Taxonomic study of Hypotrachyna subg. Everniastrum (Hale ex Sipman) Divakar, A.Crespo, Sipman, Elix & Lumbsch (Ascomycota) from China

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Abstract
The taxonomy of Hypotrachyna subg. Everniastrum (Hale ex Sipman) Divakar, A.Crespo, Sipman, Elix & Lumbsch from China is revised based on morphological, chemical and phylogenetic analyses. Four species new to science (Hypotrachyna corallifera Xin Y.Wang & Li S.Wang, sp. nov., H. longicilia Xin Y.Wang & Li S.Wang, sp. nov., H. puerensis Xin Y.Wang & Li S.Wang, sp. nov., and H. yunnana Xin Y.Wang & Li S.Wang, sp. nov.) and a new floristic record for one species (H. catawbiensis (Degel.) Divakar, A.Crespo, Sipman, Elix & Lumbsch) in China are reported, and a total of fifteen species are
INTRODUCTION

The genus *Everniastrum* Hale ex Sipman, typified by *E. cirkhatum* (Fr.) Hale ex Sipman, was first segregated from the genus *Parmelia* Ach. as section *Everniiformes* by Hue (1898), characterized by narrow lobes with marginal cilia. In 1971, section *Everniiformes* was upgraded to *Parmelia* subg. *Everniiformes* by Hale & Wirth (1971), including 16 species. In 1976, Hale transferred this subgenus into the new genus *Everniastrum* Hale, including 21 species (Hale 1976). Later, Sipman segregated *Cetrariastrum* Sipman from *Everniastrum* based on characters of the apothecia (Sipman 1980). One year later, Culbersons published a paper questioning the validity of this new genus and used *Cetrariastrum* as the correct name for *Everniastrum* (Culberson & Culberson 1981). In 1986, Sipman re-evaluated the differences between these two genera and reinstated the genus *Everniastrum* with 27 species, and *Cetrariastrum* included three species (Sipman 1986). Since that time, the genus name *Everniastrum* has been widely accepted and used worldwide. A recent molecular study transferred this genus into a subgenus, *Hypotrachyna* subg. *Everniastrum* (Divakar et al. 2013), including c. 40 species.

Ten species of *Everniastrum* have been previously reported from China (Chen 2011; Chen et al. 2015), including five species originally described from China (*Parmelia* & Culberson 1981; Wei & Jiang 1982; Chen et al. 1989; Jiang & Wei 1989; Jiang & Wei 1993), while *H. lipidifera* was excluded from subgenus *Everniastrum* and placed in the subgenus *Cetrariastrum* (Divakar et al. 2013). After examination of a large number of samples, including type specimens and fresh specimens collected at the type localities, 15 species are recognized in this study, including four species new to science (*H. cirkhatum* X.Wang & Li S.Wang, sp. nov., *H. longicilia* Xin Y.Wang & Li S.Wang, sp. nov., *H. puerensis* Xin Y.Wang & Li S.Wang, sp. nov., and *H. yunnana* Xin Y.Wang & Li S.Wang, sp. nov.) and a new floristic record for one species (*H. cirkhatum* (Degel.) Divakar, A.Crespo, Sipman, Elix & Lumbsch) in China. Detailed descriptions and figures for the new species are provided, together with a key to all known Chinese *Everniastrum* species.

MATERIAL AND METHODS

MORPHOLOGY AND CHEMISTRY

Approximately 1600 specimens at the Lichen Herbarium, Kunming Institute of Botany (KUN), were examined, and type material and related specimens were borrowed from the following herbaria: HMAS, TUR, US and W. Specimens were examined using standard microscopic techniques and hand-sectioned under a Nikon SMZ 745T dissecting microscope. Anatomical descriptions are based on observations of these preparations under a Nikon Eclipse 50I microscope, and photographs were taken using a Nikon DS-Fi2 digital camera head. The sizes of the thallus, apothecia and lobes are calculated from the smallest to the largest value.
Secondary metabolites of all the specimens were identified using spot tests and thin-layer chromatography (TLC) as described by White & James (1985) and Orange et al. (2001). Solvent C (toluene: acetic acid = 85:15) was used for TLC analysis.

### Phylogenetic Analyses

Total DNA was extracted from small fragments of fresh thallus tips or apothecia following the protocol by Ekman (1999) using the AxyPrep Multisource Genomic DNA Miniprep Kit. The nrDNA ITS region (ITS1-5.8S-ITS2) was amplified by PCR using the ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) primers. The conditions previously described by Arup (2002) were used for PCR. Specifically, 2 µL of genomic DNA was added to the following mixture: 2 µL of primer (1 µL of each primer in a 10 mM solution), 12.5 µL of 2× Taq PCr MasterMix (Aidlab) containing Taq DNA polymerase: 0.1 unit µL, MgCl2: 4 mM.

