



NOTE

Confirmation of the taxonomic status of *Lysionotus pterocaulis* (Gesneriaceae) supported by morphological and molecular evidence

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ABSTRACT: *Lysionotus serratus* var. *pterocaulis* C.Y.Wu ex W.T.Wang was initially described as a variety, but was treated as an independent species *L. pterocaulis* (C.Y.Wu ex W.T.Wang) H.W.Li in 1991, although this status was not always retained by subsequent authors. Our observation of wild living plants demonstrated that *L. pterocaulis* is obviously distinguishable from *L. serratus* D.Don through its longitudinally angled stems with wings, yellowish to white corolla, tubular to slightly funnelliform tube, and linear-lanceolate calyx lobes. Furthermore, the molecular phylogenetic analyses indicated that *L. pterocaulis* is not clustered with *L. serratus* but embedded in a different lineage. Therefore, *L. pterocaulis* is confirmed to be an independent species and an updated description is provided.

KEY WORDS: Biodiversity hotspot, limestone flora, *Lysionotus serratus*, molecular phylogeny, taxonomy.

INTRODUCTION

The genus *Lysionotus* D.Don (1822: 85) was established in 1822 based on *Lysionotus serratus* D.Don (Fig. 1), in which 31 species and seven varieties are recorded up to date according to online data (GCCC 2022; GRC 2022) and literature (Xu *et al.* 2017). China is the center of diversity of *Lysionotus*, where more than 25 species and six varieties have been recorded (Xu *et al.* 2017). New taxa continue to be described (Nong *et al.* 2010; Joe *et al.* 2017; Taram *et al.* 2019; Tian *et al.* 2020; Akhil *et al.* 2021).

Lysionotus serratus var. *pterocaulis* C.Y.Wu ex W.T.Wang (Wang, 1983) from Yunnan, China was initially described as a variety, differing from the type variety by having a winged stem, and was accepted by Wang (1990). But it was then treated as an independent species *L. pterocaulis* (C.Y.Wu ex W.T.Wang) H.W.Li (Li, 1991) (Fig. 2) in 1991 following specimen reexamination. The latter treatment, however, was not accepted, and the name was cited as a synonym for *L. serratus* var. *pterocaulis* without any justification given in subsequent literature (Wang *et al.*, 1998; Li and Wang, 2004, Wang *et al.*, 2017). Hilliard and Burt (1995), however, also suggested that its status as a variety needed reconsideration, given its occurrence in northern Vietnam, 200 km east of its other localities in Yunnan and possible differences in fruit length. The controversy of the taxonomic status could not be resolved until more robust evidence was collected.

During the investigation of the limestone flora in southeastern Yunnan in July 2020, the authors found that the corolla of *L. serratus* var. *pterocaulis* (Fig. 2) is very

different from *L. serratus* (Fig. 1), in addition to the vegetative differences already described. To further validate the taxonomic status of *L. serratus* var. *pterocaulis* and placement of this species in the genus *Lysionotus*, morphological and anatomical features were observed, and ITS and *trnL-F* sequences of this species were amplified and included in molecular phylogenetic analyses.

