

The phylogenetic position of species of *Lecanora* s. l. containing calycin and usnic acid, with the description of *Lecanora solaris* Yakovchenko & Davydov sp. nov.

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Abstract: Phylogenetic reconstructions based on ITS/5.8S and mtSSU DNA sequence data suggest a close relationship between two *Lecanora* species containing calycin and usnic acid and the *Lecanora polytropa* group. *Lecanora solaris* Yakovchenko & Davydov sp. nov. is described from the Altai Mountains in Russia. Its gross morphology resembles that of *L. somervellii* as both species have an effigurate, citrine-yellow thallus (due to the production of calycin). However, *L. solaris* is distinguished from *L. somervellii* by having a small, squamulose to marginally lobate umbilicate thallus and apothecia with a persistent margin, whereas *L. somervellii* has a large, distinctly placodioid thallus and an apothecial margin that is soon excluded.

Key words: growth form, *Lecanora polytropa*, *Lecanora somervellii*, *Lecanoraceae*, lichenized fungi, molecular phylogeny, secondary metabolites

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Introduction

Lecanora (*Lecanoraceae*, Ascomycota) in its broad sense is defined as a large, cosmopolitan genus comprising nearly 1000 currently recognized species, 250 of which have been described in the last 50 years (Lücking *et al.* 2016). Species of *Lecanora* are characterized by a crustose (or placodioid) thallus with mostly lecanorine apothecia, *Lecanora*-type asci and simple hyaline ascospores (Edwards *et al.* 2009) and can be found on rock, bark, wood, soil and detritus. The main phylogenetic lineages within *Lecanora* were recently demonstrated using molecular phylogenetic

analyses (Zhao *et al.* 2016). However it is one of the last genera of lichens that remains largely undivided into more natural units.

Secondary metabolite content has traditionally played an important role in distinguishing taxonomic groups within *Lecanora*, with species-rich groups being characterized by the presence of atranorin or usnic acids (Printzen 2001). The pulvinic acid derivative calycin has so far been found in very few *Lecanora* species as either a major or accessory compound (Obermayer & Poelt 1992; Morse & Ladd 2016). One of these species is *Lecanora somervellii* Paulson from the Himalayas, which is distinctive within the genus because of its bright yellow thallus and apothecia, due to the presence of calycin and usnic acid.

In Russia, 87 species of *Lecanora* have been reported for South Siberia (Urbanavichus 2010). During our field studies, we collected a bright yellow, calycin-containing species of *Lecanora* which differs from *L. somervellii* and is described below. The phylogenetic relationships of the two species and their position within *Lecanora* s. l. were analyzed based on ITS rDNA and mtSSU DNA sequence data.

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Materials and Methods

Specimens and phenotype studies

The specimens that constituted the core material for this study were collected by the authors from the Altai Mountains (Siberia) during field trips in 2014 and from the Magadan Region (Russian Far East) in 2012. In addition, herbarium material deposited in GZU and FR was examined.

Morphological and anatomical characters were analyzed by employing standard light microscopy methods. Cross-sections of apothecia and thalli were hand cut and observed after mounting in water. Measurements are given as follows: (smallest value recorded) $(\bar{x} - SD) - \bar{x} - (\bar{x} + SD)$ (largest value recorded), where \bar{x} is the (arithmetic) sample mean, and SD the sample standard deviation. The two extreme values and the sample mean are given to the nearest 0.5 μm .

Secondary metabolites present in the thallus were analysed by means of thin-layer chromatography (TLC) (Culberson & Kristinsson 1970) using solvent system B (hexane: methyl tert-butyl ether: formic acid, 140: 72: 18) (Culberson & Johnson 1982).

Sequences and phylogenetic reconstructions

To test the phylogenetic relationships of calycin-producing species of *Lecanora* and their relationship to other species represented in GenBank (www.ncbi.nlm.nih.gov), the ITS region of the nrDNA (ITS1, 5.8S and ITS2) and the mitochondrial small subunit of the ribosomal RNA (mtSSU) were sequenced from eight specimens (Table 1). These markers were chosen because they were also used in the most comprehensive analyses of *Lecanora* by Zhao *et al.* (2016) and many sequences are present in GenBank, whereas sequences from other loci are only available for very few species. DNA extraction, amplification and sequencing followed Davydov & Yakovchenko (2017). Sequences were aligned with those of 75 other species preferably representing type material, from Zhao *et al.* (2016) using MAFFT in Geneious 6.0 (Biomatters Ltd., New Zealand) with default settings (auto algorithm selection, gap open penalty 1.53, offset 0.123) and manually optimized. Before combining sequences into a joint ITS + mtSSU data matrix, the unambiguously alignable regions of 81 specimens for which both marker regions were obtained were used for calculations using RAxML 8.0.26 (Stamatakis 2014) to generate single-marker phylograms (not shown), which were tested for conflicts. The cladograms were similar regarding well-supported clades and lacked conflicts, therefore all sequences were combined into one matrix consisting of 1159 sites and used for RAxML and Bayesian analyses. The optimal substitution model was inferred initially assuming four independent subsets: ITS1, 5.8S, ITS2 and mtSSU using PartitionFinder version 1.1.1 (Lanfear *et al.* 2012). The Kimura 2-parameter model with a proportion of invariable sites (K80 + I) was inferred for the 5.8S partition. ITS1 and ITS2 were inferred to follow the same general time reversible model with site-specific rates modelled by a gamma-distribution and with a proportion of invariable sites (GTR+I+G).

