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# *Circinaria arida* sp. nova and the '*Aspicilia desertorum*' complex

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**Abstract:** *Circinaria arida* sp. nova is described from the Sonoran area. In the Sonoran Desert Lichen Flora 3 it was tentatively treated as *Aspicilia desertorum*. This name, however, is based on *Lecanora desertorum*, an illegitimate name introduced by Krempelhuber for both vagrant and saxicolous taxa, but usually only applied to non-vagrant, saxicolous specimens. In the analysis presented here these are shown to represent more than one taxon. For American specimens the epithet *elmorei* is available, and the combination *Circinaria elmorei* is proposed.

**Keywords:** *Aspicilia*, lichenized ascomycetes, molecular phylogeny, nuclear rDNA ITS1-5.8S-ITS2, taxonomy

#### Introduction

In the revision of the genus *Aspicilia* for the 3<sup>rd</sup> Volume of the Sonoran Desert Lichen Flora (OWE-LARSSON et al. 2008), *Aspicilia desertorum* (Pall.) Mereschk. was stated to be common in the area. However, several differences between the taxon growing in the Sonoran area and specimens referred to *A. desertorum* from other areas were pointed out. When the manuscript was prepared for the flora, nuclear rDNA ITS1-5.8S-ITS2ITS sequences were available for three specimens from the Sonoran area, but not for specimens from other areas, and the need of further molecular studies was pointed out.

One of the sequences from the Sonoran specimens was included in a phylogenetic analysis, which placed it in a group distinctly separated from the type species *A. cinerea* (L.) Körber (see NORDIN et al. 2007). Members of this group were recently transferred to *Circinaria* [e.g., *C. calcarea* (L.) A. Nordin, S. Savić & Tibell, *C. contorta* (Hoffm.) A. Nordin, S. Savić & Tibell, *C. hispida* (Mereschk.) A. Nordin, S. Savić & Tibell, and *C. leprosescens* (Sandstede) A. Nordin, S. Savić & Tibell] based on a nuLSU and mtSSU phylogeny in a study of Megasporaceae (NORDIN et al. 2010). Like the Sonoran taxon, several other taxa in this group contain aspicilin, a secondary metabolite not known outside this group. Other characteristics shared by most members of the group are a reduced number of spores in the asci, relatively large, broadly ellipsoid to rounded spores, and short conidia.

A closer examination of the nomenclature of A. desertorum (Kremp.) Mereschk. (SOHRABI & AHTI 2010) showed it to be based on the illegitimate Lecanora desertorum Kremp. (KREMPELHUBER 1867). Krempelhuber regarded the vagrant species *Lichen esculentus* Pall. as a saxicolous species, occasionally developing vagrant forms, and he included the older name among the synonyms, thereby making *Lecanora desertorum* illegitimate. In spite of that fact, the combination Aspicilia desertorum was introduced, and this name has, for a long time, mainly been applied to saxicolous specimens resembling the presumed type material of Lecanora desertorum in W. Aspicilia esculenta (Pall.) Mereschk. has generally, but rather indiscriminately, been applied to vagrant material. In the United States saxicolous, non-vagrant material morphologically similar to European 'A. desertorum' is known from the southern part of the Great Plains and the mid-west. Lecanora elmorei E.D. Rudolph is a valid name based on such material from northern New Mexico. This name seems to be the only valid name available for this taxon, but it is uncertain whether it can be applied to material from other areas.

The morphological variation of '*Aspicilia desertorum*' in America has been discussed by KUNKEL (1980), who regarded crustose and fruticose growth forms as environmental modifications of the same species. Before that WEBER (1967, 1977) also discussed environmental modifications in *Aspicilia* (and other genera). The relationship between different growth forms, however, is outside the scope of this paper but will be addressed elsewhere (see Discussion).

In this paper we present a phylogenetic analysis based on ITS sequences from the Sonoran material; material identified as *Aspicilia desertorum* from Asia, Europe, and North America; material of the vagrant *A. esculenta* and the fruticose *Circinaria hispida*; and material of a few additional *Aspicilia* species (including representatives from the genera of Megasporaceae accepted by NORDIN et al. 2010). The aim was 1) to establish the phylogenetic and taxonomic status of the Sonoran specimens; 2) to test the monophyly of '*A. desertorum*'; and 3) to evaluate the applicability of the epithet *elmorei*.

#### Material and methods

Fresh material mainly collected by the authors in America, Asia and Europe was used for DNA extraction (Tab. 1). In addition herbarium material from ANES, ASU, H, MIN, POLL, SBBG, TU, UCR, UPS, US, and the private herbarium of M. Sohrabi was studied.

