Studies in Fungi

ARTICLE

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https://doi.org/10.48130/sif-0026-0006

Studies in Fungi **2026**, 11: e008

Lecanora rhododendri sp. nov. (Lecanoraceae, Lecanorales) associated with *Rhododendron* sp. in Yunnan, China

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Abstract

During a survey of fungi on *Rhododendron* in China, *Lecanora rhododendri* sp. nov., was identified. In this paper, it is introduced with a detailed morphological description and illustrations. The species' novelty is justified on the basis of its distinct morphological characteristics and phylogenetic analyses of a concatenated internal transcribed spacer (ITS) and mitochondrial small subunit (mtSSU) sequence data using maximum likelihood and maximum parsimony criteria, as well as ITS and mtSSU nucleotide base comparisons with related taxa. A preliminary chemical test with 5% KOH and Lugol's reagent on the thallus, apothecial margin, and asci was also observed. The fungus was collected from dead branches of *Rhododendron* sp. in Yunnan, China. In the phylogenetic analyses of a combined ITS and mtSSU sequence dataset, the species formed a separate branch and has a close relationship with *Lecanora orientoafricana* in the genus *Lecanora* (Lecanoraceae, Lecanorales). Morphologically, the new isolate is typical of *Lecanora* in having a crustose thallus; apothecial, sessile ascomata with black-brown discs; *Lecanora*-type clavate asci; and hyaline, subglobose to obovoid aseptate ascospores.

Citation: Chaiwan N, Phookamsak R, Jiang H, Thiyagaraja V, Bhat J, et al. 2026. *Lecanora rhododendri* sp. nov. (Lecanoraceae, Lecanorales) associated with *Rhododendron* sp. in Yunnan, China. *Studies in Fungi* 11: e008 https://doi.org/10.48130/sif-0026-0006

Introduction

Lecanora is the second richest and cosmopolitan genus of lichenized fungi that is classified in the family Lecanoraceae (Lecanorales, Lecanoromycetes)[1]. The genus is characterized by a crustose thallus, apothecial ascomata with generally reddish-brown discs, the presence of calcium oxalate crystals in the amphithecium, atranorin in the grayish white to gray thallus, *Lecanora*-type asci that are commonly eight-spored but rarely multispored, and the production ofcolorless, almost ellipsoid to broadly ellipsoid ascospores[2–4]. Vegetative reproduction is also commonly found with the presence of soredia containing atranorin, oxalate crystals in the amphithecium, and filiform conidia[3,4]. The genus represents one of the largest and morphologically most diverse groups of lichen-forming fungi, with approximately 1,000 described species globally[5] and ~250 accepted species in Hyde et al.[1], widely distributed from the Arctic and Antarctic to subtropical and tropical regions[4,6–11]. Members of the genus are primarily crustose lichens, commonly found colonizing bark, wood, rocks, and soil in all ecosystems[4,7,9,12–15]. In addition, many species produce a wide range of secondary metabolites such as atranorin, usnic acid, and various depsides and depsidones, which are important in chemotaxonomic studies and are utilized for their antioxidant, antiviral, antimicrobial, and cytotoxic properties[16–19].

Lecanora was established by Erik Acharius in Luyken[20] and subsequently typified by *L. subfusca*. *Lecanora* species are traditionally recognized by their lecanorine apothecia, which are characterized by a thalline margin that includes algal cells and asci containing hyaline, nonseptate ascospores[2,21]. Traditionally, the core genus *Lecanora* accommodated a large majority of species in the family and thus became a taxonomic repository for crustose lichens with lecanorine apothecia. Over the last two centuries, the genus was defined in a broad sense on the basis of a few stable morphological characteristics, particularly the presence of a thalline apothecial margin and simple ascospores. This traditional circumscription led to *Lecanora* becoming a "catch-all" genus for numerous unrelated species that superficially resembled each other, contributing to taxonomic inflation and artificial groupings[21]. However, Eigler[22] demonstrated the heterogeneity of the genus and segregated the taxon into several groups according to the microscopic characteristics of the ascomata. In subsequent studies based on ascus types, many *Lecanora* taxa were reclassified in different genera, families, and subclasses[2,23–27].

Recent advances in molecular phylogenetics have confirmed that *Lecanora* in its traditional sense is polyphyletic[2,9]. Multilocus analyses using the nuclear ribosomal internal transcribed spacer (ITS), mitochondrial small subunit (mtSSU), and protein-coding genes such as *rpb*1 and *rpb*2 have revealed that many species previously placed in *Lecanora* are more closely related to other genera. This has led to the segregation of several lineages into distinct genera, including *Lecanoropsis*, *Myriolecis*, *Palicella*, *Protoparmeliopsis*, *Pulvinora*, and *Vainionora*[2,26,27–31]. Despite these advances, taxonomic delimitation within *Lecanora* remains problematic in certain species complexes, where morphological convergence and cryptic speciation are commonly found. Ongoing integrative taxonomic approaches combining morphology, chemistry, ecology, and multi-locus phylogeny are essential for resolving these issues and achieving a stable classification[32,33].

In this study, we introduce a new taxon, *Lecanora rhododendri*, collected from Yunnan, China, based on its morphology and molecular analyses coupled with preliminary chemical test (5% KOH and Lugol's reagent). The topology based on a combined ITS and mtSSU dataset revealed that *Lecanora rhododendri* sp. nov. formed a separate branch and clustered with *L. flavoviridis*, *L. darlingiae*, *L. alboflavida*, and *L. orientoafricana* within *Lecanora s. str.*

Materials and methods

Morphological examination

The sample was collected from dead and dying branches of *Rhododendron* in May 2019 in Yunnan, China. The sample was observed under a Motic SMZ-171 stereo microscope (Motic Xiamen, China) and a compound microscope (Nikon ECLIPSE 80*i*, Japan). Measurements of its morphological features (*viz.* the apothecia, amphithecia, amphithecial cortex, hypothecium, parathecium, hymenium, epihymenium, paraphyses, asci, and ascospores) were made by using the Tarosoft® Image FrameWork program. Figures were processed with Adobe Photoshop CS6 Extended v.10.0 software (Adobe Systems, USA). The herbarium material was deposited in the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS), Kunming, China, and duplicated in the Herbarium of Mae Fah Luang University (Herb. MFLU). Faces of Fungi[34] and Index Fungorum numbers[35] were obtained for the novel taxon.

