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Lichenomphalia meridionalis (Hygrophoraceae, lichenized Basidiomycota) new to Asia

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ABSTRACT. – *Lichenomphalia meridionalis*, a lichenized basidiomycete, is reported as new to Asia. It was found on Andosols along a road bank at elevations from 1,200 to 1,900 meters in Nagano and Yamanashi Prefectures, central Japan. Analyses of nrLSU sequence data showed that *L. meridionalis* formed a monophyletic clade together with Japanese and Spanish samples, and the close relationship with *L. grisella* was also confirmed by nrLSU, ITS1, and ITS2. *Lichenomphalia meridionalis* has been known exclusively from Mediterranean countries, but our results show that it could be much more widely distributed than previously thought.

KEYWORDS. – Basidiolichen, *Coccomyxa*, distribution, Japan, molecular phylogeny, taxonomy.

INTRODUCTION

Lichenomphalia Redhead, Lutzoni, Moncalvo & Vilgalys (Hygrophoraceae, Basidiomycota) is a lichenized genus of agaricoid basidiomycetes that currently includes 13 accepted species (Lücking et al. 2017). This genus is characterized by omphalinoid basidiomata with globulose *Botrydina*-type thalli or scale-like *Coriscium*-type thalli, and the fungi are associated with uni-cellular green alga of the genus *Coccomyxa* (Acton 1909, Oberwinkler 2012, Plessl 1963). Molecular phylogenetic studies have supported for the inclusion of *Lichenomphalia* in the family Hygrophoraceae (Lawrey et al. 2009, Lodge et al. 2014).

In Japan, only one species of *Lichenomphalia*, *L. hudsoniana* (H.S. Jenn.) Redhead, Lutzoni, Moncalvo & Vilgalys, has been reported previously (Asahina 1934, Yoshimura & Harada 1986). During the course of mycobiota studies of Japan, we found another species of *Lichenomphalia* in Nagano and Yamanashi Prefectures in central Japan. Based on the morphological data, the specimens were identified as *L. meridionalis* (Contu & La Rocca) P.-A. Moreau & Courtec., a species not previously known from Asia. Here we describe the morphology and anatomy of the Japanese material, and present the results of molecular analyses of newly generated ITS rDNA and nrLSU sequence data.

MATERIALS AND METHODS

Sample Collection. – Specimens were collected in May 2010, July 2012, and the period between June and October 2018 in Japan. Voucher specimens were deposited in the National Museum of Nature and Science (TNS), Tsukuba, Japan.

The locality information given in the paragraphs "*specimen(s) examined*" is basically followed by the original label of specimen housed in TNS. In the case of Japanese locality, however, the current prefecture is additionally shown in the parenthesis following the province.

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Morphological Observation. – Morphology was studied using a dissecting microscope (Olympus SZ61 and Olympus SZX16 equipped with an Olympus DP21 digital camera) and a differential interference contrast microscope (Olympus BX53 equipped with an Olympus DP73 digital camera) for dried material. Anatomy was studied from squash preparations mounted in 3% KOH and stained with 1% Congo red. Basidiospore measurements were made in a water mount and are given as minimum–average–maximum (n = the number of spores measured). The range of the length/width ratio of basidiospores is shown as "Q" and the mean value of the Q is represented as "Qx".

Culture of the Photobiont. – The photobiont of *H. Masumoto 255* (TNS) was isolated and cultured axenically. A unialgal culture of the photobiont was obtained using the methods of Nakano (1988) and Ohmura et al. (2006) with some modifications as follows. After grinding the thallus fragment with slide glasses, a mixture of the photobiont cells and fungal hyphae was spread with a sterilized spreader on sterile 2% agar plates of Bold's Basal Medium (BBM) as modified by Bischoff and Bold (1963). Petri dishes were incubated under a regime of 12 h light (20 μmol m^{-2} s^{-1}) and 12 h dark, at a temperature of 20 ± 1 °C. After two weeks, a unialgal colony was picked up from the medium under a stereomicroscope, and inoculated onto BBM agar slants. The unialgal and axenic photobiont culture (NIES-4343) was deposited at the National Institute for Environmental Studies (NIES Collection, Tsukuba, Japan).

DNA Extraction, PCR, and Sequencing. – The genomic DNA of the mycobiont was extracted from the specimens (see Table 1, Appendix A) following the method of Izumitsu et al. (2012); that of the photobiont was extracted from the isolated axenic algal cultures using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

PCR reactions with KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan) were performed with the primer set ITS1F (Gardes & Bruns 1993) and LR5 (Vilgalys & Hester 1990) for the mycobiont ITS rDNA and nrLSU (D1/D2) sequences; and with the primer set EAF3 (Marin et al. 2003) and ITS055R (Marin et al. 1998) for the photobiont ITS rDNA sequence. PCR cycling conditions were 95 °C for 5 min; 10 cycles of 98 °C for 10 sec, 55–50 °C (0.5 °C decrease per cycle) for 30 sec, 68 °C for 1 min 30 sec for the mycobiont sample (2 min for the photobiont sample); 30 cycles of 98 °C for 10 sec, 50 °C for 30 sec, 68 °C for 1 min 30 sec for the mycobiont sample (2 min for the photobiont sample); and held at 4 °C. PCR products were purified by polyethylene glycol (PEG) precipitation.

