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# Phylogenetic placement of *Phlyctis atomella* (*Phlyctidaceae*) from the Western Ghats, India

PARAYELIL A. ANSIL[1,2], KUNHIRAMAN C. RAJESHKUMAR[1,2]*, ROBERT LÜCKING[3], BHARATI SHARMA[1]

[1] Biodiversity and Palaeobiology (Fungi and Lichens) Gr., MACS Agharkar Research Institute, G.G. Agarkar Road, Pune, IN-411 004, Maharashtra, India
[2] Faculty of Science, Savitribai Phule Pune University, Pune, IN-411 005, Maharashtra, India
[3] Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, DE-14195 Berlin, Germany
*corresponding author: rajeshfungi@gmail.com

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This study examined the phylogenetic status of *Phlyctis atomella*, known from the Western Ghats, based on an integrative taxonomic approach which included morphology, anatomy, chemistry, and molecular phylogeny. Despite the existence of 26 documented species in *Phlyctis* worldwide, molecular sequence data is presently accessible solely for five of these species. Analysis based on concatenated ITS and mtSSU data suggests the placement of *P. atomella* within *Phlyctis* either as an early diverging lineage or a delineated poorly supported sister to *P. boliviensis*. This is the first molecular phylogenetic study of the crustose lichen genus *Phlyctis* based on fresh collections from India.

**Key words:** integrative taxonomy, *Phlyctis agelaea*, *Phlyctis argena*, *Phlyctis petraea*, *Phlyctis speirea*.

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Ansil P.A., Rajeshkumar K.C., Lücking R., Sharma B. (2023): Fylogenetické postavení *Phlyctis atomella* (*Phlyctidaceae*) ze Západního Ghátu v Indii. – Czech Mycol. 75(2): 139–152.

Studie zkoumá fylogenetický status druhu *Phlyctis atomella*, známého ze Západního Ghátu, na základě integrativního taxonomického přístupu, který zahrnuje morfologii, anatomii, chemii a molekulární fylogenetiku. I když ze světa je doloženo 26 druhů rodu *Phlyctis*, sekvenční data jsou v současnosti dostupná jen pro pět z těchto druhů. Analýza založená na spojených sekvencích ITS a mtSSU naznačuje postavení *P. atomella* v rodu *Phlyctis*, kde buď představuje raně odštěpenou vývojovou linii, nebo zaujímá (byť s nízkou podporou) sesterskou pozici k *P. boliviensis*. Jde o první molekulárně fylogenetickou studii korovitých lišejníků z rodu *Phlyctis*, založenou na čerstvých sběrech z Indie.

## INTRODUCTION

*Phlyctis* (Wallr.) Flot. (Flotow 1850) is a cosmopolitan genus of lichens distributed across tropical to temperate regions. The genus is characterised by a crustose, smooth to verrucose thallus with a protococcoid algal photobiont, apothecioid ascomata which are sunken or erumpent, with or without a proper exciple, unbranched or apically furcate paraphyses, 1–8-spored asci, and colourless, thin-walled, non-halonate, fusiform to ellipsoid, transversely septate to muriform septate ascospores. The presence of lichen acids such as stictic, constictic, norstictic, connorstictic, salazinic, psoromic, neopsoromic, and protocetraric acids mark the thallus chemistry (Galloway et Guzmán 1988).

The genus is currently represented by ca 26 species worldwide (Kirk et al. 2008, Benfield et al. 2009, Ma et al. 2010, Lumbsch et al. 2011, Joshi et al. 2012, Weerakoon et al. 2016, McCarthy et Elix 2017, Muscavitch et al. 2017, Poengsungnoen et al. 2019). From India, eight species are known, namely *Phlyctis atomella* (Stirt.) S. Joseph et al. (Joseph et al. 2020), *P. communis* Chitale et Makhija (Chitale et Makhija 2012), *P. karnatakana* S. Joshi et Upreti (Joshi et al. 2010), *P. monosperma* S. Joshi et Upreti (Joshi et al. 2012), *P. nepalensis* Räsänen (Räsänen 1952), *P. polyphora* Stirt. (Stirton 1881), *P. subagelaea* S. Joshi et Upreti (Joshi et al. 2010), and *P. subhimalayensis* S. Joshi et Upreti (Joshi et al. 2012). Of these, *P. communis*, *P. karnatakana* and *P. subagelaea* are reported from evergreen and semi-evergreen forests of the Western Ghats.

Joseph et al. (2020) proposed a new combination, *Phlyctis atomella* (Stirt.) S. Joseph, G.P. Sinha et S. Nayaka, based on *Platygrapha atomella* Stirt. (Stirton 1879) [syn.: *Schismatomma atomellum* (Stirt.) Zahlbr. (Zahlbruckner 1923)], with several taxonomic synonyms. As the Western Ghats of India is considered to be a lichen-rich tropical area, this study is a pioneer attempt to assess the phylogenetic placement in the genus in a thorough morphological and molecular study of *P. atomella*.

## MATERIAL AND METHODS

Sample collection. Surveys were conducted in the Mahabaleshwar (17°55'34" N, 73°39'34" E), Panchgani (17°55'56" N, 73°48'23" E) and Thoseghar areas (17°36'09" N, 73°51'19" E) in the Satara District, in Maharashtra during 2021–2022. Minimalistic sampling approaches were followed to preserve the in-situ diversity of the lichens in the habitats. Fresh thalli were collected by scrapping using a knife and transported in paper bags to the laboratory. The samples were allowed to air dry and were stored in brown-paper packs for further morpho-chemical studies. For molecular studies, fresh thalli were kept at 4 °C in the lab to avoid cross-contamination from fast-growing saprotrophic fungi.

Morphology and chemical analyses. Thallus morphology was studied using a binocular stereomicroscope (Olympus SZX16 with Digi-CAM, Tokyo, Japan). Sections through ascomata were

made using a razor blade and mounted in lactic acid (with gentle heating over a flame), 10% KOH, water, and Lugol's iodine separately for microscopy. Microscopic observations were noted using Carl Zeiss Axio imager A2 (Zeiss, Jena, Germany). Key morphological characteristics were evaluated for species-level identification using various taxonomic references (Awasthi 2000, Kirk et al. 2008, Benfield et al. 2009, Joshi et al. 2010, Ma et al. 2010, Lumbsch et al. 2011, Chitale et Makhija 2012, Joshi et Upreti 2013, Weerakoon et al. 2016, McCarthy et Elix 2017, Muscavitch et al. 2017, Poengsungnoen et al. 2019). Chemical profiles were studied by means of thin-layer chromatography (TLC) following standard protocols (Orange et al. 2001) with toluene-dioxane-acetic acid (TDA, 180:45:5) and toluene-ethyl acetate-formic acid (TEF, 139:83:8) as solvent systems. The collected specimens are deposited in the Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute, Pune, India.

