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A first report of *Acarospora rosulata* (Acarosporaceae) in Russia from Yakutia

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***Keywords*:** biodiversity, new record, lectotypification, lichens, Russia, Yakutia.

Summary. During the study of the lichen biota of Yakutia, *Acarospora rosulata* (Th. Fr.) H. Magn. was identified on the basis of morphological, anatomical, secondary metabolites and molecular data for the first time in Russia. Phylogenetic analysis based on ITS sequences revealed that the studied specimens form a well-supported clade (BS value = 86%) with specimens from Mongolia, USA, and Norway. The studied specimens are characterized by a brown, shiny, squamulose areolate thallus, sometimes with some outer lobes, numerous apothecia immersed in the areoles, a well-developed exciple, simple ellipsoid ascospores (4–5 × 1.5–2.5 μm) and the content of gyrophoric acid as the main substance. Lecanoric and hiascic acids are reported for the first time in the species. A comparative analysis of samples from Yakutia is carried out with descriptions by other authors. Since the original description of the species did not include a holotype, the article also provides a lectotypification based on the original material from Norway. A specimen stored in the herbarium of Uppsala University (UPS) was designated as the lectotype. A comparison with related species is also provided.

Первая находка *Acarospora rosulata* (Acarosporaceae) в России из Якутии

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Ключевые слова: биоразнообразие, лектотипификация, лишайники, Россия, флористические находки, Якутия.

Аннотация. В ходе изучения лихенобиоты Якутии на основании анатомии, морфологии, состава вторичных метаболитов и генетического анализа был выявлен новый для России вид лишайника – *Acarospora rosulata* (Th. Fr.) H. Magn. Филогенетический анализ, основанный на последовательностях ITS, показал, что изученные образцы формируют хорошо поддержанную кладу (BS value = 86 %) с образцами из Монголии, США и Норвегии. Изученные образцы характеризуются коричневым, блестящим, чешуйчато-ареолированным талломом, иногда с небольшими лопастями по краю, многочисленными апотециями, погруженными в ареолы, хорошо

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развитым эксципулом, простыми эллипсоидными спорами (4–5 × 1,5–2,5 мкм) и содержанием гирофоровой кислоты как основного вторичного метаболита. Впервые для вида выявлены леканоровая и хиасцевая кислоты. Проводится сравнительный анализ образцов *A. rosulata* из Якутии с описаниями других авторов. Ввиду того, что при первоописании вида не был указан голотип, в статье проводится лектотипификация на основе оригинального материала из Норвегии. В качестве лектотипа выбран образец, хранящийся в гербарии университета Упсалы (UPS). Обсуждаются отличия от морфологически близких видов.

Introduction

The genus *Acarospora* A. Massal. is the largest in the family Acarosporaceae Zahlbr. and includes about 300 species (Knudsen et al., 2023). It is characterized by polyspore asci (usually more than 100 spores), simple hyaline ascospores, areolate to squamulose thallus, aspicilioid or pseudo-lecanorine apothecia, and to the apical dome K/I –.

Acarospora rosulata was long known only from Oppland, Norway (Magnusson, 1929). However, recent studies by Knudsen et al. (2010) showed that this species is more widespread and known from Asia (Mongolia) and North America.

During the study of the Yakutia lichens biodiversity, we discovered *A. rosulata*, which is reported here for the first time for Russia. Further it is given a detailed description in the article.

Materials and methods

Field and herbarium studies. – The samples were collected during an expedition in 2023 by S. V. Chesnokov in the Bulunsky District of Yakutia. A total of four specimens were collected. Specimens from Norway stored in the LE herbarium were additionally studied. The study of anatomical and morphological features, as well as photographs, was carried out using a Stemi 508 stereoscopic microscope and an Axio Scope A.1 microscope with an Axiocam 506 camera. Measurements of microscopic features were carried out in water without adding KOH. Type material was studied based on photographs from Uppsala University (UPS). All samples are stored in the lichenological herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences (LE). The doublet specimens have been deposited in the herbarium of the Altai State University (ALTB).

