

POLYCOCCUM, VARIOSPORA, AND ZEROVIELLA, THREE NEW GENERA FOR PAKISTAN

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Two lichen species, *Variospora kudratovii* and *Zeroviella laxa*, and a lichenicolous species, *Polycoccum clauderouxii* are reported for the first time from Pakistan and represent first generic records as well. Detailed macro- and micromorphological descriptions and spot test results are provided along with ITS-based molecular phylogenetic analysis.

Key words: Khaplu, nrDNA, Teloschistaceae, xanthorioid lichen, Ziarat

INTRODUCTION

During lichen exploration, authors collected specimens of xanthorioid lichens from Khaplu, Gilgit-Baltistan and caloplacoid lichens from Ziarat, Balochistan, Pakistan. Critical studies revealed the distinctness of the specimens from all other known members of Pakistan's xanthorioid and caloplacoid lichens (Ahmad 1965, Aptroot and Iqbal 2012). Identity of the species was further confirmed with molecular analysis and by comparing the specimens with published description (Kondratyuk *et al.* 2013, 2015, 2016). As these represent new generic records for Pakistan, detailed descriptions and illustrations of our collections are provided to facilitate easy identification. The Teloschistaceae subfamilies Xanthorioideae and Caloplacoideae contain 42 and 31 genera (Kondratyuk *et al.* 2014a, b, Mishra *et al.* 2020). Previous records from Pakistan include only one genus of xanthorioid lichens, i.e. *Xanthoria* (Fr.) Th. Fr. (with six species reported) and two genera of caloplacoid lichens, i.e. *Caloplaca* Th. Fr. (with 67 species reported) and *Seiophora* Poelt (with one species reported) (Ahmad 1965, Aptroot and Iqbal 2012, Habib *et al.* 2017).

This work will bring some more insight in the lichens of Pakistan as most of the lichens of the country are very poorly known with very little information and will be helpful in compilation of taxonomic diversity of lichens of Pakistan.

MATERIAL AND METHODS

Morphological characters of the thalli were observed under the Meiji Techno, EMZ-5TR, Japan stereomicroscope. Colours were designated according to Munsell (1975). The morphology of fruiting bodies was studied separately. For micromorphological analysis, sections of the apothecia were cut and mounted on glass slides using water and 5% KOH (5/100 aqueous) as mounting media and examined under compound microscope MX4300H, Meiji Techno Co., Ltd., Japan. The spot tests were performed by applying 10% potassium hydroxide (K-test), calcium hypochloride (C-test) and KC-test to the lichen fragment and the changes were observed under a stereomicroscope. Voucher specimens were deposited in the LAH Herbarium, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan.

DNA was isolated from dried specimens using 2% CTAB protocol (Gardes and Bruns 1993). Internal Transcribed Spacer (ITS) region of nrDNA was amplified using ITS1F/ITS4 primer pair following amplification protocol of Khan *et al.* (2018). The amplified DNA fragments were visualised in 1.2% agarose gel (Sambrook and Russell 2001) and PCR products were sequenced. The nucleotide sequence comparison was performed using Basic Local Alignment Search Tool (BLAST) at National Centre for Biotechnology Information (NCBI) GenBank (Altschul *et al.* 1990). Closest sequences were downloaded from GenBank for phylogenetic analysis. Online MUSCLE was used to align the sequences with other sequences retrieved from NCBI (Edgar 2004). The start and end of alignments were trimmed to nearly equal number of sites for all sequences. Phylogenetic analysis was performed at 1,000 bootstraps in MEGA 6.0 (Tamura *et al.* 2013) by constructing maximum likelihood (ML) trees.

RESULTS AND DISCUSSION

Zeroviella laxa (Müll. Arg.) S. Y. Kondr. et Hur, Ukr. Bot. J. 72: 582, 2015 (Fig.1)

Thallus foliose, saxicolous, thick, centrally squeezed, warty, rough, rusty patches on the surface; upper surface cylindrical to subcylindrical, flattened toward lobes, with up to 1.5 mm black galls of lichenicolous fungus, yellow (7.5YR 8/4) to light orange (7.5YR 8/3, 8/4); lower surface centrally hollow, attached by margins. Lobes 0.5–1.5 mm wide, semiconvex to flattened. Pseudocyphellae well developed, numerous in the centre, up to 1 mm in diam. Apothecia not found. Spot tests: cortex K+ (violet red), C–; medulla K–, C–, KC–.