DNA isolation, polymerase chain reaction and sequencing. The DNA was isolated, PCR was performed using the Sigma REDExtract-N-Amp™ Seed PCR Kit, following the manufacturer's instructions in a thermocycler ProFlexTM PCR system (Applied Biosystems, Foster City, USA). Primers for amplification were ITS5 & ITS4 for ITS (White et al. 1990) and mrSSU1 & mrSSU3R for mtSSU (Zoller et al. 1999). Thermal cycling parameters used for amplification were: initial denaturation at 95 °C for 5 min, and 30 cycles of 94 °C for 1 min, 35 cycles at 52 °C for 30 s (ITS), 35 cycles at 50 °C for 1 min (mtSSU) and a final extension at 72 °C for 10 min. The PCR products were purified with the FavorPrep PCR Purification Kit (Favorgen Biotechcorp, Ping-Tung, Taiwan) and sequenced with the same primers using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). The sequencing reactions were run on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA).

Phylogenetic analyses. The newly generated sequences were subjected to Megablast searches in the NCBI GenBank nucleotide sequence database to identify the closest matching sequences. The phylogeny of *Phlyctis* was assessed following recent studies in the genus (Muscavitch et al. 2017), and sequences of the ITS and mtSSU gene regions, available for this particular genus, were retrieved from GenBank (Tab. 1). The individual datasets (ITS, mtSSU) were aligned and manually edited in MEGA v. 11.0.11 (Tamura et al. 2021) using MUSCLE. The selected outgroup was *Gyalecta jenensis*.

The phylogeny tool AliView v. 1.28 (Larsson 2014) was used to convert the FASTA alignment file into PHYLIP format for RAxML analyses. The markers (single gene) were first analysed separately, and a concatenated dataset was produced with ITS + mtSSU sequences. The phylogenetic analyses were conducted using the maximum likelihood (ML) and Bayesian analysis (PP) methods. Based on the J-Model test, the best fit model of nucleotide substitution GTRGAMMA+I was performed. Phylogeny was inferred using RAxML v. 8.1.11 (Stamatakis 2006, Stamatakis et al. 2008) evaluating nodal support using 1000 bootstrap (BS) pseudo-replicates. The Bayesian posterior probability analysis of the individual and concatenated ITS & mtSSU dataset were performed using MrBayes v. 3.2.7a (Ronquist et al. 2012) specifying GTRGAMMA+I as the best fitting model and allowing unlinked parameter estimation and independent rate variation. Posterior probabilities (PP) were estimated by sampling trees using a variant of the Markov Chain Monte Carlo (MCMC) method. Phylogenetic trees were sampled every 1000$^{th}$ generation (resulting in 4000 total trees) in 4,000,000 generations by running six simultaneous Markov chains. The first 1000 trees containing the burn-in phase of the analyses were discarded. The remaining 3000 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. Based on the likelihood profile, the first 25% of trees were discarded as burn-in. Only clades BS ≥ 50% under ML and PP ≥ 0.95 in the Bayesian framework were supported. Phylogenetic trees were visualised using the programme FigTree 1.4.0 (Rambaut 2014). Trees were edited using Microsoft PowerPoint. DNA sequences newly generated in this study were deposited in GenBank.

**Tab. 1.** *Phlyctis* species with GenBank accession numbers, voucher and isolate information for the sequences used in this study. Newly generated sequences are given in bold.

| Species | Specimen voucher | Isolate | Country | ITS | mtSSU |
|---|---|---|---|---|---|
| *Phlyctis agelaea* | W. Buck 47150 (NY) | NY1584 | Canada | – | MF625031 |
| *Phlyctis agelaea* | Nordin 3028 (UPS) | – | Sweden | – | AY853332 |
| *Phlyctis agelaea* | P. Lohmus s.n. 14 Sep 2006 (NY) | NY1622 | Estonia | – | MF625032 |
| *Phlyctis agelaea* | – | AFTOL-ID 1670 | – | – | HQ659176 |
| *Phlyctis argena* | Struwe 3001 (NY) | NY2991 | Sweden | MF625063 | – |
| *Phlyctis argena* | – | EDNA09-01540 | UK | FR799264 | – |
| *Phlyctis argena* | – | EDNA09-01542 | UK | FR799265 | – |
| *Phlyctis argena* | – | EDNA09-01556 | UK | FR799266 | – |
| *Phlyctis argena* | C. Lewis 1055 (NY) | NY1606 | Canada | MF625061 | – |
| *Phlyctis argena* | Anderson 162293 (QFA) | NY2930 | Canada | MF625062 | MF625042 |
| *Phlyctis argena* | Spribille 36464 (GZU) | P188 | USA | KR017093 | KR017349 |
| *Phlyctis argena* | J. Lendemer 19710 (NY) | NY1580 | USA | MF625060 | MF625033 |
| *Phlyctis argena* | J. Lendemer 27770 (NY) | NY1620 | Canada | – | MF625040 |
| *Phlyctis argena* | W. Buck 54052 (NY) | NY1602 | USA | – | MF625036 |
| *Phlyctis argena* | J. Lendemer 19633 (NY) | NY1621 | USA | – | MF625041 |
| *Phlyctis argena* | J. Lendemer 27446 (NY) | NY1617 | USA | – | MF625039 |
| *Phlyctis argena* | J. Lendemer 27185 (NY) | NY1609 | USA | – | MF625038 |
| *Phlyctis argena* | R. Hill 1632 (NY) | NY1583 | Norway | – | MF625035 |
| *Phlyctis argena* | – | AFTOL-ID 1375 | – | – | DQ986880 |
| ***Phlyctis atomella*** | **AMH 22.18** | **CRG668RALM18** | **India** | **OP244897** | **OP244893** |
| ***Phlyctis atomella*** | **AMH 22.41** | **CRG668RAMB16** | **India** | **OP244898** | **OP244894** |
| ***Phlyctis atomella*** | **AMH 21.45** | **CRG668RAT003** | **India** | **OP244899** | **OP244895** |
| *Phlyctis boliviensis* | R. Harris 58519 (NY) | NY1615 | USA | MF625065 | MF625044 |
| *Phlyctis boliviensis* | J. Lendemer 21697 (NY) | NY1577 | USA | MF625064 | MF625045 |
| *Phlyctis boliviensis* | E. Tripp 602 (NY) | NY1579 | USA | – | MF625047 |
| *Phlyctis boliviensis* | J. Lendemer 20107 (NY) | NY1578 | USA | – | MF625046 |
| *Phlyctis boliviensis* | Khitsun 502215-8 (NY) | NY3810 | USA | – | MF625048 |
| *Phlyctis petraea* | Z. Muscavitch 83 (NY) | NY4139 | USA | MF625078 | – |
| *Phlyctis petraea* | R. Harris 61265 (NY) | NY4054 | USA | MF625075 | MF625052 |
| *Phlyctis petraea* | Z. Muscavitch 20 (NY) | NY4138 | USA | MF625077 | – |
| *Phlyctis petraea* | Z. Muscavitch 84-B (S) (NY) | NY4143 | USA | MF625081 | MF625054 |
| *Phlyctis petraea* | Z. Muscavitch 84-B (N) (NY) | NY4144 | USA | MF625082 | MF625055 |
| *Phlyctis petraea* | Z. Muscavitch 84-A (NY) | NY4142 | USA | MF625080 | MF625053 |
| *Phlyctis petraea* | J. Lendemer 32260 (NY) | NY1605 | USA | MF625069 | – |
| *Phlyctis petraea* | B. Streets 5014 (NY) | NY2485 | USA | MF625072 | – |
| *Phlyctis petraea* | Z. Muscavitch 84 (NY) | NY4140 | USA | MF625079 | – |
| *Phlyctis petraea* | J. Lendemer 45857 (NY) | NY3361 | USA | MF625073 | MF625051 |
| *Phlyctis petraea* | W. Buck 63538 (NY) | NY4055 | USA | MF625076 | – |
| *Phlyctis petraea* | Shaulis s.n. 28 Apr 2012 (NY) | NY1393 | USA | MF625067 | MF625049 |
| *Phlyctis petraea* | MNRJ86239 | – | Brazil | KF625069 | – |

