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Molecular data show that *Hypotrachyna sorocheila* (Parmeliaceae) is not monophyletic

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Abstract. Lichens that reproduce by means of vegetative propagules (soredia or isidia) are generally thought to have broad geographic distributions. However, recent studies have shown that some asexually reproducing lichens with broad distribution may be comprised of multiple, independent species-level lineages. Our understanding of species diversity in asexually reproducing lichenized fungal species may be further confounded by the fact that otherwise morphologically similar taxa separated based on the presence or absence of vegetative reproductive structures may in fact be conspecific. In this study, we investigate genetic diversity of the pantropical sorediate species *Hypotrachyna sorocheila* using molecular sequence data. Specifically, we generated a three-marker dataset for *Hypotrachyna* subgen. *Everniastrum* specimens, and reconstructed a multilocus, molecular phylogeny. Our results show that sorediate samples phenotypically identifiable as *H. sorocheila* do not form a monophyletic group, but form two distinct species-level lineages. Although our data support the pantropical distribution of *H. sorocheila* s.str., including populations in East Africa, a distinct species-level clade was found in Asia and is described as a new species here, *H. himalayana* Divakar & Kirika sp. nov. This study highlights the fact that the taxonomic significance of reproductive traits may vary among lineages of lichen-forming fungi, and that there is need for careful case-by-case studies.

Keywords. New species, lichens, molecular systematics, Africa, parmelioid lichens, phylogeny, taxonomy.

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Molecular sequence data are increasingly used to test traditional, phenotype-based species delimitations in lichen-forming fungi (reviewed in Crespo & Lumbsch 2010; Leavitt et al. 2015a; Lumbsch & Leavitt 2011). While molecular data support phenotypically-based species circumscriptions in numerous cases (Kanz et al. 2015; Lendemer et al. 2015; Pino Bodas et al. 2010; Sliwa et al. 2012; Tehler & Källersjö 2001), in other instances these data have challenged traditional delimitations (Alors et al. 2015; Cornejo & Scheidegger 2015; Kraichak et al. 2015; Pérez-Ortega et al. 2016; Singh et al. 2015). Subsequent studies based on phylogenetic reconstructions may reveal previously unrecognized phenotypical differences that supported the independence of the distinct species-level clades (Arguello et al. 2007; Divakar et al. 2010; Orange 2012; Pino-Bodas et al. 2012; Schneider et al. 2016; Spribille et al. 2011).

Lichens that reproduce by means of vegetative propagules (isidia and soredia) are generally thought to have broad geographic distributions. However, some widely distributed nominal lichenized fungal species may consist of several distinct evolutionary lineages, inferred using information from DNA sequence data. Furthermore, species separated by presence or absence of soredia have commonly been shown to be conspecific (Buschbom & Mueller 2006;

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Divakar et al. 2007; Truong et al. 2013; Wirtz et al. 2012), although other studies support the independence of otherwise morphologically similar phenotypes with differing reproductive strategies as distinct species (Cornejo et al. 2009; Lücking et al. 2008). These studies imply that careful, case-by-case consideration may be required to accurately circumscribe species boundaries in well-known lichens with broad distributions.

The genus *Everniastrum* Hale ex Sipman was described by Hale (1976) to include species formerly classified in *Parmelia* subgenus *Everniiformis*. In a recent molecular phylogenetic analysis of *Hypotrachyna* s.l., the genus *Everniastrum* was found to be nested within the *Hypotrachyna* clade, subsequently reduced to synonymy with *Hypotrachyna*, and classified as a subgenus within *Hypotrachyna* (Divakar et al. 2013). Recognition at the subgeneric level has the advantage that monophyletic lineages clustered within a paraphyletic *Hypotrachyna* s.l. can be recognized without producing paraphyletic taxa (Hörandl & Stuessy 2010).

Hypotrachyna subgenus *Everniastrum* (Hale ex Sipman) Divakar et al. comprises about 33 species (Divakar et al. 2013). Species in this subgenus are characterized by long, linear thalli with subcanaliculate to involute lobes with or without long marginal cilia and rhizines. They have isolichenan in the cell walls, a pored epicortex and a palisade like upper cortex (Crespo et al. 2007; Hale 1973). In addition, all species of this genus have atranorin in the cortex and most contain salazinic acid in the medulla, often accompanied by protolichesterinic acid. Other extrolites, including alectoronic, constictic, fumarprotocetraric, gyrophoric, norstictic, and protocetraric acids, have also been reported (Culberson & Culberson 1981; Hale & Wirth 1971; Sipman 1980, 1986).