Comments: In our phylogram (Fig. 4), the sequences of *Z. laxa* formed a sister clade with East Asian *Z. mandschurica* (Zahlbr.) S. Y. Kondr. et Hur. Morphologically, *Zeroviella laxa* differs from *Z. mandschurica* in having wider

lobes (Kondratyuk *et al.* 2015). *Zeroviella laxa* is also similar to *Z. esfahanensis* S. Y. Kondr., Zarei-Darki et Hur but differs by having squeezed, thick, cylindrical to subcylindrical and warty thallus. The morphological comparison and the ITS-based phylogeny demonstrated the identity of our collection with Chinese *Z. laxa*, reported (KU056854), having exactly the same ITS sequence. Previously, *Zeroviella laxa* was reported from China in Asia. This study added this taxon as a new record to the lichen biota of Pakistan.

Material examined: Pakistan. Gilgit-Baltistan: Ghanche, Khaplu, 35.1611° N, 76.3319° E; 2,600 m a.s.l., dry temperate area, cold desert, on rock; coll.: A. Bano (KPL-01), 20 July 2018 (LAH35905).

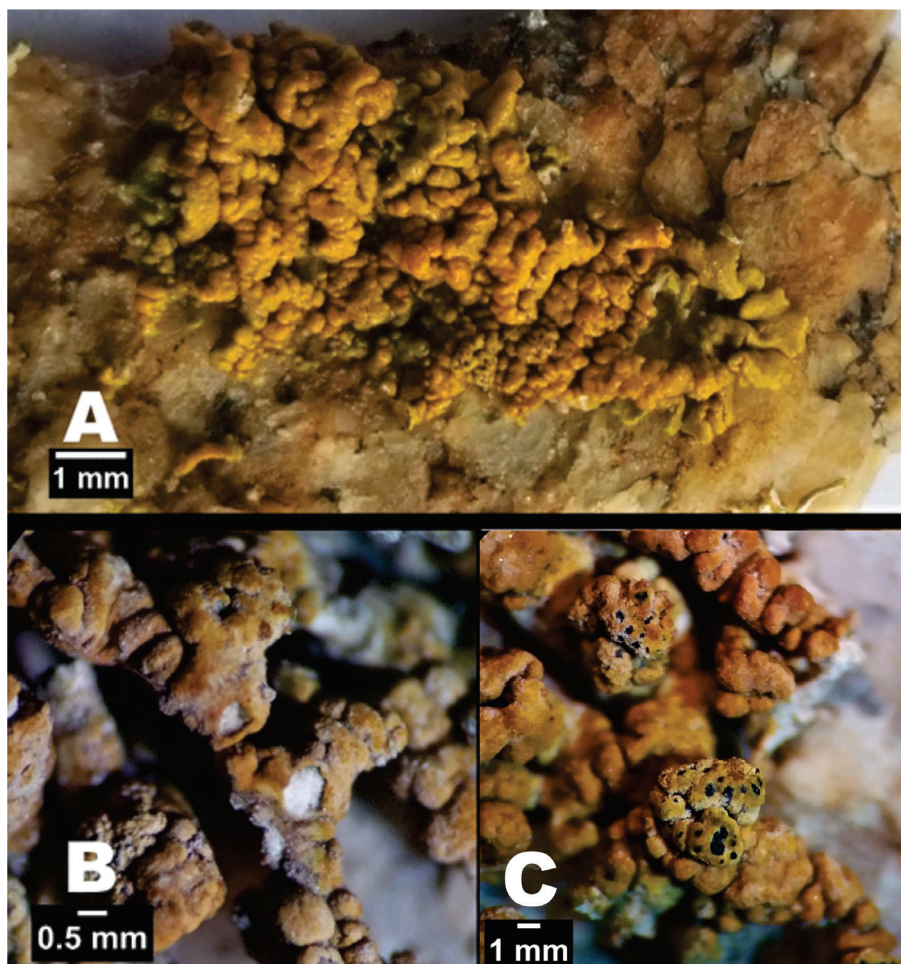


Fig. 1. *Zeroviella laxa* (LAH35905): A = thallus; B = pseudocyphellae and warty thallus; C = galls of lichenicolous fungus on thallus