| Species | Specimen voucher | Isolate | Country | ITS | mtSSU |
|---|---|---|---|---|---|
| *Phlyctis petraea* | J. Lendemer 48158 (NY) | NY4224 | USA | MF625083 | MF62056 |
| *Phlyctis petraea* | R. Harris 55969 (NY) | NY1608 | USA | MF625070 | – |
| *Phlyctis petraea* | J. Lendemer 26246 (NY) | NY1601 | USA | MF625068 | – |
| *Phlyctis petraea* | J. Lendemer 25846 (NY) | NY1603 | USA | – | MF625050 |
| *Phlyctis petraea* | B. Streets 5417 (NY) | NY3367 | USA | MF625074 | – |
| *Phlyctis petraea* | E. Byers 1751 (NY) | NY2479 | USA | MF625071 | – |
| *Phlyctis speirea* | J. Lendemer 45082 (NY) | NY3285 | USA | MF625084 | MF625059 |
| *Phlyctis speirea* | J. Lendemer 44931 (NY) | NY3282 | USA | – | MF625058 |
| *Phlyctis speirea* | J. Lendemer 18853 (NY) | NY1581 | USA | – | MF625057 |
| *Gyalecta jenensis* | – | AFTOL-ID 361 | – | HQ650712 | AY584705 |

## RESULTS

### PHYLOGENETIC RESULTS

Based on a Megablast search in the NCBI GenBank nucleotide database, the closest matches of *Phlyctis atomella* (AMH 21.45, AMH 22.18, AMH 22.41) using ITS were *P. argena* voucher BG-L-98987 from Norway [GenBank MK811759; identities = 465/566 (82%), gaps = 38/566 (6%)], *P. argena* voucher BG-L-99149 from Norway [GenBank MK811753; identities = 465/566 (82%), gaps = 38/566 (6%)], and *P. petraea* isolate NY2485 from USA [GenBank MF625072; identities = 474/578 (82%), gaps = 45/578 (7%)]. Closest matches using the mtSSU were *P. petraea* isolate NY4054 from USA [MF625052; identities = 681/750 (91%), gaps = 25/750 (3%)], *P. petraea* isolate NY4142 from USA [MF625053; identities = 681/751 (91%), gaps = 27/751 (3%)], and *P. petraea* isolate NY4144 from USA [MF625055; identities = 680/750 (91%), gaps = 27/750 (3%)].

For ITS, the analysed dataset comprised a total of 495 positions. The matrix had 174 distinct alignment patterns, with 5.88% undetermined characters or gaps. Estimated base frequencies were: A = 0.234909, C = 0.253514, G = 0.230775, T = 0.280802; substitution rates AC = 0.705948, AG = 3.439052, AT = 2.840009, CG = 0.506711, CT = 6.462049, GT = 1.000000; gamma distribution shape parameter $\alpha$ = 0.580491. The best-scoring RAxML tree had a final likelihood value of –2001.831679. Maximum likelihood and Bayesian analyses resulted in similar topologies, therefore only the ML tree is presented. *Phlyctis atomella* formed an early diverging clade sister to the clade, including *P. argena*, *P. petraea* and *P. boliviensis* (Fig. 1).

