



## *Alectoria spinosa*, a new lichen species from Hengduan Mountains, China

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### Abstract

Based on inferences from morphological, chemical and phylogenetic analysis using ITS sequences, a new species of *Alectoria* is described from the Hengduan Mountains in southwestern China, *A. spinosa* Li S. Wang & Xin Y. Wang sp. nov, it has dull yellowish fruticose thallus with abundant pseudocyphellae, which is the main character of *Alectoria*, and differs from all the other *Alectoria* species by having isidia-like spinules on the soralia. Two species of *Alectoria* are confirmed in China, their morphological and chemical traits, together with their geographical distributions in China, are discussed, and a key to the species is provided.

**Key words** – Lichenized fungi – Parmeliaceae – phylogeny – taxonomy

### Introduction

*Alectoria* Ach. (Parmeliaceae, Lecanorales; Tehler & Wedin 2008), typified by *Alectoria sarmentosa* (Ach.) Ach., is characterized by having light yellow–brown to dull yellow, fruticose, caespitose, erect to pendulous thalli, attached by a holdfast and dichotomously to irregularly branched; thalli with raised pseudocyphellae and a cortex composed of periclinal, conglutinate hyphae; medullary hyphae loose and usually verrucose, lacking a central axis; apothecia lecanorine; ascospores 1-celled, ellipsoid, turning brown or dark brown when mature, 2–4 per ascus; photobiont green algae (trebouxioid); and containing usnic, diffractaic and alectoronic acids as the major secondary compounds (Hawksworth 1970). *Alectoria* differs from *Sulcaria* Bystrek by lacking longitudinal furrows on the surface, from *Oropogon* Th. Fr. and *Bryoria* Brodo & D. Hawksw. by having brown spores and containing usnic acid in the cortex.

The genus currently contains seven species worldwide; most of the species historically reported as *Alectoria* were placed in *Bryoria* (Brodo & Hawksworth 1977, Halonen et al. 2009). *Alectoria* has a bipolar and arctic–alpine distribution (Brodo & Hawksworth 1977). One species was previously known from China (*Alectoria ochroleuca* (Hoffm.) A. Massal.; Wei 1991).

Preliminary studies on the taxonomy of *Alectoria* in China have been completed (Wu & Wang 1992, Wang et al. 2014). During these studies, an undescribed species of *Alectoria* was discovered in the Hengduan Mountains. We confirmed the placement of this new species in

*Alectoria* with morphological, chemical, and phylogenetic analyses. A description of this species and a key to both *Alectoria* species in China is provided.

## Materials & Methods

Specimens used in this study were collected from China between 1981 and 2014, and are deposited in the Herbarium of Kunming Institute of Botany, China (KUN–L). Specimens were examined using standard microscopic techniques and hand-sectioned under a Nikon SMZ 745T dissecting microscope. Anatomical descriptions are based on observations using a NIKON Eclipse 50i microscope, and structures were photographed using a Nikon digital camera head DS-Fi2. Size ranges are provided as smallest–largest single value recorded.

Secondary metabolites of all the specimens were identified using spot tests and TLC as described by Orange et al. (2001). Solvent C (toluene: acetic acid = 85:15) was used for TLC analysis.

Total DNA was extracted from small fragments of fresh thallus (Table 1.) 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. Previously described conditions by Arup (2002) were used for PCR: 2 µl of genomic DNA was added to the following mix: 2 µl of primer (1 µl for each primer of a 10 mM solution), 12.5 µl 2 × Taq PCR Mastermix (Aidlab) (containing Taq DNA Polymerase: 0.1 unit/µl; MgCl<sub>2</sub>: 4mM; dNTPs: 0.4mM) and 8.5 µl dH<sub>2</sub>O for a total volume of 25 µl. Amplifications were carried out in a thermocycler (C 1000TM), with the following profile: initial denaturation at 94°C for 5 min, followed by 30 cycles, each of three steps (94°C for 1 min, 56°C for 1 min, 72 °C for 1.5 min), and a final extension at 72°C for 7 min. PCR products were precipitated and sequenced using ABI Applied Biosystems 3730 DNA Analyzer (Foster City, California, U.S.A.) with the same primers as the original PCR amplifications..

Raw sequences were assembled and edited using SeqMan (DNASTAR packages), then sequences were automatically aligned with MUSCLE v3.6 (Edgar 2004). GBLOCKS (Castresana 2000) was used to evaluate ambiguous regions using the default settings. Bayesian inference (BI) and Maximum likelihood (ML) are used to reconstruct the phylogenetic tree, with *Gowardia arctica* as the outgroup. All characters were equally weighted and treated as unordered.

