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Standard Paper

Circinaria nimisii (*Megasporaceae*, lichenized *Ascomycota*), a new manna lichen from Greece

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Abstract

The manna lichens, a group of vagrant species with subfruticose and subfoliose thalli in the genus *Circinaria* Link, have received attention for millennia. Here, a new manna lichen species, *Circinaria nimisii* sp. nov. (*Megasporaceae*), is described and illustrated. This vagrant lichen is found on Mount Olympus in Greece and is the fourth known manna lichen in Europe. The new taxon is characterized by its subfruticose, densely-branched thallus with a muddy, earthy colour, whitish pseudocyphellae on tips of branches, mature apothecia distinctly adnate to stipitate, and paraplectenchymatous cortex tissue. Molecular sequence data from the standard barcoding marker (nrITS) also corroborate the distinction of this species from closely related congeners. Finally, *Agrestia zerovii*, previously known only from its type locality in Ukraine, is proposed as a new synonym of *Circinaria hispida*.

Keywords: *Circinaria hispida*; phylogeny; taxonomy; terricolous; vagrant lichens

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Introduction

The genus *Circinaria* Link (*Ascomycota*, *Lecanoromycetes*, *Pertusariales*, *Megasporaceae*) was resurrected by Nordin *et al.* (2010) and comprises *c.* 40 species worldwide. The genus is characterized by crustose (Chesnokov *et al.* 2018), umbilicate, fruticose, subfruticose, or spherical growth forms, a thickened medullary layer, usually with pseudocyphellae, 2–6 spores per ascus, low secondary metabolite diversity, and relatively short conidia (Sohrabi *et al.* 2013). It is distinguished from *Aspicilia* and the other genera within *Megasporaceae* by thallus morphology, presence/absence of pseudocyphellae, the number of ascospores per ascus and the size of ascospores, conidia length, and the presence/absence of some compounds such as aspicilin. This genus is distributed worldwide, with *Circinaria minuta* P. M. McCarthy & Elix recently described as a new species from Australia (McCarthy & Elix 2020).

To date, a relatively broad concept of *Circinaria* species boundaries has been adopted, emphasizing thallus anatomy and morphological differences as the main phenotypic characters for species delimitation (Nordin *et al.* 2010; Owe-Larsson *et al.* 2011; Sohrabi *et al.* 2013; Ren & Zhang 2018; Ismayil *et al.* 2019; McCarthy & Elix 2020). Studies incorporating molecular sequence data have also been critical to informing taxonomy in *Megasporaceae* and circumscribing members therein (Wheeler 2017; McCune & Di Meglio 2021). Recently, new combinations in the genus *Circinaria* have been made (Roux *et al.* 2016; Ren & Zhang 2018). However, new taxa in Wheeler's study were left unpublished and McCune & Di Meglio (2021) described some new taxa, such as *Aspicilia albonota* McCune & J. Di Meglio, *A. diploschistiformis* McCune & J. Di Meglio, *A. papilliformis* McCune & J. Di Meglio, *A. spicata* McCune & J. Di Meglio, *A. subcontinua* McCune & J. Di Meglio and *A. wyomingensis* McCune & J. Di Meglio, and united some species in *Aspicilia* despite the obvious relationship of the *Aspicilia reptans* complex to *Circinaria*. Within *Megasporaceae*, vagrant or erratic thalli (Rosentreter 1998) have received considerable attention. Some vagrant species in *Circinaria* with subfruticose and subfoliose thalli have been termed 'manna lichens' (Crum 1993; Sohrabi *et al.* 2013). Manna lichens were historically searched for by botanists across Central Asia (Eversmann 1831; Elenkin 1901), and they are interesting elements of the desert and semi-arid habitats (Crum 1993). However, manna lichens are surprisingly rare in European semi-arid regions, with only three known species in this region: *C. fruticulosa* (Eversm.) Sohrabi from Ukraine, *C. gyrosa* Sohrabi *et al.* from Spain and Ukraine, and *C. hispida* (Mereschk.) A. Nordin *et al.* from Italy, Greece, Spain and Ukraine (Kopacevskaja *et al.* 1971; Follmann & Crespo 1974; Hafellner *et al.* 2004; Sohrabi *et al.* 2013).

During the preparation of the revision of manna lichens, a set of exsiccati specimens distributed as *Aspicilia fruticulosa* in *Lichenotheca Graecensis* Fasc. 14, no. 264 (Obermayer 2004) was found to be closely related to *C. hispida* when analyzed by Sohrabi *et al.* (2013). However, this collection had distinct morphological characters that differed from the holotype of *C. hispida*. At that time, DNA sequences of these specimens were obtained (GenBank Accession nos: JQ797522, JQ797523) and

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kept under *C. hispida* s. lat. Based on a combination of morphological, chemical and molecular sequence data, this species is formally described here as *C. nimisii.* The phylogenetic relationship between the new taxon and other closely related species is assessed using maximum likelihood analysis based on an alignment of nrDNA ITS sequences.

