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RESEARCH ARTICLE

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A New Lichen-Forming Fungus, *Aspicilia humida*, from a Forested Wetland in South Korea, with a Taxonomic Key for Aspicilioid Species of Korea

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ABSTRACT

Aspicilia humida Lee is described as a new lichen-forming fungus from a wetland forest, South Korea. The new species is distinguishable from *Aspicilia aquatica* (Fr.) Körb., the most similar species, by the absence of prothallus, black disk without green color in water, olive-brown epihymenium, shorter hymenium, hymenium I + yellowish blue-green, wider paraphysial tips without a vivid pigment, smaller asci, smaller ascospores, and the presence of stictic acid. Molecular analyses employing internal transcribed spacer (ITS) and mitochondrial small subunit (mtSSU) sequences strongly support *A. humida* as a distinct species in the *A. cinerea* group. A surrogate key is provided to assist in the identification of all 28 aspicilioid species of Korea.

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1. Introduction

As the *Aspicilia* was comprising more than 300 species, the large genus has been classified into several infrageneric groups and some of them were finally splitted into some new genera or resurrected as old genera [1–6]. The genus *Megaspora* was newly introduced by the characteristics of large ascospores with thick walls and anastomosing paraphyses [7,8]. The genus *Lobothallia* was raised up from the *A. radiosa* group [9]. The old genus *Circinaria* (previously the *A. contorta/calcarea* group) was reintroduced by the characteristics of diverse thalli (crustose, foliose to subfruiticose), broad-ellipsoid to globose ascospores which are shown generally less than eight per ascus, and particularly the presence of aspicilin [3,4]. The old genus *Sagedia* was reintroduced based on the molecular analysis [3]. The genus *Teuvoa* was newly introduced by the characteristics of the lack of lobate, radiating thalli, generally absence of algal layer underlining hypothecium, the absence of secondary metabolites, and the substrate preference to barks or woods but not rocks, in comparing with *Lobothallia* [10]. The genus *Oxneriaria* (previously the *A. mashiginensis* group) was newly introduced by defining the characteristics of radiating thalli with wrinkled or lobate periphery, small ascospores, presence of substictic acid, and the habitat preference to polar and alpine areas [5]. The old genus *Aspiciliella* was reintroduced by the characteristics of consistently ellipsoid ascospores, small conidia, and the presence of norstictic acid in all species [11]. The infrageneric groups are further categorized into seven groups in *Aspicilia* s. str. [6]. The *Aspicilia* (200 spp.) is still considered the main genus of the family Megasporaceae (243 spp.) [12].

Hue, a French lichenologist, first described the aspicilioid lichens of Korea including 15 species (*Aspicilia adamanticola* Hue, *A. asteria* Hue, *A. chinnampoana* Hue, *A. dimorphodes* Hue, *A. exserta* Hue, *A. fauriana* Hue, *A. geographica* Hue, *A. leucera* Hue, *A. microsporeta* Hue, *A. stellata* Hue, *A. stenospora* Hue, *A. tofacea* Hue, *A. tumens* Hue, *A. umbrinella* Hue, and *A. vulcanica* Hue) [13]. After a century, Kondratyuk discovered *A. contorta* ssp. *hoffmanniana* S. Ekman & Fröberg ex R. Sant. (syn. *Circinaria hoffmanniana* (S. Ekman & Fröberg ex R. Sant.) A. Nordin) in 2013 [14], and Aptroot and Moon recorded *A. cinerea* (L.) Körb., *A. grisea* Arnold, *Circinaria caesiocinerea* (Nyl. ex Malbr.) A. Nordin, Savić & Tibell, and *C. leprosescens* (Sandst.) A. Nordin, Savić & Tibell in 2014 [15]. Kondratyuk focused on the aspicilioid lichens of Korea in 2016 and eight species were introduced from Korea (*A. pseudoabbasiana* S.Y. Kondr., Lőkös & Hur, *A. pseudovulcanica* S.Y. Kondr., Lőkös & Hur, *A. subepiglypta* S.Y. Kondr., Lőkös & Hur, *A. subgeographica* S.Y. Kondr., Lőkös & Hur, *A. subgoettweigensis* S.Y. Kondr., Lőkös & Hur, *A. submamillata* S.Y. Kondr., Lőkös & Hur [16], *A. geumodoensis* S.Y. Kondr., Lőkös & Hur (syn. *Rimularia geumodoensis* (S.Y.

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Kondr., Lőkös & Hur) S.Y. Kondr., Lőkös & Hur and *R. badioatra* (Kremp.) Hertel & Rambold) [17]. Particularly, Paukov reclassified both *A. dimorphodes* and *A. fauriana* to *A. intermutans* (Nyl.) Arnold, and both *A. geographica* and *A. microsporeta* to *Lecanora oreinoides* (Körb.) Hertel & Rambold in 2017 [18]. Kondratyuk detected *Rimularia gibbosa* (Ach.) Coppins, Hertel & Rambold [19] and Yakovchenko introduced two *Rimularia* species such as *R. badioatra* and *R. limborina* Nyl. in 2018 [20]. Overall 27 species of the aspicilioid lichens were recorded in Korea.

This study aimed to describe a new lichen-forming fungus in the genus *Aspicilia*. One of the field surveys for the lichen biodiversity in the forested wetlands of South Korea was carried out in a wetland forest of a high mountain, Gangwon Province in 2020, and two specimens of aspicilioid lichens were collected (Figure 1). The specimens were comprehensively analyzed in ecology, morphology, chemistry and molecular phylogeny and did not correspond to any previously known species. We describe them as a new species, *Aspicilia humida*, and this discovery contributes to the taxonomy with overall 28 taxa in the genus *Aspicilia* of Korea. The specimens are deposited in the herbarium of the Baekdudaegan National Arboretum (KBA, the herbarium acronym in the Index Herbariorum), South Korea.

