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Two new foliicolous species of *Strigula* (Strigulaceae, Strigulales) in Korea offer insight in phorophyte-dependent variation of thallus morphology

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Abstract

Foliicolous lichens grow on the surface of living leaves in tropical and subtropical forests. Compared to a large number of species of foliicolous lichens reported from other regions in tropical and subtropical Asia, only six species of the otherwise abundant genus *Strigula* have been registered from South Korea so far. Three of these, morphologically identified as *S. concreta*, *S. macrocarpa*, and *S. smaragdula*, had previously been shown to share near-identical ITS sequences, casting doubt about the usefulness of this marker for species delimitation in the genus *Strigula*. To shed light on this conundrum, we surveyed the diversity of the genus *Strigula* in the Gotjawal forest area on Jeju Island south of mainland Korea, where the climate and vegetation are suitable for foliicolous lichens. As the result of a combined analysis of phenotype and molecular data of the ITS fungal barcoding marker, we found that material morphologically similar to known species formed two strongly supported clades, representing two species new to science, *S. depressa* Woo, Lücking & Hur *sp. nov.* and *S. multiformis* Woo, Lücking & Hur *sp. nov.*, which are described herein. *Strigula multiformis* included the four previously sequenced specimens identified as *S. concreta*, *S. macrocarpa*, and *S. smaragdula*. A detailed analysis of morphological and anatomical characters revealed that all specimens of *S. multiformis* were anatomically uniform but varied in thallus morphology, mostly resembling *S. smaragdula* but with some forms similar to *S. concreta* and *S. macrocarpa*, explaining the previous misidentifications. This variation was found to be driven by leaf characters of the phorophyte species, as these apparently influence the morphology of the subcuticular thalli.

Keywords: Evergreen broad-leaved forest, Foliicolous lichens, Gotjawal forest, Jeju Island, *Strigula*

Introduction

Foliicolous lichens inhabit the surface of living leaves mainly in the tropics. More than 800 species have been recognized worldwide, with some extending into or occurring preferably in subtropical regions (Lücking 2008). In eastern Asia, a relatively large number of foliicolous lichens have been reported from tropical and subtropical regions in China and Japan, with 70 and 80 species, respectively (Thor *et al.* 2000; Aptroot *et al.* 2003; Jiang *et al.* 2017a). Of the 17 species of *Strigula* reported from China, all were found in the largely tropical provinces (south of the Tropic of Cancer) of Guangdong, Guanxi, and Yunnan, as well as Hongkong and the islands of Hainan and Taiwan, whereas only two, *S. melanobapha* (Kremp.) Santesson (1952: 188) and *S. smaragdula* Fries (1830: 550) extended further north into subtropical areas up to about 30° latitude, namely the provinces of Fujian, Guizhou, Hubei, and Hunan (Jiang *et al.* 2017b). In contrast, all nine species of *Strigula* reported from Japan (Thor *et al.* 2000) are from subtropical areas north of the Tropic of Cancer, between about 24° and 30° latitude.

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From Korea, thus far six foliicolous species belonging to *Strigula* were reported (Jayalal *et al.* 2013), a remarkable number considering that this area is at about 33° latitude, about 8° further north than e.g. the subtropical region of Florida and at the level of South Carolina, from which so far only one foliicolous species of *Strigula*, *S. smaragdula*, is known (Perlmutter *et al.* 2012). On the other hand, *S. buxi* Chodat (1912: 246) and *S. nitidula* Montagne (1845: 93) in Europe occur up to 45–48° north, and several species are found in Macaronesia at about 33° north (Roux & Sérusiaux 2004). The six species previously reported from Korea are: *S. concreta* (Fée) Santesson (1852: 177), *S. macrocarpa* Vainio (1923: 20), *S. melanobapha*, *S. nemathora* Montagne (1845: 96), *S. subelegans* Vainio (1923: 20), and *S. smaragdula* (Moon & Aptroot 2009; Jayalal *et al.* 2013). All were found on Jeju Island, likely due to its climatic conditions that favor the growth of subtropical evergreen broad-leaved forest.

Strigula is an enigmatic lichen-forming fungal genus belonging to its own family and order, Strigulaceae (Strigulales), in class Dothideomycetes and phylum Ascomycota (Hyde *et al.* 2013). The genus name *Strigula* was established by Fries (1823, 1830), although some of its species had already been described in other genera before that time (Santesson 1952). Currently, 70 species are recognized world-wide (Lücking *et al.* 2017), most of them growing on living leaves (Lücking 2008), while some also inhabit bark or rock surfaces (Harris 1995; McCarthy1997).

Because of intra-specific variation of thallus morphology, the identification of *Strigula* species solely based on morphological characteristics can lead to misidentifications (Lücking 2008). Phylogenetic analysis of molecular data is expected to better delimit *Strigula* species and unravel cryptic diversity. Thus, several new species of *Strigula* phenotypically resembling *S. antillarum* (Fée) Müller (1885: 379), *S. nitidula*, and *S. smaragdula* were discovered through molecular phylogenetic analysis in China (Jiang *et al.* 2016; 2017a, b). Among the six species reported from Korea, three species (*S. concreta, S. macrocarpa, S. smaragdula*) were previously analyzed phylogenetically and found to share near-identical ITS sequences (Jayalal *et al.* 2013), while three other species (*S. melanobapha, S. nemathora*, and *S. subelegans*) were identified based solely on phenotype (Moon & Aptroot 2009). Given that the studies in China revealed a high level of cryptic speciation in foliicolous *Strigula*, we suspected that the previously sequenced material from Korea did not actually represent the identified taxa, but other undescribed, phenotypically cryptic species. We therefore decided to explore this enigma based on a much expanded sampling on the island of Jeju, specifically the Gotjawal forest area. Our much expanded sampling not only confirmed our hypothesis that the clade previously identified with the names *S. concreta, S. macrocarpa,* and *S. smaragdula* represents a previously unrecognized species, but also revealed two additional novel lineages. Two of the three lineages are herein described as new, under the names *S. depressa* and *S. multiformis*.

Material & methods

Study area

The material was collected from Gotjawal forest locations in Jeju Island, South Korea (Fig. 1) and, after drying and processing, deposited in the Korean Lichen Research Institute (KoLRI). 'Gotjawal' is a compound word from the Jeju dialect, 'Got' meaning forest and 'Jawal' bush. It defines a place where trees and vines are tangled up and cluttered like bushes according to the Jeju Special Self-Government Province (2009: 83). The Gotjawal forest, composed of several isolated localities on the island features evergreen broad-leaved forest ideal for the growth of foliicolous *Strigula* species and other chiefly tropical lichens. The fauna and flora of this area is highly diverse and includes many endangered species, being quite distinct in composition from mainland Korea and even other areas on Jeju Island (Jayalal *et al.* 2013).

Morphological analysis

Morphological characteristics of specimens were examined using a Nikon SMZ645 stereomicroscope (Tokyo, Japan). Anatomical characteristics were examined using a Nikon Eclipse E200 compound microscope (Tokyo, Japan). Microphotographs were taken with an Olympus BX53 camera (Tokyo, Japan) and a JENOPTIK ProgRes® C14plus camera (Jena, Germany).

