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Standard Paper

Circinaria ucrainica sp. nov., a new species from sand dunes of the Lower Dnipro valley (Ukraine)

Alexander Khodosovtsev[1,2], Valerii Darmostuk[1,3], Iwona Dembicz[4], Jürgen Dengler[5,6], Ivan Moysiyenko[1] and Anna Kuzemko[2,7]

[1]Kherson State University, Kherson 73000, Ukraine; [2]M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, 01601 Kyiv, Ukraine; [3]W. Szafer Institute of Botany, Polish Academy of Sciences, 31-512 Kraków, Poland; [4]Institute of Environmental Biology, Faculty of Biology, University of Warsaw, 02-089 Warsaw, Poland; [5]Vegetation Ecology Research Group, Institute of Natural Resource Management (IUNR), Zurich University of Applied Sciences (ZHAW), 8820 Wädenswil, Switzerland; [6]Plant Ecology, Center for Ecology and Environmental Research (BayCEER), University of Bayreuth, 95447 Bayreuth, Germany and [7]Department of Botany and Zoology, Faculty of Science, Masaryk University, 625 00 Brno, Czech Republic

Abstract

During a recent expedition of the Eurasian Dry Grassland Group in the steppes of southern Ukraine, we discovered on sand dunes a new sterile crustose aspicilioid lichen with rhizomorphs. It is described here as new to science under the name *Circinaria ucrainica* (*Megasporaceae*). The new combination *Circinaria reptans* (Looman) Khodos. & Darmostuk is also proposed. *Circinaria ucrainica* is characterized by small grey areoles with a net of dark grey to brownish spicate prothalline tips and pale rhizomorphs. According to the phylogenetic analysis, the new species is closely related to the terricolous *Circinaria reptans*, but the latter has thicker rhizomorphs of 200–400 μm diameter, finely developed areoles and lacks spicate prothalline tips. Furthermore, we discuss the differences between the new species and other morphologically similar species with rhizomorphs, such as *Aspicilia spicata*, *Circinaria crespiana* and *C. reptans*. The ecological characters of soil and vegetation, including vascular plants, bryophytes and lichens, are provided for the habitat of *C. ucrainica*.

Keywords: biological crust; dry grassland; lichen; *Megasporaceae*; phylogeny

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Introduction

Dry grassland habitats often host diverse lichen communities (Biurrun *et al.* 2021) as components of 'biological crusts'. These crusts are complex assemblages of organisms including mosses, liverworts, cyanobacteria, algae, lichens, fungi and bacteria that grow on and within the uppermost layers of the soil (Eldridge 2000). Terricolous species of the genera *Aspicilia*, *Circinaria* and *Megaspora* (*Megasporaceae*) are often present in the biological crusts of arid regions. Although vagrant *Circinaria* species (Sohrabi *et al.* 2013) are attractive elements of desert habitats, only a small number of crustose aspicilioid species of this genus attached to soil were previously known (Rosentreter 1998; Sohrabi *et al.* 2010; McCune & Di Meglio 2021).

The taxonomy of aspicilioid lichens at the genus level is very complicated. For instance, the use of conidia length, which was crucial for the separation of *Aspicilia* s. str. and *Circinaria* (Nordin *et al.* 2010), has recently been criticized (McCune & Di Meglio 2021). There has also been some discussion about the genus *Circinaria*, which, in most species, is characterized by large, broadly ellipsoid to globose spores, 2–4 per ascus, and the presence of aspicilin in several species. The genus is either considered within the large clade of *Aspicilia* s. lat. (McCune & Di Meglio 2021), or partially split into smaller monophyletic clades treated as genera (e.g. Kondratyuk *et al.* 2015) although these lack statistical support. We follow the concept of Nordin *et al.* (2010) of *Circinaria* as a monophyletic clade containing the type species.

Although the lichens, lichenicolous fungi and lichen communities of the large dune areas of the Lower Dnipro valley (Kherson Region, Ukraine) have been studied in detail previously (e.g. Khodosovtsev *et al.* 2011, 2018), participants of the 15th international research expedition (known as a 'Field Workshop') of the Eurasian Dry Grassland Group (EDGG) (Moysiyenko *et al.* 2022) discovered on sandy soil, an unknown sterile, aspicilioid crustose lichen with rhizomorphs. As it did not match any previously described species, we describe it here as new to science, with detailed information on the morphology, chemistry, ecology and phylogeny based on nrITS, nrLSU and mtSSU sequences.

Materials and Methods

Taxon sampling and morphological studies

Specimens were collected in sandy habitats of the Lower Dnipro sand dunes (Ukraine) during an international research expedition