2. Materials and methods

2.1. Morphological and chemical analyses

Specimen sections were prepared manually with a razor blade under a stereomicroscope (Olympus optical SZ51; Olympus, Tokyo, Japan), scrutinized under a compound microscope (Nikon Eclipse E400; Nikon, Tokyo, Japan) and pictured using a software program (NIS-Elements D; Nikon) and a DS-Fi3 camera (Nikon) mounted on a Nikon Eclipse Ni-U microscope (Nikon). The ascospores were examined at 1000× magnification in water. The length and width of the ascospores were measured and the range of spore sizes was shown with average, standard deviation (SD), length-to-width ratio, and the number of measured spores. Thin-layer chromatography (TLC) was performed using solvent systems A and C according to standard methods [21].

2.2. Isolation, DNA extraction, amplification, and sequencing

Hand-cut sections of 10–20 ascomata with thallus from the collected specimens were prepared for DNA isolation and DNA was extracted with a NucleoSpin Plant-II Kit in line with the manufacturer's instructions (Macherey-Nagel, Düren, Germany). PCR amplifications for the internal transcribed spacer region (ITS1-5.8S-ITS2 rDNA), the mitochondrial small subunit, and the nuclear large subunit ribosomal RNA genes was achieved using the primers ITS5 and ITS4 [22], mrSSU1 and mrSSU3R [23], and LR0R and LR5 [24], respectively. The PCR thermal cycling parameters used were 95 °C (15 sec), followed by 35 cycles of 95 °C (45 sec), 54 °C (45 sec), and 72 °C (1 min), and a final extension at 72 °C (7 min) based on Ekman [25]. The annealing temperature was occasionally altered by ±1 degree in order to get a better result. PCR purification and DNA sequencing were accomplished by the Macrogen (Seoul, Korea).

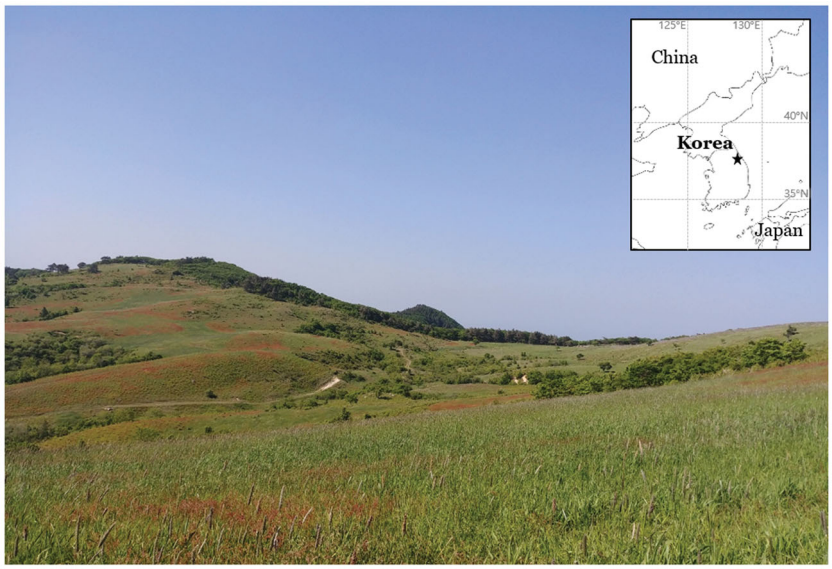

Figure 1. Specific collection site for the new species *Aspicilia humida* (black star mark).

2.3. Phylogenetic analyses

All ITS and mtSSU sequences (Table 1) were aligned and edited manually using ClustalW in Bioedit V7.2.6.1 [26]. All missing and ambiguously aligned data and parsimony-uninformative positions were removed and only parsimony-informative regions were finally analyzed in MEGA X [27]. The final alignment comprised 1163 (ITS) and 1058 (mtSSU) columns. In them, variable regions were 171 (ITS) and 120 (mtSSU). Finally, the phylogenetically informative regions were 444 (ITS) and 271 (mtSSU). Phylogenetic trees with bootstrap values were obtained in RAxML GUI 2.0 beta [28] using the maximum likelihood method with a rapid bootstrap with 1000 bootstrap replications and GTR GAMMA for the substitution matrix. The posterior probabilities were obtained in BEAST 2.6.4 [29] using the GTR 123141 (ITS) and the GTR 121323 (mtSSU) models, as the appropriate models of nucleotide substitution produced by the Bayesian model averaging methods with bModelTest [30], empirical base frequencies, gamma for the site heterogeneity model, four categories for gamma, and a 10,000,000 Markov chain Monte Carlo chain length with a 10,000-echo state screening and 1000 log parameters. Then, a consensus tree was constructed in TreeAnnotator 2.6.4 [29] with no discard of burnin, no posterior probability limit, a maximum clade credibility tree for the target tree type, and median node heights. All trees were displayed in FigTree 1.4.2 [31] and edited in Microsoft Paint. The bootstrapping and Bayesian analyses were repeated three times for the result consistency and no significant differences were shown for the tree shapes and branch values. The phylogenetic trees and DNA sequence alignments are deposited in TreeBASE under the study ID 28153. Overall analyses in the materials and methods were accomplished based on Lee and Hur [32].