The Hasegawa-Kishino-Yano model with a proportion of invariable sites and gamma-distributed, site-specific rates (HKY+I+G) was inferred as optimal for mtSSU. Bayesian inference with the Markov chain Monte Carlo (BMCMC) method (Larget & Shimon 1999) was performed using MrBayes 3.2.3 (Ronquist *et al.* 2012). We applied the above-mentioned substitution models, a variable rate prior and an unconstrained exponential branch-length prior with a mean of 0.12. The mean of the branch-length prior was calculated based on Maximum Likelihood (ML) tree reconstructions using the procedure described by Ekman & Blaaid (2011). Three parallel analyses each with 6 incrementally heated chains using the default heating factor of 0.2 were run for 40 million generations; every 200th generation was sampled until the average standard deviation of split frequencies had dropped to 0.015. This was the case after 20 million generations. The first 50% of trees was discarded as burn-in and a 50% majority-rule consensus tree was calculated from the remaining trees of the three runs with the *sumt* command implemented in MrBayes 3.2.3. The most likely tree and 1000 bootstrap replicates were calculated using RAxML 8.0.26 (Stamatakis 2014) by raxmlGUI software version 1.3.1 (Silvestro & Michalak 2012), applying the GTRGAMMA model of substitution to the subsets. Species of *Ramboldia* were used as an outgroup because this genus has been shown to be closely related to *Lecanora* s. l. (Zhao *et al.* 2016). Bootstrap support values and posterior probabilities from the BMCMC analysis were mapped onto the ML tree from RAxML because the Bayesian 50% majority-rule consensus tree had the same topology.

Results

ITS and mtSSU sequences were successfully obtained from four specimens of the putative new species, described below as *Lecanora solaris*. The phylograms are combined in Fig. 1. Four sequences of *L. solaris* are combined in a well-supported clade (MrBayes: 1.0 PP; RAxML: 100% BS). Two sequences of *L. somervellii* cluster separately in a well-supported sister clade (1.0 PP, 100% BS). Both species appear as closely related to *L. polytropa* and *L. intricata* (1.0 PP, 89% BS), with which they form a well-supported clade (the *Lecanora polytropa* group) sister to *Protoparmeliopsis*, *Rhizoplaca*, *Myriolecis* and *L. comizaeoides* (1.0 PP, 84% BS).

Lecanora solaris Yakovchenko & Davydov sp. nov.

Mycobank No.: MB 824845

Similar to *L. somervellii* but differs by its squamulose growth form and plane to moderately convex apothecial discs with a distinct, persistent thalline margin.

TABLE 1. Lichen species used in the phylogenetic analyses of calycin-producing species of *Lecanora* together with specimen information and GenBank Accession numbers. Sequences produced in the present study are in bold.