Corresponding author: Alexander Khodosovtsev; Email: khodosovtsev@gmail.com
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organized by the Eurasian Dry Grassland Group (EDGG; https://edgg.org) in June 2021 (Moysiyenko *et al.* 2022). Specimens were examined with Optica-1 and MICROMED–2 microscopes using standard microscopy techniques. Microscopic examination was carried out on material mounted in water and 10% KOH (K) (Smith *et al.* 2009). Photographs were taken with a Levenhuk C510 camera. Measurements were made in water with a precision of 0.5 µm for microscopical structures and 5 µm for anatomical layers of thalli. Dimensions are given as (min–) $\bar{x}$–SD – $\bar{x}$+SD (–max) (n), where $\bar{x}$ is the mean, SD is the standard deviation and n is the number of measurements taken. All examined specimens are deposited in the herbaria of Kherson State University, Ukraine (KHER) and the W. Szafer Institute of Botany, Polish Academy of Sciences (KRAM L). Vascular plants, bryophytes and lichens were recorded with the standardized 'biodiversity plot' sampling of EDGG (Dengler *et al.* 2016*a*). Presence of species is given for substratum grain sizes of 0.0001, 0.001, 0.01, 0.1 and 1 m^2, whereas percentage cover is indicated for 10 m^2. Nomenclature of vascular plants follows the Euro +Med Plantbase (Euro+Med 2006) at the species level.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from a small number of clean lobes using the QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. We amplified the mtDNA small subunit (mtSSU) using the primer pair mrSSU1 and mrSSU3R (Zoller *et al.* 1999), as well as nrITS (ITS1 + 5.8S + ITS2) and the nuclear large subunit rDNA (nrLSU) with the primers ITS1F and LR5 (Vilgalys & Hester 1990; Gardes & Bruns 1993). Polymerase chain reactions (PCR) were performed in a volume of 25 µl comprising 1 µl of DNA template, 0.2 µl of AmpliTaq 360 DNA polymerase (Applied Biosystems, California, USA), 2.5 µl of 10× AmpliTaq 360 PCR Buffer, 2.5 µl 25mM $MgCl_2$, 1 µl of each primer (10 µM), 2 µl GeneAmp dNTPs (10 mM; Applied Biosystems, California, USA), 0.2 µl bovine serum albumin (BSA; New England Biolabs, Massachusetts, USA), with sterile distilled water added to attain the final volume. PCR amplifications were performed using the thermocycling conditions of Rodriguez-Flakus & Printzen (2014). PCR products were visualized by running 3 µl of the PCR product on 1% agarose gels.

The newly generated sequences were checked, assembled and edited manually using Geneious Pro v. 8.0. (Biomatters, Auckland, New Zealand) and deposited in GenBank. Accession numbers are provided in Table 1.

Phylogenetic analyses and taxon selection

All generated sequences were checked by BLAST (Altschul *et al.* 1990) to verify potential contamination by unrelated fungi. BLAST searches of the obtained sequences revealed the highest similarity with *Circinaria* species (*Circinaria* aff. *arida* for the nrITS and nrLSU regions, and *C. caesiocinerea* (Nyl. ex Malbr.) A. Nordin *et al.* for mtSSU). Alignments were generated for each region using MAFFT (Katoh & Standley 2013) as implemented on the GUIDANCE2 web server (Penn *et al.* 2010). GUIDANCE2 assigns a confidence score to each ambiguous nucleotide site in the alignment and later removes regions of uncertain columns. We used the default cut-off score of 0.93 in all single gene alignments. The subsequent analyses were performed using the CIPRES Science Gateway (http://www.phylo.org/portal2/) (Miller *et al.* 2010). PartitionFinder 2 was used to select the best partition for our data and substitution models (Lanfear *et al.* 2016). A single substitution model was selected for each region (SYM + G for ITS1 and ITS2, K80 for 5.8S, TRN + I + G for nrLSU, HKY + I for mtSSU) under a greedy search algorithm and the Akaike information criterion (AIC) (Lanfear *et al.* 2012). Maximum likelihood (ML) analyses were carried out using a heuristic search as implemented in IQ-TREE on XSEDE and 100 bootstrap interactions on 1000 replicates to estimate branch support. Bayesian inference (BI) of the phylogenetic relationships was calculated using the Markov chain Monte Carlo (MCMC) approach as implemented in MrBayes v. 3.2.6 on XSEDE (Ronquist *et al.* 2012), using the partitions and substitution models obtained by PartitionFinder 2. Two independent parallel runs were started, each with four incrementally heated (0.15) chains. This MCMC was allowed to run for 100 million generations, sampling every 1000th tree and discarding the first 50% of sampled trees as a burn-in factor. The analysis was stopped after 1 million generations when the standard deviation of split frequencies had dropped below 0.01. The resulting ML and BI phylogenetic trees were visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and Inkscape (https://inkscape.org/). The tree was rooted using *Megaspora verrucosa* as the outgroup.

Thin-layer chromatography

The secondary chemistry of all samples was studied by thin-layer chromatography (TLC) following the methods of Culberson & Kristinsson (1970) and Orange *et al.* (2001).

Soil parameters

Soil samples were analyzed using the EDGG methodological approach (Dengler *et al.* 2016*a*). A mixed soil sample of the uppermost 10 cm of the soil was taken from five random locations within the 10 m^2 plot. All samples were dried at 65 °C and the following parameters were then determined in the laboratory: skeleton content (mass fraction of particles > 2 mm); the percentages of sand, clay and silt (texture class estimated with the Robinson pipette method after removing organic matter with 6% H_2O_2); pH (in a suspension of 10 g dry soil in 25 ml distilled water); electrical conductivity (EC) in the same pH extract (in a suspension of 10 g dry soil in 50 ml distilled water, µS cm^{-1}); carbon total (%); $CaCO_3$ content (%) (Schlichting *et al.* 1995; Wamelink *et al.* 2012).

