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Source: The Bryologist, 114(1):178-189. 2011.
Published By: The American Bryological and Lichenological Society, Inc.
DOI: 10.1639/0007-2745-114.1.178
URL: http://www.bioone.org/doi/full/10.1639/0007-2745-114.1.178

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Aspicilia rogeri sp. nov. (Megasporaceae) and other allied vagrant species in North America

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Abstract. A short revision of the vagrant *Aspicilia* species of North America is presented based on morphological, molecular and ecological data. Vagrant *Aspicilia* are common lichens throughout the steppes of the western United States and in southwestern parts of Canada. Species delimitation of these lichens is difficult because of the paucity of morphological characters and large degree of variation. Inferences from nuITSrDNA sequences reveals that the North American specimens of *A. fruticulosa* are not most closely related to their Eurasian populations but instead share a unique ancestor with *A. hispida.* The specimens of *A. fruticulosa* from the New World are hereby recognized as a distinct species, *A. rogeri.* Its differentiation from the similar *A. fruticulosa* and *A. hispida* is discussed. The exclusion of *A. fruticulosa* from the N. American checklist is proposed temporarily.

Keywords. *Aspicilia*, manna lichens, new species, North America, vagrant lichens.

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The North American checklist of lichen–forming fungi (Esslinger 2009) includes *Aspicilia fruticulosa* (Eversm.) Flagey. This species was first reported by Rosentreter (1993) as the only truly vagrant *Aspicilia* occurring in North America. It was compared with the similar subfruticose *A. hispida* Mereschk., which according to Rosentreter is basically attached to the substrate, at least during early stages of development, and thus only secondarily vagrant or 'erratic'. It is, however, also usually treated as one of the vagrant representatives of *Aspicilia* (see Rosentreter 1993, 1997). The great majority of the *Aspicilia* species in the checklist (and generally), including the conserved generic type *A. cinerea* (L.) Körb., occurs as firmly attached crusts on rocks, while a few are attached to soil or wood.

Vagrant *Aspicilia* species are mainly known from arid regions in Eurasia and North Africa. They are often collectively referred to as 'manna lichens' (cf. the biblical Book of Exodus 16; see also Donkin 1980, 1981). Some of the vagrant species were included in identification keys by Szatala (1957) and Poelt

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DOI: 10.1639/0007-2745-114.1.178

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(1969). The most comprehensive treatment of the vagrant *Aspicilia* to date was published by Oxner (1971), with some additional information by Andreeva (1987). Nomenclatural problems involved in this group were recently discussed by Sohrabi & Ahti (2010), who also summarized the history of the group and listed the most important publications. The vagrant *Aspicilia* are morphologically diverse and include lump–shaped, nodulose, subfruticose and foliose taxa. A large number of unresolved taxonomic problems remain, however, in this group, as in *Aspicilia* in general, and the genus is currently under revision.

Aspicilia fruticulosa was originally described from Mugodzhar Hills based on material from northwestern Kazakhstan (Eversmann 1831). This species seems to be widely distributed in Eurasian steppes, from where it has been reported by Mereschkowsky (1911) and Kulakov (2002, 2003) and recently collected by the authors (from Astrakhan, Russia by Owe–Larsson and from East Azarbaijan, Iran by Sohrabi). The species has also been reported from Turkey (Aras et al. 2007), China (Abbas 1996), Greece (Hafellner et al. 2004), Spain (Llimona & Hladun 2001) and Ukraine (Mereschkowsky 1911). When compared with specimens from Kazakhstan, Russia and Ukraine, the American specimens referred to as *A. fruticulosa* were found to differ morphologically. This discovery triggered the closer review of the taxa presented here.

Aspicilia hispida exhibits great morphological variation, but whether this variation is patterned along geographic distribution is undertain. Since *A. hispida* has some similarities with *A. fruticulosa*, a fact also pointed out by Rosentreter (1993), it was also included in this study. In America, *A. hispida* was first known as *Agrestia cyphellata* J. W. Thomson (Thomson 1960), and was later reduced to synonymy under *Agrestia hispida* (Hale & Culberson 1970) and subsequently transferred to *A. hispida* in Rosentreter (1993), a name introduced by Mereschkowsky (1911) for populations from Astrakhan, Russia.

Phylogenetic inferences from nuITS rDNA suggest that *Aspicilia hispida* and *A. fruticuolosa* are sister taxa shared a unique common ancestor with *A. calcarea* (Aras et al. 2007). The sequences produced by these authors (i.e., DQ401556–DQ401563, DQ401567–DQ401568, DQ401570–DQ401571), however, are not compatible with the ITS sequences of other *Aspicilia* studies, such as those of Ivanova & Hafellner (2002) and Nordin et al. (2007), but these do not include vagrant species. In the latter a number of distinct subgroups were identified that were also found in the analyses based on mtSSU and nuLSU (Nordin et al. 2010). Thus ITS sequences seem to be useful and informative for phylogenetic studies of *Aspicilia*.

The aim of the present study is to evaluate the morphological differences between Eurasian and American specimens of *Aspicillia fruticulosa* and to explore the relationship between these and *A. hispida* using ITS sequences and analyzed together with a sequence from *A. vagans* Oxner, another vagrant species, and sequences used by Ivanova & Hafellner (2002) and/or Nordin et al. (2007).

Material and Methods

Material of *Aspicilia fruticulosa* and *A. hispida* from B, CANL, FH, GBFS, GZU, H, IRAN LE, S, SRP (material mainly collected by R. Rosentreter), VDLG, UPS, US and the private herbarium of M. Sohrabi (hb. M. Sohrabi) was studied.