### Table 1

Specimens used in the phylogenetic analysis of ITS sequences, with name (H. = Hypotrachyna), locality, voucher and GenBank accession number. Newly obtained sequences are in **bold face.**

<table>
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<th>Voucher</th>
<th>Accession number</th>
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<td>H. sorocheila (Vain.) Divakar, A.Crespo, Sipman, Elix &amp; Lumbsch</td>
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<td>H. subsorocheila (Y.M.Jiang &amp; J.C.Wei) Divakar, A.Crespo, Sipman, Elix &amp; Lumbsch</td>
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<td>H. vexans (Zahlbr. ex W.L.Culb. &amp; C.Culb.) Divakar, A.Crespo, Sipman, Elix &amp; Lumbsch</td>
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and dNTPs: 0.4 mM) and 8.5 µL of dH₂O for a total volume of 25 µL. Amplifications were carried out in a thermocycler (C1000TM) with the following profile: initial denaturation at 94°C for 5 min, followed by 35 cycles of three steps (95°C for 1 min, 55°C for 1 min 20 s, and 72°C for 2 min) and a final extension at 72°C for 5 min. The PCR products were Sanger sequenced by Sangon Biotech (Shanghai) Co., Ltd.

All the raw sequences were assembled and edited using SeqMan (DNAstar package). The newly generated ITS sequences were complemented by sequences available in GenBank. Sequences were aligned using MAFFT version 7 with the L-INS-I alignment algorithm (Katoh et al. 2005), using the web server (http://mafft.cbrc.jp/alignment/server/) and with all parameters set to default values, and ambiguous regions were excluded using Gblocks (Talavera & Castresana 2007) with the default settings. Phylogenetic relationships were inferred using Bayesian inference (BI) and under the maximum likelihood (ML) criterion.

BI analyses were performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The optimal substitution model (GTR + G) implemented in MrBayes was determined based on the Akaike information criterion (AIC) using jModelTest 3.7 (Posada 2008). BI analyses were run for 1,000,000 Markov chain Monte Carlo (MCMC) generations and with four incrementally heated chains; MCMC started from a random tree, and trees were sampled every 1000 generations, with the first 10% of the trees discarded as burn-in. Posterior probabilities (PPs) were obtained from the 95% majority-rule consensus tree of all saved trees.

ML analyses were performed with an online version of RAxML-HPC v8.2.12 (Stamatakis 2006), with the GTR + G model. All parameters in the ML analysis were set to the default, and statistical support values were estimated by 1000 nonparametric bootstrapping pseudoreplicates. ML bootstrap support (MLBS) values were obtained from the default, and statistical support values were estimated by 1000 nonparametric bootstrapping pseudoreplicates. ML bootstrap support (MLBS) values were obtained from the 95% majority-rule consensus tree of all saved trees using FigTree v1.4.0 (Rambaut 2012).

RESULTS

PHYLOGENETIC ANALYSES

The alignment consisted of 48 ITS sequences (Table 1), of which were newly generated in this study, and included *H. kaernefeldii* Divakar, A.Crespo, Sipman, Elix & Lumbsch and *H. dubitans* (Sipman) Divakar, A.Crespo, Sipman, Elix & Lumbsch as outgroup taxa. The results of ML and Bayesian analyses revealed 11 phylogenetically supported *Everniastrum* species. All species belonging to subgenus *Everniastrum* were clustered in one clade with high support (100% MLBS and 1.00 PP), and four new species formed monotypic lineages with strong support (100% MLBS, 1.00 PP and 98% MLBS, 0.94 PP). Furthermore, these species were also supported by morphological, chemical and geographical characters, thus fully meriting recognition as new species, for which the names *H. corallifera* Xin Y.Wang & Li S.Wang, sp. nov., *H. longicilia* Xin Y.Wang & Li S.Wang, sp. nov., *H. puerensis* Xin Y.Wang & Li S.Wang, sp. nov., and *H. yunnana* Xin Y.Wang & Li S.Wang, sp. nov., were coined (Fig. 1).

TAXONOMY

Family PARMELIACEAE F.Berichtold & J.Presl

Genus *Hypotrachyna* (Vain.) Hale

1. *Hypotrachyna alectorialica* (W.L.Culb. & C.F.Culb.) Divakar, A.Crespo, Sipman, Elix & Lumbsch


SPECIMEN EXAMINED. — India. West Bengal, Darjeeling; 2 XII. 1920; Plitt (holo-, US[US]).

NOTES

For a detailed description, see Culberson & Culberson (1981).