For mtSSU, the analysed dataset comprised 713 positions. The best-scoring RAxML tree had a final likelihood value of –1852.965120. The matrix had 139 distinct alignment patterns, with 4.62% undetermined characters or gaps. Estimated

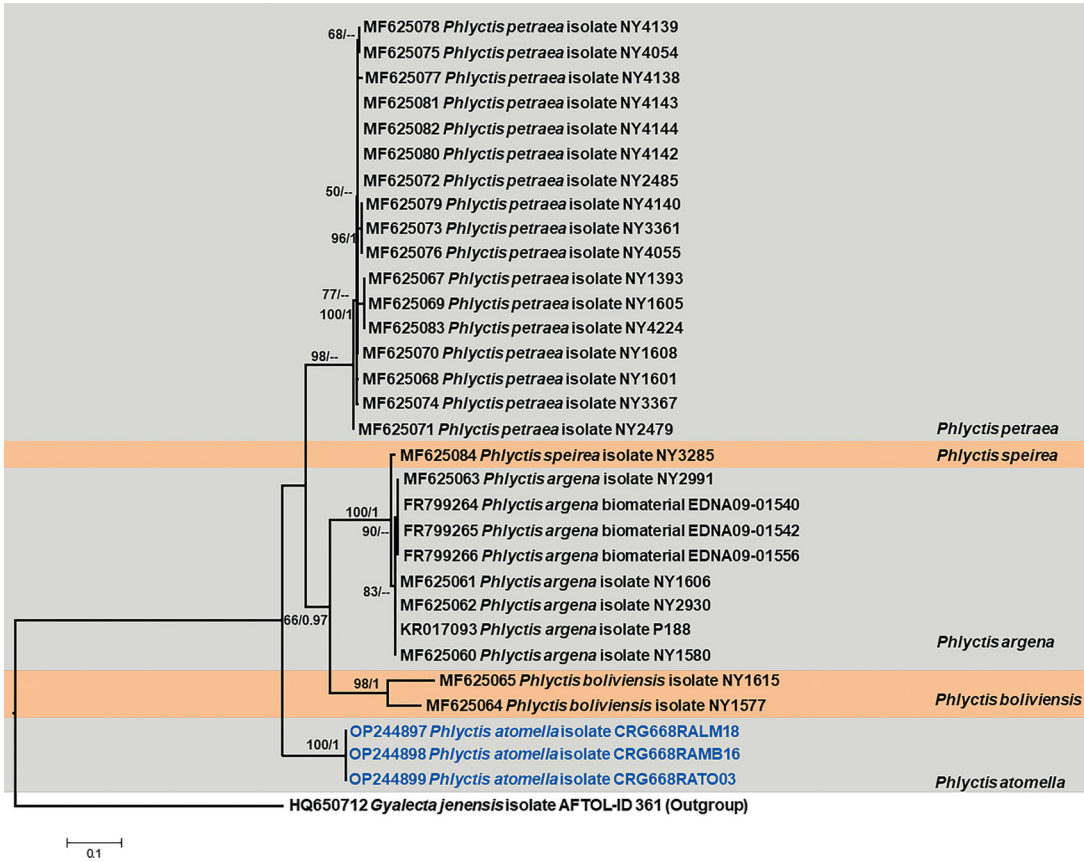


**Fig. 1.** Phylogram generated from RAxML analyses based on analyses of ITS sequence data for the genus *Phlyctis*. Bootstrap support values for ML ≥ 50% are given above the nodes, and PP ≥ 0.95 are presented. The tree is rooted with *Gyalecta jenensis* (HQ650712). The sequences generated for *Phlyctis atomella* in this study are shown in blue.

base frequencies were: A = 0.358936, C = 0.136777, G = 0.197139, T = 0.307147; substitution rates AC = 0.668027, AG = 5.269140, AT = 1.791248, CG = 0.515911, CT = 3.838832, GT = 1.000000; gamma distribution shape parameter α = 0.767221. Maximum likelihood and Bayesian analyses resulted in similar topologies, therefore only the ML tree is presented here. *Phlyctis atomella* formed a well-supported clade sister to a clade including *P. argena*, *P. agelaea*, *P. speirea*, and *P. petraea*, while *P. boliviensis* was recovered as an early diverging lineage sister to the clade containing all other species (Fig. 2).

While all species were supported (except *P. speirea* nested within *P. argena* in both datasets), the phylogenetic analysis of individual ITS and mtSSU data resulted in slightly dissimilar topologies regarding the backbone. Nevertheless, we

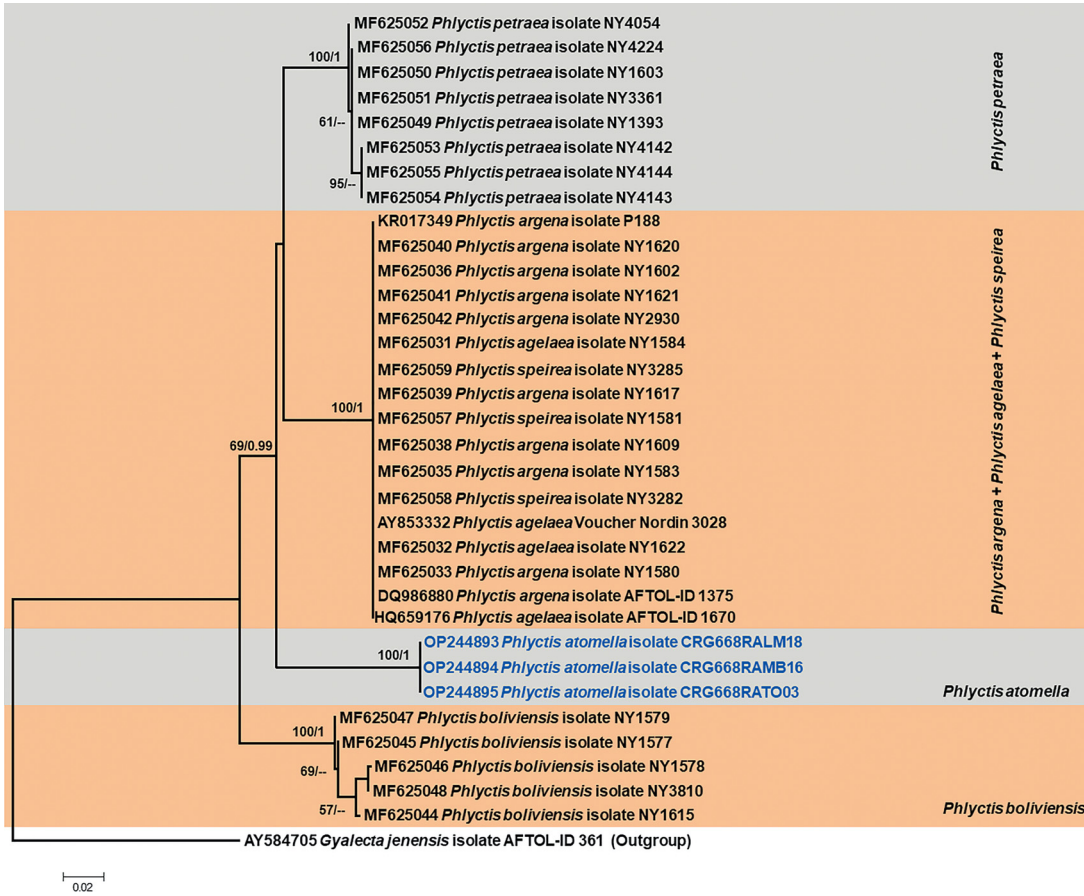


**Fig. 2.** Phylogram generated from RAxML analyses based on analyses of mtSSU sequence data for the genus *Phlyctis*. Bootstrap support values for ML ≥ 50% are given above the nodes, and PP ≥ 0.95 are presented. The tree is rooted with *Gyalecta jenensis* (AY584705). The sequences generated for *Phlyctis atomella* in this study are shown in blue.

