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Two new *Mycena* section *Calodontes* species: One newly discovered and the other new to Japan

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ABSTRACT

In 2017, two candidate species of *Mycena* were reported from Japan, with the Japanese names "*Togari-sakura-take*" and "*Mitsuhida-sakura-take*". However, to date, no taxonomic study or formal description has been undertaken for these two species. In the present study, we conducted comprehensive morphological and molecular phylogenetic examinations of "*Togari-sakura-take*" and "*Mitsuhida-sakura-take*", and compared them to known species within the genus *Mycena*. We performed phylogenetic analyses on a concatenated dataset, including the internal transcribed spacer region of ribosomal RNA, RNA polymerase II largest subunit, and translation elongation factor-1 alpha genes. "*Togari-sakura-take*" formed a clade with *Mycena subulata*, which was recently described from China, whereas "*Mitsuhida-sakura-take*" formed a distinct independent clade. We identified the former as *M. subulata* based on molecular phylogenetic analyses and morphological observations. However, the Japanese specimens displayed dextrinoid cheilocystidia and caulocystidia as well as the inamyloidity of basidiospores, which differed from the original description of *M. subulata* based on the materials from China. "*Mitsuhida-sakura-take*" was characterized by its remarkably dense lamellae and could be distinguished from known *Mycena* species by the combination of absent pleurocystidia and presence of bowling pin-shaped cheilocystidia. Here, we describe "*Mitsuhida-sakura-take*" as a new species, named *Mycena densilamellata*, in the section *Calodontes*.

Keywords: molecular phylogeny, morphology, *Mycenaceae*, new to Japan, 1 new taxon

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1. Introduction

The genus *Mycena* (Pers.) Roussel belongs to the family *Mycenaceae* Overeem and order *Agaricales* Underw. (Wijayawardene et al., 2020). This genus is one of the species-rich taxa within *Agaricales*, with approximately 600 valid species, according to the Index Fungorum database (http://www.indexfungorum.org/; accessed on Dec 5 2023) and recent studies on the phylum *Basidiomycota* (e.g., He et al., 2019). In Japan, approximately 70 *Mycena* species have been reported to date (Cha et al., 2010; Katumoto, 2010; Shirayama, 2010; Terashima et al., 2016). *Mycena* typically produces small to medium-sized mycenoid, omphalinoid, or collybioid basidioma, and possesses smooth or branched-cheilocystidia and pleurocystidia, diverticulate, and less frequently smooth hyphae of pileipellis. In addition, the lamella trama appears vinaceous to purplish brown (dextrinoid) upon staining with Melzer's reagent, whereas basidiospores are generally amyloid (Maas Geesteranus, 1980). Similar to other agarics, the taxonomy of *Mycena* is undergoing a transition from conventional research methods relying heavily on the morphological characteristics of basidiomata (Maas Geesteranus, 1980) to approaches focusing more on molecular phylogenetic analyses (Harder et al., 2010; Liu et al., 2022).

Mycena species have diverse lifestyles, primarily as saprophytes, while certain species are recognized as orchid mycorrhizal symbionts or plant pathogens (Kitahara et al., 2022; Krishnan, 2017). Some *Mycena* species are known to invade and associate with mosses (Davey et al., 2013). Recent insights into the role of *Mycena* species as symbionts of plants have been provided through the inoculation of cultured strains onto their host plants (Thoen et al., 2020) as well as field observations (Harder et al., 2023). Therefore, further taxonomic studies incorporating ecology are needed to understand the species diversity of the genus *Mycena*.

Mycena section *Calodontes* (Fr. ex Berk.) Quél. sensu Maas Geesteranus (1989) generally produces basidioma with raphanoid

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odor and diverse colors (frequently with purplish or violaceous tints), as well as smooth cheilocystidia, pleurocystidia, and smooth hyphae of pileipellis (Maas Geesteanus, 1989). This section is divided into four subsections according to their morphological characteristics (Maas Geesteranus, 1989; Maas Geesteranus & de Meijer, 1997). Subsection *Violacellae* Sing. ex Maas Geest. lectotypified by *M. violacella* (Speg.) Singer [=*Poromycena violacella* (Speg.) Singer], is unique in having inamyloid basidiospores and lacking pleurocystidia. Subsection *Purae* (Konr. and Maubl) Maas Geest., lectotypified by *M. pura* (Pers.) P. Kumm., is characterized by the presence of amyloid basidiospores, cheilocystidia, and commonly has pleurocystidia with colorless contents. Subsection *Marginatae* J.E. Lange, lectotypified by *M. pelianthina* (Fr.) Quél., also has amyloid basidiospores but is unique among the various validated subsections in forming cheilocystidia and pleurocystidia with purplish brown contents. Subsection *Generosae* Maas Geest. & de Meijer, type species, *M. generosa*, also produces amyloid basidiospores but is distinct as it lacks pleurocystidia and has cheilocystidia with much narrow necks, which are not broadly rounded at the apex.

Harder et al. (2010) attributed almost 30 species to sect. *Calodontes*. Since then, several taxonomic studies from Asia have proposed taxonomic revisions, and around 10 taxa have been added to this section: *M. cahaya* A.L.C. Chew & Desjardin, *M. polycystidiata* Z.W. Liu, Y.P. Ge, L. Zou & Q. Na, *M. rufobrunnea* Z.W. Liu, Y.P. Ge & Q. Na, *M. seminau* A.L.C. Chew & Desjardin, *M. shengshanensis* Z.W. Liu, Y.P. Ge & Q. Na, *M. sinar* A.L.C. Chew & Desjardin, *M. sirayuktha* Aravind. & Manim., *M. subulata* Z.W. Liu, Y.P. Ge & Q. Na, *M. yuezhuoi* Z.W. Liu, Y.P. Ge & Q. Na, and a new variety *M. sinar* var. *tangkaisinar* A.L.C. Chew & Desjardin (Aravindakshan & Manimohan, 2015; Chew et al., 2014; Liu et al., 2021, 2022). Despite the description of multiple new species from Asia, only four, i.e., *M. pelianthina*, *M. pura*, *M. rosea* Gramberg, and *M. subaquosa* A.H. Sm., have been reported from Japan (Hongo, 1953; Imai, 1938; Kudo & Nagasawa, 2009, 2017; Murata, 1979). Therefore, additional species are expected to be found in Japan.

Kigawa (2017) introduced two *Mycena* species, "*Mycena* sp."-1 and "*Mycena* sp."-2, which likely belong to sect. *Calodontes* based on their morphology. Kigawa (2017) suggested that these were new species and gave "*Mycena* spp."- 1 and 2 the Japanese names of "*Togari-sakura-take*" due to its awl-shaped cheilocystidia and "*Mitsuhida-sakura-take*" due to its crowded lamellae, respectively. The taxonomic positions of "*Togari-sakura-take*" and "*Mitsuhida-sakura-take*" are unclear.

The present study evaluated the taxonomic position of "*Togari-sakura-take*" ("*Mycena* sp."-1) and "*Mitsuhida-sakura-take*" ("*Mycena* sp."-2) described by Kigawa (2017) from Japan at the species and subsection levels. To this end, we used the two specimens examined by Kigawa (2017), which are stored at the Hiratsuka City Museum, and our newly collected specimens from various areas in Japan. We compared the morphology between these two species and other species of the *Mycena* section *Calodontes*. We performed phylogenetic analyses using the DNA sequences of the internal transcribed spacer (ITS) region of ribosomal RNA, RNA polymerase II largest subunit (RPB1), and translation elongation factor-1 alpha (TEF1) genes.