Material and Methods

Specimens, morphological and chemical studies

During the VI OPTIMA Meeting in 1989, Arnoldo Santos (La Laguna), a participant in the excursion, first detected this vagrant *Circinaria* population on the Plateau of Muses in the alpine belt of Mount Olympus. Accompanied by Eva Barreno (Valencia), HM visited the locality one day later, finding a large population occurring among small pebbles in open, wind-exposed steppe habitats. The collected specimens were distributed in the exsiccatae *Lichenotheca Graecensis* Fasc. 14, no. 264 (Obermayer 2004). For this study, the morphology and anatomy of this exsiccatae collection were compared with other vagrant *Circinaria* specimens from the herbaria FH, H, LE, TUR, TU and W. Specimens were examined using a dissecting microscope (Leica M50) and a compound microscope (Leica DM 2500 compact light microscope). Sections cut by hand were studied in material mounted in water. Images of the thallus were captured with a Leica Wild M3Z stereomicroscope (equipped with a Zeiss AxioCam MRc5 digital camera). Image stacking was performed using the open-source image processing software 'CombineZP'. Images of asci (with spores) and spermatia (= pycnospores) were captured with a Zeiss Axioskop microscope, equipped with the same camera as noted above. Measurements are presented as follows: (min–) $\bar{x}$ ±SD (–max) (n = number of measurements); where $\bar{x}$ is the mean and SD is the standard deviation.

For anatomical observations, a small number of branches of thalli were cut using a razor blade, and additional sections 10–16 mm thick were cut using a freezing microtome (Leica CM 3050S). Sections were mounted in lactophenol cotton blue and subsequently photographed with a Leica DM 2500 compact light microscope equipped with a digital camera. Thin-layer chromatography (TLC) was applied following Orange *et al.* (2010) using the solvent systems A, B and C. High-performance liquid chromatography (HPLC) was performed following Søchting (1997).

DNA extraction, PCR amplification and sequencing

In this study, 20 specimens were selected and used to generate molecular sequences. From these, DNA was extracted from a *c.* 1 × 1 mm^2 piece of the medulla using Qiagen's DNAeasy Blood and Tissue Kit following the manufacturer's instructions. For polymerase chain reaction (PCR), the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) were used to amplify the fungal nuclear ribosomal DNA internal transcribed spacer (ITS nrDNA, henceforth ITS). Ready-To-Go PCR beads (Pharmatica Biotech) were used for PCR amplification with 4 µl of undiluted DNA, 1 µl of each primer (10 M) and 19 µl of sterile water for dissolving the beads. The Gene Amp PCR system 9700 (Perkin-Elmer) PTC-100 and PTC-200 Thermocyclers (MJResearch) were used under the following conditions: initial denaturation for 5 min at 95 °C, followed by five cycles of 30 s at 95 °C, 30 s at 58 °C, and 1 min at 72 °C. The annealing temperature was reduced to 56 °C in the remaining 30 or 35 cycles; a final extension of 7 min at 72 °C was included in the last cycle.

Sequence alignment and phylogenetic analysis

The newly produced sequences were assembled and analyzed using Sequencher v. 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and DNASTAR software (DNASTAR, Madison, Wisconsin, USA). The final analyses included the newly generated sequences, the most similar *Circinaria* sequences available on GenBank (identity > 90%) according to a BLASTN search (Altschul *et al.* 1990), and sequences of chemically and morphologically similar species (*C. hispida*). *Aspicilia cinerea* (L.) Körb. and *A. goettweigensis* (Zahlbr.) Hue were selected as outgroups. The ITS region was aligned using MAFFT v. 7 (Katoh & Standley 2013) with the L-INS-i method (Katoh *et al.* 2005). Ambiguous positions were excluded from the analysis using Gblocks v. 0.91b (Castresana 2000), with a less stringent selection, on the Phylogeny.fr server (Dereeper *et al.* 2008).

The maximum likelihood (ML) analysis was performed on the CIPRES Science Gateway (Miller *et al.* 2010) using RAxML-HPC v. 8.2.10 (Stamatakis 2014) and the GTR + G + I model (Stamatakis 2014). We performed a non-parametric bootstrap analysis with 1000 replicates. The maximum likelihood consensus tree is shown, with bootstrap values indicated at branches (Fig. 2). The final matrix can be obtained from the corresponding author and is deposited in TreeBase (number pending).

Results and Discussion

In total, 20 new ITS sequences were generated (Table 1). The final ITS alignment comprised 95 sequences and spanned 544 aligned nucleotide position characters.

Taxonomy

Circinaria nimisii Sohrabi, H. Mayrhofer, Obermayer & S. D. Leav. sp. nov.

MycoBank No.: MB 846975

Thallus vagrant, more or less globose, similar to *Circinaria gyrosa* Sohrabi *et al.* but differs in having well-developed, horizontally orientated coralloid, flattened lobes, folds or very thick thalline lobes often forming a distinct, erected areole-like surface, attached to the central portion of the thallus; genetically distinct based on nuclear ribosomal ITS.

Type: Greece, Macedonia, Pieria, Óros Ólimbos (Mt Olympus) between refuge 'SEO' and refuge 'C', E of Mt Stefani, 45°05′N, 22°21′E, 2670 m, meadows with calcareous pebbles, on the ground (vagrant), 19 September 1989, *H. Mayrhofer* 15811 (GZU—holotype; ASU, B, C, CANB, CANL, E, G, GZU, H, HAL, HMAS, LE, M, MAF, MIN, O, TNS, UPS, MICH, PRM—isotypes).