| Species | Collection location and number or reference | GenBank Accession number | |
|---|---|--------------------------|-----------------|
| | | mtSSU | ITS |
| <i>Japewia tomoensis</i> | Canada, <i>Printzen</i> s. n. (Hb. BG) | HQ660559 | HQ650656 |
| <i>Lecanora achroa</i> | <i>Papong</i> 6458 (F) | JQ782663 | JN943714 |
| <i>L. argopholis</i> | Austria, Tirol, <i>Printzen</i> 12558 (FR-0220001) | MH520108 | MH512978 |
| <i>L. allophana</i> | Sweden, <i>Ekman</i> 3434 | AY567710 | AF070031 |
| <i>L. austrotropica</i> | <i>Papong</i> 6047 (F) | JQ782665 | JQ782706 |
| <i>L. caesiorubella</i> | <i>Lumbsch</i> 19094a (F) | JQ782666 | JN943722 |
| <i>L. californica</i> | <i>Lumbsch</i> 19914a (F) | JQ782668 | JQ782707 |
| <i>L. campestris</i> | Sweden, <i>Arup</i> (LD-L97370) | DQ787362 | AF159930 |
| <i>L. carpinea</i> | Austria, <i>Arup</i> (LD-L97007); Sweden, <i>Arup</i> (LD-03192) | DQ787364 | AY398710 |
| <i>L. cinereofusca</i> | <i>Lendemer</i> 35007 (NY-1808085) | KP224464 | KP224469 |
| <i>L. conizaeoides</i> | AFTOL-ID 1858 | KJ766418 | AF189717 |
| <i>L. elatimoides</i> | <i>Lumbsch</i> 19992d (F) | JQ782669 | JQ782709 |
| <i>L. farinacea</i> | <i>Lumbsch</i> 19971b (F) | JQ782670 | JN943726 |
| <i>L. flavopallida</i> | <i>Lumbsch</i> 19972d | JN943723 | JQ782673 |
| <i>L. flavoviridis</i> | <i>Papong</i> 6539 (F) | JQ782675 | JQ782711 |
| <i>L. formosa</i> | <i>Zhao</i> 20129045-2 | KT453819 | KT453771 |
| <i>L. gangaleoides</i> | <i>Lumbsch</i> 19923a (F) | JQ782676 | JQ782712 |
| <i>L. helva</i> | <i>Lumbsch</i> 19809h (F) | JQ782677 | JQ782713 |
| <i>L. horiza</i> | no data | KT453821 | KT453772 |
| <i>L. hybocarpa</i> | <i>Lumbsch</i> s. n. (F) | EF105417 | EF105412 |
| <i>L. imshaugii</i> | <i>Lumbsch</i> 19273b (F) | JQ782681 | JQ782717 |
| <i>L. intricata</i> | Austria, <i>Arup</i> (LD-L97031) | DQ787346 | AF070022 |
| <i>L. intumescens</i> | Norway, <i>Ekman</i> 3162 (BG) | AY300892 | AY541254 |
| <i>L. leproplaca</i> | <i>Lumbsch</i> 19558m (F) | JQ782683 | JQ782718 |
| <i>L. leprosa</i> | <i>Papong</i> 6735 (F) | JQ782682 | JQ782721 |
| <i>L. pacifica</i> | <i>Lumbsch</i> 19901c (F) | JQ782686 | JQ782722 |
| <i>L. paramerae</i> | <i>Lumbsch</i> s. n. (F) | EF105418 | EF105413 |
| <i>L. phaeocardia</i> | <i>Papong</i> 3492 (F) | JQ782687 | JQ782724 |
| <i>L. plumosa</i> | <i>Papong</i> 6965 (F) | JQ782690 | JQ782726 |
| <i>L. polytropa</i> 1 | AFTOL-ID1798 | DQ986807 | HQ650643 |
| <i>L. polytropa</i> 2 | Austria, <i>Grube</i> (GZU); Sweden <i>Arup</i> (LD-L03568) | DQ787348 | AF070017 |
| <i>L. pseudogangaleoides</i> subsp. <i>verdomii</i> | HTL19103a (F) | JQ782691 | JQ782727 |
| <i>L. queenslandica</i> | <i>Lumbsch</i> 19113 (F) | JQ782692 | JQ782728 |
| <i>L. saxigena</i> | <i>Lendemer</i> 33186 (NY-1684507) | KP224461 | KP224468 |
| <i>L. solaris</i> 1 | Russia, Republic of Altai, <i>Davydov</i> (16532) & <i>Yakovchenko</i> (LE-L-13174, holotype) | MH520107 | MH512982 |
| <i>L. solaris</i> 2 | Russia, Republic of Altai, <i>Davydov</i> (14333) & <i>Yakovchenko</i> (ALTB, paratype) | MH520109 | MH512981 |
| <i>L. solaris</i> 3 | Russia, Republic of Altai, <i>Davydov</i> (14335) & <i>Yakovchenko</i> (ALTB, paratype) | MH520110 | MH512983 |
| <i>L. solaris</i> 4 | Russia, Republic of Tuva, <i>Davydov</i> (14336) & <i>Yakovchenko</i> (ALTB, paratype) | MH520111 | MH512984 |
| <i>L. somervellii</i> 1 | Russia, Magadan Region, <i>Ohmura</i> (10109), <i>Yakovchenko</i> & <i>Zheludeva</i> (TNS-L-125468) | MH520113 | MH512979 |
| <i>L. somervellii</i> 2 | Russia, Magadan Region, <i>Ohmura</i> (10111), <i>Yakovchenko</i> & <i>Zheludeva</i> (TNS-L-125469) | MH520114 | MH512980 |
| <i>L. subimmersens</i> | <i>Papong</i> 6431 (F) | JQ782696 | JQ782732 |
| <i>L. subimmersa</i> | <i>Lumbsch</i> 19103b (F) | JQ782697 | JQ782733 |
| <i>L. sulphurea</i> | Sweden, <i>Arup</i> L96006, L01823 (LD) | DQ787356 | AF070030 |
| <i>L. symmicta</i> | <i>Printzen</i> 9999a (FR) | KJ152466 | AF070024 |
| <i>L. toroyensis</i> | <i>Papong</i> 7197 (F) | JQ782698 | JQ782734 |

(Continued)

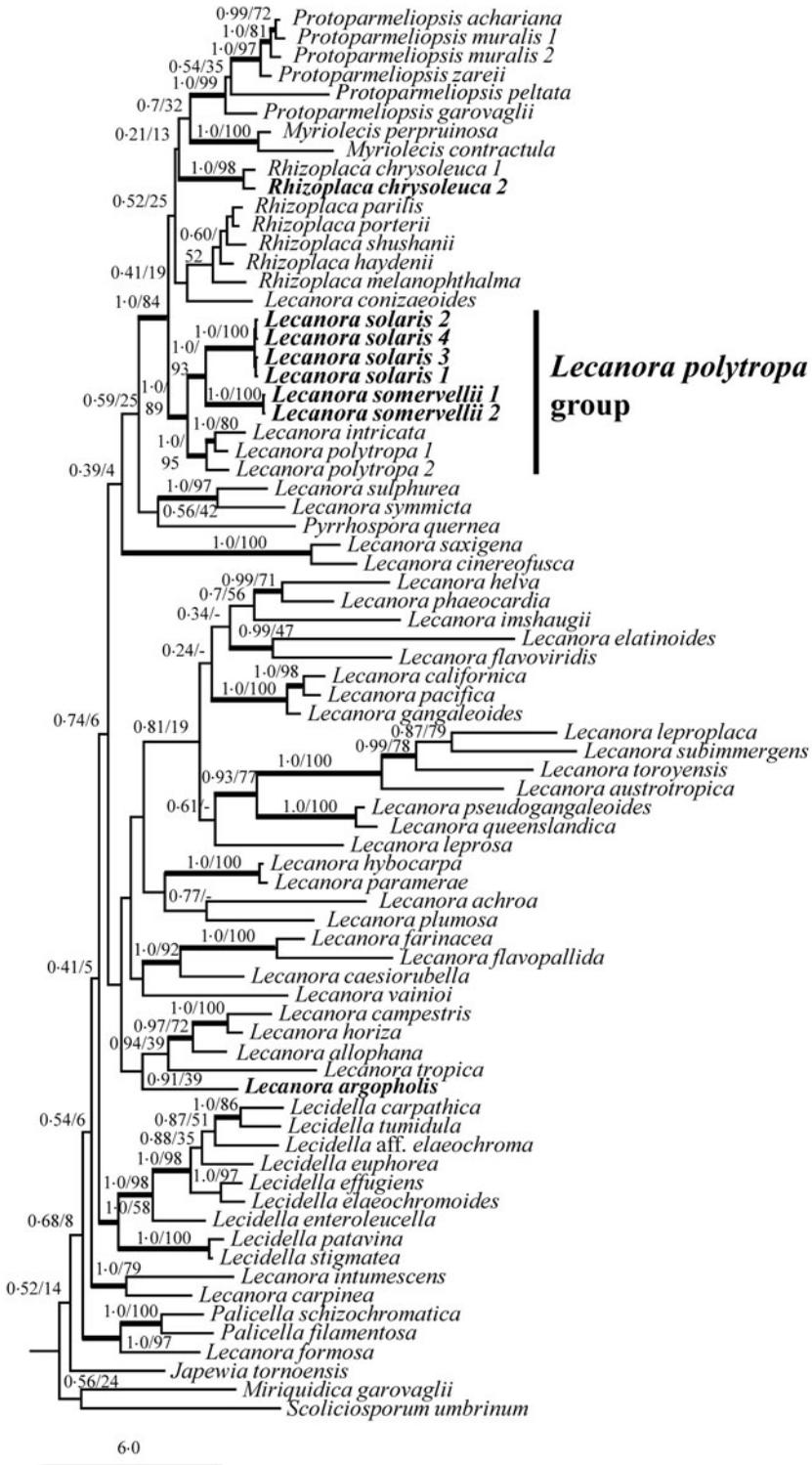
TABLE 1 (continued).