External morphology was studied under a dissecting microscope and the anatomy of the thallus, conidia and apothecia were observed using a Leica Dialux 20 compact light microscope. Photographs were taken with a digital camera on a Leica DM 2500 compact light microscope. Sections, 16–20 µm thick, were cut using a freezing microtome. The microscopic preparations were mounted in lactophenol cotton–blue or water. All microscopical measurements were made in water mounts. Chemical analyses of selected specimens were carried out using thin layer chromatography (TLC) according to Orange et al. (2001), and high performance liquid chromatography (HPLC) using methods standardized for lichen products (Søchting 1997).

DNA extraction, PCR–amplification, sequencing and alignments. From the specimens selected for the molecular work (see **Appendix 1**), DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN), following the manufacturer's protocol

except that the thallus fragments were ground with mini–pestles in 40 µl of the ATL lysis buffer included in the kit instead of using liquid nitrogen. The instructions of the manufacturer was followed, but the quantities of the ATL and AL buffers, ethanol and proteinase K were reduced to 160 µl, 180 µl, 180 µl, and 10 µl, respectively. To elute the extracted 60–120 µl DNA the AE elution buffer included in the kit was used. DNA of *Aspicilia rogeri sp. nov.* (Rosentreter 16333, 16373) was obtained with direct PCR following Arup (2006).

To amplify the ITS1–5.8S–ITS2 region, the primers ITS1–F (Gardes & Bruns 1993) combined with ITS4 (White et al. 1990), or ITS1–LM (Myllys et al. 1999) combined with ITS2–KL (Lohtander et al. 1998) were used. Ready–To–Go PCR beads in 0.2 ml tubes (GE Healthcare) were used for the PCR. 19 µl of sterile water, 4 µl of DNA extraction, and 1 µl of each primer at 10 µM concentration were added to the tubes to make up the reaction volume 25 µl. The following PCR settings were used: 5 minutes at 95°C, then 5 cycles of 30 seconds at 95°C, 30 seconds at 58°C, and 1 minute at 72°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 56°C, and 1 minute at 72°C, and finally 7 minutes at 72°C. For the primer pair, ITS1–F and ITS4 annealing at 55°C in the first 5 cycles and 53°C in the remaining 30 cycles were also used. The PCR products were purified with the GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) following the protocol enclosed with the kit. To elute the purified PCR products 20–30 µl of the elution buffer 3B included in the kit was used. The DNA concentrations of the purified PCR products were measured with a NanoDrop™ 1000 Spectrophotometer (Thermo Scientific). The PCR products were sent to the Macrogen Inc. (http://www.macrogen.com) for sequencing. Macrogen provides sequencing facilities using ABI 3730xl DNA analyzers (Applied Biosystems) and online results delivery. The primers used for sequencing were the same as in the PCR reactions. The obtained sequences were assembled and edited in SeqMan II version 4.0 (DNASTAR).

Phylogenetic analyses. *Siphula complanata* (Hook.f. & Taylor) R.Sant., was selected as an outgroup based on Miadlikowska et al. (2006). Non–coding regions such as ITS often show length variation that is problematic in homology assumptions when alignments are compiled manually or by using programs such as Clustal X (Jeanmougin et al. 1998), complemented with manual adjustments. Manual alignments are not repeatable and no objective basis to choose one alignment over the numerous possible hypotheses of homology can be defined (Giribet et al. 2002). Commonly, ambiguous regions are removed from the alignment. However, potentially valuable data is then lost and the exact delimitation of unalignable regions is arbitrary.

In order to avoid problems with the homology assumptions we used direct optimization (optimization alignment; Wheeler 1996) as an alternative approach. The analyses were performed using algorithms implemented in the program POY (Varón et al. 2008). The analyses of the original sequences were performed using version 4.1.2 of the program running on an 18 node beowulf cluster at the Finnish Museum of Natural History. Direct optimization is computationally very demanding and in order to alleviate this the ITS sequences were cut into three pieces before the analysis. This was performed within invariable regions to ensure that potential homologies between nucleotides were not a priori prevented. The analysis included an initial build of 100 Wagner trees, transformation of sequences using automatic sequence partition and static approximation with all transformation weighted equally. After this 3,000 Wagner trees were built with a local search of branch–swapping of all trees in memory using SPR and TBR algorithms with the threshold of two that sets the percentage cost for suboptimal trees more exhaustively evaluated (by an extra round of swapping) during the swap. This basic search was followed by 30 rounds of ratchet (Nixon 1999) with random upweighting of 20 percent characters by a factor of three followed by 300 iterations of tree–fusing (Goloboff 1999). Between different searches all unique optimal trees were retained. After this, the obtained implied alignments were transformed to a matrix suitable for calculation of the jackknife support values (Farris et al. 1996) using the program TNT (Goloboff et al. 2008) with 10,000 replicates.

