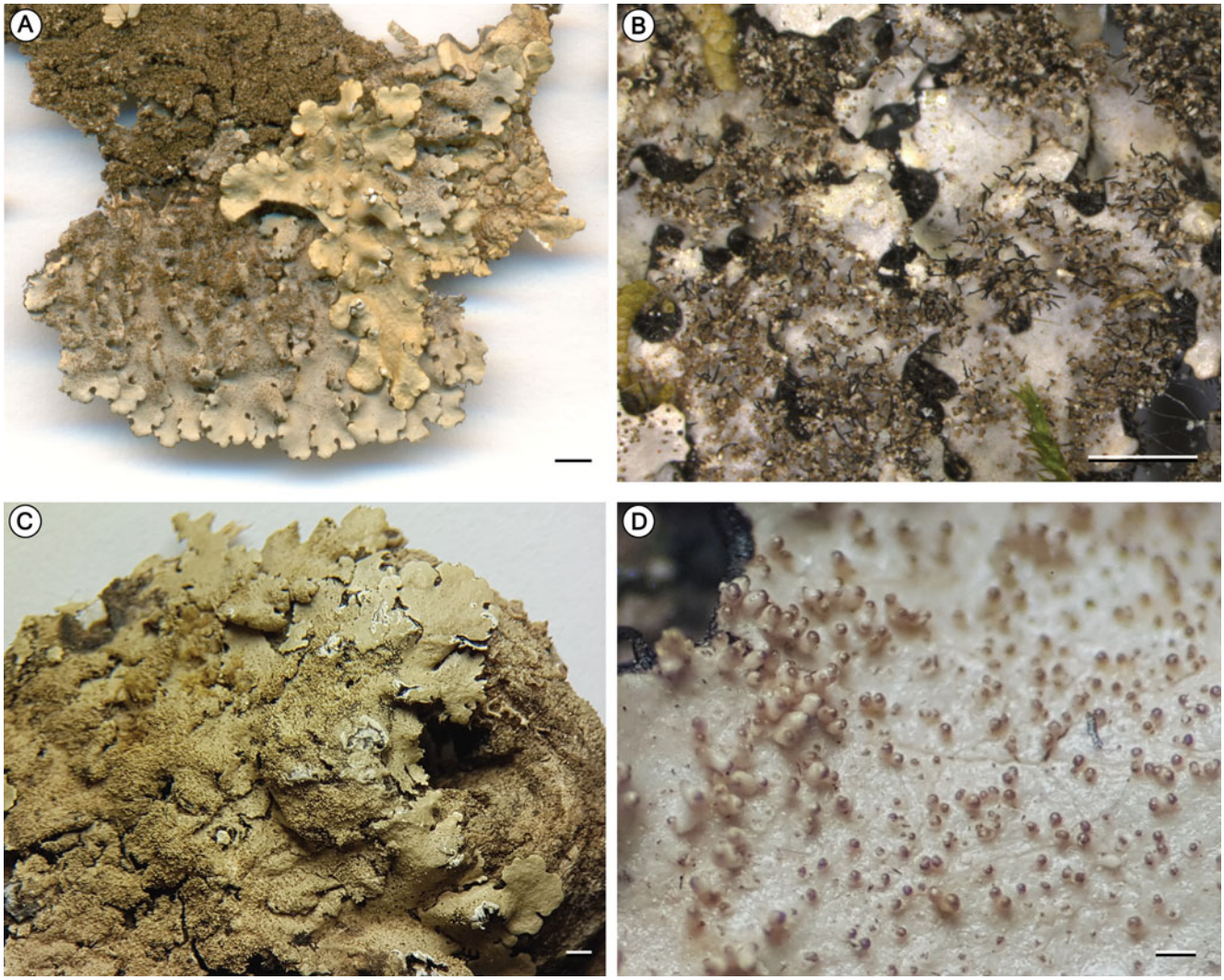

Fig. 3. Morphological features of selected *Hypotrachyna* species in Brazil. A & B, *Hypotrachyna neohorrescens*. A, thallus of the holotype (*P. Jungbluth* 3317). B, distribution and abundance of ciliated isidia on the upper surface (*P. Jungbluth* 3316). C & D, *Hypotrachyna minarum*. C, thallus (*A. S. Rodrigues* 614). D, laminal isidia lacking cilia (*A. S. Rodrigues* 594). Scales: A = 2 mm; B & C = 1 mm; D = 0.2 mm. In colour online.

corticolous, on Cerrado seasonal forest, 620 m alt., 22°15′19″S, 47° 09′17″W, 2011, *P. Jungbluth, M. M. Marcelli* & *B. R. da Hora* 2642, 2647 (PALM). *Pratânia*: Fazenda Palmeira da Serra, private reserve area of Cerrado, on seasonal forest inside sugar cane crops, corticolous, 710 m, 22°48′55″S, 48°44′36″W, 2011, *P. Jungbluth* & *S. B. Bissacot* 2690, 2709 (PALM).