Species	Code	Origin	Voucher	Accession #
Aspicilia cinerea	T467	Sweden	Nordin 5542	HQ406799
'A. desertorum' = Circinaria arida	T540	USA, Arizona	Owe-Larsson 8759	HQ406800
'A. desertorum' = C. arida		USA, Arizona	Owe-Larsson 8770	EU057905
'A. desertorum' = C. arida	T620	USA, California	Knudsen 2046	HQ406801
'A. desertorum'	SS301	Russia	Owe-Larsson 9814	HQ406802
'A. desertorum'	MS67	Turkey	John 11984B (POLL)	HQ389201
'A. desertorum'	MS74	Iran	Sohrabi 10205 (hb. Sohrabi)	HQ389203
'A. desertorum'	MS121	Russia	Owe-Larsson 9821	HQ389202
'A. desertorum' = C. elmorei	MS135	Nevada	Rosentreter 3689 (TU)	HQ389200
A. esculenta	SS303	Russia	Owe-Larsson 9824	HQ406803
Circinaria calcarea		Sweden	Nordin 5888	EU057898
C. calcarea	T529	Sweden	Nordin 5914	HQ406804
C. contorta		Sweden	Nordin 5895	EU057900
C. contorta	T444	Sweden	Tibell 23702	HQ406805
C. hispida	T829	Turkey	Candan 11 (ANES)	HQ406806
C. leprosescens		Sweden	Nordin 5906	EU057911
Lobothallia recedens	T946	Sweden	Nordin 6582a	HQ406807
Sagedia zonata		Sweden	Nordin 6035	EU057950

**Table 1.** List of sequences and vouchers with GenBank accession numbers; sequences with code numbers are new; vouchers are deposited in UPS unless otherwise indicated.

**Morphological and chemical investigations:** The material was examined with dissection and light microscopes. Sections, mainly hand-made, were studied in water and KOH (tips of paraphyses). IKI was used for the detection of amyloid reactions,  $H_2SO_4$  for calcium oxalate, and HNO<sub>3</sub> for epihymenial pigments. HPTLC was performed according to standard methods (ARUP et al. 1993, ORANGE et al. 2001). Spore measurements of *Circinaria arida* are given as (min.–) M–SD–[M]–M+SD(–max.), rounded to the nearest 0.1 µm, where 'min.' and 'max.' are the extreme values recorded, M the arithmetic mean and SD the corresponding standard deviation. Measurements of other details represent normal range with extreme values in brackets.

**Sampling:** Nuclear ITS1-5.8S-ITS2 rDNA sequences of 18 specimens representing the '*Aspicilia desertorum*' complex and other members of Megasporaceae were used in the molecular study. New sequences were produced from 13

specimens and five sequences were downloaded from GenBank (Tab. 1). In the analysis *A. cinerea*, *Lobothallia recedens* and *Sagedia zonata* were used as outgroup.

**Extractions and PCR amplifications:** Total DNA was extracted from the samples using the Qiagen DNeasy Plant Mini Kit. Lichen sample vouchers of the new sequences are listed in Table 1. PCR amplifications were conducted by using the primers ITS1-F (GARDES & BRUNS 1993) in combination with LR7 (http://www.lutzonilab.net/primers/page244.shtml) or LR1n (TIBELL 2006) to specifically amplify the fungal ITS1-5.8S-ITS2. When no or only weak bands were obtained in the first PCR, the product from this reaction was used for a nested PCR using primers ITS4 and ITS5 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR ran for 35 cycles (1 min at 94 °C, 1 min at 54 °C, 45 sec at 72 °C with a 4 sec/cycle extension at 72 °C) using ABI Taq, Promega Taq or alternatively AccuTaq premix tubes. In the samples MS67, MS74, MS121 the primer pair ITS1F (GARDES & BRUNS 1993) and ITS4 (WHITE et al. 1990) was used for the PCR amplifications, and in the sample MS135 the primer pair ITS1LM (MYLLYS et al. 1999) and ITS2KL (LOHTANDER et al. 1998).

The PCR product was purified using the Qiaquick Spin kit and protocol by Qiagen or Millipore Cleanup Plates. PCR reactions were performed using Ready-To-Go PCR beads in 0.2 tubes (Ge Healthcare). Twenty-five microlitre samples containing 19  $\mu$ l of sterile water, 4  $\mu$ l of DNA dilution, and 1  $\mu$ l of each primer at 10  $\mu$ M concentration were prepared. The following reaction conditions were used: initial denaturation for 5 min at 95 °C, followed by 5 cycles of 30 sec at 95 °C, 30 sec at 58 °C, and 1 min at 72 °C; in the remaining 30 or 35 cycles the annealing temperature was decreased to 56 °C; following the last cycle a final extensions for 7 min at 72 °C was included. For the primer pair ITS1F and ITS4 annealing at 56 °C in the first 5 cycles and 54 °C in the remaining cycles were also successfully used. Alternatively AccuPower® PCR PreMix (Bioneer) was used, adding 3  $\mu$ l diluted or undiluted DNA, 1.5  $\mu$ l of each primer (10  $\mu$ M), and water to a total volume of 20  $\mu$ l with PCR thermal cycling parameters as follows: initial denaturation for 4 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 54 °C, 45 sec. at 72 °C, and final elongation for 5 min at 72 °C.