(Fig. 1)

Thallus free (vagrant), subfruticose to tiny folding branches or flattened lobes, forming pebble-like clumps, 0.5–2 cm tall and 0.5–2 (–3) cm wide, usually spherical, rounded, sometimes irregularly shaped, rarely flat, often coralloid, plainly lobe-like branching; compact, predominantly irregularly flattened lobes dense,

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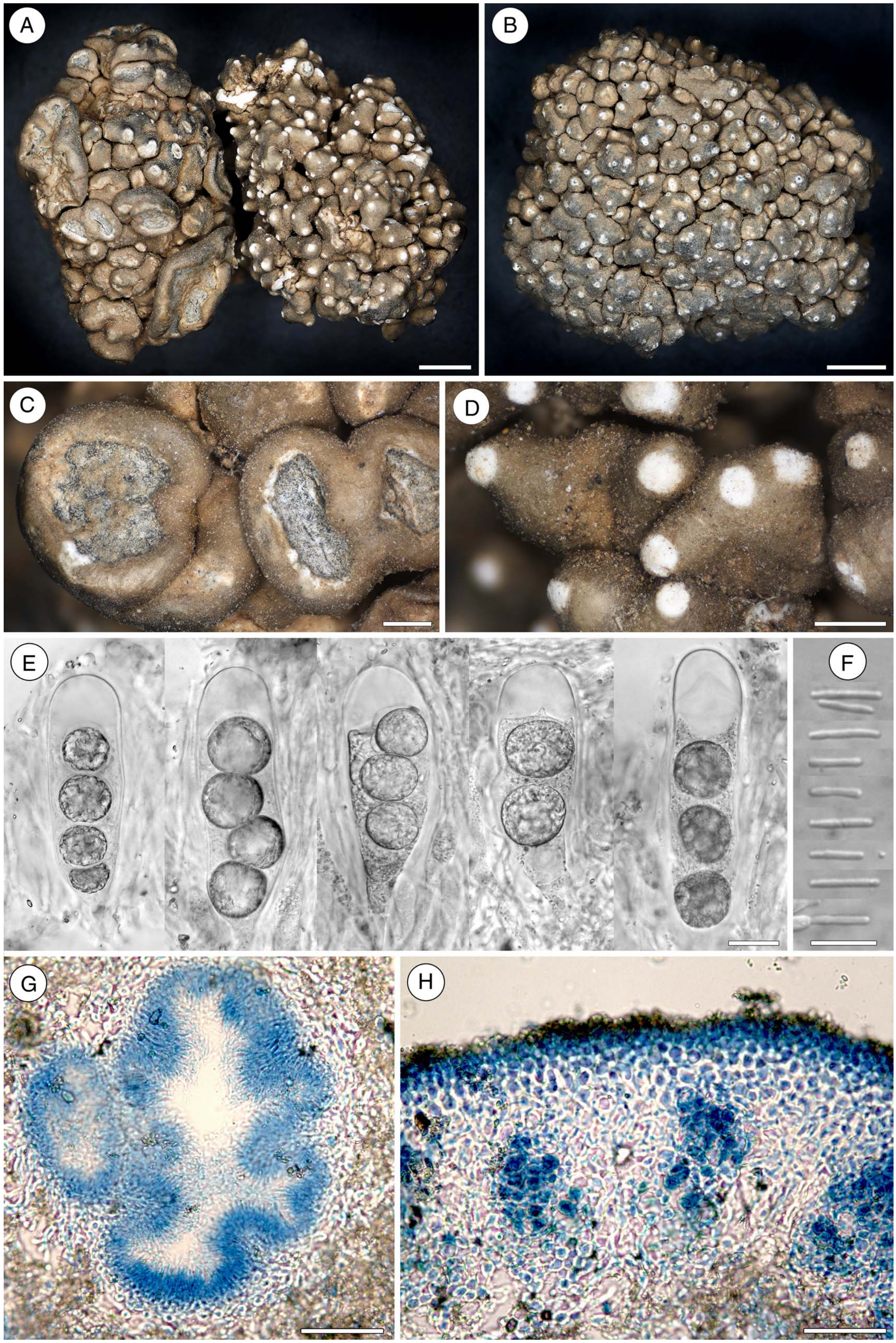

Figure 1. *Circinaria nimisii* (*H. Mayrhofer* 15811: A–F holotype in GZU; G & H isotype in H). A & B, thallus (in A with apothecia). C, aspicilioid apothecia arising from the thallus. D, phenotype with abundant pseudocyphellae forming conspicuous whitish dots. E, asci (in KOH) with thickened tholus and spores (the ascus furthest to the right has burst at the base and thus the apical tholus protrudes into the ascoplast). F, conidia (in KOH). G, section through a pycnidium (stained with lactophenol cotton blue). H, section through the thallus showing the typical cortex type with a paraplectenchymatous tissue (stained with lactophenol cotton blue). E & F photographed using differential interference contrast microscopy. Scales: A & B = 2 mm; C & D = 0.5 mm; E, G & H = 20 μm; F = 10 μm. In colour online.