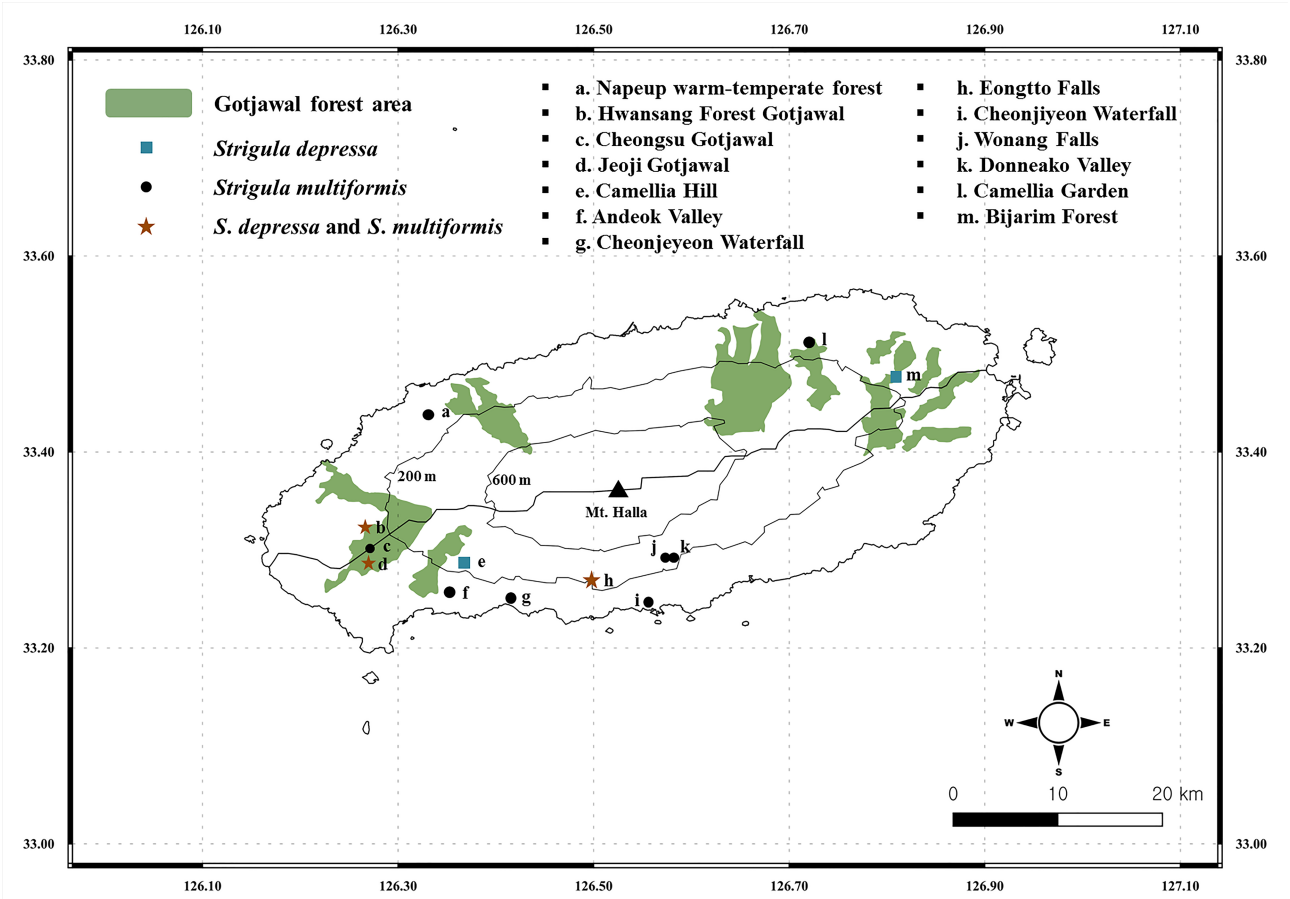

FIGURE 1. Geological distributions of *Strigula* in Jeju Island. The green region indicates Gotjawal forest. Locations of *Strigula depressa* and *Strigula multiformis* are marked with blue squares and black dots, respectively, whereas brown stars indicate the region where both *S. depressa* and *S. multiformis* were collected.

Chemical analysis

Secondary metabolites of lichen specimens were analysed by thin-layer chromatography (TLC), using solvent systems A and C according to Orange *et al.* (2010).

Molecular data and phylogenetic analysis

Genomic DNA was extracted from the fresh specimens using the KCl method (Park *et al.* 2014). The ribosomal nuclear internal transcribed spacer (ITS) region was amplified with ITS5 (5'- GGAAGTAAAAGTCGTAACAAGG -3') and ITS4 (5'- TCCTCCGCTTATTGATATGC -3') primers (White *et al.* 1990) using *AccuPower*® PCR PreMix (Bioneer, Daejeon, South Korea). PCR amplification was conducted using GeneAmp 2720 Thermal Cycler machine (Applied Biosystems, California, USA) under the following conditions: initial cycle of 5 min at 94°C, followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C, 10 min at 72°C, and then finally 10 min at 72°C. PCR products were checked by 1% agarose gel electrophoresis and purified using MEGAquick-spin™ Plus Total Fragment DNA Purification Kit (iNtRON Biotechnology, Seongnam, South Korea) following the manufacturer's instructions. Sequencing was performed using ABI Prism 3730xl analyzer (Applied Biosystems, California, USA) by Genotech (Daejeon, South Korea).

The sequences were checked, assembled, and edited using MEGA 5 (Tamura *et al.* 2011). Multiple alignment was performed using MAFFT 7 with the L-INS-i algorithm (Katoh & Standley 2013) and the few ambiguously aligned positions were adjusted manually. The phylogeny was constructed based on a maximum likelihood (ML) and Bayesian inference (BI) methods. ML analysis was conducted using RAxML 8.1.3 (Stamatakis 2014) with the universal GTRGAMMA model and 1000 bootstrap replications. For BI analysis, the best model of nucleotide substitution was tested using jModeltest v. 2.1.10 (Darriba *et al.* 2012) based on the Bayesian information criterion (BIC) with the

universal GTRGAMMA. BI analysis was conducted for 10 million generations with every 100th sampling based on Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method using MrBayes v. 3.2.6 (Ronquist *et al.* 2012) after discarding the first 25% of the sampled trees as burn-in, a final consensus tree was constructed using the 50% majority rule. *Kirschsteiniothelia tectonae* (KU144916) and *K. rostrate* (KY697280) were chosen as outgroups based on a previous study (McCarthy 1997). All newly generated sequences were deposited in GenBank (Table 1).

Results and discussion

Phylogenetic analyses

The phylogenetic tree based on the ML analysis, with Bayesian posterior probabilities added, showed that the sequenced specimens formed two monophyletic clades with high support (Fig. 2). One clade, here introduced as *S. depressa*, emerged rather basally on a long branch, whereas the other, here named *S. multiformis*, was supported sister to *Strigula smaragdula* from China. The type of the latter is from Nepal (Santesson 1952), which renders the correct identification of the Chinese specimens rather likely and supports recognition of the Korean material as new species. The most common species on Jeju Island, *S. multiformis*, is indeed morphologically similar to *S. smaragdula* and cannot be distinguished based on phenotype alone (see below). *Strigula depressa* is not closely related to any of the other sequences species; phenotypically, it would also key out as *S. smaragdula* s.lat., but the size of its ascospores and macroconidia is in the lower range of the latter and the perithecia are concentrically arranged and feature an exposed black upper portion (see below).