In *Hypotrachyna* subgen. *Everniastrum* five of the 33 described species form soredia (Sipman 1986); and *Hypotrachyna sorocheila* is the only widely distributed sorediate species in the subgenus. This taxon is known from the tropics and warm temperate regions of both the Old and New Worlds. Other sorediate species in the group include: *Hypotrachyna catawbiensis, H. columbiensis*, *H. plana* and *H. subplana*. *Hypotrachyna catawbiensis* occurs in South America, eastern North America, Papua New Guinea and East Africa; whereas *H. columbiensis*, *H. plana* and *H. subplana* are only known from South America. *Hypotrachyna plana* has been considered a synonym of *H. catawbiensis* based on morphological variation in lobe morphology, marginal rhizine size and position of soralia on lobe tips (Culberson & Culberson 1981). However, this has not been accepted by other authors (e.g., Sipman 1986).

Here we aim to elucidate evolutionary relationships in the widely distributed sorediate taxon *Hypotrachyna sorocheila* and better understand the overall diversity of sorediate species in subgenus *Everniastrum*. To this end we generated sequences of nuclear ribosomal rDNA of the internal transcribed spacer region (ITS1, 5.8S and ITS2), large subunit (nuLSU), and mitochondrial small subunit (mtSSU) of 28 samples representing nine species of *Hypotrachyna* subgen. *Everniastrum*. *Hypotrachyna kaernefeltii* and *H. dubitans* were used as outgroup. We provide a phylogenetic hypothesis for this subgenus, and describe a new sorediate taxon from the Himalayan region of Asia.

Materials and Methods

Taxon sampling. A data matrix comprised of 30 samples, representing 11 species of *Hypotrachyna* subgen. *Everniastrum* (Divakar et al. 2013) was assembled using sequences of nuclear ITS, nuLSU and mitochondrial SSU rDNA. The dataset included numerous samples of *H. sorocheila* from different geographic regions, including Africa, Asia, Australasia, and South America (**Table 1**). GenBank accession numbers and information of studied materials are shown in **Table 1**. The data sets include 30 sequences from previous publications (Divakar et al. 2006, 2010a, 2013), and 43 were newly generated for this study. Two species of *Hypotrachyna*, *H. kaernefeltii* and *H. dubitans* were used as the outgroup since they are outside subgenus *Everniastrum*.

DNA extraction and PCR amplification. Total genomic DNA was extracted from small pieces of thallus free from any visible damage or contamination using the USB PrepEase Genomic DNA Isolation Kit (USB, Cleveland, OH) and in accordance with the manufacturer's instructions. We generated sequence data from nuclear ribosomal markers, the ITS and a fragment of the nuLSU, in addition to a fragment of the mtSSU. Polymerase chain reaction (PCR) amplifications were performed using Ready-To-Go PCR Beads (GE Healthcare, Pittsburgh, PA, USA) using dilutions of 1:10 of total

Table 1. Specimens used in this study, with location, reference collection detail and GenBank accession numbers. Newly obtained sequences are in bold face.