analysed the data to test the resulting topology. The datasets under analysis included mtSSU (713 bp) and ITS (495 bp), respectively. The best-scoring RAxML tree had a final likelihood value of –3836.916844. The matrix had 258 distinct alignment patterns, with 5.88% undetermined characters or gaps. Estimated base frequencies were: A = 0.308065, C = 0.181828, G = 0.210809, T = 0.299299; substitution rates AC = 0.761870, AG = 3.903021, AT = 2.121943, CG = 0.619404, CT = 5.466822, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.647842$. Maximum likelihood and Bayesian analyses again resulted in similar topologies. Here, *Phlyctis atomella* formed a well-supported monophyletic clade sister to *P. boliviensis*, both sister to another clade including *P. argena/speirea* and

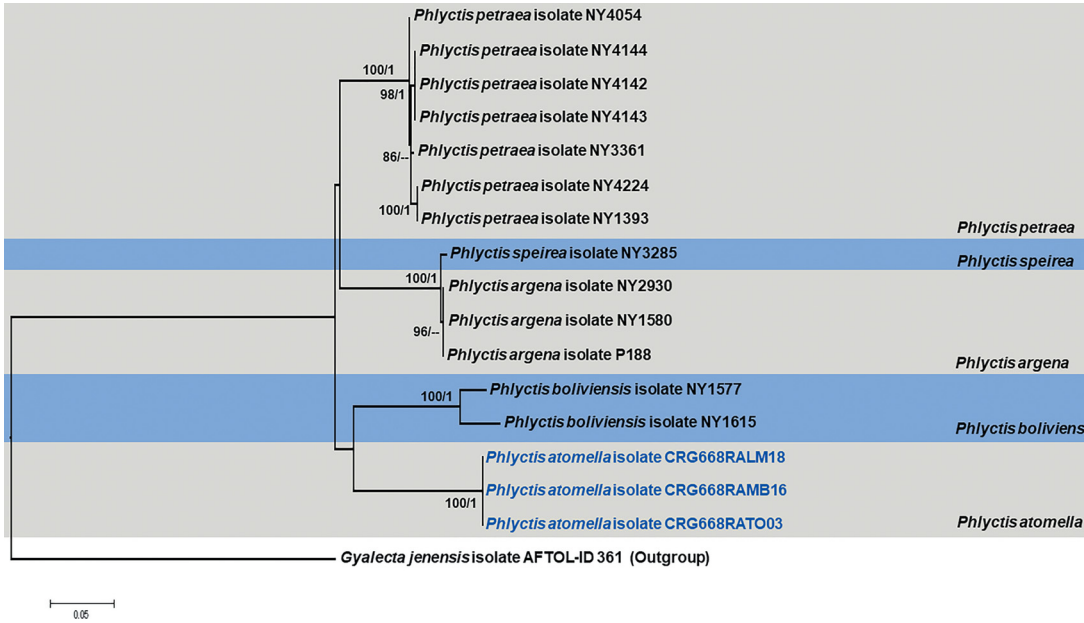


**Fig. 3.** Phylogram generated from RAxML analyses based on analyses of combined ITS and mtSSU sequence data for the genus *Phlyctis*. Bootstrap support values for ML ≥ 50% are given above the nodes, and PP ≥ 0.95 are presented. The tree is rooted with *Gyalecta jenensis* (HQ650712, AY584705). The sequences generated for *Phlyctis atomella* in this study are shown in blue.

*P. petraea*. However, the backbone was not supported, reflecting the conflict found between the ITS and mtSSU trees (Fig. 3).

## TAXONOMY

***Phlyctis atomella*** (Stirt.) S. Joseph, G.P. Sinha et S. Nayaka, Lichenologist 52(4): 330, 2020 Fig. 4

≡ *Platygrapha atomella* Stirt., Proc. Roy. phil. Soc. Glasgow 11: 317, 1879 [1878] – *Schismatomma atomellum* (Stirt.) Zahlbr., Cat. Lich. Univers. 2: 553, 1923 [1924]

= *Graphidastra himalayana* Jagadeesh et G.P. Sinha, Geophytology 39(1–2): 83, 2010

= *Platygrapha cinerea* Müll. Arg., J. Linn. Soc., Bot. 29: 224, 1892 – *Schismatomma cinereum* (Müll. Arg.) Zahlbr., Cat. Lich. Univers. 2: 555, 1923 [1924]

= *Platygrapha gregantula* Müll. Arg., J. Linn. Soc., Bot. 29: 223, 1892 – *Schismatomma gregantulum* (Müll. Arg.) Zahlbr., Cat. Lich. Univers. 2: 558, 1923 [1924]

= *Phlyctella himalayensis* Nyl., Lich. Nov. Zeland. (Paris): 73, 1888 – *Phlyctis himalayensis* (Nyl.) D.D. Awasthi, Lichenology in Indian Subcontinent, A Supplement to A Hand Book of Lichens (Dehra Dun): 15, 2000