2. Materials and methods

2.1. Collection of basidiomata specimens and cultures

Each three basidiomata of "*Mycena* spp."- 1 and 2 were collected from five areas of Japan between Sep and Oct of 2019–2021 (Table 1). Fresh basidiomata were examined macroscopically, air-dried at 45 °C for 1–2 d, and stored. To preserve specimens in a good condition for a long period with a minimum damage, we obtained five cultures that can be used repeatedly for destructive DNA analyses on 1.5% malt extract agar medium containing 1.5% malt extract (Oriental Yeast Co., Ltd., Tokyo, Japan) and 1.5% agar (Fujifilm Wako Pure Chemical Corp., Osaka, Japan) from each fresh basidioma. The dried specimens and cultures were deposited in the Fungus/Mushroom Resource and Research Center (FMRC), Tottori University (Table 1). Fungal strains deposited were cryopreserved in a vapor-phase liquid nitrogen tank at –190 °C. Deposition and utilization of Tottori University Fungal Culture Collection (TUFC) strains were supported by FMRC through the National BioResource Project of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (http://nbrp.jp). We also examined two specimens housed at the fungal herbarium of National Museum of Nature and Science (TNS; TNS-F-75029 and TNS-F-75058) for morphological and phylogenetic analyses. Furthermore, we assessed the morphology of the two specimens examined by Kigawa (2017) that were housed at the Hiratsuka City Museum (HCM-58-6667 and HCM-58-6665) (Table 1).

2.2. Morphological observations of basidiomata

The fresh basidiomata in the fields were photographed, and their forest habitats were recorded. The Online Auction Color Chart (Kramer, 2004) was used as the color standard. Microscopic characters of dried specimens were observed in Melzer's reagent, 3% potassium hydroxide or distilled water using a differential interference contrast microscope (Eclipse 80i, Nikon Co., Tokyo, Japan). The measurements of basidia [not including sterigmata (apical processes for basidiospore production)], basidioles, basidiospores, and cheilocystidia were shown as "(a)b–c(d)", where (a) indicates the 5th percentile, (b) indicates the average – standard deviation

Table 1 – *Mycena* specimens examined in this study.

Species	Specimen number in herbarium	Strain number in culture collection	Locality in Japan	Collection date	Vegetation [a]
"*Mycena* sp."-1	TUMH 65483	TUFC 102001	Daisen Town, Tottori Pref.	11 Sep 2021	*Fagus crenata*
	TUMH 65484	TUFC 102002	Tottori City, Tottori Pref.	19 Sep 2021	*Chamaecyparis obtusa*, *Betula grossa*, *Acer* sp.
	TUMH 65485	TUFC 102003	Kotoura Town, Tottori Pref.	20 Sep 2021	*Aesculus turbinata*, *F. crenata*, *Acer* sp.
	TNS-F-75058		Chichibu City, Saitama Pref.	28 Sep 2015	ND
	HCM-58-6665		Gotenba City, Shizuoka Pref.	13 Sep 2009	ND
"*Mycena* sp."-2	TUMH 65481	TUFC 101999	Ebetsu City, Hokkaido	14 Sep 2019	*Picea* spp., Betulaceae
	TUMH 65486		Narusawa Village, Yamanashi Pref.	8 Oct 2020	*Quercus crispula*, *F. crenata*, *C. obtusa*
	TUMH 65482	TUFC 102000	Tottori City, Tottori Pref.	19 Sep 2021	*C. obtusa*, *Quercus serrata*, *Carpinus* sp.
	TNS-F-75029		Chichibu City, Saitama Pref.	28 Sep 2015	ND
	HCM-58-6667		Gotenba City, Shizuoka Pref.	13 Sep 2009	ND

[a] ND: No data

(SD), (c) indicates the average + SD, and (d) indicates the 95th percentile. We measured the minimum and maximum values of sterigmata and caulocystidia. The hyphae length/width are shown as 'minimum–maximum. We measured 50 basidiospores and 30 basidia, basidioles, and cheilocystidia each. Because certain *Mycena* species in sect. *Calodontes* showed a weak amyloid reaction on their basidiospores, we determined the dyeability of basidiospores under differential interference contrast and normal mode light microscopes after treatment with Melzer's reagent for 20 min. Q and its average value (Q_m) were calculated from the length/width ratio of basidiospores. We also observed pileipellis, stipitipellis, and structures of hymenophoral trama.

2.3. DNA extraction, PCR amplification, and sequencing

Genomic DNA for PCR amplification was extracted from dried basidioma or living cultures following the method described by Hosaka and Nam (2023) or the microwave method described by Izumitsu et al. (2012). We amplified the full length of the ITS (ITS1–5.8S–ITS2) region and portions of the RPB1 and TEF1 genes. We used Dream Taq DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) for PCR amplification with the following primer pairs: ITS1F and LR5 for ITS region (Gardes & Bruns, 1993; Vilgalys & Hester, 1990), RPB1Mp_f1 and RPB1Mp_r1 for RPB1 gene (Harder et al., 2013), and tEFMp_f1 and tEFMp_r1 or EF526f/EF595f and EF1567r for TEF1 gene (Harder et al., 2013; Kauserud & Schumacher, 2001; Rehner & Buckley, 2005). PCR was performed using the standard protocol: 35 cycles at 95 °C for 30 s, 49.5 °C for 30 s, and 72 °C for 100 s in the ITS1F and LR5 primer pair; 35 cycles at 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 60 s in the RPB1MP_f1 and RPB1MP_r1 primer pair; 35 cycles at 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 60 s in tEFMP_f1 and tEFMP_r1 primer pair; 35 cycle of 95 °C for 30 s, 51 °C for 30 s, and 72 °C for 60 s in the EF526f and EF1567r primer pair. For the PCR amplifications of TEF1 gene using the primer pair EF595f and EF1567r, we used touchdown protocols (annealing temperature lowered by 1 °C per cycle), with the following PCR protocol: 35 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and directly sequenced using the same pair of bidirectional primers for TEF1 and RPB1 genes or ITS5 and ITS4 primers (White et al., 1990) for the ITS region. The nucleotide sequences were analyzed by FASMAC Co., Ltd. (http://fasmac.co.jp), and subsequent phylogenetic analyses of the sequencing data were performed using Bioedit v. 7.2.5 (Hall, 1999). The new sequences were deposited into the GenBank database with accession numbers (LC777686–LC777693, LC777726–LC777741) through the DNA Data Bank of Japan (Table 2).