**Sequencing:** Reactions for sequencing were carried out with the following primers: ITS2, ITS3, ITS4, and ITS5 (WHITE et al. 1990) and ITS1LM, (MYLLYS et al. 1999) ITS2KL (LOHTANDER et al. 1998) by MACROGEN Inc. (www.macro gen.com). Sequence contigs were assembled manually, and then aligned using ClustalW as implemented by the Bioedit software packet (http://www.mbio.nc-su.edu/RNaseP/info/programs/BIOEDIT/bioedit.html) and manually corrected. The alignment is available on request from the first author.

**Phylogenetic analysis:** The data set was processed with the maximum parsimony program PAUP\* 4.0b10 (SWOFFORD 2002). The analysis applied a heuristic search using 1000 random addition sequences, the TBR branch swapping algorithm, collapse branches if maximum branch length is zero, save multiple trees, gaps treated as missing data, characters given equal weight. Bootstrap support values were estimated using 1000 bootstrap replicates, each with 1000 random addition sequence replicates.

#### Results

A total of 119 parsimony-informative characters were used in the analysis, which resulted in a single most parsimonious tree of 319 steps, CI = 0.574, RI = 0.631, HI = 0.426, RC = 0.362. The tree is shown in Fig. 1. Bootstrap support values above 70 % are indicated above the branches.



- 1

**Fig. 1.** Single most parsimonious tree resulting from a maximum parsimony analysis of 18 ITS sequences from members of the family Megasporaceae, mainly of the '*Aspicilia desertorum*'-group. Bootstrap support values are indicated above the nodes. Branches with a values > 70 % are thickened. Names introduced in this paper (*Circinaria arida* and *C. elmorei*) are inserted to the right of the terminals.

The specimens of 'A. desertorum' from Arizona and California form a strongly supported group, distinctly separated from the specimens from other areas and grouping together with Circinaria calcarea, C. contorta, and C. leprosescens. The remaining specimens do not form a monophyletic group, but appear together with

Aspicilia esculenta and Circinaria hispida in the well supported sister group. The specimen from Turkey is more closely related to C. hispida, and one of the Russian specimens to Aspicilia esculenta than to the other 'A. desertorum' specimens. The specimen from Nevada forms a strongly supported group together with Circinaria hispida and the specimen from Turkey.

#### Discussion

The morphological and chemical differences between the specimens referred to *Aspicilia desertorum* in the Sonoran flora and '*A desertorum*' from other areas, together with the result of the present analysis, clearly indicate that they represent separate species. Since no name is available for the Sonoran species, it is described as *Circinaria arida* below.

The fact that the other representatives of 'A. desertorum' are intermixed with the two morphologically clearly distinct *Circinaria hispida* and *Aspicilia esculenta* (not formally combined into *Circinaria* here) is confusing. Judging from this analysis they might all belong to one extremely polymorphic taxon, including both vagrant and fruticose forms, as was suggested by KREMPELHUBER (1867) and KUNKEL (1980), respectively. Another possible explanation is that there are different lineages comprising both crustose and fruticose and/or vagrant taxa, where the different crustose taxa are genetically distinct but morphologically hardly distinguishable, and where the vagrant and fruticose forms are more closely related to different crustose taxa than to morphologically similar taxa. Since this group is currently being revised – a revision based on more than one gene and with both wider and denser sampling, this matter will hopefully be satisfactorily resolved in the near future.

It is obvious that the epithet '*elmorei*' cannot generally replace '*desertorum*'. If the whole group would be regarded as one species, '*esculenta*' would be the oldest epithet, but if the crustose taxa represent different species – as preliminary results from the more extensive investigation indicate, the epithet '*elmorei*' can with a high degree of confidence be applied to the specimen from Nevada, and to North American material from the Great Plains and the mid-west in general (even if it is conspecific with the fruticose form from America, originally described as *Agrestia cyphellata* J.W. Thomson). Hence the formal combination into *Circinaria* is presented below. It can, however, not be ruled out that more than one taxon is involved also in America, as seems to be the case in Europe and Asia.