Variospora kudratovii S. Y. Kondr., Kärnefelt, Elix, A. Thell, Jung Kim,
A. S. Kondr. et Hur, Acta Bot. Hung. 56: 113, 2014
(Fig. 2)

Thallus crustose, saxicolous, areolate, rugose, slightly pruinose; areoles scattered or distant, more or less aggregated, angular to irregular, determinate, 0.5–1.5 mm wide; upper surface rough, yellow (7.5YR8/4) to orange (7.5YR8/6), distinctly wrinkled, not fissured. Photobiont *Myrmecia* (Chlorophyceae), globose to subglobose, 12–20.2 μm in diam. Ascomata apothecial, initially 1–3 per areole, usually 1 adult per areole, slightly glossy, 0.2–1 mm diam. Disc orange, rounded to subrounded. Hymenium hyaline, 50–80 μm tall. Asci clavate, *Teloschistes*-type, 8-spored, 41–60 \times 10–18 μm . Ascospores bilocular, ellipsoidal, 10–16 \times 4.5–6.5 μm . Hamathecial elements: paraphysis, septate, apex swollen up to 4.5 μm wide, capitate, branched. Spot tests: thallus and apothecia K+ purple, C–, KC–.

Comments: The Pakistani specimen of *Variospora kudratovii* (Z18-3A) is morphologically similar to the type specimen of *V. kudratovii* except for having paraphyses with narrower apices (4.5 μm). *Variospora kudratovii* is also

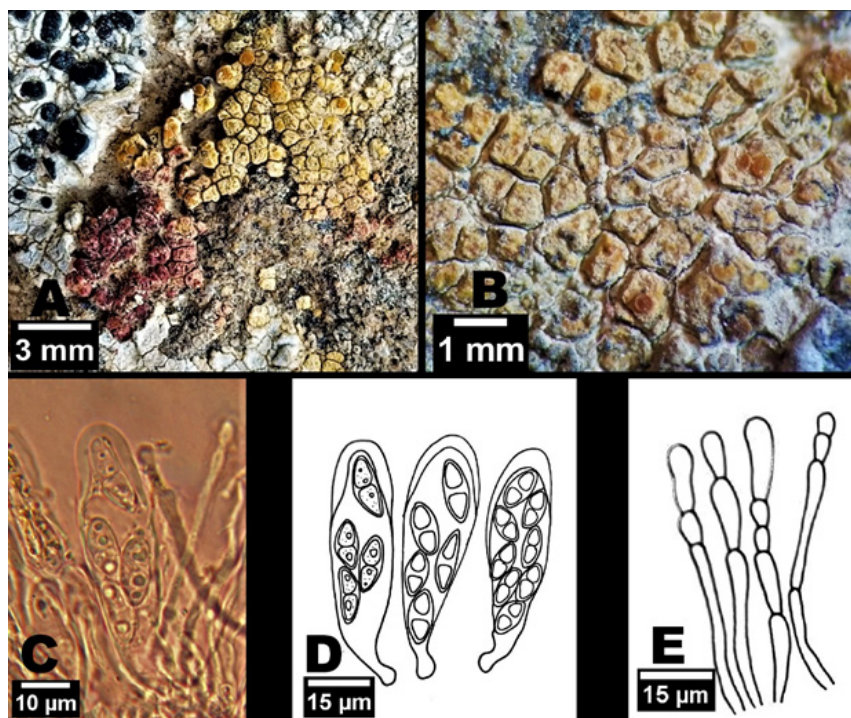


Fig. 2. *Variospora kudratovii*: A = habit; B = areoles; C–D = clavate asci and bilocular ascospores; E = paraphyses

similar to *V. dolomiticola*, but differs in having larger thalline areoles, uncracked distant areoles and larger ascospores. There is a difference of four nucleotide between the Pakistani collection of *V. kudratovii* (Z18-3A) and the Iranian specimens of *V. kudratovii* (KJ021245-KJ021247) at site 91, 92, 406 and 489, but similar at the same positions with the type specimen sequence of *V. kudratovii* (KJ021242-KJ021244). The combination of morphological studies and ITS-based molecular phylogenetic analysis confirmed the identity of the Pakistani material with *V. kudratovii*. In Asia, it has been reported earlier only from Iran (Kondratyuk *et al.* 2013). This study added *Variospora kudratovii* as a new record to the lichen biota of Pakistan.