3. Results and discussion

3.1. Phylogenetic analyses

Two independent phylogenetic trees for the genus *Aspicilia* and related genera were produced from 98 sequences (66 for ITS, and 32 for mtSSU) from GenBank and four new sequences (each two for ITS and mtSSU) from the new species (Table 1). The new species was positioned in the *A. cinerea* group in both trees. The ITS tree describes that the new species is located in a clade with *A. subfarinosa* (J. Steiner) Şenkard. & Sohrabi and *Circinaria hispida* (Mereschk.) A. Nordin, Savić & Tibell, represented by a bootstrap value of 98 and a posterior probability of 0.7 (not shown) for the branch. Other species, such as *A. abbasiana* S.Y. Kondr., Lőkös, Ismayil & S.Y. Guo, *A. blastidiata* Paukov, A. Nordin & Tibell, *A. cinerea*, *A. dudinensis* (H. Magn.) Oxner, *A. pseudoabbasiana*, *A. pseudovulcanica*, *A. subepiglypta*, *A. subdepressa* Arnold, *A. subgeographica*, *A. subgoettweigensis*, and *A. submamillata*, are closely located to the new species in the *A. cinerea* group, represented just by a bootstrap value of 79 for the branch (Figure 2). The mtSSU tree shows that the new species is solely located in the *A. cinerea* group. Closely positioned species to the new species are *A. cinerea*, *A. dudinensis*, *A. indissimilis* (H. Magn.) Räsänen, and *A. laevata* (Ach.) Arnold, represented by a bootstrap value of 95 and a posterior probability of 1 for the branch (Figure 3). The phylogenetic analyses did not designate any species identical to the new species in the genus *Aspicilia*.

3.2. Taxonomy

Aspicilia humida **B.G. Lee sp. nov.**

No: MB839181

Figure 4

3.2.1. Diagnosis

Aspicilia humida differs from *A. aquatica* by the absence of prothallus (vs. thick gray prothallus), black disk without green color in water (vs. black disk with translucent green when wet), olive-brown epihymenium (vs. olive-green epihymenium), shorter hymenium (50–60 μm vs. 150–170 μm), hymenium I + yellowish blue-green (vs. hymenium I + blue or turning dark red-brown), wider paraphysial tips without vivid pigment (4.5–6 μm wide vs. blackened tips in 2–5 μm wide), smaller asci (64–72 × 17–27 μm vs. 80–140 × 25–35 μm), smaller ascospores (10.5–23 × 6–13.5 μm vs. 20–35 × 13–20 μm), and the presence of stictic acid (vs. no substance).

3.2.2. Type

South Korea, Gangwon Province, Pyeongchang-gun, Daegwallyeong-myeon, Hoenggye-ri, a forest wetland, 37°46'0.02"N, 128°42'19.58"E, 1,047 m alt., on siliceous rock, 03 June 2020, B. G. Lee & H. J. Lee 2020-000503, with *Diplotomma alboatrum* (Hoffm.) Flot. and *Endocarpon maritimum* Y. Joshi & Hur (holotype: BDNA-L-0000703!; GenBank MW832805 for ITS, MW832823 for mtSSU, and MW832826 for LSU); same locality, on siliceous rock, 03 June 2020, B. G. Lee & H. J. Lee 2020-000511, (paratype: BDNA-L-0000711; GenBank MW832806 for ITS, MW832824 for mtSSU, MW832827 for LSU).

Thallus saxicolous, crustose, mainly areolate and partially rimose, pale gray to white, margin determinate, not pruinose, 175–300 μm thick; cortex

Table 1. Species list and DNA sequence information employed for phylogenetic analysis.

