

A new corticolous *Megaspora* (Megasporaceae) species from Armenia

Authors: Zakeri, Zakieh, Gasparian, Arsen, and Aptroot, André

Source: *Willdenowia*, 46(2) : 245-251

Published By: Botanic Garden and Botanical Museum Berlin (BGBM)

URL: <https://doi.org/10.3372/wi.46.46205>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

ZAKIEH ZAKERI¹, ARSEN GASPARYAN^{2*} & ANDRÉ APTROOT³

A new corticolous *Megaspora* (*Megasporaceae*) species from Armenia

Version of record first published online on 28 July 2016 ahead of inclusion in August 2016 issue.

Abstract: The corticolous species *Megaspora cretacea* is described as new for science. The species is characterized by a thick, cretaceous thallus and a pale bluish, rather coarse soredia covering most of the thallus. It grows on *Juniperus* bark in open arid woodlands in Armenia. A key to the three species included in the genus *Megaspora* is presented. Phylogenetic analysis based on nrITS sequences revealed that *M. cretacea* clustered within the *Megaspora* clade as sister species to *M. rimisorediata* with high support.

Key words: lichens, *Megasporaceae*, *Megaspora*, taxonomy, new species, sorediate, *Juniperus*, South Caucasus, Armenia, Khosrov Forest State Reserve, ITS

Article history: Received 29 March 2016; peer-review completed 3 May 2016; received in revised form 18 May 2016; accepted for publication 31 May 2016.

Citation: Zakeri Z., Gasparyan A. & Aptroot A. 2016: A new corticolous *Megaspora* (*Megasporaceae*) species from Armenia. – Willdenowia 46: 245–251. doi: <http://dx.doi.org/10.3372/wi.46.46205>

Introduction

According to recent phylogenetic studies, *Megasporaceae* Lumbsch is monophyletic (Nordin & al. 2010). They are mostly saxicolous crustose lichens (Valadbeigi & al. 2011). In Armenia, they are among the more common lichen families, in species diversity but especially in abundance, covering large parts of most siliceous rock faces and also present on limestone. The genus *Megaspora* (Clauzade & Cl. Roux) Hafellner & V. Wirth is closely related to the genus *Circinaria* Link (Nordin & al. 2010). It is an exception within the family, in that it is predominantly corticolous, with two species on trees, one of which is also occasionally terricolous. Both currently accepted species, *M. rimisorediata* Valadbeigi & A. Nordin and *M. verrucosa* (Ach.) Hafellner & V. Wirth (Valadbeigi & al. 2011), occur in Armenia (Gasparyan & Sipman 2013; Harutyunyan & al. 2011).

During a lichenological excursion to Armenia, organized by the second author, we collected a sorediate crustose lichen at the bases of trees of *Juniperus polycarpus* K. Koch in the Khosrov Forest State Reserve. The territory of the Reserve was already considered as a protected area in the fourth century C.E. by the Armenian king Khosrov Kotak (Khanjyan 2004). In 1958, the Khosrov Forest was officially declared as a state reserve (Anonymous 2008). The natural landscapes of phryganoid vegetation, open arid forests and montane steppes have high biological diversity and are recognized as a priority area for conservation. So far, 1849 species of vascular plants (including 24 endemic species) and 176 lichenized and lichenicolous fungi have been registered in the reserve (Anonymous 2008; Gasparyan & al. 2015).

While in the field it was not possible to recognize the collected specimens as representatives of *Megasporaceae*; rather they gave the impression of a species of the

1 Senckenberg Museum of Natural History, Am Museum 1, 02826 Görlitz, Germany.

2 Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, Königin-Luise-Str. 6–8, 14195 Berlin, Germany; *e-mail: a.gasparyan@bgbm.org (author for correspondence).

3 ABL Herbarium, G.v.d.Veenstraat 107, NL-3762 XK Soest, The Netherlands.

Caloplaca albolutescens (Nyl.) H. Olivier / *C. teicholyta* (Ach.) J. Steiner group or, less likely, a species of *Lepraria* Ach., but subsequent examination of the material revealed a few black apothecia immersed in the thallus, with large, thin-walled ascospores and a greenish epihymenium, suggesting *Megasporaceae*.