2.4. Phylogenetic analyses

The three loci datasets of ITS, RPB1, and TEF1 were used for phylogenetic analyses. The 64 sequences (Binder et al., 2010; Harder et al., 2013; Liu et al., 2021, 2022) obtained from the National Center for Biotechnology Information GenBank and the eight sequences identified by this study were analyzed (Table 2). From these sequences, we selected *Mycena rubromarginata* (Fr.) P. Kumm. and *M. plumbea* P. Karst. as outgroups (Table 2). *Mycena rubromarginata* was selected as an outgroup due to its previous use in Harder et al. (2013). *Mycena plumbea* occupies the closest position to *M. rubromarginata* among the *Mycena* species available in the GenBank, which possess all-three-loci-sequences. The sizes of DNA sequences of each locus were 645 bp (ITS), 488 bp (RPB1), and 419 bp (TEF1). We used a total of 1,552 bp sequences following alignment (including gaps). The dataset was aligned using the Muscle algorithm (Edgar, 2004) on the workbench in MEGA v. 7.0.26 software (Kumar et al., 2016). The topology of the maximum likelihood (ML) tree was estimated using RAxML GUI v. 2.0 software (Edler et al., 2021; Stamatakis, 2014). To estimate the optimal base substitution model, Model test v. 3.7 (Posada & Crandall, 1998) was carried out by using PAUP* 4 (Swofford, 1998), and the GTR+G model was selected as the best one. We assessed the reliability of branching in the tree using nonparametric bootstrap analysis with 1,000 replicates (MLBS). We also estimated the optimal base substitution model to execute Bayesian analysis. MrModeltest v. 2.3 (Nylander, 2008) was carried out using PAUP* 4 (Swofford, 1998). Following the MrModeltest results, we independently assigned the same substitution model [lset nst = 6, rates = invgamma, and prset statefreqpr = dirichlet (1, 1, 1, 1)] for each locus. We used MrBayes V.3.2.7 software (Ronquist et al., 2012) to construct phylogeny and compute Bayesian inference posterior probability (BPP) for each branch under Bayesian inference. MrBayes analysis involved two sets of four chains of Markov chain Monte Carlo composed of three heated chains and one cold chain, with the topology sampled after every 100 generations. In total, 1,500,000 generations were analyzed until the average standard deviation of the split frequencies was < 0.01. After convergence in the Markov chain Monte Carlo, the first 25% of primary topologies were discarded as burn-in; then the 50% consensus tree was constructed using remaining trees. The tree was visualized using FigTree v.1.4.4 software (tree.bio.ed.ac.uk/software/figtree/).

Because only ITS sequences were available for several species of sect. *Calodontes*, we created the ITS phylogenetic tree using *M. rubromarginata* and *M. zephirus* (Fr.) P. Kumm. as outgroups, similar to Chew et al. (2014). The 79 sequences determined by previous studies (Chew et al., 2014; Cooper, 2018; Harder et al., 2010, 2012, 2013; Liu et al., 2021, 2022; Matheny et al., 2006; Olariaga et al., 2015; Osmundson et al., 2013) and eight sequences identified in our study were analyzed using the GTR+G model and RAxML method (Table 2). In MrBayes analysis, we estimated the optimal base substitution model using KAKUSAN4 (Tanabe, 2011) and GTR+G model was selected. Bayesian analysis was performed using the same method as described previously, except that the dataset was analyzed for 1,000,000 generations.

To conduct species delimitation using genealogical concordance phylogenetic species recognition (GCPSR) (Taylor et al., 2000), we generated independent ITS, TEF1 and RPB1 trees. For molecular phylogenetic analysis of individual regions, we used the same sequences as those used to construct the concatenated three gene tree and the analysis method applied to the concatenated dataset with some minor modifications (Table 2). Briefly, we applied the GTR+G model for ML analysis and the SYM+G model for Bayesian inference in the TEF1 dataset, while the GTR+G model for both was used in the cases of ITS and RPB1. Bayesian analysis was performed for 1,500,000 generations. In the present study, we identified the species boundaries based on the concept of Taylor et al. (2000) that the transition point from concordance to conflict of the tree topologies between separate phylogenetic trees inferred from multiple genetic regions determines the limit of species.

The alignment dataset and resulting trees were deposited in TreeBase (https://treebase.org/) under the accession number TB2:S30700 for the tree in Supplementary Fig. S1, TB2:S30701 for Fig. 1, TB2:S31007 for Supplementary Fig. S3 dataset, TB2:S31008 for Supplementary Fig. S4, and TB2:S30699 for Supplementary Fig. S2.

Mycoscience

Table 2 – DNA sequences used for phylogenetic analyses.