| Species | Collection location and number or reference | GenBank Accession number | |
|--|--|--------------------------|-----------------|
| | | mtSSU | ITS |
| <i>L. tropica</i> | Papong 6440 (F) | JQ782699 | JN943720 |
| <i>L. vainioi</i> | Papong 7120 (F) | JQ782701 | JN943716 |
| <i>Lecidella</i> aff. <i>elaeochroma</i> | Zhao 20141264-2 | KT453826 | KT453752 |
| <i>L. carpathica</i> | Zhao 20140367-2 | KT453831 | KT453741 |
| <i>L. effugiens</i> | Zhao 20141269-2 | KT453832 | KT453748 |
| <i>L. elaeochromoides</i> | Zhao 20141142 | KT453836 | KT453750 |
| <i>L. enteroleucella</i> | Zhao YN0201 | KT453838 | KT453757 |
| <i>L. euphorea</i> | Zhao XL0351-2 | KT453843 | KT453745 |
| <i>L. meiococca</i> | Sweden, Ekman 3101 (BG) | AY300893 | AF517929 |
| <i>L. patavina</i> | Zhao 20140501-2 | KT453845 | KT453767 |
| <i>L. stigmatea</i> | Zhao 20141243 | KT453847 | KT453762 |
| <i>L. tumidula</i> | Zhao XL0009 | KT453854 | KT453736 |
| <i>Mirioidica garovaglii</i> | Norway, Ekman s. n. | AY567711 | KF562188 |
| <i>Myriolecis contractula</i> | AFTOL-ID 877 | DQ986898 | HQ650604 |
| <i>M. perpruinosa</i> | Austria, Wilflinget et al. 1224 (GZU), Arup (LD-97320) | DQ787344 | AF070025 |
| <i>Palicella filamentosa</i> | Germany, Cezanne & Eichler 6761 (hb. Cezanne-Eichler) | KJ152468 | GU480099 |
| <i>P. schizochromatica</i> | USA, Hauck s. n. (hb. Hauck) | HQ660555 | HQ650652 |
| <i>Protoparmeliopsis achariana</i> | Arup (LD-L03216) | DQ787342 | AF070019 |
| <i>P. garovaglii</i> | Leavitt 089 (BRY-C) | KT453818 | KT453728 |
| <i>P. muralis</i> 1 | AFTOL-ID 1882 | KJ766466 | KC791770 |
| <i>P. muralis</i> 2 | Leavitt 143 (BRY-C) | KT453822 | KT453726 |
| <i>P. peltata</i> | USA | KT453860 | KT453722 |
| <i>P. zarei</i> | B. Zarei-Darki (1111) (KW) | KP059056 | KP059049 |
| <i>Pyrrhospora quernea</i> | Sweden, Ekman 3019 (BG) | AY300908 | AF517930 |
| <i>Ramboldia arandensis</i> | Elix 28721 (CANB) | EU075527 | EU075541 |
| <i>R. brunneocarpa</i> | Elix 36756 (F) | EU075528 | EU075542 |
| <i>R. laeta</i> | Elix 36817 (F) | EU075530 | EU075544 |
| <i>R. petraeoides</i> | Elix 36816 (F) | EU075531 | EU075545 |
| <i>R. russula</i> | Lücking 17640 (F) | EU075533 | EU075547 |
| <i>R. sanguinolenta</i> | Elix 28835 (F) | EU075534 | EU075548 |
| <i>R. stuartii</i> | Elix 28664 (F) | EU075535 | EU075549 |
| <i>Rhizoplaca chrysoleuca</i> 1 | USA (BRY 55000) | KT453856 | HM577233 |
| <i>R. chrysoleuca</i> 2 | Russia, Republic of Tuva, Davydov (16455) & Yakovchenko (ALTB) | MH520112 | MH512985 |
| <i>R. haydenii</i> | USA, Leavitt 727 (BRY-C) | KT453857 | HM577304 |
| <i>R. melanophthalma</i> | Iran, Sohrabi 014624 (H) | KT453858 | JX948275 |
| <i>R. parilis</i> | USA | KT453859 | HM577309 |
| <i>R. porterii</i> | USA | KT453861 | HM577376 |
| <i>R. shushanii</i> | USA | KT453862 | HM577284 |
| <i>Scoliosporum umbrinum</i> | Norway, Ekman 3005 | AY567719 | AY541277 |