For BI, model selection was based on the Akaike information criterion (AIC) estimated by jModelTest 3.7 (Posada 2008), analyses were performed with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC). The initial burn-in was set to 50%. A 50% majority-rule consensus cladogram was computed from the remaining trees; the proportions of this tree correspond to Bayesian posterior probabilities (BPP), and clades with PP≥0.95 were considered to be significantly supported. ML tree searches and bootstrapping were performed with RAxML v7.2.6 (Stamatakis 2006.). All parameters in the ML analysis used the default setting, and statistical support values were estimated by 1000 bootstrap replicates.

## Results

Related genera *Bryoria*, *Oropogon* and *Sulcaria* were included in the phylogenetic analysis, in order to get a clear phylogenetic relationship of the new species. The phylogenetic inference places the putative new species within the clade of the genus *Alectoria*, and it formed a highly supported clade (MLBS: 100%; PP: 1.0; Fig. 1), sister to *A. ochroleuca*, *A. sarmentosa* (Ach.) Ach. and *A. imshaugii* Brodo & D. Hawksw. The three species formed a well-supported group (MLBS: 96%; PP: 1.0; *A. ochroleuca*, *A. sarmentosa* and *A. imshaugii*) without soredia on thallus and lateral spinules on the soralia, while *A. spinosa* formed another clade and differs by having spinules on soralia. Based on morphological, chemical and molecular investigations, the new species *Alectoria spinosa* is presented.

*Alectoria spinosa* Li S. Wang & Xin Y. Wang **sp. nov.**

Figs 2A–B

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Etymology – The epithet “spinosa” refers to the spinules on the soralia of the thallus.

Type – CHINA, Yunnan: Lijiang Co., Jiuhe Village, Laojunshan Mt., 26°38.186' N, 99°44.077' E, 4150 m, on the bark of *Rhododendron*, 25 June 2014, Li S. Wang 14–44046 (KUN–L 45926, Holotype)

Synopsis – Corticolous *Alectoria* with spinules growing on the soralia, light yellowish brown thallus with usnic and virensic acids.

Description – Thallus fruticose, forming clusters, tufted, 1.5 – 3.0 cm tall, yellowish brown to brown, dark brown towards the apices, the basal part black, carbonized; main branches 0.3 – 0.5 (– 1) mm in diam., cylindrical to flattened, densely branched with dichotomous branching; spinules rare, 0.1 – 0.5 mm long, branched at maturity, concolorous with the thallus; soredia granulose, isidia-like spinules growing on the soralia, 0.1 – 0.5 mm long; pseudocyphellae abundant, fissure-shaped, grayish white to light brown, 0.2 – 1.2 mm long, surface raised and sorediate when mature; apothecia and pycnidia not seen.

Branches roundish to ellipsoid in section, hollow in the center, 200 – 300 µm in diam., cortex 40 – 50 µm, hyphae of medulla loosely interwoven, partly hollow, c. 6 – 9 µm in diam., verrucose on the surface; photobiont green algae, photobiont layer 25 – 30 µm thick.

Chemistry – Medulla and cortex K ± yellow, P + slowly orange red, C –, KC + yellow, containing usnic acid, virensic acid and protocetraric acid (trace).

Known distribution – Growing on bark of *Abies delavayi* in the alpine coniferous forests above 4000 m. Endemic to Yunnan Province.

Notes – *Alectoria spinosa* is characterized by sorediate pseudocyphellae and spinules growing over soralia, which is a rather unique character in the genus *Alectoria*. It resembles the North American isidiate *A. imshaugii*, but the latter contains thamnolic and squamatic acids, subpendent thallus 5 – 8 cm long, and it has isidiiform spinules on the thallus. It might be confused with the rare sorediate morphotype of *A. sarmentosa* by having soralia, but the latter species has no spinules, the thallus is pendent (<80 cm long), and it contains alectorononic acid. *Alectoria ochroleuca* differs from this new species by the yellowish green thallus with blackened apices, without spinules and soralia on the thallus surface, and contains diffractaic acid as its main compound (Brodo and Hawksworth, 1977).

*Alectoria spinosa* might be confused with *Bryoria* and *Oropogon* due to the light yellowish brown thallus color, it does not look like typical *Alectoria*, which are pale greenish yellow. It does, however, contain usnic acid, like a typical *Alectoria*. *Alectoria spinosa* is also similar to some *Sulcaria* species with a yellowish thallus, but it lacks longitudinal furrows on the branches. The phylogenetic tree supports placement of *A. spinosa* in *Alectoria*.

Additional specimens examined – CHINA. Yunnan: Lijiang Co.: Jiuhe Village, Laojunshan Mt., 26°37' N, 99°42' E, alt. 4010 m, on bark of *Abies delavayi*, 22 May 2011, Wang & Liang 11–32083, 11–32085, 11–32102.

*Alectoria ochroleuca* (Hoffm.) Mass., Sched. Crit. Lich. Ital.: 47. 1855.