Results

The final concatenated alignment included 55 sequences of 2034 unambiguous nucleotide positions (ITS1 = 187 sites, 5.8S = 158, ITS2 = 150, nrLSU = 761, mtSSU = 778). The ML and BI analyses yielded similar topologies. Figure 1 represents the topology recovered from the BI analysis. The genus *Circinaria* formed a well-supported clade (PP = 1, BS = 95), sister to the clade containing *Lobothallia* and *Aspicilia.* The newly generated sequences of the *Circinaria* species from the sand dunes of southern Ukraine formed a highly supported separate clade with *Aspicilia reptans* (Looman) Wetmore (PP = 0.99, BS = 92). Morphologically and ecologically the latter species is similar to *C. ucrainica*, but it has thicker rhizomorphs of 200–400 µm diameter, finely developed areoles and lacks spicate prothalline tips, whereas the studied specimens grew inland on sand dunes and were characterized

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Table 1. Voucher information and GenBank Accession numbers of sequences of *Circinaria* and related species included in the phylogenetic analyses (Fig. 1).

Taxon name	Vouchers	nrLSU	mtSSU	nrITS	References
Aspicilia cinerea	Sweden, *Hermansson* 13275 (UPS)	HM060733	HM060695	EU057899	Nordin *et al.* 2010
A. epiglypta	Sweden, *Nordin* 6303 (UPS)	HM060756	HM060718	EU057907	Nordin *et al.* 2010
A. reptans 1	Canada, *Di Meglio* 261 (OSC)	MZ536844		MZ536729	McCune & Di Meglio 2021
A. reptans 2	Canada, *Di Meglio* 262 (OSC)	MZ536845		MZ536730	McCune & Di Meglio 2021
A. reptans 3	Canada, *Di Meglio* 263 (OSC)	MZ536846		MZ536731	McCune & Di Meglio 2021
A. reptans 4	USA, *Di Meglio* 303 (OSC)			MZ536740	McCune & Di Meglio 2021
A. spicata 1	USA: Oregon, *Di Meglio* 255 (OSC)			MZ536725	McCune & Di Meglio 2021
A. spicata 2	USA: Washington, *Hardman* & *Root* (*Stone*) EGL1 (OSC)	MZ536879		MZ536832	McCune & Di Meglio 2021
A. spicata 3	USA: Washington, *Root* & *Smith* (*Stone*) B1–15 (OSC)	MZ536896		MZ536830	McCune & Di Meglio 2021
A. spicata 4	USA: Washington, *Stone* NC1–10 (OSC)	MZ536898		MZ536834	McCune & Di Meglio 2021
Circinaria arida 1	USA, *Owe-Larsson* 8759			HQ406800	Owe-Larsson *et al.* 2011
C. arida 2	USA, *Owe-Larsson* 8770			EU057905	Owe-Larsson *et al.* 2011
C. arida 3	USA, *Knudsen* 2046 (UPS)			HQ406801	Owe-Larsson *et al.* 2011
C. aspera 1	Canada, *Di Meglio* 311a (OSC)	MZ536854		MZ536746	McCune & Di Meglio 2021
C. aspera 2	USA, *McCune* 35792 (OSC)			MZ536760	McCune & Di Meglio 2021
C. aspera 3	USA, *Rosentreter* 18317 (SRP)	MZ536887		MZ536820	McCune & Di Meglio 2021
C. caesiocinerea	Sweden, *Tibell* 22612 (UPS)	HM060731	HM060693		Nordin *et al.* 2010
C. calcarea 1	Sweden, *Nordin* 5888 (UPS)	HM060743	HM060705	EU057898	Nordin *et al.* 2007
C. calcarea 2	Sweden, *Nordin* 5914 (UPS)			HQ406804	Owe-Larsson *et al.* 2011
C. contorta	Finland, *Pykälä* 28872 (H)	JQ797500			Sohrabi *et al.* 2013
C. crespiana	Spain, *Rico* 1249/1 & *Florido* (H)	JX306752		JX306733	Sohrabi *et al.* 2013
C. cupreogrisea	Sweden, *Nordin* 6046 (UPS)			EU057903	Nordin *et al.* 2007
C. digitata 1	Kyrgyzstan, *Ringel* 5185-B (H)			HQ171236	Sohrabi *et al.* 2011
C. digitata 2	Kyrgyzstan, *Ringel* 5185-B (H)			HQ171230	Sohrabi *et al.* 2011
C. elmorei	USA, *Rosentreter* 3689 (TU)			HQ389200	Owe-Larsson *et al.* 2011
C. emiliae 1	Kazakhstan, *Kulakov* 3798 (UPS)	HM060729	HM060691		Nordin *et al.* 2010
C. emiliae 2	Kazakhstan, *Kulakov* 3702 (UPS)	HM060728	HM060690	JQ797512	Nordin *et al.* 2010
C. esculenta	Russia, *Owe-Larsson* 9796 (UPS)	JQ797493	JQ797485	JQ797511	Sohrabi *et al.* 2013
C. fruticulosa 1	Kazakhstan, *Lange* 5186 (H)		JQ797486	HQ171228	Sohrabi *et al.* 2011
C. fruticulosa 2	Ukraine: Crimea, *Vondrák* 5188 (CBFS)			HQ389199	Sohrabi *et al.* 2011
C. gibbosa	Sweden, *Nordin* 5878 (UPS)	HM060740	HM060702	EU057908	Nordin *et al.* 2007
C. gyrosa	Iran, *Sohrabi* 10085 (hb. M. Sohrabi)	JQ797504		JQ797540	Sohrabi *et al.* 2013
C. hispida 1	Turkey, *Candan* 11 (ANES)	HM060760	HM060722	HQ406806	Owe-Larsson *et al.* 2011
C. hispida 2	Iran, *Sohrabi* 15099 (hb. M. Sohrabi)		JQ797488	HQ171233	Sohrabi *et al.* 2011
C. leprosescens 1	*Nordin* 5906 (UPS)	HM060749	HM060711	EU057911	Nordin *et al.* 2010
C. leprosescens 2	*Nordin* 6059 (UPS)	HM060752	HM060714		Nordin *et al.* 2010
C. ucrainica **sp. nov.**	**Ukraine, *Khodosovtsev* (KRAM L- 74394)**	**PP515450**	**PP515451**	**PP515449**	**This paper**
Circinaria sp. 1	*Leavitt* 19068v2			MZ922110	
Circinaria sp. 2	*Leavitt* 19073v3			MZ922111	
Circinaria sp. 3	*Leavitt* 19031v3			MZ922112	
Circinaria sp. 4	Iran, *Sohrabi* 4758 (H)			JQ797550	Sohrabi *et al.* 2013