DNA extraction, polymerase chain reaction amplification, and sequencing

The DNA was extracted from fruiting bodies using the E.Z.N.A. Forensic DNA Kit (OMEGA® biotek). Polymerase chain reaction (PCR) was used to amplify specific gene regions: ITS was amplified using the primers ITS5 and ITS4[36], and mtSSU was amplified using the primers mtSSU1 and mtSSU3R[37]. The amplification reactions were performed in 25-μL final volumes containing 8.5 μL of sterilized ddH$_2$O, 12.5 μL of Easy Taq PCR Super Mix, 1 μL of each forward and reverse primer, and 2 μL of the DNA template. The PCR thermal cycle program for ITS and mtSSU gene amplification was 94 °C for 3 min initially, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 50 s, elongation at 72 °C for 1.30 min, and a final extension at 72 °C for 10 min. The PCR products were purified and sequenced with the same primers at Sangon Biotech, Shanghai, China. The chromatograms of the sequences were checked to ensure the quality of the sequences using BioEdit v.5[38]. DNA Star v. 5.1 was used to make consensus sequences.

Phylogenetic analyses

The newly generated ITS and mtSSU sequences were subjected to a nucleotide BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 10 August 2025) to identify closely related taxa of the new collection. The sequence data of *Lecanora* species and closely related taxa in the family Lecanoraceae were downloaded from NCBI's GenBank (Table 1) following recent publications[3,4,9,11]. Downloaded sequences were aligned with the sequences obtained in the current study using MAFFT v. 7.511 (http://mafft.cbrc.jp/alignment/server)[39]. If necessary, single gene alignments were manually improved with BioEdit v.5[38], and the combined ITS and mtSSU sequence dataset was analyzed using the maximum likelihood (ML) and maximum parsimony (MP) criteria.

The phylogenetic analyses were performed by ML using the RAxML-HPC2 package on XSEDE (8.2.8)[40,41] via the CIPRES Science Gateway platform[42], with the GTR+I+G model of nucleotide substitution, which was selected independently for each locus using MrModeltest 2.3[43]. A rapid bootstrap was set up at 1,000 replicates. The MP analysis was executed with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 with the following parameter settings: Characters unordered with equal weight, random taxon addition, branch swapping with the tree bisection–reconnection (TBR) algorithm, and branches collapsing if the maximum length was zero. Alignment gaps were treated as missing characters in the analysis of the combined dataset, where they occurred in relatively conserved regions. Trees were inferred using the heuristic search option with 1,000 random sequence additions, with maxtrees set at 1,000. Descriptive tree statistics were used for parsimony; tree length (TL), the consistency index (CI), the retention index (RI), the relative consistency index (RC), and the homoplasy index (HI) were calculated for the generated trees. Kishino–Hasegawa tests[44] were performed in order to determine whether the trees were significantly different. The ML and MP bootstrap values equal or greater than 60% are given above each node. The phylogenetic trees were visualied in FigTree v.1.4[45] and edited using Microsoft Office PowerPoint 2010 and Adobe Illustrator CS3 (Adobe Systems Inc., USA). The sequences generated in this study have been deposited in NCBI's GenBank (Table 1).

Results

Phylogenetic analyses

The result of the nucleotide BLAST search showed that the new collection is closely related to *Lecanora inversa* (voucher KRAM_L_74751) with 90.55% similarity (identities: 498/550 bp with three gaps) in the ITS, and is similar to *L. alboflavida* (vouchers KRAM : Flakus 29143, KRAM : Flakus 28262, KRAM : Rodriguez-Flakus 3997, KRAM : Flakus 29196, and KRAM : Flakus 29124) and *L. inversa* (voucher *Flavonora inversa* KRAM L 74752) with 98.14% similarity (identities: 580/591 bp with one gap) in the mtSSU. Therefore, the phylogenetic tree of 56 representative species (including the new collection) in Lecanoraceae was constructed. The combined sequence alignments comprised 80 taxa, with *Japewia aliphatica* (voucher J. Maliček 9764) and *J. tornoensis* (voucher Printzen s. n.) as the outgroup taxa. The dataset comprised 1,697 characters, including gaps (1–753 bp for the ITS, and 754–1,697 bp for the mtSSU). The RAxML analysis yielded the best scoring tree (Fig. 1) with a final ML optimization likelihood value of −18,787.258374. The sequence alignment had 1,055 distinct alignment patterns, with 34.39% undetermined characters or gaps. The estimated base frequencies were A = 0.279342, C = 0.212476, G = 0.231333, T = 0.276849, with the substitution rates AC = 1.226615, AG = 3.167475, AT = 1.707134, CG = 1.553882, CT = 5.510086, and GT = 1.000000. The gamma distribution shape parameter alpha was 0.294643, and the tree length was 5.258968. The MP dataset consisted of 885 constant characters, 157 variable parsimony-uninformative characters, and 655 parsimony-informative characters. Eighteen equally parsimonious trees were generated with a tree length of 3,628 steps (CI = 0.402,

Table 1. Taxa used in the phylogenetic analyses and their GenBank accession numbers.