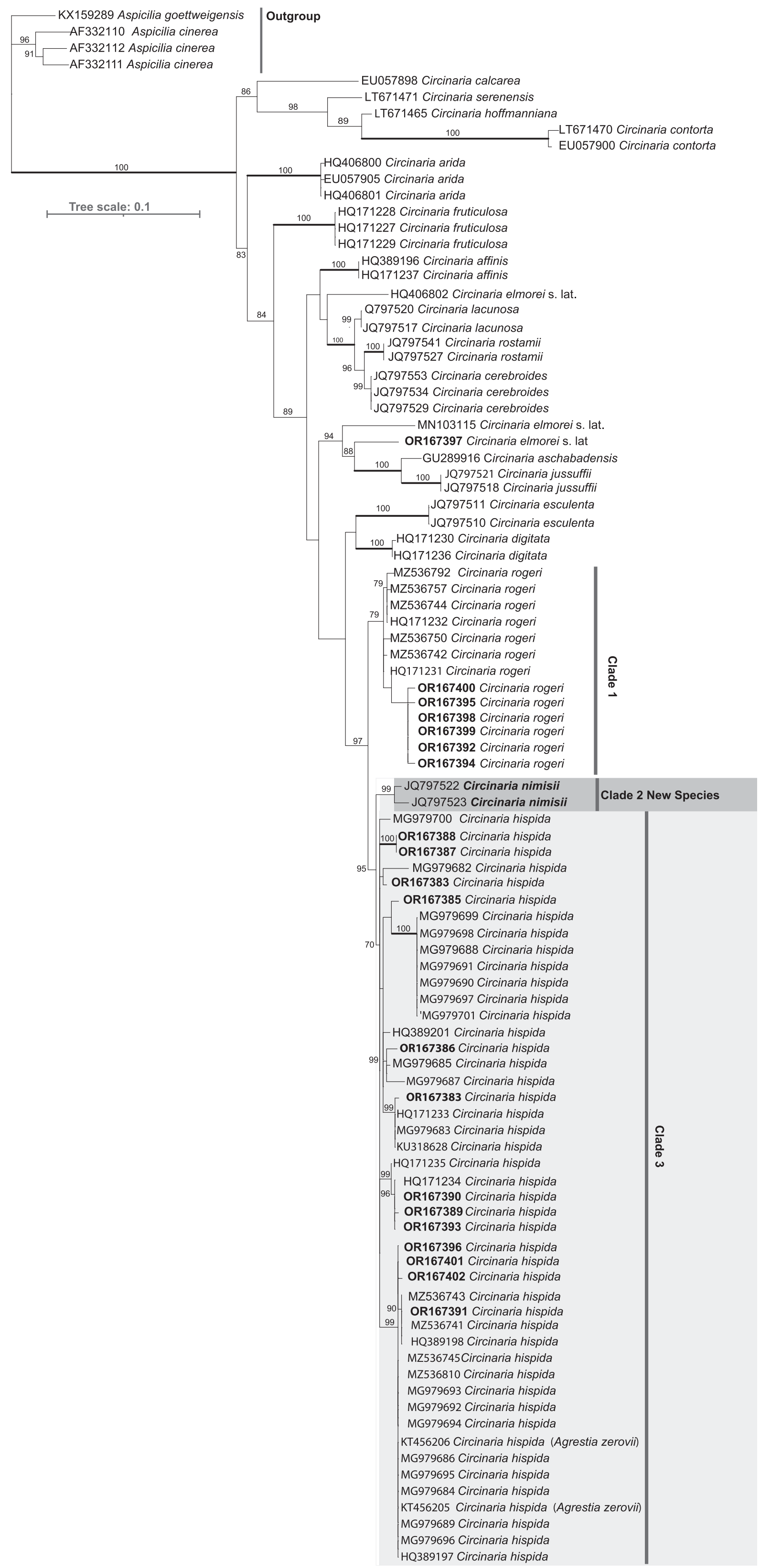

Figure 2. Phylogenetic relationships of *Circinaria* species based on a maximum likelihood (ML) analysis of the ITS nrDNA data set. ML bootstrap values ≥ 70% are adjacent to branches and branches with 100% support are shown in bold. The new species is shown in a grey box and three clades are indicated, with GenBank Accession numbers of new generated sequences shown in bold. Specimens of *Aspicilia* are outgroup. Information on the specimens used in this analysis is given in Table 1.

often forming a distinct, erected areole-like surface, attached to the central portion of the thallus, very compact, short to relatively elongate; main lobes radiating from the central axial part, usually wider up to 1.5–5 mm diam., tough in uppermost parts and more or less rimose, verrucose to verruculose, and somewhat flattened on top, up to 0.2–0.6 cm in the oldest part. *Surface* exposed side usually dull brown, brownish grey to greyish green, sometimes whitish grey, pale olive-brown to pale brown; surface on

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Table 1. Voucher details, GenBank Accession numbers and published resources for *Circinaria* specimens used in this study. New sequences are indicated in bold. *Aspicilia* specimens were used as outgroup.