Material examined: Pakistan. Balochistan: Ziarat, 30.3939° N, 67.7169° E; 2,543 m a.s.l.; dry temperate area, on rock; coll.: A. N. Khalid (Z18-3A), 2 July 2018 (LAH36070).

Polycoccum clauderouxii S. Y. Kondr., Lőkös et Hur

Acta Bot. Hung. 58: 353, 2016

(Fig. 3)

Lichenicolous, dispersed on thalline lobes when young, in mature state globose to subglobose, black, up to 1.2 mm, slightly pruinose, up to 2 mm inside thalline lobes. Pycnidia not found. Ascomata perithecioid, immersed in the galls, globose to pyriform, black, ostiolate, glossy. Paraphysis frequently branched. Asci elongate, hyaline, clavate, bilayered, 8-spored, 100–115 × 12–20 µm. Ascospores brown to blackish brown, septate, 2-celled, obclavate to cylindrical, 20–25 × 5.5–8 µm, granules 3–4.5 µm. Host: *Zeroviella laxa*, growing on rock in cold deserts.

Comments: The lichenicolous fungus *Polycoccum clauderouxii* on Pakistani material of *Zeroviella laxa* is morphologically similar to the Chinese type specimen of *P. clauderouxii*, except for having larger asci (100–115

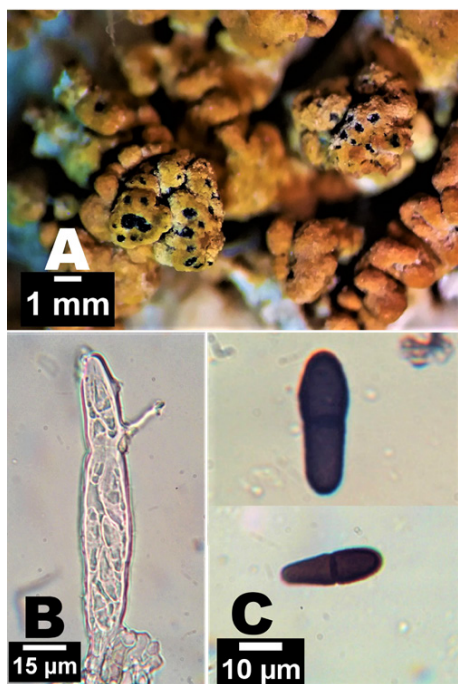


Fig. 3. *Polycoccum clauderouxii*: A = galls on the host thallus; B = elongate ascus; C = ascospores

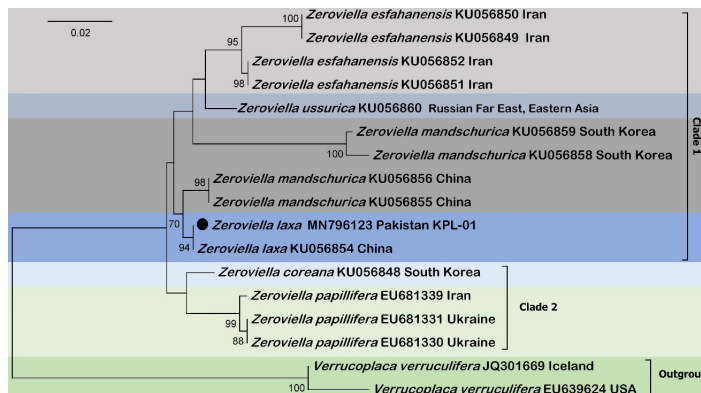


Fig. 4. Molecular phylogenetic analysis of *Zeroviella laxa*. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura 3-parameter model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The analysis involved 17 nucleotide sequences. Sequence generated from local collection is marked with ●