Taxa	Seq/DNA code	Locality	Collector(s)	Voucher	ITS	mtSSU	nuLSU
Hypotrachyna (=*Cetrariastrum*) *dubitans*	CEDU200	Peru: Ancash	Lumbsch, Wirtz & Ramirez, 19366	F (MAF-Lich 15621)	GQ919270	GQ919217	GQ919246
Hypotrachyna (=*Cetrariastrum*) *kaernefeltii*	CEAN199	Peru: Ancash	Lumbsch, Wirtz & Ramirez, 19334	MAF-Lich15620	GQ919269	GQ919217	GQ919245
Hypotrachyna (=*Everniastrum*) aff *cirrhata*	9881	Colombia; Tolima	Silano JS14_042 37745	F	**KX254119**	**KX254134**	**KX254146**
Hypotrachyna (=*Everniastrum*) *americana*	EVAM	Chile	Feuerer s.n	DNA1223(HBG, LD)	AY251418	—	—
Hypotrachyna (=*Everniastrum*) *catawbiensis*	9602	Kenya; Central	Kirika, 4325A	EA, F, MAF	**KX254120**	**KX254135**	**KX254147**
Hypotrachyna (=*Everniastrum*) *catawbiensis*	9883	Colombia; Cuaca	Salinas, 32b	F	**KX254121**	—	—
Hypotrachyna (=*Everniastrum*) *cirrhata* 1	OECIRRHA	Costa Rica: San José	Trest 149	MAF-Lich 7465	AY611070	AY611128	AY607782
Hypotrachyna (=*Everniastrum*) *cirrhata* 2	EVCI1894	Peru	Lumbsch 19342r	MAF 13976	DQ279487	DQ287795	EU562674
Hypotrachyna (=*Everniastrum*) *cirrhata* 3	ECIRRHAT	China	Crespo & al.	MAF 10374	DQ279486	DQ287794	—
Hypotrachyna (=*Everniastrum*) *columbiensis*	4768	Peru		MAF	**KX254122**	—	—
Hypotrachyna (=*Everniastrum*) *columbiensis*	9570	Peru		MAF(PY01)	**KX254123**	**KX254136**	**KX254148**
Hypotrachyna (=*Everniastrum*) *nepalensis*	ENEPAL	India: Uttarakhand	Divakar s/n	GPGC 02-000924	AY611071	AY611129	AY607783
Hypotrachyna (=*Everniastrum*) *rhizodendroidea*	ERHYZODE	China: Yunnan	Aptroot 55665	ABL	DQ279489	DQ287797	EU562676
Hypotrachyna (=*Everniastrum*) *sorocheila*	4780	India		MAF	**KX254124**	—	—
Hypotrachyna (=*Everniastrum*) *sorocheila*	9295	Kenya: Central	Kirika, 3653	EA, F	—	**KX254137**	**KX254149**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9520	Kenya: Western	Divakar et al. 19566P	MAF	**KX254125**	**KX254138**	**KX254150**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9544	Kenya; Central	Kirika, 4325B	EA, F, MAF	**KX254126**	**KX254139**	**KX254151**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9567	Kenya; Central	Kirika, 4349	EA, F, MAF	**KX254127**	**KX254140**	**KX254152**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9870	Colombia: Cundinamarca	Lomana, 299	F	—	**KX254141**	**KX254153**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9875	Colombia; Narino	Moncada, 7577	F	**KX254128**	**KX254142**	**KX254154**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9876	Colombia; Cundinamarca	Avella, 351a	F	**KX254129**	**KX254143**	**KX254155**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9878	Colombia; Cundinamarca	L. Castro, 27	F	**KX254130**	—	**KX254156**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9879	Colombia; Cundinamarca	Uribe, 24	F	**KX254131**	—	**KX254157**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9880	Colombia; Cundinamarca	Uribe, 40	F	**KX254132**	—	**KX254158**
Hypotrachyna (=*Everniastrum*) *sorocheila*	9903	New Zealand		F	**KX254133**	**KX254144**	**KX254159**
Hypotrachyna (=*Everniastrum*) *sorocheila* 1	ESORECHI	China	Crespo, Blanco & Arguello	MAF-10375	DQ279490	DQ287798	EU562677
Hypotrachyna (=*Everniastrum*) *sorocheila* 2	EVSO501	Kenya: Western Province	Divakar, Mangold & Lumbsch 19566e	F, MAF-Lich	JN943841	KR995337	JN939606
Hypotrachyna (=*Everniastrum*) *sorocheila* 3	3514	Portugal: Madeira	P.K. Divakar & M. Talavera, 6974P	MAF	**KX341977**	—	—
Hypotrachyna (=*Everniastrum*) *vexans*	9294	Kenya: Central	Kirika, 3662	EA, F	—	**KX254145**	**KX254160**
Hypotrachyna (=*Everniastrum*) *vexans*	EVEXANS	China: Yunnan	Aptroot 56597	ABL	DQ279491	DQ287799	EU562678