Taxa	Voucher details	Country	GenBank accession no.	
			ITS	mtSSU
Glaucomaria carpinea	Herbarium Malíček 14828	Czech Republic	OQ717895	–
Glaucomaria carpinea	Kondratyuk S. 21337 (KW-L)	Ukraine	MK672827	MK693683
Glaucomaria rupicola	KRAM: Flakus 29527	Bolivia	OL604012	OL604094
Glaucomaria rupicola	Leavitt 18-460c BRY-C	USA	MZ243629	–
Japewia aliphatica	**J. Maliček 9764**	**Czech Republic**	**MN547352**	**MN547343**
Japewia tornoensis	Printzen s. n.	Canada	HQ650656	HQ660559
Lecanora albella	J. Maliček 7336	Czech Republic	KY548048	KY502423
Lecanora alboflavida 0	Coppins s. n.	United Kingdom	KY548045	KY502428
Lecanora alboflavida 1	KRAM: Flakus 29124	United Kingdom	OL604044	OL604124
Lecanora alboflavida	KRAM: Flakus 29143	Bolivia	OL604042	OL604122
Lecanora alboflavida	KRAM: Rodriguez-Flakus 3997	Bolivia	OL604034	OL604116
Lecanora allophana 0	J. Malíček 9491 (hb. JM)	Finland	KY548051	KY502416
Lecanora allophana 1	J. Malíček 9626 (hb. JM)	Russia	KY548050	KY502421
Lecanora argentata	J. Malíček 1963	Czech Republic	KT630245	KT630264
Lecanora barkmaniana	Maliček 10602	Russia	MK778605	MK778530
Lecanora caesiorubella	F: Lumbsch 19094a	USA	–	JQ782666
Lecanora campestris	Arup U225	Sweden	AF159930	–
Lecanora cenisia	J. Maliček 5869	Austria	KY548047	KY502425
Lecanora crystalliniformis	Wang et al. 19-63013	China	ON807164	ON807171
Lecanora crystalliniformis	Wang et al. 17-56082	China	–	ON807172
Lecanora crystalliniformis	Wang et al. 17-56085	China	ON807163	ON807173
Lecanora crystalliniformis	Wang et al. 17-56554	China	ON807162	–
Lecanora crystalliniformis	Tang & Lui L09	China	–	ON807174
Lecanora darlingiae	Lendemer 46644	USA	–	MH481360
Lecanora expersa	J. Maliček 9624	Russia	KY548053	KY502420
Lecanora flavidomarginata	Flakus 29951	Bolivia	OL604056	OL604135
Lecanora flavidomarginata	Flakus 28943	Bolivia	OL603996	OL604077
Lecanora flavoviridis	Papong 6539	Thailand	JQ782711	JQ782675
Lecanora fulvastra	Flakus 26717	Bolivia	–	OL604089
Lecanora gangaleoides	HTL19923a	USA	MG554660	JQ782676
Lecanora glabrata	Lubeck & Kukwa 17811	Poland	MN387101	–
Lecanora helva	Papong 6444	Thailand	JQ782716	JQ782679
Lecanora horiza	n/a	Spain	KT453772	KT453821
Lecanora hybocarpa	H.T. Lumbsch s. n.	Spain	EF105412	EF105417
Lecanora intricata	KRAM: Flakus 29565b	Bolivia	OL604030	OL604112
Lecanora intumescens	J. Malíček 8480 (hb. JM)	Czech Republic	KY548040	KY502441
Lecanora intumescens	TRH L-19015	Norway	OQ945715	–
Lecanora masana	JL46185	USA	MK573928	–
Lecanora orientoafricana	**P. Kirika 2205**	**Kenya**	**JQ900619**	**JQ900617**
Lecanora phaeocardia	Papong 3492 (F)	Thailand	JQ782724	JQ782687
Lecanora pulicaris 0	J. Malíček 10262 (hb. Malíček)	Russia	MK778611	MK778539
Lecanora pulicaris 1	J. Malíček 10263 (hb. Malíček	Russia	MK778612	MK778540
Lecanora rhododendri[a]	**MFLU 20-0045**[a]	**China**[a]	**PV241604**[a]	**PV470111**[a]
Lecanora rugosella	Arup L97561	Sweden	AY398712	–
Lecanora subimmersa	HTL19103b	Australia	JQ782733	JQ782697
Lecanora subrugosa	Arup L98188	Austria	AY398711	–
Lecanora substerilis	**Maliček 202**	**Slovakia**	**KT630243**	**KT630254**
Lecanora tropica	F: Papong 6440	Thailand	JN943720	JQ782699
Lecanora vainioi	Papong 7120	Thailand	JN943716	JQ782702
Lecanora variolascens	Maliček 8422	Austria	KY548038	KY502445
Lecanora zeorina	Wang et al. 19-63051	China	ON807167	ON807168
Lecanora zeorina	Wang et al. 19-63070	China	ON807166	ON807169
Lecanora zeorina	**Wang et al. 19-62893**	**China**	**ON807165**	**ON807170**
Lecanoropsis saligna	491_DE	Germany	MT938982	MT939209
Lecanoropsis subintricata	604_JP	Japan	MT939010	MT939239
Lecidella carpathica	ZX 20140367-2	China	KT453741	KT453831
Lecidella meiococca	Ekman 3101 (BG)	Sweden	AF517929	AY300893
Palicella glaucopa 0	P. Rodriguez Flakus 2539 (FR)	Argentina	KJ152486	KJ152471
Palicella glaucopa 1	P. Rodriguez Flakus 2504b (FR)	Argentina	KJ152485	KJ152472
Parmotrema reticulatum	K.H. Moon 14834	Japan	KP942516	KP942523
Parmotrema tinctorum	TNS-L-Y.O. 5947	Japan	KF129464	KF129521
Polyozosia altunica	**Xahidin 20071910**	**China**	**MH698406**	**MH698407**
Polyozosia contractula	Brodo 31501 (DUKE)	USA	HQ650604	DQ986898

(to be continued)