All *Strigula* sequences previously reported from Korea (Jayalal *et al.* 2013) were included in the *S. multiformis* clade (Fig. 2), which demonstrates that these specimens had previously been misidentified with the names *S. concreta* (KoLRI 016406), *S. macrocarpa* (KoLRI 016366), and *S. smaragdula* (KoLRI 016344). Careful analysis of morphological variation in the *S. multiformis* clade showed that thallus morphology was strongly dependent on leaf characteristics provided by the different phorophytes, with most specimens resembling a *S. smaragdula* morphology, but on particular phorophytes the species looked more like *S. concreta* (with exposed perithecia and crenulate thallus margins) or *S. macrocarpa* (with rather large perithecia on an otherwise thin thallus; see below). As a consequence, the names *S. concreta*, *S. macrocarpa*, and *S. smaragdula* must be removed from the list of lichens reported from Korea, as their previous report was based on these misidentifications.

Given the diversity of novel species of *Strigula* revealed through recent molecular studies in China, with several new taxa morphologically similar to *S. concreta, S. subelegans* and *S. smaragdula* (Jiang *et al.* 2016, 2017a, b), we believe that further studies will also reveal additional new taxa in the Korean foliicolous lichen biota.

Strigula depressa* Woo, Lücking & Hur, *sp. nov. (Fig. 3)
MycoBank registration number: #MB831529
ITS barcoding sequence GenBank accession number (holotype): MK118881

Type:—SOUTH KOREA. Jeju-do (Province): Hwansang Forest Gotjawal; 33°19'24.54" N, 126°15'52.89" E, elev. 141 m, on leaves of *Distylium racemosum* Siebold & Zucc., 27 June 2018, *Woo 180256* (holotype: KoLRI 049975).

Diagnosis:—Differing from *Strigula smaragdula* in the concentrically arranged, erumpent perithecia that leave the rather distinctly delimited upper portion exposed and black and somewhat flattened.

Description:—Thallus subcuticular, dispersed into rounded to irregular patches, 2–5 mm across and 25–65 µm thick, with entire to minutely crenulate margins, bright green to olive green. Photobiont a species of *Cephaleuros*, cells angular-rounded, 7.5–15 × 5–12.5 µm, in irregular plates forming one to several layers. Perithecia more or less concentrically arranged, hemispherical, erumpent, laterally covered by algiferous thallus tissue but leaving a broad, somewhat flattened ostiolar area free and black, 0.15–0.25 mm diam. and 150–200 µm high. Excipulum prosoplectenchymatous, 10–25 µm thick, colorless to brown. Involucrellum carbonaceous, 30–80 µm thick, black. Paraphyses unbranched. Asci obclavate, 45–60 × 10–12.5 µm. Ascospores biseriate, fusiform, 1-septate, with constriction at the septum, 15–17.5 × 3.8–5 µm, 3–4.7 times as long as broad. Pycnidia erumpent, wart-shaped, 0.1–0.25 mm; macroconidia bacillar, 1-septate, 10–12.5 × 2.5–3.8 µm. Microconidia not seen.

Chemistry:—No lichen substances detected by TLC.

TABLE 1. Voucher and ITS sequence information of specimens deposited in GenBank. The four specimens previously identified as *S. concreta*, *S. macrocarpa*, and *S. smaragdula* are indicated in quotation marks.

	Species	Voucher	GenBank Accession No.
1	*Strigula acuticonidiarum*	HMAS:L0138045	KY100290
2	*Strigula acuticonidiarum*	HMAS:L0138051	KY100294
3	*Strigula antillarum*	HMAS:L0130573	KY100289
4	*Strigula antillarum*	HMAS:L0137209	KX216696
5	*Strigula depressa*	KoLRI049956	**MK118880**
6	*Strigula depressa*	KoLRI049975	**MK118881**
7	*Strigula depressa*	KoLRI049987	**MK118882**
8	*Strigula guangxiensis*	HMAS:L0138040	KY100301
9	*Strigula guangxiensis*	HMAS:L0138042	KY100305
10	*Strigula macrocarpa*	HMAS:L0137663	KY083729
11	*Strigula macrocarpa*	HMAS:L0137665	KY083732
12	*Strigula "concreta"*	KoLRI016406	KF553661
13	*Strigula "macrocarpa"*	KoLRI016366	KF553662
14	*Strigula multiformis*	KoLRI049962	**MK118870**
15	*Strigula multiformis*	KoLRI049964	**MK118871**
16	*Strigula multiformis*	KoLRI049986	**MK118872**
17	*Strigula multiformis*	KoLRI049991	**MK118873**
18	*Strigula multiformis*	KoLRI049941	**MK118874**
19	*Strigula multiformis*	KoLRI049947	**MK118875**
20	*Strigula multiformis*	KoLRI049959	**MK118876**
21	*Strigula multiformis*	KoLRI049983	**MK118877**
22	*Strigula multiformis*	KoLRI049806	**MK118878**
23	*Strigula multiformis*	KoLRI049808	**MK118879**
24	*Strigula "smaragdula"*	KoLRI016344	KF553663
25	*Strigula "smaragdula"*	LFF016344	KF553664
26	*Strigula sinoaustralis*	HMAS:L0137203	KX216699
27	*Strigula sinoaustralis*	HMAS:L0137204	KX216698
28	*Strigula smaragdula*	HMAS:L0138066	KY100297
29	*Strigula smaragdula*	HMAS:L0130621	KY100298
30	*Strigula smaragdula*	HMAS:L0138068	KY100299
31	*Strigula smaragdula*	HMAS:L0138067	KY100300
32	*Strigula sp.*	HMAS:L0137656	KY083734
33	*Strigula sp.*	HMAS:L0137658	KY083738
34	*Strigula prasina*	HMAS:L0137212	KX216701
35	*Strigula prasina*	HMAS:L0137213	KX216700
36	*Kirschsteiniothelia rostrata*	MFLU 15-1154	KY697280
37	*Kirschsteiniothelia tectonae*	MFLUCC 12-0050	KU144916

Newly generated sequences are in boldface.