DNA. Fungal ITS rDNA was amplified using ITS1F primers (Gardes & Bruns 1993), ITS4 and ITS4A (White et al. 1990; Larena et al. 1999); mt SSU rDNA mtSSU was amplified using the primers mrSSU1, mrSSU3R and mrSSU2R (Zoller et al. 1999); and nuLSU rDNA was amplified using LR0R and LR5 (Vilgalys & Hester 1990). PCR products were visualized on 1% agarose gel and cleaned using ExoSAP-IT (USB, Cleveland, OH, USA). Cycle sequencing of complementary strands was performed using BigDye v3.1 (Applied Biosystems, Foster City, CA, USA) and the same primers used for PCR amplifications. Sequenced PCR products were run on an ABI 3730 automated sequencer (Applied Biosystems) at the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum, Chicago, IL, USA.

Sequence editing and alignment. New sequences were assembled and edited using Geneious v8.1.7 (Biomatters Ltd, 2005-2015). Multiple sequence alignments for each locus were performed using the program MAFFT v7 (Katoh et al. 2005; Katoh & Toh 2008). For the ITS and nuLSU sequences, we used the G-INS-i alignment algorithm and '20PAM / K=2' scoring matrix, with an offset value of 0.3, and the remaining parameters were set to default values. We used the E-INS-i alignment algorithm and '20PAM / K=2' scoring matrix, with the remaining parameters were set to default values for the mtSSU sequences. We used the program Gblocks v0.91b (Talavera & Castresana 2007) to delimit and remove ambiguous alignment nucleotide positions from the final alignments using the online web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), implementing the options for a less stringent selection of ambiguous nucleotide positions.

Phylogenetic analyses. A total of 16 new nuclear ITS, 15 new nuLSU and 12 new mtSSU sequences were generated (**Table 1**). These were aligned with 30 sequences downloaded from GenBank (**Table 1**). Phylogenetic relationships were inferred using maximum likelihood (ML), and Bayesian inference (BI). Exploratory phylogenetic analyses of individual gene topologies showed no evidence of well-supported ($\geq$ 70% bootstrap values) topological conflict, and relationships were estimated from a concatenated, three-locus (ITS, nuLSU, mtSSU) data matrix using a total-evidence approach (Wiens 1998). We used the program RAxML v8.1.11 (Stamatakis 2006; Stamatakis et al. 2008) to reconstruct the concatenated ML gene-tree using the CIPRES Science Gateway server (http://www.phylo.org/portal2/). We implemented the 'GTRGAMMA' model, used locus-specific model partitions, treating all loci as separate partitions, and evaluated nodal support using 1000 bootstrap pseudoreplicates. Exploratory analyses using alternative partitioning schemes resulted in identical topologies and highly similar bootstrap support values. We also reconstructed phylogenetic relationships from the concatenated multi-locus data matrix under BI using the program BEAST v1.8.2 (Drummond & Rambaut 2007). We ran two independent Markov Chain Monte Carlo (MCMC) chains for 10 million generations, implementing a relaxed lognormal clock, a constant coalescent speciation process prior. The most appropriate model of DNA sequence evolution was selected for each marker was selected using the program PartitionFinder v1.1.1 (Lanfear et al. 2012), treating the ITS1, 5.8S, ITS2, nuLSU, and mtSSU as separate partitions. The first 2 million generations were discarded as burn-in. Chain mixing and convergence were evaluated in Tracer v1.5 (Rambaut & Drummond 2009), considering ESS values >200 as a good indicator. Posterior trees from the two independent runs were combined using the program LogCombiner v1.8.0 (Drummond et al. 2012), and the final maximum clade credibility (MCC) tree was estimated from the combined posterior distribution of trees.

