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Research article

Studies on the Gesneriaceae in Laos II: *Lysionotus calcicola*, a new species from limestone karsts in north and central Laos, supported by morphological and molecular evidence

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A new species of Gesneriaceae, *Lysionotus calcicola* Phonep., Soulad. & Souvann., is described and illustrated based on morphological and molecular evidence. This species is distributed in limestone karsts in the Luangprabang, Vientiane and Khammouane provinces in northern and central Laos. The new species can be distinguished from the hitherto known species in the genus by its deciduous shrubby habit, up to 2 m tall, and oldest stems with 4-rounded edges and longitudinal grooves, but young branches non-grooved, which are considered to be an adaptation to the severe environment in limestone karst and cliffs. ML tree based on ITS showed that the new species form a well-supported clade (94%), sister to the remaining *Lysionotus* species. Information on its distribution, ecology, phenology, vernacular name, and a provisional conservation assessment are provided.

Keywords: Didymocarpoideae, flora of Laos, Indochina, Nam Sanam-Phou Pha Marn PPA, taxonomy

Introduction

Lysionotus D.Don (1822, p. 85), belonging to the family Gesneriaceae (subfamily Didymocarpoideae), consists of 35 species distributed from northern India and Nepal eastwards through Bangladesh, Bhutan, Laos, Myanmar, Thailand, northern Vietnam and southern China, Taiwan to southern Japan (Möller et al. 2017, GRC 2024 continuously updated). In Laos, only two species, *L. levipes* (C.B.Clarke) B.L.Burt and *L. serratus* D.Don have been found (Newman et al. 2017 onwards, Souvannakhoumane et al. 2021). The genus is characterized by its sub-shrubby habit, opposite or whorled leaves, tubular funnel-shaped corolla with an inflated upper



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half, two stamens, coherent anthers with or without connective appendages, and seeds with an appendage at each end (Weber et al. 2020, Akhil et al. 2021).

Khammouane Province is one of the richest areas with karst topography in Laos. Clusters of limestone hills topped with limestone caves make the province famous, such as The Rock Viewpoint at Phou Pha Marn, Konglor Cave in Phu Hin Poun National Protected Area and Xe Bang Fai Cave in Hin Nam No National Park, and others. Supported by this topography, the Lao limestone karsts provide specific habitats for various plant species endemic to this area, such as Gesneriaceae: *Didymocarpus middletonii* Souvann., Soulad. & Tagane (Souvannakhoumane et al. 2018), *Paraboea khotamiae* Phonep. & Souvann. (Phonepaseuth et al. 2021), Poaceae: *Laobambos calcareus* Haev., Lamxay & D.Z.Li. (Haevermans et al. 2020), Polypodiaceae: *Drynaria deuansavanhii* Vongthavone & Tagane, Pteridaceae: *Hemionitis khammouanensis* Vongthavone & Phonep. (Vongthavone et al. 2025), and Vitaceae: *Tetrastigma fruticosum* Soulad., Phonep. & Tagane (Tagane et al. 2025).

Recently, an unknown species of Gesneriaceae was discovered and collected during botanical surveys in the limestone karst of the Rock Viewpoint at Phou Pha Marn, Khammouane Province. From our morphological observations, the plant was assignable to the genus *Lysionotus* D.Don based on seeds with an appendage at each end (Weber et al. 2020, Akhil et al. 2021). The new species is similar to *Lysionotus chingii* Chun ex W.T.Wang by being a subshrub with large calyx and corolla, and these two species are compared in details. In addition, the same species was collected also on the limestone of Pha Yoak Limestone Forest Reserve Area, Luangprabang Province, northern Laos and Vangvieng District, Vientiane Province, Mahaxay District, Khammouane Province, central Laos. After careful examination of these specimens, morphological comparison with closely related species, and consultation of the relevant taxonomic literature, it was concluded to be an undescribed species. In addition to the morphological examination, DNA sequences and phylogenetic analysis are extremely helpful for delimiting species (Hebert and Gregory 2005, Dick and Webb 2012). Here, we sequenced a DNA barcode region, the nuclear ribosomal internal transcribed spacer (ITS) to compare the new species with related taxa. As a result, we describe it as new to science herein, along with the line drawing, information on its distribution, ecology, phenology, vernacular name, and provisional conservation status.