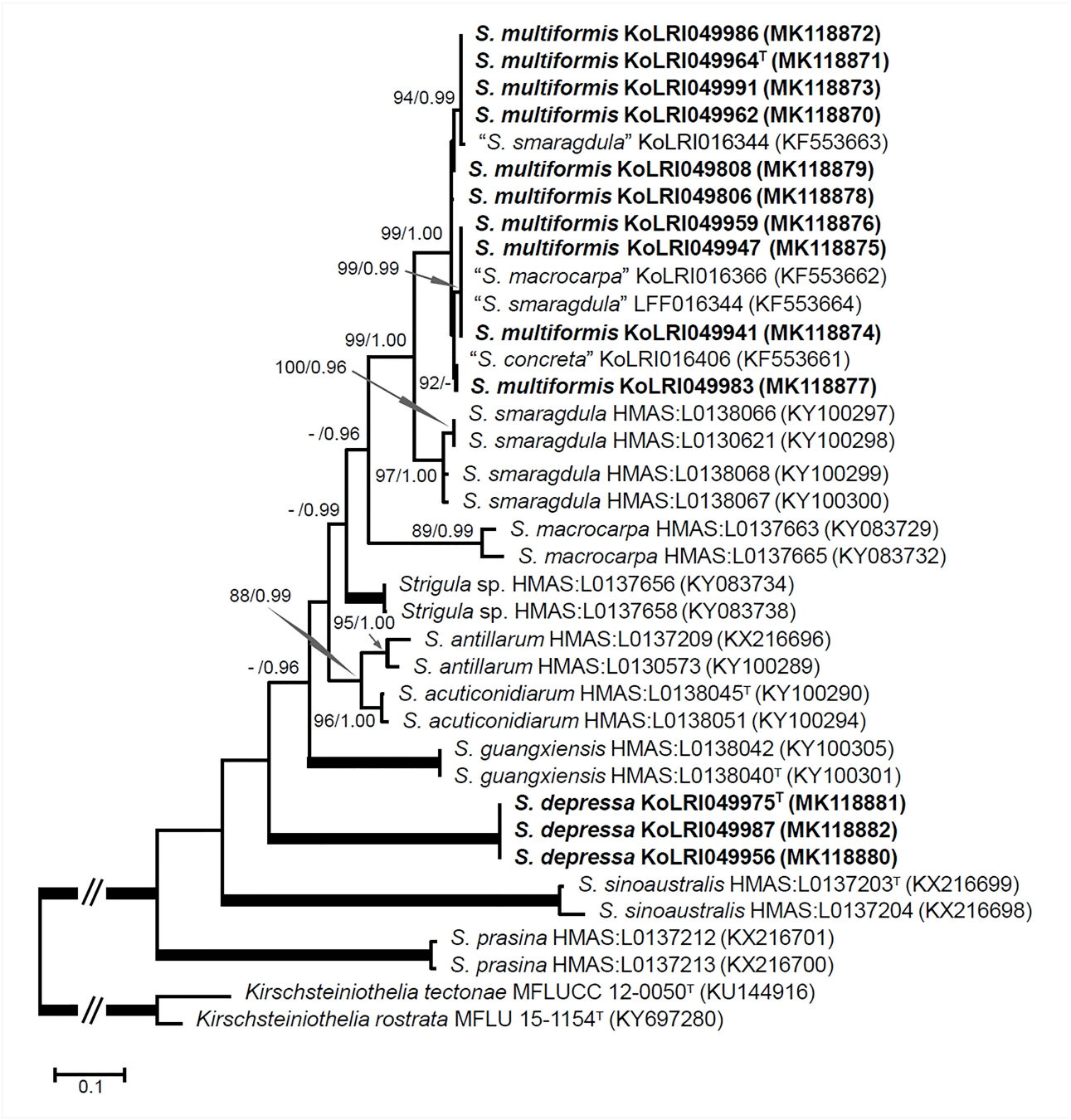

FIGURE 2. Phylogenetic tree based on maximum likelihood (ML) analysis using the ITS region. Support values are presented above branches, with ML bootstrap support (BS) >70 and Bayesian posterior probabilities (PP) > 0.95 indicated. Thickened branches indicate absolute support value (ML/PP = 100/1.00). The scale bar indicates the number of substitutions per nucleotide site. New sequences generated in this study are represented in bold. The letter "T" indicates the corresponding holotype sequence. The four previously sequenced specimens identified as *S. concreta*, *S. macrocarpa*, and *S. smaragdula* (Jayalal *et al.* 2013) are indicated in quotation marks.

Distribution and Ecology:—*Strigula depressa* was found growing on living leaves in humid and shaded areas of the evergreen broad-leaved Gotjawal forest.

Etymology:—Named for its black, largely immersed perithecia with a depressed top.

Notes:—*Strigula depressa* would key out under *S. smaragdula* s.lat. (Santesson 1952), with which it agrees in the often bright green, somewhat thickened thallus, the ascospore type, and the rather large, 1-septate macroconidia. However, it differs in the concentrically arranged perithecia which are largely immersed but have the upper, black portion exposed and somewhat flattened. The new species showed the following variation depending on leaf type (Fig. 3): specimens growing on *Machilus thunbergii* (Fig. 3E, F) most closely resembled *S. smaragdula* in the bright green, rather thick thalli with entire margins. Individuals on leaves of *Actinodaphne lancifolia* (Fig. 3G, H) produced

thinner thalli with minutely crenulate margins and slightly more greyish color, akin towards *S. concreta*. A third morphotype was found on leaves of e.g. *Distylium racemosum* (Fig. 3A, B), with rather thin, olive-green thalli somewhat resembling *Strigula nitidula*. Yet, anatomically the various morphotypes were uniform.

Specimens examined:—SOUTH KOREA. Jeju-do (Province): Bijarim Forest, elev. 170 m, 33°29'15.09" N, 126°48'30.97" E, on leaves of *Machilus thunbergii* Siebold & Zucc., 10 July 2018, *Woo 180258* (KoLRI 049977) (Fig. 3E, F). Camellia Hill, elev. 262 m, 33°17'23.71" N, 126°22'08.55" E, on leaves of *Daphniphyllum macropodum* Miq., 25 June 2018, *Woo 180237* (KoLRI 049956). Donneako Valley, elev. 271 m, 33°18'0.29" N, 126°34'55.88" E, on leaves of *Camellia japonica* L., 10 March 2018, *Hur & Woo 180268* (KoLRI 049987) (Fig. 3D). Eongtto Falls, elev. 185 m, 33°16'03.45" N, 126°29'53.59" E, on leaves of *Actinodaphne lancifolia* (S. *et* Z.) Meisn., 21 June 2018, *Woo 180225* (KoLRI 049944) (Fig. 3G, H); elev. 180 m, 33°16'02.94" N, 126°29'52.78" E, on leaves of *Ilex* sp., 21 June 2018, *Woo 180226* (KoLRI 049945) (Fig. 3C). Hwansang Forest Gotjawal, elev. 141 m, 33°19'24.54" N, 126°15'52.89" E, on leaves of *Distylium racemosum* Siebold & Zucc., 27 June 2018, *Woo 180255* (KoLRI 049974) (Fig. 3B). Jeoji Gotjawal, elev. 114 m, 33°17'23.58" N, 126°16'26.63" E, on leaves of *Machilus* sp., 27 June 2018, *Woo 180254* (KoLRI 049973).

