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New species of genus *Oxneriaria* S.Y.Kondr. & Lókös
(lichenized Ascomycota, Megasporaceae)
from Khyber Pakhtunkhwa, Pakistan

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New species of genus *Oxneriaria* S.Y.Kondr. & Lökös (lichenized Ascomycota, Megasporaceae) from Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Oxneriaria immersa H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. and *O. insignis* R.Zulfiqar & Khalid, sp. nov. (Megasporaceae) are described here as new to science, both characterized by their elongating/spreading marginal areoles. The distinguishing features of *O. immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. are poriform-aspiculosporoid apothecial discs, a smaller hymenium and hypothecium (90-100 and 50-70 µm, respectively) and larger ascospores (20-25 × 10-15 µm). The key characters of *O. insignis* R.Zulfiqar & Khalid, sp. nov. are distinctly lecanorine apothecial discs, a taller hymenium and hypothecium (190-230 and 100-160 µm, respectively) and smaller ascospores (15-21 × 9-13 µm). Detailed morpho-anatomical descriptions and a nrITS phylogeny of both species along with a worldwide key to the genus *Oxneriaria* S.Y.Kondr. & Lökös are provided.

RÉSUMÉ

Nouvelles espèces du genre Oxneriaria S.Y.Kondr. & Lökös (Ascomycota lichénisé, Megasporaceae) de Khyber Pakhtunkhwa, Pakistan.

Oxneriaria immersa H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. et *O. insignis* R.Zulfiqar & Khalid, sp. nov. (Megasporaceae) sont décrits ici comme nouveaux pour la science, tous deux caractérisés par leurs aréoles marginales allongées/étalées. Les caractères distinctifs d'*O. immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. sont des disques apothéciaux poriformes-aspiculosporoides, un hyménium et un hypothécium plus petits (90-100 et 50-70 µm, respectivement) et des ascospores plus grandes (20-25 × 10-15 µm). Les caractères clés d'*O. insignis* R.Zulfiqar & Khalid, sp. nov. sont des disques apothéciaux nettement lécanoriques, un hyménium et un hypothécium plus grands (190-230 et 100-160 µm, respectivement) et des ascospores plus petites (15-21 × 9-13 µm). Des descriptions morpho-anatomiques détaillées, une phylogénie nrITS des deux espèces ainsi qu'une clé mondiale du genre *Oxneriaria* S.Y.Kondr. & Lökös sont fournies.

KEY WORDS

Pakistan,
Kohistan,
Swat Valley,
Oxneriaria,
phylogeny,
new species.

MOTS CLÉS

Pakistan,
Kohistan,
Swat,
Oxneriaria,
phylogénie,
espèces nouvelles.

INTRODUCTION

The genus *Oxneriaria* S.Y.Kondr. & Lökös, currently comprises 13 species worldwide (Zulfiqar *et al.* 2023). Its members often show a radiating thallus with wrinkled or lobate peripheral zone, rather small ascospores, and the presence of substictic acid (Moniri *et al.* 2017). The members of the genus are distributed mainly in cold polar and high-altitude localities of the northern hemisphere. They grow on siliceous rock, limestone, schistose limestone and serpentine rocks. The genus *Oxneriaria* (for the former *Aspicilia mashiginensis* group) is found to be a member of the *Sagedia* clade and positioning in distant position from the *Aspicilia/Circinaria* clade after three gene phylogeny based on nrITS, 28S nrLSU and 12S mtSSU sequences (Moniri *et al.* 2017).

For Pakistan, the genus was previously unknown due to the lack of extensive surveys and lichenological research. Recently, Zulfiqar *et al.* (2023) published the first generic record of *Oxneriaria* from Pakistan, comprising two new species viz., *O. iqbali* R.Zulfiqar, H.S.Asghar, K.Habib & Khalid and *O. kohistaniensis* R.Zulfiqar, K.Habib & Khalid, from various localities. Another new species, *O. pakistanica* M.S.Iqbal, Usman, K.Habib & Khalid, was described by Iqbal *et al.* (2023).

In this paper, two more species of the genus *Oxneriaria* are being described as new to science from Pakistan, *O. immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. and *O. insignis* R.Zulfiqar & Khalid, sp. nov. This raises the number of *Oxneriaria* species reported from Pakistan from three to five and shows their wide distribution in Pakistan.

MATERIAL AND METHODS

COLLECTION AND PRESERVATION

Specimens were collected during surveys of different sites in the districts Kohistan and Swat, KP, Pakistan in the years 2020 and 2021, focused on additions to the lichen biota of Pakistan. The specimens are deposited in Herbarium LAH, Institute of Botany, University of the Punjab, Lahore.

MORPHOLOGICAL AND CHEMICAL CHARACTERIZATION

Specimens were examined macro- and micro-morphologically with a Meiji Techno EMZ-5TR stereomicroscope and a Swift M4000-D compound microscope. Anatomical characterization and measurements were carried out by preparing and observing slides with hand-cut apothecial sections mounted in water and 5% KOH. Ascospore measurements were taken at 100 \times magnification. Secondary chemistry was analysed using spot tests and thin-layer chromatography using Solvent System C, following Orange *et al.* (2001).