In the framework of a phylogenetic study of Asian *Megasporaceae*, the first author sequenced the material and found that it clusters inside *Megaspora* as a sister species to *M. rimisorediata*. Therefore, we describe it as a new species in this genus.

Megaspora rimisorediata has a restricted distribution. It was described from Iran (Valadbeigi & al. 2011) and later found also in S Armenia (Gasparyan & Sipman 2013; Gasparyan & al. 2015). *Megaspora verrucosa* has been reported from Europe, Africa, Asia, North and South America, New Zealand and Antarctica (Smith & al. 2009).

Currently, Armenia is the centre of diversity of the genus, with all three currently known species present. The new species has been reported from two localities. Further comprehensive studies are required to explore distributional and ecological patterns of the new species.

Material and methods

Identification and descriptive work was carried out in Soest and BGBM using an Olympus SZX7 stereomicroscope and an Olympus BX50 compound microscope with interference contrast, connected to a Nikon Coolpix digital camera. Sections were mounted in tap water, in which also all measurements were taken. The specimens from this study are preserved in ABL and B (herbarium codes after Thiers 2016+). The chemistry of the type specimen was investigated by thin-layer chromatography (TLC) using solvent A (Orange & al. 2001).

DNA extraction — We used nuclear ITS1-5.8S-ITS2 rDNA sequences of specimens in the molecular study because it has been shown that among the regions of the ribosomal cistron, the internal transcribed spacer (ITS) region has the highest probability of successful identification for a range of fungi (Schoch & al. 2012; Divakar & al. 2015). Total DNA was extracted from freshly collected material according to Park & al. (2014). We followed the instructions given in that paper except for the following steps: we used a 1 × 1 mm² piece of medulla and mixed it with bead-beader without liquid nitrogen; instead of chloroform we used Roti®-C/I (chloroform/isoamyl alcohol at a ratio of 24:1); and at the end we used only 30 µL TE buffer instead of 100 µL because of the low quantity of DNA.

PCR amplifications and sequencing — The primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990) was used for the PCR amplifications. PCR amplifications were performed in a 12.5 µL volume contain-

ing 2 µL undiluted DNA, 0.5 µL of each primer (10 mM), 6.4 µL of sterile water, 1 µL dNTP (2 mM), 1 µL s-buffer, 1 µL MgCl₂, 0.1 µL Taq-polymerase. Thermal cycling parameters were initial denaturation for 5 min at 95 °C, followed by 30 cycles of 30 sec at 95 °C, 30 sec at 54 °C, and 1 min at 72 °C; following the last cycle a final extensions for 3 min at 72 °C was included. Amplification product was viewed by electrophoresis on 1% agarose gels and stained with ethidiumboromide and was purified by adding 2 µL ExoSAP-IT™ (Exonuclease 1-shrimp alkaline phosphatase) to 5 µL of the PCR products, followed by a heat treatment of 15 min at 37 °C and 15 min at 80 °C. The PCR product was sequenced in both directions by Bik-F Laboratory in Frankfurt am Main. For the reconstruction of a phylogenetic tree, all ITS sequences of *Megasporaceae* from Valadbeigi & al. (2011) were used as well as seven accessible sequences of *Megaspora* from NCBI GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). Two sequences were obtained from the new species and submitted to the NCBI GenBank (Table 1). The sequences were aligned through the Muscle V4 program web server (Edgar 2004) with the default settings. The aligned sequences were adjusted manually in PhyDE software (Müller & al. 2010). Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) was used to eliminate ambiguously aligned positions, applying settings allowing for smaller final blocks, gap position within the final blocks and less strict flanking position (Castresana 2000).