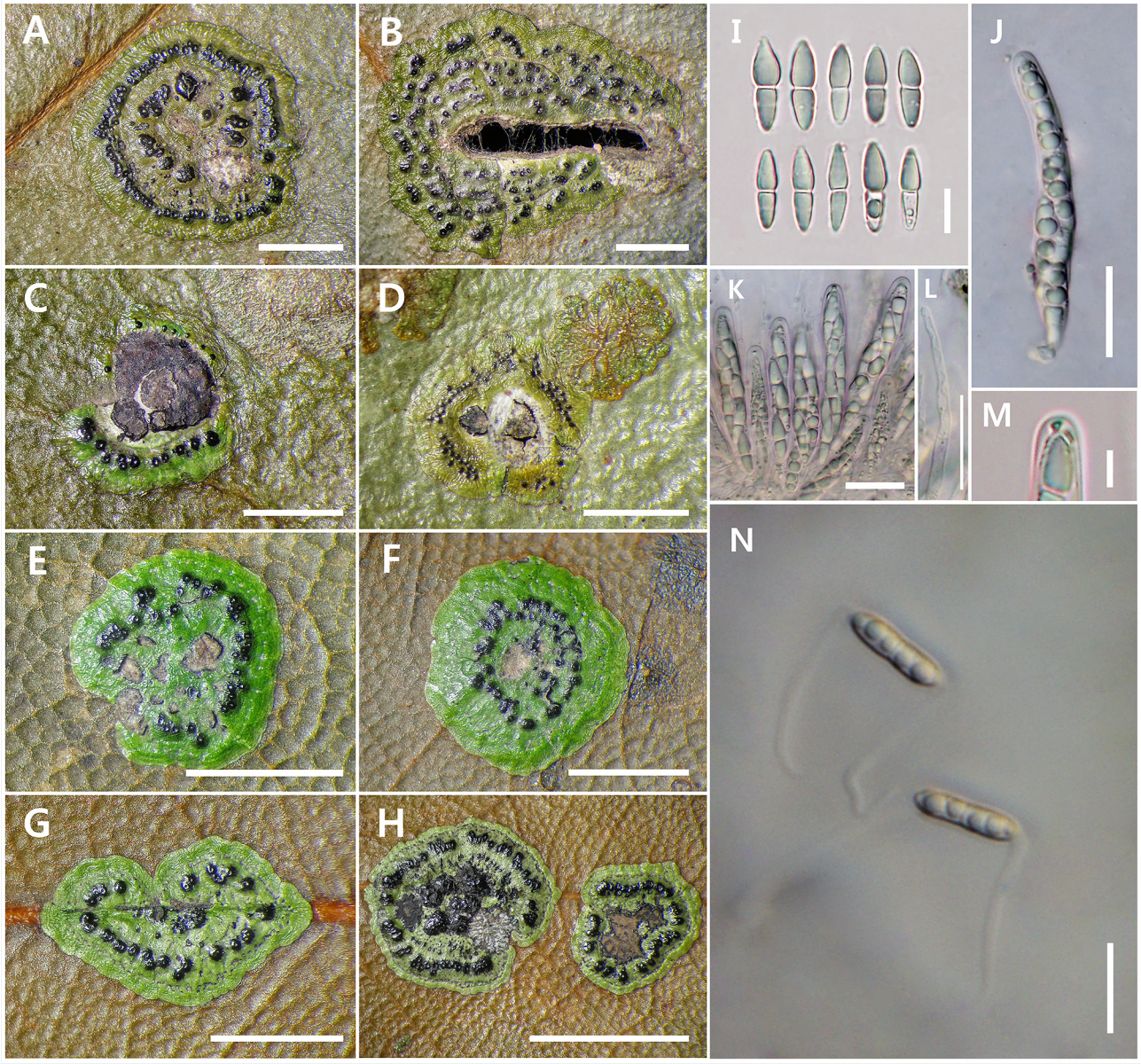

FIGURE 3. Morphological characteristics of *Strigula depressa* (A: holotype). A–H; growing on various phorophytes, namely *Distylium racemosum* (A, B), *Ilex* sp. (C), *Camellia japonica* (D), *Machilus thunbergii* (E, F) and *Actinodaphne lancifolia* (G, H). I; ascospores. J; ascus. K; asci. L; paraphyses. M; ascus apex. N; macroconidia with gelatinous appendaxes. Scale bars: A–H = 2 mm; I & N = 10 µm; J, K, and L = 20 µm; M = 5 µm.

Strigula multiformis* Woo, Lücking & Hur, *sp. nov. (Figs 4, 5)
MycoBank registration number: #MB831530
ITS barcoding sequence GenBank accession number (holotype): MK118871

Type:—SOUTH KOREA. Jeju-do (Province): Napeup warm-temperate forest, 33°25'59.49" N, 126°19'49.24" E, elev. 89 m, on leaves of *Machilus thunbergii* Siebold & Zucc., 26 June 2018, *Woo 180245* (holotype: KoLRI 049964).

Diagnosis:—Similar to *Strigula smaragdula* but differing in the slightly smaller ascospores and conidia.

Morphology:—Thallus subcuticular, continuous or dispersed into rounded to irregular, partly confluent patches, 2–7 mm across and 50–100 µm thick, with entire to crenulate margins, bright green to rarely somewhat olive-green, often with concentric color variation, with the inner parts paler to whitish. Photobiont a species of *Cephaleuros*, cells angular-rounded, 5–12.5 × 2.5–7.5 µm, in irregular plates forming one to several layers. Perithecia hemispherical, erumpent, typically covered by algiferous thallus tissue up to the ostiolum, rarely the upper portion exposed and black 0.3–0.7 mm diam. and 150–225 µm high. Excipulum prosoplectenchymatous, 10–12.5 µm thick, colorless. Involucrellum carbonaceous, 30–80 µm thick, black. Paraphyses unbranched. Asci obclavate, 50–75 × 7.5–12.5 µm. Ascospores biseriate, fusiform, 1-septate, with constriction at the septum, 15–20 × 3.8–5 µm, 3–4 times as long as broad. Pycnidia erumpent, wart-shaped, 0.1–0.17 mm; macroconidia bacillar, 1-septate, 15–17.5 × 2.5–3.8 µm. Microconidia fusiform, non-septate, 3–4 × 1.5–2 µm.