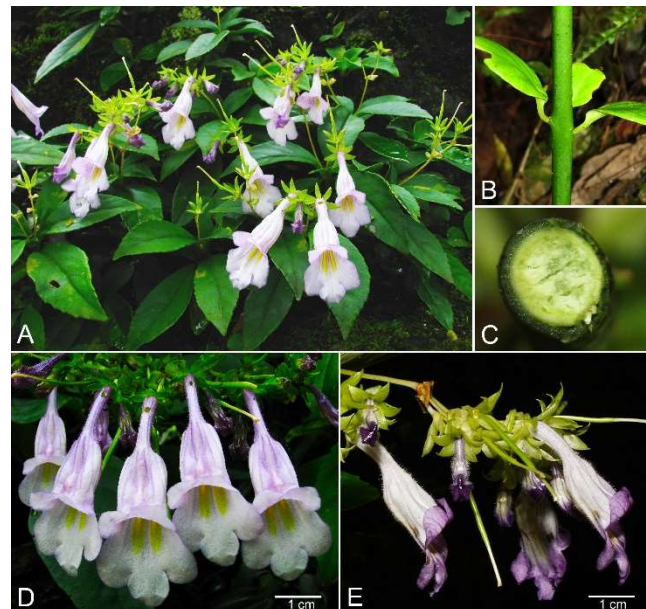


Fig 1. *Lysionotus serratus*. A: Habit. B: Stem. C: Cross section of stem. D: Flowers, frontal view. E: Flowers and pistils, lateral view.