Chemical analysis. – The presence of secondary metabolites was determined by color reactions (“spot” test) with undiluted Lugol’s solution (I), 10 % KOH solution (K) and with a sodium hypochlorite (C) solution (Smith et al., 2009).

LC-ESI-MS/MS analysis. – For the analysis 5 mg of lichen was ground in a mortar. The resulting powder was extracted twice with 0.5 ml of acetone. The extraction was carried out with constant stirring for 24 hours at a temperature of 20–25 °C. The obtained extracts were centrifuged at 15000 g for 5 min and then kept at 4 °C until analysis. LC-ESI-MS analysis was performed with a Shimadzu LC-30 Nexera chromatograph. For chromatographic separation, a Phenomenex Keenetex C18 column (100 × 2.1 mm, 1.7 μm) was used. The mobile phase consisted of (A) aqueous formic acid (0.1 %) and (B) acetonitrile. Analyses were performed at 40 °C with a flow rate of 0.3 ml/min in the linear gradient elution mode with a change in the proportion of B from 30 to 100 % over 20 min. The volume of injected sample was 2 μl. Spectra of eluting substances were recorded in UV at 250 nm. After chromatographic separation, the samples were analyzed using a triple quadrupole mass-selective detector LCMS-8030 Shimadzu (Japan) with electrospray ionization (ESI) in the negative mode. The voltage on the capillary was 3.5 kV, nebulizing gas flow rate 3 L/min, drying gas (nitrogen) temperature 250 °C, drying gas flow rate 15 L/min. Mass spectra were recorded in the range 100–800 m/z. MS/MS spectra were obtained by collision-induced dissociation of precursor ions with an energy of 15 eV. The resulting chromatograms were processed using the MZmine 4 v. 4.1.0 software. To identify lichen substances, we compared of their polarity related to their retention time (Rt), obtained m/z values of pseudo-molecular and fragment ions with the authentic standards from the Komarov Botanical Institute of the Russian Academy of Sciences (BIN RAS) collection.

DNA extraction, PCR amplification and DNA sequencing. – DNA extraction was performed using a commercial Phytosorb kit (Synthol, Russia). The following primers were used for the PCR: ITS1f (Gardes, Bruns, 1993) and ITS4 (White et al., 1990). The amplification program: initial denaturation 94 °C – 5 min, then 5 cycles (94 °C – 30 s, 55 °C – 30 s, 72 °C – 60 s), 30 cycles (94 °C – 30 s, 52 °C – 30 s, 72 °C – 60 s), elongation 72 °C – 300 s (Westberg et al., 2015). The obtained amplicons were purified using the Cleanup S-cup kit (Eurogen, Russia), after which they were sequenced at the BIN RAS. The obtained sequences were compared with sequences from the NCBI database (NCBI. URL: https://www.

ncbi.nlm.nih.gov/) using the BLASTn algorithm (Altschul et al., 1990; BLAST. URL: http://www.ncbi.nlm.nih.gov/BLAST/) to check the sequence identity and check for possible contamination.

Sequence alignment and phylogenetic analysis. – Chromatograms were edited and aligned using the ClustalW algorithm in the Unipro UGENE (Okonechnikov et al., 2012). The new sequence was uploaded to NCBI (GenBank). Our resulting ITS sequences were aligned along with sequences members of the genus *Acarospora* available from GenBank (Table 1).

The alignment consisted of 14 sequences with 527 sites, of which 414 were conserved. We constructed single-locus ITS nrDNA phylogeny using the Maximum Likelihood (ML) method. For the ML analysis, following substitution models were selected: TIM3e+G4 for ITS1, ITS2 and 5.8S. Phylogenetic tree was constructed using IQ-TREE with 1000 bootstraps (Nguyen et al., 2015; Chernomor et al., 2016; IQ-TREE. URL: www.iqtree.org) and visualized using iTOL (Letunic, Bork, 2021; iTOL. URL: https://itol.embl.de/).

Results

Molecular study. The three newly sequenced specimens of *Acarospora rosulata* grouped together with three existing *A. rosulata* specimens, forming a single well-supported clade (BS value = 86 %) (Fig. 1).