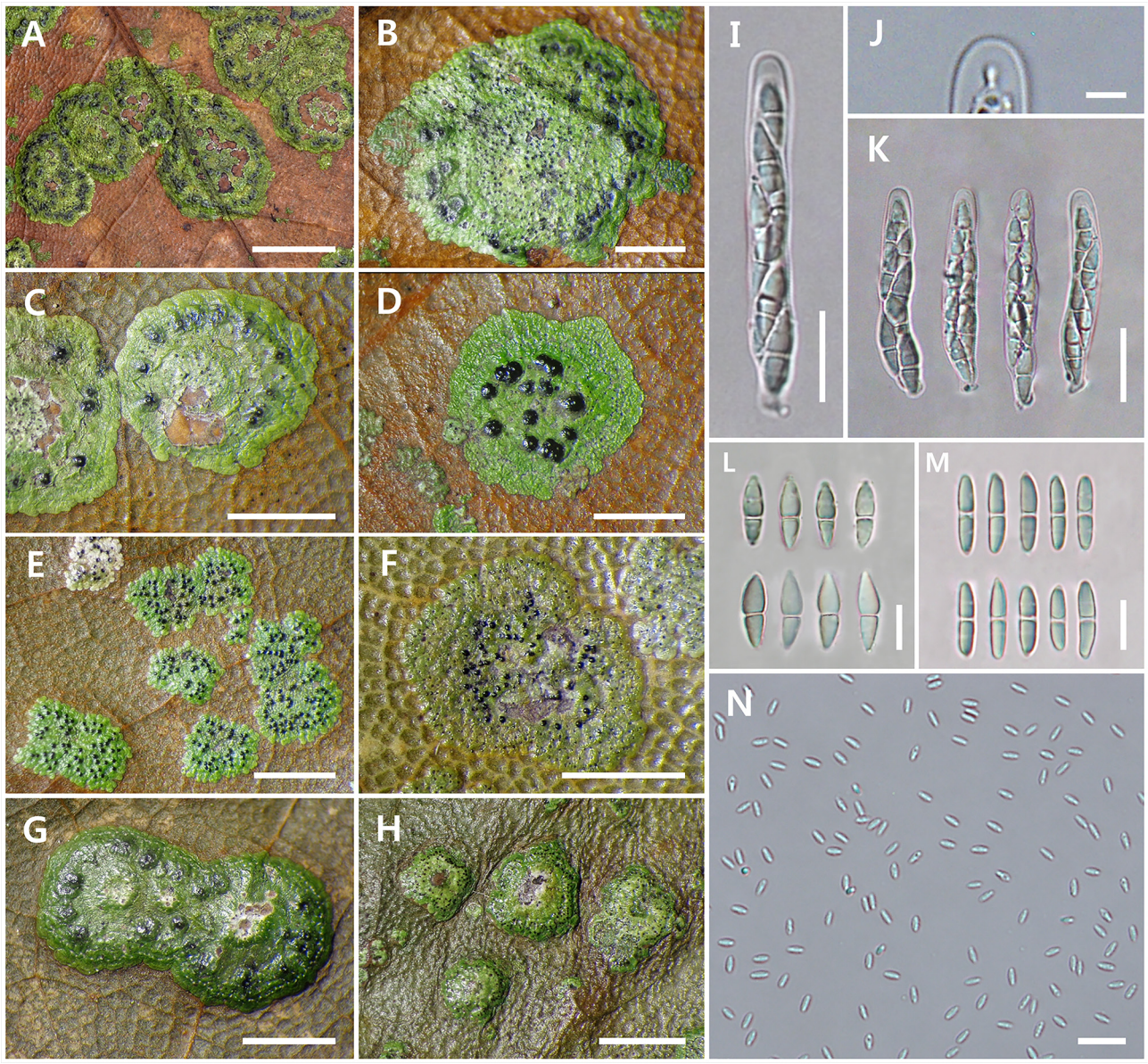

FIGURE 4. Morphological characteristics of *Strigula multiformis* (A, B: holotype; C–H: paratypes). I; ascus. J; ascus apex. K; asci. L; ascospores. M; macroconidia. N; microconidia. Scale bars: A = 5 mm; B–H = 2 mm; I = 20 µm; J = 4 µm; K–N = 10 µm.

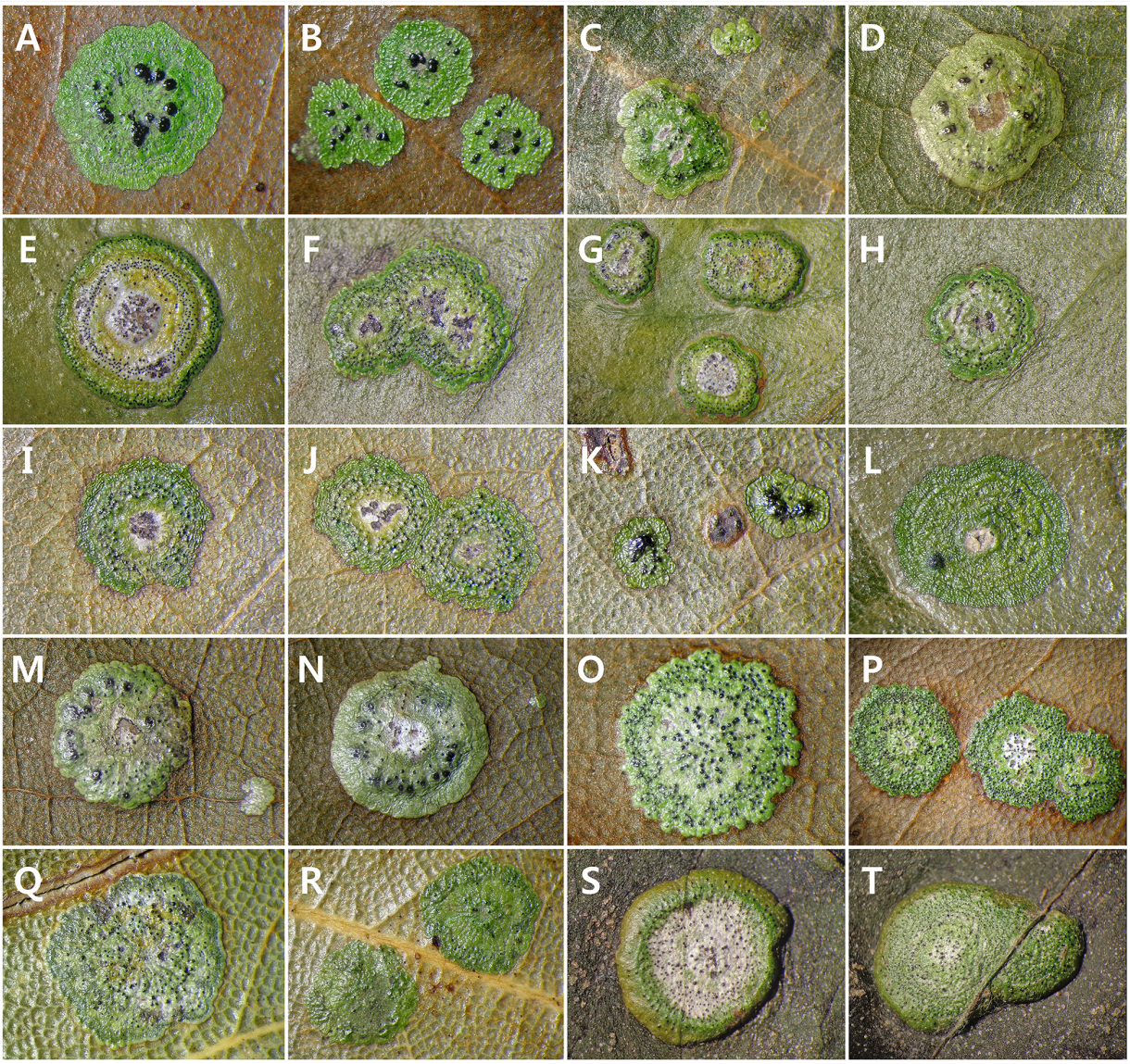

FIGURE 5. Various thallus shapes of *Strigula multiformis* influenced by leaf surface. *Actinodaphne lancifolia* (A, B); *Aucuba japonica* (C, D); *Camellia japonica* (E–H); *Litsea japonica* (I–L); *Machilus japonica* (M, N); *Machilus thunbergii* (O, P); *Machilus* sp. (Q, R); *Piper kadsura* (S, T).

Chemistry:—No lichen substances detected by TLC.

Distribution and Ecology:—*Strigula multiformis* is the most common *Strigula* species growing on living leaves in humid and shaded areas of the evergreen broad-leaved Gotjawal forest.

Etymology:—Named for its variable thallus morphology, influenced by leaf type.