Phylogenetic analyses — MrModeltest (Nylander 2004) was used to determine the most appropriate model using AIC, with GTR + I + G found to be the best-fitting model of nucleotide evolution. Bayesian inference of phylogeny with Markov chain Monte Carlo sampling was performed on the Bayesian inference of phylogeny with Markov chain Monte Carlo sampling was performed on the 477 unambiguously aligned nucleotide positions. Bayesian analyses were conducted with MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003) using the GTR model of nucleotide substitution including a proportion of invariable sites and a discrete gamma distribution with six rate categories. Two independent runs, each with four Metropolis-Coupled Markov Chain Monte Carlo chains and a temperature of 0.2 were initiated and run for 1 000 000 generations, with tree and parameter sampling every 100 generations. Burn-in was set to discard 25 % of samples. Maximum parsimonious trees (MPs) were reconstructed in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) using the heuristic search option with 100 random sequence additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Alignment gaps were treated as missing and all characters were unordered and of equal weight. The robustness of the trees obtained was evaluated by 1000 bootstrap replications with ten random sequence additions. Molecular Evolutionary Genetics Analysis software (MEGA ver-

Table 1. Voucher specimens and NCBI GenBank accession numbers of the ITS sequences used in the phylogenetic analyses.

| Taxon | Locality, voucher | GenBank acc. no. |
|--------------------------------|-----------------------------------------------------|------------------|
| <i>Aspicilia cinerea</i> | Sweden, Dalarna, <i>Hermansson 13275</i> (UPS) | EU057899 |
| <i>Aspicilia indissimilis</i> | Sweden, <i>Nordin 5943</i> (UPS) | EU057909 |
| <i>Aspicilia laevata</i> | Sweden, <i>Tibell 23659</i> (UPS) | EU057910 |
| <i>Circinaria calcarea</i> | Sweden, <i>Nordin 5888</i> (UPS) | EU057898 |
| <i>Circinaria contorta</i> | Sweden, <i>Nordin 5895</i> (UPS) | EU057900 |
| <i>Circinaria leproscens</i> | Sweden, <i>Nordin 5906</i> (UPS) | EU057911 |
| <i>Lobothallia melanaspis</i> | Norway, <i>Own-Larsson 8943a</i> (UPS) | JF825524 |
| <i>Lobothallia radiosa</i> | Sweden, <i>Nordin 5889</i> (UPS) | JF703124 |
| <i>Megaspora cretacea</i> | Armenia, <i>Aptroot 73835</i> (B) | KX253974 |
| <i>Megaspora cretacea</i> | Armenia, <i>Gasparyan 600199170</i> (B) | KX253975 |
| <i>Megaspora rimisorediata</i> | Iran, <i>Valadbeigi 2250</i> (TARI) | JF825525 |
| <i>Megaspora rimisorediata</i> | China, Xinjiang, <i>XJU 20116002</i> | KT443790 |
| <i>Megaspora rimisorediata</i> | China, Xinjiang, <i>XJU 20136001</i> | KT443789 |
| <i>Megaspora rimisorediata</i> | China, Xinjiang, <i>XJU 20111617</i> | KT443788 |
| <i>Megaspora rimisorediata</i> | China, Xinjiang, <i>XJU 91815043</i> | KT443787 |
| <i>Megaspora verrucosa</i> | Austria, <i>Trinkaus</i> (GZU) | AF332121 |
| <i>Megaspora verrucosa</i> | Austria, <i>Hafellner 48544 & Ivanova</i> (GZU) | AF332122 |
| <i>Megaspora verrucosa</i> | China, Xinjiang, <i>XJU 200753</i> | KT443786 |
| <i>Megaspora verrucosa</i> | China, Xinjiang, <i>XJU 20000724</i> | KT443785 |
| <i>Megaspora verrucosa</i> | U.S.A., Colorado, <i>St. Clair C54042</i> (BRY) | KC667053 |
| <i>Sagedia mastrucata</i> | Norway, Troms, <i>Nordin 5708</i> (UPS) | EU057913 |
| <i>Sagedia simoensis</i> | Sweden, <i>Own-Larsson 9000</i> (UPS) | EU057926 |
| <i>Sagedia zonata</i> | Sweden, <i>Nordin 5932</i> (UPS) | EU057949 |
| <i>Ochrolechia parella</i> | France, Brittany, <i>Feige</i> (ESS-20864) | AF329174 |

sion 7.0) was used to reconstruct the Maximum Likelihood phylogenetic tree based on the GTR + I + G model (Nei & Kumar 2000; Kumar & al. 2016). Initial

tained by Bayesian and maximum likelihood phylogenetic inference. The majority rule consensus tree of Bayesian analysis is shown here (Fig. 1) with posterior probabilities

tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A GTR model of nucleotide substitution including a proportion of invariable sites and a discrete gamma distribution with five rate categories (GTR + I + G) were used in Maximum Likelihood approach.