No.	Species	ID (ITS)	ID (mtSSU)	Voucher
1	*Aspicilia abbasiana*	KM609324		WDLC-20111154
2	*Aspicilia aschabadensis*	GU289916		Borisova1934 (LE)
3	*Aspicilia berntii*	EU502747		Nordin 6392 (UPS)
4	*Aspicilia bicensis*	KU341407		Anderson 16123 (NY)
5	*Aspicilia blastidiata*	KX129963		AGP20111009-01
6	*Aspicilia brucei*	JF703111		UPS:Owe-Larsson 9147
7	*Aspicilia calcitrapa*	JF703113		MARSSJ:Roux 24309
8	*Aspicilia cinerea*	EU057899	HM060695	Hermansson 13275 (UPS)
9	*Aspicilia cinerea*	HQ406799	HM060696	Nordin 5542 (UPS)
10	*Aspicilia cinerea*	HQ650637	DQ986890	AFTOL-ID 647
11	*Aspicilia coronata*	EU057901		Lattman 13.V.2006 (UPS)
12	*Aspicilia desertorum*	HQ406802	HM060689	Owe-Larsson 9814 (UPS)
13	*Aspicilia dudinensis*	EU057906	HM060710	Nordin 6036 (UPS)
14	*Aspicilia dudinensis*	MN906265		McCune 36017 (OSC)
15	*Aspicilia dudinensis*		HM060719	UPS:Nordin 5971
16	*Aspicilia epiglypta*	MH248866	MH248867	Nordin 7037 (UPS)
17	*Aspicilia fluviatilis*	HQ259264		UPS:Nordin 6188
18	*Aspicilia granulosa*	HQ259265		UPS:Nordin 6174
19	***Aspicilia humida***	**MW832805**	**MW832823**	**BDNA-L-0000703**
20	***Aspicilia humida***	**MW832806**	**MW832824**	**BDNA-L-0000711**
21	*Aspicilia indissimilis*	EU057909	HM060708	Nordin 5943 (UPS)
22	*Aspicilia laevata*	EU057910	HM060692	Tibell 23659 (UPS)
23	*Aspicilia prestensis*	JF703122		UPS:Roux 24883a
24	*Aspicilia pseudoabbasiana*	KY249599		KoLRI 018579
25	*Aspicilia pseudovulcanica*	KY249601		KoLRI 012338
26	*Aspicilia pseudovulcanica*	KY249602		KoLRI 012420
27	*Aspicilia pseudovulcanica*	KY249603		KoLRI 023829
28	*Aspicilia subdepressa*	JF703123		MARSSJ:Roux 24653
29	*Aspicilia subepiglypta*	KY249607		KoLRI 012535_2
30	*Aspicilia subfarinosa*	MN989235		Sipman & Raus 63720
31	*Aspicilia subgeographica*	KY249611		KoLRI 018704
32	*Aspicilia subgoettweigensis*	KY249616		KoLRI 007090
33	*Aspicilia submamillata*	KY249620		KoLRI 011146
34	*Aspicilia subradians*	HQ259267		UPS:Nordin 5984
35	*Aspicilia verrucigera*	EU057939		Tibell 22669 (UPS)
36	*Aspiciliella intermutans*	MH248863	MH248869	Roux 25790 (CR)
37	*Circinaria affinis*	HQ389194		Abbas 20081364 (H)
38	*Circinaria caesiocinerea*	HQ650636	DQ986892	AFTOL-ID 653
39	*Circinaria calcarea*	EU057898		Nordin 5888 (UPS)
40	*Circinaria calcarea*		AY853310	Wedin 6500 (UPS)
41	*Circinaria cerebroides*	JQ797534		Ringel 5138 (H)
42	*Circinaria contorta*	HQ650638	DQ986876	AFTOL-ID 1358
43	*Circinaria cupreogrisea*	EU057903		Nordin 6046 (UPS)
44	*Circinaria esculenta*	HQ406803		Owe-Larsson 9824 (UPS)
45	*Circinaria esculenta*	JQ797511	JQ797485	Owe-Larsson 9796 (UPS)
46	*Circinaria fruticulosa*	HQ389199		Vondrak 5188 (GBFS)
47	*Circinaria fruticulosa*		KC020253	ASPFRU 2361
48	*Circinaria gibbosa*	EU057908	HM060702	Nordin 5878 (UPS)
49	*Circinaria gyrosa*	JQ797528	JQ797487	Sohrabi 10401 A
50	*Circinaria hispida*	HQ171233		Sohrabi 15099
51	*Circinaria jussuffii*	JQ797518		Esnault 2033 (GZU)
52	*Circinaria lacunosa*	JQ797517		Abbas 940003 (H)
53	*Circinaria leprosescens*	EU057911	HM060711	Nordin 5906 (UPS)
54	*Circinaria sphaerothallina*	JQ797545		Sohrabi 9369
55	*Lobothallia alphoplaca*	JQ797516	JQ797480	Sohrabi 3677
56	*Lobothallia alphoplaca*	KT456207	KT456211	SK A20
57	*Lobothallia melanaspis*	JF825524		Owe-Larsson 8943a (UPS)
58	*Lobothallia melanaspis*		HM060688	UPS:Nordin 6622
59	*Lobothallia praeradiosa*	MK347501	MK348229	UFU L-1264
60	*Lobothallia recedens*	HQ406807		Nordin 6035 (UPS)
61	*Lobothallia recedens*		HM060724	UPS:Nordin 6582
62	*Oxneriaria dendroplaca*	HQ259259	HM060706	UPS:Nordin 5952
63	*Oxneriaria mashiginensis*	EU057912	HM060694	Nordin 5790 (UPS)
64	*Oxneriaria permutata*	EU057918	HM060709	Nordin 6027 (UPS)
65	*Oxneriaria rivulicola*	EU057922	HM060715	Nordin 5957 (UPS)
66	*Oxneriaria supertegens*	EU057936	HM060704	Owe-Larsson 9002 (UPS)
67	*Oxneriaria verruculosa*	EU057940	HM060703	Owe-Larsson 9007 (UPS)
68	*Oxneriaria virginea*	HQ259270		UPS:Nordin 6017a
69	*Sagedia mastrucata*	EU057913	HM060698	Nordin 5708 (UPS)
70	*Sagedia nunatakkorum*	KT630250		Malicek-228
71	*Sagedia simoensis*	EU057926	HM060701	Owe-Larsson 9000 (UPS)
72	*Sagedia zonata*	EU057946	HM060700	Owe-Larsson 8942 (UPS)
73	*Teuvoa tibetica*	GU289915		Obermayer 04386
	Overall	**68**	**34**	

DNA sequences for the new species *Aspicilia humida* (in bold) were generated in this study. All others were obtained from GenBank. The species names are followed by GenBank accession numbers and voucher information. ITS, internal transcribed spacer; mtSSU, mitochondrial small subunit; Voucher, voucher information.