#### **Taxonomic part**

#### Circinaria arida Owe-Larss., A. Nordin & Tibell sp. nova

Thallus saxicola, crustaceus, areolatus vel verrucosus, plerumque fuscus ad olivaceofuscus, interdum parte olivaceous, cinereofuscus, cinereus vel albocinereus. Apothecia immersa; margo thallinus plusminusve elevatus, vulgo orbe albido vel albocinereo; epihymenium olivaceofuscum vel fuscum, interdum olivaceum, raro viride; hymenium hyalinum, (100-)130-190(-220) µm altum; paraphyses submoniliformes ad moniliformes; asci clavati, typo Aspicilia; ascosporae hyalinae, simplices, globosae vel subglobosae,  $(14-)19.1-[22.8]-26.5(-36) \times (13-)16.7-[19.4]-22.1(-28)$  µm; conidia filiformia, recta vel leviter curvata, (5-)6-11(-13) x

0.8–1(–1.5) μm. Vulgo aspicilinum continens. –Type: U.S.A. Arizona, Yavapai Co., ca. 5 km SE of Campe Verde, 34°26′46′′N, 111°41′27′′W, 1200 m, on SW exposed vertical rock (breccia), III. 2003, *Owe-Larsson* 8770 (UPS–holotype).

#### (Colour Plate 1F)

**Description:** Thallus areolate to vertucose, (0.5-)1-6 cm in diam., (0.1-)0.2-0.7(-1.2) mm thick; areoles angular to rounded or irregular, flat to finally  $\pm$ convex, (0.2-)0.4-1.5(-2.3) mm in diam., contiguous and separated by distinct cracks, or sometimes  $\pm$  dispersed, especially at the thallus edge; prothallus rarely present, then very sparsely developed along the thallus edge, fimbriate or forming a narrow dark zone, black to brown-black or olive-black, 0.1–0.4(–0.7) mm wide; surface usually brown to olive-brown, sometimes partly olive, ochre, gray-brown, gray or gray-white (from pruina), dull to  $\pm$  shiny; upper cortex (15–)20–45(–70)  $\mu$ m thick, uppermost part  $\pm$  brown or rarely olive-brown to green, 5–15(–30)  $\mu$ m thick, with cells (4-)5-7(-9) µm in diam.; cortex covered with an epineeral layer or crystals, (2-)5-22(-40) µm thick; medulla white, I –, algal layer continuous; photobiont chlorococcoid, cells  $\pm$  spherical, 5–20(–28) µm in diam. Apothecia aspicilioid, usually rather common to common, (0.1–)0.2–0.7(–1.4) mm in diam., 1-2(-5) per areole, round to sometimes angular, elongated or irregular; disc black, concave, rarely flat, usually with a thin, white pruina; thalline margin flat to usually  $\pm$  elevated and prominent in older apothecia, usually with a white to gray rim, sometimes concolorous with thallus; exciple  $(15-)20-60(-100) \mu m$  wide, I + blue entirely or only medially, sometimes also I -; uppermost cells brown, ± globose, 5-7(-9) µm in diam.; epihymenium olive-brown to brown, sometimes olive, rarely green, with crystals, N + green to blue-green, K + brown to greenbrown; hymenium hyaline, I + persistent blue, (100-)130-190(-220) μm; paraphyses submoniliform to moniliform, with (1-)2-4(-6) upper cells  $\pm$  globose to sometimes subglobose, rarely subcylindrical, (3-)4-6(-7) µm wide, in lower part (1-)1.5-2(-3) µm wide, slightly branched and anastomosing; subhymenium and hypothecium pale, I + persistently blue, together (20-)30-60(-80) µm thick. Asci clavate,  $(60-)80-130(-165) \times (17-)20-32(-40) \mu m$ , 2-4(-6)-spored. Ascospores hyaline, simple, globose to subglobose,  $(14-)19.1-[22.8]-26.5(-36) \times$  $(13-)16.7-[19.4]-22.1(-28) \mu m$  (n = 335). Pycnidia rare to rather common, 1-2 (-5) per areole, immersed, (80-)120-180(-250) µm in diam., rarely aggregated, with a black, punctiform to rarely elongated ostiole, usually surrounded by a white rim, (40-)50-100(-120) µm in diam.; conidia filiform, straight or slightly curved, (5-)6-11(-13) x 0.8-1(-1.5) µm. Chemistry and spot tests: Cortex and medulla I-, K-, P-, C-. Aspicilin present in 86 % of the specimens (all specimens cited investigated by TLC).

**Notes:** *Circinaria arida* is characterized by a brown to olive-brown or graybrown, rather thin thallus, usually lacking a prothallus, with contiguous to dispersed, finally  $\pm$  convex areoles. Furthermore, the disc of the apothecia usually has a white pruina and the thalline margin a white rim, the asci have 2–4(–6) globose to subglobose spores, the conidia are short, and aspicilin is usually present.