Typification note. While searching for type material, a reference to a holotype was discovered in Knudsen et al. (2010). When examining the protologue, no reference to a holotype was found, leading us to conclude that the specimen was erroneously designated as the holotype (Turland et al., 2018).

However, this specimen is the only original material that was found, which is why it was chosen for lectotypification.

Table 1. GenBank accession numbers and additional information for the specimens used in the phylogenetic analysis. Newly generated sequence is in bold

Species	Location	Collection data (herbarium)	GenBank Accession number
A. bullata Anzi	Austria	Obermayer 1987-09-09 (GZU 1-90)	GU184108
A. bullata Anzi	Germany	Huneck-Poelt 21 v 1993 (GZU)	GU184110
A. bullata Anzi	Italy	Reeb and Roux VR 8-VII-98/6 (DUKE)	GU184114
A. bullata Anzi	Iran	Maassouni Safavi 1850 (B 60 0133511)	GU184113
A. rosulata (Th. Fr.) H. Magn.	Mongolia	Reeb and Zavarzin VR 30-VII-04/4 (DUKE)	GU184115
A. rosulata (Th. Fr.) H. Magn.	USA, California	Knudsen 929 (UCR)	GU184116
A. rosulata (Th. Fr.) H. Magn.	Norway	Westberg 08-193 (S)	GU184118
A. rosulata (Th. Fr.) H. Magn.	**Russia, Yakutia**	**Chesnokov 16.08.2023 (LE L-26088)**	**PQ736105**
A. rosulata (Th. Fr.) H. Magn.	**Russia, Yakutia**	**Chesnokov 27.08.2023 (LE L-26090)**	**PQ736514**
A. rosulata (Th. Fr.) H. Magn.	**Russia, Yakutia**	**Chesnokov 27.08.2023 (LE L-26091)**	**PQ736523**
A. badiofusca (Nyl.) Th. Fr.	Spain	Reeb and Roux VR 2-IX-00/23 (DUKE)	GU184121
A. badiofusca var. *badiofusca* (Nyl.) Th. Fr.	France	Reeb and Roux VR 9-VII-98/11 (DUKE)	GU184120
A. macrospora ssp. *macrospora* A. Massal. ex Bagl.	Norway	Timdal 3186, 04.03.1982 (O, Lichens 33416)	GU184123
A. cervina A. Massal.	France	Reeb and Roux VR 6-VII-98/11 (DUKE)	GU184124

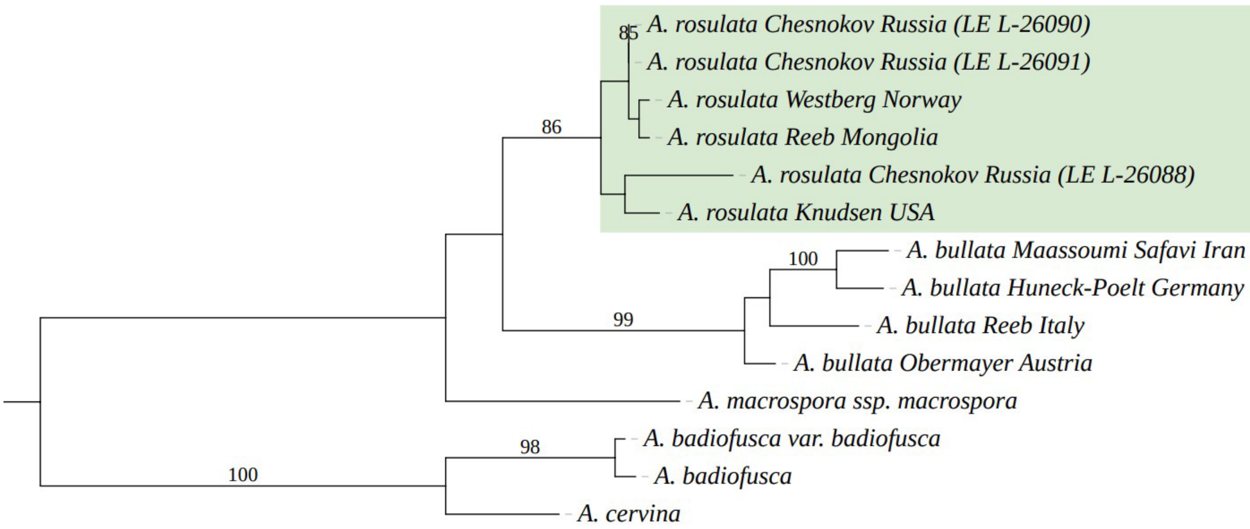

Fig. 1. Phylogenetic tree of *Acarospora species* inferred from the ML analysis (bootstrap > 85 %) of the ITS nrDNA.