Table 1. (continued)

Taxa	Voucher details	Country	GenBank accession no.	
			ITS	mtSSU
Polyozosia perpruinosa	Arup U176	Sweden	AF070025	–
Protoparmeliopsis achariana	Arup U155	Sweden	AF070019	DQ787342
'*Protoparmeliopsis crystalliniformis*'	BDNA-L-0000298	South Korea	MW832796	MW832802
'*Protoparmeliopsis crystalliniformis*'	**BDNA-L-0000349**	**South Korea**	**MW832797**	**MW832803**
Protoparmeliopsis garovaglii	Leavitt 089 (BRY-C)	USA	KT453728	KT453818
Protoparmeliopsis garovaglii	M. Haji Moniri (KW-L)	Iran	MK672841	MK693689
Protoparmeliopsis garovaglii	19-63070	China	ON807161	ON807175
Protoparmeliopsis garovaglii	19-63467	China	ON807160	ON807176
Protoparmeliopsis kopachevskae	KoLRI 040276	South Korea	MK672847	–
Protoparmeliopsis muralis	SK 765	Romania	KP059048	KP059054
Protoparmeliopsis muralis	Schmull s. n.	Germany	HQ650653	HQ660556
Protoparmeliopsis nashii	Knudsen 19030	n/a	ON447553	ON367825
Protoparmeliopsis peltata	n/a	USA	KT453722	KT453860
Protoparmeliopsis peltata	14619	Iran	ON807159	ON807177
Protoparmeliopsis zareii	SK 480	Iran	KP059049	KP059055
Pulvinora stereothallina	**E. A. Davydov no. 14817 & L. S. Yakovchenko (LE-L15134)**	**Russia**	**MW257118**	**MW257159**
Pulvinora stereothallina	E. A. Davydov no. 14820 (ALTB)	Russia	MW257112	MW257152

[a] The new taxon is indicated in bold. The ex-type strains are indicated by black bold and the unavailable sequence is indicated by "–".

RI = 0.675, RC = 0.271, HI = 0.598), of which the first tree was shown to be the most parsimonious tree according to the Kishino–Hasegawa test.

Phylogenetic trees inferred from the ML and MP analyses showed a similar topology and concurred with previous studies[9]. Most described *Lecanora* species (such as *Lecanora s. str.* in Li et al.[9]) clustered within the *Lecanora s. str.* clade in this study, comprising *L. alboflavida* (vouchers: Coppins s. n., KRAM: Rodriguez-Flakus 3997, KRAM: Flakus 29143, and KRAM: Flakus 29124), *L. barkmaniana* (voucher: Maliček 10602), *L. crystalliniformis* (vouchers: Wang et al. 17-56554, Tang & Lui L09, Wang et al. 17-56082, Wang et al. 19-63013, and Wang et al. 17-56085), *L. crystalliniformis* (as *Protoparmeliopsis crystalliniformis'* vouchers: Lee & Kim 2020-000149 and Lee 2020-000098), *L. darlingiae* (voucher: Lendemer 46644), *L. flavidomarginata* (vouchers: Flakus 28943 and Flakus 29951), *L. flavoviridis* (voucher: Papong 6539), *L. fulvastra* (voucher: Flakus 26717), *L. masana* (voucher: JL46185), *L. orientoafricana* (voucher: Kirika 2205 [F]), *L. vainioi* (voucher: Papong 7120), *L. variolascens* (voucher: Maliček 8422), *L. zeorina* (vouchers: Wang et al. 19-62893, Wang et al. 19-63051, and Wang et al. 19-63070). The new species, *L. rhododendri* (MFLU 20-0045), was also included in this clade and is basal to *L. orientoafricana*, *L. alboflavida*, *L. darlingiae*, and *L. flavoviridis*.

All species of *Lecanora subfusca* group *s.str.* in Li et al.[9] also formed a distinct subclade and was basal of *Lecanora s. str.* in the present study, comprising *L. allophana* (vouchers: J. Malíček 9491 [hb. JM] and J. Malíček 9626 [hb. JM]), *L. argentata* (voucher: J. Malíček 1963), *L. campestris* (voucher: Arup U225), *L. cenisia* (voucher: J. Malíček 5869), *L. expersa* (voucher: J. Malíček 9624), *L. gangaleoides* (voucher: HTL19923a), *L. glabrata* (voucher: Lubeck & Kukwa 17811), *L. helva* (voucher: Papong 6444), *L. horiza*, *L. hybocarpa* (voucher: H. T. Lumbsch s. n.), *L. phaeocardia* (voucher: Papong 3492), *L. pulicaris* (vouchers: J. Malíček 10263 [hb. Malíček] and J. Malíček 10262 [hb. Malíček]), *L. rugosella* (voucher: Arup L97561), *L. subimmersa* (voucher: HTL19103b), *L. subrugosa* (voucher: Arup L98188), *L. substerilis* (voucher: Maliček 202), and *L. tropica* (voucher: F: Papong 6440). However, *Lecanora albella* group *s. lat. viz. L. albella* (voucher: Malíček 7336) and *L. caesiorubella* (voucher: F: Lumbsch 19094a) formed a separate lineage, distant from the *Lecanora s. str.* clade and clustered with *Lecidella*, *Lecanora intumescens* (vouchers: J. Malíček 8480 [hb. JM] and TRH L-19015) and *Glaucomaria*.

The genus *Palicella*, namely *P. glaucopa* (vouchers: P. Rodriguez Flakus 2539 (FR) and P. Rodriguez Flakus 2504b (FR)), constituted an independent lineage and was closely related to *Lecanora subfusca* group *s.str.* Other representative genera, namely *Glaucomaria*, *Lecidella*, *Parmotrema*, *Polyozosia*, *Protoparmeliopsis*, and *Pulvinora*, formed well-resolved clades basal to *Lecanora* and *Palicella*, except for *Lecanoropsis* in the present study. Two *Lecanoropsis* species, namely *L. subintricata* (voucher: 604 JP) and *L. saligna* (voucher: 491 DE), formed separate branches, were basal to *Lecanora intricata* (voucher: KRAM: Flakus 29565b), and clustered with the genera *Protoparmeliopsis* and *Polyozosia* with strong support values (100% ML and 94% MP; Fig. 1).