(*Continued*)

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Table 1. (*Continued*)

Taxon name	Vouchers	nrLSU	mtSSU	nrITS	References
Circinaria sp. 5	Iran, *Sohrabi* 10117B (hb. M. Sohrabi)			JQ797544	Sohrabi *et al.* 2013
Circinaria sp. 6	Iran, *Sohrabi* 9380b (IRAN)			JQ797547	Sohrabi *et al.* 2013
Circinaria sp. 7	Iran, *Sohrabi* 9380b (IRAN)			JQ797548	Sohrabi *et al.* 2013
Circinaria sp. 8	Iran, *Sohrabi* 10092A (IRAN)			JQ797549	Sohrabi *et al.* 2013
Circinaria sp. 9	Iran, *Sohrabi* 9357 (IRAN)			JQ797530	Sohrabi *et al.* 2013
Circinaria sp. 10	Iran, *Sohrabi* 9347 (IRAN)			JQ797546	Sohrabi *et al.* 2013
Lobothallia alphoplaca 1	USA, *Leavitt & Leavitt* 849 (BRY – C54920)	KC667060		JX306739	Sohrabi *et al.* 2013
L. alphoplaca 2	USA, *Leavitt* et al. 447 (BRY – C54921)	KC667061		JX306737	Sohrabi *et al.* 2013
L. farinosa	France: Rhône-Alpes, *Roux* 25286 (UPS)	HM060761	HM060723		Nordin *et al.* 2010
L. melanaspis	Sweden, *Nordin* 6622 (UPS)	HM060726	HM060688	HQ259272	Nordin *et al.* 2011
L. radiosa	Switzerland, *Lumbsch* (F)	DQ780306	DQ780274		Schmitt *et al.* 2006
Megaspora verrucosa 1	Turkey, Kinalioglu 1679 (B)	JQ797497	JQ797482		Sohrabi *et al.* 2013
M. verrucosa 2	Sweden, *Nordin* 6495 (UPS)	HM060725	HM060687		Nordin *et al.* 2010
M. verrucosa 3	USA, *St. Clair* 18429 (BRY – C54042)	KC667062		KC667053	Sohrabi *et al.* 2013

by narrow spicate prothalline tips and rhizomorphs. Therefore, taking into account the differences in morphological, ecological and molecular data, we describe a new *Circinaria* species growing on sandy soil and also propose a new combination for *Aspicilia reptans*.

Taxonomy

Circinaria ucrainica Khodos. & Darmostuk sp. nov.

MycoBank No.: MB 853496

Differing from *Aspicilia spicata* by the presence of finely developed dark grey to brown spicate prothalline tips, 50–150 µm diam., (0.5–)1–2.5(–3) mm long, poorly developed grey areoles, a thinner epicortex and the presence of aspicilin.

Type: Ukraine, Kherson Region, Kherson District, near Oleshky, Landscape Reserve 'Sagy', 46°36′40.7″N, 32°51′28.8″E, 12 m, 11 June 2021, *A. Khodosovtsev* (KHER 15091—holotype; KHER 15092, 15093, KRAM L- 74394—isotypes). GenBank Accession nos: PP515449 (nrITS), PP515450 (nrLSU), PP515451 (mtSSU).

(Fig. 2)

Thalli crustose, cushion-like, relatively large, 2–10 cm diam. and 0.2–0.5 cm high, consisting of dispersed or rarely overlapping areoles, interconnected by abundant mycobiont rhizomorphs. *Areoles* rarely finely developed and overlapping, (120–)150–200(–220) µm thick, more or less isodiametric, 0.2–1.0 mm wide or elongated, 0.1–0.2 × 0.5–1.0 mm, flat, slightly convex or almost cylindrical, grey or greyish green, sometimes with a brown tinge above and whitish below. *Spicate prothalline tips* formed on the edges of areoles, (2–)3–5(–7) per areole, dark grey to brown, 50–150 µm diam., horizontally or vertically oriented, (0.5–)1–2.5(–3) mm long, single or dichotomously branched, fan-shaped, extended and bluish at the tips. *Rhizomorphs* hyaline, 100–150 µm diam., up to 10 mm long, branched and forming a loose network. *Epicortex* hyaline, consisting of dead cells, without crystals, (5–)7–9(–12) µm deep ($n = 10$). *Upper cortex* hyaline or light brown in external parts, (20–)30–50(–65) µm ($n = 20$) thick, paraplectenchymatous, consisting of rounded cells with lumina (4–)5.0–8.0(–9.0) µm ($n = 30$) diam., completely covered by cylindrical areoles (Fig. 2D). *Algal plectenchyma c.* 30–50 µm thick, more or less continuous or with clusters deep in medulla; algae *Trebouxia*-type, (8–)9.5–13.5(–20) µm diam. *Medulla* white, loose, prosoplectenchymatous, *c.* 80–120 µm thick. *Lower cortex* not developed. *Rhizomorphs* and *spicate prothalline tips* covering hyaline layers *c.* 10–15 µm thick, without algae, prosoplectenchymatous, with lumina (1.5–)2.0–4.0(–4.5) µm ($n = 15$) wide.