Type: Russia, Republic of Altai, Kosh-Agachsky District, Sailjugem Range, right bank of the Bayan-Chagan River 2.5 km S of its junction with the Karasu River, on

siliceous rocks, 49°31'55"N, 88°46'45"E, 2630 m a.s.l., 15 June 2014, E. A. Davydov 16532 & L. S. Yakovchenko (LE-L-13174—holotype; ALTB—isotype).

FIG. 1. The phylogenetic relationships of calycin-producing species of *Lecanora* and their relationship to other species represented in GenBank using ITS and mtSSU sequences. The reliability of each branch was tested by Maximum Likelihood (ML) and Bayesian methods. Numbers at tree nodes indicate bootstrap values of Bayesian inference with the Markov chain Monte Carlo (BMC) posterior probabilities (left of slash) and ML (right of slash). Thicker branches indicate BMC posterior probability values ≥ 0.95 or ML bootstrap values $\geq 70\%$. GenBank Accession numbers are given in Table 1. Sequences produced in the present study are marked in bold. Out-group not shown.



(Fig. 2B–F)

Thallus squamulose, \pm circular, up to 10–15 mm diam.; squamules (0.2–)0.7–1.3–1.9 (–3.5) mm diam., crowded, moderately to strongly convex, rounded initially but soon becoming incised and irregular in outline, sometimes peltate. *Surface* bright yellow, shiny, initially smooth, later strongly rugose, epruinose. *Vegetative propagules* absent. *Cortex* 42.5–57.5 μ m, of compacted irregularly-oriented hyphae, up to 3.5 μ m thick, with elongate lumina becoming rounded towards the uppermost part of the cortex and covered by cortical crystals. Cortical crystals \pm rounded, irregular in shape, angular or bacilliform. *Algal layer* continuous, 135–215 μ m thick, algae chlorococcoid, 17.5–22.5 μ m diam.; medulla of loose hyphae of up to 5 μ m diam.

Apothecia lecanorine, common, concentrated in centre of the thallus, at first arising singly on areoles that are then obscured, crowded to overlapping (1–4 apothecia per squamule), immersed initially soon becoming sessile and strongly constricted at the base, (0.4–)0.9–1.2–1.5 (–2.0) mm diam., with raised margin; *disc* initially plane, later moderately convex, initially smooth, later smooth or scarcely rugose, rounded to irregular in outline, concolorous with the thallus to ochre-yellow, epruinose; *thalline margin* initially distinct, later disappearing, concolorous with disc, somewhat shiny, epruinose, (62.5–)79.0–97.0–115.0 (–125.0) μ m thick. *Cortex of thalline margin* same as the thalline cortex; *medulla* reaching into the thalline margin, consisting of loose hyphae of up to 5 μ m diam., with green algal cells of up to 22.5 μ m diam. *Hymenium* (42.5–)48.0–52.0–55.0 (–57.5) μ m tall, hyaline; *subhymenium* (12.5–)20.5–27.0–33.5 (–42.5) μ m tall, hyaline, with oil drops; *epihymenium* (12.5–)13.5–18.0–22.0 (–25.0) μ m tall, medium brown, densely incrustated by crystals, which penetrate down into the hymenium. *Hypothecium* (50.0–)

70.0–91.5–113.5 (–137.5) μ m tall, hyaline with sulphur yellow pigment (calycin) in uppermost part, opaque, composed of compact, elongated, irregularly-oriented hyphae of 2.5–3.5 μ m diam.; *paraphyses* simple to branched near the tips and in the mid-hymenium, septate, 2.0–2.5 μ m wide, with clavate tips 3–5 μ m wide. *Asci* clavate, 8-spored, (32.5–)36.5–40.5–44.0 (–51.5) \times (12.5–)14.0–15.0–16.5 (–19.0) μ m, *Lecanora*-type; *ascospores* simple, hyaline, ellipsoid to narrowly ellipsoid, (7.5–)10.0–11.5–13.0 (–14.0) \times (4.5–)5.0–5.5–6.0 (–7.5) μ m.

Pycnidia rare, 90–110 μ m diam., immersed, walls colourless, conidiogenous cells elongate-ampulliform; *conidia* filiform, colourless, simple, (9.5–)12.0–16.0–20.0 (–22.0) \times c. 0.8 μ m.

Chemistry. Thallus K+ weakly reddish, KC–, C–, Pd–; calycin as well as usnic and rangiformic acids by TLC.