DNA EXTRACTION, PCR AMPLIFICATION

AND SEQUENCING

Genomic DNA was extracted directly from a portion of the thallus with apothecia from each specimen using a modified 2% CTAB method (Gardes & Bruns, 1993). Extracted DNA was used for PCR amplification of the ITS nrDNA marker using primers

pair, *i.e.*, ITS1F forward primer (5'CTTGGTCATTTAGAG-GAAGTAA3') (Gardes & Bruns 1993) and ITS4 reverse primer (5'TCCTCCGCTTATTGATATGC3') (White *et al.* 1990).

The amplified DNA fragments (PCR products) were visualized with the help of a 1% agarose gel using an ethidium bromide through gel documentation system (Sambrook & Russel 2006). The amplified products were then sequenced commercially.

PHYLOGENETIC ANALYSIS

Forward and reverse sequences of ITS region were obtained and final sequences of each specimen were assembled using BioEdit v. 7.2.5 (Hall *et al.* 2011) and matched with other online DNA sequences available through BLAST at NCBI (<https://www.ncbi.nlm.nih.gov/guide/>). A comprehensive representation of currently available sequences used for the phylogenetic analyses are presented in Appendix 2, together with voucher numbers, GenBank accession numbers and country of origin. The BLAST search showed that the sequences most similar to the new species belong in *Oxneriaria*. Related sequences of *Oxneriaria* species were then selected from the NCBI database for the phylogenetic analysis. *Megaspora verrucosa* (Ach.) Arcadia & A.Nordin (KP314363) was chosen as an outgroup.

The final alignment was made by using MAFFT version 7 (Katoh *et al.* 2019). All sequences were trimmed at their ends to nearly equal number of sites using BioEdit v. 7.2.5. The final Maximum Likelihood phylogram was made in RAxML-HPC2 using XSEDE tool (8.2.10) with 1000 bootstrap values. The evolutionary history was inferred using the Maximum Likelihood (ML) method by bootstrap testing of 1000 replicates. Phylogenetic trees were visualized using FigTree v. 1.4.2 (Rambaut 2012). Newly generated sequences were deposited in GenBank (Appendix 2).

RESULTS

The data matrix includes 502 characters, of which 338 sites were conserved, 156 were variable, 115 were parsimony informative and 41 were singleton sites. The newly generated sequences of *Oxneriaria immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. (LAH37898, LAH37911) and *O. insignis* R.Zulfiqar & Khalid, sp. nov. (LAH37900, LAH37899) formed a sister clade to *O. iqbali* (ON392706, ON392709, ON392710) recently described from Pakistan (Zulfiqar *et al.* 2023). Moreover they were separated into two sister clades, supported by a strong bootstrap value (BS = 100%), which shows that they represent two separate novel species (Fig. 1).

Oxneriaria immersa
H.S.Asghar, R.Zulfiqar & Khalid, sp. nov.
(Fig. 2)

Characterized by distinctly elongating/spreading marginal areoles, absence of prothallus, poriform-aspicilioid apothecial discs, larger ascospores (20–25 \times 10–15 μm) and the presence of norstictic acid.

HOLOTYPE. — **Pakistan.** Khyber Pakhtunkhwa Province, Kohistan, Dassu, on calcareous rocks, 841 m a.s.l., 35°35'N,