Cycle sequence reactions were conducted using BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems) using following primers: ITS1F and ITS4 (White et al. 1990) for the mycobiont ITS rDNA (ITS1-5.8S-ITS2), LR0R (Rehner & Samuels 1994) and LR5 for the mycobiont nrLSU (D1/D2), and TW81 and AB28 (Goff & Moon 1993) for the photobiont ITS rDNA (ITS1-5.8S-ITS2). DNA sequences were analyzed using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems).

Sequence alignment and phylogenetic analyses. – The newly generated DNA sequences were aligned with MAFFT version 7 using the −AUTO option (Katoh et al. 2017) and manually refined with Seaview v.4.6.1 (Gouy et al. 2010). The refined alignment was submitted to TreeBASE (http:// www.treebase.org; Accession number for the mycobiont nrLSU: S24064, for the mycobiont ITS1: S24718, for the mycobiont ITS2: S24721, and the photobiont ITS rDNA: S24053). All new sequences obtained in this study were registered in GenBank (see Table 1, Appendix A).

The phylogenetic analyses for the mycobiont nrLSU (D1/D2), ITS1, and ITS2, and for the photobiont ITS rDNA (ITS1-5.8S-ITS2) were performed using maximum-likelihood (ML) and Bayesian methods. The optimum substitution models for each data set were estimated using Kakusan4 software (Tanabe 2011) based on the Akaike information criterion (AIC; Akaike 1974) for the ML analysis and based on the Bayesian information criterion (BIC; Schwarz 1978) for the Bayesian analysis. The ML analysis was performed with RAxML v8.2.10 on the CIPRES Science Gateway v3.3 web portal (Miller et al. 2010; Stamatakis 2014) based on the models selected with the AICc4 parameter. GTR+Gamma model was implemented for all datasets. Bootstrap proportions (BPs) were obtained using 1,000 bootstrap replications. The Bayesian analysis was performed with MrBayes v.3.2.6 on the CIPRES Science Gateway (Miller et al. 2010; Ronquist et al. 2012), using substitution models containing the BIC4 parameter. SYM+Gamma was implemented for the mycobiont nrLSU, JC69 for the mycobiont ITS1, HKY85 for the mycobiont ITS2, and GTR+Gamma for the photobiont ITS rDNA. Two simultaneous and independent Metropolis-coupled Markov Chain Monte Carlo (MCMC) runs were performed for 2,000,000 generations with the tree sampled for every 1,000 generations of the analyses. Convergence of the MCMC procedure