In the Sonoran flora (OWE-LARSSON et al. 2008) *Circinaria arida* was treated as *Aspicilia desertorum* (see above). However, European and Asian specimens referred to that species, as well as American specimens from Idaho, Nevada, and New Mexico (inclusive of the type of *Lecanora elmorei*), differ by having a thick thallus and large apothecia, and by the arrangement of the algal layer, especially in the thalline margin of the apothecia, where the algae are arranged in glomerules interrupted by hyphal tissue. Furthermore they lack aspicilin.

Like *Circinaria arida* two other species occurring in the same area, *C. contorta* and *Aspicilia praecrenata* (not combined into *Circinaria* here) contain aspicilin and have 2–6 globose to subglobose spores per ascus. *Circinaria contorta*, however, differs by its white to grey or green-grey, rather thin thallus with  $\pm$  dispersed, flat areoles, and is also distinctly separated from *C. arida* in the ITS phylogeny presented above (Fig. 1). *Aspicilia praecrenata*, on the other hand, differs by its terricolous habit, the buff to light brown or ochre thallus colour, and the  $\pm$  crenulate thalline margin of the apothecia.

*Circinaria calcarea* has not been found in the Sonoran area, although the name has frequently been used for North American material. *Circinaria calcarea* differs from *C. arida* by its white to grey, contiguous, rimose-areolate thallus, delimited by a prothallus, and the lack of aspicilin

**Ecology and distribution:** On siliceous rocks, boulders, or small stones on the ground, sometimes on volcanic, calciferous or calcareous rock, in deserts, chaparral, scrubland or open areas with scattered trees, at 240–2170 m. So far known the distribution is restricted to a contiguous area in southwestern U.S.A. (Arizona, southern California, New Mexico) and northernmost Mexico (Baja California, Sonora and Chihuahua).

Additional specimens examined: U.S.A. Arizona: Apache Co., W end of Querino Wash, just S of Interstate Hwy 40, 6 miles W of Houck, 35°14'N, 109°16'W, 1900 m, S-facing, Juniperus community, III. 1986, Ryan 19194 (ASU); Coconino Co., Grand Canyon National Park, North Rim, 0.3 km from Imperial Point along the rim trail (site 29), 36°16'N, 111°58'W, ca. 2680 m, on siliceous rock, VIII. 1991, Boykin 2463 (ASU); Grand Canyon National Park (site 23), North Kaibab Trail, east slope across from the bridge to Ribbon Falls, 36°09'10"N, 112°03'W, ca. 1210 m, on siliceous rock, Boykin 2726 (ASU); Grand Canyon National Park, along the North Kaibab Trail 6 km N of Phantom Ranch, 36°08'30"N, 112°04'W, ca. 975 m, on sandstone, XI. 1991, Nash 30674 (ASU); Grand Canyon National Park, along Hermit's Rest Trail into the canyon (South Rim), 5000-6000 feet, rocky ledges with pinyon pine, yucca and sage brush, VI. 1966, Wetmore 14975 (MIN); Grand Canyon National Park, South Rim, on Rowes Well Road near edge of park, 7000 ft, in open area with ponderosa pine, pinyon pine, sage brush and cactus, VI. 1966, Wetmore 15031 (MIN); Gila Co., North of Payson, 34°15'24"N, 111°20'20"W, ca. 1572 m, on rock, IV. 1997, Biringer 114 (ASU); 0.5 km N of Coolidge Dam at San Carlos Lake, desert transition area with Acacia NW hillside, 33°10'50"N, 110°31'50"W, ca. 870 m, on rock, Nash 35908 (ASU); Graham Co., 0.6 miles up dirt road N of Hwy 666, 12 miles E of Safford, 32°48'N, 109°32'W, ca. 1010 m, Larrea-Opuntia community, III. 1986, Ryan 19237 (ASU); Maricopa Co., McDowell Mountains Regional Park (Ft. McDowell Quadrangle, 15 minute series: T. 4N, R. 6E, Section 1), 1700 ft, upper Sonoran Desertscrub, under Larrea divaricata, II. 1974, Lane 360 & Nash (ASU); Coon Bluff area, E of McDowell Mountain, ca. 33°33'N, 111°35'W, dry hillside, on rocks, II. 1972, Lehto s.n. (ASU); Sierra Estrella regional park, 20 miles WSW of Phoenix, 1600 feet, on granite, XI. 1972, Nash 5563 & Weber (ASU); White Tank Mountains, County Regional Park, 1900 ft, on granite, Nash 9827 (ASU); Junction of I-8 and Freeman Rd, 1800 feet, on rock, I. 1975, Nash 9918 (ASU); Vulture Mountains, 7.5 mi. SW of Wickenburg, 33°53'N, 112°47'W, 2900 ft, on rhyolite, II. 1975, Nash 10003 (ASU); Along the Apache Trail (Rte 88) ca. 5 km W of Canyon Lake, upper Sonoran Desert, 33°31'N, 111°27'W, 700 m, on rhyolite, XII. 1988, Nash 25996 (ASU); Tonto National Forest, 5 km SSW of Canyon Lake along route 88 (Apache Trail), 33°30'15"N, 111°27'30"W, ca. 730 m, on rhyolite, X. 1996, Nash 38999 (ASU); South end of the White Hills, 32°47′N, 112°50′30″W, ca. 300 m, upland Sonoran Desert, on volcanic rock, Nash 42742 (ASU); Mohave Co., 2.5 km W of Burro Creek Crossing, Hwy US 93, 34°35'30"N,