Acarospora rosulata (Th. Fr.) H. Magn., 1924, Göteborg. Vetensk.-och Vitter.-Handl., Ser. 4, 28(2): 121.

≡ *Acarospora fuscata* * *discreta* f. *rosulata* Th. Fr., 1871, Lich. Scand. 1: 218 (Fig. 2).

Lectotype (designated here) (Mycobank Typification no.: MBT 10023767): [Norway] "Oppland, Vågå, Th. Fries, 1863" (UPS L-67470 (116799) [digital image!], cited on the label as "holotype") (Fig. 3).

We provide a detailed description of the species based on the studied specimens from Yakutia and Norway stored in herbarium LE and literature data (Magnusson, 1929; Knudsen et al., 2010). Differences from descriptions by other authors are presented in parentheses.

Thallus squamulose areolate, contiguous to dispersed, forming clusters sometimes with some outer lobes: specimens from Yakutia – up to 12 cm in diam., specimens from Norway – up to 1 cm in diam. (up to 7 cm, Knudsen et al., 2010). Areoles or squamules up to 3.5 mm in diam., rounded or irregularly shaped, sometimes lobed, 2–3 lobes per squamule, broadly attached. The thickness of the areoles is up to 1.5 mm according to Knudsen et al. (2010), however this feature differs significantly between Asian and European material: in specimens from Yakutia it is up to 1 mm, in specimens from Norway it is 0.2–0.4 mm. Upper surface light to dark brown, shiny or slightly shiny, smooth or rough, wrinkled, fissured, sometimes with whitish pruina (epruinose, according to Knudsen et al., 2010). Lower surface light or brown. Epicortex uneven, up to 40 µm thick (n = 71). Upper cortex 25–55 µm thick (n = 72) (30–80 µm thick, Knudsen et al., 2010), with red-brown upper layer up to 20 µm thick, paraplectenchymatous, consisting of cells 2–7 µm in diam. (specimens from Yakutia – 3–7 µm in diam. (n = 41); specimens from Norway – 2–5 µm in diam. (n = 31)) (1.5–3 µm in diam., Magnusson, 1929, 3–6 µm in diam., Knudsen et al., 2010). Algal layer 50–140 µm thick (n = 71), continuous, dense, with algae 6–15 µm in diam. (n = 54). Medulla up to 450 µm thick (n = 76) (specimens from Norway – 100–250 µm thick (n = 32)) (90–120 µm thick, Magnusson, 1929; up to 600 µm thick, Knudsen et al., 2010), prosoplectenchymatous. Lower cortex about 15 µm thick, pale, indistinct or ecorticate.

Apothecia 0.2–1.1 mm in diam. (n = 68) (specimens from Norway – 0.2–0.5 mm in diam. (n = 31)), 1 to several per areole, usually numerous (up to 36), initially punctiform, then immersed. Disc concave, round to irregular, reddish-brown or darker than thallus, smooth or slightly rough. Exciple specimens from Yakutia up to 120 µm thick (n = 72), specimens from Norway – 20–40 µm thick (n = 34) (10–15 µm thick, Magnusson, 1929; 20–40 µm thick, Knudsen et al., 2010), often with brownish top. Hymenium 80–150 µm high (n = 68), hyaline, epithecium 10–15 µm thick (n = 68), brown. Paraphyses 1.5–2.5 µm thick (n = 85), conglutinated, with brown cups, apices widened up to 3.5 µm in diam., septate, sometimes with oil drops. Asci 60–80 × 20–30 µm (n = 72), clavate, containing 100–200 spores. Ascospores 4–5 × 1.5–2.5 µm (n = 78) (3–4 × 1.7 µm, Magnusson, 1929), hyaline, simple, ellipsoid to broadly ellipsoid. Subhymenium 25–70 µm thick (n = 64) (specimens from Yakutia – 25–50 µm thick (n = 34)). Hypothecium 9–20 µm thick, yellowish.