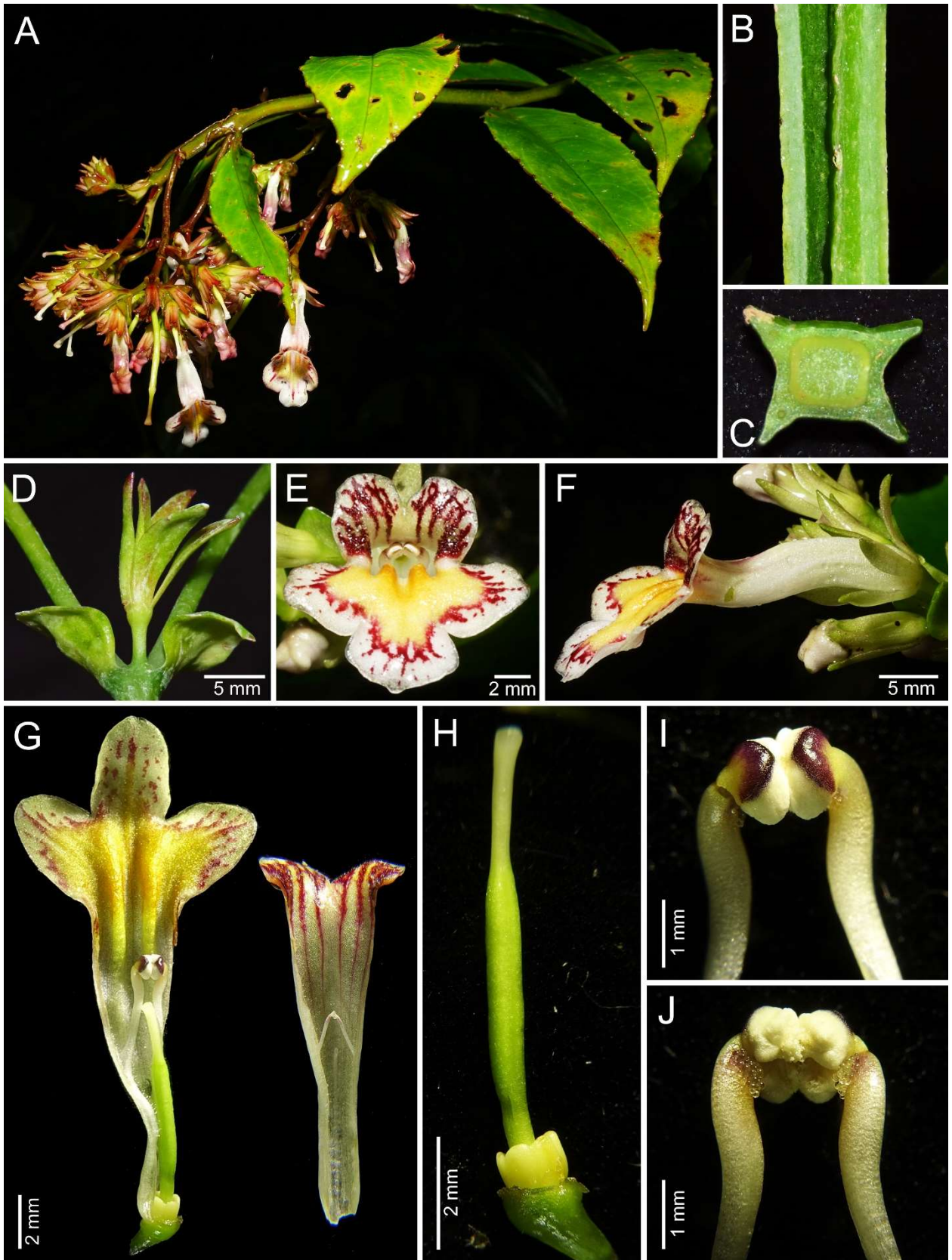


Fig 2. *Lysionotus pterocaulis*. **A:** A part of plant. **B:** Stem. **C:** Cross section of stem. **D:** Bracts and calyx. **E:** Flower, frontal view. **F:** Flower, lateral view. **G:** Opened corolla showing stamens and pistil. **H:** Pistil, **I:** Stamens, face view. **J:** Stamens, dorsal view.



Table 1. Species of *Lysionotus* and related taxa sampled and GenBank accession numbers of sequences used in this study. Newly generated sequences of taxa are highlighted in bold and listed with the voucher information.

Species	GenBank accession numbers		voucher information
	ITS	<i>trnL-F</i>	
<i>Anna mollifolia</i> (W.T.Wang) W.T.Wang & K.Y.Pan	KY288030	FJ501543	
<i>A. ophiorrhizoides</i> (Hemsl.) Burt & Davidson	KM063147	HQ632937	
<i>A. submontana</i> Pellegr.	FJ501362	FJ501542	
<i>Hemiboea longzhouensis</i> W.T.Wang ex Z.Y.Li	MN334638	HQ632888	
<i>H. subcapitata</i> Clarke	MN334657	FJ501535	
<i>Lysionotus aeshynanthoides</i> W.T.Wang	MW507480	MW523021	Yunnan, Xichou, C.Y. Zou <i>et al.</i> ZCY875 (IBK)
<i>L. chingii</i> Chun ex W.T.Wang	AB547216	FJ501498	
<i>L. denticulosus</i> W.T.Wang	AB547217	KM232652	
<i>L. fengshanensis</i> Yan Liu & D.X.Nong	MW507484	MW523017	Guangxi, Fengshan, W.B. Xu 14069 (IBK)
<i>L. forrestii</i> W.W.Smith	--	FJ501495	
<i>L. gracilis</i> W. W.Smith	MW507476	MW523011	Yunnan, Lushui, W.B. Xu 14186 (IBK)
<i>L. heterophyllus</i> Franch.	MW507483	MW523022	Guangxi, Tianlin, Y. Qin s.n. (IBK)
<i>L. longipedunculatus</i> (W.T.Wang) W.T.Wang	KY288028	MW523013	Guangxi, Jingxi, Z.R. Liu & W.B. Xu 14059 (IBK)
<i>L. microphyllus</i> W.T.Wang	HQ327463	--	
<i>L. oblongifolius</i> W.T.Wang	AB547218	--	
<i>L. pauciflorus</i> Maxim.	AB514441	MK746232	
<i>L. petelotii</i> Pellegr.	AB498587	MW523020	Yunnan, Xichou, C.Y. Zou <i>et al.</i> ZCY313 (IBK)
<i>L. pterocaulis</i> (C.Y.Wu ex W.T.Wang) H.W.Li	MW507481	MW523019	Yunnan, Maguan, C.Y. Zou <i>et al.</i> ZCY749 (IBK)
<i>L. pubescens</i> C.B.Clarke	--	MW523015	Yunnan, Tengchong, W.B. Xu 14185 (IBK)
<i>L. sangzhiensis</i> W.T. Wang	KJ475422	KM232653	
<i>L. serratus</i> D.Don	MW507479	MW523018	Yunnan, Maguan, C.Y. Zou <i>et al.</i> ZCY793 (IBK)
<i>L. sessilifolius</i> Hand.-Mazz.	MW507477	MW523012	Yunnan, Gongshan, W.B. Xu 14190 (IBK)
<i>L. sp.</i>	MW507478	MW523014	Yunnan, Lushui, W.B. Xu 14188 (IBK)
<i>L. wilsonii</i> Rehd.	MW507482	--	Sichuan, Tianquan, W.B. Xu 13918 (IBK)
<i>Raphiocarpus sinicus</i> Chun	MW507485	MW523016	Guangxi, Fangchenggang, Y.B. Liao <i>et al.</i> 14051(1BK)