Our new species, *Lecanora rhododendri* (MFLU 20-0045), formed an independent branch basal to *L. orientoafricana*, *L. alboflavida*, *L. darlingiae*, and *L. flavoviridis* with significant support in the ML analysis (93% ML) but low support in the MP analysis (32% MP; Fig. 1). These species also clustered with *L. masana* with 69% support in the ML analysis.

Lecanora rhododendri Chaiwan, sp. nov. (Fig. 2)

Index Fungorum number: IF901032; *Faces of Fungi number*: FoF17546

Etymology: The epithet "rhododendri" refers to the host genus, *Rhododendron*, from which the fungus was collected.

Holotype: MFLU 20-0045

Associated with dead and dried branches of *Rhododendron* sp. *Thallus* crustose, areolate to squamulose, verrucose, thin to thick, whitish; margin distinct; roundish to concave, greenish gray to brownish black. **Photobiont** trebouxioid. **Apothecia** 0.6–2.0 mm in diameter, lecanorine, numerous, rounded, dispersed or aggregated, sessile, strictly constricted at the base, rising singly on the areoles; **disc** black-brown, velvety, in one plane, pruinose; margin inconspicuously merged with the thallus, prominent, thick, smooth, entire to flexuose. **Amphithecial cortex** uniform, gelatinous, inspersed with crystals, hyaline, 45–63 μm thick, with the hyphae growing out

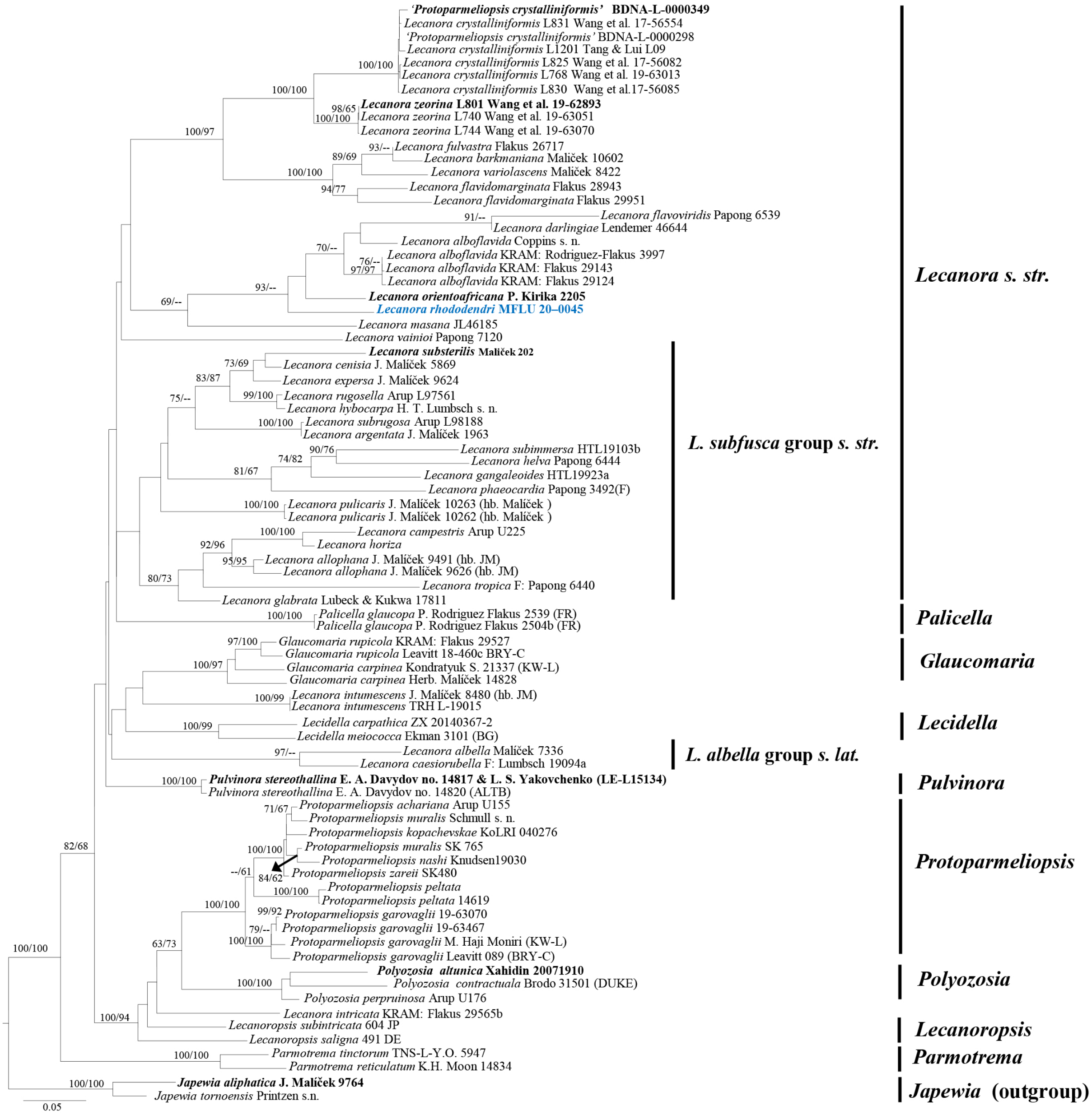

Fig. 1 Phylogram generated from RAxML based on combined ITS and mtSSU sequence data. Bootstrap support values for ML and MP equal to or greater than 60% are indicated as ML/MP at the nodes. The newly generated species in this study is indicated in blue bold text and the ex-type strains are indicated by black bold text. The scale bar represents the expected number of nucleotide substitutions per site.