Results

Phylogeny

The maximum parsimony analysis resulted 12 most parsimonious trees with 513 steps, consistency index (CI) = 0.591, retention index (RI) = 0.690, rescaled consistency index (RC) = 0.407 and homoplasy index (HI) = 0.409. The Maximum Likelihood analysis resulted a tree with the highest log likelihood (-2014.3274). Majority rule consensus tree for maximum parsimony analysis was congruent with the tree ob-

Table 2. The main distinguishing characteristics of *Megaspora cretacea*, *M. rimisorediata* and *M. verrucosa*.

| | <i>Megaspora cretacea</i> | <i>Megaspora rimisorediata</i> | <i>Megaspora verrucosa</i> |
|----------------------|----------------------------------------------------------------|-------------------------------------------------------------------------------|----------------------------------------------------------|
| Thallus | whitish grey, irregularly delimited to almost lobate | ochraceous to bluish grey, dense net of elongate cracks over thallus | white to grey-white, continuous to areolate to verrucose |
| Soredia | pale bluish grey, covering most of thallus, c. 100 µm in diam. | dark bluish green, produced along sides of elongate cracks, 50–70 µm in diam. | absent |
| Hymenium | not interspersed, c. 150 µm high | not interspersed, to 150 µm high | interspersed at times, 200–250 µm high |
| Paraphyses | unbranched | branched and anastomosing | branched but not anastomosing |
| Asci | 125–140 × 25–31 µm | c. 145 × 46 µm | 200–230 × 45–50 µm |
| Ascospores per ascus | 4 | 4–8 | 8 |
| Ascospores | 27–31 × 18–21 µm | 35–42 × 23–27 µm | 30–60 × 21–42 µm |
| Substrate | bark of <i>Juniperus</i> sp. | bark of <i>Juniperus</i> sp., <i>Quercus</i> sp. | soil, mosses, plant remains on calcareous rocks, bark |