Taxa	Country, voucher (herbarium)	Reference	GenBank ITS
Aspicilia cinerea	Sweden, Uppland, *Hafellner* 37308 (GZU)	Ivanova & Hafellner 2002	AF332111
A. cinerea	Austria, Styria, *Hafellner* 40563 (GZU)	Ivanova & Hafellner 2002	AF332110
A. cinerea	Austria, *Kocourkova* & *Hafellner* 46364(GZU)	Ivanova & Hafellner 2002	AF332112
A. goettweigensis	Austria, *Vondrák* 14026 (PRA)	Paukov *et al.* 2017	KX159289
Circinaria affinis	Russia, *Kulakov* 1408b (POLL)	Sohrabi *et al.* 2013	HQ389196
C. affinis	Russia, *Kulakov* 1408 (M)	Sohrabi *et al.* 2013	HQ171237
C. arida	USA, *Knudsen* 2046 (UPS)	Owe-Larsson *et al.* 2011	HQ406801
C. arida	USA, *Owe-Larsson* 8759 (UPS)	Owe-Larsson *et al.* 2011	HQ406800
C. arida	USA, *Owe-Larsson* 8770 (UPS)	Nordin *et al.* 2007	EU057905
C. aschabadensis	Turkmenistan, *A. Borisova* 1934 (LE)	Sohrabi *et al.* 2010	GU289916
C. calcarea	Sweden, *Nordin* 5888 (UPS)	Nordin *et al.* 2010	EU057898
C. cerebroides	Kyrgyzstan, *Ringel* 5184 (H)	Sohrabi *et al.* 2013	JQ797553
C. cerebroides	Kyrgyzstan, Inner Tian-Shan, *Ringel* 5138 (H)	Sohrabi *et al.* 2013	JQ797534
C. cerebroides	Kyrgyzstan, *H. Ringel* & *C. Jashhof* 5180	Sohrabi *et al.* 2013	JQ797529
C. contorta	Sweden, *Nordin* 5895 (UPS)	Nordin *et al.* 2010	EU057900
C. contorta	Sweden, L-204675 (UPS)	Roux *et al.* 2016	LT671470
C. digitata	Kyrgyzstan, *H. Ringel* 5185-B (H)	Sohrabi *et al.* 2011*a*	HQ171236
C. digitata	Kyrgyzstan, *H. Ringel* 5185 (H)	Sohrabi *et al.* 2011*a*	HQ171230
C. elmorei s. lat.	USA, *McCune* (29308)	This paper	**OR167397**
C. elmorei s. lat.	Russia, *Owe-Larsson* 9814 (UPS)	Owe-Larsson *et al.* 2011	HQ406802
C. elmorei s. lat.	China (Niu10-0067)	Direct submission	MN103115
C. esculenta	Russia, *Owe-Larsson* 9796 (UPS)	Sohrabi *et al.* 2013	JQ797511
C. esculenta	Russia, *Owe-Larsson* 9796 (UPS)	Sohrabi *et al.* 2013	JQ797510
C. fruticulosa	Russia, *Kulakov* 8 (hb. V. John, No: 9913)	Sohrabi *et al.* 2013	HQ171227
C. fruticulosa	China, *Abdulla Abbas* 940001 (H)	Sohrabi *et al.* 2013	HQ171229
C. fruticulosa	Kazakhstan, *Markus Lange* 5186 (H)	Sohrabi *et al.* 2011*a*	HQ171228
C. hispida	Spain, Zaorejas 8	Molins *et al.* 2018	MG979699
C. hispida	Iran, *Sohrabi* 10426 (hb. M. Sohrabi)	This paper	**OR167383**
C. hispida	USA, Utah, *Leavitt* 758	This paper	**OR167401**
C. hispida	USA, Wyoming, *Leavitt* 675	This paper	**OR167402**
C. hispida	USA, Colorado, *Leavitt* 760	This paper	**OR167393**
C. hispida	Spain, Maranchon 6	Molins *et al.* 2018	MG979687
C. hispida	USA, Idaho, *Leavitt* 672	This paper	**OR167396**
C. hispida	Turkey, MS209 (*Candan* 22) (hb. M. Sohrabi)	This paper	**OR167386**
C. hispida	USA, *Ann DeBolt* 2230	This paper	**OR167391**
C. hispida	Spain, Maranchon 4	Molins *et al.* 2018	MG979685
C. hispida	USA, *Muscha* & *Rosentreter* No. 121 (SRP)	Sohrabi *et al.* 2013	HQ171234
C. hispida	USA, Colorado, *Leavitt* 760-2	This paper	**OR167390**
C. hispida	USA, *Rosentreter* 16233 & *Cochrane* (SRP)	Sohrabi *et al.* 2013	HQ389198
C. hispida	Russia, *N. N. Ochirova* s. n. 2003 (LE)	Sohrabi *et al.* 2011*a*	HQ171235
C. hispida	Italy, *J. Hafellner* 59364	This paper	**OR167383**

(*Continued*)

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Table 1. (*Continued*)