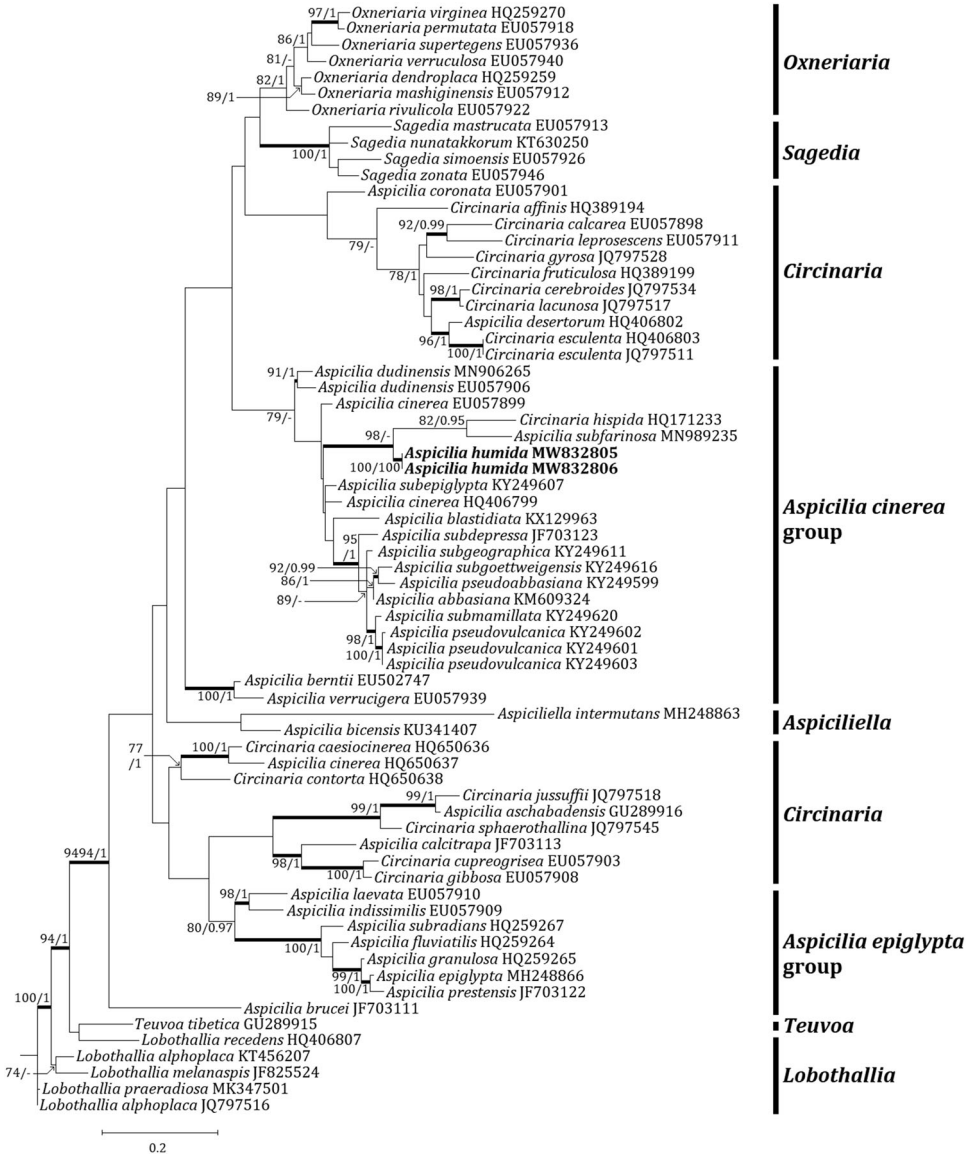

Figure 2. Phylogenetic relationship among available species in the genus *Aspicilia* based on a maximum likelihood analysis of the dataset of ITS sequences. The tree was rooted with six *Lobothallia* and *Teuvoa* sequences. Maximum likelihood bootstrap values $\geq$ 70% and posterior probabilities $\geq$ 95% are shown above internal branches. Branches with bootstrap values $\geq$ 90% are shown in bold. The new species *Aspicilia humida* is presented in bold, and all species names are followed by the Genbank accession numbers. Reference Table 1 provides the species related to the specific GenBank accession numbers and voucher information.

hyaline, 25–30 μm thick; medulla 25–30 μm thick; photobiont coccoid, algal layer 35–50 μm thick, cells globose to subglobose, 5–15 μm. Small crystals in cortex, medulla and between algal cells, not dissolving in K. Prothallus inconspicuous.

Apothecia abundant, generally rounded but subangular or even irregular when several apothecia contiguous or coalescent, emerging single to several per an areole, adnate when mature, not constricted at the base, 0.2–1.7 mm diam. Disk flat or somewhat concave, smooth or slightly rugose, not pruinose, black from the beginning and partially paler when old, 100–130 μm thick; lecanorine, thalline margin present and same color to thallus or slightly darker, proper margin indistinct. Amphithecium well-developed, with small crystals in both cortical layer and medulla, crystals extending to the base, not dissolving in K, 90–100 μm wide laterally, 50–60 μm wide at periphery. Parathecium inconspicuous, hyaline but olive-brown at periphery, 10–15 μm wide laterally, 15–25 μm wide at periphery, disappearing to the base. Epihymenium olive green to brown, smooth and not granular, brown pigment dissolving in K, 10–15 μm high. Hymenium hyaline, 50–60 μm high, I + yellowish blue-green. Hypothecium hyaline, 25–50 μm high. Oil droplets present mainly in hypothecium and also along paraphyses in hymenium. Paraphyses septate, anastomosing, 2–2.5 μm wide, simple or branched at tips, tip cells somewhat bead-like (moniliform), bead-like formation clearer in staining, swollen but not pigmented, 4.5–6 μm wide. Asci clavate, 8-spored, 64–72 $\times$ 17–27 μm