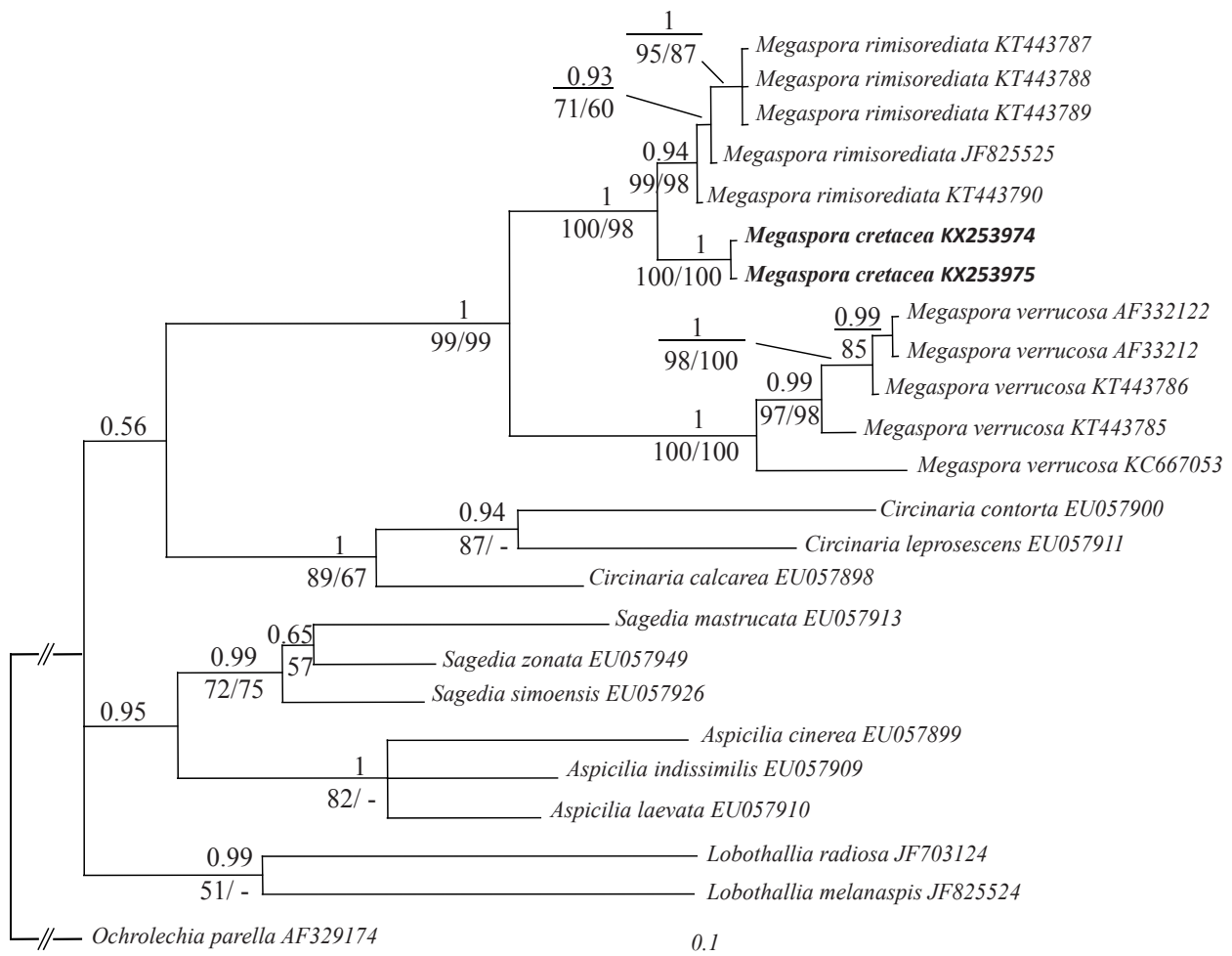


Fig. 1. Phylogenetic relationships of family *Megasporaceae* showing the consensus tree of the Bayesian analysis of the ITS dataset. Bayesian posterior probabilities are shown above the branches and MP/ML bootstrap values ≥ 50 are shown below the lines adjacent to the branches. Distance of outgroup and ingroup root is shortened three times.

of Bayesian analysis and bootstrap numbers of Maximum Parsimony and Maximum Likelihood analysis.

The molecular phylogenetic results confirmed affiliation of the new species to the genus *Megaspora*. It clusters in a phylogenetic tree in *Megaspora*, as sister to *M. rimisorediata* (PP = 1; MP/ML BS = 100/100). The phylogenetic trees resulting from the three different analyses also confirmed *Megaspora* clade as a monophyletic group even after adding the new species samples (*M. cretacea*) with a high posterior probability and bootstrapping values (PP = 1; MP/ML BS = 99/99). Monophyly of species *M. verrucosa* and *M. rimisorediata* were confirmed with a high supporting values (PP = 1; MP/ML BS = 100/100 for *M. verrucosa* and PP = 0.94; MP/ML BS = 99/98 for *M. rimisorediata*).

Taxonomy

Megaspora cretacea Gasparayan, Zakeri & Aptroot, **sp. nov.** – MycoBank #817072 – Fig. 2A–C.

Holotype: Armenia, Ararat, Vedi, Urtsadzor, Khosrov Forest State Reserve, 40°00'42"N, 44°54'04"E, 1600 m, on

Juniperus polycarpus bark, 17 Jun 2015, A. Aptroot 73835 (B 600200932; isotypes: ABL, GLM).

Diagnosis — *Megaspora* with thallus whitish grey, cretaceous, fully soresiate with soredia c. 0.1 mm in diam.; apothecia sparse, immersed; ascospores 4 per ascus, broadly ellipsoid, 27–31 \times 18–21 μ m, hyaline, thin-walled.