Material and methods

Field surveys and morphological investigation

An unknown *Lysionotus* was initially collected during our field survey in the limestone karst of The Rock Viewpoint at Phou Pha Marn, Khammouane Province, in July 2022 and re-collected in September 2022. Additional specimens were collected from Mahaxay District, Khammouane Province, in August 2022, and Vangvieng District, Vientiane Province

in August 2022 and September 2023. One specimen of the same species, which was gathered from Luangprabang Province in July 2014 and deposited at QBG, was confirmed during our examinations. We verified the validity of the new species by referring to taxonomic literature (Pellegrin 1931, Chun 1946, Wang et al. 1998, Weber et al. 1998, 2020, Souvannakhoumane et al. 2021, Lu et al. 2023), and available related digital images of type specimens from online resources (A, K, P, PE and US). Floral measurements were made based on fresh specimens for the description below. The terminology used in the description follows Beentje (2016). The preliminary conservation assessment is based on the guidelines of the IUCN Standards and Petitions Committee (2024). Types are deposited in FOF, BKF and HNL.

DNA extraction, sequencing and phylogenetic analysis

Total genomic DNA was extracted from silica-gel dried leaf samples collected in the field by using the modified CTAB protocol (Doyle and Doyle 1987). Amplification and sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region, was performed according to published protocols following Clement et al. (2004). The PCR products were purified using the ExoSAP-IT (USB Products Affymetrix, Inc., Cleveland, USA) and used as templates for direct sequencing. Reaction mixtures for sequencing were prepared using the SuperDye ver. 3.1 Cycle Sequencing Kit (Edge BioSystems, California, USA). The reaction mixtures were analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems, CA, USA).

Two samples of the new species, collected from Phou Pha Marn karst and Vangvieng karst, were used in this study and ITS data newly generated. Besides these, we downloaded 30 ITS sequences of related taxa coverage in subfamily Didymocarpoideae from GenBank obtained from published phylogenetic studies (Möller et al. 2011, Ranasinghe et al. 2024) (Table 1) for phylogenetic analysis: one sequence of *Allostigma* W.T.Wang, Anna Pellegr., *Cyrtandra* J.R.Forst. & G.Forst., *Glabrella* Mich.Möller & W.H.Chen, *Hemiboea* C.B.Clarke, *Pseudochirita guangxiensis* (S.Z.Huang) W.T.Wang, *Raphiocarpus* Chun, *Ridleyandra* A.Weber & B.L.Burt, two of *Chayamaritia* D.J.Middleton & Mich.Möller, *Codonoboea* Ridl., *Loxostigma* C.B.Clarke, *Petrocosmea* Oliv., three of *Didymocarpus* Wall., *Henckelia* Spreng., and six of *Lysionotus* D.Don (all in the same tribe Trichosporeae) were used as ingroups. Two sequences of *Rhychogossum obliquum* Blume & *Stauranthera grandifolia* Benth, both in the tribe Epitemateae were used as outgroups (Ranasinghe et al. 2024). In total, 33 sequences of 17 genera were included in this study. The sequence alignment was performed with ClustalW with default parameters implemented in MEGA11 (Tamura et al. 2021) followed by manually checking in Aliview (Larsson 2014). ML tree construct and analysis using the IQ-TREE web server (Nguyen et al. 2015) and employing one-thousand bootstrap pseudoreplicates via the ultrafast bootstrap (Hoang et al. 2018).

Table 1. Species of Gesneriaceae with 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.