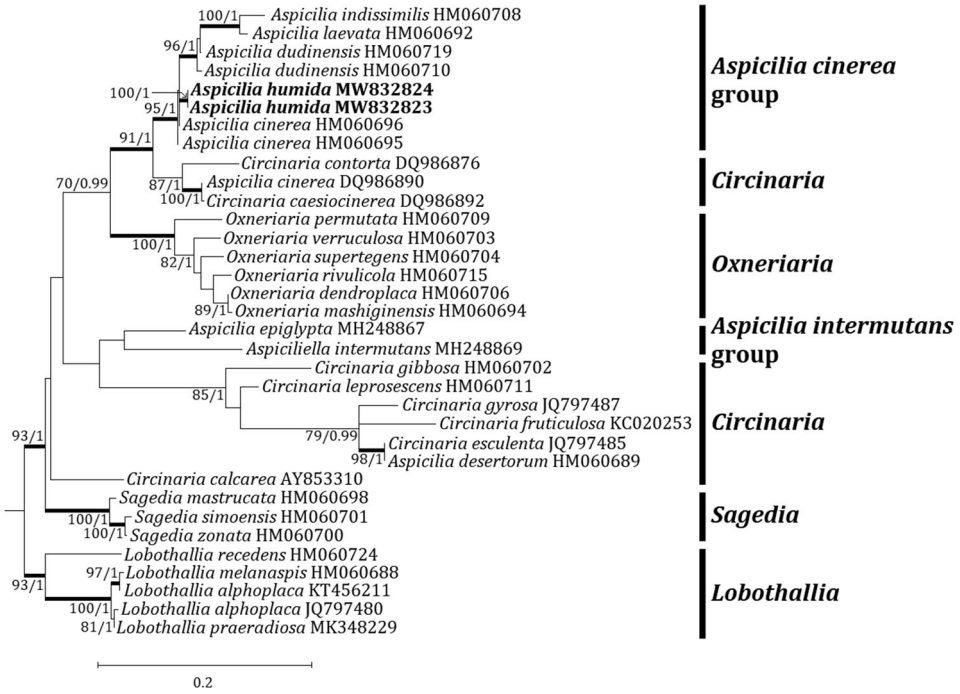

Figure 3. Phylogenetic relationships among available species in the genus *Aspicilia* based on a maximum likelihood analysis of the dataset of the mitochondrial small subunit (mtSSU) sequences. The tree was rooted with five *Lobothallia* sequences. Maximum-likelihood bootstrap values $\geq$ 70% and posterior probabilities $\geq$ 95% are shown above internal branches. Branches with bootstrap values $\geq$ 90% are shown in bold. The new species *Aspicilia humida* is presented in bold, and all species names are followed by the GenBank accession numbers. Reference Table 1 provides the species related to the specific GenBank accession numbers and voucher information.

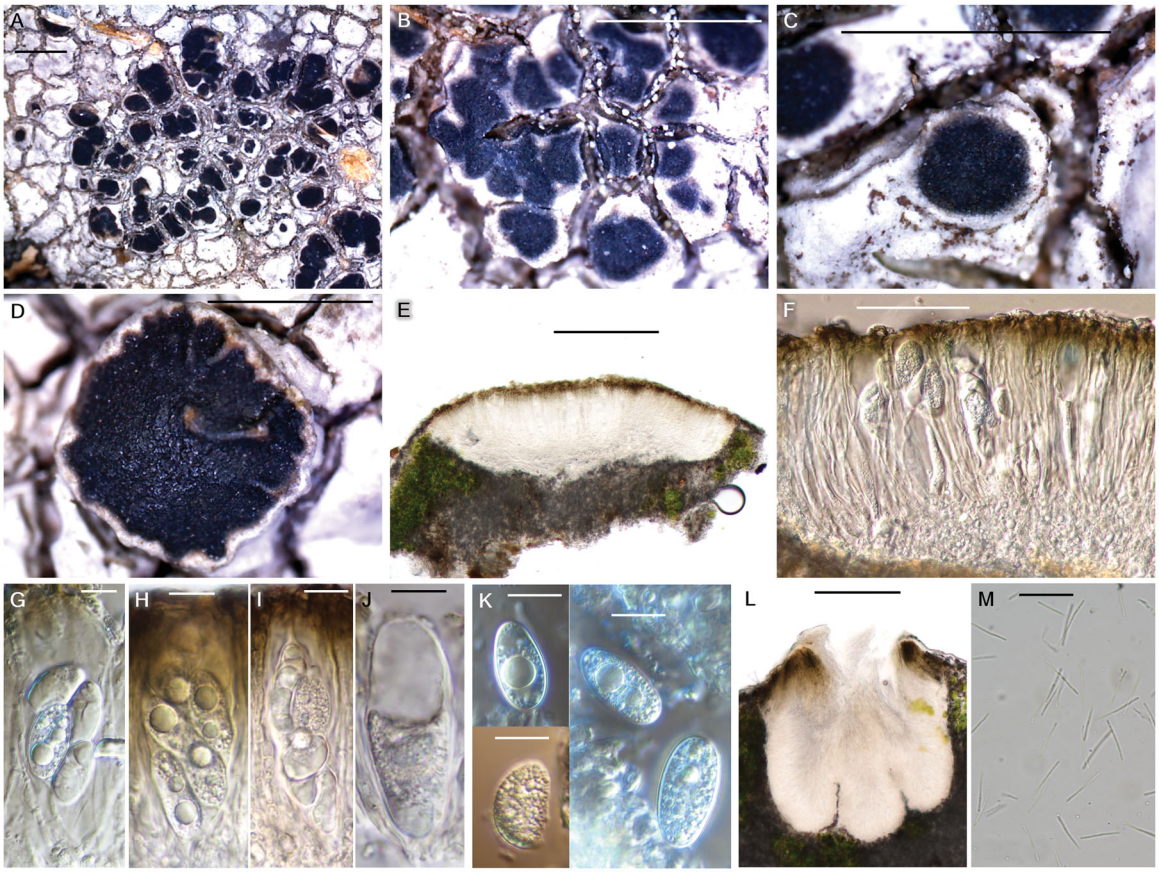

Figure 4. *Aspicilia humida* (BDNA-L-0000703, holotype for A–K; BDNA-L-0000711, paratype for L & M) in morphology. (A–D): Habitus and apothecia emerging single to several per an areole; (E): Adnate apothecia without constriction at the base in section; (F): Epihymenium in olive-brown pigment; (G–J): Clavate asci with eight spores; (K): Ellipsoid or globose ascospores with no septation; (L): Immersed pycnidia; (M): Thread-like pycnoconidia. Bars: A–D 1 mm; E 200 μm; F 50 μm; G–K 10 μm; L 100 μm; M 10 μm.