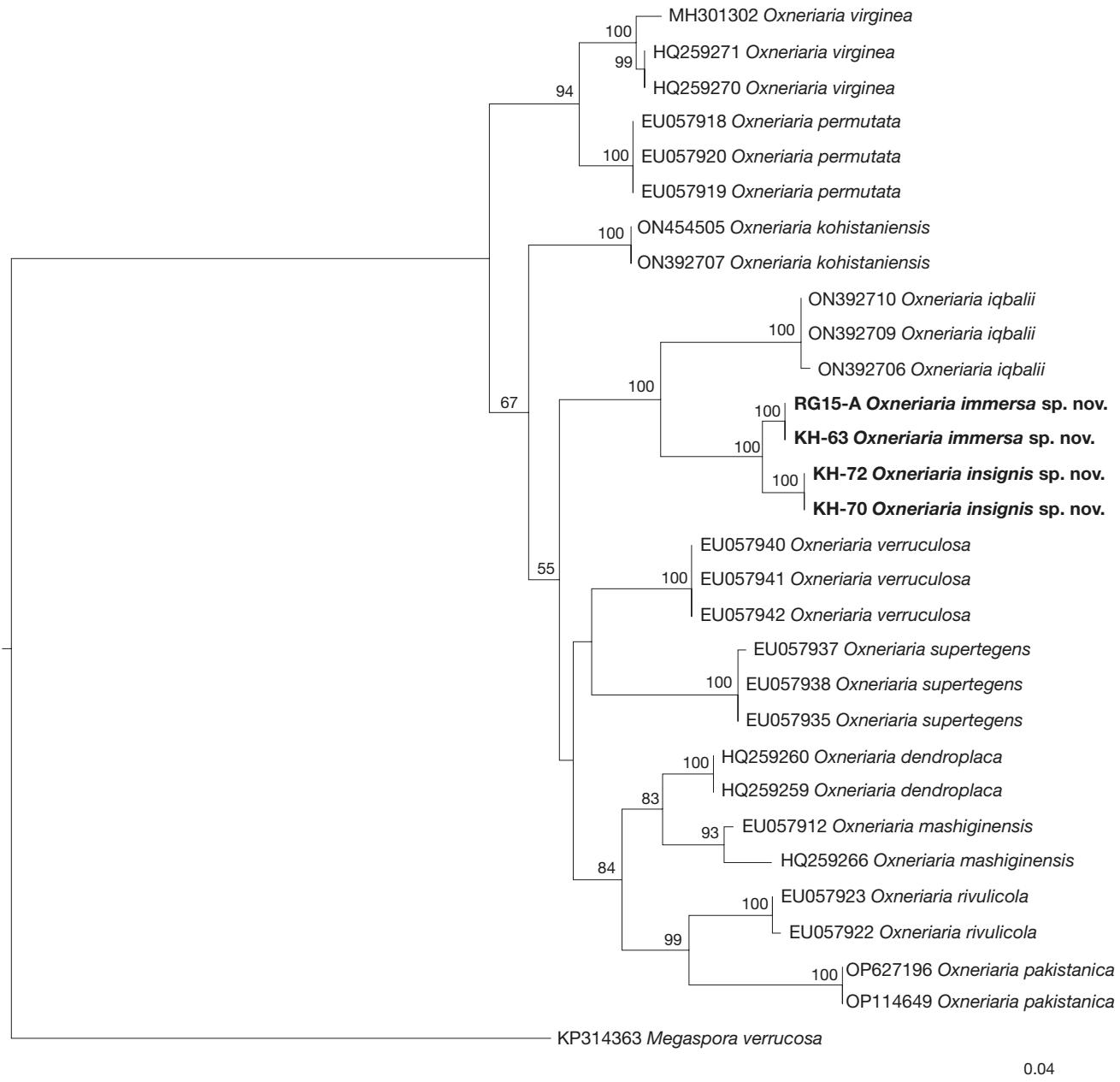


FIG. 1. — Molecular phylogenetic analysis by maximum likelihood (ML) method based on ITS sequences. Bootstrap values >50% based on 1000 replicates for ML are shown at the branches and novel sequences generated during this study are in **bold**.

73°37'E, 12.VII.2020, K. Habib & A.N. Khalid, *KH-63* (holo-, LAH[LAH37898]; GenBank[[OQ249532](#)]).

PARATYPE. — **Pakistan.** Khyber Pakhtunkhwa, Swat, Miandam, on calcareous rocks, 1800 m a.s.l., 35°41'N, 72°48'E, 15.IX.2021, M. Usman, *RG-15-A* (para-, LAH[LAH37911]; GenBank[[OQ152021](#)]).

ETYMOLOGY. — The specific epithet 'immersa' (Latin) refers to the immersed apothecial discs.

CHEMISTRY. — K+ (yellow turning red), C-, KC-. TLC: norstictic acid.

HABITAT AND ECOLOGY. — The holotype (LAH37898) was found on calcareous rocks in a dry, temperate area, in an open situation, exposed to sun and rain. The temperature typically varies from

-8 to 28°C and the annual rainfall varies between 700 and 800 mm. The topography of the area has extreme variations in elevation. The type specimen was found at an elevation of 841 m a.s.l. whereas the paratype (LAH37911) was found at an elevation of 1800 m a.s.l., in thick hilly forests of Miandam, Swat Valley, with mean maximum and minimum temperature of 30°C and -2°C, respectively.

MYCOBANK. — MB849311.

DESCRIPTION

Thallus crustose-areolate, well-defined, up to 4 cm across, pruinose, 200-400 µm thick in section. Areoles: rounded to irregular, plane to convex, 0.3-1 mm in diameter, marginally elongated/spreading giving a lobate view, up to 1.5 mm long.