2. *Hypotrachyna catalwienisis* (Degel.) Divakar, A.Crespo, Sipman, Elix & Lumbsch


SELECTED SPECIMENS EXAMINED. — China. Yunnan Prov.: Wuding Co., Shizh Ma; 102°22′43″N, 25°31′55″E; 2192 m, on bark; 30.VI.2011; E. D. Liu 11-2344; Wenshan Co., Baoshu Mt; 20°21.822′N, 103°54.624′E; 2870 m, on bark; 22.VII.2011; L. S. Wang 11-32165; Nanjian Co., Fenghuang Mt; 24°53′56.34″N, 100°19′49.66″E; 2340 m, on Pinus bark; 14.VI.2012; L. S. Wang 12-34296; Ci., Aziying Vil.; 25°19.960′N, 102°50.956′E; 2190 m, on bark; 14.VI.2012; L. S. Wang 12-37158.

ECOLOGY AND DISTRIBUTION. — In China, this species usually grows on the bark of *Pinus* in the Hengduan Mts, at elevations of 2100–2800 m. Elsewhere, it has been reported from North and South America, South Asia and East Africa and is widely distributed in the tropics (Culberson & Culberson 1981; Sipman 1980).
Morphology

Thallus

2-5 cm long, lobes 0.5-1 mm wide, lobe tips dichotomously branched, flat or involute, internodes 3-6 mm long.

Upper surface

Smooth, greyish green to pale ivory.

Lower surface

Smooth to slightly wrinkled, black in colour, turning brownish near the lobe tips.

Cilia

Sparse, 1-2 mm long, simple or sparingly branched.

Rhizines

Sparse or absent.

Soredia

Subterminal or laminal, erumpent and capitulate.

Isidia

Absent.

Apothecia

Not seen.

Pycnidia

Not seen.

Fig. 1. — Phylogenetic tree generated from maximum likelihood (ML) analysis based on ITS sequence data. ML bootstrap support values greater than 70% and Bayesian posterior probabilities (PPs) greater than 0.95 are given above the nodes. New species are indicated in bold. “TYPE” refers to the sequences of types, “TOPO” for specimens that come from the type locality. Scale bar: 0.02 substitutions.
This species is characterized by having cilia on the lobe margin and a bare lower surface. The type specimen was collected from Nepal and published with a very brief description (Fries 1825). The brief description makes the taxonomic range of this species narrower and needed to clarify the range of this species and the cryptic taxa mistakenly identified as this species.

4. *Hypotrachyna corallifera* Xin Y. Wang & Li S. Wang, sp. nov. (Fig. 2)

**ECOLOGY AND DISTRIBUTION.** — This species occurs in Yunnan and Taiwan, similarly to *H. vexans*, and usually grows on the bark of *Quercus* or *Pinus*, rarely on moss, at elevations of 1400–2300 m.

**SELECTED MATERIALS EXAMINED.** — China, Yunnan Prov.: Shizong Co., Junzishan Mt; 23°30.602’N, 120°48.719’E; 2283 m, on Juniperus bark; 26.IX.2015; L. S. Wang 15-49365 (holo-, KUN[KUN]).

**ETYMOLOGY.** — The epithet “*corallifera*” refers to the coralliform isidia.

**MORPHOLOGY**

**Thallus**

2–6 cm in diameter, lobes slim and involute, 0.5–1 mm wide, internodes 1–2 mm long, dichotomously branched, tips usually pointed and curving downward.

**Upper surface**

Smooth, greisy green to brownish grey (if stored in herbarium), densely isidiate.

**Isidia**

Numerous, 0.5–1.2 mm long, usually growing in the centre or at the tip of the lobe, forming tufts, coralliform-branched.

**Lobules**

Present among the isidia.

**Apothecia**

Not seen.

**Lower surface**

Smooth and bare, slightly wrinkled, dark brown in the centre, turning pale yellowish near the tips, rhizines absent.

**Cilia**

Sparse or absent, 0.8–2 mm long, simple or dichotomously branched.
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Soredia
Absent.

Pycnidia
Absent.

CHEMISTRY
Cortex
K+ yellow.

Medulla
K+ yellow to red, C-, P+ orange red, containing atranorin and salazinic acid (detected by TLC).

NOTES
This species is characterized by dense and coralloid-branched isidia on the upper surface and lobules growing between the isidia. Another isidiate species in China, *H. vexans*, differs from this new species by its small, laminal, granulose,
marginal, and usually simple isidia without branches, often with lateral black cilia (Fig. 5D). Furthermore, *H. vexans* has flatter and wider lobes and lacks lobules. The phylogenetic tree based on specimens from the type locality (Taiwan Prov.) also supported this species as being different from *H. vexans*. *H. fragilis* has a type of isidia similar to that in this new species but differs by its absence of lobules, its long and fragile isidia (up to 3 mm), mainly growing on the lobe tips and margins, and a different geographic distribution (South America).