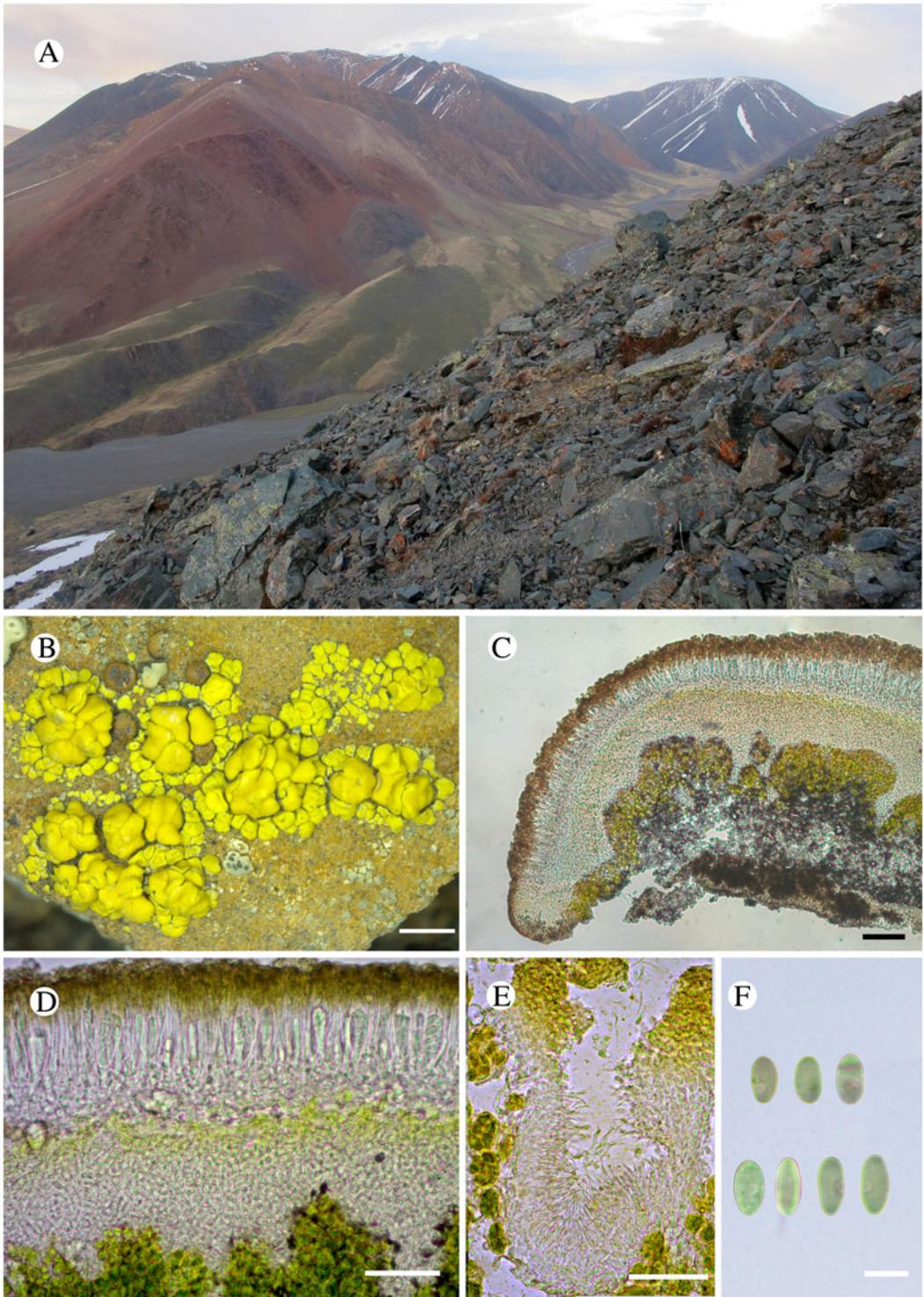
Etymology. The name refers to the bright yellow “sunny” colour of the lichen.

Substratum and ecology. The species grows on hard volcanic or weakly calcareous overhanging rocks in open pioneer communities within high mountain vegetation at elevations between 2630–3100 m (Fig. 2A). The species was scarce in this habitat. The following species co-occurred with *Lecanora solaris*: *Acarospora* cf. *elevata* H. Magn., *Acarospora* sp., *Aspicilia* sp., *Lecanora* sp., *Protoparmeliopsis peltata* (Ramond) Arup *et al.*, *Carbonea vorticos*a (Flörke) Hertel.

Distribution. *Lecanora solaris* is so far known only from the Altai Mountains where it was collected in the Sailjugem Range and the Mongun-Taiga massif.

Notes. The examination of the neotype of *L. somervellii* revealed that *L. solaris* differs in the thallus morphology: *L. somervellii* has a large, well-developed, placodioid thallus

FIG. 2. A–F, *Lecanora solaris*; A, type locality, Sailjugem Range, right bank of the Bayan-Chagan River; B, holotype (LE-L-13174); C, section of apothecium; D, section of apothecium showing clearly the layer of calycin; E, pycnidia with broadly falcate conidia; F, ascospores. Scales: B = 2 mm; C = 100 μ m; D = 50 μ m; E = 25 μ m; F = 10 μ m.



and lecanorine apothecia which soon become strongly convex with an excluded thalline margin; whereas *L. solaris* has a squamulose thallus with flattened to moderately convex, lecanorine apothecia with a persistent thalline margin. According to our field observations in the Magadan Region the two species differ also in their habitat; *L. somervellii* occurs on sunny exposed siliceous rocks while *L. solaris* favours shaded, weakly calcareous rocks. Morphologically, *L. solaris* also resembles species of *Pleopsidium* but can easily be distinguished anatomically by having 8-spored asci. Sterile samples differ from *Pleopsidium* by the small size and shape of the thallus, which is never placodioid and large, as well as by its habitat: *Pleopsidium* species occur on exposed, well-insolated surfaces of siliceous rocks.