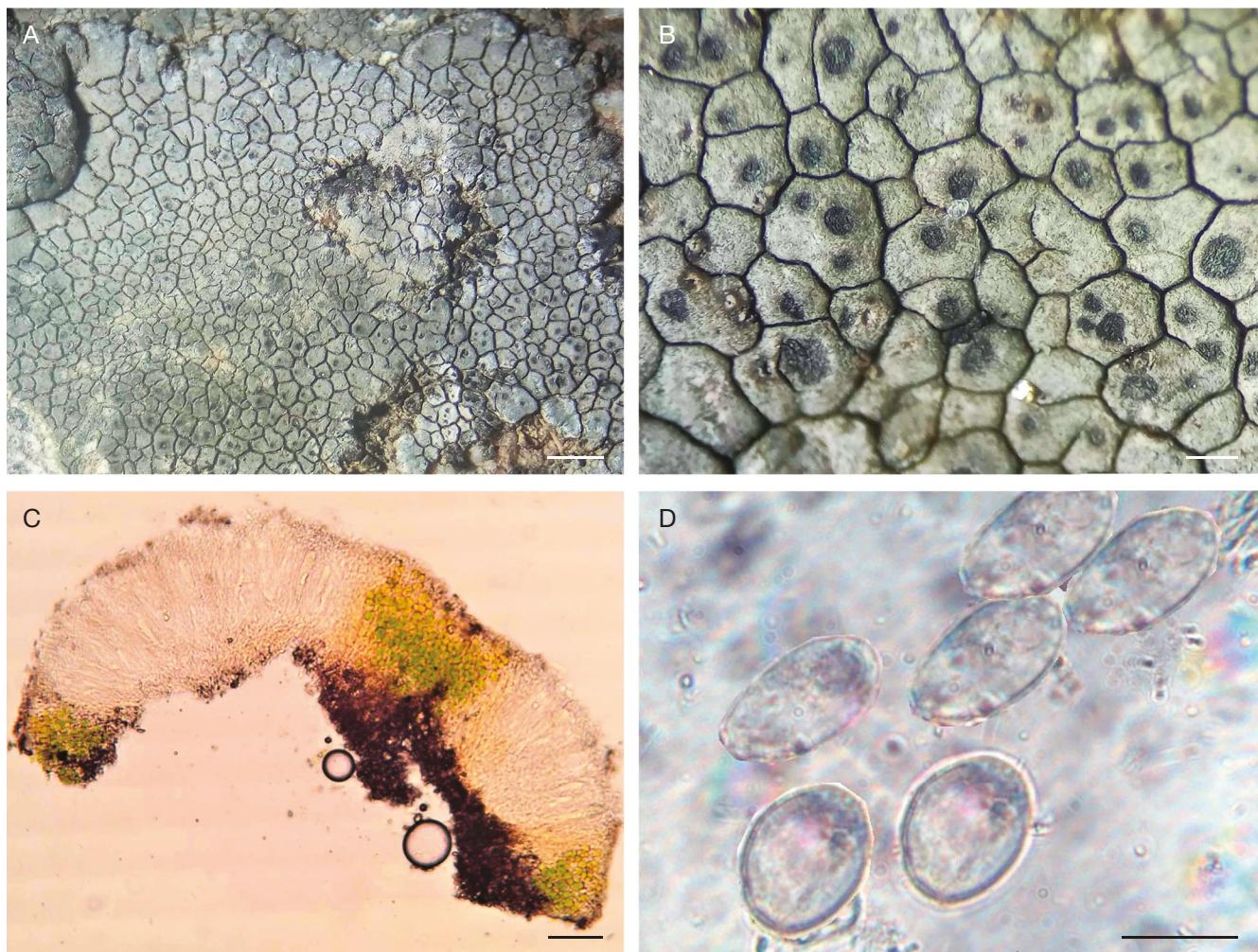


FIG. 2. — *Oxneriaria immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. (holotype): **A**, crustose-areolate thallus; **B**, apothecia; **C**, cross section of an apothecium; **D**, ascospores. Scale bars: A, 1 cm; B, 0.5 mm; C, 50 µm; D, 15 µm.

Prothallus: absent. Color: dark grey when dry, greenish grey when wet. Upper cortex: dark brown, 10-15 µm thick, cells rounded, 5-9 µm in diameter. Algal layer: 40-70 µm thick, photobiont chlorococcoid, 9-12 µm in diameter. Medulla: 200-250 µm thick, white, hyphae hyaline, 3-4 µm wide. Apothecia: poriform-aspicilioid, 1-3 per areole. Disc: black, smooth, shiny, epruinose, plane to concave, 0.1-0.7 mm in diameter. Margins: indistinct. Epiphyllum: brown, 10-15 µm tall. Hymenium: hyaline, 90-100 µm tall. Hypothecium: hyaline, 50-70 µm tall. Ascii: hyaline, clavate, 90-130 × 15-20 µm. Ascospores: hyaline, subglobose to ellipsoid, 20-25 × 10-15 µm.

NOTES

Morphologically *Oxneriaria immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. resembles *O. virginea* (Hue) S.Y.Kondr. & Lökös but differs in having poriform-aspicilioid apothelial discs (vs poriform or slightly protruding), larger ascospores 20-25 × 10-15 µm (vs smaller, 14-22 × 9-12 µm) and a different chemistry, i.e., the presence of norstictic acid (vs no substance detected or once only with stictic acid) (Nordin et al. 2011; Halici et al. 2018).

Oxneriaria immersa H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. is also morphologically close to *O. kohistaniensis* but differs in having a crustose-areolate thallus (vs verrucose-areolate), elongating/radiating marginal areoles (vs non radiating), a smaller hymenium 90-100 µm (vs 100-150 µm), sub-moniliform (vs simple) paraphyses and larger ascospores 20-25 × 10-15 µm (vs smaller, 15-20 × 9-12 µm) (Zulfiqar et al. 2023). From *Oxneriaria permutata* (Zahlbr.) S.Y.Kondr. & Lökös the new taxon differs in having an absence of prothallus (vs presence), a slightly taller hypothecium 50-70 µm (vs 40-50 µm), larger ascospores 20-25 × 10-15 µm (vs smaller, 15-22 × 9-12 µm), large conidia 15-35 µm (vs 16-18 µm) and a different chemistry, presence of norstictic acid (vs no substance detected) (Nimis 2016).

Another, phylogenetically, related taxon is *Oxneriaria iqbalii*, which has similar thallus coloration and nearly identical size of the ascospores but the new taxon differs in having elongating/radiating marginal areoles (vs non radiating), a smaller hymenium 90-100 µm (vs 130-160 µm) and the presence of norstictic acid (vs no substance detected) (Zulfiqar et al. 2023). See also Appendix 1.

