





Standard Paper

Circinaria persepolitana (Megasporeaceae), a new lichen species from historic stone surfaces in Persepolis, a UNESCO World Heritage Site in Iran

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Abstract

Persepolis, a UNESCO World Heritage Site in south-western Iran, dates back to more than 2500 years ago, and is colonized by a great diversity of lichen-forming fungi. A survey of the lichen-forming fungi revealed a species abundant in different areas of the cultural site, which turned out to be a new species of the genus *Circinaria*. The new species, *Circinaria persepolitana*, is introduced and described on the basis of morphological and molecular data. *Circinaria persepolitana* is characterized by having a crustose thallus, rimose to areolate, usually with bullate areoles, with an olive green to olive-brown surface and angular to elongate areoles in the marginal zone. Phylogenetic analyses including other species of the genus showed that the new species is phylogenetically close to *C. mansourii*, *C. ochracea* and *C. reptans*. We propose a new combination of *Circinaria reptans* (Looman) Sohrabi, Owe-Larsson & Paukov. The bioweathering capacity of the new species was also analyzed by scanning electron microscopy, examining the interface between the lichen thallus and the lithic substratum to assess its potential threat to the conservation of heritage surfaces. We found this species to be a potential biodeteriogenic agent, as thalli were closely attached to the lithic substratum and biogeophysical and biogeochemical changes at the rock surface could be associated with the colonization.

Keywords: biodeterioration; cultural heritage; lichens; lithobionts; microbial communities; new species; Persia

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Introduction

The genus of lichen-forming fungi *Circinaria* Link, comprising c. 40 species, is the second largest in the family Megasporeaceae, after *Aspicilia* A. Massal. (Wijayawardene *et al.* 2020; Paukov *et al.* 2021). The genus shows a wide variety of growth forms, including fruticose, crustose and umbilicate species. Whereas crustose species of the genus have long been included within the genus *Aspicilia* A. Massal., fruticose species, usually vagrant, have been included in the genera *Agrestia* J. W. Thomson, *Sphaerothallia* Eversm. or *Chlorangium* Link (Sohrabi *et al.* 2013a). *Circinaria* species have received great attention in recent years, especially the vagrant taxa (Nordin *et al.* 2010; Sohrabi *et al.* 2013a; McCune & Di Meglio 2021), and several new species have been described from Iran (Sohrabi *et al.* 2013a), China

(Ismayil *et al.* 2015, 2019), Greece (Sohrabi *et al.* 2023), Italy (Nascimbene *et al.* 2023) and the USA (Owe-Larsson *et al.* 2011; McCune & Di Meglio 2021). Crustose species of the genus are primarily epilithic, and have grey to olive-brown thalli, with or without a grey prothallus. Recent molecular studies have greatly improved our understanding of the infrageneric relationships and taxonomic value of the morphological characters of the genus (Nordin *et al.* 2010; Sohrabi *et al.* 2013a; McCune & di Meglio 2021).

Persepolis, capital of the Achaemenid Empire (c. 550–330 BC), located in south-western Iran and surrounded by the southern Zagros Mountains of the Iranian plateau, is one of 27 UNESCO World Heritage Sites in Iran (<https://whc.unesco.org/en/list/>). The monumental stone of its construction, including those displaying bas-reliefs of incalculable artistic and archaeological value (Mousavi 2012), is colonized by a large number of lichen-forming fungi, including several species of the genus *Circinaria*. Species of this genus also occur on stone surfaces in other UNESCO World Heritage Sites in Iran such as Pasargadae (Sohrabi *et al.* 2017), which is part of the Sassanid Archaeological Landscape in Fars Province,

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and Bistone in Kermanshah and Sousha in Khuzestan Province.

During a recent lichenological survey of Persepolis, an apparently new species of *Circinaria* colonizing the bas-reliefs and stone walls was found. In this study, we describe the new taxon, *Circinaria persepolitana*, based on morphological observations and molecular analyses of representative specimens. Given the high abundance of *C. persepolitana* at the historic site of Persepolis and the need to avoid biodeterioration of this emblematic site for its conservation, we also analyzed the interactions established by the lichen thalli of this species with the lithic substratum and their capacity to deteriorate the colonized stone surface.

During our research, we also conducted a phylogenetic analysis of several species belonging to the *Circinaria* genus, including *C. persepolitana*, *C. mansourii* (Sohrabi) Sohrabi and *Aspicilia reptans* (Looman) Wetmore. Our aim was to re-evaluate and determine the status of these species. Our analysis revealed that the species we were investigating is closely related to *C. mansourii* and *A. reptans*. Based on our findings, we make the new combination *Circinaria reptans* and propose a correction to its nomenclature.

Material and Methods

Sampling sites

We collected two types of samples from two locations during our research. One was directly obtained from bare rock suitable as a lichen substratum while the other included rock colonized by lichens. The locations were at Kuh-e Rahmat but *c.* 5 km apart. The first location was at the Naghsh-e-Rajab limestone outcrop (29.9661°N, 52.8867°E) and the second was within the historic site or palace complex (29.9358°N, 52.8919°E).

To compare the mineral composition of rocks, we collected small stone pieces (1 cm²) from the historic area with permission from the general office of Persepolis. These were analyzed by X-ray diffraction (XRD). We collected samples identified as no. 109 (where the holotype of the new species was also recorded) from the Naghsh-e-Rajab limestone outcrop, located outside the historic site's limits. Additionally, we gathered substratum samples (no. 106) in powder form from the Hall of a Hundred Columns and sample no. 108, comprising small stone pieces measuring *c.* 1 cm², from the Unfinished Gate.

To compare the DNA of lichen samples found at the historic site with the type specimens (collected at the Naghsh-e-Rajab limestone outcrops), we sampled small lichen fragments, *c.* 1 mm² in size, from significant areas of the Persepolis site, including the Apadana Palace, Hadish Palace, Tachara Palace, Hall of a Hundred Columns, and the Unfinished Gate.

X-ray diffraction (XRD). Samples 109, 108 and 106 were ground to powder using an agate mortar and then analyzed using an x-ray diffractometer (Philips PW 1730, The Netherlands). The analysis involved Cu K-alpha radiation and a scan range of 3–35°2 θ , with a scan step of 0–02°2 θ and 1 s per step. Mineral identification was aided by connecting a graphical terminal to the JCPDS—International Center for Diffraction Data 'Powder Diffraction File' (PDF) database.

Morphology and anatomy

The present study is based mainly on material collected by the first author (MS) and managed according to Obermayer (2002);

specimens were later deposited in the ICH herbarium at the Iranian Research Organization for Science and Technology (IROST) in Tehran. Field photographs were taken with a Canon PowerShot SX60 HS camera.

Morphological observations of specimens were performed with a Wild M7 stereomicroscope (Wild Heerbrugg, Switzerland). Thallus measurements were made at $\times 25$ magnification and rounded to the nearest 0.1 mm. Anatomical features (Ryan *et al.* 2002; Owe-Larsson *et al.* 2007) were measured in hand-cut sections and observed on material mounted in water under a PZO DIC microscope (Warsaw, Poland). Measurements were taken at $\times 1000$ magnification using an oil immersion lens and a Wild vernier micrometer (scale of 1 μ m), to an accuracy of 0.5 μ m for microscopic structures and 5 μ m for the anatomical layers of thalli, according to the formula $(\min -) \bar{x} - SD - \bar{x} + SD (-\max) (n)$, where \bar{x} is the mean, SD is the standard deviation and *n* is the number of measurements.

Chemical analyses

Spot tests, UV and TLC. Spot tests with 10% potassium hydroxide (K), 5.25% sodium hypochlorite (C) and Lugol's iodine (I) following Nylander (1866), and an ethanol solution of 5% para-phenylenediamine (PD) following Orange *et al.* (2010) were conducted to examine the chemical characteristics of the cortex and medulla. Reagent C was also applied after pretreatment with K on the same thallus fragment (KC). Long-wave ultraviolet light (350 nm) was used to observe lichen thalli. Thin-layer chromatography was performed following Orange *et al.* (2010), using solvent systems A, B and C.

Liquid chromatography and tandem mass spectrometry (LC-MS/MS) analysis. The metabolic profile was analyzed following the protocol described by Norouzi *et al.* (2020). In brief, the compounds were separated using a Waters Alliance e2695 separation module (Milford, MA, USA) equipped with an Atlantis T3 C18 column (2.1 mm \times 100 mm, 3 μ m; Milford, MA, USA). The temperature of the column was set at 30 °C. The extract was dissolved in methanol, followed by filtration (PTFE membrane filters, 0.45 μ m; Simplepure, China). A 20-min gradient elution programme was used on 10 μ l of the sample with the following conditions: 95% A (water + 0.1% formic acid, v/v), gradually reduced to 5% A within 20 min and followed by 95% B (acetonitrile) for 5 min. The eluent flow rate was set at 0.25 ml min⁻¹.

The MS/MS analysis was conducted on a Waters Micromass Quattro micro API mass spectrometer (Milford, MA, USA) in negative ionization mode. The following conditions were adopted: source temperature 120 °C, dissociation temperature 300 °C, capillary voltage 3.5 kV, cone voltage 30 V, collision energy 30 eV. N₂ was used for both nebulizing and gas drying. Data acquisition, processing and visualization were performed using MassLynx v. 4.1 and MZmine v. 2.37. Finally, the MS/MS spectra database of lichen substances (LDB), provided by Olivier-Jimenez *et al.* (2019) and hosted by the GNPS public spectral library (Wang *et al.* 2016), was used to confirm the identity of most of the detected compounds.