Apothecia, *pycnidia*, *pseudocyphellae* and *vegetative diaspores* absent.

Chemistry. TLC: aspicilin. Spot tests negative.

Etymology. The species is named after the Ukraine, where the Eurasian Dry Grassland Group (https://edgg.org) organized a research expedition to the steppe and coastal habitats in May–June 2021, during which the species was discovered.

Ecology. The species grows in the sand dunes of the Lower Dnipro valley, Ukraine, where it can cover a significant area (up to 20%). It is part of the biological crust dominated by the moss *Syntrichia ruraliformis* (up to 40%), along with other lichens such as *Cladonia fimbriata* (L.) Fr., *C. foliacea* (Huds.) Willd., *Placynthiella uliginosa* (Schrad.) Coppins & P. James s. lat., *Xanthoparmelia camtschadalis* (Ach.) Hale and the mosses *Bryum caespiticium* Hedw. and *Ceratodon purpureus* (Hedw.) Brid. Vascular plants in the biodiversity plot were represented by 32 species with the highest cover by *Poa bulbosa* L. (15%), *Helichrysum arenarium* (L.) Moench (5%) and *Stipa borysthenica* Klokov ex Prokudin (5%) (Table 2).

The rhizomorphs of *Circinaria ucrainica* grew closely attached to sand grains of stable small dunes. The main soil parameters in the habitat are: proportion of skeleton – 0.6%; soil texture class – sand; proportion sand – 99.0%; proportion silt – 1.0%; proportion of clay – 0.02%; pH (H_2O) – 6.97; electrical conductivity – 64.4 µS cm^{-1}; carbon total – 0.33%; $CaCO_3$ content – 12.5%.