Results and Discussion

We generated 43 new DNA sequences including ITS, nuLSU and mtSSU of the following *Hypotrachyna* subgen. *Everniastrum* species: *H. cawtabiensis, H. columbiensis, H. sorocheila* and *H. vexans*. The matrix of the concatenated data set included 2160 unambiguously aligned nucleotide position characters, 500 unambiguously aligned nucleotide position characters in ITS, 835 in nuLSU and 783 in mtSSU. The ITS PCR product obtained ranged between 600 to 800 bp. Differences in size were due to the presence or absence of insertions of about 200 bp identified as group I introns (Gutierrez et al. 2007) at the 3′ end of the SSU rDNA, which were excluded from the final analyses. The ML and BI analyses were identical in their topology and therefore here only the ML tree with support values of both analyses is shown (**Fig. 1**). Alignments and trees associated with

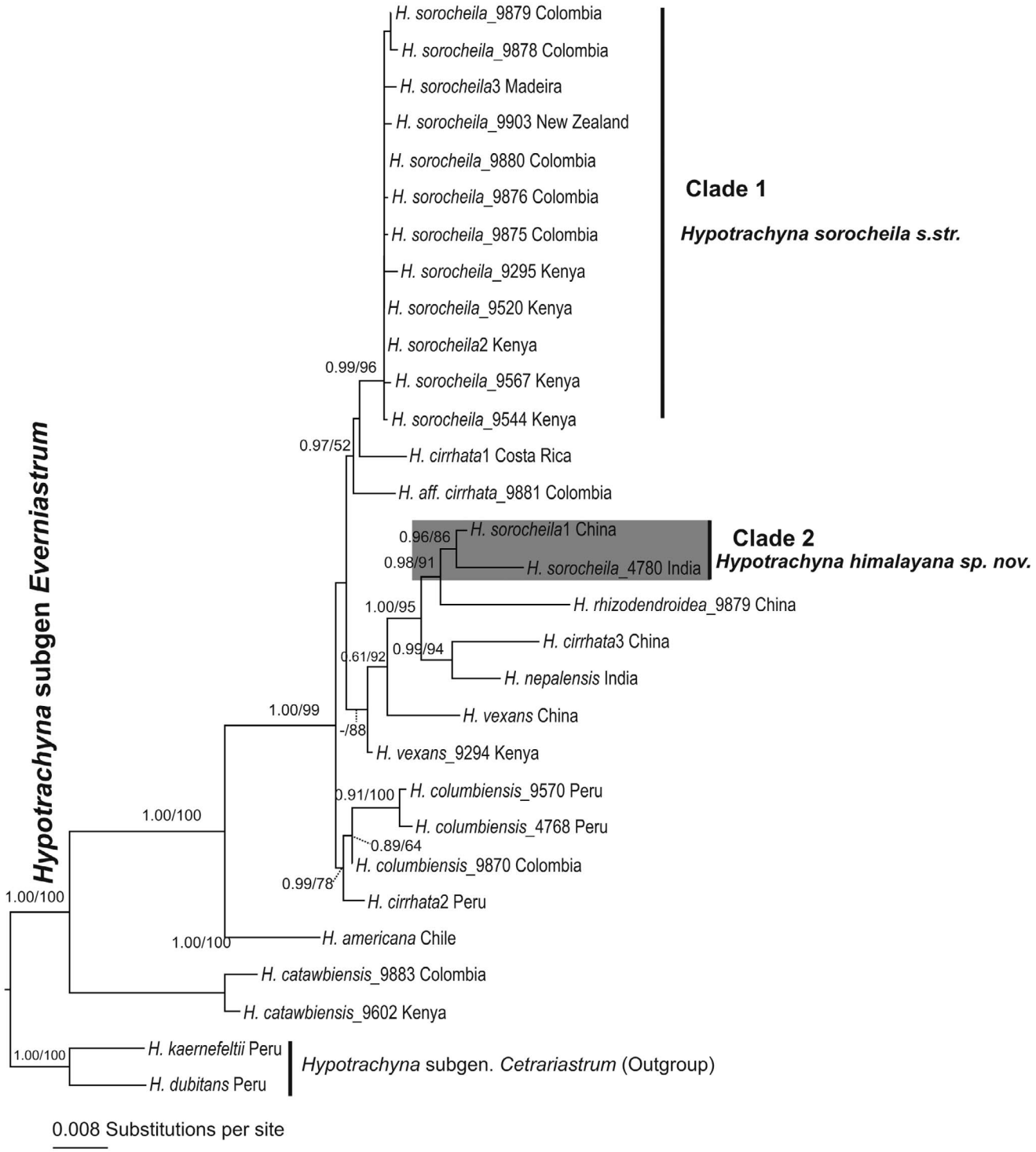

Figure 1. Phylogenetic relation among *Hypotrachyna* subgen *Everniastrum* taxa based on a maximum-likelihood (ML) analysis of a concatenated, three locus dataset, ITS, nuLSU and mtSSU rDNA. Since ML and Bayesian inference topologies were identical, only the ML topology is reported here. Posterior probabilities ≥0.95/ ML bootstrap values ≥70% are given above the branches.

this study are available in Treebase (http://purl.org/phylo/treebase/phylows/study/TB2:S19305).