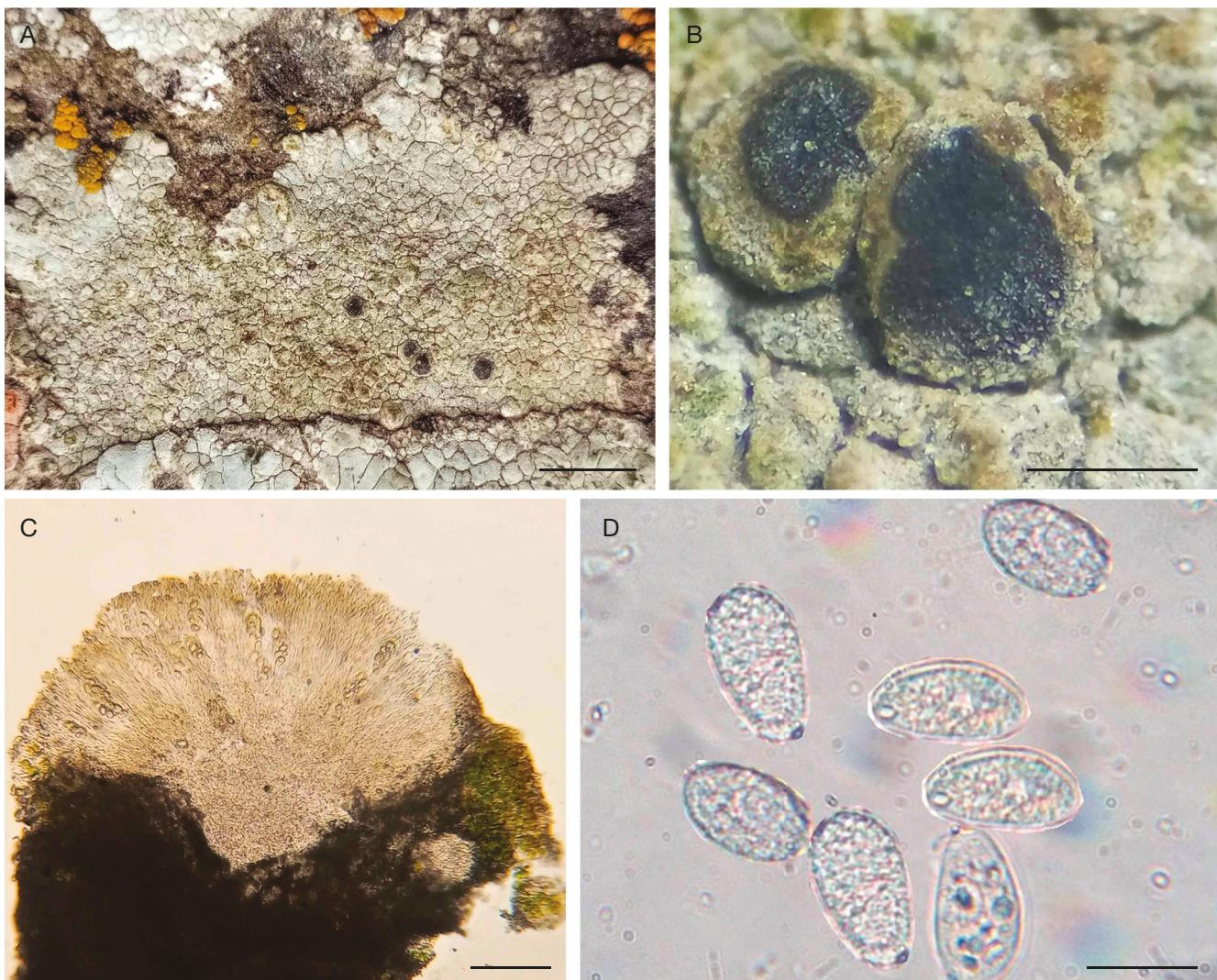


FIG. 3. — *Oxneriaria insignis* R.Zulfiqar & Khalid, sp. nov. (holotype): **A**, crustose-areolate thallus; **B**, apothecia; **C**, cross section of an apothecium; **D**, ascospores. Scale bars: A, 1 cm; B, 0.5 mm; C, 150 µm; D, 15 µm.

Oxneriaria insignis
R.Zulfiqar & Khalid, sp. nov.
(Fig. 3)

Characterized by distinctly lecanorine apothecial discs, taller hymenium and hypothecium (190–230, 100–160 µm) respectively and smaller ascospores (15–21 × 9–13 µm).

HOLOTYPE. — **Pakistan.** Khyber Pakhtunkhwa Province, Kohistan, Dassu, on calcareous rocks, 841 m a.s.l., 35°35'N, 73°37'E, 12.VII.2020, K. Habib & A.N. Khalid, KH-72 (holo-, LAH[LAH37900]; GenBank[[OQ249530](#)]).

PARATYPE. — **Pakistan.** Khyber Pakhtunkhwa, Kohistan, Razika Seo Valley, on calcareous rocks, 1067 m a.s.l., 35°26'N, 73°27'E, 9.IX.2020, K. Habib & A.N. Khalid, KH-70 (para-, LAH[LAH37899]; GenBank[[OQ249531](#)]).

ETYMOLOGY. — The specific epithet ‘insignis’ (Latin) refers to the distinct lecanorine apothecial discs.

CHEMISTRY. — Thallus, K+ (yellowish green), C-, KC-; Medulla, K+ (yellow), C-, KC-. TLC: cryptostictic acid.

HABITAT AND ECOLOGY. — The holotype (LAH37900) was found on calcareous rocks, in a dry, temperate area, in an open situation exposed to sun and rain. The temperature typically varies from –8 to 28°C with an annual rainfall varying between 700 and 800 mm. The topography of the area has extreme variations in elevation. This species was found at an elevation of 841 m a.s.l. The paratype (LAH37899) was found at an elevation of 1067 m a.s.l., having the same habitat as the holotype.