DNA extraction, amplification and sequencing

DNA extraction was performed on a total of seven specimens of the new putative taxon, including two recently collected herbarium specimens (of which Sohrabi 32109 was chosen as the

holotype), as well as five thalli collected directly from surfaces of historical monuments. In addition, we also carried out DNA extraction of three specimens of *Circinaria mansourii*. Areoles were excised from thalli with a razor blade, placed in microcentrifuge tubes and stored at -80°C for 1 h after which they were pulverized using a Qiagen TissueLyser II and glass beads. DNA was extracted using E.Z.N.A.[®] Forensic DNA Kit (Omega Bio-Tek), following the manufacturer's instructions. The fungal nrITS region was amplified using the primers ITS1-F (White *et al.* 1990) and ITS4_KYO2 (Toju *et al.* 2012). Amplification reactions were prepared for a 15 μl final volume containing 7.5 μl of MyTaq[™] Red Mix (Bioline) which includes the MyTaq[™] DNA polymerase, 0.5 μl of each of the primers at 10 μM , 5.5 μl of H_2O , and 1 μl of template. PCR conditions for the amplification of the nrITS were as follows: 5 min at 95°C ; 10 cycles of 30 s at 95°C , 30 s at 66°C , 1 min 30 s at 72°C ; 34 cycles of 30 s at 95°C , 30 s at 56°C , 1 min 30 s at 72°C ; 10 min at 72°C . Seven sequences of the nrITS region of the putative new species were obtained.

Alignment and phylogenetic analysis

For this study, a total of 10 new ITS sequences were generated and 36 additional sequences were downloaded from GenBank. A summary, including material and locality information, names of collectors and where specimens were deposited, is given in Table 1. Species nomenclature follows Nordin *et al.* (2010), Owe-Larsson *et al.* (2011), Sohrabi *et al.* (2013a, b) and Paukov *et al.* (2017).

ITS sequences were aligned with BioEdit v. 7.1.3.0 (Hall 1999) and MAFFT v. 7 (<https://mafft.cbrc.jp/alignment/server/>) using the progressive G-INS-1 method (Katoh *et al.* 2019) and later corrected by hand. The optimal substitution model was inferred using the ModelTest-NG algorithm (Darriba *et al.* 2020) implemented in raxmlGUI v. 2.0 software (Kozlov *et al.* 2019; Edler *et al.* 2021); the general time reversible model (GTR + G) was selected. Bayesian inference with the Markov chain Monte Carlo (BMCMC) method (Larget & Simon 1999) was performed using BEAST v. 2.6.6 (Bouckaert *et al.* 2019). Two independent runs of BEAST were made with a chain length of 10^7 , and every 1000th generation was recorded. Tree files from two independent runs were combined in LogCombiner v. 2.6.6, a part of the BEAST 2 package (<http://beast2.cs.auckland.ac.nz/>). A maximum clade credibility tree with mean node heights was inferred with a 50% burn-in fraction and a posterior probability of 0.5. Tree files were visualized with FigTree v. 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). The most likely tree and 1000 rapid bootstrap pseudoreplicates were calculated using RAxML v. 8.0.26 (Stamatakis 2014) with raxmlGUI v. 2.0 software (Edler *et al.* 2021), applying the GTRGAMMA model of substitution. Since both phylogenetic inference methods returned identical tree topologies, the final topology shown in Fig. 1 corresponds to that recovered using maximum likelihood (ML) inference. Bootstrap support values and BMCMC posterior probability were noted on the best-scoring tree. *Aspicilia goettweigensis* (Zahlbr.) Hue, a representative of the large monophyletic genus *Aspicilia* s. str. after Nordin *et al.* (2010) within the family *Megasporaceae*, was selected as outgroup for phylogenetic reconstruction.

Scanning electron microscopy study

Rock fragments (sample no. 106) colonized by *Circinaria persepolitana* were prepared following the procedure developed by

Wierzchos & Ascaso (1994) for the observation of the lichen-lithic substratum interface by scanning electron microscopy using the back-scattered electron mode (SEM-BSE). Lichen-colonized rock fragments were fixed in glutaraldehyde and osmium tetroxide solutions, dehydrated in a graded ethanol series, and embedded in LR-White resin. Blocks of resin-embedded rock samples were finely polished, carbon coated, and observed using an FEI INSPECT scanning electron microscope (Oregon, USA) at the Museo Nacional de Ciencias Naturales (CSIC). Microprobe analyses were performed using an Oxford Instruments INCA X-Ray Energy Dispersive Spectrometer (EDS) microanalytical system (Oxford, UK) during SEM observation. To obtain high-resolution images of crystal morphology, small fragments of rocks harbouring the lichen thallus were coated with gold (10 nm) and analyzed with a TESCAN Mira3 FESEM (Brno, Czechia). The microscope was operated at an acceleration voltage of 0.2 to 1 kV using the Beam Deceleration Mode.

Results and Discussion

The topology of the phylogenetic tree recovered by ML analysis is shown in Fig. 1. The supported relationships among the species agree with previously published phylogenetic reconstructions of the group (Owe-Larsson *et al.* 2011; Sohrabi *et al.* 2013a, b). The Bayesian tree returned an identical topology to that obtained by maximum likelihood using RAxML.

The recovered phylogram based on the analyses of nrITS sequences showed low support for most of the relationships within the genus *Circinaria* (Fig. 1). A well-supported basal clade (BS = 95, PP = 1) included the species *Circinaria contorta* (Hoffm.) A. Nordin *et al.*, *C. hoffmanniana* (S. Ekman & Fröberg ex R. Sant.) A. Nordin and *C. serenensis* (Cl. Roux & M. Bertrand) A. Nordin. The rest of the relationships among species were not supported, with the exception of the clade containing *C. cerebroides* (Mereschk.) Sohrabi, *C. rostamii* Sohrabi and *C. lacunosa* (Mereschk.) Sohrabi (95/1), the clade containing *C. hispida* (Mereschk.) A. Nordin *et al.* and *C. rogeri* (Sohrabi) Sohrabi (85/1), and the clade containing the new species, *C. ochracea* Thüs & Nascimbene, *C. mansourii* and *Aspicilia reptans* (Looman) Wetmore (100/1). The specimens of *C. persepolitana* formed a well-supported clade (95/1) sister to the clade formed by *C. ochracea*, *C. mansourii* and *Aspicilia reptans* (99/1). A high level of molecular diversity was found in *C. persepolitana* compared to other species in the group (Fig. 1).

Taxonomy

Circinaria persepolitana Sohrabi & Pérez-Ort. sp. nov.

Mycobank No.: MB 847202

A species of *Circinaria* with an areolate crustose thallus. Areoles rimose or squamuliform to verrucose, or bullate-areolate in the centre, hollow and cracking into popcorn-like tufts, and angular or elongated in the margins, with diverging and branching prothallus-like extensions in the grey rim. On calcareous rocks (limestone). The only known lichen substance found in this species is α -alectoronic acid. By its bullate hollow areoles, the new species resembles *Aspicilia goettweigensis*. The latter species differs from *Circinaria persepolitana* by containing the stictic acid complex.

Table 1. Voucher information and GenBank Accession numbers for ITS sequences of *Circinaria* specimens used in this study. New sequences are indicated in bold.

Species	Voucher information	GenBank Accession number	Reference
<i>Aspicilia goettweigensis</i>	Austria, <i>Vondrák</i> 14026 (PRA)	KX159289	Paukov et al. 2017
<i>Circinaria affinis</i>	Russia, <i>Kulakov</i> 1408 (M)	HQ171237	Sohrabi et al. 2011a
<i>C. affinis</i>	Russia, <i>Kulakov</i> 1408b (POLL)	HQ389196	Sohrabi et al. 2011a
<i>C. arida</i>	USA, <i>Owe-Larsson</i> 8759 (UPS)	HQ406800	Owe-Larsson et al. 2011
<i>C. arida</i>	USA, <i>Knudsen</i> 2046 (UPS)	HQ406801	Owe-Larsson et al. 2011
<i>C. calcarea</i>	Sweden, <i>Nordin</i> 5888 (UPS)	EU057898	Nordin et al. 2007
<i>C. calcarea</i>	Sweden, <i>Nordin</i> 5914 (UPS)	HQ406804	Owe-Larsson et al. 2011
<i>C. calcarea</i>	Sweden, <i>Nordin</i> 6310 (UPS)	LT671467	Roux et al. 2016
<i>C. calcarea</i>	France, <i>Roux</i> et al. 25256 (UPS)	LT671468	Roux et al. 2016
<i>C. cerebroides</i>	Kyrgyzstan, <i>Ringel & Jashhof</i> 5180 (H)	JQ797529	Sohrabi et al. 2013a
<i>C. cerebroides</i>	Kyrgyzstan, <i>Ringel</i> 5138 (H)	JQ797534	Sohrabi et al. 2013a
<i>C. cerebroides</i>	Kyrgyzstan, <i>Ringel</i> 5184 (H)	JQ797553	Sohrabi et al. 2013a
<i>C. contorta</i>	Sweden, <i>Nordin</i> 5895 (UPS)	EU057900	Nordin et al. 2007
<i>C. contorta</i>	Sweden, <i>Fröberg</i> 09-44i (UPS)	LT671470	Roux et al. 2016
<i>C. esculenta</i>	Russia, <i>Owe-Larsson</i> 9796 (UPS)	JQ797510	Sohrabi et al. 2013a
<i>C. esculenta</i>	Russia, <i>Owe-Larsson</i> 9796 (UPS)	JQ797511	Sohrabi et al. 2013a
<i>C. fruticulosa</i>	Russia, <i>Kulakov</i> 1408 (hb. V. John)	HQ171227	Sohrabi et al. 2011a
<i>C. fruticulosa</i>	Kazakhstan, <i>Lange</i> 5186 (H)	HQ171228	Sohrabi et al. 2011a
<i>C. fruticulosa</i>	China, <i>Abbas</i> 940001 (H)	HQ171229	Sohrabi et al. 2011a
<i>C. hispida</i>	Iran, <i>Sohrabi</i> 15099 (hb. M. Sohrabi)	HQ171233	Sohrabi et al. 2011a
<i>C. hispida</i>	Russia, <i>Ochirova</i> s. n. (LE)	HQ171235	Sohrabi et al. 2011a
<i>C. hoffmanniana</i>	Sweden, <i>Nordin</i> 5917 (UPS)	LT671465	Roux et al. 2016
<i>C. jussuffii</i>	Algeria, <i>Esnault</i> 2033 (GZU)	JQ797518	Sohrabi et al. 2013a
<i>C. jussuffii</i>	Morocco, <i>Vězda</i> 2381 (H)	JQ797521	Sohrabi et al. 2013a
<i>C. lacunosa</i>	China, <i>Abbas</i> 940003 (H)	JQ797517	Sohrabi et al. 2013a
<i>C. lacunosa</i>	Kazakhstan, <i>Piregoudov</i> s. n. (LE)	JQ797520	Sohrabi et al. 2013a
<i>C. mansourii</i>	Russia, <i>Paukov</i> 3049 (UFU)	OM273290	This study
<i>C. mansourii</i>	Russia, <i>Paukov</i> 3285 (UFU)	OM273291	This study
<i>C. mansourii</i>	Russia, <i>Paukov</i> 3503 (UFU)	OM273292	This study
<i>C. mansourii</i>	Iran, <i>Sohrabi</i> 15077 (IRAN—holotype)	MS015088	Sohrabi et al. 2013b
<i>C. ochracea</i>	Italy, <i>Nascimbene</i> SMNS-STU-F-0002797 (STU)	OQ073918	Nascimbene et al. 2023
<i>C. ochracea</i>	Italy, <i>Nascimbene</i> 7208a (BOLO)	OQ073919	Nascimbene et al. 2023
<i>C. persepolitana</i>	Iran, Naghsh-e-Rajab <i>Sohrabi</i> 32109 (ICH—holotype)	OR365438	This study
<i>C. persepolitana</i>	Iran, Persepolis WHS, Hadish Palace	OR365439	This study
<i>C. persepolitana</i>	Iran, Persepolis WHS, Unfinished Gate	OR365440	This study
<i>C. persepolitana</i>	Iran, Naghsh-e-Rajab, <i>Sohrabi</i> 32099 (IRAN)	OR365441	This study
<i>C. persepolitana</i>	Iran, Persepolis WHS, Apadana Palace	OR365442	This study
<i>C. persepolitana</i>	Iran, Persepolis WHS, Tachara Palace	OR365443	This study
<i>C. persepolitana</i>	Iran, Persepolis WHS, Hall of a Hundred Columns	OR365444	This study
<i>C. reptans</i>	USA, <i>Di Meglio</i> 203 (OSC)	MZ536721	McCune & Di Meglio 2021
<i>C. reptans</i>	Canada, <i>Di Meglio</i> 263 (OSC)	MZ536731	McCune & Di Meglio 2021
<i>C. reptans</i>	USA, <i>Di Meglio</i> 303 (OSC)	MZ536740	McCune & Di Meglio 2021
<i>C. reptans</i>	USA, <i>McCune</i> 35788 (OSC)	MZ536761	McCune & Di Meglio 2021