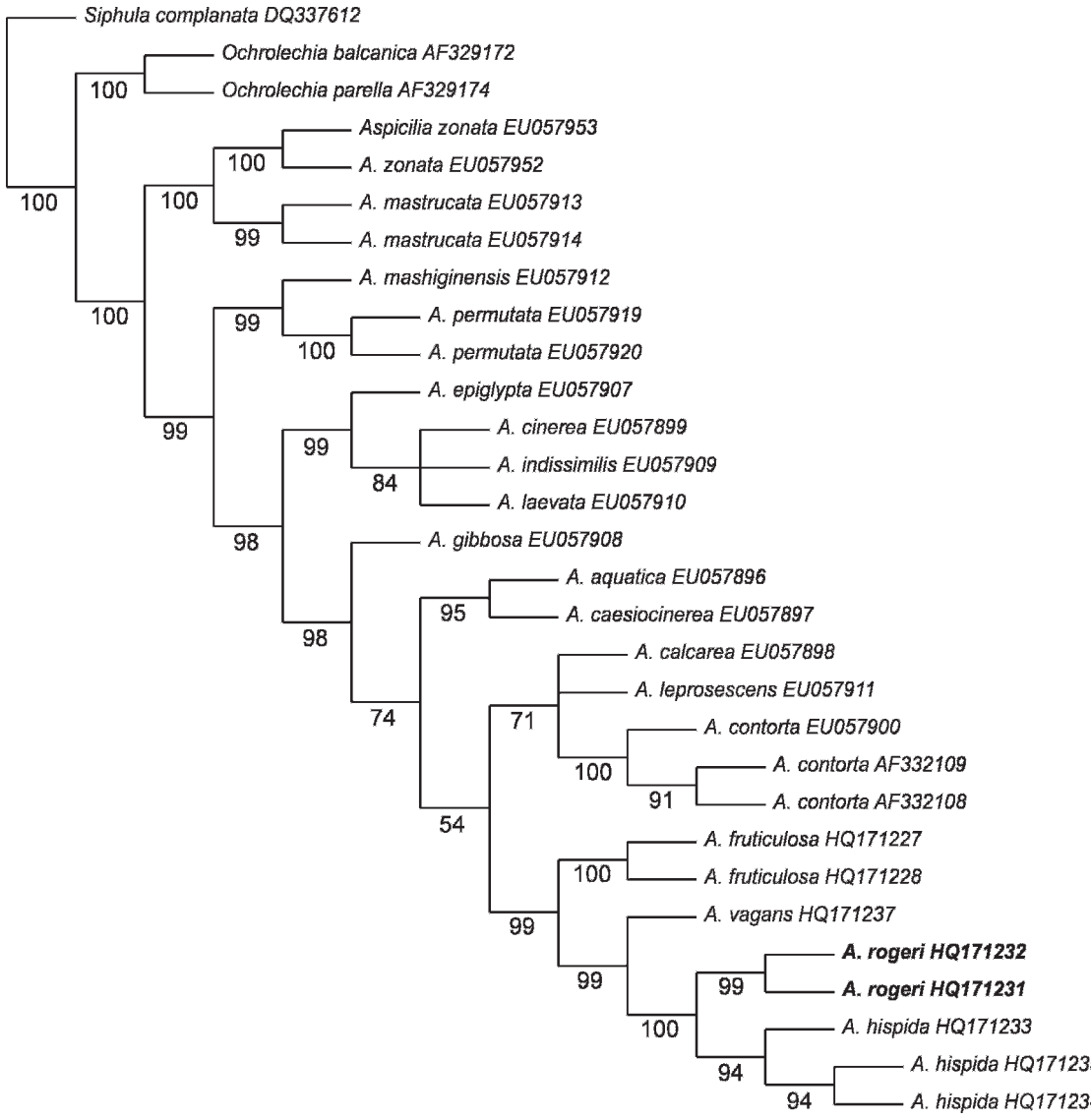

Figure 1. Majority rule (50%) consensus tree showing the jackknife values. The tree is identical with the single parsimonious tree obtained with direct optimization except for the nodes leading to *Aspicilia indissimilis* and *A. laevata*, and a node leading to *A. calcarea* and three terminals of *A. contorta*. These nodes had jackknife support values $<$ 50%, and thus they are shown as collapsed.

Results and Discussion

Direct optimization resulted in one parsimonious optimization with a tree length of 825 steps (**Fig. 1**). The American representatives of *Aspicilia fruticulosa* compose a robust sister-group to *A. hispida* and are not most closely related to the Eurasian specimens of *A. fruticulosa* or to *A. vagans*, the other vagrant species included in the analysis. The American representatives *of A. fruticulosa* differ morphologically from *A. fruticulosa* and *A. hispida* (**Table 1**) and are hence here recognized as a distinct species, *A. rogeri*. Togther with *A. vagans* these species compose a monophyletic group, whose affinities remain ambiguous.

The name *Aspicilia fruticulosa* is included in American checklist of lichen–forming fungi (Esslinger 2009) and based on this study and an extensive number of examined specimens, it becomes

Table 1. Comparison of *Aspicilia rogeri* with similar *Aspicilia* species: *A. fruticulosa* and *A. hispida*.

Character /Species	*A. fruticulosa*	*A. rogeri*	*A. hispida*
Thallus shape	lump–shaped	lump–shaped	tufted, cladonoid
Substrate	vagrant	vagrant	attached to soil secondarily vagrant
Branching pattern	predominantly dichotomous	predominantly irregular	predominantly irregular
Branch tips	not tapering, concave, not blackened	tapering, blunt, whitish with black centre (pycnidia, erupted or not)	tapering, pointed blackened
Pycnidia	not found	common, conspicuous mainly at branch tips	rare, inconspicuous along branches
Pseudocyphellae	mainly apical obscure	mainly apical conspicuous	along branches conspicuous

clear that the Eurasian *A. fruticulosa* has not been collected from North America. Therefore, the exclusion of *A. fruticulosa* from the N. American checklist is proposed temporarily.