Taxon name [a]	Specimen/Isolate numbers	Locality	Type	GenBank accession numbers [b]			Reference [b]	
				ITS	RPB1	TEF1	ITS	RPB1, TEF1
M. aff. *holoporphyra*	DED 8334	Africa		MH414547	ND	ND	Cooper, 2018	ND
M. aff. *pura*	TL8052	Ecuador		FN394623	KF723687	KF723641	Harder et al., 2010	Harder et al., 2013
M. aff. *pura*	TL9433	Ecuador		FN394622	KF723688	KF723642	Harder et al., 2010	Harder et al., 2013
M. aff. *pura*	TL9450	Ecuador		KJ144653	KF723689	KF723643	Harder et al., 2010	Harder et al., 2013
M. aff. *pura*	TL9678	Ecuador		FN394621	KF723690	KF723644	Harder et al., 2010	Harder et al., 2013
M. brunnea	CBH386	Denmark		FN394565	KF723679	KF723633	Harder et al., 2010	Harder et al., 2013
M. brunnea	CBH187	Sweden		FN394564	KF723678	KF723632	Harder et al., 2010	Harder et al., 2013
M. brunnea	M0160144	Sweden	Holotype	KT222187	ND	ND	Olariaga et al., 2015	ND
M. brunneoviolacea	BAP594	Africa	Holotype	MH414546	ND	ND	Cooper, 2018	ND
M. cahaya	KLU M 1221	Malaysia	Holotype	KF537248	ND	ND	Chew et al., 2014	ND
M. densilamellata	TUFC 101999	Japan	Ex-holotype	LC777686	LC777726	LC777734	This study	This study
M. densilamellata	TUMH 65486	Japan		LC777687	LC777727	LC777735	This study	This study
M. densilamellata	TUMH 65482	Japan		LC777688	LC777728	LC777736	This study	This study
M. densilamellata	TNS-F-75029	Japan		LC777689	LC777729	LC777737	This study	This study
M. diosma	LK1191/2002	Germany		FN394619	KF723700	KF723654	Harder et al., 2010	Harder et al., 2013
M. diosma	CBH400	Denmark		FN394617	KF723699	KF723653	Harder et al., 2010	Harder et al., 2013
M. dura	10315	Austria	Holotype	FN394560	KF723694	KF723648	Harder et al., 2013	Harder et al., 2013
M. dura	ARAN-Fungi 1387	Spain		KT222189	ND	ND	Olariaga et al., 2015	ND
M. dura	ARAN-Fungi 1966	Spain		KT222190	ND	ND	Olariaga et al., 2015	ND
M. dura	ARAN-Fungi 2950	Spain		KT222188	ND	ND	Olariaga et al., 2015	ND
M. lammiensis	165927	Finland		FN394552	KF723697	KF723651	Harder et al., 2010	Harder et al., 2013
M. luteovariegata	TL5614	Denmark		FN394602	KF723666	KF723620	Harder et al., 2010	Harder et al., 2013
M. luteovariegata	CBH226	Denmark	Epitype	FN394604	KF723664	KF723618	Harder et al., 2010	Harder et al., 2013
M. luteovariegata	DB2005/152	Denmark		FN394603	KF723665	KF723619	Harder et al., 2010	Harder et al., 2013
M. pearsoniana	JV06890	Denmark		FN394612	KF723692	KF723646	Harder et al., 2010	Harder et al., 2013
M. pearsoniana	LK880/2002	Germany		FN394613	KF723693	KF723647	Harder et al., 2010	Harder et al., 2013
M. pearsoniana	CBH068	Germany		FN394614	KF723691	KF723645	Harder et al., 2010	Harder et al., 2013
M. pearsoniana	FCME25817	USA	Epitype	JN182198	ND	ND	Harder et al., 2012	ND
*M. pearsoniana*_II	TL3966	Denmark		FN394615	ND	ND	Harder et al., 2013	ND
*M. pearsoniana*_II	TENN61865	USA		JN182201	ND	ND	Harder et al., 2012	ND
M. pelianthina	CBH016	Denmark		FN394547	KF723696	KF723650	Harder et al., 2010	Harder et al., 2013
M. pelianthina	CBH015	Denmark		FN394549	KF723695	KF723649	Harder et al., 2010	Harder et al., 2013
M. polycystidiata	FFAAS0417	China	Holotype	ON427731	ON468456	ON468469	Liu et al., 2022	Liu et al., 2022
M. polycystidiata	FFAAS0418	China		ON427732	ON468457	ON468470	Liu et al., 2022	Liu et al., 2022
M. polycystidiata	FFAAS0421	China		ON427733	ON468458	ON468471	Liu et al., 2022	Liu et al., 2022
M. polycystidiata	FFAAS0422	China		ON427734	ON468459	ON468472	Liu et al., 2022	Liu et al., 2022
*M. pura*_I	CBH039	Denmark		FN394588	KF723680	KF723634	Harder et al., 2010	Harder et al., 2013
*M. pura*_II	CBH366	Denmark		FN394572	KF723673	KF723627	Harder et al., 2010	Harder et al., 2013
*M. pura*_II	CBH404	Denmark		FN394566	KF723674	KF723628	Harder et al., 2010	Harder et al., 2013
*M. pura*_II	CBH169	Denmark		FN394579	KF723672	KF723626	Harder et al., 2010	Harder et al., 2013
*M. pura*_II	CBH105	Denmark		FN394581	KF723671	KF723625	Harder et al., 2010	Harder et al., 2013
*M. pura*_III	CBH019	Denmark		FN394605	KF723675	KF723629	Harder et al., 2010	Harder et al., 2013
*M. pura*_III	CBH022	Denmark		FN394574	KF723676	KF723630	Harder et al., 2010	Harder et al., 2013
*M. pura*_III	KK	Slovakia		FN394606	KF723677	KF723631	Harder et al., 2010	Harder et al., 2013
*M. pura*_IV	JV06979	Denmark		FN394585	KF723668	KF723622	Harder et al., 2010	Harder et al., 2013
*M. pura*_IV	CBH410	Denmark		FN394595	KF723667	KF723621	Harder et al., 2010	Harder et al., 2013
*M. pura*_IV	TL12786	Sweden		FN394591	KF723670	KF723624	Harder et al., 2010	Harder et al., 2013
*M. pura*_IV	TL4571	Denmark		FN394583	KF723669	KF723623	Harder et al., 2010	Harder et al., 2013
*M. pura*_VI	BAP132	USA		FN394561	KF723660	KF723614	Harder et al., 2010	Harder et al., 2013
*M. pura*_VII	IS/10/11/2000	USA		FN394611	KF723661	KF723615	Harder et al., 2010	Harder et al., 2013
*M. pura*_VIII	CBH402	Denmark		FN394599	KF723663	KF723617	Harder et al., 2010	Harder et al., 2013
*M. pura*_VIII	CBH216	Denmark		FN394598	KF723662	KF723616	Harder et al., 2010	Harder et al., 2013
*M. pura*_IX	CBH166	Denmark		FN394607	KF723701	KF723655	Harder et al., 2010	Harder et al., 2013
*M. pura*_IX	CBH358	Denmark		FN394608	KF723702	KF723656	Harder et al., 2010	Harder et al., 2013
*M. pura*_IX	CBH371	Denmark		KF913023	KF723704	KF723658	Harder et al., 2013	Harder et al., 2013
*M. pura*_IX	CBH367	Denmark		KF913022	KF723703	KF723657	Harder et al., 2013	Harder et al., 2013
*M. pura*_X	BAP165A	USA		FN394563	KF723698	KF723652	Harder et al., 2010	Harder et al., 2013
M. rosea	TL12393	Denmark		FN394555	KF723684	KF723638	Harder et al., 2010	Harder et al., 2013
M. rosea	CBH409	Germany		FN394551	KF723683	KF723637	Harder et al., 2010	Harder et al., 2013
M. rosea	UP2	UK		FN394550	KF723686	KF723640	Harder et al., 2010	Harder et al., 2013
M. rosea	CBH383	Denmark		FN394553	KF723682	KF723636	Harder et al., 2010	Harder et al., 2013
M. rosea	CBH097	Denmark		FN394556	KF723681	KF723635	Harder et al., 2010	Harder et al., 2013
M. rosea	TL12409	Denmark		FN394557	KF723685	KF723639	Harder et al., 2010	Harder et al., 2013
M. rufobrunnea	FFAAS0414	China		ON427728	ON468453	ON468466	Liu et al., 2022	Liu et al., 2022
M. rufobrunnea	FFAAS0415	China		ON427729	ON468454	ON468467	Liu et al., 2022	Liu et al., 2022
M. rufobrunnea	FFAAS0416	China	Holotype	ON427730	ON468455	ON468468	Liu et al., 2022	Liu et al., 2022
M. seminau	KLU M 1223	Malaysia	Holotype	KF537250	ND	ND	Chew et al., 2014	ND
M. seminau	KLU M 1226	Malaysia		KF537252	ND	ND	Chew et al., 2014	ND
M. shengshanensis	FFAAS0424	China	Holotype	ON427739	ON468464	ON468477	Liu et al., 2022	Liu et al., 2022
M. shengshanensis	FFAAS0425	China		ON427740	ON468465	ON468478	Liu et al., 2022	Liu et al., 2022
M. sinar	KLU M 1222	Malaysia	Holotype	KF537249	ND	ND	Chew et al., 2014	ND
M. sinar	KLU M 1220	Malaysia		KF537247	ND	ND	Chew et al., 2014	ND
M. sinar var. *tangkaisinar*	KLU M 1225	Malaysia	Holotype	KF537251	ND	ND	Chew et al., 2014	ND
M. subulata	FFAAS0419	China		ON427735	ON468460	ON468473	Liu et al., 2022	Liu et al., 2022
M. subulata	FFAAS0420	China		ON427736	ON468461	ON468474	Liu et al., 2022	Liu et al., 2022
M. subulata	FFAAS0423	China	Holotype	ON427737	ON468462	ON468475	Liu et al., 2022	Liu et al., 2022
M. subulata	FFAAS0426	China		ON427738	ON468463	ON468476	Liu et al., 2022	Liu et al., 2022
M. subulata	TUMH 65483	Japan		LC777690	LC777730	LC777738	This study	This study
M. subulata	TUMH 65484	Japan		LC777691	LC777731	LC777739	This study	This study
M. subulata	TUMH 65485	Japan		LC777692	LC777732	LC777740	This study	This study
M. subulata	TNS-F-75058	Japan		LC777693	LC777733	LC777741	This study	This study
M. yuezhuoi	FFAAS0346	China		MW581492	MW868168	MW882251	Liu et al., 2021	Liu et al., 2021
M. yuezhuoi	FFAAS0344	China		MW581490	MW868166	MW882249	Liu et al., 2021	Liu et al., 2021
M. yuezhuoi	FFAAS0345	China	Holotype	MW581491	MW868169	MW882250	Liu et al., 2021	Liu et al., 2021
M. yuezhuoi	FFAAS0347	China		MW581493	MW868167	MW882252	Liu et al., 2021	Liu et al., 2021
M. plumbea	AFTOL-ID 1631	USA		DQ494677	DQ447928	GU187729	Matheny et al., 2006	Matheny et al., 2006 Binder et al., 2010
M. rubromarginata	JV09362	Denmark		FN394624	KF723705	KF723659	Harder et al., 2010	Harder et al., 2013
M. zephirus	54m	Italy		JF908462	ND	ND	Osmundson et al., 2013	Binder et al., 2010