(Continued)

Table 1. (Continued)

Species	Voucher information	GenBank Accession number	Reference
<i>C. rogeri</i>	USA, Rosentreter 16333 (SRP)	HQ171232	Sohrabi et al. 2011b
<i>C. rogeri</i>	USA, Rosentreter 16373 (SRP)	HQ171231	Sohrabi et al. 2011b
<i>C. rostamii</i>	Iran, Sohrabi 9364 (IRAN)	JQ797541	Sohrabi et al. 2013a
<i>C. rostamii</i>	Iran, Sohrabi 10212 (IRAN)	JQ797527	Sohrabi et al. 2013a
<i>C. serenensis</i>	France, Bertrand & Roux L-205589 (UPS)	LT671471	Roux et al. 2016

Type: Iran, Fars, Marvdasht, Naghsh-e-Rajab limestone outcrops, northern slope, in the quarry of stone monuments of Persepolis, 1635 m, on grey limestone, GPS: 29.9661°N, 52.8867°E, 15 June 2019, M. Sohrabi 32109 (ICH—holotype; IRAN, hb. M. Sohrabi—isotypes). GenBank Accession: OR365438.

(Figs 2 & 3)

Thallus crustose, (1–)2–10(–12) cm diam., (0.2–)0.5–1(–3) mm thick. *Areoles* rimose or squamuliform (slightly vertically elevated areoles) to verrucose or bullate-areolate, angular to irregular, flat to ±convex, sometimes rounded, (0.2–)0.5–1.5(–2.5) mm diam., contiguous or dispersed, rarely overlapping. Central areoles somewhat papillate, in older parts verrucose, hollow and cracking into popcorn-like tufts, more or less squamuliform areolate. *Thallus* edge usually formed by elongated areoles different from the central areoles. *Prothallus* usually distinct, branching, sparsely developed along the thallus edge, more or less fimbriate, forming a white-grey margin, 0.1–0.4(–1) mm wide. *Surface* usually greyish olive-brown, sometimes partly olive-brown, ochre, grey-brown, white-grey, dull, slightly pruinose. *Pseudocyphellae* not seen. *Epinecral upper layer* hyaline, consisting of dead cells, without crystals, (5–)7–9(–11) µm ($n = 15$). *Cortex* uneven, paraplectenchymatous, (10–)30–45(–60) µm thick ($n = 20$), uppermost part ±brown or rarely olive-brown, with cells (5–)7–9(–12) µm diam. ($n = 15$), (5–)10–22(–40) µm thick ($n = 18$), covered with crystals. *Medulla* white, containing crystals of calcium oxalate c. 1–3 µm diam., I–; algal layer discontinuous. *Photobiont* chlorococcoid (*Trebouxia*-type), cells spherical to subspherical, (10–)15–25(–30) µm diam. ($n = 30$).

Apothecia and *pycnidia* not observed.

Chemistry. Spot tests: cortex and medulla I–, K–, P–, C–. TLC: no lichen substances detected. LC-MS: α-alectoronic acid, [M–H][–]m/z^b: 543.99, chemical formula: C₂₈H₃₂O₉ [M–H+CH₃OH], calculated mass: 543.223, R_t (min)^a: 17.17, MS² ions, m/z (relative intensity)^c: 528, 516, 513, 497, 487, 471, 358, 256, 146. In addition, seven unidentified lichen substances were detected; however, further investigation with more material is needed.

Etymology. The species is named after Persepolis, capital of the Persian Achaemenid Empire from the reign of Darius I (the Great, r. 522–486 BC) until its destruction in 330 BC and a UNESCO World Heritage Site in Iran, where the type collection was obtained.

Ecology, habitat and distribution. This species typically grows on large limestone boulders in various locations on Kuh-e Rahmat (Mountain of Mercy) in Fars Province, south-western Iran,

where several archaeological sites can be found. It is also distributed in Naghsh-e Rajab, an archaeological site located just west of the historic city of Istakhr and c. 5 km north of the Persepolis site. The species can be found in the northern sector of the Persepolis site, including areas such as the walls of the Gate of All Nations (Xerxes Gate), Apadana Palace, Throne Hall (Hall of a Hundred Columns), Treasury, Council Hall, Palace of Artaxerxes III, Imperial Stables, Tripylon (Three-gated Hall), Tachara (Palace of Darius), Hadish (Palace of Xerxes), and the Palace of Artaxerxes I. Additionally, it can be found on calcareous rocks, both on vertical and horizontal surfaces exposed to the sun, in mountainous regions near the UNESCO World Heritage Site of Persepolis (Fig. 2 G & H). *Circinaria persepolitana* is often a predominant species (e.g. in the type locality) in suitable habitats, usually with co-dominant species such as *Acarospora cervina* A. Massal., *A. laqueata* Stizenb., *Calogaya biatorina* (A. Massal.) Arup et al., *C. decipiens* (Arnold) Arup et al., *Circinaria scabridula* (H. Magn.) Sohrabi, *Lobothallia radiosa* (Hoffm.) Hafellner s. lat. and *Protoparmeliopsis usbekica* (Poelt) S. Y. Kondr.

Remarks. *Circinaria persepolitana* is a well-delimited species easily distinguished from other saxicolous species of *Circinaria* by its convex to squamuliform areolate thallus, thin angular to elongated marginal areoles, the grey prothallus-like extensions, branched at the margin, fimbriate or forming a grey rim, and by its exceptional lichen chemistry (α-alectoronic acid by LC-MS analysis) and negative reactions to K, C, I, P and UV in both medulla and cortex. *Circinaria persepolitana* is also characterized by areoles that become hollow and crack into popcorn-like tufts of tissue or upright squamules with the white exposed medulla similar to *Aspicilia goettweigensis*. The species differs from *Circinaria contorta*, a common species of the genus in the region, by having a different thallus morphology. *Circinaria contorta* has generally scattered, rounded, ultimately ±convex to squamuliform areoles and contains aspicilin. The thalli of *Circinaria contorta* are usually fertile with crateriform apothecia and pruinose discs.

In the phylogenetic tree, *Circinaria persepolitana* is subdivided into two supported subclades. Specimens from both subclades differ by 13–18 nucleotides in nrITS, but inhabit the same ecotopes and there are no obvious morphological differences in specimens forming both subclades. We therefore interpret them as two intra-specific lineages within *C. persepolitana*. However, we do not exclude the possibility that future studies may show the coexistence of two cryptic species.