Taxa	Country, voucher (herbarium)	Reference	GenBank ITS
C. hispida	Iran, East Azerbaijan, *Sohrabi* 10212b (hb. M. Sohrabi)	Sohrabi *et al.* 2013	HQ389197
C. hispida	USA, Wyoming, *Perry* 516 (OSC)	Molins *et al.* 2018	MZ536810
C. hispida	USA, Colorado, *Leavitt* 759	This paper	**OR167389**
C. hispida	Spain, Maranchon 3	Molins *et al.* 2018	MG979684
C. hispida	Spain, Guadalajara, Zaorejas	Molins *et al.* 2018	KU318628
C. hispida	Spain, Zaorejas 4	Molins *et al.* 2018	MG979695
C. hispida	Spain, Maranchon 5	Molins *et al.* 2018	MG979686
C. hispida	Spain, Zaorejas	Molins *et al.* 2018	MG979700
C. hispida	Spain, Maranchon 10	Molins *et al.* 2018	MG979691
C. hispida	Spain, Zaorejas 6	Molins *et al.* 2018	MG979697
C. hispida	Spain, Maranchon 8	Molins *et al.* 2018	MG979689
C. hispida	Spain, Zaorejas 7	Molins *et al.* 2018	MG979698
C. hispida	Spain, Zaorejas 7	Molins *et al.* 2018	MG979688
C. hispida	Spain, Zaorejas	Molins *et al.* 2018	MG979694
C. hispida	Spain, Zaorejas 3	Molins *et al.* 2018	MG979692
C. hispida	Spain, Zaorejas 2	Molins *et al.* 2018	MG979693
C. hispida	Spain, Maranchon 10	Molins *et al.* 2018	MG979690
C. hispida	Spain, Zaorejas 10	Molins *et al.* 2018	MG979701
C. hispida	Spain, Maranchon 10	Molins *et al.* 2018	MG979682
C. hispida	USA, *Di Meglio* 304 (OSC)	Molins *et al.* 2018	MZ536741
C. hispida	USA, *Di Meglio* 307 (OSC)	Molins *et al.* 2018	MZ536743
C. hispida	USA, *Di Meglio* 310 (OSC)	McCune & Di Meglio 2021	MZ536745
C. hispida	Iran, *Sohrabi* 15099 (hb. M. Sohrabi)	Sohrabi *et al.* 2011*a*	HQ171233
C. hispida	Spain, Zaorejas 4	Molins *et al.* 2018	MG979683
C. hispida	Spain, Zaorejas 6	Molins *et al.* 2018	MG979696
C. hispida	Turkey, *Candan* 22 (hb. M. Sohrabi)	This paper	**OR167385**
C. hispida (crustose)	Ukraine, Alupka, *Vondrák* 5671a	This paper	**OR167387**
C. hispida (vagrant)	Ukraine, Alupka, *Vondrák* 5671b	This paper	**OR167388**
C. hispida (*Agrestia zerovii*)	Ukraine, *M. Kryvokhyzhaya* 2 (KW-L)	Kondratyuk *et al.* 2015	KT456205
C. hispida (*Agrestia zerovii*)	Ukraine, *M. Kryvokhyzhaya* 2 (KW-L)	Kondratyuk *et al.* 2015	KT456206
C. hispida (crustose)	Turkey, *John* 11984B (POLL)	Owe-Larsson *et al.* 2011	HQ389201
C. hoffmanniana	Sweden, L-163189 (UPS)	Roux *et al.* 2016	LT671465
C. jussuffii	Algeria, *Esnault* 2033 (GZU)	Sohrabi *et al.* 2013	JQ797518
C. jussuffii	Morocco, *Vězda*, *Lich. Sel. Exs*. no. 2381 (H)	Sohrabi *et al.* 2013	JQ797521
C. lacunosa	China, *Abbas* 940003 (H)	Sohrabi *et al.* 2013	JQ797517
C. lacunosa	Kazakhstan, *Piregoudov* s. n. (LE)	Sohrabi *et al.* 2013	JQ797520
C. nimisii	Greece, *Mayrhofer* 15811A (GZU)	Sohrabi *et al.* 2013	JQ797522
C. nimisii	Greece, *Mayrhofer* 15811B (hb. M. Sohrabi)	Sohrabi *et al.* 2013	JQ797523
C. rogeri	USA, Utah, *Leavitt* 680	This paper	**OR167398**
C. rogeri	USA, Colorado, *Di Meglio* 558 (OSC)	McCune & Di Meglio 2021	MZ536750
C. rogeri	USA, *Rosentreter* 16333 (SRP)	Sohrabi *et al.* 2011*a*	HQ171232
C. rogeri	USA, *Di Meglio* 305 (OSC)	McCune & Di Meglio 2021	MZ536742

(*Continued*)

Table 1. (*Continued*)

Taxa	Country, voucher (herbarium)	Reference	GenBank ITS
C. rogeri	USA, *Rosentreter* 16373 (SRP)	Sohrabi *et al.* 2011*a*	HQ171231
C. rogeri	USA, Colorado, *Di Meglio* 309 (OSC)	McCune & Di Meglio 2021	MZ536744
C. rogeri	USA, Colorado, *Leavitt* 749	This paper	**OR167392**
C. rogeri	USA, Oregon, *McCune* 34833 (OSC)	McCune & Di Meglio 2021	MZ536757
C. rogeri	USA, Utah, *Leavitt* 681	This paper	**OR167399**
C. rogeri	USA, Idaho, *Leavitt* 674	This paper	**OR167395**
C. rogeri	USA, Idaho, *Leavitt* 673	This paper	**OR167394**
C. rogeri	USA, Idaho, *McCune* 38174 (OSC)	McCune & Di Meglio 2021	MZ536792
C. rogeri	USA, Utah, *Leavitt* 682	This paper	**OR167400**
C. rostamii	Iran, *Sohrabi* 10212 (IRAN)	Sohrabi *et al.* 2013	JQ797527
C. rostamii	Iran, *Sohrabi* 9364 (IRAN)	Sohrabi *et al.* 2013	JQ797541
C. serenensis	France, *Bertrand* & *Cl. Roux* L-205589 (UPS)	Roux *et al.* 2016	LT671471

the covered side usually darkish green to dark brown, greenish brown or almost dark green-brown (sometimes reddish brown when ferriferous oxides are present in the soil). *Branch tips* not tapering, pulvinate, not blackened. *Pseudocyphellae* very common, ±white spots along the folding branches or flattened lobes and usually seen on top of folding branches or flattened lobes; *cortex* one layer, (15–)20–40(–65) µm thick, paraplectenchymatous, ±brown, *c.* 2–3 cells thick, cells (4–)6–10(–12) µm diam., inner part indistinct, mixed with prosoplectenchymatous tissue of medulla and sometimes making a distinct layer with (30–)40–80(–90) µm tall epinecral layer (fluctuation of algal cells in the medulla layer make it uneven, without a distinct border and difficult to distinguish from the proper cortex layer), 1–5(–12) µm thick. *Photobiont* chlorococcoid, cells ±round, 5–22 µm diam., clustered in small groups, each group up to 80–180 × 50–110 µm wide. *Medulla* white, often muddy, depending on lump size, 0.3–10 mm, I−, containing calcium oxalate crystals.