**SELECTED MATERIALS EXAMINED.** — *China*, Yunnan Prov.: Dali Ci., Cangshan Mt; 25°34′N, 100°13′E; 3750 m, on bark; 22.X.2002; J. C. Wei WY203 (HMAS–Neotype, selected in Chen et al. 2015); Cangshan Mt; TV station; 25°41′144″N, 100°6′7″E; 2800 m, on Pinus bark; 22.X.2002; L. S. Wang 02-22486; Nanjian Co., Dazhong Mt; 24°50′15″30″N, 100°25′16′50″E; 2750 m, on bark; 20.XII.2012; L. S. Wang 12-37774.

**NOTES**

For a detailed description, see Jiang & Wei (1993).

This species is characterized by containing diffractaic acid, which is unique among the known *Everniastrum* species. The species has been reported from only Yunnan Province. Diffractaic acid was not found in our specimens (45 specimens – some of them are listed under *H. nepalensis*, *H. sinensis* and *H. yunnana* Xin Y. Wang & Li S. Wang, sp. nov.) collected from the type locality (Dali Ci., Cangshan Mt), and we suppose that two chemotypes exist in Yunnan, a rare type with additional diffractaic acid and a more common type with atranorin and protolichesterinic and salazinic acids.

Apart from its chemical composition, this species can be distinguished by its large ascospores (up to 32 µm long), apothecia growing on the terminal part of the lobes, with a distinct rugose podetium, and long marginal cilia (up to 2.5 mm long).

6. *Hypotrachyna himalayana* Divakar & Kirika


**SELECTED SPECIMENS EXAMINED.** — *Colombia*, prope Bogota; 8500 ft s.w. J. Weir 5 pt. p. (H. sorocheila, holotype, BM[BMI]).

*China*, Yunnan Prov., Lijiang Co., Ganheba Vill.; 26°52′28.68″N, 100°14′9.545″E; 3550 m, on bark; 22.IX.2015; J. C. Wei 9282 (HMAS). Xundian Co., Hekou Vill., Xuzhao Temple; 25°38′23.97″N, 100°14′9.545″E; 3000 m, on bark; 22.IX.2002; J. C. Wei WY203 (HMAS–Neotype, selected in Chen et al. 2015); Yuxi Co., Xingyi temp.; 25°10′60″N, 102°37′41″E; 2600 m, on bark; 27.XI.2011; L. S. Wang 29–47382.

**NOTES**

For a detailed description, see Kirika et al. (2016).

This species can be distinguished by the lip-shaped soralia growing on the lower surface of the lobe tips and by containing atranorin and salazinic acid as the main compounds. It is distinguished from other sorediate species in China as follows. It differs from *H. cattawbensis* by its anchor-shaped soralia on the lobe tips, while in *H. cattawbensis*, the soralia are usually laminar. Furthermore, it contains gyrophoric acid (medulla K-, C+ red) instead of salazinic acid (medulla K+ yellow turning red, C-). *H. subsorocheila* has wider and shorter lobes, the upper surface is pustulate and forms soralia, and salazinic and gyrophoric acids are absent (medulla K-, C-); it has characters identical to those in *H. sorocheila* but is distinct in its position on the phylogenetic tree and its geographic distribution, as it is restricted to Asia, whereas *H. sorocheila* is mainly distributed in South America and currently not known from Asia (Kirika et al. 2016). Moreover, these two species formed two distinct clades in the tree (Fig. 1), consistent with the result of Kirika. Thus, the previous report of *H. sorocheila* in China (Chen et al. 2015) should be revised as *H. himalayana*.

7. *Hypotrachyna longicilia* Xin Y. Wang & Li S. Wang, sp. nov.

(Fig. 3)

**ETYMOLOGY.** — The epithet “longicilia” refers to the long and curved cilia.

**DIAGNOSIS.** — This species is characterized by dense, long and curved marginal cilia up to 3.5 mm long, which are dichotomously to irregularly branched. It resembles *E. diffracticum* in its long cilia but differs in lacking diffractaic acid, its longer cilia, its smaller ascospores and its subtropical distribution.

**TYPE.** — *China*, Yunnan Prov., Yuanyang Co., Xiguanyin Mt; 23°10′60″N, 102°37′41″E; 2600 m, on bark; 27.XI.2011; D. Liu (holo-, KUN[KUN]).

**ECOLOGY AND DISTRIBUTION.** — This species is common in the southern part of the Hengduan Ms and of Taiwan Province, with a subtropical distribution. It usually grows on the bark of Pinus or Salix, at elevations of 1900-2800 m.