Taxon	Voucher	Country of origin	GenBank accession number (ITS)
<i>Allostigma guangxiense</i> W.T.Wang	M.Moeller-MMO 08-1280 (E)	China	HQ632977
<i>Anna ophiorrhizoides</i> (Hemsl.) B.L.Burt & R.A.Davidson	M.Moeller-MMO 08-1280 (E)	China	HQ633034
<i>Chayamaritia banksiae</i> D.J.Middleton	M.Newman et al. LAO1428 (E)	Laos	KP325426
<i>Chayamaritia smitinandii</i> (B.L.Burt) D.J.Middleton	D.J.Middleton MID5652 (E)	Thailand	KP325425
<i>Codonoboea albomarginata</i> (Hemsl.) Kiew	A. Weber 840805-1/12 (WU)	Peninsular Malaysia	HQ632961
<i>Codonoboea pumila</i> (Ridl.) C.L.Lim	EDNA10-00268	–	JF912570
<i>Cyrtandra wallichii</i> (C.B.Clarke) B.L.Burt	Poopath MP187 (BKF)	Thailand	MN843378
<i>Didymocarpus middletonii</i> Souvann., Soulad. & Tagane	Tagane et al. L1198 (FU)	Laos	LC373522
<i>Didymocarpus newmanii</i> B.L.Burt	Palee 622 (CMU)	Thailand	DQ912674
<i>Didymocarpus purpureobracteatus</i> W.W.Sm.	Moller 01-70 (E)	China	DQ912676
<i>Glabrella longipes</i> (Hemsl. ex Oliv.) Mich. Möller & W.H.Chen	QZJ0978	China	OL753128
<i>Hemiboea bicornuta</i> (Hayata) Ohwi	Zhong_Shiwen	–	KY288050
<i>Henckelia anachoreta</i> (Hance) D.J.Middleton & Mich.Möller	Isolate MD2	–	OR758859
<i>Henckelia anachoreta</i> (Hance) D.J.Middleton & Mich.Möller	P.W. Li 2013143	–	MN627938
<i>Henckelia dielsii</i> (Borza) D.J.Middleton & Mich.Möller	J.M. Li 118223 (HEAC)	China	KR336987
<i>Loxostigma fimbriosepalum</i> K.Y.Pan	Y.Z. Wang 991005 (PE)	China	KU985104
<i>Loxostigma griffithii</i> (Wight) C.B.Clarke	FCY2013021	–	OL753124
<i>Lysionotus apicidens</i> (Hance) T.Yamaz.	G.Kokubugata 11862 (TNS)	–	AB514557
<i>Lysionotus calcicola</i> Phonep., Soulad. & Souvann.	Souladeth, P. et al. PPM034 (FOF)	Laos	This study
<i>Lysionotus calcicola</i> Phonep., Soulad. & Souvann.	Phonepaseuth, P. & Souvannakhoummane, K. VV23022 (FOF)	Laos	This study
<i>Lysionotus calcicola</i> Phonep., Soulad. & Souvann.	Phonepaseuth, P. & Souvannakhoummane, K. VV23022.2 (FOF)	Laos	This study
<i>Lysionotus chingii</i> Chun ex W.T.Wang	M.Moeller-06-983B (E)	China	AB547216
<i>Lysionotus denticulosus</i> W.T.Wang	M.Moeller-06-855B (E)	China	AB547217
<i>Lysionotus oblongifolius</i> W.T.Wang	M.Moeller-06-937B (E)	China	AB547218
<i>Lysionotus pauciflorus</i> Maxim.	M.Moeller-01-123-3 (E)	China	AB547214
<i>Lysionotus serratus</i> D.Don	Isolate LS	–	OQ940524
<i>Petrocosmea kerrii</i> Craib	Isolate P2 cult. RBGE 19715592	–	FJ501334
<i>Petrocosmea kerrii</i> Craib var. <i>crinita</i> W.T.Wang	QZJ-2007-084 (PE)	China	KR006462
<i>Pseudochirita guangxiensis</i> (S.Z.Huang) W.T.Wang	M.Moeller-MMO06-798 (E)	China	HQ633003
<i>Raphiocarpus petelotii</i> (Pellegr.) B.L.Burt	Qiu, Z.-J. et al. strain 50	–	JN092447
<i>Ridleyandra petiolata</i> (Ridl.) A.Weber	M.A.Mohd.Hairuf, FRI 60092 (KEP)	Peninsular Malaysia	HQ633032
<i>Rhynchoglossum obliquum</i> Blume	isolate HNBT05	–	KJ475424
<i>Stauranthera grandifolia</i> Benth.	isolate HNBT04	–	KJ475410

Results and discussion

Morphological observation

The new species is a lithophytic deciduous shrub with 4-rounded angles with longitudinally grooved oldest stems, opposite leaves, a funnellform corolla, and lateral staminodes in a pair that are similar to fertile stamens, but smaller and without pollen, appearing to have 4 fertile stamens. In habit it is also similar to the genus *Raphiocarpus*, but it differs in having 2 fertile stamens and a capitate stigma (versus 4 fertile stamens and bilobed stigma in *Raphiocarpus*). Like other

species of *Lysionotus*, it has seeds with an appendage at each end, but it is different from other species of the genus by being a deciduous subshrub and having 4-rounded angled, longitudinally grooved stems (Fig. 1–3).

Phylogenetic analysis

In the ML tree based on ITS the three samples of the new species form a well-supported clade (94%), sister to the remaining *Lysionotus* species (96%). Among the sampled taxa, *Hemiboea* form the sister to *Lysionotus* (66%). The new