The species

Aspicilia rogeri Sohrabi, *sp. nov.* **Fig. 2**

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Thallus liber, subfruticosus, flavovirens, olivaceus, olivaceofuscus vel cinereus. Apothecia primo immersa, dein adnata ad substipitata; margo thallinus plusminusve elevatus, vulgo orbe albido vel albocinereo; epihymenium olivaceofuscum vel fuscum, interdum olivaceum, raro viride; hymenium hyalinum, 100–140 µm altum; paraphyses submoniliformes ad moniliformes; asci clavati, typo Aspicilia; ascosporae hyalinae, simplices, globosae vel subglobosae, 19–34 × 17–30 µm. Conidia filiformia, recta vel leviter curvata, 7–16 × 0.8–1.5 µm. Materiae chemicae secundariae absentes.

Type: U.S.A. oregon: Wallowa Co., The Nature Conservancy's Zumwalt Prairie Nature Preserve, NE of Summer Camp, Barren rocky ephemeral seepage area surrounded by Oregon Palouse Prairie, 1380 m (4528 ft.), 45.578° N, 116.983° W, 7 August 2007, *Rosentreter 16333* (holotype: SRP (fertile specimen!), isotypes: H (fertile specimen!) & IRAN).

Illustrations. Brodo et al. (2001: 169, color plate; as *A. hispida*, and after fourth print as *A. fruticulosa*), McCune & Rosentreter (2007: 53, colour plate; as *A. fruticulosa*), and Rosentreter et al. (2007: 45, color plate: as *A. fruticulosa*). Additional color photos are presented at the Myco-Lich website (www.myco-lich.com) edited by Sohrabi et al. (2010a).

Description. Thallus free, subfruticose, dichotomously to irregularly branched, forming shrubby, more or less spherical to elongated or rarely flattened lumps, 0.5–2.0 × 0.5–1.5 (–2.5) cm (**Fig. 2A**). Branches compact, cylindrical, short to relatively elongated, at base often slightly flattened 1.5–4.0(–6.0) mm wide; tips blunt, pale, with central black spots (probably erupted pycnidia) (**Fig. 2B**). Surface yellowish green, olive–green to darkish green or olive–brown, sometimes greyish green, dull (paler in parts not exposed to light). Pseudocyphellae pale (± white), usually on the apical parts of the branches. Cortex two layered (**Fig. 2D**), outer part (10–)20–25(–30) µm thick, paraplectenchymatous, ± brown, c. 2–3 cells thick, cells (4–)5–7(–8) µm in diam.; inner part prosoplectenchymatous (30–)40–80(–90) c. 2–4 times as thick as the outer layer; cortex covered with a thin epinecral layer 1–5(–12) µm thick. Photobiont chlorococcoid, cells ± round, 5–15 µm in diam., clustered in small groups, each group up to 60–120 × 40–90 µm, Apothecia (**Fig. 2C**) aspicilioid when young, later becoming adnate to stipitate, rare, up to 1.5(–2.5) mm in diam., usually occurring in broader parts of the main branches; disc black to brown–black, commonly pruinose, concave to slightly convex, occasionally subdivided