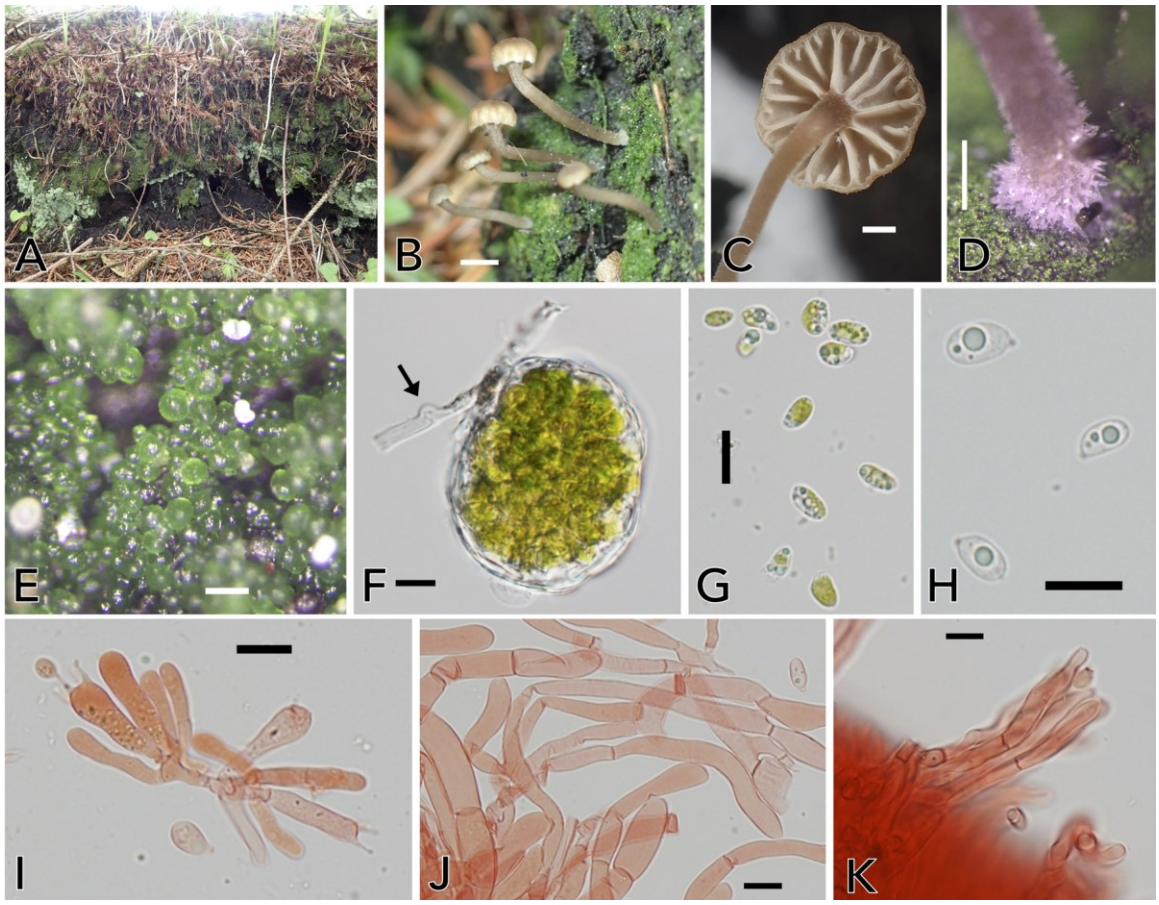

Figure 1. *Lichenomphalia meridionalis* collected in Japan. A, habitat. B, *L. meridionalis in situ*. (*H. Masumoto 255*, TNS). C, lamella and lamellula. (*H. Masumoto 276*, TNS). D, hairs at the base of stipe. E, thallus of "*Botrydina*-type." F, detail of thallus with interconnecting hyphae with a clamp. (*H. Masumoto 255*, TNS). G, *Coccomyxa subellipsoidea*, the photobiont of *L. meridionalis*. H, basidiospores. I, tetrasporic (left) and bisporic (right) basidia. J, hyphae of the pileipellis. K, detail of stipe hairs. Scales: 2 mm (B), 1 mm (C, D), 100 µm (E), 10 µm (F–K).

was assessed from the average standard deviation of split frequencies (<0.01) and effective sample size scores (all >100) using MrBayes and Tracer v. 1.7.1 (Rambaut et al. 2018), respectively. The first 25% of the trees were discarded as burn-in, and the remaining trees were used to calculate the 50% majority-rule trees and to determine the posterior probabilities (PP) for individual branches.

RESULTS

***Lichenomphalia meridionalis* (Contu & La Rocca) P. A. Moreau & Courtec.**, Documents Mycologiques 34 (135–136): 50. 2008. ≡ *Omphalina meridionalis* Contu & La Rocca, Fungi non Delineati 9: 32. 1999. TYPE: **ITARY. SARDINIA**: Prov. Sassari, M. Limbara, Monte Muroni, zona parafuoco, 10.i.1999, *M. Contu s.n.* (IB[n.v.], holotype).

DESCRIPTION OF JAPANESE MATERIAL. – The vegetative thallus consists of green to dark-green tiny globules (20–80 µm diam.) (Fig. 1E), known as "*Botrydina*-type thallus" (Redhead & Kuyper 1987). The globule contains the photobiont cells (5–8 µm diam.) inside, and the surface consists of appressed pseudoparenchymatous hyphal tissue with angular cells (5–12 µm diam.). The globules are aggregated with interconnecting hyphae (2–4 µm wide) (Fig. 1F).

Pileus 3.5–9.0 mm diam., convex to plano-convex, translucent-striate, edges crenate, surface glabrescent to pruinose, and initially dark brown then brown and fading to light brown (Fig. 1B). Lamellae