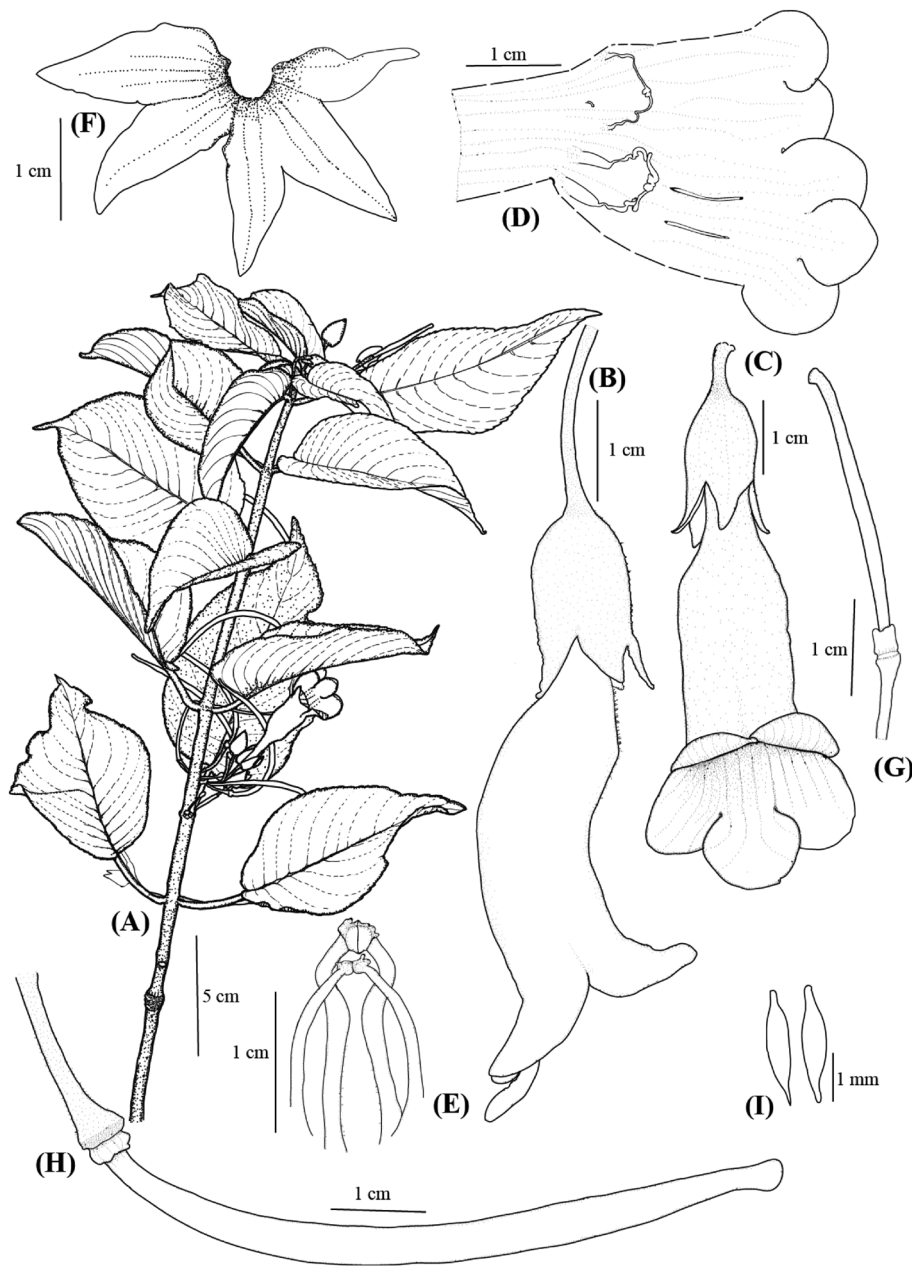


Figure 1. *Lysionotus calcicola* Phonep., Soulad. & Souvann. (A) Habit, (B) flower lateral view, (C) flower top view, (D) corolla opened out showing stamens and staminodes, (E) stamens and staminodes, (F) calyx, (G) pistil, (H) fresh fruit, (I) seeds. Drawn from Souladeth et al. PPM034 (FOF) by K. Souvannakhoummane.

species falls far distant from its morphologically closest congener, *L. chingii* (Fig. 4).

Taxonomic treatment

Lysionotus calcicola Phonep., Soulad. & Souvann. sp. nov. (Fig. 1–3)

Diagnosis

A species similar to *Lysionotus chingii* Chun ex W.T.Wang of China in having papery leaves, glabrous peduncles, white

funnel-shaped corollas, and subulate seed appendages, but distinguished by its 4-rounded-angled and longitudinally grooved stems (versus terete), sparsely puberulent lamina (versus glabrous), corolla sparsely short glandular hairy on both surfaces (versus glabrous on outer surface), and sparsely glandular hairy pistil (versus glabrous). For further comparison see Table 2.

Type: Laos, Khammouane Province: Khoumklam District, in limestone karst of The Rock Viewpoint at Phou Pha Marn, 478 m a.s.l., 2 July 2022, P. Souladeth et al. PPM034 (holotype: FOF, isotypes: BKF, HNL).