MYCOBANK. — MB849310.

DESCRIPTION

Thallus crustose-areolate, up to 3 cm across, dull, section in thallus 250–350 µm thick. Areoles: rounded to irregular, plane to convex, 0.3–0.8 mm in diameter, more or less distinctly elongated at the margins, up to 1 mm long, 0.5 mm wide. Color: whitish grey with brownish tinge when dry, greenish grey when wet. Upper cortex: paraplectenchymatous, 10–15 µm thick, cells rounded, 7–9 µm in diameter. Algal layer: 70–90 µm thick, continuous, even, photobiont chlorococcoid, 6–9 µm in diameter. Medulla: 150–250 µm thick, white, hyphae hyaline, 3–4 µm

KEY TO WORLDWIDE GENUS *OXNERIARIA* S.Y.KONDR. & LÖKÖS

1. Thallus zonate 2
- Thallus rimose-areolate/verrucose 3
2. Thallus whitish grey to grey with brownish tinge, prothallus present, hymenium length unknown, ascospores $13.6-[17.1]-22.6 \times 7.9-[9.6]-12.4 \mu\text{m}$ *O. rivulicola* (H.Magn.) S.Y.Kondr. & Lökös
3. Apothecia lecanorine, marginally elongated areoles 4
- Apothecia poriform/aspiciloid, marginally non-elongated areoles 5
4. Thallus whitish grey with brownish tinge, prothallus absent, hymenium $190-230 \mu\text{m}$ tall, ascospores $15-21 \times 9-13 \mu\text{m}$ *O. insignis* R.Zulfiqar & Khalid, sp. nov.
- Thallus whitish, prothallus present, hymenium $90-100 \mu\text{m}$ tall, ascospores $14-17 \times 9-13 \mu\text{m}$ *O. hayrenii* (H.Magn.) S.Y.Kondr. & Lökös
5. Apothecia smaller 6
- Apothecia larger 15
6. Secondary metabolites absent 7
- Secondary metabolites present 8
7. Thallus whitish grey to grey, prothallus absent, hymenium $130-160 \mu\text{m}$ tall, ascospores $20-26 \times 10-14 \mu\text{m}$ *O. iqbalii* R.Zulfiqar, H.S.Asghar, K.Habib & Khalid
- Thallus grey-white to pale blue-grey, prothallus present, hymenium $100-115 \mu\text{m}$ tall, ascospores $15-22 \times 9-12 \mu\text{m}$ *O. permutata* (Zahlbr.) S.Y.Kondr. & Lökös
8. Conidia absent 9
- Conidia present 10
9. Thallus whitish, prothallus indistinct/absent, hymenium length unknown, ascospores $14.7-[17.9]-21.5 \times 9.0-[11.1]-12.4 \mu\text{m}$ *O. virginea* (Hue) S.Y.Kondr. & Lökös
10. Conidia shorter 11
- Conidia larger 14
11. Paraphyses simple 12
- Paraphyses submoniliform 13
12. Thallus off-white to whitish grey or pale-grey, prothallus absent, hymenium $100-150 \mu\text{m}$ tall, ascospores $15-20 \times 9-12 \mu\text{m}$ *O. kohistaniensis* R.Zulfiqar, K.Habib & Khalid
13. Thallus whitish grey, grey to blue-grey, prothallus present, hymenium $70-80 \mu\text{m}$ tall, ascospores $12-17 \times 7.5-9 \mu\text{m}$ *O. verruculosa* (Kremp.) S.Y.Kondr. & Lökös
- Thallus brown to dark greenish-grey, prothallus present, hymenium height, ascospores $13.6-[15.7]-19.2 \times 7.9-[8.9]-11.9 \mu\text{m}$ *O. dendroplaca* (H.Magn.) S.Y.Kondr. & Lökös
14. Thallus dark grey to greenish grey, prothallus absent, hymenium $90-100 \mu\text{m}$ tall, ascospores $20-25 \times 10-15 \mu\text{m}$ *O. immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov.
15. Soralia present 16
- Soralia absent 17
16. Thallus pale to dark grey or brownish grey, prothallus present, unknown hymenium height, ascospores $13.6-[15.4]-18.1 \times 9.0-[9.8]-11.3 \mu\text{m}$ *O. mashiginensis* (Zahlbr.) S.Y.Kondr. & Lökös
17. Thallus whitish grey to grey, prothallus absent, hymenium $100-155 \mu\text{m}$ tall, ascospores $10-18 \times 7-10 \mu\text{m}$ *O. pakistanica* M.S.Iqbal, Usman, K.Habib & Khalid
- Thallus whitish, pale grey or grey-brown, prothallus present, hymenium $115-140 \mu\text{m}$ tall, ascospores $15-25 \times 10-16 \mu\text{m}$ *O. supertegens* (Arnold) S.Y.Kondr. & Lökös

wide. Apothecia: lecanorine, confluent, usually one per areole. Disc: black, plane to weakly concave, pruinose, 0.5-1 mm in diameter. Margins: thick, persistent, concolorous with thallus. Epiphymenium: brown, 10-15 μm tall. Hymenium: hyaline,

190-230 μm tall. Hypothecium: hyaline, 100-160 μm tall. Thalline exciple: 80-100 μm thick. Proper exciple: indistinct. Ascii: 8-spored, hyaline, clavate, $85-115 \times 30-40 \mu\text{m}$. Ascospores: broadly ellipsoid to ovoid, hyaline, $15-21 \times 9-13 \mu\text{m}$.