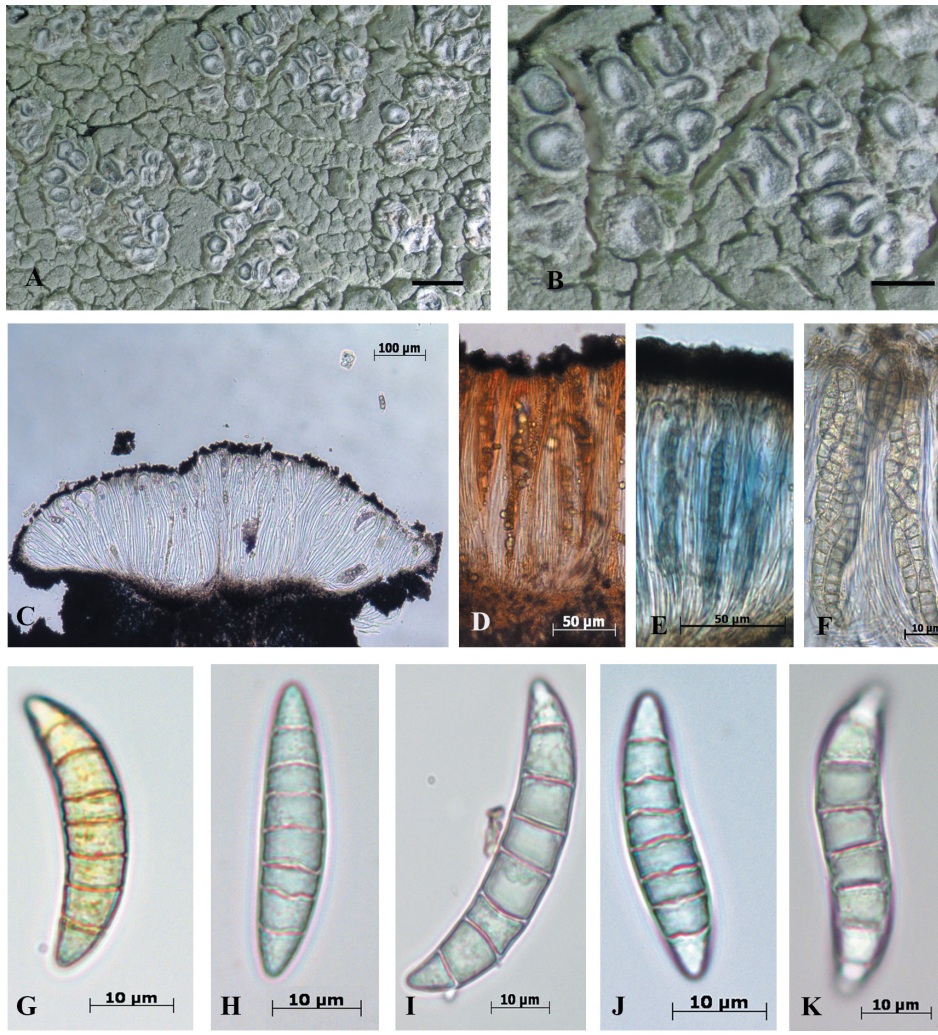


**Fig. 4.** *Phlyctis atomella* (AMH 22.18): **A** – thallus, **B** – apothecia, **C** – section of apothecium showing hymenium, **D** – I+ orange-red hymenium, **E** – KI+ blue hymenium, **F** – asci containing 8 ascospores, **G** – I– ascospore, **H–K** – ascospores. Scale bars: A = 1 mm, B = 500 µm, C = 100 µm, D–E = 50 µm, F–K = 10 µm. Photos by P.A. Ansil.

Description of fresh material. Thallus crustose, corticolous, moderately thick, pale yellowish, greyish, whitish, greyish green, uneven to subleprose, ecorticate, matt, irregular, cracked; photobiont a green protococcoid alga. Apothecia numerous, single to aggregated (in a group of 3–7), round to irregular, granular, erumpent, 0.3–1 mm in diam.; disc greyish black, concave when dry, plane when hydrated, densely pruinose to epruinose, 0.2–0.8 mm diam. Epithecium

densely granular, brown, opaque, 14–26 µm thick. Hymenium hyaline, clear, 50–230 µm high, I– to I+ orange-red, KI+ initially hyaline slowly becoming pale blue. Hypothecium brownish yellow to brown, 40–60 µm high. Paraphyses slender, becoming branched and anastomosing towards apex, 1–2 µm thick. Asci 8-spored, cylindrical-clavate, thin-walled, 60–120 × 13–20 µm. Ascospores hyaline, transversely 7-septate, fusiform, falcate to sigmoid, I–, 30–65(78) × 4–11 µm, without perispore.

C h e m i s t r y. Thallus K+ yellow, quickly turning red, containing norstictic acid.

**Specimens examined**

I n d i a. Maharashtra: Satara District, Panchgani, elev. 1279 m, 17°55'56" N, 73°48'23" E, 15 February 2022, P.A. Ansil et K.C. Rajeshkumar (AMH 22.18). – Thosaeghar, elev. 1049 m, 17°36'09" N, 73°51'19" E, 30 September 2022, P.A. Ansil et K.C. Rajeshkumar (AMH 21.45). – Mahabaleshwar, elev. 1373 m, 17°55'34" N, 73°39'34" E, 15 February 2022, P.A. Ansil et K.C. Rajeshkumar (AMH 22.41).

## DISCUSSION

### Dissimilar topologies in phylogenetic analyses

Despite the existence of 26 documented species in *Phlyctis* worldwide, molecular sequence data is presently accessible solely for five of these species (this study includes data of a sixth species). In the given ITS phylogeny, the six different species of *Phlyctis* are comparatively well resolved. However, mtSSU phylogeny could not resolve *P. argena*, *P. agelaea*, and *P. speirea* clades, which is a major difference we have noted in the topology. The same unresolved topology can be seen in the mtSSU analysis in Muscavitch et al. (2017) studying North American *Phlyctis*.

ITS sequences generally have a good resolution at the species-level in phylogenetic analyses of fungi (including lichenised fungi), but they may not provide sufficient resolution for higher taxonomic levels. Contrarily, mitochondrial genes, including mtSSU, can provide a higher resolution at higher taxonomic levels compared to ITS, making them suitable for resolving relationships between fungal orders, classes, and even phyla. The mtSSU gene may not provide the same level of resolution at the species level as ITS. However, we used a combination of ITS and mtSSU to achieve higher resolution and robust phylogeny.

### Morphological variability

Based on specimens examined in this study, *Phlyctis atomella* is characterised by a whitish to greyish green, ecorticate, K+ yellow turning red thallus, fusiform, straight to slightly curved to sigmoid, I–, hyaline, transversely 7-septate ascospores, 30–65(78) × 4–11 µm in size, and norstictic acid.