MATERIALS AND METHODS

Morphological observation

A plant of *Lysionotus* with winged stem was collected by the authors from Maguan County, southeastern Yunnan in April 2020. After consulting the relevant literature (Wang, 1983; Wang, 1990; Li, 1991, Wang *et al.*, 1998, Li and Wang, 2004), as well as herbarium specimens of E, IBK, IBSC, KUN and PE, and comparing with all described species of *Lysionotus*, we identified it as *L. serratus* var. *pterocaulis* preliminarily. To further confirm our identification, we checked carefully the holotype and isotype sheets of *L. serratus* var. *pterocaulis* and checked the geographic distribution of this species.

The measurements, color, shape and other details given here in the updated description of *L. pterocaulis* are based on observations of fresh material, photos from the field and specimens. Comparisons with *L. serratus* were based on photos taken in the field, as well as on specimens studied in herbaria (IBK, KUN and PE).

Sampling and DNA sequencing

The fresh leaves of 12 species of *Lysionotus* and *Raphiocarpus sinicus* Chun were collected and quickly dried with silica gel. The dried leaves were used for extracting genomic DNA, polymerase chain reaction (PCR) amplification and sequencing.

The modified CTAB extraction method (Doyle & Doyle 1987) was used to extract total genomic DNA. The nuclear ribosomal internal transcribed spacer (ITS) and chloroplast *trnL-F* intron-spacer fragments were selected for phylogenetic analyses. For the polymerase chain reaction (PCR) reaction, 10 µL enzyme mixture, 1 µL forward primer, 1 µL reverse primer and 1 µL template were mixed, and double distilled water was added up to 20 µL. The following reaction procedure was adopted for amplification: 5 min at 95°C, followed with 35 cycles of 1min at 94°C, 1 min at 55°C for ITS (52°C 1 min for *trnL-F*), and 2 min at 72°C with 10 min at 72°C for the final elongation and end with 10 min at 16°C. The sequences were manually checked and edited according to the corresponding chromatograms generated by sequencing from both directions. A total of 10 ITS and 12 *trnL-F* sequences were newly acquired. The newly acquired sequences have been submitted to GenBank, and their voucher information is listed in Table 1.

Phylogenetic analysis

Besides the newly generated data, we downloaded 23 sequences (13 ITS sequences and 10 *trnL-F* sequences) of *Lysionotus*, *Anna* Pellegr. (Pellegrin, 1930), and *Hemiboea* C.B.Clarke (Clarke, 1888) from GenBank (Table 1). In total, 45 sequences of 25 species were included in the