In the inferred multilocus topology, all the samples of *Hypotrachyna* subgen. *Everniastrum* formed a strongly supported monophyletic group as in a previous study (Divakar et al. 2013). While some species in this subgenus formed monophyletic groups, others did not, including *H. cirrhata, H.*

sorocheila and *H. vexans* (**Fig. 1**). The polyphyly of *H. cirrhata* has been shown in previous studies (Divakar et al. 2006, 2013), whereas non-monophyly of *H. sorocheila* and *H. vexans* is shown here for the first time. An Asian sample of the latter species formed a strongly supported monophyletic group with samples of other species collected in Asia, and the Kenyan sample of *H. vexans* formed a sister relation to this Asian clade, resulting in paraphyly of *H. vexans* as currently circumscribed. *Hypotrachyna vexans* is an isidiate species described from Taiwan (Culberson & Culberson 1981; Divakar & Upreti 2005; Hale 1976) and the specimen from Kenya may belong to an undescribed species. However, additional sampling is necessary in order to better understand the delimitation of this species.

Samples of *Hypotrachyna sorocheila* were recovered in two distinct, well-supported clades. Clade '1' included samples from Colombia, Kenya, Madeira, and New Zealand, whereas clade '2' included samples from China and India. Clade '2' clustered in the Asian clade (**Fig. 1**). Clade '1' formed a sister-group relationship with two South American samples of an apotheciate species *H. cirrhata* and clade '2' formed a sister-group relationship with the Asian apotheciate species *H. rhizodendroidea*. Interestingly, both sorediate lineages formed sister-group relationships with apotheciate taxa.

While *Hypotrachyna sorocheila* is common in South America and East Africa, it is rarely collected in Asia (Culberson & Culberson 1981; Sipman 1980, 1986). In India the species is known from two localities in West and East Himalayas (Divakar & Upreti 2005). *Hypotrachyna sorocheila* is here reported for the first time from Madeira. It is a sorediate species that was originally described from Colombia. Since all Colombian material belongs to clade '1', we here consider this clade as *H. sorocheila* s.str. and propose below an epitype to fix the use of this name. Consequently, a new species is described below to accommodate samples from Asia (clade '2'). Furthermore, our results demonstrate that samples from the Neo- and Paleotropics belong to a single lineage, supporting the pantropical distribution of *H. sorocheila* s.str.

Hypotrachyna catawbiensis and *H. columbiensis* are the only other sorediate species in this subgenus. These two species clustered in separate lineages. The neotropical *H. columbiensis* formed a well supported monophyletic group sister to a *H. cirrhata* sample from Peru. *Hypotrachyna catawbiensis* had a sister-group relationship to all other species in the subgenus. The species is known from the New World and East Africa. The samples from Kenya and Colombia clustered together indicating that this species is comprised of geographically disjunct populations. While the disjunct and wide distribution pattern of sorediate taxa in this subgenus has been discussed previously (Culberson & Culberson 1981; Sipman 1980, 1986), this has not been tested using molecular data before.

Our results suggest that sorediate morphs in *Hypotrachyna* subgen. *Everniastrum* belong to independent lineages distinct from esorediate taxa. Our results confirm that some sorediate species-level lineages in this subgenus can be widely distributed (e.g., *H. catawbiensis* and *H. sorocheila*). Studies have shown that species distributed in different continents may belong to distinct lineages (e.g., Alors et al. 2016; Argüello et al. 2007; Divakar et al. 2010b; Leavitt et al. 2015b). In some cases, sorediate morphs in other genera have been shown to be conspecific with esorediate counterparts (Buschbom & Mueller 2006; Divakar et al. 2007; Truong et al. 2013; Wirtz et al. 2012). In other instances, sorediate taxa have been shown to represent lineages distinct from esorediate counterparts that are otherwise morphologically similar (Cornejo et al. 2009; Lücking et al. 2008), as observed in *Hypotrachyna* subgen. *Everniastrum* in this study. Hence, the taxonomic significance of a reproductive trait varies among lineages of lichen-forming fungi (Leavitt et al. 2015b; Tehler et al. 2009).