Circinaria persepolitana is phylogenetically close to two terricolous species that grow in arid regions of Eurasia and North America, *Aspicilia reptans* and *C. mansourii*. Both species differ from *C. persepolitana* by their centrally warted and peripherally lobed thalli, which grow on soil and plant detritus, the presence

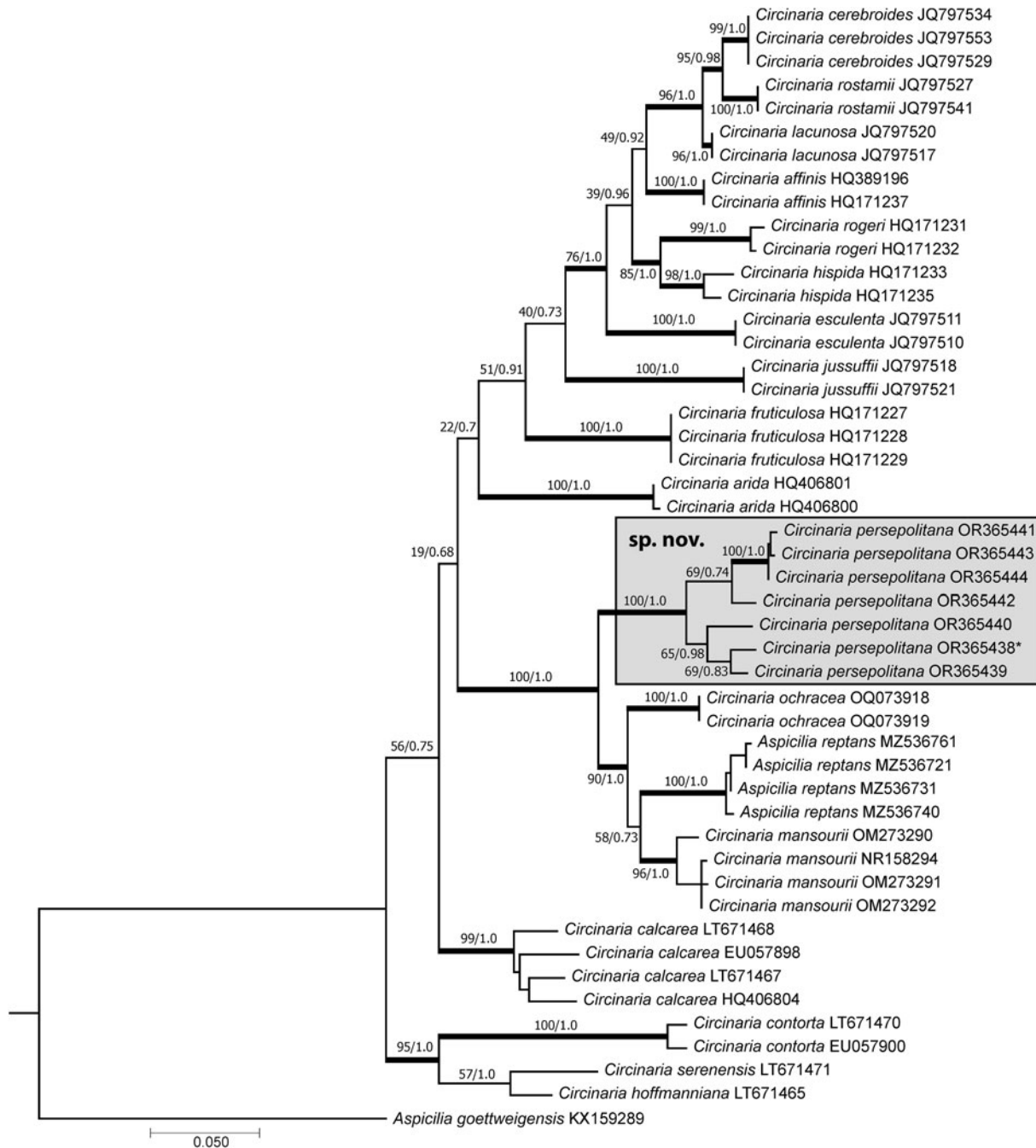


Figure 1. Maximum likelihood (ML) phylogeny of selected *Circinaria* ITS sequences. The reliability of each branch was tested by ML and Bayesian methods. Values at tree nodes indicate ML bootstrap percentages (left) and Bayesian inference with the Markov chain Monte Carlo (BMC) posterior probabilities (right). Thicker branches indicate ML bootstrap values $\geq 70\%$ or BMC posterior probability ≥ 0.95 . Accession numbers and voucher information are provided in Table 1. *Aspiciella goettweigensis* was used as an outgroup. Branch lengths represent the estimated number of substitutions per site assuming the respective models of substitution. The type specimen is marked with an asterisk.

of rhizomorphs on the lower surface of the thalli, and by containing aspicilin as a secondary metabolite. *Circinaria mansourii*, like *C. persepolitana*, has long prothallus-like extensions, which are attached to the tips of lobes. Both *A. reptans* and *C. mansourii* lack a saxicolous habit and have convex to squamuliform areoles and α -alecoronic acid. The recently described *Circinaria ochracea* (Nascimbene *et al.* 2023) belongs to the same branch of the phylogenetic tree as *C. persepolitana*, *C. mansourii* and *Aspiciella reptans* but differs ecologically by growing in a water-splash zone and it has a uniform crustose thallus with flat ochraceous

areoles. The new species is also similar to *Circinaria crespiana* (V. J. Rico) Sohrabi & V. J. Rico, a Mediterranean species known from Spain and Italy, grows among mosses, on siliceous rocks and on soil. It has large squamulose thalli attached to the substratum with rhizomorph-like extensions (Sanders & Rico 1992), and large, appressed apothecia up to 3 mm in diameter.

Note. Due to restrictions in collecting any kind of material from UNESCO World Heritage Sites, the holotype specimen and specimen Sohrabi 32099 were collected from calcareous rocks

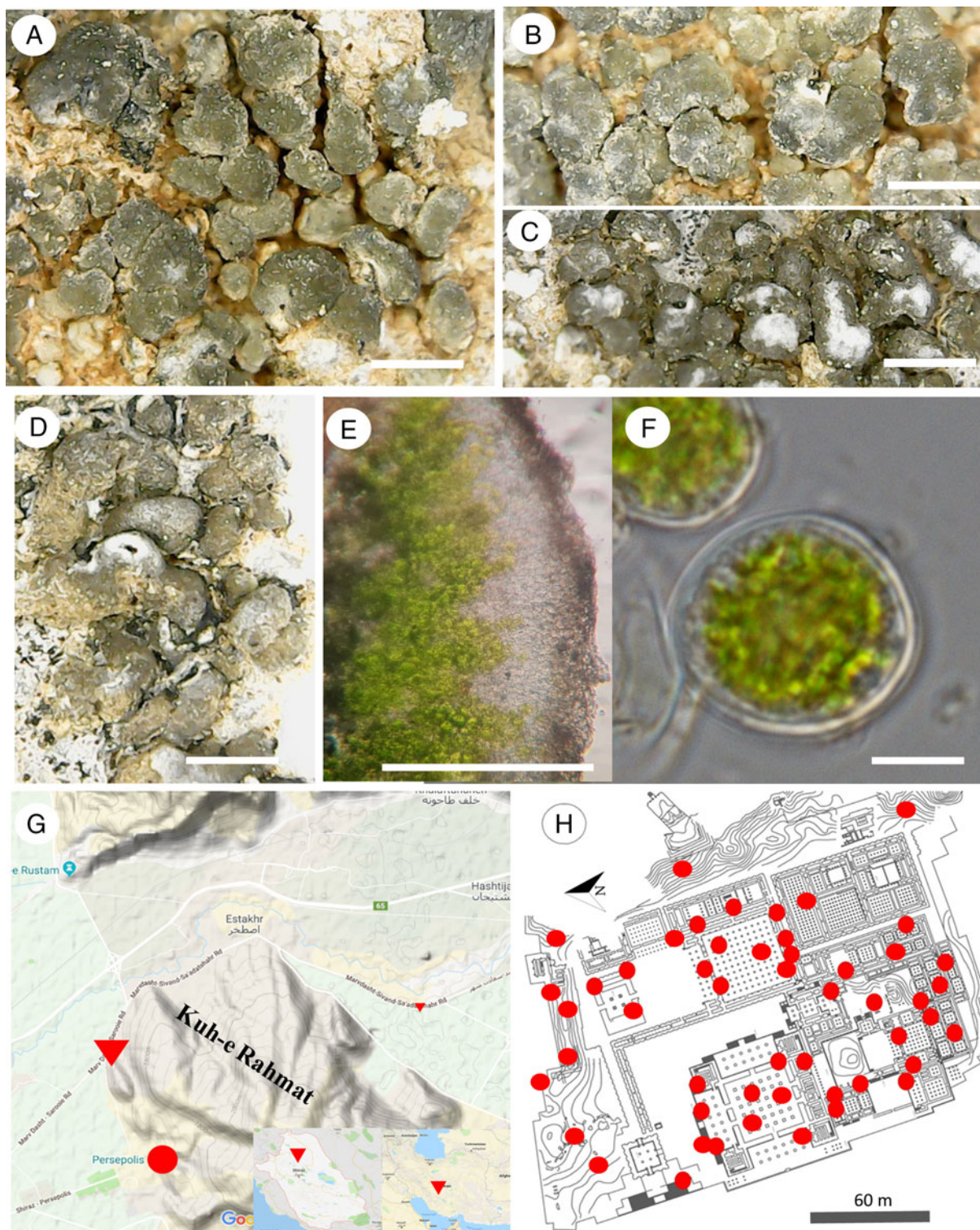


Figure 2. *Circinaria persepolitana* (A–F) and its distribution in south-western Iran (G & H). A, projections of bullate-areolate surfaces in the central part of the thallus. B, projections of angular to irregular areoles. C & D, papillate areoles, somewhat verrucose, hollow and cracking into popcorn-like tufts, more or less squamuliform areolate. E, cross-section of the green photobiont algal layer, and the white and grey mycobiont layer. F, chlorococcoid algal cell (*Trebouxia*-type). G, location of the Persepolis UNESCO World Heritage Site and distribution of *C. persepolitana* in the area, with the holotype locality indicated with a triangle. H, distribution map of *C. persepolitana* in the historic site of Persepolis. Scales: A–D = 1 mm; E = 200 μ m; F = 10 μ m. In colour online.

(limestone) outside Persepolis, from Naghsh-e-Rajab, c. 5 km north of the historic site. X-ray diffraction (XRD) analyses conducted on samples 106, 108 and 109 (Fig. 4), revealed that the rock composition in the locality where the type specimen was

collected (Naghsh-e Rajab, sample 109) and that of the historical substratum (Persepolis, sample 108) exhibited a remarkable degree of similarity, displaying an analogous pattern of calcite and subordinate quartz and clay minerals.