**SELECTED SPECIMENS EXAMINED.** — *China*, Yunnan Prov., Gongshan Co., Dongshaofang Vill.; 27°42.164″N, 98°29.671″E; 2500 m, on litter; 21.II.2005; L. S. Wang 00–19064; *China*, Yunnan Prov., Wangxian Co., Xingyi Temp.; 25°34′N, 100°13′E; 3750 m, on bark; 22.X.2002; L. S. Wang 12-37774.

**MORPHOLOGY.**

*Thallus*

3-10 cm in diameter, lobes 0.8-1.2 mm wide, flat or slightly involute, rather slim and long, internodes 5-8(15) mm long, dichotomously branched, tips usually pointed.

*Upper surface*

Smooth, greyish green to pale grey.
Lower surface
Strongly wrinkled, black in the centre, turning dark brown near the lobe tips.

Cilia
Rather dense, 1.5-3(-3.5) mm long, curved, dichotomously to irregularly branched.

Rhizines
Absent.

Soredia and isidia
Absent.

Lobules
Absent.

Apothecia
Subterminal, disc yellowish brown to reddish brown, 1-3(-5) mm in diameter, podetium usually smooth, ascus 8-spored, spores kidney-shaped, 20-22 × 5-7 µm.
8. *Hypotrachyna mexicana* (Egan) Divakar, A.Crespo, Sipman, Elix & Lumbsch


**SELECTED SPECIMENS EXAMINED.** — *Mexico*, Jalisco: Combre de Tejamanil, Mpio. de Jalapa; 2200 m, on trees; 25.X.1971; *González*, 1971 (holo-, US[US]).

*China*, Yunnan Prov., Fengshan Co., Fengshan Mt; 1650 m, on *Camellia* bark; 11.VII.1982; *L. S. Wang* 11-22378.

**CHEMISTRY**

**Cortex** K+ yellow.

**Medulla** K+ yellow turning red, C-, P+ orange. Containing atranorin and salazinic acid (detected by TLC).

**NOTES**

This species can be distinguished from all the other Chinese species by its long, curved and branched cilia; the cilia usually attach to the substrate or to nearby lobes. Furthermore, the lobes are rather long and slim, forming long internodes (up to 15 mm long). *H. diffractaica*, having long cilia, might be confused with this species, but *H. longicilia* Xin Y.Wang & Li S.Wang, sp. nov., has much longer (up to 3.5 mm) and denser cilia, shorter ascospores, and a subtropical distribution.

Specimens of *H. cirrhata* with long cilia resemble this species, but the lobes of *H. longicilia* Xin Y.Wang & Li S.Wang, sp. nov., are much slimmer and longer (internodes up to 15 mm long), and the cilia are dense, usually curved and with branches, whereas in *H. cirrhata*, the cilia are usually sparse, short, simple and without branches.


**SELECTED SPECIMENS EXAMINED.** — *China*, Yunnan Prov., Wenshan Co., Bozhu Vil., Bozhu Mt; 20°21.822'N, 103°54.624'E; 2870 m, on bark; 22.VII.2011; *L. S. Wang* 11-32167.

**CHEMISTRY**

**Cortex** K+ yellow.

**Medulla** K+ yellow, KC+ pale red, and salazinic acid (detected by TLC).

**NOTES**

This species is characterized by the presence of dense rhizines on the lower surface, marginal cilia usually simple and less than 1 mm long, slim lobes, and the presence of protocetraric and salazinic acids. *H. subnepalensis* (holo-, US[US]) is morphologically identical but differs in containing fumarprotocetraric acid.

*H. nepalensis* is widely distributed in Asia, growing in the mountains of Yunnan, Nepal, India, Thailand and Java (Culberson & Culberson 1981), and usually grows on shrubs and trees. Local people from northwestern Yunnan use this species as a medicine (raw material for antibiotics). We used specimens from the type locality in Nepal and the nearby region of China for an estimation of the phylogenetic position of the species (Fig. 1). This phylogenetic analysis confirmed the monophyly of the species, providing a reference for its delimitation.

10. *Hypotrachyna puerensis* Xin Y.Wang & Li S.Wang, sp. nov.

(Fig. 4)

**MYCOBANK NUMBER.** — MB 832373.

**ETYMOLOGY.** — The epithet “pueresa” refers to the type locality, Pu’er city in southern Yunnan Province.

**DIAGNOSIS.** — This species is unique in its light brown to yellowish white colour, bare lower surface, absence of marginal cilia, and protocetraric acid and atranorin contents (medulla K-). It resembles *H. lipidifera* in the absence of salazinic acid and the bare lower surface, but the latter species has sparse marginal cilia and does not produce protocetraric acid.

**TYPE.** — *China*, Yunnan Prov., Pu’er Ci., roadside forest along the old road from Pu’er to Kunming; 22°52’08.47″N, 100°59’25.61″E; 1548 m, on bark; 20.XII.2013; *X. Y. Wang* 13-41509.