($n = 5$). Ascospores constantly simple, ellipsoid or somewhat globose, 10.5–23 × 6–13.5 μm (mean = 17.3 × 9.8 μm; SD = 2.6 (L), 1.5 (W); L/W ratio 1.2–2.7, ratio mean = 1.8, ratio SD = 0.3; $n = 106$). Pycnidia immersed, ostiolar region slightly projected, rounded, black, 250–275 × 200–230 μm. Pycnoconidia thread-like, straight, slightly curved or v-shaped, 5.5–28 × 0.5–1.0 μm (mean = 15.1 × 0.7 μm; SD = 3.7 (L), 0.1 (W), $n = 110$)

3.2.3. Chemistry

Thallus K–, KC–, C–, Pd–. Medulla K + yellow, I–. UV + gray to dull white. Stictic acid was detected by TLC.

3.2.4. Distribution and ecology

The species occurs on a siliceous rock nearby a stream in an open wetland forest of a high mountain. The species is currently known from the type collections.

3.2.5. Etymology

The species epithet indicates the lichen's geography, namely a humid wetland.

3.2.6. Notes

The new species is similar to *A. aquatica*, *A. vulcanica* and *A. pseudovulcanica* in having white to gray thallus with negative reaction in K among saxicolous species. However, the new species differs from *A. aquatica* by the absence of prothallus, black disk without green color in water, olive-brown epihymenium, shorter hymenium, hymenium I + yellowish blue-green, wider paraphysial tips without vivid pigment, smaller asci, smaller ascospores, and the presence of stictic acid [33,34].

The new species is different from *A. vulcanica* by apothecia emerging one to several per single areole, larger apothecia, narrower paraphyses, shorter and wider asci, and the substrate preference to siliceous rock [13,16].

The new species is distinguished from *A. pseudovulcanica* by thicker, larger apothecia without pruina, shorter hymenium, smaller ascospores [16]. Reference Table 2 provides the key characteristics distinguishing *A. humida* from the compared species above.

The new species is further compared with *A. straminella* Hue and *A. verrucigera* Hue in having grayish, areolate thallus and K + yellow medulla in saxicolous species. However, *A. straminella* is different from the new species by thicker and straw-gray thallus, smaller apothecia, taller hymenium and wider ascospores [13,16]. *Aspicilia verrucigera* differs from the new species by thicker and darker

Table 2. Comparison of *Aspicilia humida* with closely-related species.

Species	*Aspicilia humida*	*Aspicilia aquatica*	*Aspicilia pseudovulcanica*	*Aspicilia vulcanica*
Thallus growth form	mainly areolate, partially rimose	areolate to rimose	areolate or slightly cracked to continuous	rimose-areolate
Thallus color	pale gray to white	white to gray, often with a yellow or olive tinge	whitish gray to light gray	whitish to white-gray
Thallus thickness (μm)	175–300	200–400	150–200	120–160
Prothallus	inconspicuous	black to gray	–	–
Apothecia per areole	one to several	1 to 2(–4)	–	1 to 2
Apothecia (mm in diam.)	0.2–1.7	0.2–0.5	0.5–0.9	0.3–0.4
Pruina	absent	absent	white pruina on disk	white pruina on disk
Disk color	black without green in water	black with translucent green in water	gray to gray-black	(gray to gray-black)
Epihymenium color	olive green to brown	green to olive	dirty greenish brown	colorless
Hymenium height (μm)	50–60	150–200	110–130	(110–140)
Hymenium in Iodine	yellowish blue-green	blue, or turning dark red-brown	–	–
Paraphyses	2–2.5	1.5–2	–	c. 4
Paraphysial tip width (μm)	4.5–6	2–5	5–6	5–7
Paraphysial tip pigment	inconspicuous	blackened	brownish	colorless
Asci (μm)	64–72 × 17–27	85–140 × 25–35	–	80–90 × 14–12
Ascospores (μm)	10.5–23 × 6–13.5	20–35 × 13–20	18–23 × 10–12	17–20 × 7–10
Substance	stictic acid	no substance	stictic acid	–
Substrate	siliceous rocks nearby streams	siliceous rocks nearby streams	siliceous rocks	volcanic rock
Reference	BDNA-L-0000703 (holotype), BDNA-L-0000711 (paratype)	[33,34]	[16]	[13,16]

The morphological and chemical characteristics for several species close to the new species are referenced from the previous literature. All information on the new species is produced from type specimens (BDNA-L-0000703 and BDNA-L-0000711) in this study. The brackets for *Aspicilia vulcanica*, i.e. disk color and hymenium height, are assumed from previous literature.

thallus occasionally with brown color, smaller apothecia, taller hymenium, wider ascospores and the presence of norstictic acid [16,33].

3.9 Key to aspicilioid species of Korea (28 taxa)

Overall 28 species have been recorded for the aspicilioid lichens including the genera *Aspicilia*, *Circinaria*, *Lecanora*, and *Rimularia* in Korea, except for synonyms. For synonyms after taxonomic revision, *A. adamanticola* is corresponded to *A. cinerea* [18], *A. contorta* ssp. *hoffmanniana* is reclassified to *C. hoffmanniana* [35], *A. geographica* and *A. microsporeta* are conspecific to *L. oreinoides* [18], *A. dimorphodes* and *A. fauriana* are indistinguishable to *A. intermutans* [18], and *A. geumodoensis* is reclassified to *R. geumodoensis* [18]. This key is revised from Kondratyuk's work [16] only for Korea territory, and seven more species are included such as *A. grisea*, *A. humida*, *A. intermutans*, *C. caesiocinerea*, *C. hoffmanniana*, *L. oreinoides*, and *R. limborina*. *A. exserta* is corrected from *A. excerta* of Kondratyuk's work [16].