Chemistry. HPLC revealed the presence of gyrophoric acid (major compound), lecanoric acid, hiascic acid (in trace amount), atranorin (in minor amount) (Fig. 4).

Ecology and distribution. Europe (Oppland, Norway), western North America, Asia (Mongolia) and Russia (Yakutia). On siliceous rocks, sandstone, schist, rarely on calcareous rocks.

Specimens examined: "Russia, Republic of Sakha (Yakutia), Bulunsky District, right bank of the river Olenek, 3.3 km northwest from the mouth of the river Ballaganagh, 72°16´59.4″N, 123°05´29.5″E, 27 m a. s. l., sandstone cliffs on the shore, on sandstone. 27 VIII 2023. Leg. S. V. Chesnokov" (LE L-26089, dupl. in ALTB; LE L-26090, LE L-26091); "Russia, Republic of Sakha (Yakutia), Bulunsky District, env. Tiksi, 2.35 km west of the weather station, 71°35´39.0″N, 128°52´07.2″E, 46 m a. s. l., shale outcrops overgrown with dryad, arctous and lichens, on shale. 16 VIII 2023. Leg. S. V. Chesnokov" (LE L-26088).

Additional specimens examined: "Norway: Gudbrandsdalen, Lom, c. 400 m. s. m., ad saxa erratica. Leg. A. H. Magnusson" (Keissler, Krypt. Exs. 2867) (LE L-27490, LE L-27491).