Notes:—*Strigula multiformis* is the most common foliicolous species of the genus in the subtropical forest of Korea. Depending on the phorophyte and leaf type, its thallus morphology varies considerably, although it usually closely resembles *S. smaragdula* (Fig. 4). It can be distinguished from the latter by the slightly smaller ascospores and conidia, although the diagnostic value of these measures remains to be tested in a thorough phylogenetic revision of *S. smaragdula* s.lat. Therefore, at present we consider this a largely cryptic speciation.

The rich material of *Strigula multiformis* allowed for a comprehensive analysis of morphological variation of sequenced specimens depending on leaf type (Fig. 5). On most phorophytes, the species closely resembles a typical *S. smaragdula*, with thickened thallus with somewhat crenulate margins, with often numerous and densely arranged pycnidia, and with characteristic color variation, the inner portions becoming yellowish or paler to whitish. Such thalli were found on e.g. *Aucuba japonica* (Fig. 5C, D), *Camellia japonica* (Fig. 5E–H), *Machilus japonica* (Fig. 5M, N), and *M. thunbergii* (Fig. 5O, P). On *Machilus* sp. (Fig. 5Q, R), the thalli appeared thinner and more crenulate. Quite thin thalli with distinctly crenulate margins, with more exposed perithecia and pycnidia, resembling *S. concreta* or *S.*

nitidula, were found on *Actinodaphne lancifolia* (Fig. 5A, B) and *Litsea japonica* (Fig. 5I–L). On *Piper kadsura* (Fig. 5S, T), the thalli appeared more inflated, with a whitish center, and forming depressions on the leaf underside.

Notably, on leaves of *Actinodaphne lancifolia*, the two new species, *Strigula depressa* and *S. multiformis*, produce rather similar thalli (Fig. 3G, H, 5A, B), although they are phylogenetically only distantly related, a remarkable case of convergent morphogenesis triggered by leaf characteristics. This situation is remarkably similar to what was recently found in the New Zealand endemic *Strigula novae-zelandiae* (Nag Raj) Sérusiaux (1998: 150). Ford *et al.* (2019) discovered three morphotypes on leaves of *Beilschmiedia tarairi*: small thalli with crenulate-lobulate margins and pycnidia aggregated in radiating lines (morphotype A), larger thalli with crenulate-lobulate margins and pycnidia more irregularly dispersed (morphotype B), and thalli with entire margins and pycnidia aggregate in radiating lines (morphotype C). Using ITS data, the authors found that morphotypes A and B represent ontogenetic stages of *S. novae-zelandiae* s.str., whereas morphotype C belonged to an only distantly related species, newly described as *S. oleistrata* M. Ford, Blanchon & de Lange (Ford *et al.* 2019: 272). Also here, the phorophyte appears to shape the morphology of two phylogenetically distinct species in a remarkably convergent manner, resulting in a shared unique, yet phylogenetically cryptic disposition of the pycnidia.

The underlying causes of this phenomenon are not known but seem to relate to characteristics of the leaf cuticle and patterns of leaf venation. Thus, Lücking (2008) reported similar variation for *Strigula concreta* and *S. nemathora*, depending on the nature of the phorophyte. Given the diversity of phorophytes in the tropics, coupled with the apparently high degree of cryptic speciation of these lichen fungi, this paints a complex picture of diversification and morphological plasticity in foliicolous representatives of *Strigula*, rendering accurate identification based on morphology alone difficult.

Specimens examined:—SOUTH KOREA. Jeju-do (Province): Andeok Valley, elev. 124 m, 33°15'23.06" N, 126°21'16.61" E, on leaves of *Aucuba japonica* Thunb., 20 June 2018, *Woo 180218* (KoLRI 049937) (Fig. 5C); elev. 33 m, 33°15'21.4" N, 126°21'16.8" E, on leaves of *Machilus* sp., 28 Feburary 2018, *Hur & Woo 180008* (KoLRI 046897) (Fig. 4F; Fig. 5Q); elev. 92 m, 33°15’24.28” N, 126°21’07.74” E, 20 June 2018, *Woo 180223* (KoLRI 049942) (Fig. 4G); elev. 91 m, 33°15'24.72" N, 126°21'07.62" E, on leaves of *Machilus thunbergii* Siebold & Zucc., 20 June 2018, *Woo 180222* (KoLRI 049941) (Fig. 5O, P). Bijarim Forest, elev. 131 m, 33°29'27.09" N, 126°48'37.15" E, on leaves of *Actinodaphne lancifolia* (Siebold & Zucc.) Meisn., 10 July 2018, *Woo 180259* (KoLRI 049978) (Fig. 4D; Fig. 5A, B). Camellia Garden, elev. 85 m, 33°30'52.82" N, 126°42'34.19" E, on leaves of *Aucuba japonica* Thunb., 10 July 2018, *Woo 180265* (KoLRI 049984) (Fig. 5D); elev. 108 m, 33°30'43.87" N, 126°42'43.22" E, on leaves of *Camellia japonica* L., 10 July 2018, *Woo 180262* (KoLRI 049981) (Fig. 4H); elev. 77 m, 33°30'52.90" N, 126°42'31.77" E, 10 July 2018, *Woo 180264* (KoLRI 049983) (Fig. 5H). Cheongsu Gotjawal, elev. 115 m, 33°17'58.79" N, 126°16'13.24" E, on leaves of *Machilus* sp., 25 June 2018, *Woo 180240* (KoLRI 049959) (Fig. 4E). Cheonjeyeon Waterfall, elev. 45 m, 33°15'09.43" N, 126°33'30.58" E, on leaves of *Camellia japonica* L., 20 June 2018, *Woo 180224* (KoLRI 049943) (Fig. 5G); elev. 0 m, 33°14'43.93" N, 126°33'30.58" E, on leaves of *Camellia japonica* L., 21 June 2018, *Woo 180228* (KoLRI 049947) (Fig. 5E, F); elev. 6 m, 33°14'44.51" N, 126°33'29.61" E, on leaves of *Litsea japonica* Mirb., 21 June 2018, *Woo 180229* (KoLRI 049948) (Fig. 5K). Hwansang Forest Gotjawal, elev. 132 m, 33°19'23.00" N, 126°15'52.27" E, on leaves of *Litsea japonica* Mirb., 27 June 2018, *Woo 180257* (KoLRI 049976) (Fig. 5L). Jeoji Gotjawal, elev. 167 m, 33°19'12.25" N, 126°17'10.93" E, on leaves of *Machilus* sp., 24 March 2018, *Hur & Woo 180124* (KoLRI 046808); elev. 167 m, 33°19'12.25" N, 126°17'10.93" E, on leaves of *Machilus japonica* Siebold & Zucc., 24 March 2018, *Hur & Woo 180122* (KoLRI 049806). Napeup warm-temperate forest, elev. 94 m, 33°26'01.54" N, 126°19'52.89" E, on leaves of *Litsea japonica* Mirb., 26 June 2018, *Woo 180249* (KoLRI 049968) (Fig. 5I, J); elev. 90 m, 33°26'00.25" N, 126°19'50.52" E, on leaves of *Machilus* sp., 26 June 2018, *Woo 180247* (KoLRI 049966) (Fig. 4C); elev. 97 m, 33°26'02.99" N, 126°19'46.86" E, on leaves of *Machilus thunbergii* Siebold & Zucc., 11 March 2018, *Hur & Woo 180272* (KoLRI 049991) (Fig. 5R); elev. 97 m, 33°26'02.99" N, 126°19'46.86" E, 22 June 2018, *Woo 180267* (KoLRI 049986); elev. 91 m, 33°26'02.49" N, 126°19'46.82" E, on leaves of *Piper kadsura* (Choisy) Ohwi, 26 June 2018, *Woo 180243* (KoLRI 049962) (Fig. 5S, T). Wonang Falls, elev. 287 m, 33°18'02.63" N, 126°34'46.14" E, on leaves of *Machilus japonica* Siebold & Zucc., 22 June 2018, *Woo 180235* (KoLRI 049954) (Fig. 5M, N).