**ECOLOGY AND DISTRIBUTION.** — This species is found in the southern Yunnan subtropical forest. It usually grows in the broadleaf forest at elevations of 1500-2300 m.

**SELECTED SPECIMENS EXAMINED.** — *China*, Yunnan Prov., Pu’er Ci., roadside forest along the old road from Pu’er to Kunming; 22°52’08.47″N, 100°59’25.61″E; 1548 m, on bark; 20.XII.2013; *X. Y. Wang* 13-41509.
Morphology

Thallus
2-6 cm in diameter, lobes 0.6-1 mm wide, slim and short, internodes 3-6 mm long, dichotomously branched, tips usually pointed.

Upper surface
Smooth, greyish green to pale grey, tip part usually black.

Lower surface
Smooth, sometimes wrinkled, brown in the centre, turning light brown to yellowish white near the lobe tips.

Cilia
Absent or very rare along the mature lobes.

Rhizines
Absent in the tips, growing sparsely near the lobe centre.

Soredia and isidia
Absent.

Lobules
Absent.
**Apothecia**
Not seen.

**Pycnidia**
Not seen.

**CHEMISTRY**
Cortex
K+ yellow.

**Medulla**
K-, C-, P+ red, containing atranorin and protocetraric acid (detected by TLC).

**Note**
This species can be separated from all the other Chinese species by the bare lower surface tip and bare margin, the absence of salazinic acid (medulla K-), and the presence of protocetraric acid. It resembles *H. mexicana* in the whitish lower surface and the presence of protocetraric acid, but the latter species has sparse to dense marginal cilia, wider lobes (up to 2.5 mm wide) and no rhizines on the lower surface. This species might be confused with specimens of *H. cirrhata* with sparse cilia, but the latter species always contains salazinic acid (medulla K+ red) and grows at higher elevations, whereas *H. puerensis* has a more subtropical distribution. In the phylogenetic tree (Fig. 1), *H. puerensis* Xin Y.Wang & Li S.Wang, sp. nov., has a more subtropical distribution.

In the phylogenetic tree (Fig. 1), *H. puerensis* Xin Y.Wang & Li S.Wang, sp. nov., formed a monophyletic clade, sister to the *H. vexans* clade but not close to *H. cirrhata* or *H. vexans*.


**Notes**
For a detailed description, see *Wei & Jiang* (1982).

This species can be separated from all other known species of *Everniatrum* by its special cilia type — short, thick, fruticose, irregularly and richly branched (Fig. 5A), the lower surface covered with sparse rhizines, and atranorin and salazinic acid as the main compounds. It was first reported from Xizang Province and usually grows on shrubs at elevations of 2300-3800 m. However, no specimens examined in this study, including those collected from the type locality, had exactly the same morphological characters as the type specimen. The phylogenetic analysis also supported this view (Fig. 1): our specimens (GenBank Nos: MN335186, MN335188) with branched cilia were clustered within the *H. nepalensis* clade, indicating that these specimens with branched cilia were not *H. rhizodendroides*. The concept of this species needs further study with fresh material having the proper type of cilia from the type locality.

12. *Hypotrachyna sinensis* (J.B.Chen & J.C.Wei) Divakar, A.Crespo, Sipman, Elix & Lumbsch


**SELECTED SPECIMENS EXAMINED.** — **CHINA**, Hubei Prov., Shennongjia Forest Reserve; 31°26'59.99"N, 110°24.2.046"E; 2200 m, on *Pinus* bark; 15.VII.1984; **J. B. Chen** 10688 (holo-, HMAS[HMAS]). Yunnan Prov., Dali Co., Cangshan Mt; 25°41'N, 100°06'E; 3400 m, on *Pinus* bark; 12.VI.2005; **L. S. Wang** 05-24650. Binchuan Co., Jizushan Mt; 25°57.847"N, 100°22.151"E; 2620 m, on bark; 13.III.2012; **L. S. Wang** 12-33460. Deqing Co., Yubeng Vil.; 28°23.922"N, 98°46.155"E; 3500 m, on bark; 14.XII.2012; **D. L. Niu** 12-3647.

**Notes**
For a detailed description, see *Chen et al.* (1989).

This species is unique in having lobules on the upper surface (Fig. 5B). This character separates *H. sinensis* from all the other species in the subgenus *Everniastrum*. Furthermore, its upper surface is rather rugose, and the species contains atranorin and salazinic acid as the main compounds. It was first reported from Hubei Province, Shennongjia, and new collections from Yunnan Province were found during this study. It usually grows on bark, rarely on rock, at elevations of 2200–3400 m, and it is currently known only from China.