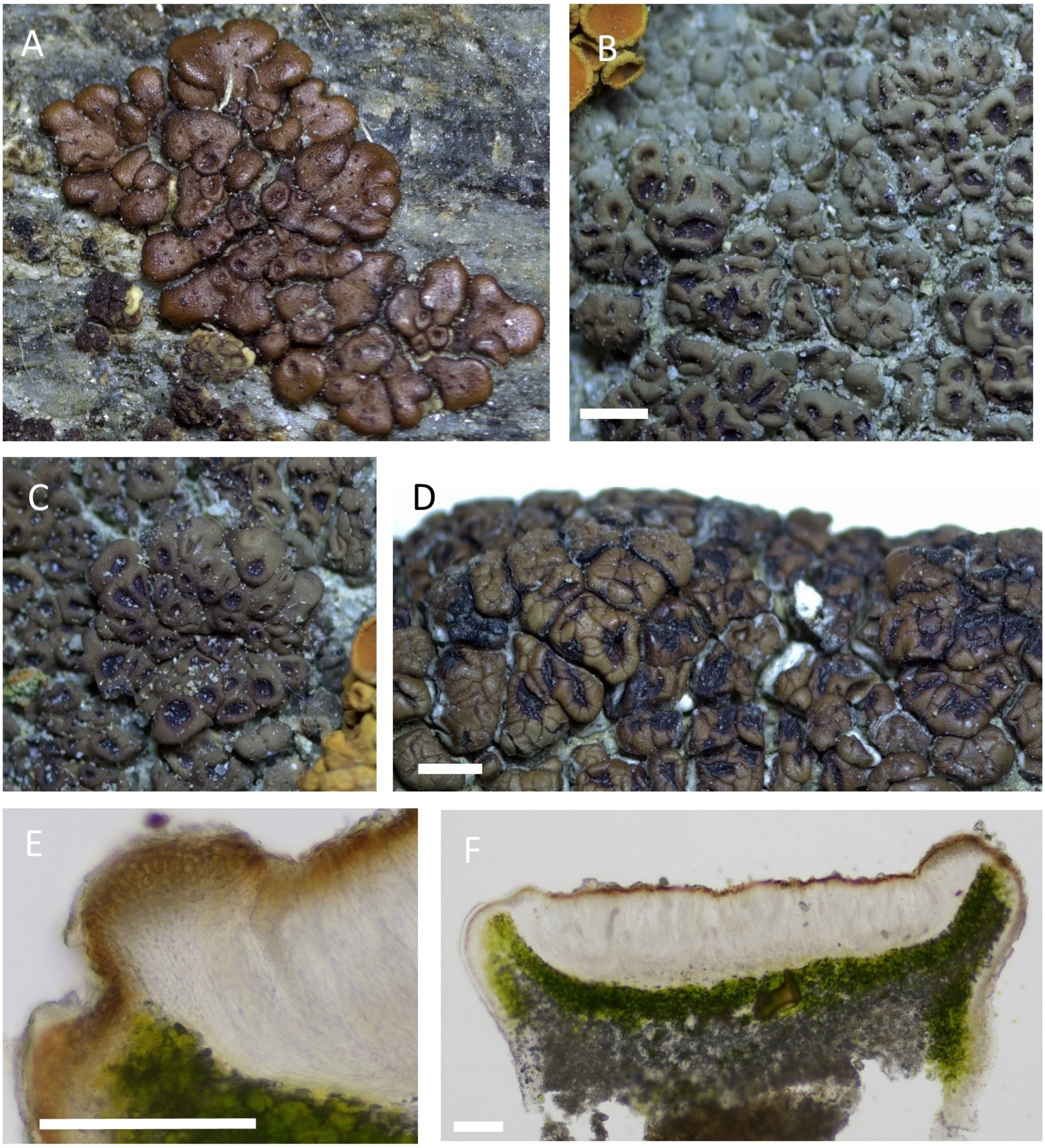

Fig. 2. *Acarospora rosulata*: A–D – thallus; E – exciple; F – thallus section. Scale bars: A–D = 1 mm; E–F = 100 µm.

Fig. 3. Lectotypus of *Acarospora rosulata*. Norway, Oppland, Vågå, Th. Fries, 1863 (UPS L-67470).