113°32'W, ca. 1035 m, IV. 1947, Darrow 4319 (ASU); 38 miles E of Kingman, near Interstate Hwy 40, 35°10'N, 113°25'W, ca. 1500 m, I. 1986, Ryan 16129 (ASU); Pima Co., Tucson Mountains, 32°15'N, 111°05'W, ca. 762 m, along stream bed, on caliche, XI. 1936, Darrow 1310 (ASU); North slope of "A" Mountain, Tucson Mountains, 32°13'N, 111°01'W, ca. 762 m, on basalt, XI. 1938, Darrow 1414 (ASU; UPS); Saguaro Nat. Mon., Tucson Mt. Section, 2 miles south of Panther Peak, half mile SE of Cam Both Picnic Area, 2580 ft, along gulleys in cactus scrub, VI. 1986, Wetmore 55320 (MIN): Saguaro Nat. Mon., Tucson Mt. Section, Apache Peak, 3000 ft, on rocky east slopes and peak with mesquite, cactus and scrub, VI. 1986, Wetmore 55330 (MIN); Saguaro Nat. Mon., Tucson Mt. Section, King Canyon, 3200 ft, along stream near picnic area and on ridges and north facing rocks in mesquite and cactus, VII. 1986, Wetmore 55363 (MIN); Pinal Co., Boyce Thomson Southwestern Arboretum, 33°16'N, 111°10'W, 732 m, on rhyolite, common in shady areas, extensive when surface horizontal, IV. 1973, Huemmer s.n. (ASU); Between Boyce Thompson Arboretum and Picket Post Mountain, 33°16'N, 111°10'W, ca. 735 m, upper Sonoran Desert, XII. 1988, Nash 26340 (ASU); 5 km SW of Superior, just S of Picketpost Mt., near Southwestern Arboretum, 33°24'N, 111°09'W, ca. 840 m, desert mountains, NW-slope, I. 1953, Weber S1912 (ASU); Between Boyce Thompson Arboretum and Picket Post Mountain (just W of Superior), 33°16'N, 111°10'W, 670–800 m, upper Sonoran Desert on N facing hillside with Simmondsia, Cercidium, Lycium, XII. 1988, Wetmore 63342 (MIN); Santa Cruz Co., Coronado National Forest, hills just south of Pena Blanca Lake along state route 289, 3800 ft, on rock, XII. 1972, Nash 6087 (ASU); Yavapai Co., Coconino National Forest, 0.5 km N of McGuireville, 34°38'30"N, 111°49'W, 1070 m, Canotia and other desert vegetation, on limestone, II. 1994, Kantrud 29 (ASU); Coconino National Forest, adjacent to Clear Creek campground, 34°31'N, 111°46°W, ca. 1000 m, gypsum region, on volcanic rock, II. 1993, Nash 34136 (ASU); Ca. 5 km SE of Campe Verde, 34°28'08"N, 111°43'13"W, 1200 m, on small stone on the ground, III. 2003, Owe-Larsson 8759 (UPS); Ca. 5 km SE of Campe Verde, 34°26'46"N, 111°41'27"W, 1200 m, on SW exposed vertical rock (breccia), III. 2003, Owe-Larsson 8774 (UPS); Along Arizona highway 279 southeast of town of Cottonwood, 3500 ft, in rocky barrens with few scrub trees, VI. 1966, Wetmore 15043 (MIN); Yuma Co., Dirt road to Horse Tanks, Kofa Wildlife Refuge, E of Hwy 95, 33°15'N, 114°14'W, ca. 610 m, Sonoran Desert, I. 1986, Rvan 16046 (ASU); California: Los Angeles Co., Santa Catalina Isl., 0.8 km NW of Catalina airfield at junction of road to Avalon and a secondary road to the coast, 33°24'15"N, 118°24'45"W, 440 m, on steep north facing hillside and ridgetop with oaks, XII. 1993, Wetmore 73284 (MIN); Riverside Co., San Jacinto Mountains, between Cahuilla Vista and Pinyon Flats, 33°35.130'N, 116°25.843'W, 1200 m, granite boulders in drainage in juniper-scrub, XII. 2004, Knudsen 2046 & P. Griffith (UCR); Pinyon Flats, Plateau below St. Rosa Mountain, 33°35.027'N, 116°26.214'W, 1235 m, open Pinyon-Juniper habitat, on N side of exposed rocky outcrop, X. 2004, Owe-Larsson 9168, 9170 & 9171 & Knudsen (UPS); Plateau below St. Rosa Mountain, Carizzo Road 1061, 33°36.617'N, 116°25.207'W, 1061 m, desert, on exposed boulder, X. 2004, Owe-Larsson 9172 & Knudsen (UPS); San Bernardino National Forest, San Jacinto Mountains, around Pinyon Flats Campground above Palm Desert, 4300 ft, on ridgetops with juniper, oak, agave and cactus, probably burned, VI. 1966, Wetmore 14558 (MIN); San Bernardino Co., Granite Mountains, Beryl Allinson Trail, Granite Cove, Mohave Natural Preserve, Sweeney Granite Mountains Desert Research Center (University of California), 1.3 mi. NW of Kelbaker Rd., 4.1 mi. NE of U.S. 40, 75 mi. E of Barstow, 34°47'10"N, 115°38'45"W, 4000 ft, granite boulders and scattered shrubs, X. 1998, Tucker 36221 & Tucker (SBBG); San Luis Obispo Co., Los Padres National Forest, Panza Range (or north end of Sierra Madre Range), about 1 mi. N on unmarked Forest Service Rd at ranch entrance on State Hwy 166, 25 mi. E of junction of 188 and U.S. Hwy 101, Big Rocks, narrow wooded canyon with conglomerate boulders, cliffs, and sloping rock slabs, scattered oaks (Quercus agrifolia), and chaparral, mostly Chamise (Adenostoma), on boulders, X. 1995, Tucker 34142 & Bratt (SBBG); Santa Barbara Co., Bluff Gate, Buckhorn Road, 4800 ft, sandstone, II. 1980, Bratt 234 (SBBG); Santa Cruz Island, W of Sauces Gate, Ridge Road, on rock, IX. 1997, Bratt 10109 (SBBG); Santa Cruz Island, 4.5 km east of navy radar station at east pinery, 34°00'15"N, 119°37'30"W, 240-335 m, in oak stand on north facing hillside and ridge in grassland, I. 1994, Wetmore 74076 (MIN); Santa Rosa Island, north slope of Black Mt., 33°58'45"N, 120°04'30"W, 300-320 m, on north facing slope in grove of oaks (Quercus tomentella), I. 1994, Wetmore 73599 (ASU); Kern Co., Los Padres National Forest near town of Frazier Park, 4500 ft, Pinyon pine and oak, VI. 1966, Wetmore 14772 (MIN); Tulare Co., Sequoia National Park, Kern Canyon, Kern Hot Springs, south bank of Rock Creek at canyon wall, N and