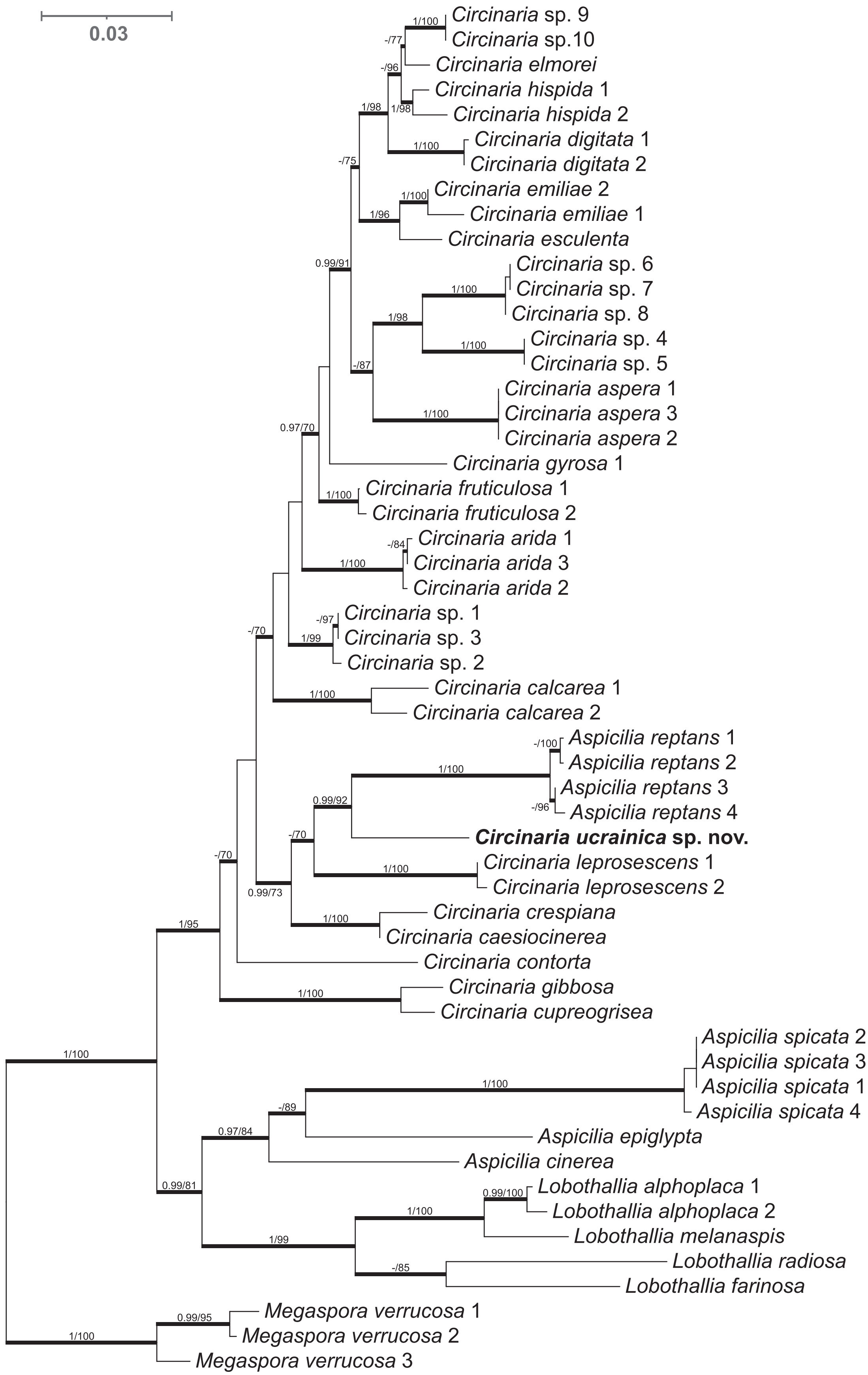

Figure 1. Phylogenetic placement of *Circinaria ucrainica* inferred from Bayesian inference (BI) analyses of the combined nrITS, nrLSU and mtSSU data set. *Megaspora verrucosa* was used as the outgroup. Bold branches represent either maximum likelihood (ML) bootstrap values ≥ 70 and/or Bayesian posterior probabilities ≥ 0.97. BI/ML values are indicated on branches. The new species is shown in bold.

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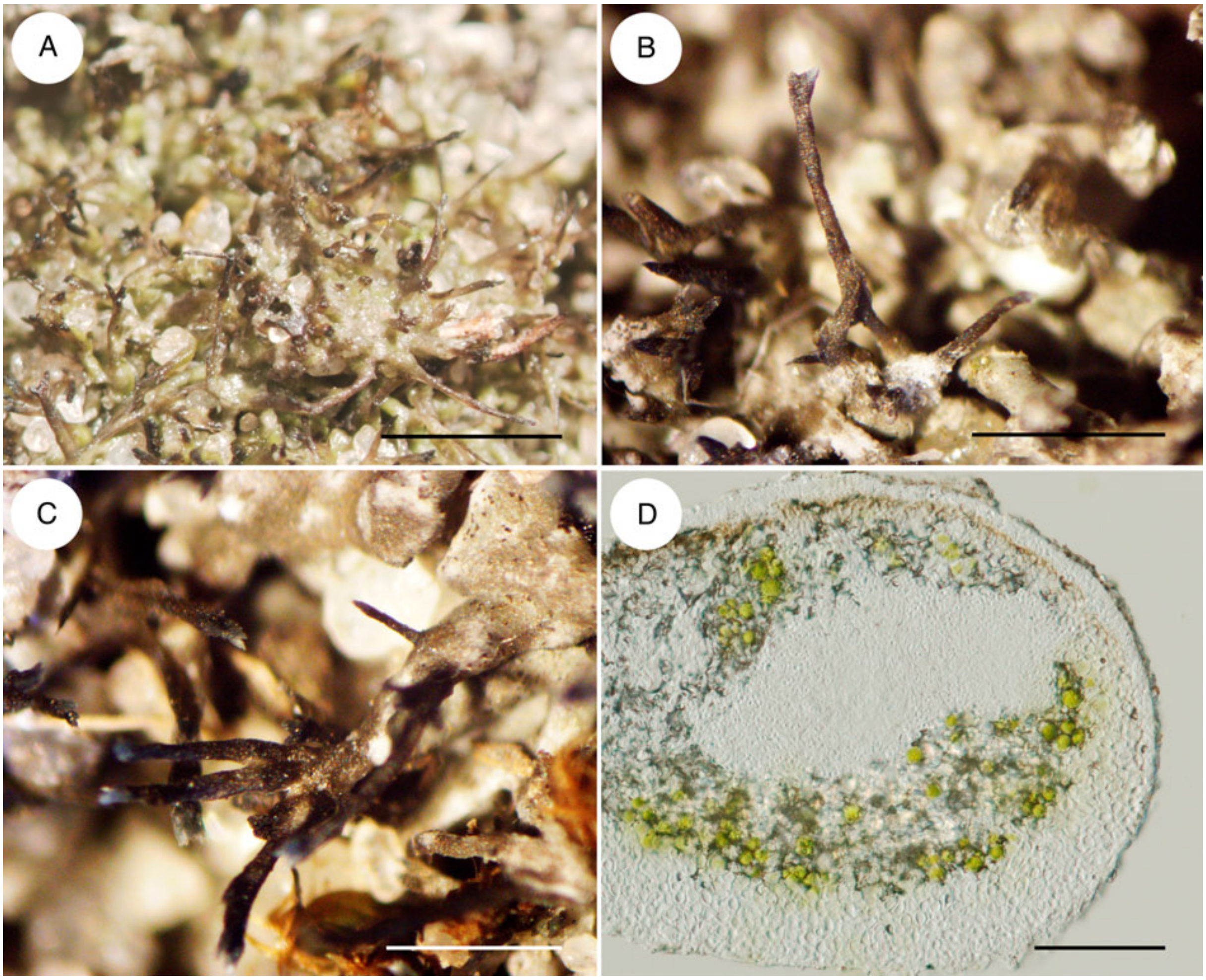

Figure 2. *Circinaria ucrainica*. A, general habitat of wet lichen thalli. B, spicate prothalline tips. C, elongated areoles with spicate prothalline tips. D, cross-section through almost cylindrical areoles. Scales: A = 2 mm; B & C = 1 mm; D = 100 μm. In colour online.