**SELECTED SPECIMENS EXAMINED.** — **CHINA**, Yunnan Prov., Lijiang Co., Yuhucun Vil.; 28°6'5.115"N, 85°34'11.99"E; 3650 m, on *Rhododendron* bark; 14.VI.1996; **J. C. Wei** 1555-1 (holo-, HMAS[HMAS]). Lijiang Co., Yuhucun Vil.; 28°6'5.115"N, 85°34'11.99"E; 3650 m, on *Rhododendron* bark; 14.VI.1996; **J. C. Wei** 1555-1 (holo-, HMAS[HMAS]).

**Notes**
For a detailed description, see *Jiang & Wei* (1989).

*H. subsorocheila* can be distinguished by its pustular upper surface, forming granulose soralia, which are concolorous with the thallus or turning blackish and spread from the tip to the centre of the lobes; the lobes are usually wide and short (Fig. 5C). Salazinic acid is absent. Two other sedispecies, *H. himalayana* and *H. sorocheila*, also have soralia, but the soralia are powdery and white in these species and grow only on the lower surface of the lobe tips; the lobes are slim and anchor-shaped, and they contain salazinic acid. Furthermore, our phylogenetic analysis (Fig. 1) revealed that *H. subsorocheila* (GenBank Nos: MN335193, MN335194) was not clustered with *H. sorocheila* or *H. himalayana*.
14. Hypotrachyna yunnana Xin Y. Wang & Li S. Wang, sp. nov. (Fig. 6)

**MycoBank number.** — MB 832374.

**Etymology.** — The epithet "yunnana" refers to the type locality, Yunnan Province.

**Diagnosis.** — This species is unique in its thick and wide lobes, which are up to 5 mm wide. The lobe upper surface is covered with bundles of cilia, the margin of lobes usually shows a thick black rim covered with dense, shrubby, and dichotomously branched cilia, and the lower surface is covered with simple or branched rhizines.

**Type.** — China, Yunnan Prov., Nanjian Co., Wuliangshan Mt; 24°52.276′N, 100°34.5139′E; 2348 m, on Vaccinium bark; 19.XII.2012; L. S. Wang 12-37635 (holo-, KUN[KUN]).

**Ecology and Distribution.** — This species is found in Yunnan only, mainly in the northwest, growing on bark of Abies, Pinus, Vaccinium or sometimes on soil over rock, at elevations of 2200-3100 m.

**Selected specimens examined.** — China, Yunnan Prov., Nanjian Co., Wuliangshan Mt; 24°45.213′N, 100°30.131′E; 2340 m, on bark; 22.III.2012; L. S. Wang 12-33039. Nanjian Co., Fenghuang Mt; 24°53′43.60′N, 100°19′52.22′E; 2360 m, on Vaccinium bark; 14.VI.2012; L. S. Wang 12-34276. Dali Co., Cangshan Mt; 25°41.111′N, 100°06.244′E; 3410 m, on soil over rock; 14.VIII.2011; L. S. Wang 11-32263. Gongshan Co., Yeniu Valley; 27°48.045′N, 98°49.518′E; 2950 m, on Abies bark; 30.V.2000; L. S. Wang 00-19359.

**Morphology**

**Thallus**

6-15 cm in diameter, lobes rather thick and wide, 2-5 mm wide, internodes 5-10 mm long, dichotomously branched, tips usually pointed.

**Upper surface**

Rugose, greyish green to brownish grey (if stored in herbarium), marginal part with thick, black rim, bundled cilia sparsely growing on the upper surface, marginal cilia dense and shrubby, 0.3-0.5 mm long, dichotomously branched.

**Lower surface**

Smooth and shiny, slightly wrinkled, black in the centre, turning dark brown near the tips.
**Rhizines**
Dense in the centre, turning moderate near the tips, simple or dichotomously branched, short and black, 0.3-0.6 mm long.

**Soredia and isidia**
Absent.

**Lobules**
Absent.

**Apothecia**
Rare, subterminal, disc chestnut brown, 2-8 mm in diameter, ascus 8-spored, spores kidney-shaped, 5-7 × 20-23 µm.