[a] Roman numeral of *Mycena pura* followed Harder et al. (2013). *Mycena pura* V and *M. pura* XI were currently renamed to *M. luteovariegata* and to *M. brunnea* (Harder et al., 2013; Olariaga et al., 2015).
[b] ND: No data.

doi: 10.47371/mycosci.2024.02.002

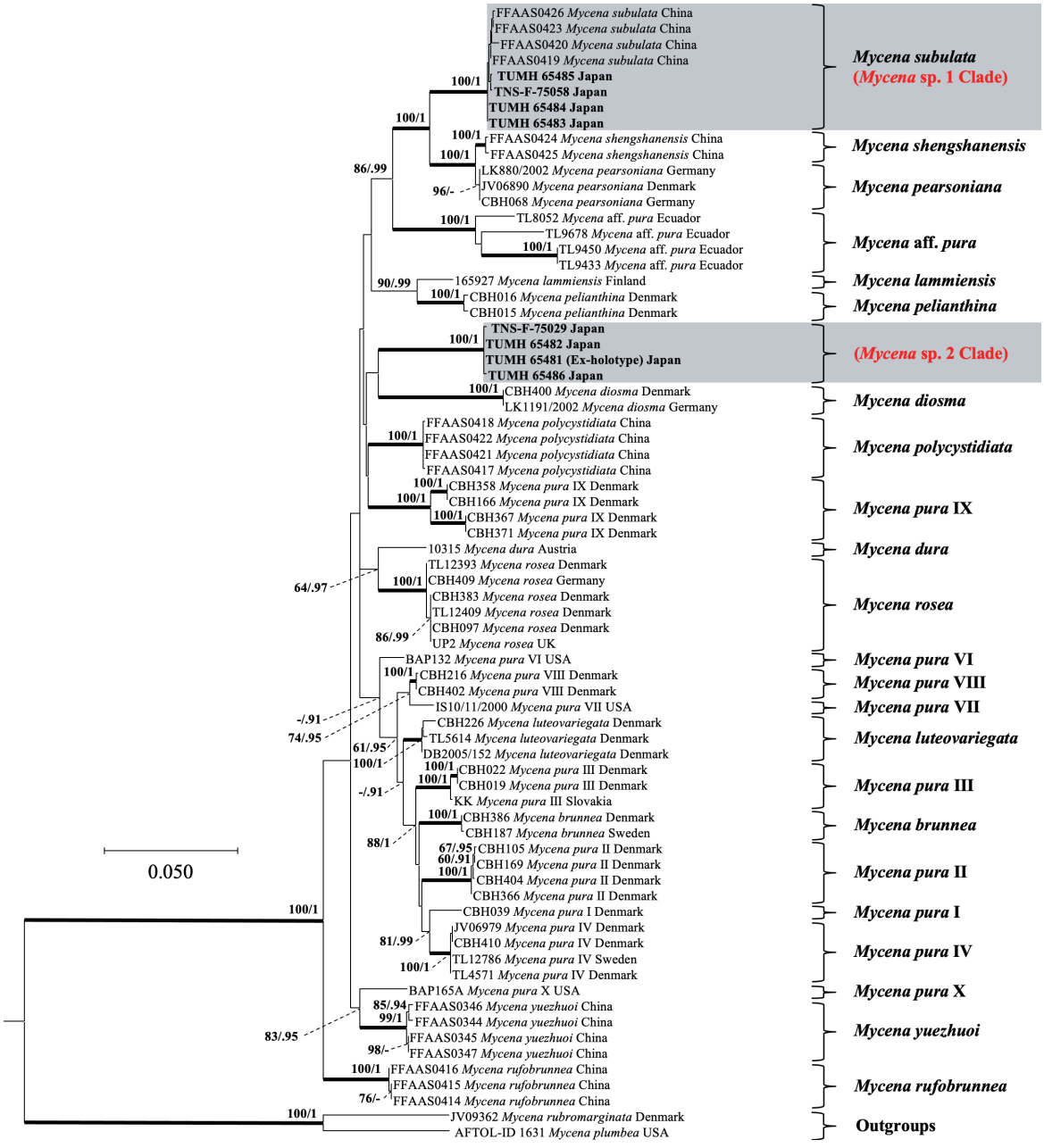

Fig. 1 – Maximum likelihood (ML) tree of the *Mycena* sect. *Calodontes* inferred from concatenated multigene (ITS, RPB1, TEF1) sequences using RAxML. Statistical supports at the nodes are ML bootstrap support (MLBS)/Bayesian posterior probability (BPP). Thick nodes indicate strong support (MLBS≥90 % and BPP≥0.95) and omission of the support value shows less than 60 % in MLBS and 0.90 in BPP. Sequences determined in this study are shown in bold letters and the other sequences were obtained from the NCBI database.

3. Results

3.1. Morphology

Both "*Mycena* spp."- 1 and 2 exhibited basidiomata with a reddish tone (pinkish or purplish), smooth cheilocystidia, as well as smooth hyphae of both pileipellis and stipitipellis. These features match the characters of sect. *Calodontes* sense Maas Geesteranus (1989). "*Mycena* sp."-1 had inamyloid basidiospores and lacked pleurocystidia corresponding with characteristics of subsect. *Violacellae*. This fungus resembles *M. subulata* reported from China (Liu et al., 2022) in having awl-shaped cheilocystidia, but differs from Chinese specimens having amyloid basidiospores. Moreover, Japanese specimens had dextrinoid cheilocystidia and caulocystidia. "*Mycena* sp."-2 had amyloid basidiospores and cheilocystidia with a broadly rounded apex, similar to the characteristics of subsect. *Purae* sensu Maas Geesteranus and de Meijer (1997). This fungus had 32–44 lamellae reaching the stipe, bowling-pin shaped cheilocystidia, and amyloid basidiospores, but lacked pleurocystidia. These features are unique among species of sect. *Calodontes*. Morphological comparisons with other phylogenetically related or morphologically similar species are discussed in the "Discussion" part.