NOTES

From the phylogenetically related *Oxneriaria immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov., *O. insignis* R.Zulfiqar & Khalid, sp. nov. differs in having lecanorine apothecial discs (vs poriform-aspiciloid), a taller hymenium and hypothecium 190–230 µm, 100–160 µm (vs 90–100 µm, 50–70 µm), respectively, simple paraphyses (vs sub-moniliform) and smaller ascospores 15–21 × 9–13 µm (vs 20–25 × 10–15 µm) (Appendix 1). Phylogenetically, *O. insignis* R.Zulfiqar & Khalid, sp. nov. also makes a distinct branch from *O. immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov., further reveals its novelty (Fig. 1).

Oxneriaria insignis R.Zulfiqar & Khalid, sp. nov. also resembles *O. supertegens* (Arnold) S.Y.Kondr. & Lökös but the new taxon differs in having distinctly lecanorine apothecial discs (vs lecanorine-aspiciloid), absence of prothallus (vs presence), a taller hymenium 190–230 µm (vs 115–140 µm), simple paraphyses (vs sub-moniliform), smaller ascospores 15–21 × 9–13 µm (vs 15–25 × 10–16 µm) and a different chemistry, cryptostictic acid (vs aspicilin) (Nimis 2016).

From *Oxneriaria iqbalii*, the new taxon differs in having elongating/radiating marginal areoles (vs non radiating), lecanorine apothecial discs (vs aspiciloid), a taller hymenium and hypothecium 190–230 µm, 100–160 µm (vs 130–160 µm, 40–50 µm), respectively, smaller ascospores 15–21 × 9–13 µm (vs 20–26 × 10–14 µm) and a different chemistry, cryptostictic acid (vs no substance detected) (Zulfiqar *et al.* 2023).

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REFERENCES

- GARDES M. & BRUNS T. D. 1993. — ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2 (2): 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- HALICI M. G., BARTAK M. & GÜLLÜ M. 2018. — Identification of some lichenised fungi from James Ross Island (Antarctic Peninsula) using nrITS markers. *New Zealand Journal of Botany* 56 (3): 276–290. <https://doi.org/10.1080/0028825X.2018.1478861>
- HALL T., BIOSCIENCES I. & CARLSBAD C. 2011. — BioEdit: an important software for molecular biology. *GERF Bulletin of Biosciences* 2: 60–61.
- IQBAL M. S., USMAN M., HABIB K. & KHALID A. N. 2023. — *Oxneriaria pakistanica* sp. nov. (Megasporaceae, Pertusariales, Ascomycota) from Darel Valley, Gilgit Baltistan, Pakistan. *Phytotaxa* 579 (2): 125–131. <https://doi.org/10.11646/phytotaxa.579.2.6>
- KATOH K., ROZEWICKI J. & YAMADA K. D. 2019. — MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20 (4): 1160–1166. <https://doi.org/10.1093/bib/bbx108>
- MONIRI M., GROMAKOVA A. B., LÖKÖS L. & KONDRAKYUK S. Y. 2017. — New members of the Megasporaceae (Pertusariales, lichen-forming Ascomycota): *Megaspora iranica* spec. nova and *Oxneriaria* gen. nova. *Acta Botanica Hungarica* 59 (3–4): 343–370. <https://doi.org/10.1556/034.59.2017.3-4.5>
- NIMIS P. L. 2016. — ITALIC – the information system on Italian lichens. Version 5.0. University of Trieste, Department of Biology, Trieste. Available at: <http://dryades.units.it/italic> (accessed on 31 December 2022).
- NORDIN A., BJÖRN O. L. & TIBELL L. 2011. — Two new *Aspicilia* species from Fennoscandia and Russia. *The Lichenologist* 43 (1): 27–37. <https://doi.org/10.1017/S0024282910000629>
- ORANGE A., JAMES P. & WHITE F. J. 2001. — *Microchemical Methods for the Identification of Lichens*. British Lichen Society, London, 101 p.
- RAMBAUT A. 2012. — FigTree v1.4.2. Available at: <http://tree.bio.ed.ac.uk/software/figtree>.
- SAMBROOK J. & RUSSELL D. W. 2006. — Purification of nucleic acids by extraction with phenol: chloroform. *Cold Spring Harbor Protocols* 2006: pdb-prot4455. <https://doi.org/10.1101/pdb.prot4455>
- WHITE T. J., BRUNS T., LEE S. & TAYLOR J. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in INNIS N., GELFAND D., SNINSKY J. & WHITE T. J. (eds), *PCR Protocols: A Guide to the Methods and Applications*. Academic Press, New York: 315–322.
- ZULFIQAR R., ASGHAR H. S., HABIB K. & KHALID A. N. 2023. — Two new species of the genus *Oxneriaria* (lichenized Ascomycota: Megasporaceae) from Pakistan. *Plant Systematics and Evolution* 309 (1): 2. <https://doi.org/10.1007/s00606-022-01836-w>

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APPENDICES

APPENDIX 1. — A brief comparison of *Oxneriaria immersa* H.S.Asghar, R.Zulfiqar & Khalid, sp. nov. and *O. insignis* R.Zulfiqar & Khalid, sp. nov. with related taxa.