NW facing cliff face near bank of creek, willow, cottonwood, cedar, pine and fir, 10 VIII. 1985, *Blakeman 537* (MIN); Ventura Co., Approx. 4 mi. south of Lockwood Valley Road, Mutah Flat Road, open area in Pine/chaparral area, on pebbles, VI. 1986, *Bratt 5083* (SBBG); **New Mexico**: Dona Ana Co., Organ Mts., 15 miles east of Las Cruces near San Agustin Pass along US 70, 6000 ft, on west side of pass in pinyon pines, juniper and oak, VI. 1966, *Wetmore 14434* (MIN); McKinley Co., Cibola National Forest at Quaking Aspen Campground SE of Gallup, 7300 ft, in open area with Ponderosa pines, VII. 1966, *Wetmore 15137* (MIN); San Miguel Co., Pagosa Canyon just NE of McAllister Lake (next canyon E of Fr. Arsene's 'Canon Sud'), Las Vegas Quadr. 1963, 39°32'N, 105°08'W, 1950–2000 m, NE-facing rimrock, IX. 1973, *Weber, Kunkel & LaFarge s.n.* (MIN).

MEXICO. **Baja California**: 23 km E of El Rosario along Rte 1, Northern Vizcaino region of the Sonoran Desert, 30°02'N, 115°31'W, 200 m, on steep hillside with *Ambrosia, Idria columnaris, Opuntia molesta, Pachycereus pringlei*, I. 1989, *Wetmore 63750* (MIN); **Chihuahua**: Microndas de Santa Cruz, ca. 84 mi. S of Chihuahua City along Rte. 45, 27°41'N, 105°10'W, 4350 feet, *Larrea*-ocotillo-cacti habitat, on volcanic rock, VI. 1976, *Nash 13837* (ASU); **Sonora**: 17 km east of Esqueda, 30°39'30'N, 109°27'30''W, 1090 m, on north facing rocks along desert wash with scattered mesquite, III. 1992, *Wetmore 69901* (MIN).