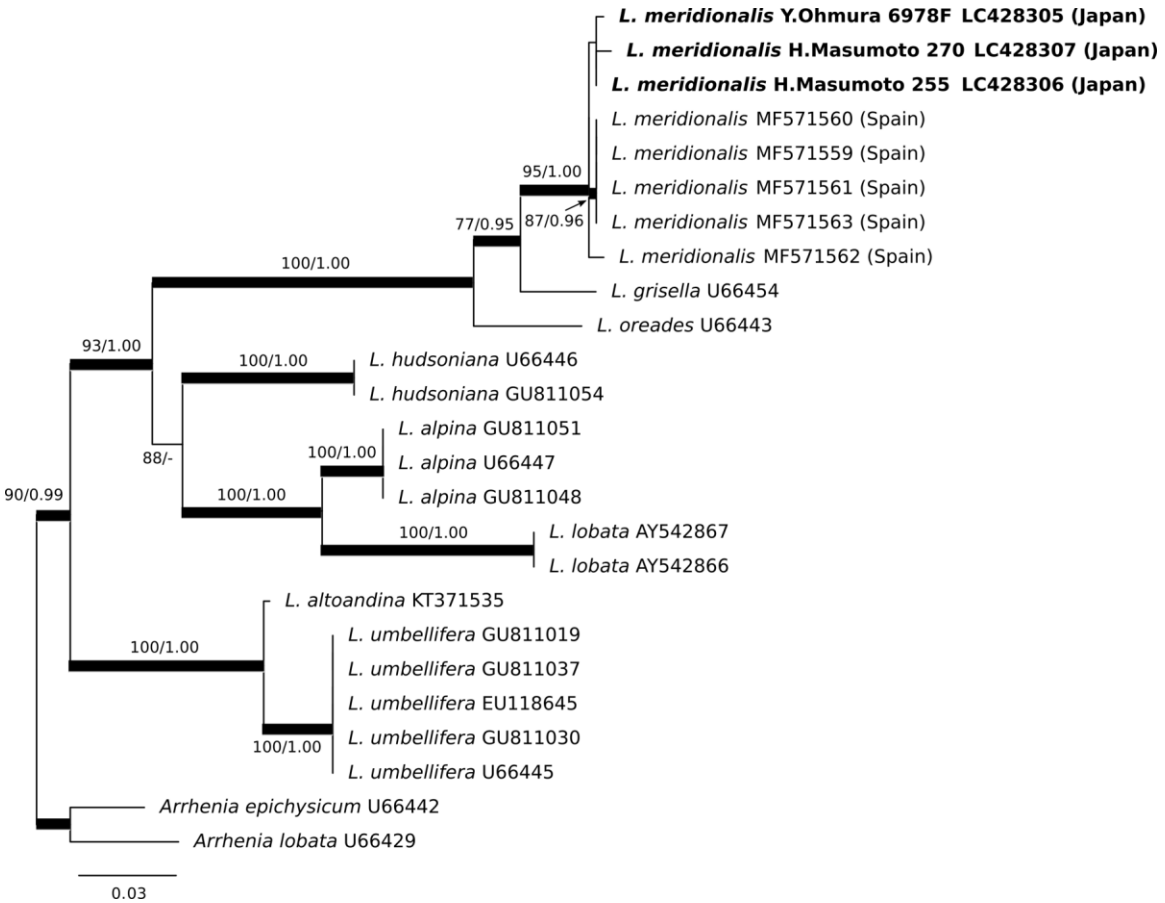

Figure 2. ML tree for the *Lichenomphalia meridionalis* and the related species based on the nrLSU (D1/D2). Branches with ML bootstrap proportion (BP) ≥70% and Bayesian posterior probabilities (PP) ≥0.95 are thickened and support values are presented at the branches as BP/PP. A hyphen ("-") indicates values lower than 70% BP or 0.95 PP. The scale bar represents the nucleotide substitutions per site. Two outgroup species were chosen based on Lodge et al. (2014).

arcuate-decurrent, distant, whitish to grayish white, always paler than pileus, with some lamellulae, occasionally forking at the margin (Fig. 1C). Stipe 5.0–10.0 × 0.6–1.1 mm, usually longer than pileus diam., brown to dark brown, concolorous with pileus or darker, pubescent along the length of the stipe, white tomentose at the base with mycelial patch associated with the globules of the vegetative thallus. Basidia 22–35 × 4–7 µm, predominantly 4-spored but occasionally 2-spored, clavate to subcylindrical, sterigmata 2.6–4.5 µm (Fig. 1I). Basidiospores 4.8–6.9–8.9 × 2.8–3.9–5.3 µm, Q = 1.2–2.4 (Qx = 1.8; n = 70), ellipsoid to elongate, thin-walled, hyaline, non-amyloid, with prominent apiculus (Fig. 1H). Cystidia absent. Pileipellis a cutis of cylindrical hyphae with clavate ends, 3.5–9.0 µm wide, faintly encrusting pigment, not 'zebloid' stripe (Fig. 1J). Stipitipellis of parallel, cylindrical hyphae 3–5 µm wide, with hairs in tufts up to 100 µm from stipe (Fig. 1D and 1K). Clamps absent in all tissues, except for interconnecting hyphae between globules (Fig. 1F).

ECOLOGY AND DISTRIUBTION. – In Japan, the species was found on Andosols along roadsides in montane to subalpine habitat at elevations between 1200 and 1900 meters (Fig. 1A). The fruiting body occurred solitary or dispersed in a colony during early summer and autumn but disappeared in mid-summer at the habitats. This species was previously known exclusively from Mediterranean regions of Europe such as Italy (Sardinia, the type locality), Spain (Segovia, Gerona, Guadalajara, and Madrid), France (Corsica), and Greek (Lesvos) (Contu & La Rocca 1999; Barrasa & Esteve-Raventós 2000; Barrasa & Rico 2001; Telleria et al. 2016; Roux et al. 2017). It is here reported as new to Asia.

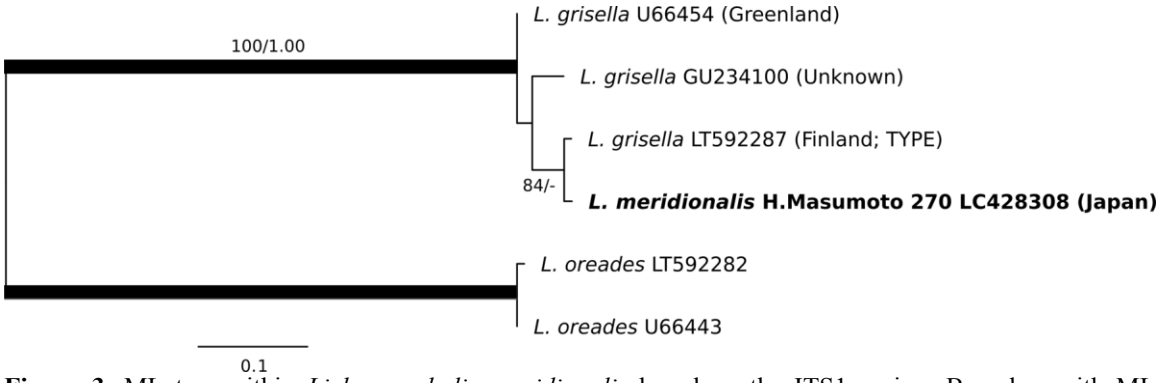

Figure 3. ML tree within *Lichenomphalia meridionalis* based on the ITS1 region. Branches with ML bootstrap proportion (BP) ≥70% and Bayesian posterior probabilities (PP) ≥0.95 are thickened and support values are presented at the branches as BP/PP. A hyphen ("-") indicates values lower than 70% BP or 0.95 PP. The scale bar represents the nucleotide substitutions per site. *Lichenomphalia oreades* was chosen as outgroup.