3.2. Phylogenetic analyses and species delimitation

Both "*Mycena* spp."- 1 and 2 formed clades in the position of sect. *Calodontes* in all phylogenetic trees inferred from each of the

ITS, RPB1, and TEF1 sequences and their concatenated data (Fig. 1; Supplementary Figs. S1–S4). "*Mycena* sp."-1 clustered with Chinese *M. subulata* specimens with high MLBS/BPP values in the concatenated dataset (100/1) and it the individual ITS (98/1), RPB1 (99/1), and TEF1 (100/1) trees. High degrees of DNA homology were observed between Chinese *M. subulata* and Japanese "*Mycena* sp."-1 specimens (ITS, 97.9–99.8%; RPB1, 98.8–100%; TEF1, 98.2–99.5%). According to the species delimitation using the GCPSR concept, we compared tree topologies at the node connecting eight specimens, including Chinese *M. subulata* and Japanese "*Mycena* sp." -1. The topologies coincided within ITS, RPB1, and TEF1 phylogenetic trees (= concordance). However, the topologies below that node were different among the trees (= conflict) (Fig. 2; Supplementary Figs. S2–S4). Consequently, *M. subulata* and "*Mycena* sp."-1 were regarded as conspecific. Although Harder et al. (2013) suggested the possibility of a recombination event occurring in the ITS region in some species of sect. *Calodontes*, no such traces were detected in the multiple alignment of the ITS sequences of Japanese and Chinese *M. subulata* specimens (Supplementary Fig. S5). "*Mycena* sp."-2 formed a distinct clade clustering without any other species, as indicated by the MLBS/BPP values of 100/1 for the concatenated, 100/1 for ITS, 100/1 for RPB1, and 100/1 for TEF1 trees (Fig. 1; Supplementary Figs. S1–S4). "*Mycena* sp."-2 formed a sister clade with European *M. diosma* in concatenated and TEF1 trees without MLBS/BPP support (Fig. 1; Supplementary Fig. S3). Topological discordance was observed in the relation between "*Mycena* sp."-2 and *M. diosma* in ITS and RPB1 trees (Supplementary Figs. S1, S2, S4). In addition, DNA homology between *M. diosma* and "*Mycena* sp."-2 specimens was 84.1–84.8% in the ITS, 94.3–94.5% in the RPB1, and 89.4% in the TEF1. Therefore, we concluded that "*Mycena* sp."-2 and *M. diosma* are different species. Although we failed to obtain sequences from Kigawa's original specimens, the other sequences derived from specimens with similar morphological characteristics to Kigawa's descriptions (Kigawa, 2017) clustered into the "*Mycena* sp."-1 or "*Mycena* sp."-2 clade (Fig. 1; Supplementary Figs. S1–S4). In the three regions where GCPSR were performed, almost all species-clades were reproduced in each tree. However, in the ITS region, *M. luteovariegata* and *M. pura* IX did not constitute monophyly, which did not match the results of TEF1 and RPB1 trees, as reported by Harder et al. (2013) (Supplementary Figs. S2–S4).

3.3. Taxonomy

Based on the phylogenetic and morphological analyses, we concluded that "*Mycena* sp."-1 is *M. subulata,* which has been described from China, whereas "*Mycena* sp."-2 is a new species. The morphological characteristics of these species are described below.

Mycena subulata Z.W. Liu, Y.P. Ge & Q. Na, MycoKeys 93: 46 (2022) Fig. 3

Japanese name: Togari-sakura-take (Kigawa, 2017).

Macromorphology: Basidiomata (Fig. 3A, B) small- to medium-sized, mycenoid to collybioid. Pileus (Fig. 3C) 15–55 mm in diam, subumbonate to convex; surface light pink (oac487) to brown-pink (oac730); margin striate when wet, gradually disappear as it dry, glabrous, hygrophanous, whitish (oac909) to light pink (oac550) or pale orange (oac682). Pileal context 2 mm in thickness. Lamellae (Fig. 3D, E) 26–33 reaching the stipe, closed to crowded, adnate to subdecurrent, lateral veins and lamellulae are present, light pink (oac549) to pale orange (oac682). Stipe (Fig. 3F) 20–65 mm long, 2–3 mm diam in center, 3–6 mm diam at basal part, smooth, rarely squarrose, light pink (oac550) to pink (oac486) or orange-brown (oac681); base slightly enlarged, tomentose; mycelial cords present or absent, yellow (oac6) to orange (oac811). Odor raphanoid. Taste unknown.

Micromorphology: Basidiospores (Fig. 3G, H) 6–7.5(–8) × (4–)4.5(–5.5) µm, Q = (1.32–)1.38–1.70(–1.84), Q_m = 1.43–1.72 (200 spores from 4 collections), ellipsoid to elongate, rarely pip-shaped, thin-walled, smooth, inamyloid, subhyaline. Basidia (Fig. 3I) (19–)20–24(–26) × (6–)7–8.5 µm, clavate, thin-walled, smooth, inamyloid, 2–4 spored, subhyaline; basal clamps partially present. Sterigmata 1.5–6.5 µm in length, thin-walled, smooth, inamyloid, subhyaline. Basidioles (17.5–)18.5–23(–25) × (5.5–)6–7.5(–8.5) µm, clavate, thin-walled, smooth, inamyloid, subhyaline; basal clamps partially present. Cheilocystidia (Fig. 3J–M) has two shapes; awl-shaped cystidia (33–)39.5–43(–63) × (4.5–)5–8.5(–10.5) µm, thick-walled, smooth, dextrinoid, subhyaline; clavate to obclavate cystidia (26–)27.5–38(–41.5) × (7.5–)8–11.5(–13) µm, thin-walled, smooth, inamyloid, subhyaline; basal clamps partially present. Pleurocysitidia absent. Hymenophoral trama subregular, hyphae 2–8 µm regular thin-walled, smooth, dextrinoid, subhyaline; clamp connections partially present. Pileipellis tomentocutis, hyphae 2–6 µm diam, cylindrical, thin-walled, smooth, weakly dextrinoid, subhyaline; clamp connections partially present. Pileocystidia not observed. Pileitrama pseudoparenchymatous, hyphae 9–25 µm diam, cylindrical, thin-walled, smooth, dextrinoid, subhyaline; clamp connections partially present. Stipitipellis parallel, hyphae 2–4 µm diam, cylindrical, smooth, thin-walled, weakly dextrinoid, subhyaline; clamp

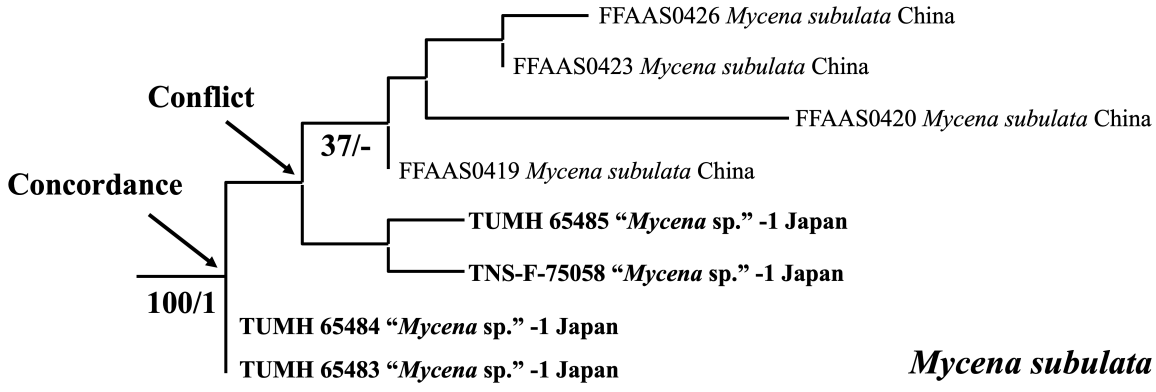

Fig. 2 – Species delimitation based on the genealogical concordance phylogenetic species recognition (GCPSR) concept. Topology of the three-loci-concatenated tree was employed. "Concordance" shows the node that all three independent trees (ITS, TEF1 and RPB1) corresponded to each other, and "Conflict" shows the node that those independent trees did not correspond to each other. Statistical supports at the nodes are ML bootstrap support (MLBS)/Bayesian posterior probability (BPP). (see also Supplementary Figs. S2–S4).