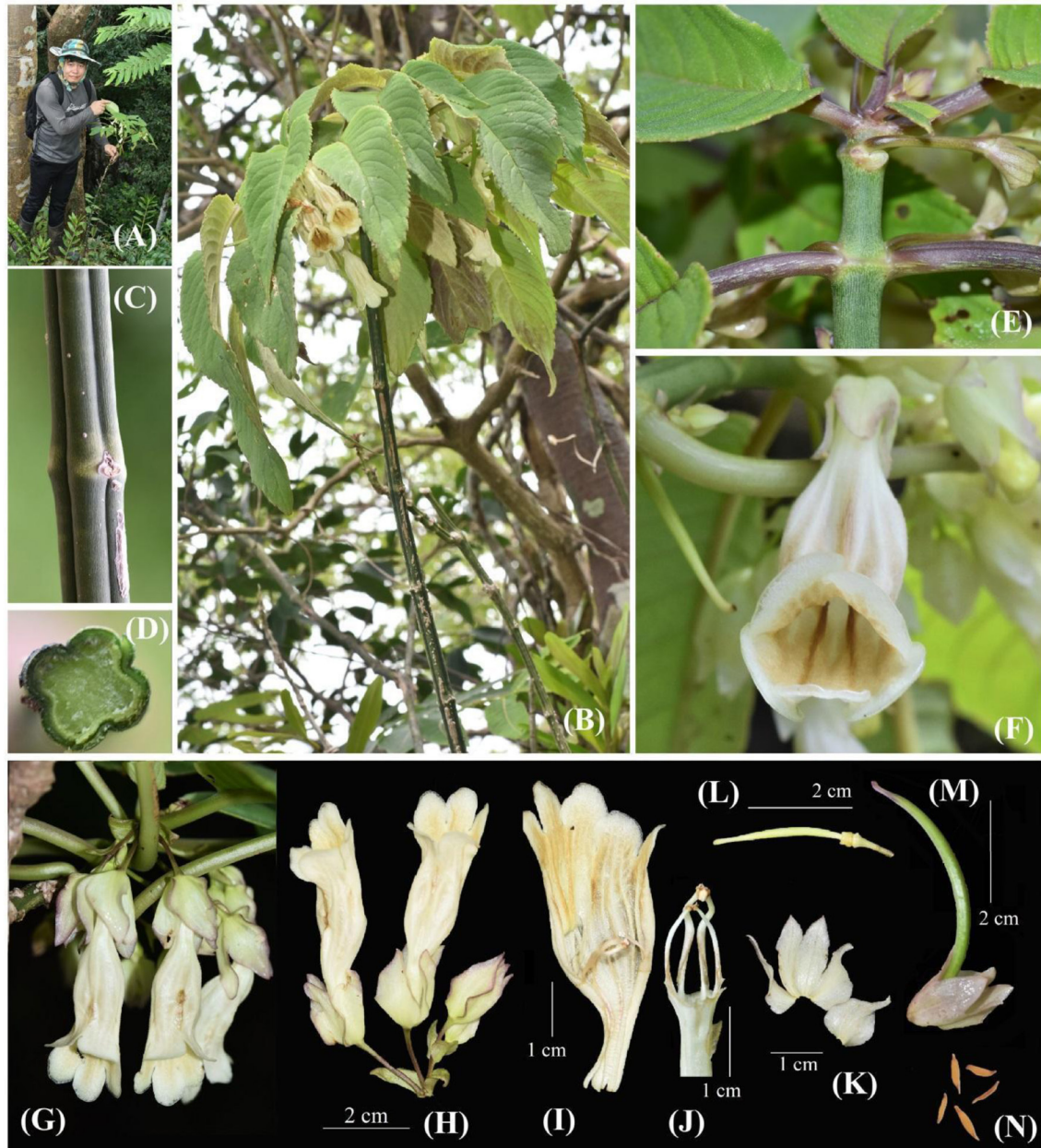


Figure 2. *Lysionotus calcicola* Phonep., Soulad. & Souvann. (A–B) Habitat and habit, (C) 4-angled stem, (D) cross section of stem, (E) arrangement of leaves, (F) flower, front view, (G–H) inflorescences, (I) corolla opened out, (J) stamens and base of corolla tube, (K) calyx opened (L) pistil, (M) young fruit with calyx, (N) seeds. Photos from Souladeth et al. PPM034 (FOF). (A–F) and (L) by P. Phonepaseuth, (G–K) by K. Souvannakhoummane, (N) by D. Kongxaisavath.

Etymology

The specific epithet refers to the Latin word ‘*calcicola*’ meaning ‘growing on calcareous substrate’, which is the habitat where this plant is found.

Description

Subshrub, lithophytic, perennial, deciduous, 60–200 cm tall. Stems succulent, 55–195 cm long, 0.6–0.8 cm in diameter, 4-rounded-angled, longitudinally grooved except in young branches, dark green when young, brownish gray when old,

glabrescent to glabrous; internodes 4–10 cm. Leaves opposite, slightly anisophyllous in a pair; petioles 3.5–10 cm long, adaxially sunken, abaxially rounded, light green to reddish green, sparsely puberulent; lamina papery, ovate to elliptic, 12.0–22.0 × 5.5–11.0 cm, 2.0–2.2 times as long as wide, with adaxial surface dark green, sparsely puberulent, and abaxial surface pale green, puberulent, more densely so on veins, acute to acuminate at apex, obliquely cordate at base, with sub-entire to irregularly serrate margin; midrib prominent abaxially; lateral veins 9–12 pairs, prominent abaxially.