Description — *Thallus* whitish grey, crustose, ecorticate, to 0.2 mm thick, irregularly delimited to almost lobate, occupying areas up to 5 cm in diam. *Medulla* white, cretaceous. *Soralia* covering most of thallus surface, pale bluish grey; soredia c. 100 μ m in diam. *Photobiont* chlorococcoid. *Apothecia* sparse, dispersed, immersed in thallus, round, 0.3–0.5 mm in diam.; disc black, concave; margin black, raised above disc, incurved, c. 0.1 mm wide, with some crenations. *Hymenium* IKI+ blue, c. 150 μ m high, not interspersed with oil droplets. *Subhymenium* hyaline. *Epithymenium* greenish, colour unchanged in KOH. *Hypothecium* hyaline. *Paraphyses* 2–2.5 μ m thick, not branched. *Asci* clavate, 125–140 \times 25–31 μ m. *Ascospores* 4 per ascus, broadly ellipsoid, 27–31 \times 18–21 μ m, hya-

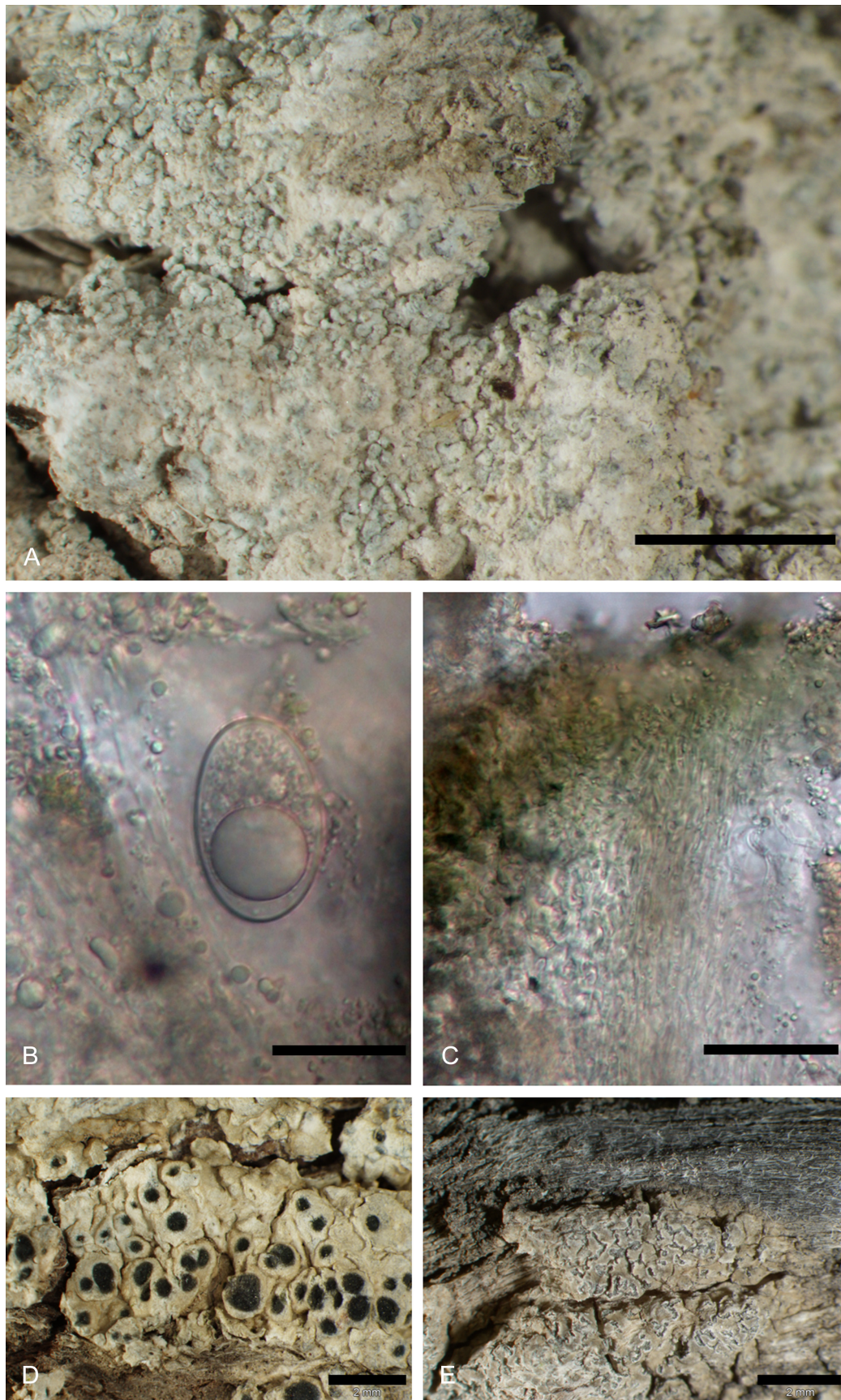


Fig. 2. A–C: *Megaspora cretacea*, holotype; A: thallus with soredia and apothecia; B: ascospore; C: hymenium (with excipulum at left). – D: *M. verrucosa* thallus with apothecia. – E: *M. rimisorediata* thallus with net of cracks and soredia. – Scale bars: A, D, E = 2 mm; B, C = 20 µm.

line, thin-walled (less than 1 µm). *Pycnidia* not observed. *Conidia* not observed.

Chemistry — Thallus KOH-, C-, Pd-, UV-. TLC: No lichen substances detected.

Distribution and ecology — The species is known from two separate localities within the Khosrov Forest State Reserve, Armenia. It occurs on bases of trees of *Juniperus polycarpus* K. Koch in arid, open, montane forests. The forest ecosystems in the Khosrov Forest State Reserve, at 1400–2300 m, are generally dominated by oak trees (*Quercus macranthera* Fisch. & C. A. Mey. ex Hohen.) and sparse juniper (*J. polycarpus*) formations, accompanied by *Fraxinus excelsior* L., *Sorbus aucuparia* L., and species of *Acer* L. and *Pyrus* L. (Khanjyan 2004).

Etymology — The epithet is derived from word *cretaceus* (resembling chalk) in reference to the colour and texture of the thallus.