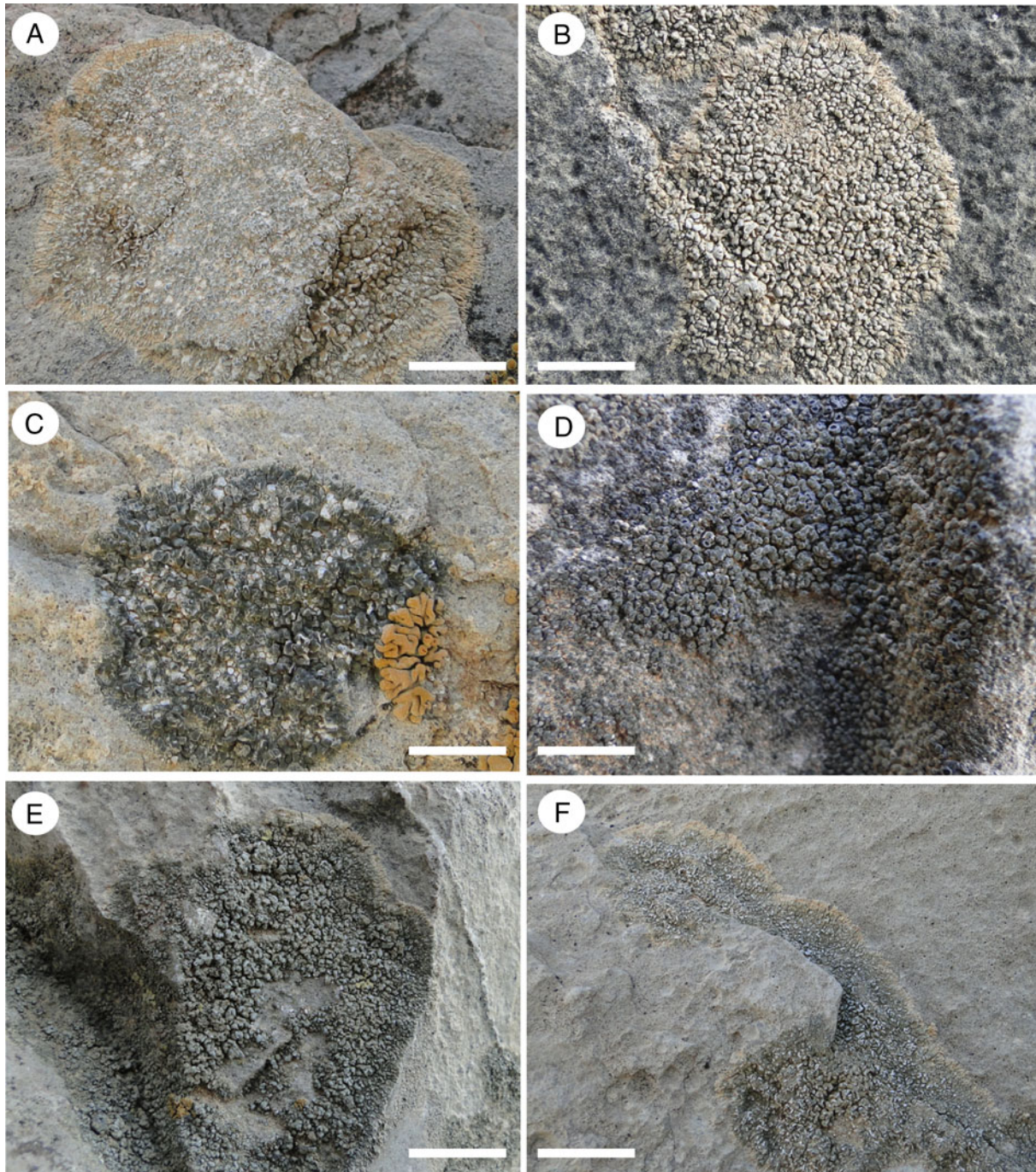


Figure 3. *Circinaria persepolitana* on historic rock surfaces. A, diffuse thallus margin with some areoles peeled off. B, verrucose thallus. C, hollow squamuliform areolate thallus, with visible white medulla. D & E, thallus with rimose-areolate to bullate-areolate, dispersed or rarely overlapping areoles, verrucose to squamuliform (slightly vertically elevated areoles). F, the outer part of the thallus with grey prothallus-like extensions. Scales = 1 cm. In colour online.

Additional specimens examined. *Circinaria contorta*. **Iran:** Mazandaran: Nour, Roian, Koop Village, open forest area surrounding the village, 36.333778°N, 51.859027°E, 1500 m, saxicolous, on calcareous rock, 14 x 2002, Mehdi Mofid 3074 (hb. M. Sohrabi).

Circinaria mansourii. **Iran:** Golestan: Golestan National Park, Mirzabaylou towards Almeh valley, 37.35°N, 56.2°E, 1300 m, 2008, Sohrabi 15077 & Ghobad-Nejhad (hb. M. Sohrabi-MS016192—*isotypes*).—**Russia:** Astrakhanskaya Oblast: Akhtubinskiy District, Bogdinsko-Baskunchakskiy, ‘Bolshoye

Bogdo’ mountain, 48.133889°N, 46.836389°E, 2018, A. G. Paukov 3049; *ibid.*, 2019, A. G. Paukov 3285, 3503 (UFU).

Nomenclatural notes. In their work on the *Aspicilia reptans* group in western North America, McCune & Di Meglio (2021) proposed the synonymization of *A. reptans* and *Circinaria mansourii*. Study of additional material of *C. mansourii* from the Astrakhan oblast in Russia shows that it has morphological and anatomical features that merit keeping *C. mansourii* as

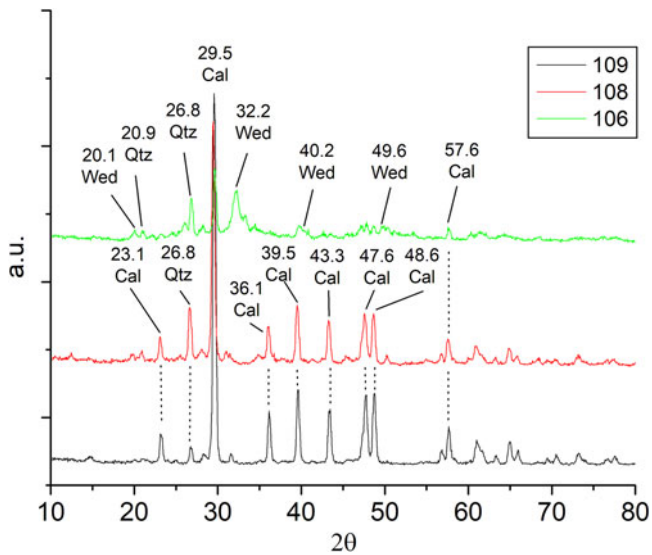


Figure 4. X-ray diffraction analysis of rock samples indicating the substratum of *Circinaria persepolitana* in three distinct locations. Sample 106 corresponds to the lichen-lithic substratum (with thallus of *C. persepolitana*) found in the Hall of a Hundred Columns. Sample 108 corresponds to the substratum without a lichen thallus, sampled from the Unfinished Gate. Sample 109 corresponds to the substratum of the holotype specimen (without lichen thallus) located in Naghsh-e-Rajab limestone outcrops. Qtz = quartz peak; Wed = weddellite peak (a mineral form of calcium oxalate); Cal = calcite peak; a.u. = arbitrary scale; 2θ (theta angle) = the angle of diffraction, measured in degrees. In colour online.

a separate taxon. It differs from *A. reptans* in having many 'prothalline spicate tips' and an algal layer similar to 'type 2' (McCune & Di Meglio 2021) with vertical projections 30–40 μm wide towards the cortex, connected at their bases to form a more or less continuous, but sometimes fragmented, algal layer. Rhizomorphs are abundant in the specimens studied and they are moderately fertile with 1–3-spored asci and ascospores 22–25 \times 15–20 μm . Conidia are similar to those reported for *A. reptans*, 6–9 μm . Furthermore, in the phylogenetic tree, *C. mansourii* forms a statistically supported sister group with *Aspicilia reptans*.

McCune & Di Meglio (2021) made no taxonomic rearrangements in the genus *Aspicilia*; however, according to our phylogenetic reconstruction, *A. reptans* is placed within *Circinaria*. Therefore, we propose a new combination here:

***Circinaria reptans* (Looman) Sohrabi, Owe-Larsson & Paukov comb. nov.**

Mycobank No.: MB 847207

Lecanora reptans Looman, *Bryologist* 65, 301 (1962).—*Aspicilia reptans* (Looman) Wetmore, *Mycotaxon* 23, 243 (1985); type: Canada, Saskatchewan, Webb, near Swift Current, on soil in eroded grassland, elevation 792 m a.s.l., 12 April 1959, Looman 596114 (WIS—holotype).

New records

Circinaria mansourii has previously been recorded from the Iranian provinces of Golestan and East Azerbaijan (Lumbsch *et al.* 2011). It is found on soil and dead plant debris, often on dead tufts of *Poa bulbosa* L. in arid communities. The study of

lichens in the Bogdinsko-Baskunchakskiy Nature Reserve revealed the presence of the species in the north of the Caspian Region, in Astrakhanskaya Oblast, Russia (Loktionov *et al.* 2016). Here it grows in herbaceous communities almost exclusively on the basal parts of dead *Poa bulbosa*.

Weathering capacity

Areoles of *Circinaria persepolitana* (sample 106) were observed closely attached to the rock surface, which appeared markedly disintegrated (white arrows in Fig. 5A). Mycobiont hyphae formed rounded cavities in the attachment area (black arrows in Fig. 5A and white arrow in Fig. 5B) or penetrated, generating fissures within the stone (arrow in Fig. 5C). As a result of these interactions with the colonized rock, and the associated biogeochemical and biogeophysical alterations, mineral fragments accumulate within the lichen thallus structure (Fig. 5D (arrows) & 5E). These mineral fragments from the rocky substratum (black asterisk in Fig. 5E, EDS spectra in 5F) appeared intermixed with calcium-rich crystals (white asterisk in Fig. 5E, EDS spectra in 5G, SEM-SE images in 5H & I). The XRD analysis of the thallus-rock interface of *C. persepolitana* sample 106 (Fig. 4) confirmed the presence of weddellite ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$), the dihydrate calcium oxalate.