Apothecia aspicilioid, round to somewhat irregular, adnate to stipitate, rare, up to 0.5–1.5(–2) mm wide, among the folding branches or flattened lobes in older parts. *Disc* black to brown-black, pruinose, concave to convex when young, becoming flattered when old. *Thalline margin* flat to ±elevated and prominent in older apothecia, entire, concolorous with thallus or with a thin to thickened white rim. *True exciple* (25–)35–85(–95) µm wide, ±I + medially blue, uppermost cell brown, ±globose, 4–5(–7) µm diam.; *epihymenium*: K+ colour fading from brown to light yellowish green, N+ pale green; *hymenium* hyaline, occasionally with a few oil drops, (100–)110–140(–150) µm tall; *paraphyses* moniliform to submoniliform, with upper cells ±globose, 4–7 µm wide, in the lower part 5–9 × 2–3 µm wide, branched; *hypothecium* and *subhymenium* pale, (35–)45–65(–85) µm thick, I+ blue. *Asci* broadly clavate, (80–)90–100(–110) × 25–35 µm, with a thick apical dome (thollus) (20–30 µm tall), 2–4 (–5)-spored. *Ascospores* hyaline, simple, globose to subglobose, (13–)15–*19.1*–20(–23) × (12–)14–*18.6*–20(–22) ($n = 30$).

Pycnidia usually on top of folding branches or flattened lobes, immersed, single, stretched flask-shaped, internal wall colourless, frequently with black to brownish ostiole. *Conidia* filiform, straight to very slightly curved, (8–)10–14(–17) × 1–1.3 µm.

Chemistry. All spot tests (K, C, KC, CK, P, I) were negative in both the cortex and medulla; UV−. TLC and HPLC: no substances detected.

Etymology. The species is named in honour of Prof. Pier Luigi Nimis, an Italian lichenologist who supported the first author at the initial stages of Iranian lichenology, and author of the ITALIC (versions 1–7) lichen website which has made significant contributions to the development of lichenology in the world.

Ecology and distribution. *Circinaria nimisii* occurs terricolously between small pebbles of limestone and scattered cushions of perennial grasses and herbs. It grows in the alpine belt at open wind-exposed locations with steppe-like conditions. It is so far known only from the Plateau of Muses on Mount Olympus in Greece.

Remarks. *Circinaria nimisii* is a distinct subfruticose species, separated from related species by ITS sequence data as well as morphological and anatomical characters (see Table 2). The gross morphology of the thallus in *C. nimisii* resembles that of *C. gyrosa* and *C. rogeri* (Sohrabi) Sohrabi. *Circinaria nimisii* is characterized by its muddy or earthy colour and subfruticose thallus with whitish pseudocyphellae on tips of folding branches or flattened lobes, 2–4 spored asci, ascospores smaller than those in *C. gyrosa*, *C. hispida* and *C. rogeri*, with oil drops, and conidia that are somewhat longer than in *C. hispida*. *Circinaria fruticulosa* differs from *C. nimisii* by its blackish olive, greyish brown and vagrant, convoluted, aggregated verrucose thallus, well-developed branches more or less rounded to cylindrical, radiating in different directions from the central part, apothecial disc with white rim, and filiform, needle-shaped conidia. *Circinaria affinis* (Eversm.) Sohrabi differs from *C. nimisii* by its confluent thallus with verrucae and by lacking branches. *Circinaria digitata* differs from *C. nimisii* by its thallus morphology, especially with its relatively finger-like, elongated and branched thallus (Sohrabi *et al.* 2011*a*).

Based on ITS sequence data, *C. nimisii* is most closely related to *C. hispida* (Fig. 2). However, *C. hispida* s. str. is directly attached to soil (Rosentreter 1998; Sohrabi *et al.* 2011*b*), while

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Table 2. A comparison of *Circinaria nimisii* with morphologically similar species.

Character	*C. nimisii*	*C. rogeri*	*C. gyrosa*	*C. fruticulosa*	*C. affinis*	*C. hispida*
Thallus	subfruticose, more or less spherical to elongated to irregular lobes, rimose-verrucose surface	subfruticose, elongated, angular to rounded or irregular surface	subfruticose, spherical to elongated lobes, rimose-verrucose surface	subfruticose, subglobose, with numerous regular branches	subfruticose, almost subglobose, confluent, verrucose surface	tufted, cladonoid, numerous elongated, angular to irregular surface
Substratum	vagrant	vagrant	vagrant	vagrant	vagrant	attached to the soil, erratic
Areoles	with elevated areole-like angular to rounded or irregular lobes	no areoles	with elevated areole-like lobes	no areoles	areole-like lobes, rimose-verrucose	no areoles
Branch pattern	compact, predominantly irregular	predominantly irregular	predominantly irregular	predominantly dichotomous	compact, no branch observed	predominantly irregular
Branch tips	not tapering, pulvinate, not blackened	tapering, blunt, whitish, with a black centre, (pycnidia, erupted or not)	not tapering, pulvinate, not blackened	not tapering, concave, not blackened	not tapering, somewhat concave to flat, not blackened	tapering, pointing, blackened
Pseudocyphellae	along areole-like lobes conspicuous	along branches conspicuous	mainly apical conspicuous	mainly apical obscure	on areole-like lobes conspicuous	along branches conspicuous
Apothecia	aspicilioid, round to somewhat irregular, adnate to stipitate,	aspicilioid when young, later becoming adnate to stipitate	aspicilioid, round to sometimes angular, elongated or irregular	cryptolecanorine, disc brown to black	aspicilioid, deeply immersed, single, disc black to brown, rounded to fissure-like	aspicilioid when young, later adnate to substipitate, rare
Pycnidia	(1–)2 per areole, immersed	at tips of branchlets, sometimes on pseudocyphellae	(1–)2 per areole, immersed	at tips of branchlets, sometimes on pseudocyphellae	common, immersed, sometimes occurring on the pseudocyphellae	rare, inconspicuous, along branches
Reference	This study	Sohrabi *et al.* 2011	Sohrabi *et al.* 2013*a*	Sohrabi *et al.* 2013*a*	Sohrabi *et al.* 2013*a*	Sohrabi *et al.* 2013*a*