Figs 2C–D

≡ *Usnea ochroleuca* Hoffm., Descr. Adunbr. Pl. Lich. 2(1): 7. 1791.

Description – see Wang et al. (2014).

Notes – This species is characterized by the dull yellowish green surface with blackened apices; conspicuous white pseudocyphellae, verrucose medullary hyphae and usnic acid. It is similar to *Sulcaria sulcata* (Lév.) Bystrek, but the latter species differs in having longitudinal furrows on the surface and in lacking usnic acid.

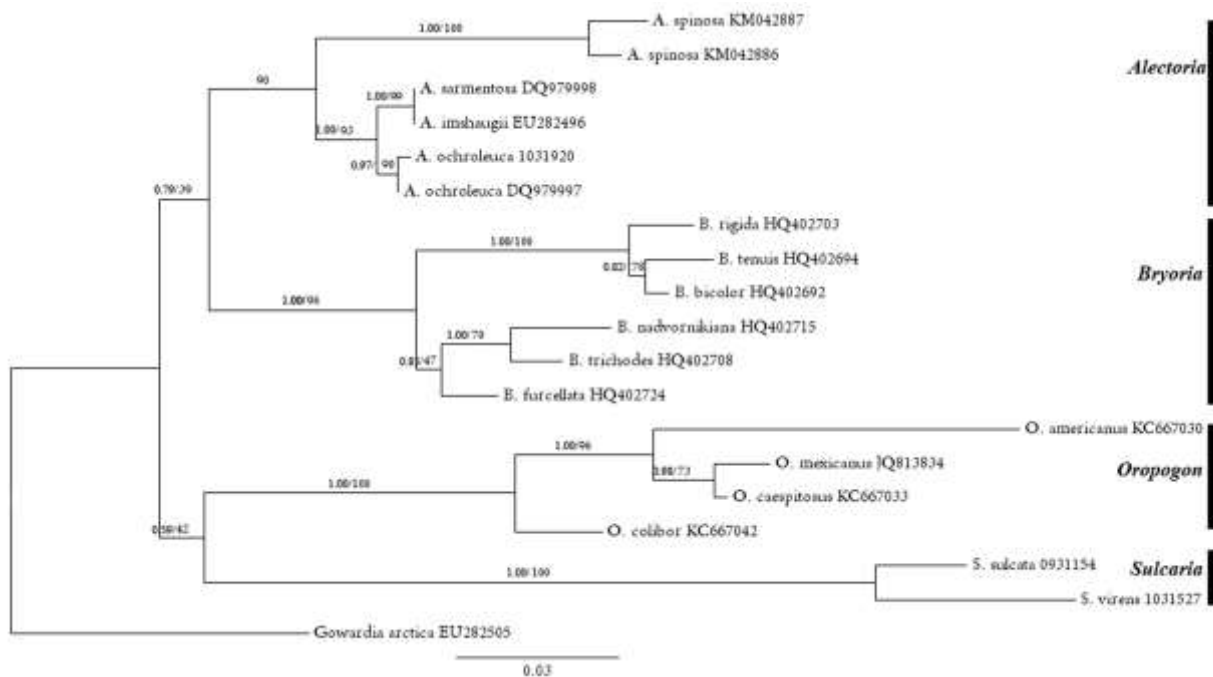
Selected specimens examined – CHINA. Yunnan. Deqin Co.: Mt. Baima, 4000 – 4800 m, under *Rhododendron* shrub, Li S. Wang 94–15361, 93–13500, 81–22849, 85–8898, 81–22850, 85–8891, 84–41a. Xizang: Demula Co.: alt. 5000m, 1976, Zang 9419; Yadong Co., alt. 4760m, on rock, 1975, Zang 44. Inner Mongolia: Daxinganling, alt. 1460m, on rock, 1951, Li S. Wang 1905.