MOLECULAR ANALYSES OF THE MYCOBIONT. – In the BLAST search of the nrLSU (D1/D2), the sequences of Japanese *Lichenomphalia meridionalis* (GenBank accession nos. LC428305–LC428307) showed 99% identity (492–494/498 bp with no gaps) with the sequences of Spanish *L. meridionalis* (MF571559–MF571563). In addition, the BLAST identities for nrLSU between Japanese *L. meridionalis* (LC428306) and *L. grisella* (U66454) or *L. oreades* (U66443) were 97% (819/842 bp containing one gap) and 95% (804/843 bp containing four gaps) respectively. ML and Bayesian phylogenetic analyses were performed using an aligned sequence dataset comprising 465 bp from nrLSU (D1/D2) (Fig. 2). The highest log likelihood of the ML tree of nrLSU was –1701.842261, and the Bayesian likelihood score was –1742.409. The topology recovered by Bayesian analysis was identical to that of the ML tree. In the ML tree of the nrLSU, the sequences of Japanese specimens formed a monophyletic clade [support value: 95 (BP)/1.00 (PP)] together with the sequences registered as Spanish *L. meridionalis* in GenBank (MF571559–MF571563) (Fig. 2).

We tried to sequence ITS rDNA from all Japanese *L. meridionalis* samples, but most of them failed due to unexplained polymorphisms. Only one ITS rDNA sequence (LC428308) was obtained from one sample (*H. Masumoto 270*, TNS). In the BLAST search of the ITS1, the sequence of Japanese *L. meridionalis* (LC428308) showed 99% identity (159/161 bp with no gaps) with the sequence of Finnish *L. grisella* (LT592287) obtained from the type material in Lücking et al. (2017). In addition, the BLAST identity of two other ITS1 sequences of *L. grisella* (GU234100 and U66454) was 93% (151/163 bp with eight gaps) and 89% (146/164 bp with eight gaps), respectively. There was no sequence of the ITS1 registered as *L. meridionalis* in GenBank. ML and Bayesian phylogenetic analyses were performed using an aligned sequence dataset comprising 149 bp from the ITS1 (Fig. 3). The highest log likelihood of the ML tree of the ITS1 was –426.685432, and the Bayesian likelihood score was –444.726. The topology recovered by Bayesian analysis was identical to that of the ML tree. In the ML tree of the ITS1, the sequence of Japanese *L. meridionalis* constitutes a well-supported monophyletic clade with three *L. grisella* sequences [support value: 100 (BP)/1.00 (PP)]. However, the phylogenetic relationships of each sequence within the clade were not well resolved (Fig. 3).

In the BLAST search of the ITS2, the sequence of Japanese *L. meridionalis* (LC428308) showed 99% identity (200–201/203 bp with one gap) with the sequences registered as Spanish *L. meridionalis* (MH379110–MH379114). In addition, the BLAST identities for the ITS2 between Japanese *L. meridionlis* (LC428308) and two other sequences of *L. grisella* (U66454 and GU234100) was 97% (185/191 bp with one gap) and 94% (179/190 with no gaps), respectively. The sequence of the ITS2 obtained from the type material of *L. grisella* was not registered in GenBank. ML and Bayesian phylogenetic analyses were performed using an aligned sequence dataset comprising 196 bp from the ITS2 (Fig. 4). The highest log likelihood of the ML tree of the ITS2 was –516.114335, and the Bayesian likelihood score was –534.712. The topology recovered by Bayesian analysis was identical to that of the ML tree. In the ML tree of the ITS2, the sequences of Japanese *L. meridionalis* formed a well-supported monophyletic clade with the

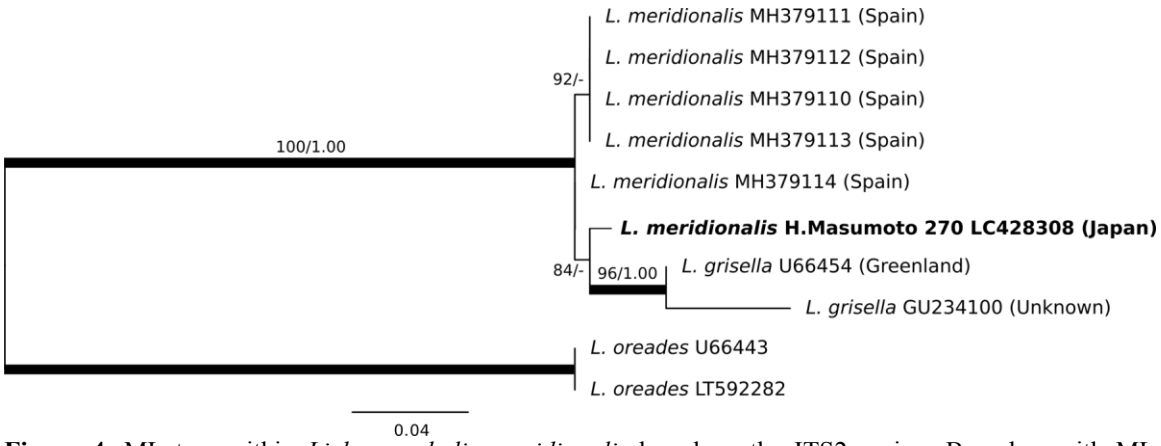

Figure 4. ML tree within *Lichenomphalia meridionalis* based on the ITS2 region. Branches with ML bootstrap proportion (BP) ≥70% and Bayesian posterior probabilities (PP) ≥0.95 are thickened and support values are presented at the branches as BP/PP. A hyphen ("-") indicates values lower than 70% BP or 0.95 PP. The scale bar represents the nucleotide substitutions per site. *Lichenomphalia oreades* was chosen as outgroup.