*Phlyctis atomella* was proposed as a new combination by Joseph et al. (2020) for *Platygrapha atomella* by synonymising *Graphidastra himalayana*, *Platygrapha cinerea*, *Platygrapha gregantula*, and *Phlyctella himalayensis*. The fresh material collected in this study shows a slight yellowish tinge in the thallus colour, which is not an adequate character to propose a new species, as the major colour of the thallus in *P. atomella* is greyish or whitish. Also, the thallus colour in the earlier amended protologue of *Graphidastra himalayana* was mentioned to be whitish to whitish grey (Jagadeesh Ram et Sinha 2010). Moreover, *P. atomella* has a wide ascospore size range where the samples from the present study fit in. Although the freshly studied samples have slightly broader ascospores, this is not considered to be a key distinguishing characteristic to propose a new species. Even though the sample shows slightly larger ascocarps, their shape and pruinose disc is identical to that of *P. atomella*. The thallus has also shown to have the same chemistry. Hence, we consider the fresh material studied to be *P. atomella*, and the newly generated sequence data is assigned to this species.

**Similar species and distinguishing characters**

*Phlyctis atomella* resembles several other species morphologically, chemically, and/or in ascospore type.

*Phlyctis karnatakana* S. Joshi et Upreti, described from India (Joshi et al. 2010, Joshi et Upreti 2013), with the same chemistry and with 7-septate ascospores, has an uneven-verrucose thallus, a KI– hymenium and much smaller (0.3–0.4 mm diam.) ascomata, mostly aggregated apothecia with indistinct pruina only, and much smaller ascospores (20–30 × 3.5–7 µm). *Phlyctis lueckingii* Weerakoon et Aptroot from Sri Lanka (Weerakoon et al. 2016) largely agrees with *P. atomella* in habit, including dispersed apothecia with a pruinose disc. However, it has smaller apothecia, a KI– hymenium, and, like *P. karnatakana*, much smaller ascospores (27–29 × 5.5–6.5 µm). It is unclear how *P. lueckingii* differs from *P. karnatakana*; they agree in all important morphological, anatomical and chemical details, including the usually clustered apothecia and ascospore size and septation. Weerakoon et al. (2016) did not mention *P. karnatakana* in their discussion and were possibly unaware of this taxon.

*Phlyctis communis* Chitale et Makhija, also described from India (Chitale et Makhija 2012), agrees with the new species in the norstictic acid chemistry and transversally septate ascospores, but the ascospores are smaller [18–33(45) × 6–9 µm] and have 7–15 septa; also, salazinic acid is present in addition to stictic acid. Another species with a norstictic acid chemistry but ascospores with more numerous septa (7–11), also has slightly longer ascospores (55–86 × 5–7 µm), is *P. longifera* (Nyl.) G.J. Galloway et D. Guzmán, described from Chile (Galloway et Guzmán 1988).

*Phlyctis atomella* is similar to *P. brasiliensis* Nyl., which differs in having narrower, ca 4.5 µm wide ascospores. It can also be compared to three taxa described from New Zealand, *P. oleosa* Stirt., *P. uncinata* Stirt., and *P. subuncinata* Stirt. (Galloway 1985, 2007). *Phlyctis oleosa* has a corticate, glossy thallus and larger ascospores (55–80 × 13–18 µm), whereas *P. uncinata* possesses larger ascospores (55–100 × 5–10 µm) with 7–13 septa, and *P. subuncinata* produces stictic and constictic acids.

Even though *Phlyctis agelaea* (Ach.) Flot., *P. argena* (Ach.) Flot., *P. petraea* R.C. Harris, Musc., Ladd et Lendemer, and *P. speirea* G. Merr are phylogenetically comparable to *P. atomella*, *P. agelaea*, *P. argena* and *P. speirea* differ morphologically from *P. atomella* in having muriform ascospores. The ascospore form of *P. petraea* is unknown because mature apothecia of this species have never been observed.

## Key to the species of *Phlyctis* reported from India

1 Lichen substances absent, thallus K–, P–; ascospores 5–7 septate, 20–40 × 2–4 µm ..... ***P. subhimalayensis***
1' Lichen substances present, thallus K–, K+ yellow, K+ yellow turning red, or P+ yellow to orange-red ..... 2
2 Ascospores muriform ..... 3
2' Ascospores transversally septate ..... 5
3 Ascospores 3–8 per ascus, 60–110 × 7.5–9.5 µm ..... ***P. polyphora***
3' Ascospores 1 per ascus ..... 4
4 Thallus greyish green, K–; ascospores 45–53 × 12–16 µm; unknown grey spot in Rf class 5 ..... ***P. nepalensis***
4' Thallus whitish grey, K+ yellowish, P+ orange-red; ascospores 60–130 × 12–30 µm; contains fumarprotocetraric acid ..... ***P. subagelaea***
5 Ascospores 1 per ascus, 130–180 × 30–40 µm, 15-septate; thallus P+ yellow, K–, with psoromic acid ..... ***P. monosperma***
5' Ascospores 8 per ascus, up to 105 × 18–24 µm, variously septate; thallus P–, K+ yellow turning red, with norstictic (and salazinic) acids ..... 6
6 Ascospores 7–15-septate, 18–33(45) × 6–9 µm; with norstictic and salazinic acids .. ***P. communis***
6' Ascospores 7-septate; with norstictic acid only ..... 7
7 Thallus whitish grey; ascospores 20–30 × 5–7 µm ..... ***P. karnatakana***
7' Thallus ash grey to greyish green; ascospores 30–65(78) × 4–11 µm ..... ***P. atomella***

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## References


Awasthi D.D. (2000): Lichenology in Indian subcontinent: A supplement to A hand book of lichens. – 123 pp., Bishen Singh Mahendra Pal Singh, Dehra Dun.

Benfield B., James P.W., Hitch C.J. (2009): The lichens of Great Britain and Ireland, 2nd ed. – 695 pp., British Lichen Society, London.

Chitale G., Makhija U. (2012): A new species of the lichen genus *Phlyctis* from Maharashtra, India. – Mycotaxon 120: 75–79. DOI: https://doi.org/10.5248/120.75

Flotow J.V. (1850): Lichenologische Beiträge zur Flora Europaea. – Botanische Zeitung 8: 569–575.