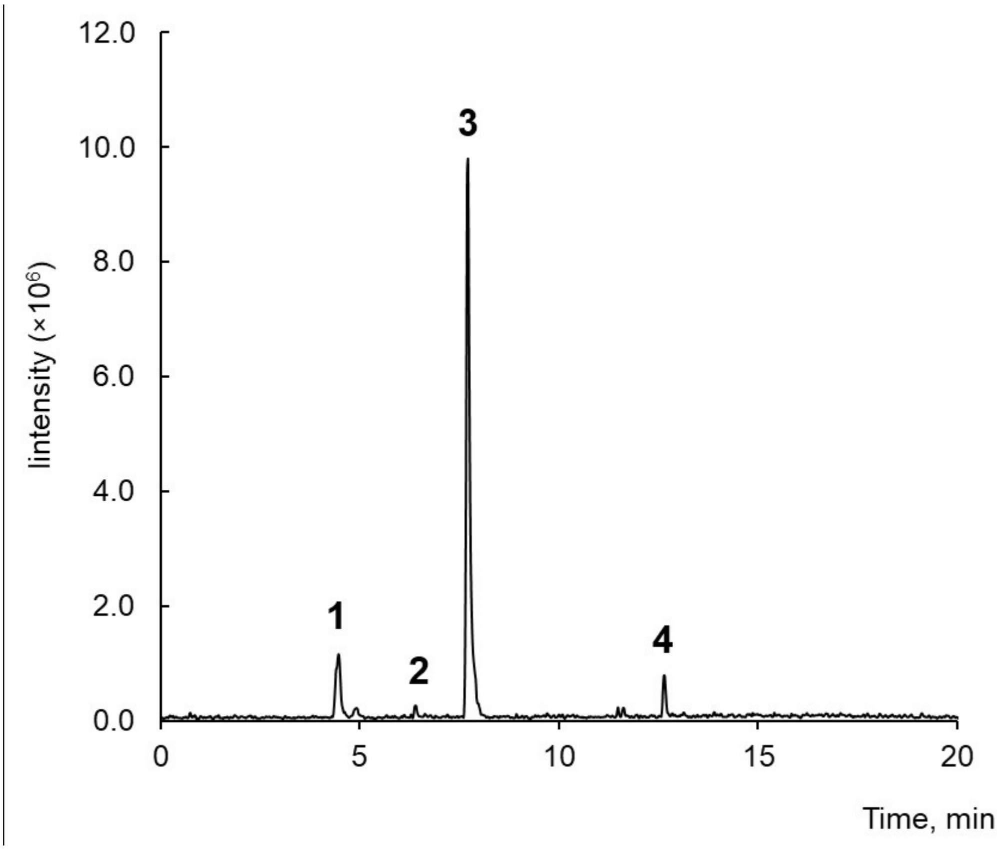

Fig. 4. LC-MS chromatogram of acetone extracts of *Acarospora rosulata:* 1= lecanoric acid; 2 = hiascic acid; 3 = gyrophoric acid; 4 = atranorin.

Discussion

According to Magnusson (1929), *Acarospora rosulata* has morphological similarities with *A. veronensis* A. Massal. However, they are easily distinguished by spot test with C (*A. rosulata* has a positive red reaction). *Acarospora rosulata* belongs to group of brown-colored *Acarospora* with a positive C+ reaction.

Acarospora rosulata is most closely related to *A. bullata* Anzi. They are clearly distinguished by the fact that in *A. rosulata*, the apothecial disc is smooth, rough, or fissured, while in *A. bullata*, the disc is distinctly wrinkled. Furthermore, *A. rosulata* is clearly distinguished by molecular data.

In this study, Ilya Prokopiev was the first to identify secondary metabolites for *A. rosulata*, such as lecanoric acid, hiascic acid (in trace amount) and minor amount of atranorin. We attribute these results to the use of a more precise HPLC analysis method. However, these compounds likely have no taxonomic significance, as lecanoric acid is a common secondary metabolite in brown-colored *Acarospora* (Golubkova, 1988), and the other components are present in low concentrations. Further studies of secondary metabolites in the *Acarospora* genus are needed to confirm their taxonomic significance, as they are currently insufficient.

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REFERENCES / ЛИТЕРАТУРА

Chernomor O., von Haeseler A., Minh B. Q. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.* 65: 997–1008. https://doi.org/10.1093/sysbio/syw037

Gardes M., Bruns T. D. 1993. ITS primers with enhanced specificity for basidiomycetes. Application for the identification of mycorrhizae and rust. *Molec. Ecol.* 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Knudsen K., Valérie Reeb, Westberg M., Srikantha R., Bhattacharya D. 2010. *Acarospora rosulata* in Europe, North America and Asia. *The Lichenologist* 42(3): 291–296. https://doi.org/10.1017/S002428290990715

Letunic I., Bork P. 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 49(W1): W293–W296. https://doi.org/10.1093/nar/gkab301

Magnusson A. H. 1929. A monograph of the genus *Acarospora. Kungl. Svenska Vetenskapsakad. Handl.* 3(7): 1–400.

Nguyen L. T., Schmidt H. A., von Haeseler A., Minh B. Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Okonechnikov K., Golosova O., Fursov M., Team U. 2012. Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics* 28(8): 1166–1167. https://doi.org/10.1093/bioinformatics/bts091

Turland N. J., Wiersema J. H., Barrie F. R., Greuter W., Hawksworth D. L., Herendeen P. S., et al. (eds.) 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress. (Shenzhen, China, July 2017). *Regnum Vegetabile.* Vol. 159. Glashütten: Koeltz Botanical Books. 288 pp. https://doi.org/10.12705/Code.2018

Westberg M., Millanes A. M., Knudsen K., Wedin M. 2015. Phylogeny of the Acarosporaceae (Lecanoromycetes, Ascomycota, Fungi) and the evolution of carbonized ascomata. *Fungal Divers.* 73: 145–158. https://doi.org/10.1007/s13225-015-0325-x

White T. J., Bruns T. D., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenies. In: *PCR Protocols: A Guide to Methods and Applications*. San Diego: Academic Press. Pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wijayawardene N. N., Hyde K. D., Dai D. Q., Sánchez-García M., Goto B. T., Magurno F. 2022. Outline of Fungi and fungus-like taxa–2021. *Mycosphere* 13(1): 53–453. https://doi.org/10.5943/mycosphere/13/1/2