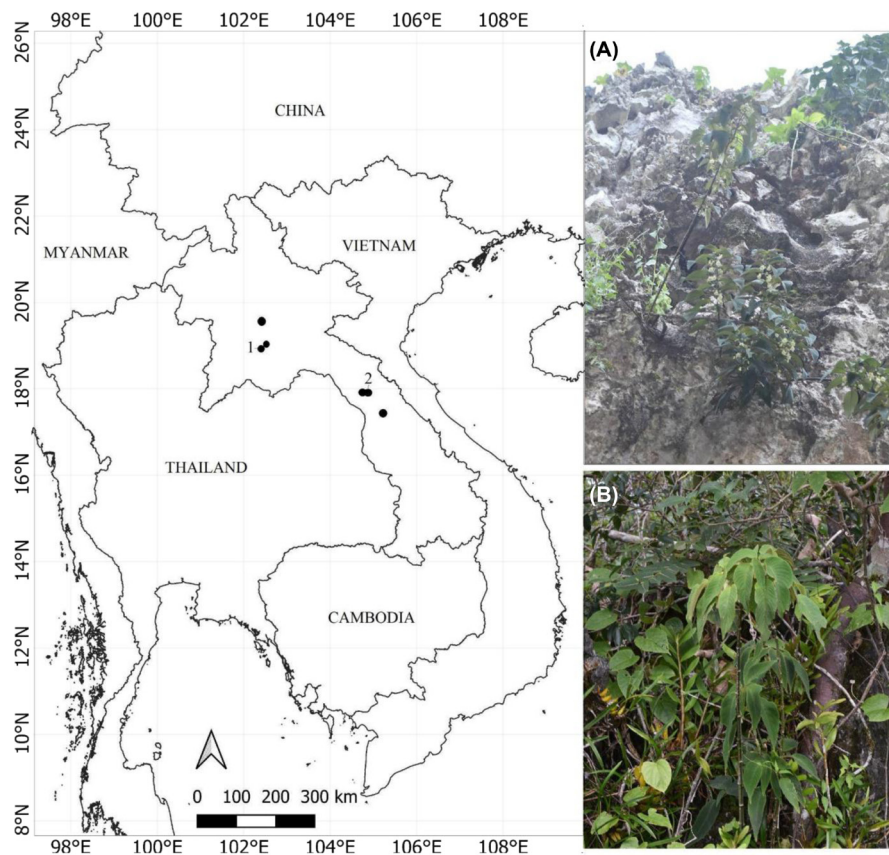


Figure 3. Map shows distribution of *Lysionotus calcicola* Phoney., Soulad. & Souvann. (1) population in north and (2) population in central Laos with the addition of habitat images. (A) Plant in full sun karst environment in Vangvieng district, (B) in semi-shade habitat in type locality. Photograph by P. Phoneyaseuth.

Inflorescences axillary, on twigs behind leaves, with 2–3(–4) flowers per cyme; peduncle 2–6 cm long, lightly green, sparsely puberulent; bracts 2, elliptic to broadly elliptic, 8–12 × 5–6 mm, lightly green to reddish-green, puberulent, acute at apex, with ciliate margin. Flowers pendulous; pedicels 6–16 mm long, lightly green to greenish-red, sparsely covered with multicellular hairs. Calyx zygomorphic of 5 lobes fused to 1/3–2/3 from base, its outer surface white to lightly green, sometimes reddish-green along margin of the lobes, sparsely puberulent, and inner surface glabrous; upper lobe 1, broadly ovate, 1.5 × 1.0–1.2 cm; lateral lobes 2, equal, broadly ovate, 1.5 × 0.8–1.0 cm; lower lobes 2, equal, broadly elliptic, 1.7–1.8 × 0.5–0.7 cm. Corolla funnel-shaped, 4.2–5.5 cm long, whitish to pale brown, with two darker-coloured prominent ridges ventrally near throat on corolla floor, sparsely covered with short glandular (capitate) hairs on both surfaces; limb 2-lipped; tube with 1.0–1.6 cm long narrow part and 2.8–3.0 cm long dilated part; upper lip 2-lobed with lobes 5.5–6.5 × 4.5–5.5 mm; lower lip 3-lobed with lateral lobes 8–10 × 7–9 mm, and middle lobe 7.0–8.5 × 5.0–7.0 mm. Stamens 2, cohered, divergent; anther thecae confluent; anterior pair inserted at 12–16 mm from corolla base; filaments 12–18 mm long, lightly yellow to white, glabrous except for base with sparse short glandular hairs; anthers 0.8–1.2 × 0.6–1.0