C. nimisii is strictly vagrant. Furthermore, morphological differences exist between C. nimisii and the vagrant morphotype of C. hispida s. str. (*sensu* Mereschkowsky 1911; Sohrabi *et al.* 2013). *Circinaria hispida* s. str. is a subfruticose lichen, forming tiny, bushy thalli with narrow cylindrical branches and black apices at the branch tip, along with scattered whitish pseudocyphellae throughout the thallus, with two types of cortex: paraplectenchymatous and prosoplectenchymatous. The crustose morphotypes of *C. hispida* are more similar to *C. nimisii* in terms of how they both colonize pebble surfaces, and *C. nimisii* can be further diagnosed by the presence of the one-layered cortex (paraplectenchymatous). DNA sequence data from the standard DNA barcode (ITS), consistently separates *C. nimisii* from the morphologically similar *C. hispida* specimens (Fig. 2).

Phylogeny. In the phylogenetic analysis of the ITS multiple sequence alignment, *C. nimisii* was recovered within the sphaerothallioid species group, with close affinities to *C. hispida* (Clade 3), *C. rogeri* (Clade 1) and *C. digitata* (HQ171230, HQ171236). The new species is strongly supported as a distinct clade (Clade 2) in the ITS phylogeny (Fig. 2).

Additional specimens of C. hispida *examined for morphological comparison.* **Kazakhstan:** Aklushenskaya District, Bayzhanshal limestone ridge, 1957, *Andreeva* (LE). *Akmola Region*: near Kökshetau Mts, S direction, 1957, *Andreeva* (LE).—**Kyrgyzstan:** *Naryn Region*: northern side of Naryn-Twell Valley of River Naryn, 23 km to the E of Naryn Mountain, 2250 m, 1970, *Bredkina* (LE).—**Iran:** *Hamadan Province*: foothills above Gholi-Abad, *c.* 60 km N of Hamadan, 1800 m, 1974, *Alava* 14749-d (TUR). *East Azerbaijan Province*: Marand District, 32 km N of Marand towards Jolfa, 1440 m, 2007, *Sohrabi* et al. 10102 (hb. M. Sohrabi); *ibid.*, Zonuz, 20 km N of Marand towards Jolfa, 1800 m, 2007, *Sohrabi* et al. 10064 (hb. M. Sohrabi); Jolfa District, 1 km S of Daran Village, E of Hadishahr, 1700 m, 2007, *Sohrabi* et al. 10136 (hb. M. Sohrabi).—**Italy:** *Piedmont Region*: Province of Cuneo, Alpi Liguri, Cima di Pertega, W above the village of Úpega, just E below the summit, *c.* 2400 m, 2000, *Hafellner & Hafellner* 59353 (GZU, TSB); *ibid.*, Alpi Cozie, crest SW above Colle dell'Agnello, *c.* 2830 m, 2000, *Hafellner* 59364 (GZU).—**Greece:** Mt Parnassus, Fterolaka, near the cableway, 1850 m, 1989, *Tretiach & Roux* (TSB).—**Mongolia:** *Zavkhan Province* [correctly *Govi-Altai*]: Taishir sum, right bank of Zavkhan River, *c.* 2 km N of Taishir Town, 1978, *Biazrov* 8373 (LE).—**Russia:** *Astrakhan Oblast*: near Lake Baskunchak, 1926, *Tomin* (H); *ibid.*, 1926, *Tomin* 37 (FH, H); *ibid.*, 1927, *Tomin* 56 (FH, H); Regio Astrachanensis per declive (in parte superiore) montis Bogdo ad terram inter gramina, fruticulos lapidesque crescit, saepe libere vagature, 1926, *Savicz*, in *Savicz: Lich. Ross.* no. 97 (FH, GZU, H, W).—**Turkey:** *Malatya Province*: Malatya, Pınarbaşı mesire alanı ve çevresi, 931 m, 2003, *Candan* 11 (H).

Circinaria hispida (Mereschk.) A. Nordin, S. Savić & Tibell

In Nordin *et al.*, *Mycologia* **102**, 1346 (2010) (fig. 5a–c in Sohrabi *et al.* (2013)).—Basionym: *Aspicilia hispida* Mereschk., *Trudy Obshch. Estestvoisp. Imp. Kazansk. Univ.* **43** (5), 10, 35 (1911); type: Russia, 'Ad terram argilloso-calcaream montis Bogdo prope lacu, Baskuntschak in gub Astrachan', 50–120 m, 1910, *Mereschkowsky* in Mereschkowsky: *Lich. Ross. Exs.* no. 34 (TU—lectotype designated by Sohrabi & Ahti (2010); LE L1988, W—isolectotypes).

Syn. nov.: *Agrestia zerovii* S. Y. Kondr. *et al.*, MB 813878; type: Ukraine, Kharkiv Oblast (= region), Dvorichansky District, in the vicinity of Dvorichana settlement, Korobchyno protected territory (= zakaznik), 49°50′00.5″N, 37°40′27.7″E, the upper part of the slope, W from the road, below plantation, leg. *Gromakova*, *A. B.*, s. n., 27.05.2012, (KW-L 70479—holotype); the same locality (KW-L 70480, KHER, CWU—isotypes).

Note. Morphological characteristics and the ITS sequence variation of *Agrestia zerovii* S. Y. Kondr. *et al.*, published in Kondratyuk *et al.* (2015), fall within the typical variation of *C. hispida*. Therefore, we propose that *A. zerovii* is considered a new synonym of *C. hispida*. We also propose that the identification of EU057905 in Nordin *et al.* (2007) is corrected in GenBank.

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