Additional specimen examined — ARMENIA: Ararat, Vedi, Urtsadzor, Khosrov Forest State Reserve, 39°59'07"N, 44°53'51"E, 1390 m, on *Juniperus polycarpus* bark, 17 Jun 2015, A. Gasparyan (B 600199170).

Discussion

Megaspora cretacea is a morphologically distinctive species, from which the two other species of the genus, *M. verrucosa* (Fig. 2D) and *M. rimisorediata* (Fig. 2E), can be separated as follows (Table 2): *M. verrucosa* has no soredia, whereas *M. cretacea* and *M. rimisorediata* are both sorediate; the closely related *M. rimisorediata* differs from *M. cretacea* by the presence of a dense net of elongate cracks over the thallus, dark bluish green soredia, branched paraphyses and larger ascospores.

Key to the species of *Megaspora*

1. Soredia absent *M. verrucosa*
– Soredia present **2**
2. Thallus ochraceous to bluish grey with a dense net of elongate cracks; soredia produced along sides of elongate cracks, dark bluish green
..... *M. rimisorediata*
– Thallus whitish grey, irregularly delimited to almost lobate; soredia covering most of thallus, pale bluish grey *M. cretacea*

Acknowledgements

The authors would like to express their gratitude to the staff of the Khosrov Forest State Reserve for kind support

during field work and to the Ministry of Nature Protection for permission to collect the specimens. The authors are also grateful to staff and volunteers of the Young Biologists Association NGO, especially Hripsime Atoyán, Vanuhi Hambarzumyan and Maria Antonosyan for field assistance during the excursion. A.G. acknowledges financial support from the DAAD (Deutscher Akademischer Austauschdienst, German Academic Exchange Service) and the project “Developing Tools for Conserving the Plant Diversity of the Transcaucasus” financed by the Volkswagen Foundation. A.A. thanks the Stichting Hugo de Vries-fonds for a travel grant. Leo Spier is thanked for performing TLC. The authors also thank Anders Nordin and Robert Lücking for their reviews of an earlier version of this paper.