mm, subglobose to broadly ellipsoid, white to lightly brown. Staminodes 3; posterior lateral pair inserted at 10–12 mm from the corolla base; filaments 10–15 mm long, color and hairy as stamens; antheroids 0.5–0.9 × 0.4–0.8 mm, color and hairy as anthers; posterior central staminode 1.0–1.5 mm long, color and hairy as stamens, with capitate apex. Disc annular, lightly green, weakly 5-lobed, 2–3 mm high, glabrous. Pistil 25–31 mm long; ovary narrowly ellipsoid, 18–22 mm long, lightly green, glabrous; style 6–8 mm long, lightly green, sparsely glandular hairy; stigma 1 mm long, capitate. Fruits linear or filiform, 4–5 cm long, 0.3–0.4 cm in diameter, slightly curved, green when young, dark brown when mature, glabrous. Seeds numerous, 1.6–2.4 × 0.2–0.4 mm, narrowly ellipsoid to filiform, light brown; appendages subulate, 0.3–0.5 mm long.

Phenology

Flowering and fruiting in July–September.

Distribution

Lysionotus calcicola is currently known only from Laos (Luangprabang, Vientiane and Khammouane provinces) (Fig. 3).

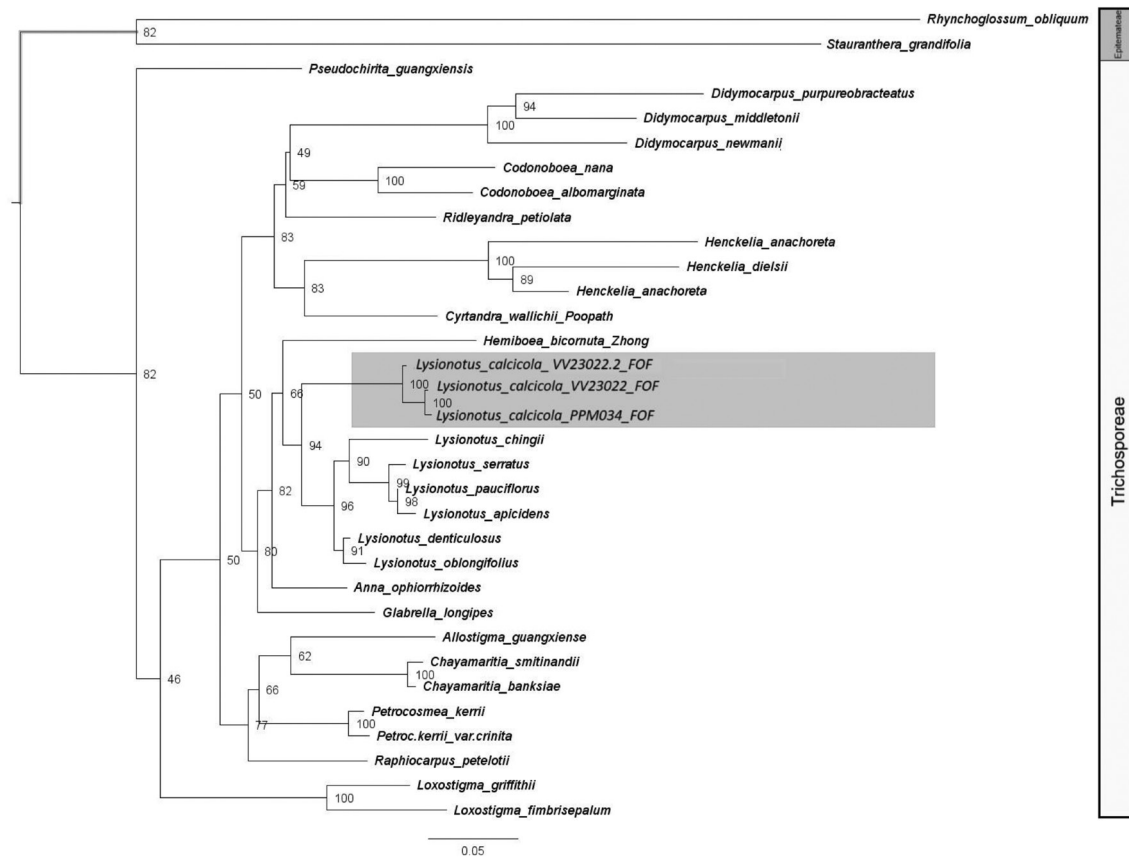


Figure 4. ML tree of *Lysionotus* species and related genera based on the nuclear ribosomal internal transcribed spacer (ITS) regions showing the position of new species in highlight. Numbers on the branches indicate support values (1000 ultrafast bootstrap replicates).