## Key to *Alectoria* in China

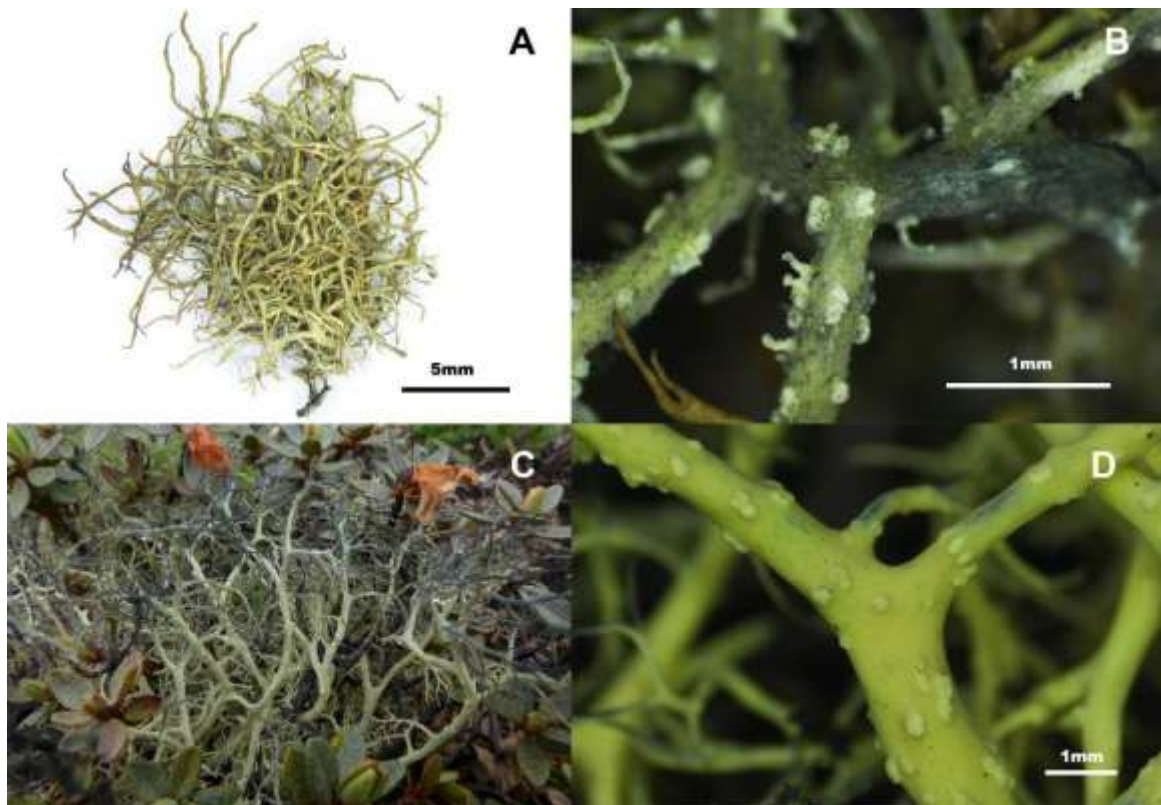
1. Thallus without soredia, medulla with diffractaic acid (CK + gold yellow) ... *Alectoria ochroleuca*  
 1. Thallus sorediate, without diffractaic acid, contains virensic acid (CK –)..... *Alectoria spinosa*

**Table 1** Specimens used in this study, newly obtained sequence numbers are in bold.

Taxon	Locality	Voucher specimen	Accession No.
<i>Alectoria imshaugii</i>	Finland	Helsinki 00014, BM	EU282496
<i>A. spinosa</i>	China, Yunnan	11-32085, KUN-L	<b>KM042886</b>
<i>A. spinosa</i>	China, Yunnan	11-32083, KUN-L	<b>KM042887</b>
<i>A. nigricans</i>	Sweden	Lundqvist 8377, UPS	DQ979996
<i>A. ochroleuca</i>	Austria	Wedin Aug. 1988, BM	DQ979997
<i>A. ochroleuca</i>	China, Yunnan	10-31920, KUN-L	<b>KM979761</b>
<i>A. sarmentosa</i>	Sweden	Wedin 6350, UPS	DQ979998
<i>Bryoria bicolor</i>	not known	J. Hermansson 14110, UPS	HQ402692
<i>B. furcellata</i>	Canada: Alberta	Colberg & Prokopetz s.n., UBC	HQ402724
<i>B. nadvornikiana</i>	China, Yunnan	06-26535, KUN-L	HQ402715
<i>B. rigida</i>	China, Yunnan	06-26208, KUN-L	HQ402703
<i>B. tenuis</i>	not known	S. Velmala 64, H	HQ402694
<i>B. trichodes</i>	Russia: Kamchatka	D. Himelbrant EL 6, H	HQ402708
<i>Oropogon americanus</i>	Costa Rica	Unknown	KC667030
<i>O. caespitosus</i>	Mexico	A_6396	KC667033
<i>O. colibor</i>	Costa Rica	6715_1	KC667042
<i>O. mexicanus</i>	Mexico	Esslinger 18532, TLE	JQ813834
<i>Sulcaria sulcata</i>	China, Yunnan	09-31154, KUN-L	<b>KM979759</b>
<i>S. virens</i>	China, Yunnan	10-31527, KUN-L	<b>KM979760</b>
<i>Gowardia arctica</i>	Unknown	Mattsson 5142, UPS	EU282505



**Fig. 1** – Phylogenetic tree inferred from Maximum Likelihood (ML) analysis based on the ITS data. Support values are posterior probabilities (from Bayesian Inference) followed by ML bootstrap frequencies.



**Fig. 2** – A Thallus of *Alectoria spinosa*. B Spinules on the soralia of *A. spinosa*. C Habit of *A. ochroleuca*. D Pseudocyphellae of *A. ochroleuca*.

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