The space between *C. persepolitana* areoles was frequently occupied by fungal growths associated with numerous mineral fragments of different sizes (Fig. 6A), which also induced rock surface disintegration (Fig. 6A & B). In these communities dominated by fungi (Fig. 6C), colonies of cyanobacteria were also observed (asterisk in Fig. 6B) as well as heterotrophic bacterial colonies (arrows in Fig. 6B–D).

Final Remarks

The identification of biodeteriogenic micro-organisms in historical monuments is essential for protecting the cultural heritage today and transferring it to future generations. In this regard, molecular biology techniques make it possible to identify with certainty the micro-organisms that colonize monumental and historic stone surfaces. Among them, phylogenetic analyses can play a key role in the identification and taxonomic classification of these organisms, but they have hardly been used in the context of cultural heritage management.

The new species *Circinaria persepolitana*, which has great potential for contributing to the biodeterioration of bas reliefs in Persepolis (Fig. 7), has been described here. This biodeteriogenic potential has been attributed because of its capacity to induce biogeophysical and biogeochemical actions on the lithic substratum. In fact, lichens are important biodeteriogen agents, especially in cultural heritage assets made with carbonate rocks (Ascaso *et al.* 2002; de los Ríos *et al.* 2009). In addition, the establishment of *C. persepolitana* thalli facilitated the colonization of heterotrophic bacteria and cyanobacteria, which also can be involved in biodeterioration processes (de los Ríos *et al.* 2002, 2004; Nir *et al.* 2022). Mineral dissolution and disintegration of stone surfaces are frequent phenomena associated with the establishment and development of lichen thalli on limestone from archaeological sites, threatening its conservation (Sohrabi *et al.* 2017; Matteucci *et al.* 2019; Nir *et al.* 2022). These biodeterioration processes can be particularly harmful in areas with bas reliefs because the sculptural artwork may disappear. Hence, the survey of *Circinaria persepolitana* and long-term monitoring of new

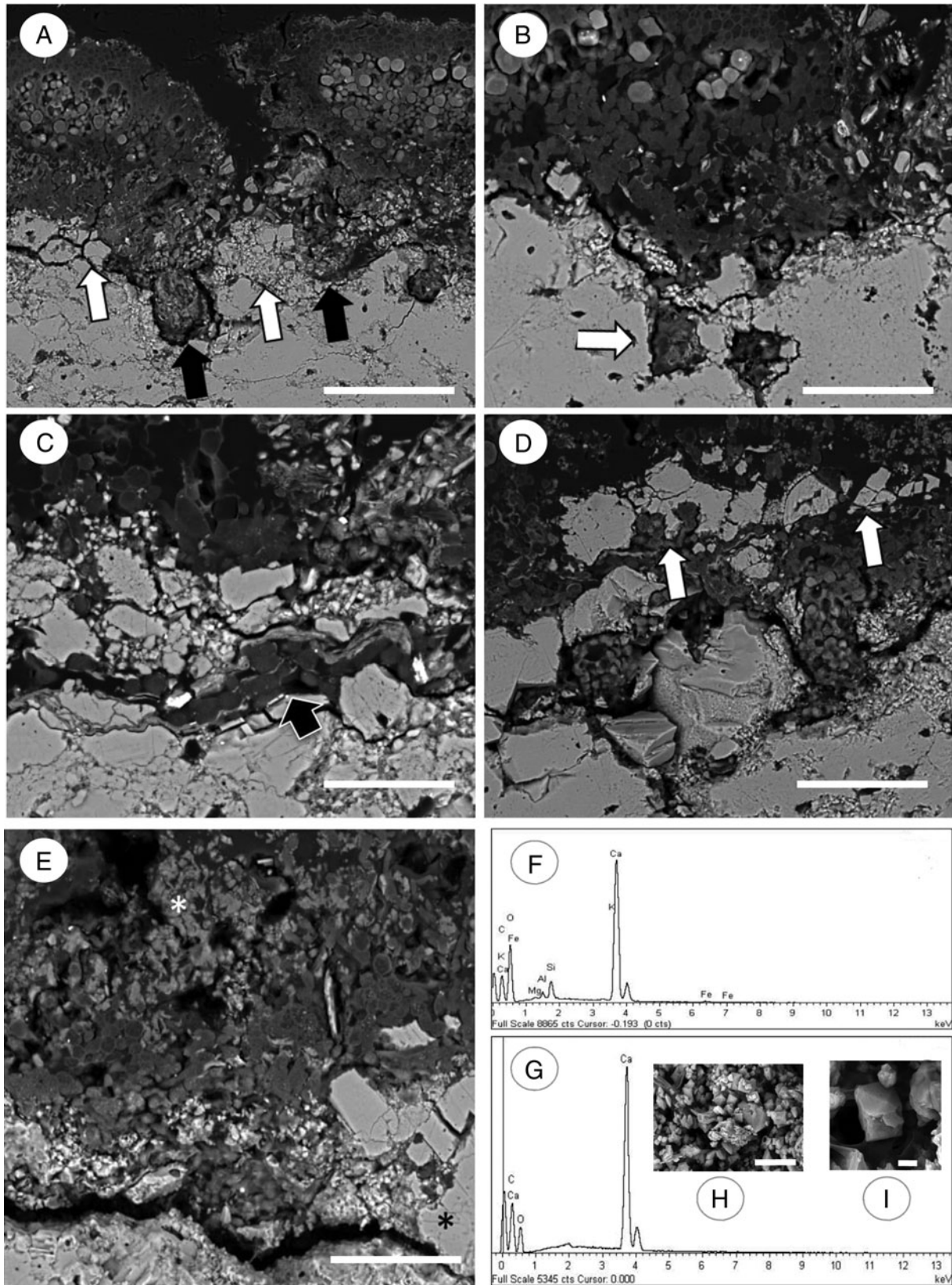


Figure 5. Scanning electron microscopy of the *Circinaria persepolitana*-lithic substratum interface using the back-scattered electron mode (SEM-BSE). A–E, *Circinaria persepolitana*-lithic substratum interface with beige limestone. A, lichen-rock interface showing penetration of mycobiont hyphae within the lithic substratum (black arrows) and disintegration of stone surface (white arrows). B, detail of the rounded cavities formed by dissolution under *C. persepolitana* thalli (white arrow). C, detail of fissures in the lithic substratum induced by the penetration of mycobiont hyphae (black arrow). D, detail of the lower part of the thallus harbouring mineral fragments retrieved from the stone surface (white arrows). E, detail of the accumulation of calcium oxalate crystals (white asterisk) within the medulla together with mineral fragments (black asterisk). F, EDS spectra of limestone fragments (black asterisk in E). G, EDS spectra of calcium-rich crystals (white asterisk in E). H & I, SEM-BSE images of calcium oxalate crystals from the thalli. Scales: A = 100 μm ; B–D = 50 μm ; H = 10 μm ; I = 2 μm .

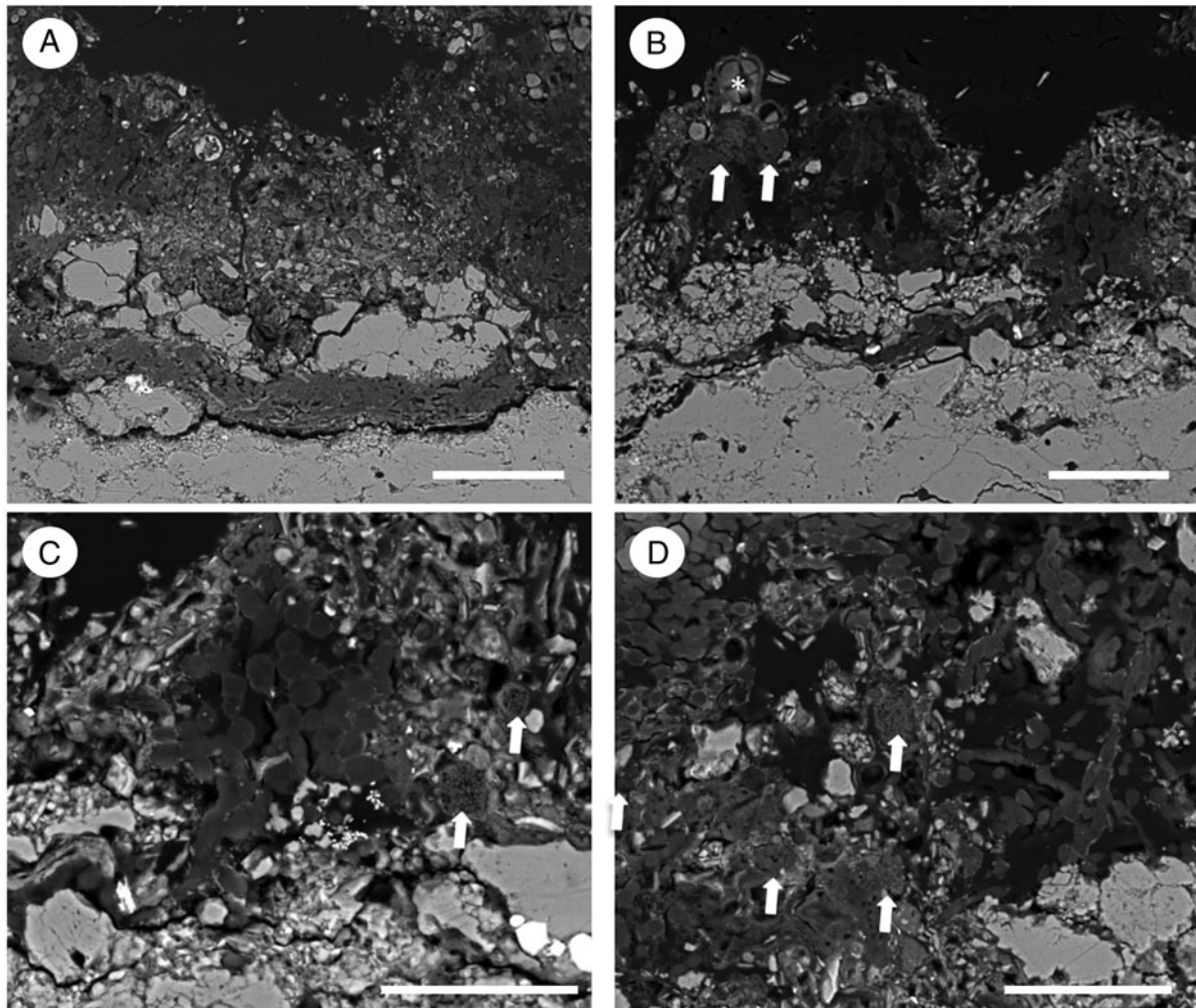


Figure 6. Scanning electron microscopy using the back-scattered electron mode (SEM-BSE) showing images of areas in between *Circinaria persepolitana* areoles. A, area extensively colonized by fungal hyphae. B, area showing association of cyanobacteria (asterisk) and heterotrophic bacteria colonies at the surface of the fungal growth (white arrows). C, detail of free-living fungi and heterotrophic bacteria colonies (white arrows) in the proximity of the stone surface. D, detail of the presence of several heterotrophic bacterial colonies (white arrows) amongst fungal hyphae. Scales: A = 100 μm ; B & D = 50 μm ; C = 40 μm .

establishments in different areas of Persepolis is essential for preserving these valuable bas reliefs.