Galloway D.J. (1985): Flora of New Zealand: Lichens. – P.D. Hasselberg, Government Printer, Wellington.

Galloway D.J. (2007): Flora of New Zealand: Lichens, including lichen-forming and lichenicolous fungi. Revised second edition, Vols 1 and 2. – Manaaki Whenua Press, Landcare Research, Lincoln.

Galloway D.J., Guzmán G. (1988): A new species of *Phlyctis* from Chile. – The Lichenologist 20(4): 393–397. DOI: https://doi.org/10.1017/S0024282988000507

Jagadeesh Ram T.A.M., Sinha G.P. (2010): *Graphidastra himalayana* (*Roccellaceae*), a new lichen species from eastern Himalaya, India. – Geophytology 39: 83–86.

Joseph S., Sinha G.P., Nayaka S. (2020): Taxonomic status of the genus *Schismatomma* (lichenized *Ascomycota*: *Arthoniales*) in India. – The Lichenologist 52(4): 329–331. DOI: https://doi.org/10.1017/s0024282920000249

Joshi S., Upreti D.K. (2013): The lichen genus *Phlyctis* (*Phlyctidaceae*) in India. – Geophytology 42: 151–157.

Joshi S., Upreti D.K., Mishra G.K., Divakar P.K. (2010): Two new species of the lichen genus *Phlyctis* in India. – The Bryologist 113(4): 724–727. DOI: https://doi.org/10.1639/0007-2745-113.4.724

Joshi S., Upreti D.K., Nayaka S. (2012): Two new species in the lichen genus *Phlyctis* (*Phlyctidaceae*) from India. – The Lichenologist 44(3): 363–369. DOI: https://doi.org/10.1017/S0024282911000879

Kirk P.M., Cannon P.F., Minter D.W., Stalpers J.A. (2008): Ainsworth and Bisby's Dictionary of the Fungi, 10th ed. – 771 pp., CABI Publishing, Wallingford. DOI: https://doi.org/10.1079/9780851998268.0000

Larsson A. (2014): AliView: a fast and lightweight viewer and editor for large data sets. – Bioinformatics 30: 3276–3278. DOI: https://doi.org/10.1093/bioinformatics/btu531

Lumbsch H.T., Ahti T., Altermann S., De Paz G.A., Aptroot A., Arup U., Peña A.B., Bawingan P.A., Benatti M.N., Betancourt L., Bjork C.R. (2011): One hundred new species of lichenized fungi: a signature of undiscovered global diversity. – Phytotaxa 18: 1–127. DOI: https://doi.org/10.11646/phytotaxa.18.1.1

Ma R., Li H.M., Wang H.Y., Zhao Z.T. (2010): A new species of *Phlyctis* (*Phlyctidaceae*) from China. – Mycotaxon 114: 361–366. DOI: https://doi.org/10.5248/114.361

McCarthy P.M., Elix J.A. (2017): A new species of *Phlyctis* (lichenized *Ascomycota*, *Phlyctidaceae*) from Australia. – Australasian Lichenology 81: 2–5.

Muscavitch Z.M., Lendemer J.C., Harris R.C. (2017): A review of the lichen genus *Phlyctis* in North America (*Phlyctidaceae*) including the description of a new widespread saxicolous species from eastern North America. – The Bryologist 120(4): 388–417. DOI: https://doi.org/10.1639/0007-2745-120.4.388

Orange A., James P.W., White F.J. (2001): Microchemical methods for the identification of lichens. – 101 pp., British Lichen Society, London.

Poengsungnoen V., Buaruang K., Vongshewarat K., Sangvichien E., Boonpragob K., Mongkolsuk P., Lumbsch H.T. (2019): Three new crustose lichens from Thailand. – The Bryologist 122(3): 451–456. DOI: https://doi.org/10.1639/0007-2745-122.3.451

Rambaut A. (2014): FigTree v. 1.4.2. – http://tree.bio.ed.ac.uk/software/figtree [accessed 10 July 2022]

Räsänen V. (1952): Lichenes novi. VII. – Archivum Societatis Zoologicae Botanicae Fennicae 'Vanamo' 6(2): 80–86.

RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D.L., DARLING A., HÖHNA S., LARGET B., LIU L., SUCHARD M.A., HUELSENBECK J.P. (2012): MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. – Systematic Biology 61(3): 539–542. DOI: https://doi.org/10.1093/sysbio/sys029

STAMATAKIS A. (2006): RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. – Bioinformatics 22(21): 2688–2690. DOI: https://doi.org/10.1093/bioinformatics/btl446

STAMATAKIS A., HOOVER P., ROUGEMONT J. (2008): A rapid bootstrap algorithm for the RAxML web servers. – Systematic Biology 57(5): 758–771. DOI: https://doi.org/10.1080/10635150802429642

STIRTON J. (1879): New and rare lichens from India and the Himalayas. – Proceedings of the Royal Philosophical Society of Glasgow 11: 306–322.

STIRTON J. (1881): On vegetable parasites on the Tea plant, more especially that of Assam. – Proceedings of the Royal Philosophical Society of Glasgow 13: 181–193.

TAMURA K., STECHER G., KUMAR S. (2021): MEGA11: molecular evolutionary genetics analysis version 11. – Molecular Biology and Evolution 38(7): 3022–3027. DOI: https://doi.org/10.1093/molbev/msab120

WEERAKOON G., WOLSELEY P.A., ARACHCHIGE O., DA SILVA CÁCERES M.E., JAYALAL U., APTROOT A. (2016): Eight new lichen species and 88 new records from Sri Lanka. – The Bryologist 119(2): 131–142. DOI: https://doi.org/10.1639/0007-2745-119.2.131

WHITE T.J., BRUNS T., LEE S.J.W.T., TAYLOR J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., eds, PCR Protocols: A guide to methods and applications, pp. 315–322. Academic Press, San Diego. DOI: https://doi.org/10.1016/b978-0-12-372180-8.50042-1

ZAHLBRUCKNER A. (1923): Catalogus Lichenum Universalis, Vol. 2: 1–815. – Verlag von Gebrüder Borntraeger, Leipzig.

ZOLLER S., SCHEIDEGGER C., SPERISEN C. (1999): PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. – The Lichenologist 31(5): 511–516. DOI: https://doi.org/10.1006/lich.1999.0220