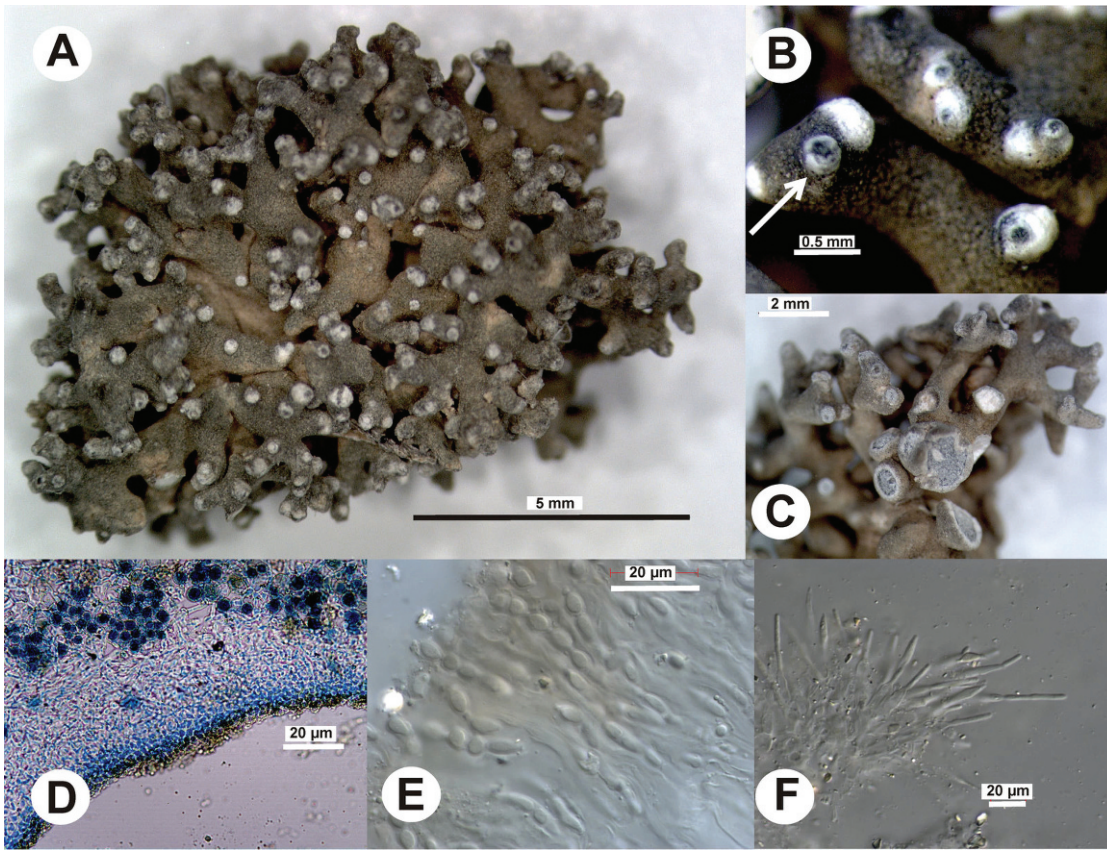

Figure 2. *Aspicilia rogeri* (*Rosentreter 4874*). **A**. Free (vagrant) subfruticose thallus. **B**. Pycnidia with black ostioles surrounded by white rims. **C–F.** Fertile specimen (holotype) **C.** Apothecium. **D**. Two–layered cortex, outer part paraplectenchymatous, inner part prosoplectenchymatous. **E**. Paraphyses: moniliform to submoniliform, with ± globose upper cells. **F.** Conidia on elongated conidiogenous cells.

by white strands (soluble particles in K and N); thalline margin flat to terete ± elevated and prominent in older apothecia, entire, concolorous with thallus or with a thin to thick white rim; proper exciple (15–)35–75(–105) µm wide, medially usually I+ blue, uppermost cells brown, ± globose, 4–5(–7) µm in diam; epihymenium brown to light yellowish green, K+ brown, N+ pale green; hymenium hyaline, occasionally with few oil droplets, (100–)110–125(–140) µm tall; paraphyses (**Fig. 2E**) moniliform to submoniliform, with upper cells ± globose, 4–5 µm wide, in lower part 2–3 µm wide, branched; hypothecium pale, 45–65(–75) µm thick. Asci broadly clavate, 90–110(–120) × 28–32 µm, *Aspicilia*–type, 2–4(–5) spored; ascospores hyaline, simple, globose to subglobose, (19–)21.2–28.2(–34) × (17–)18.8–23.2(–30) µm (n=30). Pycnidia usually on top of branchelets (**Fig. 2B**), rarely on other parts of the thallus, immersed, single, flask–shaped to very slightly folded, cavity enclosed by more or less elongate hyphae, internal wall colorless, frequently with black to brownish ostiole, often surrounded by a white rim; conidia filiform, straight to slightly curved (7–) 9.6–13.8 (–16) × (0.8–)1–1.3(–1.5) µm, (n=87) (**Fig. 2F**).

Chemistry. Cortex and medulla K–, C–, KC–, P–, I–. No substances detected by TLC or HPLC. Rosentreter et al. (2007) reported that chemistry of the medulla is K+ red in some North American *A. fruticulosa*. However, we did not find any samples with a positive K reaction.

Etymology. The new taxon is named in honour of the American ecologist Roger Rosentreter, who has

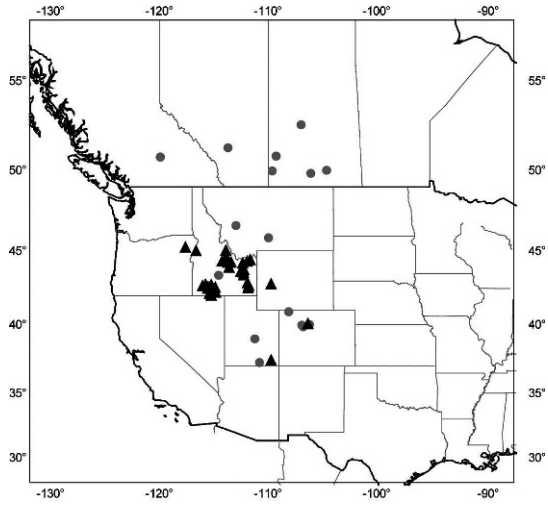

Figure 3. Distribution map of *Aspicilia rogeri* (triangles) and *A. hispida* (circles) in North America.

made a significant contribution to the knowledge of soil crust lichens, as well as has kindly provided invaluable specimens for us.

Ecology and habitats. *Aspicilia rogeri* is a rare and locally common species, frequently found at elevations of 1000–2000 m. It is obligatory vagrant on calcareous soils in shrub steppe and prefers open habitats that are ephemerally moist in winter or spring but dry most of the year. So far, the species is known from the calcareous badlands in western North America in black sagebrush, *Artemisia nova* or other *Artemisia* habitats. Other plant species in these habitats include *Artemisia arbuscula*, *A. frigida*, *A. longiloba*, *A. tridentata* subsp. *wyomingensis*, *Agropyron spicatum*, *Achnatherum hymenoides*, *Atriplex confertifolia*, *A. nuttallii*, *Elymus* spp., *Eriogonum caespitosa*, *Haplopappus acaulis*, *Phlox hoodii*, *Petrophytum caespitosum*, *Poa secunda*, *Stipa spp.* and *Tanacetum nuttallii*. Associated lichen species include *Aspicilia hispida* and other terricolous species as reported in McCune & Rosentreter (2007).

Distribution. *Aspicilia rogeri* is so far only known from western North America (Colorado, Idaho, Oregon, Utah and Wyoming; **Fig. 3**). An online distribution map of *A. rogeri* based on this study, is presented at the Myco-Lich website (www.myco-lich.com) edited by Sohrabi et al. (2010a).