Conversely, the description of *C. persepolitana* confirms the valuable contribution of archaeological areas to lichen diversity in man-made habitats. Archaeological areas in Latium (central Italy) have been shown to host a rich lichen flora, and most of the saxicolous species reported for Latium occur solely on heritage surfaces (Nimis *et al.* 1987). Although *C. persepolitana* has also been observed in natural mountainous areas surrounding Persepolis, its presence in the anthropized plain, largely characterized by an agricultural landscape, seems rather limited to the monumental area. To explain the lichen richness of the archaeological sites in Latium, a relationship with the provenance of different lithic materials from heterogeneous regions of the Roman Empire has been hypothesized (Nimis *et al.* 1987). The observation of *C. persepolitana* in the site of Kuh-e-Rahmat, where the building stones used in Persepolis were quarried (Torabi-Kaveh *et al.* 2019), similarly suggests a possible human-driven origin for the observed colonization in the monumental site. The current absence of apothecia and pycnidia, however, appears somehow in

contrast to the widespread colonization observed through the UNESCO site, and suggests some limits to a potential further expansion of the species in the area. The abundant production and release of spores was shown to be a common trait of species displaying the highest frequencies on heritage surfaces, at least in the decades following cleaning interventions (Morando *et al.* 2019). By contrast, the spread but limited number of large thalli of *C. persepolitana* seem to testify to a very old colonization event and/or transport to the site. Similarly, *Pyrenodesmia erodens* (Tretiach *et al.*) Søchting *et al.* had been observed as a deteriogenic lichen species on monuments, but no apothecia were found on the heritage surfaces and its description became possible only with fertile specimens from a natural outcrop (Tretiach *et al.* 2003). To our knowledge, several black fungal taxa have been isolated and described from monumental stone surfaces (Sert *et al.* 2007, 2011; Labuda *et al.* 2008; Sert & Sterflinger 2010), but there are no descriptions of new species directly from UNESCO World Heritage monuments.

Despite the threat to stone conservation posed by lichen colonization, this study suggests the need to address strategies that

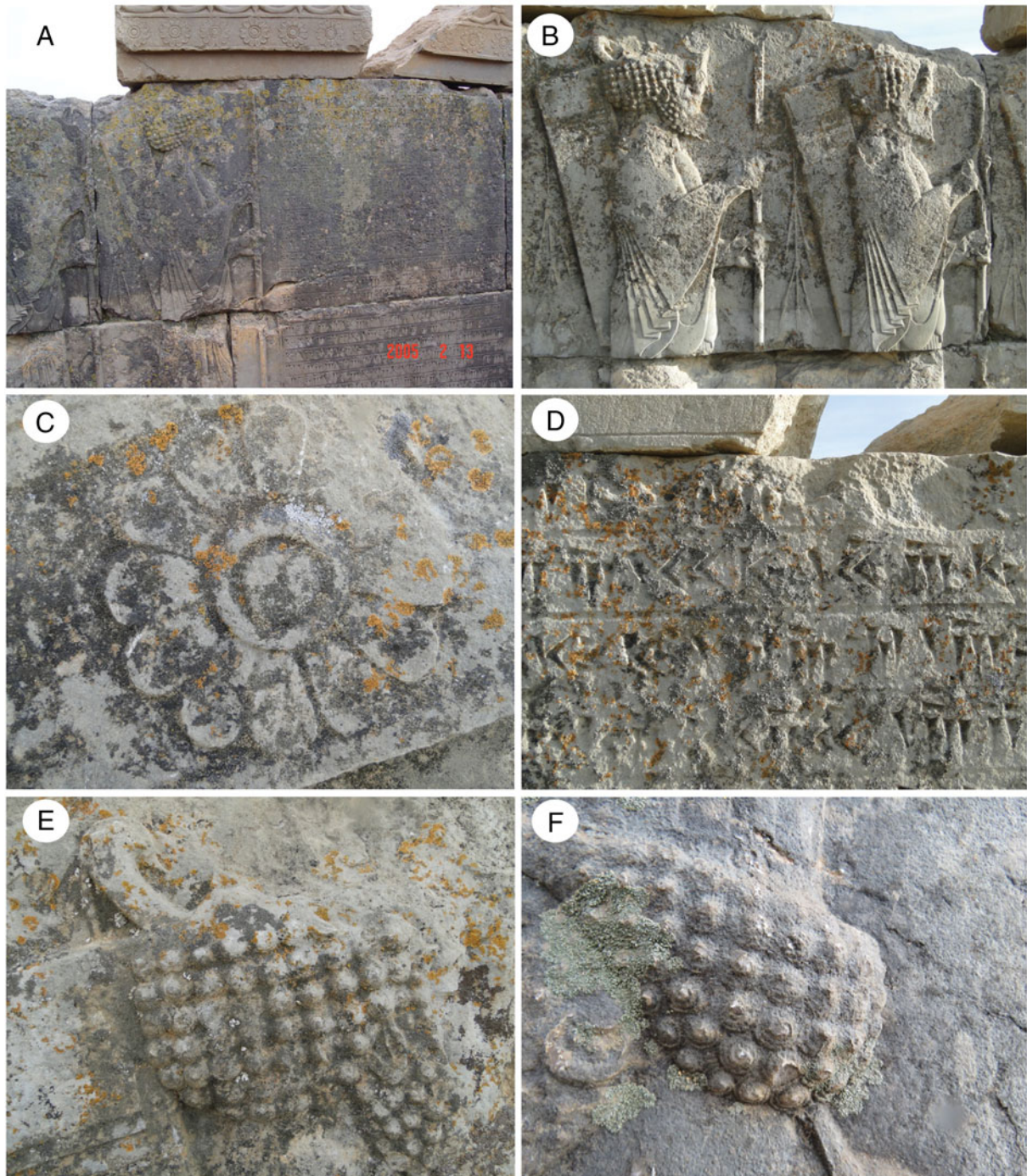


Figure 7. Colonization of *Circinaria persepolitana* on historic rock reliefs of Persepolis in south-western Iran. A & B, Achaemenid warriors and cuneiform reliefs in the northern wall of the Hadish Palace. C, a lotus flower, with visible colonization. D, colonization of old Persian cuneiform. E & F, detail from the outer Achaemenid warriors from Persepolis. In colour online.

combine the safeguarding of heritage surfaces with caring for the biological value of this species. Indeed, lichen diversity may represent an additional element and a potential object of valorization in the context of the archaeological site (Seaward 2004). Although the removal of this species seems generally advisable to limit its deteriorogenous impact on the stone surfaces in Persepolis (see above), limits to the expansion of its colonization (related to the exclusive occurrence of sterile thalli)

suggest the possibility of a local conservation of some thalli, such as on surfaces without reliefs or other decorations, and therefore allowing visitors to see them. Conserving the habitat and substrate of new species and preserving historic surfaces while reducing biodeterioration pose the greatest challenge for environmentalists and restorers. We must establish new regulations to manage the overlapping natural habitats and UNESCO historic sites.