We observed two different morphs of *L. solaris*: squamulose (typical) and umbilicate (Davydov 14336 & Yakovchenko). The umbilicate morph differs in the thallus margin having a lobate, umbilicate (not uniformly squamulose) growth form; a smooth to strongly echinate (not rugose) thallus surface and apothecial discs that become more strongly rugose with age. Both morphs were collected at the same locality and under similar ecological conditions. The ITS sequences of both morphs were identical but the mtSSU sequence of the umbilicate morph (MH520111) differs by five substitutions from that of the squamulose morphs, including a specimen from the same locality. The short and unsupported branches within the *L. solaris* clade, however, indicate that these differences reflect intraspecific variability. The mtSSU sequence of the umbilicate morph also includes an insertion at position 638 (ACCC-GCG-GCAAAGCATCAGTGAGCC) which is lacking in all sequences of squamulose specimens, and very similar to the homologous part of the sequence of *Rhizoplaca chrysoleuca* (Sm.) Zopf (ACCTTGCGTTGCAAAGCATCAGTGAGTGCC: differences marked in bold). However, this insertion is extremely variable among *Lecanora* and *Rhizoplaca* species and was excluded from the alignment.

Additional specimens examined. Russia: Republic of Altai: Kosh-Agachsky District, Sailjugem Range, watershed of Bayan-Chagan and Sarzhemoty Rivers, 4 km S of its junction, 49°32'00"N, 88°45'01"E, 2750 m a.s.l., in crevices of rocks, 2014, E. A. Davydov 14333 & L. S. Yakovchenko (ALTB); *ibid.*, right bank of the Bayan-Chagan River, 2.5 km S of its junction with the Karasu River, 49°32'01"N, 88°46'36"E, 2680 m a.s.l., in crevices of rocks, 2014, E. A. Davydov 14334 & L. S. Yakovchenko (ALTB). *Republic of Tuva:* Mongun-Taiginsky District, Mongun-Taiga massif, headwaters of the Mugur River, 27.5 km W of Mugur-Aksy, 50°18'22"N, 90°04'26"E, 3000–3100 m a.s.l., alpine meadows and mountain tundras with stones, on rocks, 2014, E. A. Davydov 14335, 14336 & L. S. Yakovchenko (ALTB).

Lecanora somervellii Paulson

J. Bot., Lond. 63: 192 (1925); type: Nepal, Langtang area, huge rocks near Kyangjin, 3750 m, 8–10 September 1986, *J. Poelt* N86-L257 [GZU—neotype!, selected by Obermayer & Poelt, *Lichenologist* 24: 112 (1992)].

This species is characterized by its effigurate, citrine-yellow thallus (due to the production of calycin). The specimens collected from the Magadan Region (Yakovchenko *et al.* 2018) are a good match to the protologue and description by Obermayer & Poelt (1992). The chemical substances detected by TLC were calycin, usnic acid, rangiformic acid, norrangiformic acid, and an unidentified fatty acid (Rf class 3 in solvent B). *Lecanora somervellii* is known from the Himalayas, both in Nepal and Tibet, where it grows on steep to overhanging sides of very hard siliceous rocks at an altitudinal range of *c.* 3750 m to *c.* 5540 m (Paulson 1925; Obermayer & Poelt 1992).

Specimens examined. Nepal: Central Himalaya: Langtang area, huge rocks near Kyangjin, elev. *c.* 3750 m, 8–10 September 1986, *J. Poelt* N86-L257 (GZU).—*Russia: Magadan Region:* small mountain *c.* 120 km NE of Atoka, 61°11'47.7"N, 153°58'10.5"E, elev. 1130 m, on rock, 11 August 2012, Y. Ohmura 10109, 10111, L. S. Yakovchenko & E. Zheludeva (TNS-L-125468, 125469).

Discussion

A similarity between *Lecanora somervellii* and *L. polytropa* f. *illusoria* (Ach.) Leight. was already noted in the protologue of *L. somervellii* by Paulson (1925), who nevertheless

assumed a closer relationship for his new species with *Aspicilia*. Our phylogeny based on ITS and mtSSU and the six-gene phylogeny of Zhao *et al.* (2016) both support the hypotheses that the calycin-producing *L. solaris* and *L. somervellii* represent sister taxa within the *L. polytropa*-group and that *Myriolecis*, *Protoparmeliopsis*, *Rhizoplaca* and the *L. polytropa*-group are closely related to each other. Apart from showing a close relationship between *L. formosa* and *Palicella* (also inferred by Zhao *et al.* (2016)), our phylogeny does not allow further conclusions on the relationships within *Lecanora* s. l. because its backbone is not supported.

The discovery of *L. solaris* in the Altai Mountains returns us to the problem of the neotypification of *L. somervellii* by Obermayer & Poelt (1992). As the holotype of *L. somervellii* was lost (or at least not found) and Paulson's original material was missing, a neotype was selected for *L. somervellii* from the material collected by Poelt in Nepal in 1986. The neotype is consistent with the protologue of the species published by Paulson (1925), in which the unique citrine-yellow colour of *L. somervellii* and its 8-spored asci are noted. However, other characters of the neotype material were not consistent with the protologue (Obermayer & Poelt (1992)). For example, Paulson described the thallus of *L. somervellii* as squamulose with a white lower surface and the apothecia as lecanorine, plane to immersed or slightly convex and with a persistent thalline margin. The neotype, on the other hand, has a distinctly placodioid thallus with closely attached marginal lobes and apothecia with a thalline margin only in the juvenile stage. Hence, morphologically *L. solaris* better fits the original description of Paulson (1925). However, since Paulson's original material is not available, we must follow the neotypification by Obermayer & Poelt (1992). Furthermore, no material similar to *L. solaris* has as yet been found in the Himalayas.