Species/ Characters	<i>O. immersa</i> sp. nov.	<i>O. insignis</i> sp. nov.	<i>O. iqbalii</i>	<i>O. kohistaniensis</i>	<i>O. permutata</i>	<i>O. supertegens</i>	<i>O. virginea</i>
Thallus type	crustose-areolate	crustose-areolate	crustose-areolate	verrucose-areolate	rimose-areolate	rimose, rarely distinctly areolate	crustose-areolate
Marginal areoles	elongated/spreading giving a lobate view	more or less indistinctly elongated at margins	non-radiating	non-radiating	more or less elongate and indistinctly radiating	—	elongated at the periphery and give a lobate view
Apothecia	poriform-aspiciloid	lecanorine	aspiciloid	poriform-aspiciloid	lecanorine-aspiciloid	lecanorine-aspiciloid	poriform, slightly protruding
Prothallus	absent	absent	absent	absent	present	present	indistinct/absent
Hymenium (height)	90-100 µm	190-230 µm	130-160 µm	100-150 µm	100-115 µm	115-140 µm	—
Hypothecium (height)	50-70 µm	100-160 µm	40-50 µm	60-70 µm	40-50 µm	—	—
Paraphyses (type)	submoniliform	simple	submoniliform	simple	submoniliform	submoniliform	—
Size of ascospores	20-25×10-15 µm	15-21×9-13 µm	20-26×10-14 µm	15-20×9-12 µm	15-22×9-12 µm	15-25×10-16 µm	14-22×9-12 µm
Conidia Chemistry	15-35 µm norstictid acid	15-20 µm cryptostictic acid	15-30 µm no substance detected	15-20 µm cryptostictic acid, 2'-O-methylsulphurellin	16-18 µm no substance detected	17-40 µm aspicilin	absent no substance detected/once only with stictic acid
References	this paper	this paper	Zulfiqar et al. (2023)	Zulfiqar et al. (2023)	Nimis (2016)	Nimis (2016)	Nordin et al. (2011); Halici et al. (2018)

APPENDIX 2. — Taxa used in the phylogenetic analyses. The sequences generated in the present study are marked with *.

Taxa	ITS GenBank accession number	Voucher number	Country
<i>Megaspora verrucosa</i>	KP314363	ZT2013098	Svalbard
<i>Oxneriaria dendroplaca</i>	HQ259259	Nordin 5952 (UPS)	Sweden
<i>Oxneriaria dendroplaca</i>	HQ259260	Nordin 6366 (UPS)	Sweden
<i>Oxneriaria immersa</i> sp. nov.*	OQ249532	LAH37898	Pakistan
<i>Oxneriaria immersa</i> sp. nov.*	OQ152021	LAH37911	Pakistan
<i>Oxneriaria insignis</i> sp. nov.*	OQ249530	LAH37900	Pakistan
<i>Oxneriaria insignis</i> sp. nov.*	OQ249531	LAH37899	Pakistan
<i>Oxneriaria iqbalii</i>	ON392710	LAH37155	Pakistan
<i>Oxneriaria iqbalii</i>	ON392709	LAH37156	Pakistan
<i>Oxneriaria iqbalii</i>	ON392706	LAH37157	Pakistan
<i>Oxneriaria kohistaniensis</i>	ON454505	LAH37151	Pakistan
<i>Oxneriaria kohistaniensis</i>	ON392707	LAH37152	Pakistan
<i>Oxneriaria mashiginensis</i>	EU057912	Nordin 5790 (UPS)	Sweden
<i>Oxneriaria mashiginensis</i>	HQ259266	Tibell 23557 (UPS)	Sweden
<i>Oxneriaria pakistanica</i>	OP627196	LAH37501	Pakistan
<i>Oxneriaria pakistanica</i>	OP114649	LAH37495	Pakistan
<i>Oxneriaria permutata</i>	EU057920	Nordin 6038 (UPS)	Sweden
<i>Oxneriaria permutata</i>	EU057921	Nordin 6039 (UPS)	Sweden
<i>Oxneriaria permutata</i>	EU057918	Nordin 6027 (UPS)	Sweden
<i>Oxneriaria rivulicola</i>	EU057922	Nordin 5957 (UPS)	Sweden
<i>Oxneriaria rivulicola</i>	EU057923	Nordin 5960 (UPS)	Sweden
<i>Oxneriaria supertegens</i>	EU057936	Owe-Larsson 9002 (UPS)	Norway
<i>Oxneriaria supertegens</i>	EU057937	Owe-Larsson 9011 (UPS)	Norway
<i>Oxneriaria supertegens</i>	EU057938	Nordin 6023 (UPS)	Sweden
<i>Oxneriaria verruculosa</i>	EU057940	Owe-Larsson 9007 (UPS)	Norway
<i>Oxneriaria verruculosa</i>	EU057941	Owe-Larsson 9003 (UPS)	Norway
<i>Oxneriaria verruculosa</i>	EU057942	Nordin 5942 (UPS)	Sweden
<i>Oxneriaria virginea</i>	HQ259270	Nordin 6017a (UPS)	Sweden
<i>Oxneriaria virginea</i>	HQ259271	Ebbestad SVL1-1 (UPS)	Svalbard
<i>Oxneriaria virginea</i>	MH301302	JR 0.009	Antarctica