Discussion. *Aspicilia rogeri* is a distinct vagrant species, separated from related species by ITS sequence data as well as morphological and anatomical characters. In the field it is easily mistaken for *A. fruticulosa*. However, *A. rogeri* has tapering branches, with a black tissue or pycnidia surrounded by a pale zone at the tip. In *A. fruticulosa* the branch tips are more or less concave and occasionally depressed, and lacks pycnidia. The two species also differ in the branching pattern: the branches in *A. fruticulosa* are more uniform and dichotomous, whereas in *A. rogeri* the branches often irregular and only rarely dichotomous. Moreover, the thallus of *A. rogeri* is looser and more fragile. So far pycnidia and conidia have not been observed in *A. fruticulosa*.

According to our phylogenetic analyses *Aspicilia hispida* is closely related to *A. rogeri*. It is commonly found in the same habitats as *A. rogeri* in western North America. *Aspicilia hispida* is attached to the soil, and cannot thus be described as truly vagrant. It is well characterized by its branches, which are longer and cylindrical, and more or less "*Cladonia*–like." *Aspicilia hispida* is also differentiated by the sharp, pointed black apices of the small branches, and rounded to elongated white–spotted pseudocyphellae on the branches.

Additional specimens examined. U.S.A: IDAHO: Custer Co., 20 miles N of Howe, 1889 m, 27 May 1988, *Rosentreter 4874* (SRP); COLORADO: Grand Co., 6 km NW of Kremmling on Hwy 40, 2300 m, 17 June 1995, *Rosentreter 9334* (SRP); Lake Co., Drake Flats, about 2.5 air miles E of Plush, 1554 m, *K. Yanski 467* (SRP); UTAH: San Juan Co., E side of Summit Road, 2 miles N of US Hwy 160, just SE of old drill pool, 2103 m, 27 September 1985, *Anderson 15971* (CANL); WYOMING: Sublette Co., NW of Pig Piney, ca. 1170 m, 14 September 2007, *Levy–Boyd* & *Rosentreter 16373* (SRP).

Aspicilia hispida Mereschk., Trudy Obshch. Estestvoisp. Imp. Kazansk. Univ. 43 (5): 10, 35. 1911.

Illustrations. Color photos from Eurasian and American representatives of *A. hispida* based on this study are presented at the Myco-Lich website (www.myco-lich.com) edited by Sohrabi et al. (2010a).

Description. Thallus subfruticose erect, usually basally attached or imbedded in soil, occasionally

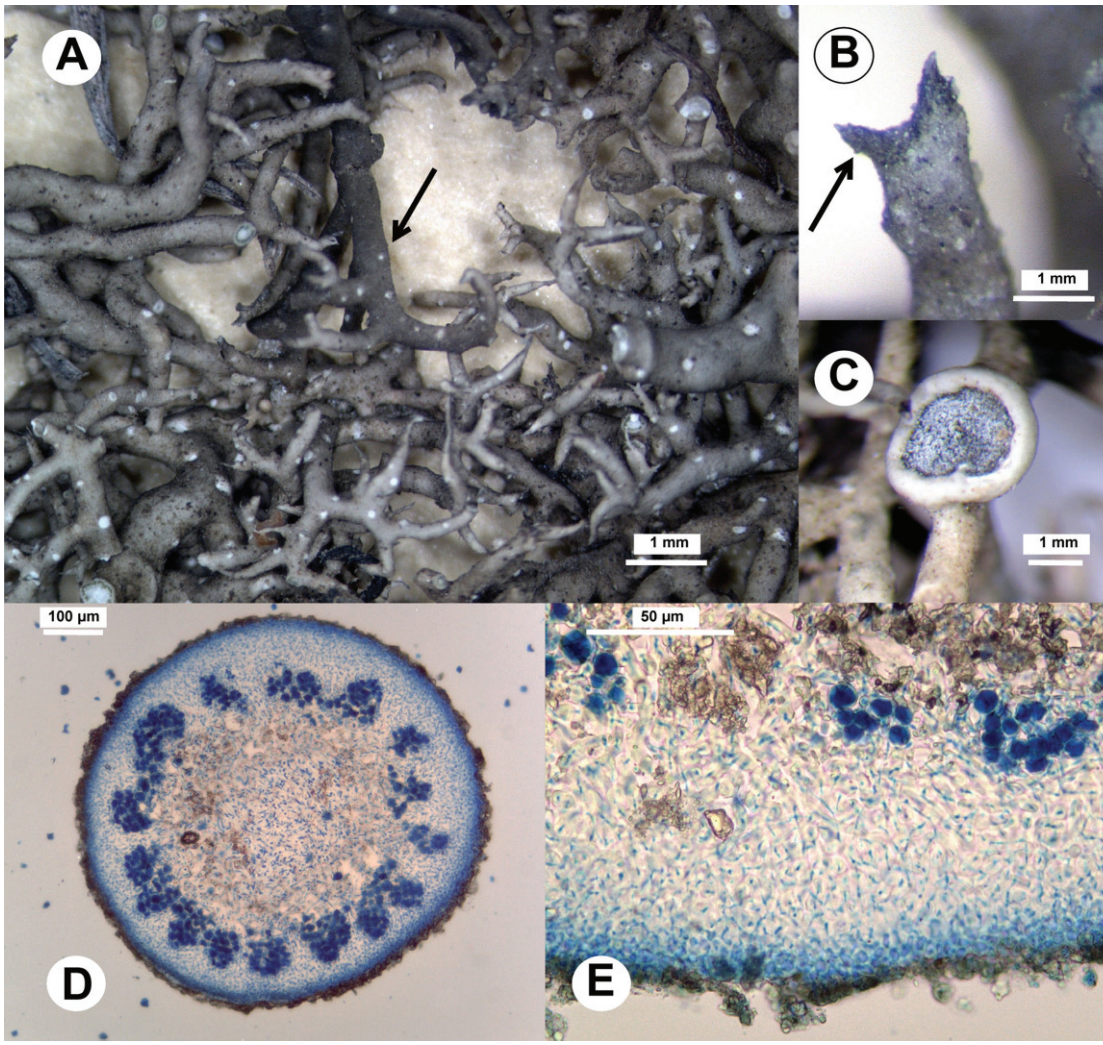

Figure 4. *Aspicilia hispida* (*Spribille* & *Wagner 25348*). **A.** Subfruticose thallus with narrow and elongated branches. **B.** Branchlet with black tips. **C.** Apothecium with pruinose disc **D.** Cross section of branch showing algal cells clustered in small groups. **E.** Two–layered cortex, outer part paraplectenchymatous, inner part prosoplectenchymatous.