Discussion

The Cerrado is characterized by a patchy landscape with frequently burned areas which have poor or absent lichenized mycobiota, since it takes *c.* 20 years for significant coverage and diversity to develop again (Marcelli *et al.* 1998). Species of *Hypotrachyna* subgenus *Parmelinopsis* are especially frequent in these environments (Jungbluth 2006); however, their diversity may be underestimated due to the lack of studies based on morphology and molecular tools (Lendemer & Allen 2020).

Using the nuITS and mtSSU regions, specimens phenotypically similar to *H. horrescens* and *H. mcmulliniana* collected in south-eastern Cerrado were recovered as new species of the *Parmelinopsis* subgenus. *Hypotrachyna neohorrescens* sp. nov. and *H. horrescens* produce frequent ciliated isidia on the upper surface, laciniae 0.5–1.7 mm wide, ellipsoid spores 12.5–19 × 8.8–11.3 µm in *H. neohorrescens* and 16–19 × 10–12 µm in *H. horrescens* according to Hale (1976) (the studied lectotype did not have apothecia), and the same chemistry (atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid, 5-*O*-methylhiascic acid and gyrophoric acid) (Table 2). Thus, morphological and chemical characters did not distinguish these species, although they belong to different clades and accumulate significant genetic divergence (0.0359–0.0551 s/s in the nuITS; Fig. 2). Phenotypically cryptic or 'near-cryptic' species are commonly found in molecular studies of *Parmeliaceae*, which have revealed higher levels of undescribed diversity among the lichenized fungi in recent decades (Crespo & Lumbsch 2010; Altermann *et al.* 2014; Singh *et al.* 2015; Leavitt *et al.* 2016; Lutsak *et al.* 2020). *Hypotrachyna neohorrescens* and *H. mcmulliniana* also share many morphological features; however, they can be distinguished by the lacinia size (0.5–1.7 mm vs 1.0–4.0 mm wide) and the size of the ascospores ((12.5–)16.0(–19.0) × (8.8–)10.2(–11.3) µm vs 9.9–13.8 × 5.1–8.1 µm). Furthermore, the known geographical distribution of *H. neohorrescens* is the south-eastern Cerrado (South America), while

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H. mcmulliniana is widespread throughout south-eastern North America (Table 2; Lendemer & Allen 2020).

Phylogenetic reconstructions based solely on the nuITS marker did not recover reciprocal monophyly of *H. neohorrescens* and *H. mcmulliniana* (Supplementary Material Fig. S1, available online). Conversely, the two species were separated in distinct clades with the nuITS and mtSSU regions concatenated (Fig. 2). The nuITS genetic distances, widely used as a tool for taxon delimitation in *Parmeliaceae* (Leavitt *et al.* 2014; Divakar *et al.* 2016; Del-Prado *et al.* 2019), do not appear to be universally efficient in discriminating species of the subgenus *Parmelinopsis*. The mean genetic distance between *H. neohorrescens* and *H. mcmulliniana* was 0.018 s/s, ranging from 0.014–0.020 s/s, at the threshold between intra- and interspecific distances of *Parmeliaceae* (0.015–0.017 s/s; Del-Prado *et al.* 2010). Similarly, *H. afrorevoluta* (Krog & Swinscow) Krog & Swinscow and *H. appalachensis* Lendemer & J. L. Allen present nuITS distances between 0.016–0.018 s/s, indicating that some species of the subgenus *Parmelinopsis* may have a recent origin and cannot be differentiated using only the nuITS region.

Considering the different datasets collected in this study, we propose *Hypotrachyna neohorrescens* as a new species and reinforce the need for more taxonomic and molecular studies to unveil the diversity of *Hypotrachyna* subgenus *Parmelinopsis* in the neotropical region, especially in threatened biodiversity hotspots such as the Cerrado.

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