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References

Aptroot, A., Ferraro, L., Lai, M.J., Sipman, H. & Sparrius, L. (2003) Foliicolous lichens and their lichenicolous ascomycetes from Yunnan and Taiwan. *Mycotaxon* 88: 41–47.

Chodat, R. (1912) Lichens épiphylles sur les buis de la forêt de Coudré (Lac Leman). *Bulletin de la Société Botanique de Genève* 4: 246.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.
https://doi.org/10.1038/nmeth.2109

Ford, M., Blanchon, D.J., Veale, A., Doyle, E.J., Rolfe, J.R. & de Lange, P.J. (2019) Hidden in plain sight – a new *Strigula* species segregated from *Strigula novae-zelandiae* (Lichenized Ascomycota: Strigulaceae). *Phytotaxa* 424: 267–281.
https://doi.org/10.11646/phytotaxa.424.5.1

Fries, E.M. (1823) *Systema Mycologicum. Lund* 2. pp. 276–620.

Fries, E.M. (1830) Eclogae Fungorum praecipue ex herbariis germanorum de scriptorum. *Linnaea* 5: 497–553.

Harris, R.C. (1995) *More Florida lichens*. New York Botanical Garden, 192 pp.

Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansa, H.A., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P.W., Dai, D.Q., Diederich, P., Dissanayake, A.J., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu, Y.X., Lücking, R., Monkai, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I., Shearer, C.A., Seutrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirre-Hudson, B., Alias, S.A., Aptroot, A., Bahkali, A.H., Bezerra, J.L., Bhat, J.D., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., De Hoog, S., Kang, J.C., Knudsen, K., Li, W.J., Li, X., Liu, Z.Y., Mapook, A., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A,. Scheuer, C., Schumm, F., Taylor, J.E., Tian, Q., Tibpromma, S., Wanasinghe, D.N., Wang, Y., Xu, J.C., Yan, J., Yacharoen, S. & Zhang, M. (2013) Families of Dothideomycetes. *Fungal Diversity* 63: 1–313.
https://doi.org/10.1007/s13225-013-0263-4

Jayalal, U., Oh, S.O., Lücking, R., Joshi, S., Kim, J.A., Park, J.S. & Hur, J.S. (2013) Contributions to the foliicolous lichens flora of South Korea. *Mycobiology* 41: 202–209.
https://doi.org/10.5941/MYCO.2013.41.4.202

Jeju Special Self-Government Province. (2009) *Jeju Dialect Dictionary*. 914 pp.

Jiang, S.H., Wei, X.L. & Wei, J.C. (2016) *Strigula sinoaustralis* sp. nov. and three *Strigula* spp. new to China. *Mycotaxon* 131: 795–803.
https://doi.org/10.5248/131.795

Jiang, S.H., Wei, X.L. & Wei, J.C. (2017a) Two new species of *Strigula* (lichenised Dothideomycetes, Ascomycota) from China, with a key to the Chinese foliicolous species. *Mycokeys* 19: 31–42.
https://doi.org/10.3897/mycokeys.19.11174

Jiang, S.H., Wei, X.L. & Wei, J.C. (2017b) A new species and two new records of *Strigula* (lichenized Ascomycota) from China. *Mycoscience* 58: 391–397.
https://doi.org/10.1016/j.myc.2017.05.003

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
https://doi.org/10.1093/molbev/mst010

Lücking, R. (2008) Foliicolous lichenized fungi. *Flora Neotropica Monograph* 103: 1–867.

Lücking, R., Hodkinson, B.P. & Leavitt, S.D. (2017) The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *The Bryologist* 119: 361–417.
https://doi.org/10.1639/0007-2745-119.4.361

McCarthy, P.M. (1997) New and interesting saxicolous species of *Strigula*. *Lichenologist* 29: 513–523.
https://doi.org/10.1006/lich.1997.0109

Moon, K.H. & Aptroot, A. (2009) Pyrenocarpous lichens in Korea. *Bibliotheca Lichenologica* 99: 297–314.
https://doi.org/10.5248/133.127

Montagne, J.F.C. (1845) Criptogamia o plantas cellulares. *In: Ramon de la Sagra: Historia Física, Política y Natural de la Isla de Cuba*. IX. A. Bertrand, Paris.

Müller, J. (1885) Pyrenocarpeae Cubenses a cl. C. Wright lectae. *Botanische Jahrbücher* 6: 375–421.

Orange, A., James, P.W. & White, F.J. (2010) *Microchemical Methods for the Identification of Lichens*. British Lichen Society, London. 101 pp.

Park, S.Y., Jang, S.H., Oh, S.O., Kim, J.A. & Hur, J.S. (2014) An easy, rapid, and cost-effective method for DNA extraction from various lichen taxa and specimens suitable for analysis of fungal and algal strains. *Mycobiology* 42: 311–316.

https://doi.org/10.5941/MYCO.2014.42.4.311

Perlmutter, G.P., Lendemer, J.C., Guccion, J.C., Harris, R.C., Hodkinson, B.P., Kubilius, W.P., Lay, E. & Schaefer, H.P. (2012) A provisional survey of lichen diversity in south-central South Carolina, U.S.A., from the 19th Tuckerman Lichen Workshop. *Opuscula Philolichenum* 11: 104–119.

Roux, C. & Sérusiaux, E. [with collaboration by Bricaud, O. & Coppins, B.J.] (2004) Le genre *Strigula* (Lichens) en Europe et en Macaronésie. [The genus *Strigula* (Lichens) in Europe and Macronesia]. *Bibliotheca Lichenologica* 90: 1–96.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2014) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
https://doi.org/10.1093/sysbio/sys029

Santesson, R. (1952) Foliicolous lichens I. A revision of the taxonomy of the obligately foliicolous, lichenized fungi. *Symbolae Botanicae Upsaliensis* 12 (1): 1–590.

Sérusiaux, E. (1998) Further observations on the lichen genus *Strigula* in New Zealand. *The Bryologist* 101: 147–152.
https://doi.org/10.1639/0007-2745(1998)101[147:FOOTLG]2.0.CO;2

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.
https://doi.org/10.1093/molbev/msr121

Thor, G., Lücking, R. & Matsumoto, T. (2000) The foliicolous lichens of Japan. *Symbolae Botanicae Upsaliensis* 32: 1–72.

Vainio, E.A. (1923) Lichenes Insularum Philippinarum IV. *Annales Academiae Scientiarum Fennicae* Series A, 19: 1–84.

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, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols, a guide to methods and applications.* Academic, San Diego, pp. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1