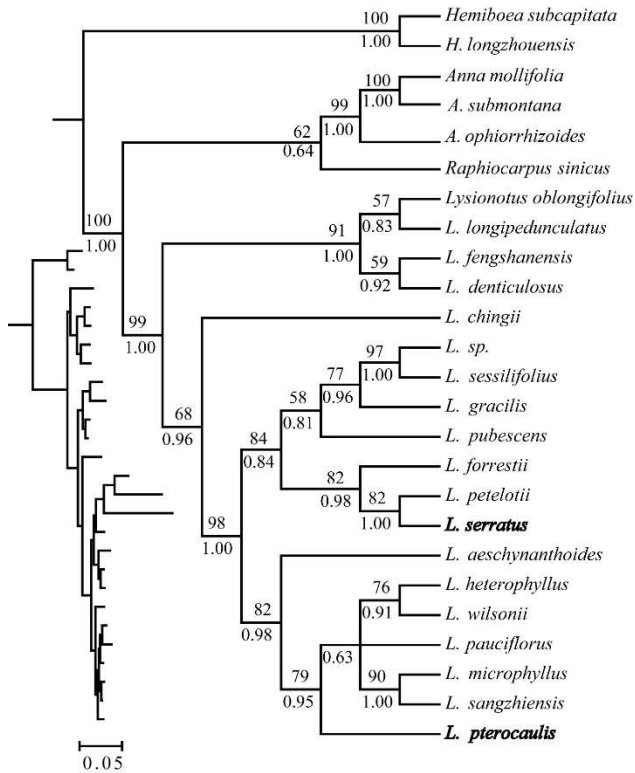


Fig 3. Phylogenetic tree inferred by ML and BI analyses based on the combined ITS and chloroplast *trnL-F* regions, with a phylogram showing branch lengths illustrated on the left lower side of the main figure of the cladogram. BI posterior probability/ML bootstrap support values (>0.5 or 50%) are shown below and above the corresponding branches in the cladogram. ***Lysionotus pterocaulis*** and ***L. serratus*** are highlighted in bold.

phylogenetic reconstruction. The taxa of *Anna*, *Hemiboea* and *Raphiocarpus* Chun (Chun, 1946) were selected as the outgroups with the *Hemiboea* species (the most distant outgroup taxa) assigned as the tree root based on the previous phylogenetic studies (Möller *et al.*, 2011, Weber *et al.*, 2011).

DNA sequences were aligned using the program MUSCLE 3.8.31 (Edgar 2004) and adjusted manually in Bioedit 5.0.9 (Hall, 1999). We reconstructed the phylogeny using maximum likelihood (ML) and Bayesian inference (BI). The trees were reconstructed based on combined data, as the trees based on each of the two loci showed largely unresolved relationships and no strong topological conflict (with bootstrap support value bigger than 80%) in *Lysionotus* was observed, except that the species *L. longipedunculatus* groups with *L. sessilifolius* with maximum support in the *trnL-F* tree while it is nested within a different clade in the ITS tree. ML analyses were performed using RAxML-VI-HPC (Stamatakis 2006) with the substitution model GTR+G based on jModelTest (Posada, 2008), and 1000 rapid bootstrap searches (BS). Bayesian analyses (BI) were conducted in MrBayes 3.2.6 (Fredrik *et al.*, 2012), with the optimal substitution models of HKY+G and

K81uf+I+G for the ITS and *trnL-F* data as selected by jModelTest (Posada 2008) according to the Akaike Information Criterion (AIC). All BI analyses were run for 100,000,000 generations with four chains in two parallel runs and sampled every 5000 generations with a burn-in of the first 5000 trees. The convergence of the two parallel runs was guaranteed by the splitting frequency being less than 0.005. All other parameters were set as default.

RESULTS AND DISCUSSION

Morphological analysis

Lysionotus serratus var. *pterocaulis* was published first in 1983 as a variety, and only with a vegetative description. Here, the detailed description of *L. pterocaulis* is supplemented based on the collections from southeastern Yunnan, China and Cao Bang, northern Vietnam, and photos are also provided.

Lysionotus pterocaulis is very similar to *Lysionotus serratus* in leaf shape, but differs from the latter by the stems being longitudinally angled, winged along angles (vs. cylindrical or slightly angular, without wing along angles); the corolla yellowish to white, outside glabrous, 2.5–3.1 cm long overall, tube tubular to slightly funnellform, 18–21 × 3–5 mm (vs. purplish to white, outside sparsely puberulent, 3.5–4.5 cm long, tube slender funnellform, 22–30 × 8–10 mm); the calyx lobes linear-lanceolate, 6–8 × 1.5–2 mm (vs. lanceolate to narrowly ovate, 4–8 × 1.5–4 mm). Based on the morphological observation, we confirm that *L. pterocaulis* must be treated as an independent species.