sequences of five Spanish *L. meridionalis* and two *L. grisella*. However, the phylogenetic relationships between the sequence of Japanese *L. meridionalis* and others within the clade were not well resolved (Fig. 4).

MOLECULAR ANALYSIS OF THE PHOTOBIONT. – The ITS rDNA (ITS1-5.8S-ITS2) sequence of algal culture (NIES-4343) isolated from the thalli of Japanese *Lichenomphalia meridionalis* (*H. Masumoto 255*, TNS) showed 97% BLAST identity (620/636 bp containing seven gaps) with that of *Coccomyxa subellipsoidea* SAG 216-13 (GenBank accession no. AY328523) which originated from thalli of *Lichenomphalia umbellifera* (Zoller & Lutzoni 2003). Following the BLAST result, ML and Bayesian phylogenetic analyses based on the ITS rDNA sequences of the isolated photobiont and the related *Coccomyxa* species were performed using an aligned sequence dataset comprising 518 bp from ITS rDNA (Fig. 5). The highest log likelihood of the ML tree of the ITS rDNA was –3471.566684, and the Bayesian likelihood score was –3488.11. The topology recovered by Bayesian analysis was identical to that of the ML tree. In the ML tree, the photobiont sequence of *L. meridionalis* formed a monophyletic clade [support value: 100 (BP)/1.00 (PP)] with *C. subellipsoidea* sequences (Fig. 5). Therefore, the photobiont of *L. meridionalis* examined in this study was inferred to be *C. subellipsoidea* (Fig. 1G).

Specimens examined. – **JAPAN. KAI PROVINCE (YAMANASHI PREFECTURE)**: Mt. Fuji, Minamitsuru-gun, on soil, ca. 1200 m elev., 14.v.2010, *I. Asai s.n.* (herb. *Y. Ohmura-6978F*, TNS). **SHINANO PROVINCE (NAGANO PREFECTURE)**: Sugadairakogen, Ueda-city, 36.53475277N 138.34248055E, on soil along a road bank, 1330 m elev., 7.vii.2012, *Y. Ohmura 9116* (TNS), 20.vi.2018, *H. Masumoto 255* (TNS), 12.vii.2018, *H. Masumoto 264* (TNS), 2.x.2018, *H. Masumoto 276* (TNS); Mt. Nekodake, Sugadairakogen, Ueda-city (N 36.54596, E 138.38718), on soil along a road bank, 1969 m elev., 27.viii.2018, *H. Masumoto 270* (TNS).

DISCUSSION

The occurrence of *Lichenomphalia meridionalis* in Japan was confirmed by the morphological and molecular phylogenetic data obtained in this study. The basidiospore size of Japanese specimens [4.8–6.9–8.9 × 2.8–3.9–5.3 µm (Q = 1.2–2.4; Qx = 1.8)] is consistent with the protologue of *L. meridionalis* [5.2–7.5 × 3.7–4.5 µm (Q value not shown; estimated Q = 1.2–2.0)] (Contu & La Rocca 1999). On the other hand, the basidiospore size in the emended description of *L. meridionalis* by Barrasa & Esteve-Raventós (2000) [7.6–9.3–11 × 4–5–6 µm (Q = 1.5–2.3; Qx = 1.9)] was slightly larger than the original description and Japanese materials. However, there was almost no difference in the Q value of the basidiospores and other macroscopic and microscopic features between our Japanese materials and those descriptions. Therefore, we regard the differences of the basidiospore size as infraspecific variation.