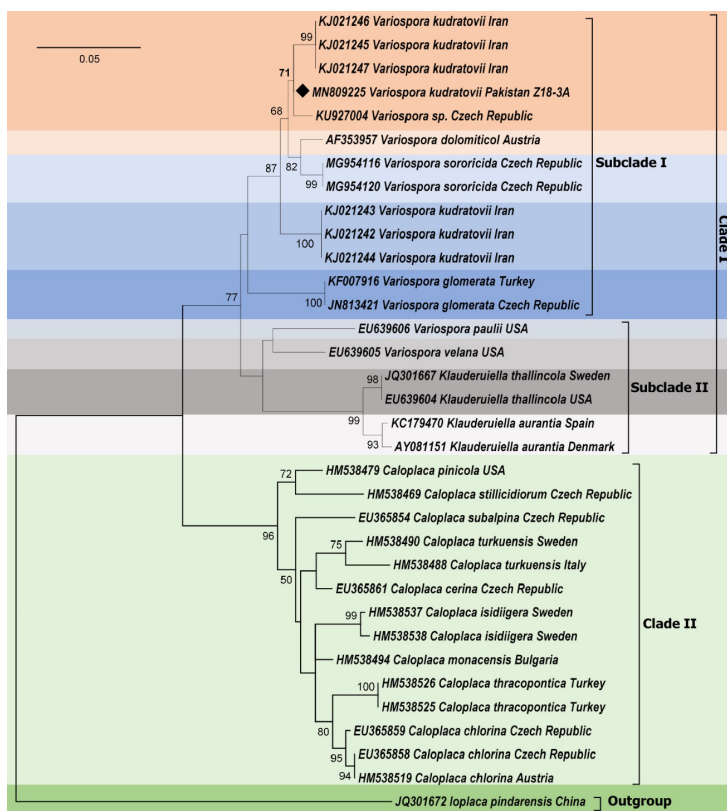


Fig. 5. Molecular phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model. The analysis involved 34 nucleotide sequences. All positions containing gaps and missing data were eliminated. Sequence generated from local collection is marked with ◆

vs. 65–70 μm) and ascospores (22–25 vs. 16–21 μm). Our Pakistani specimen differs from *Polycoccum clauzadei* Nav.-Ros. et Cl. Roux (described from France colonising on a *Xanthoria* host; Navarro-Rosinés and Roux 1998) by its larger galls on a *Zeroviella* host thalli, 8-spored asci, and longer ascospores.

Previously *Polycoccum clauderouxii* was known from the type locality, China, on *Zeroviella laxa* growing on soil in mountain deserts. This study describes *Polycoccum clauderouxii* found on *Zeroviella laxa*, growing on rock in cold desert. This fungus is reported here for the first time outside the type locality and is an addition to the fungi of Pakistan.

Material examined: Pakistan. Gilgit-Baltistan: Ghanche, Khaplu; 35.1611° N, 76.3319° E; 2,600 m a.s.l.; dry temperate area, cold desert, on *Zeroviella laxa*; coll.: A. Bano (KPL-01), 20 July 2018, (LAH35905).

PHYLOGENETIC ANALYSIS

The aligned data set of *Zeroviella laxa* comprised of 538 characters containing gaps, of which 422 sites were conserved, 113 variable, 95 parsimony informative and 18 were singleton. *Verrucoplaca verruculifera* (Vain.) S. Y. Kondr., Kärnefelt, Elix, A. Thell, Jung Kim, M. H. Jeong, N. N. Yu, A. S. Kondr. et Hur (EU639624 & JQ301669) was chosen as an outgroup. The Pakistani collection, *Zeroviella laxa* (KPL-01) clustered with the Chinese *Z. laxa* (KU056854) with 94% bootstrap support (Fig. 4) and formed a sister clade with the East Asian *Z. mandschurica* (Zahlbr.) S. Y. Kondr. et Hur.

The aligned data set of *Variospora kudratovii* included 542 characters including gaps, of which 372 sites were conserved, 163 variable, 109 parsimony informative and 53 singletons. *Ioplaca pindarensis* (Räsänen) Poelt et Hinter (JQ301672) was chosen as an outgroup. The Pakistani collection of *V. kudratovii* (Z18-3A) clustered with Iranian specimens of *V. kudratovii* (KJ021245-KJ021247) with 71% bootstrap support (Fig. 5) and formed a sister clade with *V. dolomiticola* (Hue) Arup, Søchting et Frödén and *Caloplaca sororicida* Steiner et Poelt.

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