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References

- Ascaso C, Wierzychos J, Souza-Egipsy V, de los Ríos A and Rodrigues JD (2002) In situ evaluation of the biodeteriorating action of microorganisms and the effects of biocides on carbonate rock of the Jeronimos Monastery (Lisbon). *International Biodeterioration and Biodegradation* **49**, 1–12.
- Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, de Maio N, *et al.* (2019) BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **15**, e1006650.
- Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B and Flouri T (2020) ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* **37**, 291–294.
- de los Ríos A, Wierzychos J and Ascaso C (2002) Microhabitats and chemical microenvironments under saxicolous lichens growing on granite. *Microbial Ecology* **43**, 181–188.
- de los Ríos A, Galván V and Ascaso C (2004) In situ microscopical diagnosis of biodeterioration processes at the convent of Santa Cruz la Real, Segovia, Spain. *International Biodeterioration and Biodegradation* **54**, 113–120.
- de los Ríos A, Cámara B, García M, Rico V, Galván V and Ascaso C (2009) Deteriorating effects of lichen and microbial colonization of carbonate building rocks in the Romanesque churches of Segovia (Spain). *Science of the Total Environment* **407**, 1123–1134.
- Eidler D, Klein J, Antonelli A and Silvestro D (2021) raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* **12**, 373–377.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.
- Ismayil G, Wen XM and Abbas A (2015) New records of *Circinaria* Link. genus and *Circinaria contorta* species in Xinjiang, China. *Arid Zone Research* **32**, 1229–1232.
- Ismayil G, Abbas A and Guo SY (2019) A new saxicolous *Circinaria* species (*Megasporaceae*) from northeast China. *Bryologist* **122**, 23–30.
- Katoh K, Rozewicki J and Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**, 1160–1166.
- Kozlov AM, Darriba D, Flouri T, Morel B and Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **35**, 4453–4455.
- Labuda R, Eliáš P, Sert H and Sterflinger K (2008) *Alternaria jesenskae* sp. nov., a new species from Slovakia on *Fumana procumbens* (*Cistaceae*). *Microbiological Research* **163**, 208–214.
- Larget B and Simon DL (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* **16**, 750–759.
- Loktionov AP, Voloboeva OV and Nurmuhambetova DE (2016) Characteristics of flora of the National natural park 'Bogdinsko-Baskunchakskiy'. *Yeststvennyye Nauki (Natural Sciences)* **3**, 29–38 [In Russian].
- Lumsch HT, Ahti T, Altermann S, Amo de Paz G, Aptroot A, Arup U, Bárcenas Peña A, Bawingan PA, Benatti MN, Betancourt L, *et al.* (2011) One hundred new species of lichenized fungi: a signature of undiscovered global diversity. *Phytotaxa* **18**, 1–127.
- Matteucci E, Scarcella AV, Croveri P, Marengo A, Borghi A, Benelli C, Hamdan O and Favero-Longo SE (2019) Lichens and other lithobionts on the carbonate rock surfaces of the heritage site of the tomb of Lazarus (Palestinian territories): diversity, biodeterioration, and control issues in a semi-arid environment. *Annals of Microbiology* **69**, 1033–1046.
- McCune B and Di Meglio J (2021) Revision of the *Aspicilia reptans* group in western North America, an important component of soil biocrusts. *Monographs in North American Lichenology* **5**, 1–94.
- Morando M, Matteucci E, Nascimbene J, Borghi A, Piervittori R and Favero-Longo SE (2019) Effectiveness of aerobiological dispersal and microenvironmental requirements together influence spatial colonization patterns of lichen species on the stone cultural heritage. *Science of the Total Environment* **685**, 1066–1074.
- Mousavi A (2012) *Persepolis: Discovery and Afterlife of a World Wonder*. Boston, Berlin: Walter de Gruyter.
- Nascimbene J, Nimis PL, Klüßendorf J and Thüs H (2023) Freshwater lichens, including new species in the genera *Verrucaria*, *Placopyrenium* and *Circinaria*, associated with *Lobothallia hydrocharis* (Poelt & Nimis) Sohrabi & Nimis from watercourses of Sardinia. *Journal of Fungi* **9**, 380.
- Nimis PL, Monte M and Tretiach M (1987) Flora e vegetazione lichenica di aree archeologiche del Lazio. *Studia Geobotanica* **7**, 3–161.
- Nir I, Barak H, Kramarsky-Winter E, Kushmaro A and de los Ríos A (2022) Microscopic and biomolecular complementary approaches to characterize bioweathering processes at petroglyph sites from the Negev Desert, Israel. *Environmental Microbiology* **24**, 967–980.
- Nordin A, Tibell L and Owe-Larsson B (2007) A preliminary phylogeny of *Aspicilia* in relation to morphological and secondary product variation. *Bibliotheca Lichenologica* **96**, 247–266.
- Nordin A, Savić S and Tibell L (2010) Phylogeny and taxonomy of *Aspicilia* and *Megasporaceae*. *Mycologia* **102**, 1339–1349.
- Norouzi H, Azizi A, Gholami M, Sohrabi M and Boustie J (2020) Chemotype variations among lichen ecotypes of *Umbilicaria aprina* as revealed by LC-ESI-MS/MS: a survey of antioxidant phenolics. *Environmental Science and Pollution Research* **27**, 40296–40308.
- Nylander W (1866) Circa novum in studio lichenum criterium chemicum. *Flora (Regensburg)* **49**, 198–201.
- Obermayer W (2002) Management of a lichen herbarium. In Kranner I, Beckett RP and Varma A (eds), *Protocols in Lichenology*. Berlin, Heidelberg: Springer, pp. 507–523.
- Olivier-Jimenez D, Chollet-Krugler M, Rondeau D, Beniddir MA, Ferron S, Delhaye T, Allard PM, Wolfender JL, Sipman HJM, Lücking R, *et al.* (2019) A database of high-resolution MS/MS spectra for lichen metabolites. *Scientific Data* **6**, 294.
- Orange A, James PW and White FJ (2010) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.
- Owe-Larsson B, Nordin A and Tibell L (2007) *Aspicilia*. In Nash TH, III, Gries C and Bungartz F (eds), *Lichen Flora of the Greater Sonoran Desert Region, Vol. 3*. Tempe, Arizona: Lichens Unlimited, Arizona State University, pp. 61–108.
- Owe-Larsson B, Nordin A, Tibell L and Sohrabi M (2011) *Circinaria arida* sp. nova and the '*Aspicilia desertorum*' complex. *Bibliotheca Lichenologica* **106**, 235–246.
- Paukov A, Nordin A, Tibell L, Frolov I and Vondrák J (2017) *Aspicilia goettweigensis* (*Megasporaceae*, lichenized *Ascomycetes*) – a poorly known and overlooked species in Europe and Russia. *Nordic Journal of Botany* **35**, 595–601.
- Paukov A, Davydov E, Nordin A, Owe-Larsson B, Sohrabi M and Teptina A (2021) Estimation of the global diversity of *Megasporaceae*, Abstracts of the 9th International Association for Lichenology Symposium (IAL 9), 1–6 August 2021, Brazil, p. 127.
- Roux C, Bertrand M and Nordin A (2016) *Aspicilia serenensis* Cl. Roux et M. Bertrand sp. nov., espèce nouvelle de lichen (groupe d'*A. calcarea*, *Megasporaceae*). *Bulletin de la Société Linnéenne de Provence* **67**, 165–182.
- Ryan BD, Bungartz F and Nash TH, III (2002) Morphology and anatomy of the lichen thallus. In Nash TH, III, Ryan BD, Gries C and Bungartz F (eds), *Lichen Flora of the Greater Sonoran Desert Region, Vol. 1*. Tempe, Arizona: Lichens Unlimited, Arizona State University, pp. 8–47.

- Sanders WB and Rico VJ** (1992) Lichenizing rhizomorphs and thallus development in the squamulose lichen *Aspicilia crespiana* Rico *ined.* (*Lecanorales*, *Ascomycetes*). *Botanica Acta* **105**, 449–456.
- Seaward MRD** (2004) Lichens as subversive agents of biodeterioration. In St Clair LL and Seaward MRD (eds), *Biodeterioration of Stone Surfaces*. Dordrecht: Springer, pp. 9–18.
- Sert HB and Sterflinger K** (2010) A new *Coniosporium* species from historical marble monuments. *Mycological Progress* **9**, 353–359.
- Sert HB, Sümbül H and Sterflinger K** (2007) *Sarcinomyces sideticae*, a new black yeast from historical marble monuments in Side (Antalya, Turkey). *Botanical Journal of the Linnean Society* **154**, 373–380.
- Sert HB, Sümbül H and Sterflinger K** (2011) Two new species of *Capnobotryella* from historical monuments. *Mycological Progress* **10**, 333–339.
- Sohrabi M, Ahti T and Litterski B** (2011a) *Aspicilia digitata* sp. nov., a new vagrant lichen from Kyrgyzstan. *Lichenologist* **43**, 39–46.
- Sohrabi M, Stenroos S, Högnabba F, Nordin A and Owe-Larsson B** (2011b) *Aspicilia rogeri* sp. nov. (*Megasporaceae*) and other allied vagrant species in North America. *Bryologist* **114**, 178–189.
- Sohrabi M, Stenroos S, Myllys L, Sochting U, Ahti T and Hyvönen J** (2013a) Phylogeny and taxonomy of the ‘manna lichens’. *Mycological Progress* **12**, 231–269.
- Sohrabi M, Leavitt SD, Rico VJ, Halıcı MG, Shrestha G and Stenroos S** (2013b) *Teuvoa*, a new lichen genus in *Megasporaceae* (*Ascomycota*: *Pertusariales*), including *Teuvoa junipericola* sp. nov. *Lichenologist* **45**, 347–360.
- Sohrabi M, Favero-Longo SE, Pérez-Ortega S, Ascaso C, Haghghat Z, Talebian MH, Fadaei H and de los Ríos A** (2017) Lichen colonization and associated deterioration processes in Pasargadae, UNESCO world heritage site, Iran. *International Biodeterioration and Biodegradation* **117**, 171–182.
- Sohrabi M, Leavitt SD, Obermayer W and Mayrhofer H** (2023) *Circinaria nimisii* (*Megasporaceae*, lichenized *Ascomycota*), a new manna lichen from Greece. *Lichenologist* **55**, 367–376.
- Stamatakis A** (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.
- Toju H, Tanabe AS, Yamamoto S and Sato H** (2012) High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS ONE* **7**, e40863.
- Torabi-Kaveh M, Heidari M, Mohseni H and Ménendez B** (2019) Role of petrography in durability of limestone used in construction of Persepolis complex subjected to artificial accelerated ageing tests. *Environmental Earth Sciences* **78**, 1–18.
- Tretiach M, Pinna D and Grube M** (2003) *Caloplaca erodens* [sect. *Pyrenodesmia*], a new lichen species from Italy with an unusual thallus type. *Mycological Progress* **2**, 127–136.
- Wang M, Carver JJ, Phelan V, Sanchez LM, Garg N, Peng Y, Nguyen DD, Watrous J, Kapon CA, Luzzatto-Knaan T, et al.** (2016) Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature Biotechnology* **34**, 828–837.
- White TJ, Bruns T, Lee SB and Taylor JW** (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds), *PCR Protocols: a Guide to Methods and Applications*. San Diego: Academic Press, pp. 315–322.
- Wierzchos J and Ascaso C** (1994) Application of back-scattered electron imaging to the study of the lichen-rock interface. *Journal of Microscopy* **175**, 54–59.
- Wijayawardene NN, Hyde KD, Al-Ani LKT, Tedersoo L, Haelewaters D, Rajeshkumar KC, Zhao RL, Aptroot A, Leontyev D and Saxena RK** (2020) Outline of Fungi and fungus-like taxa. *Mycosphere* **11**, 1060–1456.