1. Paraphysis cells not bead-like, disk umbonate or gyrose **2**
   Paraphysis cells bead-like (moniliform), disk generally smooth **5**
2. Thallus C– or C ± pink (not or containing ± gyrophoric acid), hymenium up to 150 μm, ascospores 18–30 × 10–18 μm ..... ***Rimularia limborina***
   Thallus C + red (containing gyrophoric acid), hymenium up to 120 μm, ascospores 10–25 × 7–13 μm **3**
3. Thallus lighter, whitish gray, ascospores 20–25 × 9–13 μm..... ***Rimularia geumodoensis***
   Thallus darker, pinkish brown to gray brown, ascospores 10–25 × 7–13 μm **4**
4. Thallus pinkish brown to dark gray-brown, hymenium 70–100 μm, apothecia 0.2–0.4 mm diam., disk concave to umbonate, ascospores 10–22 × 7–13 μm..... ***Rimularia badioatra***
   Thallus pale gray-brown to beige, hymenium up to 75 μm, apothecia 0.5–0.8 mm diam., disk slightly convex, ascospores 16–25 × 10–13 μm..... ***Rimularia gibbosa***
5. Thallus or medulla K + red or K + yellow turning to red (containing norstictic acid) **6**
   Thallus or medulla K– red or K + yellow (containing aspicilin or stictic acid, but not containing norstictic acid) **16**
6. Thallus with farinose-erose soredia..... ***A. grisea***
   Thallus without soredia **7**
7. Apothecia rare or solitary per areole when present **8**
   Apothecia occurring one to several per areole **12**
8. Thallus whitish **9**
   Thallus grayish to brownish **11**
9. Thallus thin, apothecia 0.2–0.3 mm diam..... ***A. tofacea***
   Thallus thick, apothecia 0.5–1.5 mm diam. ... **10**
10. Asci 120 × 24 μm, ascospores 15–20 × 9–10 μm..... ***A. exserta***
    Asci 80 × 16 μm, ascospores 17–20 × 7–8 μm..... ***A. leucera***
11. Apothecia 0.3–0.5 mm diam., ascospores 16–20 × 6–7 μm..... ***A. stenospora***
    Apothecia 0.4–1.0 mm diam., ascospores 12–19 × 7–11 μm..... ***A. tumens***
12. Thallus white without grayish or brownish color..... **13**
    Thallus white-gray or gray to gray-brown..... **14**
13. Thallus thin 0.16–0.22 mm thick, apothecia occurring one to two per areole, 0.4–0.8 mm diam. ..... ***A. chinnampoana***
    Thallus thick 0.5–0.6 mm thick, apothecia occurring one to several per areole, 0.3–0.5 mm diam. ..... ***A. stellata***
14. Apothecia 0.2–0.3 mm diam., occurring three to five per areole, conidia 15–20 × 0.7–0.9 μm..... ***A. subepiglypta***
    Apothecia 0.4–1.2 mm diam., occurring one to three per areole, conidia 7–16 × 1 μm..... **15**
15. Apothecia occurring one to two per areole, ascospores 12–22 × 6–13 μm, conidia 11–16 × 1 μm ..... ***A. cinerea***
    Apothecia occurring two to three per areole, ascospores 22–28 × 12–14 μm, conidia 7–11 × 1 μm ..... ***A. intermutans***
16. On calcareous or volcanic rocks..... **17**
    On siliceous rocks..... **18**
17. On calcareous rocks, thallus with pruina, apothecia prominent, black, asci 4-spored..... ***Circinaria hoffmanniana***
    On volcanic rocks, thallus without pruina, apothecia immersed, flesh-colored (beige), asci 8-spored ..... ***A. vulcanica***
18. Thallus with soredia or isidia, containing aspicilin ..... ***Circinaria leprosescens***
    Thallus without soredia or isidia..... **19**
19. Thallus areolate, thick (0.4–0.6 mm thick) **20**
    Thallus areolate to rimose or subsquamulose, thin or thick..... **23**
20. Thallus with pruina **21**
    Thallus without pruina **22**
21. Apothecia 0.2–0.5 mm diam., disk blackish with grayish pruina ..... ***A. subgoettweigensis***
    Apothecia 0.4–1.0 mm diam., disk gray to brownish with white pruina .. ***A. submamillata***
22. Thallus dark gray to lead-gray, apothecia 0.25–0.3 mm diam., ascospores 17–22 × 8–12 μm, conidia 13–17 × 0.7–0.8 μm.... ***A. pseudoabbasiana***

Thallus light gray to slightly brownish gray, apothecia 0.9–1 mm diam., ascospores 19–22 × 12–16 μm, conidia 3.5–5.5 × 0.7–0.8 μm ..... ***A. subgeographica***

23. Thallus with pruina **24**
    Thallus without pruina **26**
24. Thallus thick (up to 0.4 mm thick), white to cream white, or pale yellow, apothecia occurring one to three per areole, ascospores 9–14 × 4–6.5 μm ..... ***Lecanora oreinoides***
    Thallus thin, white, light gray or pale brown, apothecia occurring one to two per areole, ascospores 16–24 × 9–14 μm **25**
25. Apothecia 0.2–0.5 mm diam., thallus white or pale brown ..... ***A. umbrinella***
    Apothecia 0.5–0.9 mm diam., thallus white-gray to light gray..... ***A. pseudovulcanica***
26. Thallus subsquamulose in center, ascospores 14–30 × 7–16 μm, containing aspicilin..... ***Circinaria caesiocinerea***
    Thallus areolate to rimose only, ascospores 10–23 × 6–14 μm, not containing aspicilin.....**27**
27. Thallus bluish gray, apothecia mostly solitary per areole, rarely two, 0.2–0.5 mm diam., asci 100–132 × 22–26 μm, ascospores 16–20 × 10–14 μm..... ***A. asteria***
    Thallus pale gray to white, apothecia occurring one to several per areole, 0.2–1.7 mm diam., asci 64–72 × 17–27 μm, ascospores 10.5–23 × 6–13.5 μm, containing stictic acid ..... ***A. humida***

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