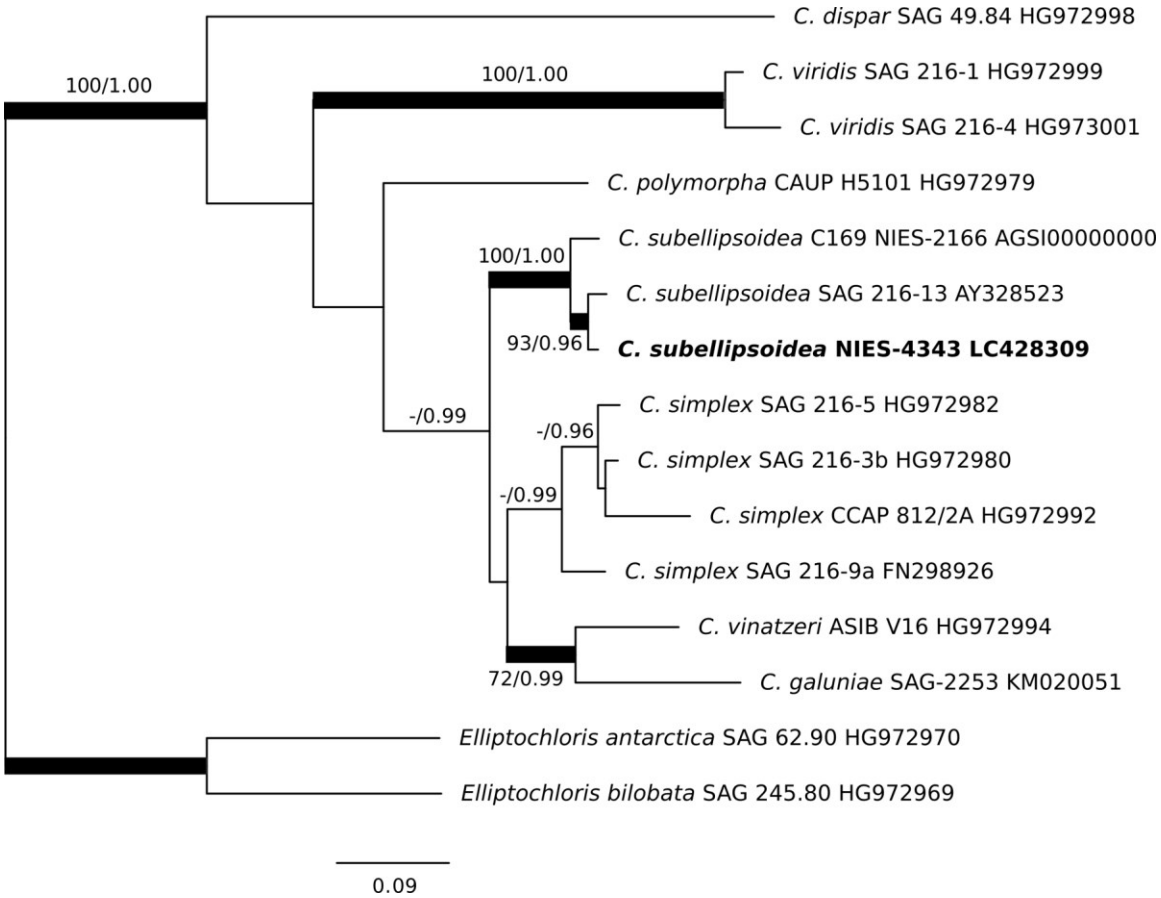

Figure 5. ML tree for the isolated photobiont from *Lichenomphalia meridionalis* and *Coccomyxa* species based on the ITS rDNA sequences. Branches with ML bootstrap proportion (BP) ≥70% and Bayesian posterior probabilities (PP) ≥0.95 are thickened and support values are presented at the branches as BP/PP. A hyphen ("-") indicates values lower than 70% BP or 0.95 PP. The scale bar represents the nucleotide substitutions per site. Two outgroup species were chosen based on Darienko et al. (2015).

Lichenomphalia meridionalis resembles *L. grisella* in morphology, and the close relationship is supported by the molecular data. These species are distinguished by the number of basidial sterigmata and the pigmentation of pileipellis hyphae (Barrasa et al. 2009; Lücking et al. 2017). That is, *L. meridionalis* has four-spored basidia (Contu & La Rocca 1999) or a mixture of four to two-spored basidia (Barrasa & Esteve-Raventós 2000), whereas *L. grisella* is characterized by two-spored basidia (Lücking et al. 2017). In addition, the pileilellis hyphae of *L. meridionalis* do not have brown striped pigmentation, whereas those of *L. grisella* is encrusted by the brown striped pigmentation. The molecular phylogenetic tree based on nrLSU using Japanese and Spanish materials supports the monophyly of *L. meridionalis* (Fig. 2) although each tree based on ITS1 and ITS2 could not solve the phylogenetic relationship between *L. meridionalis* and *L. grisella* because of the less information in each ITS region (Fig. 3 and 4).

In Japan, another species of *Lichenomphalia*, *L. hudsoniana* (H.S. Jenn.) Redhead, Lutzoni, Moncalvo & Vilgalys, is known (Asahina 1934; Yoshimura & Harada 1986). It is readily distinguished from *L. meridionalis* by the squamulose (*Coriscium*-type) thallus, larger basidiomata (pileus 10–15 mm diam.) and occurrence in alpine habitats (Kurokawa 2006). Elsewhere in eastern Asia, *L. velutina* (Quél.) Redhead, Lutzoni, Moncalvo & Vilgalys, which resembles *L. meridionalis* in gross morphology, was reported from Yunnan, China (Liu et al. 2018). However, the Chinese *L. velutina* is different from *L. meridionalis* in having 'zebloid' brown stripes on some hyphae of the fruiting bodies and in that it grows in an alpine habitat (vs. montane to subalpine habitat of *L. meridionalis*). Since the identity of the ITS rDNA between the Chinese sample and our data of *L. meridionalis* (LC428308) was low at 83% (sharing 416/503 bp with 38 gaps), the sequence of this specimen was not used for phylogenetic analysis in this study. In addition to *L. grisella* above, there are also some *L. meridionalis*-like species such as *L. cinereispinula*

Neville & Fouchier and *L. pararustica* (Clémençon) Elborne, but no sequence data are yet been available. Further collections of these morphologically similar species are needed to examine whether they are phylogenetically distinct from *L. meridionalis*.