Habitat and ecology

Lysionotus calcicola grows in semi-shaded to open limestone crevices, at elevations of 285–890 m a.s.l. In the habitat where we collected the above type specimen, the plant was associated with *Dendrobium moschatum* (Banks) Sw., *Coelogyne* sp. (Orchidaceae), *Davallia denticulata* (Burm.) Mett. (Davalliaceae) and *Paraboea* sp. (Gesneriaceae). In Vangvieng District, it was found on open limestone rocks, and the plants possessed a variation in leaf colour, green to reddish-green. It is growing along with *Begonia viscosa* Aver. & H.Q.Nguyen

(Begoniaceae), the unusual deciduous cane-like species which is considered adapted to karst limestone environment.

Provisional IUCN conservation assessment

Thus far, six populations are known from northern to central of Laos. From our field observations, the number of mature individuals is an estimated total 60, as around 10 mature individuals occur in each population. The type locality is located within the protected area of Nam Sanam-Phou Pha Marn Provincial Protected Area in central Laos and under

Table 2. Morphological comparison of *Lysionotus calcicola* and *L. chingii* (Wang et al. 1998).

Characteristics	<i>L. calcicola</i>	<i>L. chingii</i>
Life form	lithophytic	epiphytic or lithophytic
Habit	subshrubs, 60–200 cm tall	subshrubs, up to 90 cm tall
Stem	4-rounded angled, longitudinally grooved, glabrescent to glabrous	rounded, glabrous
Lamina	slightly anisophyllous, ovate to elliptic, margins slightly serrate to entire, apex acute to acuminate, sparsely puberulent	isophyllous, elliptic to narrowly elliptic to oblong, glabrous, margins entire to denticulate
Petiole	sparsely puberulent	glabrous
Calyx lobes	zygomorphic, fused at 1/3–2/3 from base	zygomorphic, fused 1/2 from base
Corolla	4.2–5.5 cm long, whitish to pale brown, sparsely covered with short glandular hairs on both side	4 cm long, cream or tinged with green, glabrous outside, inside puberulent basally
Pistil	sparsely glandular hairy	glabrous
Distribution	north to central of Laos	Guangxi of China and north of Vietnam

care and well-preserved for ecotourism of a private company. The population in Mahaxay is close to the type locality and share the same limestone karst range. The two populations in Luangprabang and Vientiane in the north of Laos also share the same limestone karst range and are around 420 km far from the type locality in central of Laos. The area of occupancy (AOO) is calculated as 24 km². However, the populations in northern Laos are not located in protected areas, and some potential risks, such as agriculture land-use change, and mining activities, have been confirmed. Therefore, the most appropriate provisional assessment of the IUCN conservation status of this species is as Endangered (EN D (IUCN 2024)). Further information on its distribution and number of individuals/populations is needed to accurately assess the status and conserve this species.

Additional specimens examined

Laos, Luangprabang Province, Nan District, Pha Yoak Village, Pha Yoak, Limestone Forest Reserve Area, 890 m a.s.l., 18 July 2014, C. Maknoi et al. L3-071 (QBG). Vientiane Province, Vangvieng District, Blue lagoon 2 karst, 285 m a.s.l., 20 Aug. 2022, P. Phonpaseuth et al. P069 (FOF); *ibidem*, 9 Sept. 2023, P. Phonpaseuth & K. Souvannakhoummane VV23022 (FOF); Phangoun mountain, 17 July 2022, S. Lanorsavanh & K. Souvannakhoummane 17722 (HNL, FOF). Khammouane Province, Khounkham District, in limestone karst of Phou Pha Marn the Rock viewpoint, Nam Sanam-Phou Pha Marn PPA, 470 m a.s.l., 1 July 2022, P. Souladeth et al. PPM03 (HNL, FOF); *ibidem*, 21 July 2023, K. Souvannakhoummane & T. Vongthavone PPM026 (FOF); *ibidem*, 7 Sept. 2023, S. Tagane et al. Z527 (FOF, KAG, VNM); Mahaxay District, Vern Village, 20 Aug. 2022, S. Lanorsavanh et al. LP167 (HNL).

Vernacular name

Dok Nga Xang Hin (ດອກງາຊ້າງຫິນ), proposed here. ‘Dok Nga Xang’ in Laos refers to a given name for the genus *Lysionotus*, and ‘Hin’ refers to limestone karst, the type of habitat where this plant is found.

Propagation and cultivation

Lysionotus calcicola has cylindrical stems and a shrubby habit; this would be an adaptation to limestone karsts and cliffs, which are severe environments for its survival. When cultivated in the experimental field at the National University of Laos, it was easily propagated from cuttings. After we cut stems and they had been kept with a little moisture and shadow in the nursery, roots and new leaves were generated in a month. As it is easily propagated, tolerant to dry conditions, and has large beautiful white flowers, this species has a potential to be cultivated as an ornamental plant.

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Author contributions

Keouudone Souvannakhoummane: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (equal); Writing – review and editing (equal).

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Data availability statement

There are no additional data for this paper.

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