Here we add evidence supporting the distinction of a number of sorediate species in *Hypotrachyna* subgen. *Everniastrum*. Our results show different distribution patterns in these sorediate taxa, with some that are widely distributed (e.g., *H. catawbiensis* and *H. sorocheila*) and others with more restricted distributions in tropical regions (e.g., *H. columbiensis* and *H. himalayana* sp. nov.).

Taxonomy

Hypotrachyna himalayana Divakar & Kirika, *sp.nov*. **Fig. 2**

Mycobank MB 817198

The species is morphologically similar to H. sorocheila but differs in geographic distribution restricted to Asia, molecular phylogenetic tree topology and

Figure 2. *Hypotrachyna himalayana* habit (MAF-Lich 10375).

consists of samples grouped in clade '2', within the Asian clade (Fig. 1).

Type: CHINA. YUNNAN: Jianchuan Co., ridge on trail to Lao Sueri Shan, *Rhododendron* forest-scrub with scattered *Abies*, on tree trunk, 26.37N, 99.43E, alt. 3980m, 19 Oct. 2002, A. Crespo, O. Blanco & A. Arguello (holotype: MAF-Lich 10375). Genbank accession number: ITS DQ279490, nuLSU EU562677 and mtSSU DQ287798.

Description. Thallus loosely attached to the substratum, suberect, ca. 3.0 cm across, dichotomously lacinate lobate. Lobes sublinear, elongate, tapering at apices, 1–2.5 mm wide. Margin flat to involute especially near apices, ciliate. Cilia sparse, simple, ca. 0.8 mm long. Upper surface gray, smooth, sorediate. Soralia granular, subterminal, sorediate apices involute. Medulla white. Lower surface black with narrow brown to dark brown colored zone near tip of lobes, canaliculated, smooth or sometimes transversely wrinkled in lower parts, sparsely rhizinate near margins. Rhizines absent or a few, simple to branched, black, to 1 mm long. Apothecia and pycnidia not seen in the specimen examined.

Chemistry. Cortex K+ yellow; medulla K+ yellow turning red, C–, KC–, P+ orange-red; atranorin and salazinic acid.

Etymology. The taxon name is based on its occurrence in the Himalayan region.

Remarks. This taxon is morphologically most similar to *Hypotrachyna sorocheila*; however, in the current phylogenetic reconstruction it appears to be more closely related to the apotheciate species *H. rhizodendroidea* known from Asia (**Fig. 1**, **Table 1**). It is worth emphasizing that no morphological differences have yet been found distinguishing *H. sorocheila* and the new segregate, *H. himalayana*; however, the latter lacks galbinic acid and protolichesterinic acid (Culberson & Culberson 1981). *Hypotrachyna himalayana* is the only sorediate species known so far from Asia in the *Hypotrachyna* subgen. *Everniastrum*. It occurs corticolous in *Rhododendron* forest and scattered *Abies* and other tree trunks at higher elevations ranging from 3000 to 4000 m in Himalayan regions of China and India. At the moment it is known from only three localities of the Himalayan region.

Hypotrachyna sorocheila (Vain.) Divakar et al. (2013)

Type: COLOMBIA. "prope Bogota," 8500′ s.m., J. Weir 5 pr.p. (holotype: BM)

Remarks. A full description of *Hypotrachyna sorocheila* is found elsewhere (Culberson & Culberson 1981; Sipman 1980). Morphologically, it is similar to *H. himalayana* but in the phylogenetic tree it appears close to two samples of *H. cirrhata* from South America. The species grows on trees and mossy shrubs in South America, Australasia, Africa and Madeira. So far *H. sorocheila* is not known from Asia.

Acknowledgments

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