Among the yellow-coloured *Lecanora* species, only *L. somervellii* and *L. solaris* are so far known to contain calycin and usnic acid. Other species, such as *Lecanora sulphurella* Hepp, *L. fulvastra* Kremp. and *L. inaurata*

C. A. Morse & Ladd, either contain only usnic acid as a yellow pigment or produce atranorin and/or chloroatranorin as major secondary compound(s) in addition to calycin (Follmann & Huneck 1976; Leuckert & Mayrhofer 1985; Lumbsch 1994; Morse & Ladd 2016).

This and previous phylogenetic studies (Peréz-Ortega *et al.* 2010; Zhao *et al.* 2016), as well as phenotypic characters (Obermayer & Poelt 1992; Arup & Grube 1998), indicate that *Lecanora* species with calycin and usnic acid, and the *L. polytropa* group, do not belong in *Lecanora* s. s. Our data suggest that *L. somervellii* and *L. solaris* should be included in the *L. polytropa* group *sensu* Zhao *et al.* (2016). Apart from the calycin production, both species appear phenotypically similar to *L. polytropa* in their concave or plane, marginate, broadly attached apothecia arising singly on areoles and soon becoming convex, immarginate, constricted below and clustered in the centre of the thallus obscuring the areoles. Well-developed thalli of *L. polytropa* may sometimes form squamulose thalli similar to those of *L. solaris*. The *L. polytropa* group in this new sense is strongly supported as monophyletic and probably requires taxonomic recognition at the generic level. However, additional data are necessary to fully understand the species composition of this clade before it can be accepted as a separate genus.

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REFERENCES

- Arup, U. & Grube, M. (1998) Molecular systematics of *Lecanora* subgenus *Placodium*. *Lichenologist* **30**: 415–425.
- Culbertson, C. F. & Johnson, A. (1982) Substitution of methyl tert.-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *Journal of Chromatography* **238**: 483–487.
- Culbertson, C. F. & Kristinsson, H. A. (1970) A standardized method for the identification of lichen products. *Journal of Chromatography* **46**: 85–93.
- Davydov, E. A. & Yakovchenko, L. S. (2017) *Rhizocarpon smaragdulum*, a new monosporic yellow-thalline species and some additional species of the genus *Rhizocarpon* from the Altai Mountains (Siberia). *Lichenologist* **49**: 457–466.
- Edwards, B., Aptroot, A., Hawksworth, D. L. & James, P. W. (2009) *Lecanora*. In *The Lichens of Great Britain and Ireland* (C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James & P. A. Wolseley, eds): 465–502. London: British Lichen Society.
- Ekman, S. & Blaalid, R. (2011) The devil in the details: interactions between the branch-length prior and likelihood model affect node support and branch lengths in the phylogeny of the *Psoraceae*. *Systematic Biology* **60**: 541–561.
- Follmann, G. & Huneck, S. (1976) Mitteilungen über Flechteninhaltsstoffe CXII. Neue Flechtenanalysen 5. *Philippia* **3**: 9–19.
- Lanfear, R., Calcott, B., Ho, S. Y. W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**: 1695–1701.
- Large, B. & Simon, D. (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* **16**: 750–759.
- Leuckert, C. & Mayrhofer, H. (1985) Chemische Flechtenanalysen IV. *Herzogia* **7**: 99–104.
- Lücking, R., Hodkinson, B. P. & Leavitt, S. D. (2016) The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – approaching one thousand genera. *Bryologist* **119**: 361–416.
- Lumbsch, H. T. (1994) Calycin in *Lecanora fulvastra*. *Lichenologist* **26**: 94–96.
- Morse, C. A. & Ladd, D. (2016) *Lecanora inaurata*, a new member of the *L. subfusca* group from central North America. *Lichenologist* **48**: 377–385.
- Obermayer, W. & Poelt, J. (1992) Contributions to the knowledge of the lichen flora of the Himalayas III. On *Lecanora somervellii* Paulson (lichenized Ascomycota, *Lecanoraceae*). *Lichenologist* **24**: 111–117.
- Paulson, R. (1925) Lichens of Mount Everest. *Journal of Botany* **63**: 189–193.
- Pérez-Ortega, S., Spribille, T., Palice, Z., Elix, J. A. & Printzen, C. (2010) A molecular phylogeny of the *Lecanora varia* group, including a new species from western North America. *Mycological Progress* **9**: 523–535.
- Printzen, C. (2001) Corticolous and lignicolous species of *Lecanora* (*Lecanoraceae*, *Lecanorales*) with usnic or isousnic acid in the Sonoran Desert Region. *Bryologist* **104**: 382–409.
- Ronquist, R., 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**: 539–542.
- Silvestro, D. & Michalak, I. (2012) raxmlGUI: a graphical front-end for RAXML. *Organisms Diversity and Evolution* **12**: 335–337.
- Stamatakis, A. (2014) RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
- Urbanavichus, G. P. (2010) *A Checklist of the Lichen Flora of Russia*. St. Petersburg: Nauka.
- Yakovchenko, L. S., Zheludeva, E. V., Ohmura, Y. & Davydov, E. A. (2018) *Lecanora somervellii* – new lichen for Russia from Magadan Region. *Turczaninowia* **21** (4): 35–39. [in Russian]
- Zhao, X., Leavitt, S. D., Zhao, Z. T., Zhang, L. L., Arup, U., Grube, M., Pérez-Ortega, S., Printzen, C., Śliwa, L., Kraichak, E., et al. (2016) Towards a revised generic classification of lecanoroid lichens (*Lecanoraceae*, Ascomycota) based on molecular, morphological and chemical evidence. *Fungal Diversity* **78**: 293–304.