### *Circinaria elmorei* (E.D. Rudolph) Owe-Larss., A. Nordin & M. Sohrabi comb. nova

Basionym: *Lecanora elmorei* E.D.Rudolph, Ann. Missouri Bot. Gard. 40: 65 (1953) – Type: U.S.A. New Mexico, Chaco Canyon, on sandstone, 6200 ft, I. 1952, *T. & F Elmore s.n.* (US).

(Colour Plate 1G)

**Description:** Thallus  $\pm$  vertucose, 2.5 cm in diam., (0.3–)0.5–1.3(–2) mm thick; areoles rounded or irregular,  $\pm$  convex, (0.2-)1.0-2.5(-3.5) mm in diam., contiguous and separated by distinct, deep cracks; prothallus not present; surface brown to olive-brown, dull; upper cortex 30–50  $\mu$ m thick, uppermost part ± brown, 10-15(-20) µm thick, with cells 5-8(-10) µm in diam.; cortex covered with an epinecral layer or crystals, 10–20 µm thick; medulla white, I-, algal layer arranged in glomerules interrupted by hyphal tissue, especially in the thalline margin; photobiont chlorococcoid, cells  $\pm$  round, 7–15(–18) µm in diam. Apothecia aspicilioid, common, (0.2-)0.4-1.4(-1.8) mm in diam., 1(-3) per areole, round to elongated or irregular; disc black, concave, usually with a thin white pruina; thalline margin  $\pm$  elevated and prominent in older apothecia, concolorous with thallus, rarely with a white to gray thin rim; exciple  $30-50 \mu m$  wide, I + blue or partly I + blue medially, uppermost cells brown,  $\pm$  globose, 5–6(–7) µm in diam.; epihymenium olive-brown to brown, with crystals, N + green, K + brown; hymenium hyaline, I + persistent blue, 170 µm; paraphyses submoniliform to moniliform, with 2–4 upper cells  $\pm$  globose, 4–5(–5.5) µm wide, in lower part 1.5-2 µm wide, slightly branched and anastomosing; subhymenium and hypothecium pale, I + persistently blue, together 40–50  $\mu$ m thick; asci clavate, 95–110  $\times$  24–32 µm, 2–4-spored; ascospores hyaline, simple, globose to subglobose, 24–  $28 \times 18-28 \ \mu m \ (n = 7)$ . Pycnidia rare, 1(-5) per areole, immersed, 90–180  $\mu m$  in diam., with a black, punctiform ostiole, usually surrounded by a white rim, 60-90  $\mu$ m in diam.; conidia filiform, straight, (6–)7–10(–12) x 0.8–1  $\mu$ m. Chemistry and spot tests: cortex and medulla I-, K-, P-, C-. No substances detected by TLC.

**Ecology and distribution:** On sandstone at the type locality and rhyolite pebbles in Nevada, in both cases in canyons; otherwise, both ecology and distribution poorly known.

Additional specimen examined: U.S.A. Nevada: Pershing Co., South of Winnemucca, Humboldt mountains, Johnson canyon, T31N R33E, Sec. 12 NE1/4 30, 5200 ft, on pebbles of rhyolite, *Artemisia arbuscula, Poa* habitat, VII. 1985, *Rosentreter 3689* (TU).

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WHITE, T.J., BRUNS, T.D., LEE, S.B. & TAYLOR, J.W. (1990): Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. – In: INNIS, N., GELFAND, D., SNINSKY, J. & WHITE, T. (eds.), PCR-Protocols and Applications: A Laboratory Manual, 315–322. – Academic Press, New York. **Colour Plate 1. A–C**, Dark-fruited *Caloplaca* species from Cape Verde (scalebars = 1 mm). **A**, *C. caesioisidiata*, holotype with isidiate thallus and pruinose apothecia. **B**, *C. caesiosorediata*, holotype with scattered soralia and apothecia. **C**, *C. catillarioides*, holotype with areolate thallus and numerous apothecia. **D–E**, Habitus of *Buellia tomnashiana*. **D**, showing the thallus and apothecia on and alongside the leaf vein (arrow; scalebar = 2 mm). **E**, showing the minute granular thallus and apothecia (scalebars = 0.5 mm). **F–G**, *Circinaria* species of the '*Aspicilia desertorum*' complex (scalebars = 3 mm). **F**, habitus of *Circinaria arida*, holotype. **G**, habitus of *Circinaria elmorei*, holotype. **H–I**, A new *Cliostomum* species from south-west Africa (scalebars = 2 mm). **H**, thalli of *Cliostomum namibicum* with flesh coloured apothecia and pycnidia, associated with *Combea mollusca*. **I**, *Chrysothrix granulosa*, a species commonly associated with *Cliostomum namibicum*.