basally. **Amphithecium** with small to large crystals (= melacarpella-type). **Hypothecium** hyaline, 25–30 µm high. **Parathecium** hyaline, with grayish crystals, 5–7 µm thick. **Hymenium** hyaline, 55–70 µm high, clear, yellowish in KOH. **Epihymenium** brownish, 10–12 µm thick. **Paraphyses** septate, sparingly branched, apically slightly swollen at tips, hyaline. **Asci** 63–85 × 10–14 µm, eight-spored, unitunicate, clavate, Lecanora-type, apically round, with I+ in Lugol's iodine. **Ascospores** 20–30 × 5–10 µm, hyaline, simple, subglobose to obovoid, aseptate. Asexual morph: Undetermined.

Chemistry: Thallus and apothecial margin K+ yellow in 5% KOH and I+ blue in Lugol's reagent.

Material examined: China, Yunnan, Zhaotong City, 24˚21894' N, 100˚29927' E, 2465 m above sea level on the bark of dead and dried branches of *Rhododendron* sp., 6 May 2019, H.L. Li & O. Janson, RD-023 (MFLU 20-0045, holotype); *ibid.*, KUN-HKAS 150900 (isotype).

Notes: In the present study, *Lecanora rhododendri* sp. nov., has a close phylogenetic relationship with *L. orientoafricana* (voucher P. Kirika 2205) and also clustered with *L. alboflavida* (vouchers KRAM: Rodriguez-Flakus 3997, KRAM: Flakus 29143, and KRAM: Flakus 29124) and *L. masana* (voucher JL46185). Morphologically, *L. rhododendri* is typical of *Lecanora* in having a crustose, areolate, whitish thallus with an indeterminate, smooth to irregularly wrinkled margin; sessile, lecanorine apothecia with black-brown, velvety, planar, pruinose discs, with small to large crystals of amphithecium; eight-spored *Lecanora*-type asci; and hyaline, subglobose to obovoid, aseptate ascospores[2,12]. *Lecanora orientoafricana* differs from the new species in having smaller apothecia (0.4–1.4 mm in diameter vs. 0.6–2.0 mm), with light red-brown to brown, matt, planar or concave, sparsely grayish pruinose discs, smaller asci (50–60 × 10–12 vs. 63–85 × 10–14 µm), and smaller ascospores (12.5–15.5 × 6.0–8.5 µm vs. 20–30 × 5–10)[46]. *Lecanora alboflavida* can be distinguished

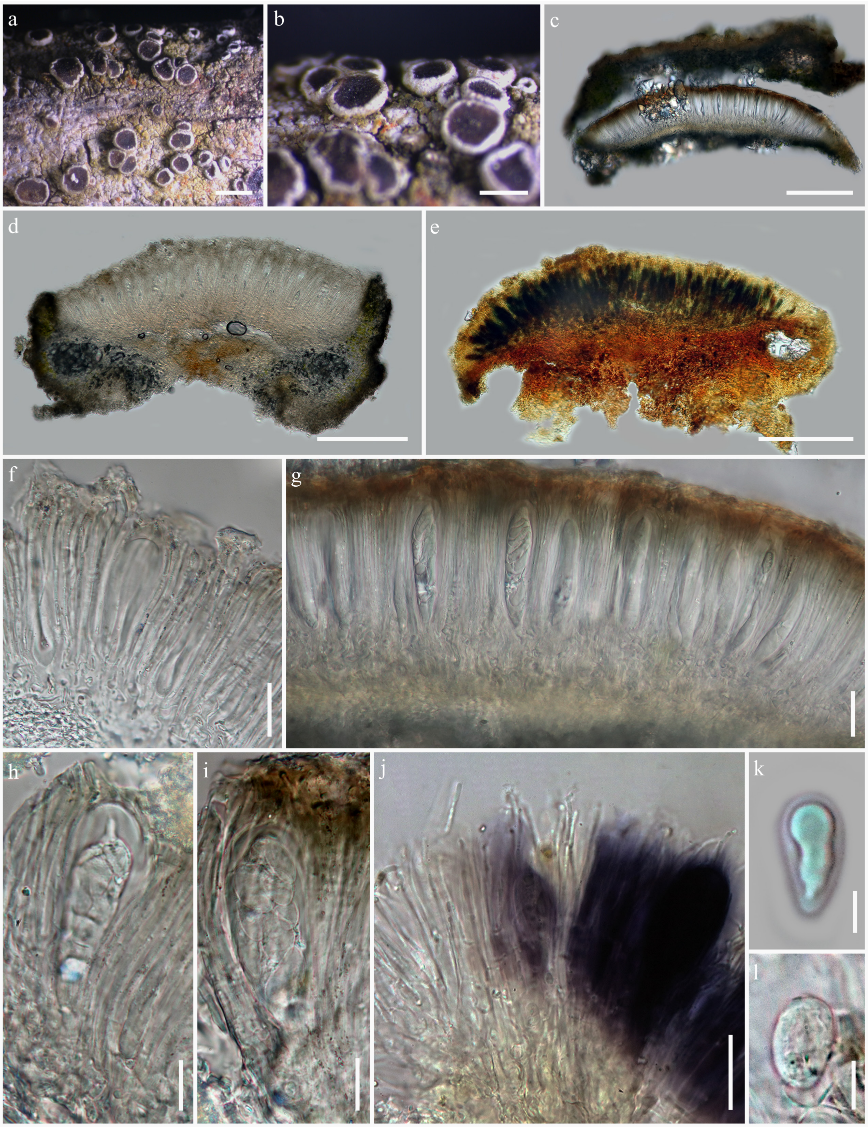

Fig. 2 *Lecanora rhododendri* (MFLU 20-0045, holotype) (a), (b) Appearance of lecanorine apothecia on the host's surface. (c), (d) Section of the apothecia in water. (e) Apothecium stained with 5% KOH. (f) Paraphyses. (g) Close-up of the asci in an apothecium. (h), (i) Asci embedded in the paraphyses. (j) Asci and paraphyses stained with Lugol's reagent. (k), (l) Ascospores. Scale bars: (a) = 1 mm, (b)–(e) = 500 μm, (f)–(j) = 20 μm, (k), (l) = 10 μm.