References

- Anonymous. 2008: “Khosrov Forest” State Reserve management plan 2010–2014. – Yerevan: Ministry of Nature Protection.
- Castresana J. 2000: Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. – *Molec. Biol. Evol.* **17**: 540–552.
- Divakar P. K., Leavitt S. D., Molina M. C., Del-Prado R., Lumbsch H. T. & Crespo A. 2016: A DNA barcoding approach for identification of hidden diversity in *Parmeliaceae* (Ascomycota): *Parmelia* sensu stricto as a case study. – *Bot. J. Linn. Soc.* **180**: 21–29.
- Edgar R. C. 2004: MUSCLE: multiple sequence alignment with high accuracy and high throughput. – *Nucl. Acids Res.* **32**: 1792–1797.
- Gardes M. & Bruns T. D. 1993: ITS primers with enhanced specificity for *Basidiomycetes*-application to the identification of mycorrhizae and rusts. – *Molec. Ecol.* **2**: 113–118.
- Gasparyan A., Aptroot A., Burgaz A. R., Otte V., Zakeri Z., Rico V. J., Araujo E., Crespo A., Divakar P. K. & Lumbsch H. T. 2015: First inventory of lichens and lichenicolous fungi in the Khosrov Forest State Reserve, Armenia. – *Fl. Medit.* **25**: 105–114.
- Gasparyan A. & Sipman H. J. M. 2013: New lichen records from Armenia. – *Mycotaxon* **123**: 491.
- Harutyunyan S., Wiesmair B. & Mayrhofer H. 2011: Catalogue of the lichenized fungi in Armenia. – *Herzogia* **24**: 265–296.
- Khanjyan N. 2004: Specially protected nature areas of Armenia. – Yerevan: Ministry for Nature Protection.
- Kumar S., Stecher G. & Tamura K. 2016: MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. – *Molec. Biol. Evol.* **33**: 1870–1874.
- Müller J., Müller K., Neinhuis C. & Quandt D. 2010: PhyDE: Phylogenetic Data Editor, v0.9971. – Published at <http://www.phyde.de> [accessed 29 Mar 2016].
- Nei M. & Kumar S. 2000: Molecular evolution and phylogenetics. – New York: Oxford University Press.

- Nordin A., Savić S. & Tibell L. 2010: Phylogeny and taxonomy of *Aspicilia* and *Megasporaceae*. – *Mycologia* **102**: 1339–1349.
- Nylander J. A. A. 2004: MrModeltest v2. Program distributed by the author. – Uppsala: Evolutionary Biology Centre, Uppsala University.
- Orange A., James P. W. & White F. J. 2001: Microchemical methods for the identification of lichens. – London: British Lichen Society.
- Park S.-Y., Jang S.-H., Oh S.-O., Kim J. A & Hur J.-S. 2014: An easy, rapid, and cost-effective method for DNA extraction from various lichen taxa and specimens suitable for analysis of fungal and algal strains. – *Mycobiology* **42**: 311–316.
- Ronquist F. & Huelsenbeck J. P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. – *Bioinformatics* **19**: 1572–1574.
- Schoch C. L., Seifert K. A., Huhndorf S., Robert V., Spouge J. L., Levesque C. A., Chen W. & Fungal Barcoding Consortium 2012: Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. – *Proc. Natl. Acad. Sci. U.S.A.* **109**: 6241–6246.
- Smith C. W., Aptroot A., Coppins B. J., Fletcher A., Gilbert O. L., James P. W. & Wolseley P. A. (ed.) 2009: The lichens of Great Britain and Ireland. – London: British Lichen Society.
- Swofford D. L. 2003: PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. – Sunderland: Sinauer Associates.
- Thiers B. 2016+ [continuously updated]: Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. – Published at <http://sweetgum.nybg.org/science/ih/> [last accessed 14 Jul 2016].
- Valadbeigi T., Nordin A. & Tibell L. 2011: *Megaspora rimisorediata* (*Pertusariales*, *Megasporaceae*), a new sorediate species from Iran and its affinities with *Aspicilia* sensu lato. – *Lichenologist* **43**: 285–291.
- White T. J., Bruns T., Lee S. & Taylor J. W. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – Pp. 315–322 in: Innis M. A., Gelfand D. H., Sninsky J. J. & White T. J. (ed.), *PCR Protocols: a guide to methods and applications*. – New York: Academic Press.

Willdenowia

Open-access online edition www.bioone.org/loi/will  BioOne

Online ISSN 1868-6397 · Print ISSN 0511-9618 · Impact factor 0.500

Published by the Botanic Garden and Botanical Museum Berlin, Freie Universität Berlin

© 2016 The Authors · This open-access article is distributed under the CC BY 4.0 licence