In this study, the photobiont of *L. meridionalis* was isolated from the Japanese samples and identified as *Coccomyxa subellipsoidea* by molecular phylogenetic analyses (Fig. 5). Given that several *Lichenomphalia* species occurring in Canada, Greenland, and Iceland are associated with *C. subellipsoidea* (Zoller & Lutzoni 2003), it is not unexpected that *L. meridionalis* would also associate with that photobiont.

The discovery of *Lichenomphalia meridionalis* in Asia is biogeographically interesting because this species has been previously reported exclusively from Mediterranean regions of Europe. Since the fruiting body of *L. meridionalis* is inconspicuous and ephemeral, further careful investigations are needed to document distribution of this species.

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APPENDIX A – VOUCHER METADATA FOR SEQUENCES USED IN THIS STUDY

Table 1. GenBank accession numbers of samples used for molecular analyses in this study. New sequences are shown in bold face.

Taxon	Origin; voucher or strains	ITS	LSU	Reference
Arrhenia epichysicum	Unknown; Redhead 5223, Redhead 3140 (DAOM)	–	U66442	Lutzoni (1997)
A. lobata	France; Lutzoni & Lamoure 910824-1 (DUKE)	–	U66429	Lutzoni & Vilgarys (1995)
Coccomyxa dispar	Germany; SAG 49.84	HG972999	–	Darienko et al. (2015)
C. galuniae	Germany; SAG 2253	KM020051	–	Unpublished
C. polymorpha	France; CAUP H5101	HG972979	–	Darienko et al. (2015)
C. simplex	Switzerland; SAG 216-5	HG972982	–	Darienko et al. (2015)
C. simplex	Switzerland; SAG 216-3b	HG972980	–	Darienko et al. (2015)
C. simplex	U.K.; CCAP 812/2A	HG972992	–	Darienko et al. (2015)
C. simplex	Czech Republic; SAG 216-9a	FN298926	–	Pröschold et al. (2011)
C. subellipsoidea	Antarctica; C-169 (NIES-2166)	AGSI00000000	–	Blanc et al. (2012)
C. subellipsoidea	Austria; SAG 216-13	AY328523	–	Zoller & Lutzoni (2003)
C. subellipsoidea	Japan; NIES-4343	**LC428309**	–	This study
C. vinatzeri	Italy; ASIB V16	HG972994	–	Darienko et al. (2015)
C. viridis	France; SAG 216-1	HG972999	–	Darienko et al. (2015)
C. viridis	Switzerland; SAG 216-4	HG973001	–	Darienko et al. (2015)
Elliptochloris bilobata	Austria; SAG 245.80	HG972969	–	Darienko et al. (2015)
Hemichloris antarctica	Antarctica; SAG 62.90	HG972970	–	Darienko et al. (2015)
Lichenomphalia alpina	Canada; FL 930816-8 (DUKE)	–	U66447	Lutzoni (1997)
L. alpina	U.S.A.; GAL1264	–	GU811048	Geml et al. (2012)
L. alpina	Norway; GAL2689	–	GU811051	Geml et al. (2012)
L. altoandina	Chile; 160478 (SGO) [TYPE]	–	KT371535	Sandoval-Leiva et al. (2017)
L. grisella	Greenland; FL 930812-1 (DUKE)	U66454	U66454	Lutzoni (1997)

L. grisella	Unknown; KH73	GU234100	–	Geml et al. (2012)
L. grisella	Finland; H6042076 [TYPE]	LT592287	–	Lücking et al. (2017)
L. hudsoniana	Canada; FL 920728-4a (DUKE)	–	U66446	Lutzoni (1997)
L. hudsoniana	U.S.A.; GAL1209	–	GU811054	Geml et al. (2012)
L. lobata	Ecuador; Palice 2327	–	AY542866	Palice et al. (2005)
L. lobata	Ecuador; Palice 3275	–	AY542867	Palice et al. (2005)
L. meridionalis	Japan; H. Masumoto 255 (TNS)	–	**LC428306**	This study
L. meridionalis	Japan; H. Masumoto 270 (TNS)	**LC428308**	**LC428307**	This study
L. meridionalis	Japan; Y. Ohmura 6978F (TNS)	–	**LC428305**	This study
L. meridionalis	Spain; AH:23331	MH379110	MF571559	Unpublished
L. meridionalis	Spain; AH:37721	MH379111	MF571560	Unpublished
L. meridionalis	Spain; AH:27351	MH379112	MF571561	Unpublished
L. meridionalis	Spain; AH:25359	MH379113	MF571562	Unpublished
L. meridionalis	Spain; AH:39237	MH379114	MF571563	Unpublished
L. oreades	Canada; Lutzoni 930822-6 (DUKE)	U66443	U66443	Lutzoni (1997)
L. oreades	U.S.A.; FH00543609 [TYPE]	LT592282	–	Lücking et al. (2017)
L. umbellifera	Canada; Lutzoni 930817-2 (DUKE)	–	U66445	Lutzoni (1997)
L. umbellifera	Sweden; Rova 2501 (GB) / JR 2501	–	EU118645	Larsson (2007)
L. umbellifera	Sweden; DU0011879	–	GU811030	Geml et al. (2012)
L. umbellifera	U.S.A.; GAL5374	–	GU811019	Geml et al. (2012)
L. umbellifera	Norway; Gulden 302/86	–	GU811037	Geml et al. (2012)