Specimen examined. **Ukraine:** Kherson region, Kherson district, near Oleshky, Landscape Reserve 'Sagy', 46°36′40.6″N, 32°51′28.3″E, 12 m, biodiversity plot UAS046, 2 vi 2021, *I. Dembicz, J. Dengler* & *I. Moysiyenko* (KHER 15094—paratype).

New combination

Circinaria reptans (Looman) Khodos. & Darmostuk comb. nov.

MycoBank No.: MB 853497

Basionym: *Lecanora reptans* Looman, *Bryologist* **65**, 301 (1963).

Discussion

Rhizomorphs and spicate prothalline tips are rare and specific structures in *Aspicilia* s. lat. Rhizomorphs are linear mycelial aggregates produced from the lower cortex of crustose lichens (Sanders & Rico 1992; Sanders & Ascaso 1997; Sanders 1999; McCune & Di Meglio 2021). They are mainly characteristic of species that overgrow loose substrata, such as mosses or soil. In *Megasporaceae*, these structures are rare and found only in a small number of species of *Aspicilia* s. lat. (incl. *Circinaria*) (McCune & Di Meglio 2021). In Europe, *Circinaria crespiana* (V.J. Rico) Sohrabi & V.J. Rico was described on mosses from the Mediterranean (Rico 1999). It has thick underground rhizomorphs, up to 1.5 mm wide (in *Circinaria ucrainica* up to 0.15 mm), lacks spicate prothalline tips and has better developed squamulose areoles, up to 5 mm in width (in *C. ucrainica* up to 1 mm). Another species with rhizomorphs is *Circinaria reptans*, reported from calcareous soil and plant detritus in North America (Canada, USA) and Asia (Iran) (Lumbsch *et al.* 2011; McCune & Di Meglio 2021). This species is similar to *C. ucrainica* but has thicker rhizomorphs of 200–400 μm diameter, finely developed areoles and lacks spicate prothalline tips. Recently, rhizomorphs were found in *Aspicilia diploschistiformis* McCune & J. Di Meglio, *A. papilliformis* McCune & J. Di Meglio, *A. subcontinua* McCune & J. Di Meglio and *A. wyomingensis* McCune & J. Di Meglio (McCune & Di Meglio 2021), but these species lack spicate prothalline tips like those found in *Circinaria ucrainica*. These species resolved in different clades in the phylogenetic analysis provided by McCune & Di Meglio (2021), phylogenetically different from the *Circinaria reptans* clade.

The term 'spicate prothalline tips' was introduced by McCune & Di Meglio (2021) to describe fungal structures formed by the prothallus. These structures were found in *Aspicilia californica* Rosentr. and *A. filiformis* Rosentr. from arid habitats in the United States (Sanders 1999). However, these species differ

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Table 2. Diversity and cover of vascular plants, mosses and lichens adjacent to the EDGG biodiversity plot UAS046 with *Circinaria ucrainica* (L = lichen, M = mosses). The table is ordered according to Dengler *et al*. (2016*b*). Organisms are arranged in the order in which they are identified in the corner of the site.

	*Edge length of square (m)											
	NW corner						SE corner					
Species	0.01	0.03	0.1	0.32	1.0	%3.16	0.01	0.03	0.1	0.32	1.0	%3.16
[M]*Bryum caespiticium*	+	+	+	+	+	1						1
[M]*Ceratodon purpureus*	+	+	+	+	+	2						–
[M]*Syntrichia ruraliformis*	+	+	+	+	+	40				+	+	10
Poa bulbosa		+	+	+	+	15	+	+	+	+	+	10
[L]*Cladonia fimbriata*						–		+	+	+	+	1
Artemisia marschalliana			+	+	+	2	+	+	+	+	+	0.5
[L]*Cladonia foliacea*				+	+	12			+	+	+	3
Draba verna						0.01					+	0.01
Helichrysum arenarium				+	+	5			+	+	+	10
Lomelosia argentea				+	+	0.3						–
Sedum aetnense				+	+	0.01	+	+	+	+	+	0.5
Stipa borysthenica				+	+	5				+	+	12
Thymus borysthenicus						–				+	+	8
Bassia laniflora					+	0.001						–
Carex colchica					+	0.01						0.01
[L]*Cladonia sp.*					+	0.05						–
Dianthus platyodon					+	0.05					+	1
Euphorbia seguieriana					+	0.5						0.01
Festuca beckeri					+	0.7					+	1
[L]*Placynthiella uliginosa* s. lat.					+	0.5		+	+	+	+	5
Koeleria glauca						–					+	0.1
Scirpoides holoschoenus						–					+	3
[L]*Xanthoparmelia camtschadalis*						–					+	0.1
Allium guttatum						0.01						–
Odontarrhena tortuosa						0.05						0.01
Cerastium gracile						0.02						0.01
Centaurea breviceps						0.1						–
[L]***Circinaria ucrainica***						0.5					+	20
Crepis ramosissima						0.001						–
Cynodon dactylon						3						–
Filago arvensis						0.001						–
Minuartia viscosa						0.05		+	+	+	+	2
Salix rosmarinifolia						0.5					+	4
Jakobaea borysthenica						0.3						–
Corynephorus canescens						–						0.2
Erygeron canadensis						–						0.02
Holosteum umbellatum						–						0.01
Silene borysthenica						–						0.1
Veronica dillenii						–						0.02