vagrant or appearing to be vagrant since the thallus is brittle and easily broken, about 5–20 mm tall and 5–20(–30) mm broad, forming small tufts; branching irregular to dichotomous, main branches variable in width, (0.3–)0.5–1.5(–2) mm in diam (**Fig. 4A**), but distinctly tapering and pointed at the tips (**Fig. 4B**); surface gray, green–gray, olive–gray, yellow–gray or brown–gray to green, olive, olive–brown or almost brown, dull, at branch tips black (**Fig. 4B**). Pseudocyphellae whitish, round to elongated, 0.1–0.8 mm in diam., common along the branches (**Fig. 4A**). Cortex two layered (**Fig. 4E**); outer part (25–)30–40(–45) µm thick, paraplectenchymatous, ± brown, c. 3–5 cells thick, cells (4–)5–7(–8) µm in diam.; inner part prosoplectenchymatous, hyaline,c. 2–3 times as thick as the outer layer; cortex covered with a thin epinecral, amorphous layer 1–10(–15) µm thick. Photobiont chlorococcoid, cells ± round, 5–15(–20) µm in diam., clustered in small groups (**Fig. 4D**). Apothecia (**Fig. 4C**) aspicilioid when young, later adnate to substipitate, rare, 0.3–2(–3) mm in diam., occurring in broad parts of the main branches, disc black to brown–black, sometimes with a gray pruina, concave when young, in older apothecia plane to slightly convex; thalline margin flat to ± elevated and prominent in older apothecia,

entire, concolorous with thallus or with a thin, white rim; proper exciple:(45–)60–90(–105) wide; epihymenium N+ green; hymenium hyaline, (95–)110–130(–145) μm tall; paraphyses moniliform to sub–moniliform, upper cells ± globose, 4–6 μm wide, in lower part 2–3 μm wide, branched; hypothecium pale, 45–65(–75) μm thick; asci broadly clavate, *Aspicilia*–type, 85–95(–110) × 25–32 μm, 2–4 spored; ascospores hyaline, simple, subglobose, (19–)21–24(–26) × (18–)19–23(–24) μm (n=30). Pycnidia rare, along the branches, with black ostiole; conidia filiform, straight to slightly curved, 8–12(–14) × 0.8–1.2 μm.

Ecology and habitats. On ± calciferous soil in arid steppe or steppe–like habitats, usually growing in open stony slopes. Vagrant forms accumulate in wind–deposited drifts. An example of a steppe element found in temperate and subtropical, semi–arid regions of the Northern Hemisphere.

Distribution. Widespread, so far known from southern Europe (Hafellner 2004), Russia (Kulakov 2002, 2003), Ukraine (Mereschkowsky 1911), Middle Asia (Andreeva 1987) and Iran (Sohrabi et al. 2010b). In North America it is known from Canada (Saskatchewan) and USA (eastern Oregon to eastern Montana and northern Great Plains, south to Utah, Colorado and Arizona; Owe–Larsson et al. 2007). An online distribution map of *A. hispida* based on this study is presented at the Myco-Lich website (www.myco-lich.com) edited by Sohrabi et al. (2010a).

Discussion. *Aspicilia hispida* is characterized by its narrow subfruticose thallus with whitish pseudocyphellae along the branches. Fertile specimens are rarely observed and have so far only been reported by Thomson (1960), Brodo (1976) and Rosentreter et al. 2007 (p. 46, photo color plate). Two other terricolous species, *A. californica* and *A. filiformis*, are subfruticose, prostrate and lack pseudocyphellae.

Selected specimen used for distribution map. *Aspicilia hispida* Mereschk. Canada: alberta: Bighill Creek valley 1.5 miles NE of Cochrane, Nell, 26 February 1967, *Bird 18450* (canl); british columbia: Kamloops area, NW of Tranquille, along trail towards E end of Dewdrop Range, open rather exposed ridge, 100 m, 22 May 1988, *Goward & Knight 88–188* (h); saskatchewan: Matador, N shore of Lake Diefenbaker, due S of IBP Station, 2000 ft, 30 April 1969, *Sheard & Reid 1827 & 1845* (canl); U.S.A: utah: Box Elder Co., Curlew Valley proper, Snowville, 1350 m, October 1973, *Lange & Schulze,* A. Vězda: Lich. Sele. EXI. No: 1265 (h, s); colorado: Montezuma Co., Mesa Verde National Park, 7000 ft, 30 May 1959, *Weber & Erdman*, in Weber's Lichen Exs. No: 144 (s, fh); idaho: Owhyee Co., 3 miles W of Hwy 95 and 14 air miles SW of Marsing, 4500 ft, 21 May 1987, *DeBolt 705* (us); montana: Sweet Grass Co., Just E of Springdale, hills just W of Mendenhall Creek Road; 1259 m, 29 October 2007, *Spribille* & *Wagner 25348* (gzu).