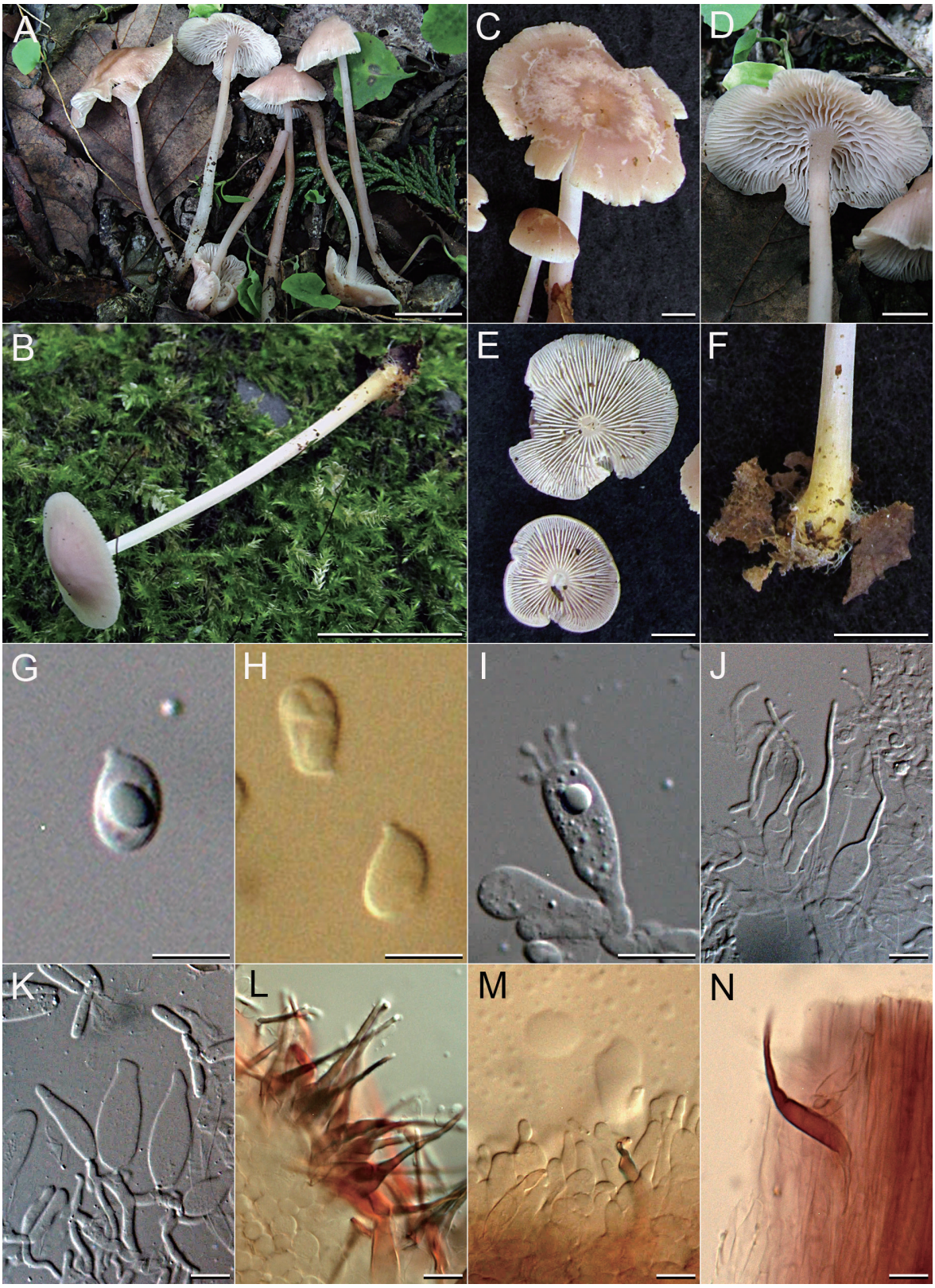

Fig. 3 – *Mycena subulata*. Macroscopic (A–F) and microscopic (G–N) images. A, B: Basidiomata (A: TUMH 65484; B: TUMH 65483), C: Pileus (TUMH 65485), D, E: Lamellae (D; TUMH 65484, E; TUMH 65485), F: Stipe (TUMH 65483). G: Basidiospore in 3% KOH (TUMH 65484), H: Basidiospores in Melzer's reagent (TUMH 65484), I: Basidium (TUMH 65485), J: awl-shaped cheilocystidia (TUMH 65484), K: Obclavate cheilocystidia (TNS-F-75058). L: awl-shaped cheilocystidia dyeing with Melzer's reagent (TUMH 65483), M: Obclavate cheilocystidia dyeing with Melzer's reagent (TNS-F-75058), N: Caulocystidium dyeing with Melzer's reagent (TUMH 65485). *Bars*: A, B 15 mm; C–F 5 mm; G, H 5 μm; I–N 10 μm.

connections partially present. Stipitrama parallel, hyphae 2–13 μm diam, cylindrical to ellipsoid, thin-walled, dextrinoid, subhyaline; clamp connections partially present. Caulocystidia (Fig. 3N) rarely present, 36–60 × 5–9 μm, awl-shaped, thick-walled, smooth, dextrinoid, subhyaline; basal clamps partially present.

Ecology: Cool temperate region of Japan, Sep, solitary to gregarious, on litter layer in *Cryptomeria japonica* (L.f.) D. Don, *Fagus crenata* Blume, *Aesculus turbinata* Blume, and/or *Acer* sp. Heilongjiang Province, China, scattered on the litter layer in *Pinus koraiensis* Siebold et Zucc., *Larix gmelinii* (Rupr.) Rupr. ex Kuzen. and *Tilia* sp. mixed forests.

Materials examined: JAPAN. Tottori Pref., Daisen Town, on the leaf litter in a summer green forest of *Qurcus*, 11 Sep 2021, leg. K. Nagamune (Specimen: TUMH 65483; Culture: TUFC 102001); Tottori City, Shikano Town, on the ground in a mixed forest of *Acer*, *Chamaecyparis*, *Cerasus*, 19 Sep 2021, leg. K. Nagamune (Specimen: TUMH 65484; Culture: TUFC 102002); Kotoura Town, on the ground in a summer green forest of *Fagus*, *Aesculus*, and *Acer*, 20 Sep 2021, leg. R. Sugawara (Specimen: TUMH 65485; Culture: TUFC 102003): Saitama Pref., Chichibu City, Nakatsugawa, 28 Sep 2015, leg. K. Hosaka (Specimen: TNS-F-75058): Shizuoka Pref. Gotenba City, 13 Sep 2009, leg. S. Kigawa (Specimen: HCM-58-6665).