Phylogenetic analysis

The combined matrix used for phylogenetic reconstruction had a length of 1751 characters (*trnL-F*: 963 characters, ITS: 788 characters) including 188 parsimony informative sites (*trnL-F*: 62 characters, ITS: 126 characters), 531 variable but parsimony uninformative sites (*trnL-F*: 299 characters, ITS: 232 characters) and 1220 constant sites (*trnL-F*: 664 characters, ITS: 556 characters). The parameters of consistency index (CI), retention index (RI) and homoplasy index (HI) were 0.763, 0.708 and 0.237 for the combined data (0.850, 0.735 and 0.150; 0.733, 0.756 and 0.267 for the *trnL-F* and ITS, respectively).

All *Lysionotus* taxa form a clade in the trees (BS = 99%; PP = 1.00) (Fig. 3). *L. pterocaulis* and *L. serratus* are embedded in two distinct lineages. In one lineage (BS = 84%; PP = 0.84), *L. serratus* is closest to *L. petelotii* Pellegr. with moderate to maximum support (BS = 82%; PP = 1.00), followed with *L. forrestii* (BS = 82%; PP = 0.98). *L. pterocaulis* is nested within the other lineage (BS = 82%; PP = 0.98), in which, it groups with a sublineage consisting of *L. heterophyllus* Franch., *L. wilsonii* Rehd., *L. pauciflorus* Maxim., *L. microphyllus* W.T. Wang and *L.*



sangzhiensis W.T.Wang with moderate support (BS = 79%; PP = 0.95). Phylogenetic analyses revealed that these two species are not closely related to each other, also supporting the recognition of species rank of *L. pterocaulis*.

TAXONOMIC TREATMENT

Lysionotus pterocaulis (C.Y.Wu ex W.T.Wang) H.W.Li in Z. Y. Wu, Fl. Yunnan, 5: 553, pl. 172: 7–11. 1991; W. W. Wang *et al.* in Z. Y. Wu & P. H. Raven, Fl. China, 18: 393. 1998; Z. Y. Li & Y. Z. Wang, Plants of Gesneriaceae in China, 410. 2005.

Basionym: *Lysionotus serratus* var. *pterocaulis* C.Y.Wu ex W.T.Wang in Guihaia 3(4): 277. 1983 et Fl. Reip. Pop. Sin. 69: 553. 1990; W. W. Wang *et al.* in Z. Y. Wu & P. H. Raven, Fl. China, 18: 393. 1998; Z. Y. Li & Y. Z. Wang, Plants of Gesneriaceae in China, 410. 2005.

Type: Pingbian County, Xinnong Township, Shitouzhai, alt. 1280 m, 28 July 1953, *P. Y. Mao 02631* (Holotype KUN!, Isotype PE!).

Morphology: Subshrubs. Stems 50–100 cm long, glabrous. Stems longitudinally angled, winged along angles. Petiole ca. 5 mm long; leaf blade elliptic to lanceolate, ovate, 6–11 × 2–4.5 cm, herbaceous or papery, glabrous, base cuneate to rounded, margin dentate to serrate or crenulate, apex acuminate; lateral veins 5–7 on each side of midrib, flat to prominent. Cymes 9–17-flowered; peduncle 6–10 cm long, glabrous; bracts ovate to broadly ovate, 5–8.5 × 4–7.5 mm. Pedicel 6–10 mm long. Calyx 5-sect from base; segments 6–8 × 1.5–2 mm, glabrous. Corolla yellowish to white, 2.8–3.1 cm long, outside glabrous, inside sparsely puberulent; tube tubular or slightly funnelliform, 1.8–2.1 × 0.3–0.5 cm; the limb distinctly 2-lipped, adaxial lip 2-lobed to near middle, lobes broadly ovate, 3–4 × ca. 4 mm, abaxial lip 3-lobed to near middle, lobes broadly ovate to oblong, 4–5 × 3–4 mm. Stamens 5–6 mm long; filaments 4–5 mm long, sparsely puberulent; anthers ellipsoid, ca. 1.2 mm long, connective appendage gland-dotted; staminodes 3, glabrous, lateral ones 3–4 mm long, adnate to ca. 1.5 cm above the corolla tube base, apex slightly expand; middle one ca. 1.0 mm long, adnate to ca. 1.5 cm above the corolla tube base. Disc ringlike, margin repand, ca. 1 mm high. Pistil ca. 8 mm long, glabrous. Capsule linear, 6–11 cm long, glabrous. Seed ca. 1 mm long, appendages hairlike, ca. 2 mm long.