* *Astragalus varius* was found only in 100 m^2

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from *C. ucrainica* by their fruticose growth. The recently described crustose *Aspicilia spicata* McCune & J. Di Meglio with rhizomorphs (McCune & Di Meglio 2021) has finely developed spicate prothalline tips and is morphologically very close to *C. ucrainica*. However, *A. spicata* has finely developed, beaded-lobate or stringy to reticulate-lobate or warty-areolate brown areoles with a thick epicortex (in contrast to *C. ucrainica*, with poorly developed greyish areoles and a thin epicortex). *Aspicilia californica*, *A. filiformis* and *A. spicata* belong to the *Aspicilia filiformis* clade (McCune & Di Meglio 2021), which is phylogenetically distant from the *Circinaria reptans* clade. *Circinaria aspera* (Mereschk.) Sohrabi & Şenkard. rarely has rhizomorphs and spicate prothalline tips (McCune & Di Meglio 2021) but differs by a dimorphic thallus with crustose and fruticose parts (in contrast to the poorly developed thallus of *C. ucrainica*), rounded or isidioid prothalline tips (vs spicate tips in *C. ucrainica*), as well as a thicker epicortex, 12–50 µm (vs (5–)7–9(–12) µm in *C. ucrainica*). *Aspicilia albonota* McCune & J. Di Meglio has very small spicate prothalline tips, but this species has no rhizomorphs and forms pseudocyphellae on the areoles (McCune & Di Meglio 2021). The poorly studied *Aspicilia terrestris* Tomin (Tomin 1956), from arid salt habitats of Kazakhstan (Lake Inder) and Russia (Lake Baskunchak), lacks spicate prothalline tips and is morphologically similar to *Circinaria reptans*.

Based on current knowledge, *Circinaria ucrainica* has a very narrow distribution range but is quite abundant locally. It has been found only on a small number of dunes, even though 140 relevés of terricolous bryophyte and lichen communities had previously been conducted in the area without encountering the species (Khodosovtsev *et al.* 2011). Phytosociologically, the plots with *Circinaria ucrainica* can, in accordance with the 'EuroVegChecklist' (Mucina *et al.* 2016), be assigned to the class *Koelerio-Corynephoretea canescentis*, the order *Festucetalia vaginatae*, and the alliance *Festucion beckeri*. According to the '*Prodrome of the Vegetation of Ukraine*' (Dubyna *et al.* 2019), the assignment is somewhat different due to a different system of higher units of psammophyte vegetation. There it belongs to the class *Festucetea vaginatae* but the same order and alliance as in the EuroVegChecklist. In terms of floristic composition, the stand is closest to the association *Centaureo brevicipiti-Festucetum beckeri*. If we consider the mosaic of cryptogam communities within the 'biological crust', then the crust can be attributed to the class *Ceratodonto purpurei-Polytrichetea piliferi*, the alliance *Cladonion rei* and close to the association *Syntrichietum ruraliformis* (Khodosovtsev *et al.* 2011). This is especially evident at small scales within the biodiversity plot (Table 2). Following the EUNIS habitat classification (Schaminée *et al.* 2018; Chytrý *et al.* 2020), the site belongs to the habitat type 'R11-Pannonian and Pontic sandy steppe', while in the national habitat classification of Ukraine (Kuzemko *et al.* 2018) it corresponds to 'T1.1.2-Sandy grasslands on neutral substrata'.

While most lichen species have large distribution ranges, some vagrant and terricolous *Circinaria* species from arid habitats are local endemics. For example, *Circinaria tominii* (Oxner) Sohrabi was collected only from two nearby localities in the Czuensi Desert (Altai, Russia) (Sohrabi *et al.* 2013) and *C. aschabadensis* (J. Steiner) Sohrabi is known from a single locality in the Kopet-Dagh Desert (Turkmenistan). *Circinaria ucrainica* is probably a local endemic of the dunes of the Lower Dnipro and needs to be protected. The population grows in the Landscape Reserve 'Sagy', but it is situated only 150–200 m from the main roads to Oleshky, Kakhovka and Crimea, where the Russian invasion of Ukraine took place in 2022. The single known population of the species is under threat of complete destruction and possible extinction.

The international research expeditions of the Eurasian Dry Grassland Group, conducted since 2009 in grassland and other non-forest ecosystems throughout the Palearctic, do not only yield standardized high-quality biodiversity data of vascular plants, but also of terricolous bryophytes and lichens, and sometimes animal taxa, together with soil and other environmental variables (Dengler *et al.* 2016*a*, *b*). Careful sampling of survey plots of defined areas by international experts, particularly for understudied taxa such as lichens, provides not only valuable references for species diversity in different grassland types and regions (Dengler *et al.* 2016*b*; Biurrun *et al.* 2021), but has repeatedly led to first records of lichen species for countries or other larger geographical areas. *Circinaria ucrainica* is already the second species new to science reported on an EDGG expedition, after the spider *Pulchellodromus navarrus* found on the EDGG expedition in Navarre, Spain (Kastrygina *et al.* 2016).

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Author ORCIDs. Alexander Khodosovtsev, 0000-0002-5906-9876; Valerii Darmostuk, 0000-0003-1430-1755; Iwona Dembicz, 0000-0002-6162-1519; Jürgen Dengler, 0000-0003-3221-660X; Ivan Moysiyenko, 0000-0002-0689-6392; Anna Kuzemko, 0000-0002-9425-2756.

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