from the new species in having a thick, smooth to more often postulate, continuous, minutely granular-verrucose or minutely areolate, sorediate, greenish-gray to gray thallus, and rarely producing apothecia, asci and ascospores; if present, immature apothecia are scattered, planar on the surface of the thallus, circular or distorted, with a thick margin, with planar, subpruinose, light brown discs[3,47]. Unfortunately, there are only a few reports of *L. alboflavida*. A comprehensive morphological study was carried out by Laundon[47], who synonymized the species under the genus *Ochrolechia*. *Lecanora masana* has a grayish-white, epruinose thallus without sorediate or isidia; lecanorine, sessile apothecia with yellow to yellow-green discs; an amphithecium without crystals; eigh-spored, clavate, *Lecanora*-type asci; and broadly ellipsoid ascospores[48].

Lecanora orientoafricana was reported growing on bark in a degraded montane forest dominated by *Podocarpus falcatus*, *Rapanea melanophloes,* and *Faurea saligna* at an altitude of 3,240 m in Kenya[46]. *Lecanora alboflavida* is rarely known, with very rare oceanic species, mostly occurring on the acidic bark of old trees from the British Isles, Norway, France, and Macaronesia[3,49]; however, the type specimen (Taylor's saxicolous specimen from 1836) was described on transition rocks[3,47]. *Lecanora masana* is endemic to high elevations in the southern Appalachian Mountains and is narrowly distributed in the southeastern United States[50]. The species was reported in different habitats, including spruce–fir forests, northern hardwood forests, and shrub balds, growing on the bark of *Abies fraseri*, *Betula* spp., *Picea rubens*, and ericaceous shrubs[50]. However, our new species occurred on the bark of dead and dried branches of *Rhododendron* sp. at high altitudes (2,465 m above sea level) in Yunnan, China, where the forests are dominated by *Rhododendron* species.

The ITS nucleotide pairwise comparison revealed that *Lecanora rhododendri* (MFLU 20-0045) differs from *L. orientoafricana* (voucher P. Kirika 2205, ex-type) in 57/550 bp with three gaps (10.36%), *L. alboflavida* (voucher KRAM : Flakus 29124) in 55/515 bp with three gaps (10.68%), *L. alboflavida* (voucher Coppins s. n.) in 65/553 bp with four gaps (11.75%), and *L. masana* (voucher JL46185) in 100/550 bp with seven gaps (18.18%). The mtSSU nucleotide pairwise comparison revealed that the new species differs from

L. orientoafricana (voucher P. Kirika 2205, ex-type) in 22/606 bp with 6 gaps, *L. alboflavida* (voucher KRAM : Flakus 29124) in 91/866 bp with 49 gaps (10.51%), and *L. alboflavida* (voucher Coppins s. n.) in 76/828 bp with 49 gaps (9.19%). On the basis of its morphological distinctiveness, phylogenetic analyses, and the nucleotide-based pairwise comparison, *L. rhododendri* is introduced here as a new species.

Discussion

The discovery of *Lecanora rhododendri* sp. nov. enhances the recognition of cryptic and previously overlooked species diversity within this complex genus. As highlighted in recent studies, traditional morphological classifications have often underestimated the number of distinct lineages because of convergent features such as thalline apothecial margins and simple ascospores[2,32]. The combination of detailed morphological assessment and molecular phylogenetic analysis is therefore essential to accurately delimit species boundaries in *Lecanora*[33,51]. Using the phylogenetic results coupled with the morphological characteristics, the chemical study of KOH and Lugol's reagent test, and DNA polymorphism, we propose a new species in this study, which improves our current understanding of *Lecanora* fungi. The novelty of *L. rhododendri* could be justified on the basis of a combination of diagnostic morphological features, including the thallus, ascospore dimensions, and pigmentation of the apothecial disc. These distinctions are consistent across multiple collections and supported by clear phylogenetic separation from the related species *L. alboflavida*, *L. masana,* and *L. orientoafricana*[2,28,46].

Lecanora rhododendri sp. nov. is characterized by sessile, lecanorine apothecia with planar, pruinose discs; small to large crystals of the amphithecium; hyaline hypothecia lacking chlorinated xanthones; eight-spored, *Lecanora*-type asci; and hyaline, aseptate ascospores. Unfortunately, mature ascospores of *L. rhododendri* are rarely found; only a few immature ascospores could be observed in the present study. Most species within the genus *Lecanora* exhibit an amyloid reaction at the ascal apex, characterized by a distinct blue coloration in Lugol's iodine (I+), particularly in species possessing the *Lecanora*-type ascus. This positive reaction serves as a key diagnostic feature and is valuable for confirming generic or species-level placement. The presence or absence of this reaction can also aid in distinguishing *Lecanora* from morphologically similar taxa that lack an amyloid response. The ascal apex of *L. rhododendri* exhibits an amyloid reaction (I+), showing a positive response when tested with Lugol's iodine. Additionally, the thallus of *Lecanora* species exhibits a KOH reaction, showing a positive response when gelatinous distinctly delimited from the medulla and contains only crystals (granules) which are soluble in KOH but remain gelatinous[52,53]. A preliminary chemical study showed that the thallus and apothecial margin of *L. rhododendri* turned to K+ yellow in 5% KOH and I+ blue in Lugol's reagent. These diagnostic features are in agreement with typical members of *Lecanora s. str.* However, additional morphological and chemical studies are necessary to fully understand the circumscription of species in this group.