Distribution and habitat: *Lysionotus pterocaulis* is found from southeastern Yunnan, China and Cao Bang, northern Vietnam (Fig. 4), and grows in the forests of limestone karst, on rock surface or as an epiphyte in mosses on trunk, at an elevation between 1100 m and 1700 m.

Phenology: Flowering from July to September, fruiting from September to October.

Conservation status: *Lysionotus pterocaulis* is not

common in the wild and is found in fragmented habitats. We assessed the total ‘area of occupancy’ (AOO) as less than 1000 km², and less than 10 populations have been recorded. Therefore, we recommend that this species should be classified in the category of ‘Vulnerable’ (VU B2a) according to the IUCN red list criteria (IUCN 2022).

Additional specimens examined: CHINA. Yunnan: Pingbian County (Ping-pien Hsien), 7 July 1934, *H. T. Tsai 60704* (PE!); *ibid.*, 17 August 1934, *H. T. Tsai 61508* (PE!); Pingbian County, Baiyan Township, alt. 1320 m, 6 July 1953, *P. Y. Mao 02390* (PE!). Pingbian County, Shi-tuen, alt. 1500 m, 27 September 1939, *C. W. Wang 82121* (PE!). Jinping County, Adeboqing, E103°8'49", 22°57'10", alt. 1498 m, 26 April 2012, *Jinping Exped. 5325300271* (IMDY!). Maguan County, Gulinqing, alt. 1200–1700 m, 1–10 October 1985, *Z. R. Xu GL5346* (E!); Maguan County, Gulinqing township, E104°01'08", 22°56'19", alt. 1683 m, 2 April 2020, *C. Y. Zou & al. ZCY749* (IBK); *ibid.*, 22 October 2020, *W. B. Xu 14228* (IBK); Maguan County, Bazhai township, 7 October 2021, *Q. Yuan & W. B. Xu 15254* (IBK). Hekou County, Lianhuatan, 28 November 1992, *Y. Z. Wang 92078* (PE). VIETNAM. Cao Bang: Nam Kep to Cao Ouac, E106°16', N22°40', alt. 1200 m, July 1924, *Petelot 717* (P).

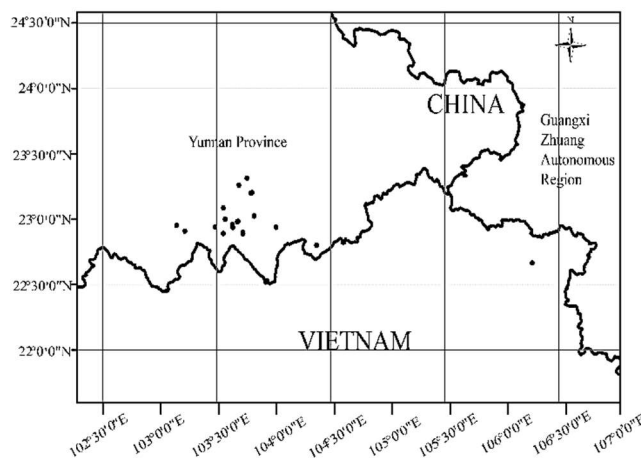


Fig 4. Map showing the distribution of *Lysionotus pterocaulis* in China and Vietnam.

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