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**KEY TO *HYPOTRACHYNA* SUBGENUS *EVERNIASTRUM* (HALE EX SIPMAN) DIVAKAR, A.CRESPO, SIPMAN, ELIX & LUMBSCH SPECIES FROM CHINA**

1. Lower surface, especially near the tips bare, without rhizines ................................................................. 2
   — Lower surface covered with rhizines ........................................................................................................... 10

2. Marginal cilia absent, lower surface yellowish white .......... *H. puerensis* Xin Y.Wang & Li S.Wang, sp. nov.
   — Marginal cilia present, sparse or dense .................................................................................................. 3

3. Soredia or isidia present ............................................................................................................................... 4
   — Soredia or isidia absent ............................................................................................................................ 8

4. Soredia present ............................................................................................................................................. 5
   — Isidia present ........................................................................................................................................... 7

5. Soralia at the tip on the lower surface, whitish and lip-shaped, medulla K+ red, with salazinic acid ..........
   — Soralia pustulate, terminal to laminal on the upper surface, medulla K-, lacking salazinic acid .......... 6

6. Medulla C+ pink, with gyrophoric acid .........................................................................................................
   — Medulla C-, without gyrophoric acid ................................................................................................. 14

7. Isidia simple, granular to cylindrical, lobules absent ............................................................................. 11
   — Isidia long and coralloid-branched, forming tufts, mixed with lobules ............................................. 14

8. Upper surface covered with lobules ........................................................................................................ 11
   — Upper surface bare ............................................................................................................................... 9

9. Cilia long and curved, irregularly branched, up to 3.5 mm long ............................................................. 13
   — Cilia simple without branches, less than 2 mm long ................................................................. 13

10. Upper surface rough, covered with tufts of cilia .......... *H. yunnana* Xin Y.Wang & Li S.Wang, sp. nov.
    — Upper surface smooth, without cilia ................................................................................................... 11

11. Cilia branched ........................................................................................................................................ 12
    — Cilia simple or absent ........................................................................................................................ 13

12. Cilia long and branched, with diffractaic acid ................................................................................................
    — Cilia short and shrubby-branched, without diffractaic acid ............................................................... 13

    — Medulla K+ red, with salazinic acid ................................................................................................... 14

14. Alectorialic acid present .......................................................................................................................... 14
    — Alectorialic acid absent ..................................................................................................................... 14
**Pycnidia**
Numerous, on the upper surface, black, c. 0.1 mm in diameter.

**Chemistry**

**Cortex**
K+ yellow.

**Medulla**
K+ yellow to red, C-, P+ orange red, containing atranorin and salazinic acid (detected by TLC).

**Notes**
This species can be separated from all the other species in subgenus *Everniastrum* by the presence of cilia on the...
upper surface, the thick and wide lobes (up to 5 mm wide) and the dense short cilia on the margin of the lobes. It might be mistaken for *H. sinensis* because of its rugose upper surface, but the latter species has a bare lower surface and sparse simple cilia on the margin; furthermore, *H. sinensis* has lobules on the upper surface. *H. nepalensis*, when exhibiting dense rhizines, might be confused with this species, but *H. nepalensis* has simple and sparse marginal cilia, and the upper surface is smooth without cilia. *H. rhizodenendroidea*, having branched marginal cilia, is similar to this new species, but it has fruticose, irregularly branched cilia and a sparsely rhizinate lower surface.

15. *Hypotrachyna vexans* (Zahlbr. ex W. L. Culb. & C. E. Culb.) Divakar, A. Crespo, Sipman, Elix & Lumbsch


**NOTES**

For a detailed description, see Culberson & Culberson (1981).

This species is characterized by a sparsely to densely isidiate upper surface, and the isidia are usually short or granulose (Fig. 5D), lacking rhizines on the lower surface and having sparse marginal cilia. *H. fragilis* also has isidia on the upper surface but differs by its long and fragile isidia (up to 3 mm long), usually coralloid-branched, and lobes that are wide and short, usually flat and appressed. In contrast, in *H. vexans*, the lobes are long and usually involute, and the lobe tips mostly curve downward. *H. subvexans*, with similar isidia, can be distinguished by the absence of salazinic acid in the thallus (medulla K-) and wide lobes up to 3 mm wide. This suggests that there are cryptic species hidden under these names and that the species diversity is higher than presently assumed. In this study, specimens from type localities were freshly collected and sequenced for *H. cirrhata*, *H. nepalensis* and *H. vexans*, providing a phylogenetic foundation for the delimitation of these species.

**SPECIES DIVERSITY IN THE HENGDUAN MTS**

The four new species described in this study have unique taxonomic, chemical and geographical characters and are supported by phylogenetic analysis with high support values. However, due to the lack of fresh specimens, there are still some groups of species with special morphological characters but without support from phylogenetic data awaiting confirmation. More floristic surveys are needed for further study: sampling of different populations of the same species and sampling of fresh, reliably identified material of described species to clarify their phylogenetic relationships. Although the subgenus *Everniastrum* has already received considerable attention in China, new species are still being discovered, showing the high species diversity of lichens in the Hengduan Mts area.

The discovery of four new species and a new floristic record for a species in this well-studied subgenus suggests that there are more cryptic species hidden within *Everniastrum*. Further studies are needed, involving more specimens and sequences from all over the world, especially from the type localities, to identify the proper species boundaries of this subgenus and to discover the species hidden under the traditional species names.

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