Selected specimens used for comparison. ***Aspicilia fruticulosa*** (Eversm.) Flagey, Kazakhstan:"Ad terram in viciniis Sarepta (Gub. Saratowsk)", 1864, *Becker s.n,* Elenkin 1901: Lich. Fl. Ross. No. 24f. (h, le); Tarbagatai, nordwesl. vorgebirge, ca 40 km E Stadt Tarbagatai, 1000 m, 01 August 2001, *Lange 5186* (h); Akmolinskaya Oblast (=Akmola Province), 20 km SE of the Tengiz Lake, banks of the river Kulanotpes, 4 km NNW of the town Kulanutpes, 340 m, 16 July 2007, *Wagner L–0070*, in Lichenotheca Graecensis, Fasc. 17: 321 (gzu). Russia: Volgograd Region, Kalachovsky district, vicinity of Bolshegolubinsky garden. northern slope of river Bolshaya Golubaya, 24 July 1994, *V.G. Kulakov.* (le, vdlg); ukraine: Crimean Peninsula, Alupka, Aj–Petrinskaja jajla ca. 1 km SE of Mt. Bedene–kyr, c. 1100 m, 11 February 2006, *Vondrák 5188* (gbfs).

Aspicilia hispida Mereschk., Kazakhstan: Aklushenskaya District, Bayzhanshal, 12 May 1957, *Andreeva* (le). Iran: golestan: Golestan National Park, Mirzabaylou towards Almeh valley, 1300 m, 20 May 2008; *Sohrabi (15068) & Ghobad-Nejhad* (hb. M. Sohrabi); Italy: Piemonte, Prov. Cuneo: Alpi Cozie, crest SW above Colle dell Agnello, ca. 2830 m, 25 July 2000, *Hafellner 59364.* (gzu, 09–2003); 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!, w); Ukraine: Krym (Crimea), Supra Terram stepporum prope simpheropolin, in Pininsula Taurica, 1910, *Mereschkowsky s.n,* Lich. Ros. Exs. No: 35 (le).

Acknowledgments

We are grateful to T. Ahti (Helsinki), H. Sipman (Berlin), W. Obermayer (Graz), I. Brodo (Ottawa) and J. Hyvönen (Helsinki) for their help and discussions. We also wish to thank the herbarium curators, who made many collections available to us. We would also like to thank U. Søchting (Copenhagen) for his kind help in DNA extraction from some vagrant species. The Iranian Ministry of Science and Technology financially supported the studies of Mohammad Sohrabi at the University of Helsinki. Societas pro Fauna et Flora Fennica supported Sohrabi's travel to Sweden. Soili Stenroos wishes to thank the Academy of Finland (grant 211171) for financial support. We are indebted to anonymous reviewers for critical advice and helpful suggestions.

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ms received March 26, 2010; accepted November 10, 2010.

Appendix 1. Voucher and GenBank accession numbers for ITS sequences used in the analysis. New sequences shown in bold.

Aspicilia aquatica, Sweden, *Hermansson* 11467 (ups), EU057896; *A. caesiocinerea,* Sweden, *Tibell* 22612 (ups), EU057897; *A. calcarea,* Sweden, *Nordin* 5888 (ups), EU057898; *A. cinerea,* Sweden, *Hermansson 13275* (ups), EU05789; *A. contorta,* Sweden, *Nordin* 5895 (ups), EU057900; Austria, *Hafellner & Hafellner* 43516 (gzu), AF332109; Austria, *Wilfling* s. n. (gzu), AF332108; *A. epiglypta,* Sweden, *Nordin* 6303 (ups), EU057907; *A. fruticulosa,* Kyrgyzstan, *Lange* 5186 (h), **HQ171228;** Russia, *Kulakov* s.n. (hb. V. John, 9913), **HQ171227;** *A. gibbosa,* Sweden, *Nordin* 5878 (ups), EU057908; *A. hispida,* Iran, *Sohrabi* 15099 (hb. Sohrabi), HQ171233; Russia, *Ochirova* s. n. (le) , **HQ171235;** U.S.A, *Muscha* 121 (srp), **HQ171234;** *A. indissimilis,* Sweden, *Nordin* 5943 (ups), EU057909; *A. laevata,* Sweden, *Tibell* 23659 (ups), EU057910; *A. leprosescens,* Sweden, *Nordin* 5906 (ups), EU057911; *A. mashiginensis,* Sweden, *Nordin* 5790 (ups), EU057912; *A. mastrucata,* Sweden, *Nordin* 5708 (ups), EU057913; Sweden, *Nordin* 5481 (ups), EU057914; *A. permutata,* Sweden, *Nordin* 6029 (ups), EU057919; *A.*

permutata, Sweden, *Nordin* 6038 (ups), EU057920; *A. rogeri,* U.S.A, *Rosentreter* 16333 (srp), **HQ171231;** U.S.A, *Rosentreter* 16373 (srp), **HQ171232;** *A. vagans,* Russia, *Kulakov* s.n. (hb V. John 9911), **HQ171237;** *A. zonata,* Sweden, *Nordin* 5949 (UPS), EU057953; *A. zonata,* Sweden, *Nordin* 6006 (ups), EU057952; *Ochrolechia balcanica,* Greece, *Schmitt* (ess-20968), AF329172; *O. parella,* France, *Feige* (ess-20864), AF329174; *Siphula complanata* Australia, *Kantvilas* (ho 517570), DQ337612.