The monophyly of *Lecanora rhododendri* placed the species in *Lecanora s. str. Lecanora rhododendri* formed a stable lineage as a sister to *L. orientoafricana* (voucher P. Kirika 2205) and clustered with *L. alboflavida* (vouchers Coppins s. n., KRAM: Rodriguez-Flakus 3997, KRAM: Flakus 29143, and KRAM: Flakus 29124) and *L. masana* (voucher JL46185), as well as *L. darlingiae* (voucher Lendemer 46644) and *L. flavoviridis* (voucher Papong 6539). This phylogenetic result concurred with teh finding of Li et al.[9,54] that these sorediate species (*L. alboflavida*, *L. darlingiae*, and *L. orientoafricana*) and a species with a dark hypothecium (*L. flavoviridis*) always clustered into a monophyletic clade. Notably, *L. alboflavida* (vouchers Coppins s. n.) formed a distinct branch with other collections of *L. alboflavida* and clustered with *L. darlingiae* (voucher Lendemer 46644) and *L. flavoviridis* (voucher Papong 6539) in the present study; the intraspecific variation of *L. alboflavida* (vouchers Coppins s. n.) needs to be clarified, pending further study. Unfortunately, the phylogenetic position of many *Lecanora* species and their allied genera in Lecanoraceae could not be resolved in the present study, concuring with other previous studies that *Lecanora* is highly polyphyletic and its phylogeny at higher taxonomic levels remained unresolved[2,3,55,56]. This unstable phylogenetic position might have occurred because of the phylogenetic markers (ITS and mtSSU) and the numbers of available taxa on the tree. Members of the *Lecanora* group also strongly demand future taxonomic changes based on multilocus genetic data (e.g., ITS, mtSSU, *rpb1*, and *rpb2*).

Lecanora species can thrive in extreme conditions, such as higher altitudes, dry areas, and low-nutrient environments. Their ability to survive in these areas allows them to occupy ecological niches that would otherwise be unavailable to other organisms[28,52,53]. In terms of its ecology and distribution, the new species, *L. rhododendri*, appears to have a restricted habitat preference, being confined to a high elevation (2,465 m above sea level) on corticolous *Rhododendron* sp. in Yunnan, China. This niche specificity may contribute to its previous misidentification or exclusion from broader surveys, particularly those focused on more common lowland taxa. The biogeographical pattern observed here reflects similar trends reported for narrowly endemic lichens in mountainous or isolated environments[57,58]. Approximately, about 119 *Lecanora* species have been reported from China thus far[9,54,59–70], distributed across low to high elevation gradients (altitudes of 500–4,500 m). Among them, 25 species were classified within *Lecanora s. str.* However, these species lack the molecular data required for a comprehensive systematic revision to clarify their placement in *Lecanora*[9].

In this study, *Lecanora rhododendri* is introduced. It was found at an altitude of 2,465 m in forests dominated by *Rhododendron* sp. in Yunnan, China. We propose the recognition of this taxon as a new species within *Lecanora*, based on phylogenetic evidence, its morphological characteristics, and DNA polymorphism. Its discovery underscores the importance of continued taxonomic exploration in understudied habitats and highlights the role of integrative approaches in revealing hidden fungal diversity.

Author contributions

The authors confirm their contributions to the paper as follows: conceptualization, data curation, and formal analysis: Chaiwan N, Phookamsak R, Jiang H, Thiyagaraja V; funding acquisition: Promputtha I, Phookamsak R; investigation, methodology, and writing – original draft: Chaiwan N, Phookamsak R, Jiang H, Thiyagaraja V; project administration: Chaiwan N, Phookamsak R; supervision: Promputtha I, Phookamsak R; writing – review and editing: Phookamsak R, Jiang H, Promputtha I, Bhat J, Dawoud TM. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article. Sequences generated in this study were submitted to GenBank.

Acknowledgements

Napalai Chaiwan would like to thank the Chiang Mai University (CMU) Proactive Researcher grant of Chiang Mai University (Grant No. EX010059) and Chiang Mai University. The authors thank Li Huili and Owen Janson for their help with collecting the specimens. We also thank Shaun Pennycook from Manaaki Whenua - Landcare Research for his assistance in naming the new species. Prof. David L Hawksworth from Plant and Fungal Comparative Biology, Royal Botanic Gardens, Kew is thanked for his assistance in finding literature in Lecanora masana; Hongbo Jiang appreciates the Postdoctoral Directional Training Foundation of Yunnan Province (Grant No. E33O38E261) under Yunnan Provincial Department of Human Resources and Social Security, Yunnan, China, and the 1st batch of the national (Chinese) postdoctoral overseas attraction program in 2023 from Ministry of Education of China (MOE). Rungtiwa Phookamsak sincerely acknowledges the Talent Introduction Plan of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan Revitalization Talent Support Program 'Young Talent' Project (Grant No. YNWR-QNBJ-2020-120), Yunnan Revitalization Talent Support Program: High-end Foreign Expert Project (Grant No. XDYC-GDWZ-2024-0016), Independent Research of Department of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences (Grant No. Y537731261), Yunnan Provincial Department of Human Resources and Social Security, Yunnan Province Foreign Expert Project (Grant No. 202505AO120002) and Yunnan Intelligence Union Program for Young Scientists, Yunnan, China (Grant No. 202503AM140005). Jayarama D. Bhat and Turki M. Dawoud gratefully acknowledge the financial support under the Distinguished Scientist Fellowship Programme (DSFP), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 8 September 2025; Revised 8 January 2026; Accepted 19 January 2026; Published online 13 March 2026

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