Note: Many short and branched spinous seta-like structures were formed on the cultured mycelia (Supplementary Fig. S6). These structures resembled the acanthocytes discovered in the cultured mycelia of *Stropharia rugosoannulata* Farl. ex Murrill as a possible apparatus for defense against nematodes (Yang et al., 2021). A glassy substance covered the seta-like hyphae and could be easily detached and fragmented on slide preparation.

Mycena densilamellata Nagamune, S. Kigawa & N. Endo, sp. nov. Fig. 4

MycoBank no.: MB 850080

Type: JAPAN, Hokkaido, Ebetsu City, Nopporo Shinrin Kouen Prefectural Natural Park, on the ground of mixed forest where *Picea*, and *Alnus* were dominated, 14 Sep 2019, leg. R. Sugawara (holotype, TUMH 65481; isotype, TNS-F-82703)

Ex-holotype culture: TUFC 101999 (polysporic strain)

Gene sequences from ex-holotype culture: LC777686 (ITS), LC777726 (TEF1), LC777734 (RPB1)

Etymology: *densus* (Latin) + *lamellatus* (Latin), referring to the densely formed lamella at the gill of the pileus.

Japanese name: Mitsuhida-sakura-take (Kigawa, 2017).

Macromorphology: Basidiomata (Fig. 4A, B) small- to medium-sized, collybioid. Pileus (Fig. 4C) 20–35 mm in diam, umbonate to subumbonate when young, convex with age; surface purplish (oac427) to dark purplish (oac514); margin striate when wet, gradually disappear as it dry, glabrous, hygrophanous, whitish (oac909) to cream (oac900). Pileal context 2 mm thickness. Lamellae (Fig. 4D, E) 32–44 reaching the stipe, crowded, adnexed to adnate, subdecurrent, lateral veins and lamellulae present, pale lilac (oac438). Stipe (Fig. 4F) 30–70 mm long, 2–4 mm diam in center, 4–7 diam at basal part, smooth or slightly longitudinal striate, pinkish (oac473) to reddish brown (oac525); base slightly enlarged, tomentose, concolorous or yellow (oac6); mycelial cords present or absent, whitish (oac909). Odor raphanoid. Taste unknown.

Micromorphology: Basidiospores (Fig. 4G–I) 5.5–6.5(–7) × 3–4(–4.5) μm, Q = (1.50–)1.58–1.89(–2.02), Q_m = 1.64–1.80 (200 spores from 4 collections), ellipsoid to cylindrical, rarely pip-shaped, thin-walled, smooth, amyloid, subhyaline. Basidia (Fig. 4J) (17–)19.5–26(–27.5) × (5.5–)6–7(–7.5) μm, clavate or constricted in the middle, thin-walled, smooth, inamyloid, subhyaline, 2–4 spored; basal clamps partially present. Sterigmata 1–6 μm length, thin-walled, smooth, inamyloid, subhyaline. Basidioles (16.5–)18–26(–27.5) × (4–)4.5–6.5(–7.5) μm, clavate, thin-walled, smooth, inamyloid, subhyaline; basal clamps partially present. Cheilocystidia (Fig. 4K–M) (27–)29.5–44(–48) × (7–)8–14(–17) μm, clavate, rarely protruding tip, bowling-pin shaped, thin-walled, smooth, inamyloid, subhyaline; basal clamps partially present. Pleurocystidia absent. Hymenophoral trama subregular, hyphae 3–6 μm diam, thin-walled, smooth, dextrinoid, subhyaline; clamp connections partially present. Pileipellis tomentocutis, hyphae 1–4 μm diam, cylindrical, thin-walled, smooth, inamyloid, subhyaline; clamp connections partially present. Pileocystidia not observed. Pileitrama pseudoparenchymatous, hyphae 4–17 μm diam, cylindrical to ellipsoid, thin-wallled, smooth, dextrinoid, subhyaline; clamp connections partially present. Stipitipellis parallel, hyphae 1–2 μm diam, cylindrical, smooth, thin-walled, weakly dextrinoid, subhyaline; clamp connections partially present. Stipitrama parallel, hyphae 3–10 μm diam, cylindrical to slightly expanded, thin-walled, dextrinoid, subhyaline; hyphae, clamp connections partially present. Caulocystidia (Fig. 4N) rarely present, 24–32.5 × 6–8 μm, obclavate to clavate, thin-walled, smooth, inamyloid, subhyaline; basal clamps partially present.

Ecology: Cool temperate region of Japan, Sep to Oct, gregarious to scattered on litter layer in *Quercus serrata* Murray, *Chamaecyparis obtusa* (Siebold et Zucc.) Endl., *Fagus crenata, Alnus* sp. *Picea abies* (L.) H. Karst. and *Picea glehnii* (F. Schmidt) Mast.

Other materials examined: JAPAN. Yamanashi Pref., Minamitsuru-gun, Narusawa Village, on the leaf litter in a mixed forest of *Quercus*, *Fagus*, and *Chamaecyparis*, 8 Oct 2020, leg. R. Sugawara (Specimen: TUMH 65486): Tottori Pref., Tottori City, Shikano Town, on the ground in a mixed forest of *Chamaecyparis*, *Quercus*, and *Carpinus*, 19 Sep 2021, leg. K. Nagamune (Specimen: TUMH 65482; Culture: TUFC 102000): Saitama Pref., Chichibu City, Nakatsugawa, 28 Sep 2015, leg. K. Hosaka (Specimen: TNS-F-75029): Shizuoka Pref. Gotenba City, 13 Sep 2009, leg. S. Kigawa (Specimen: HCM-58-6667).

Note: Many short and branched acanthocyte-like structures were formed on the cultured mycelia (Supplementary Fig. S7) as observed in "*Mycena* sp."-1 cultures (see above).

4. Discussion

"*Mycena* sp."-1 constituted a clade with the Chinese specimens of *M. subulata* (Fig. 1; Supplementary Figs. S1–S4) and showed similar characteristics of narrowly fusiform, long, and narrow protuberance of cheilocystidia (Fig. 3). The delimitation of species according to the GCPSR concept (Fig. 2) strongly suggested conspecificity. *Mycena subulata* has not been reported previously from Japan. Here, we assign the Japanese name "*Togari-sakura-take*", to the species, consistent with Kigawa (2017). However, our specimens from Japan did not show the amyloid basidiospores, which differed from the original description by Liu et al. (2022). Although the Japanese specimens showed dextrinoid cheilocystidia, Liu et al. (2022) did not include this characteristic in the original description. It is necessary to observe more specimens (including the type specimen) to determine whether these features exhibit regional differences among the Japanese samples. In the detailed analysis of the molecular data, seven mutation sites in the ITS were detected between Chinese and Japanese *M. subulata* (Supplementary Fig. S5). However, these base differences did not distinguish between Chinese and Japanese specimens on the phylogenetic trees (Supplementary Figs. S1, S2).

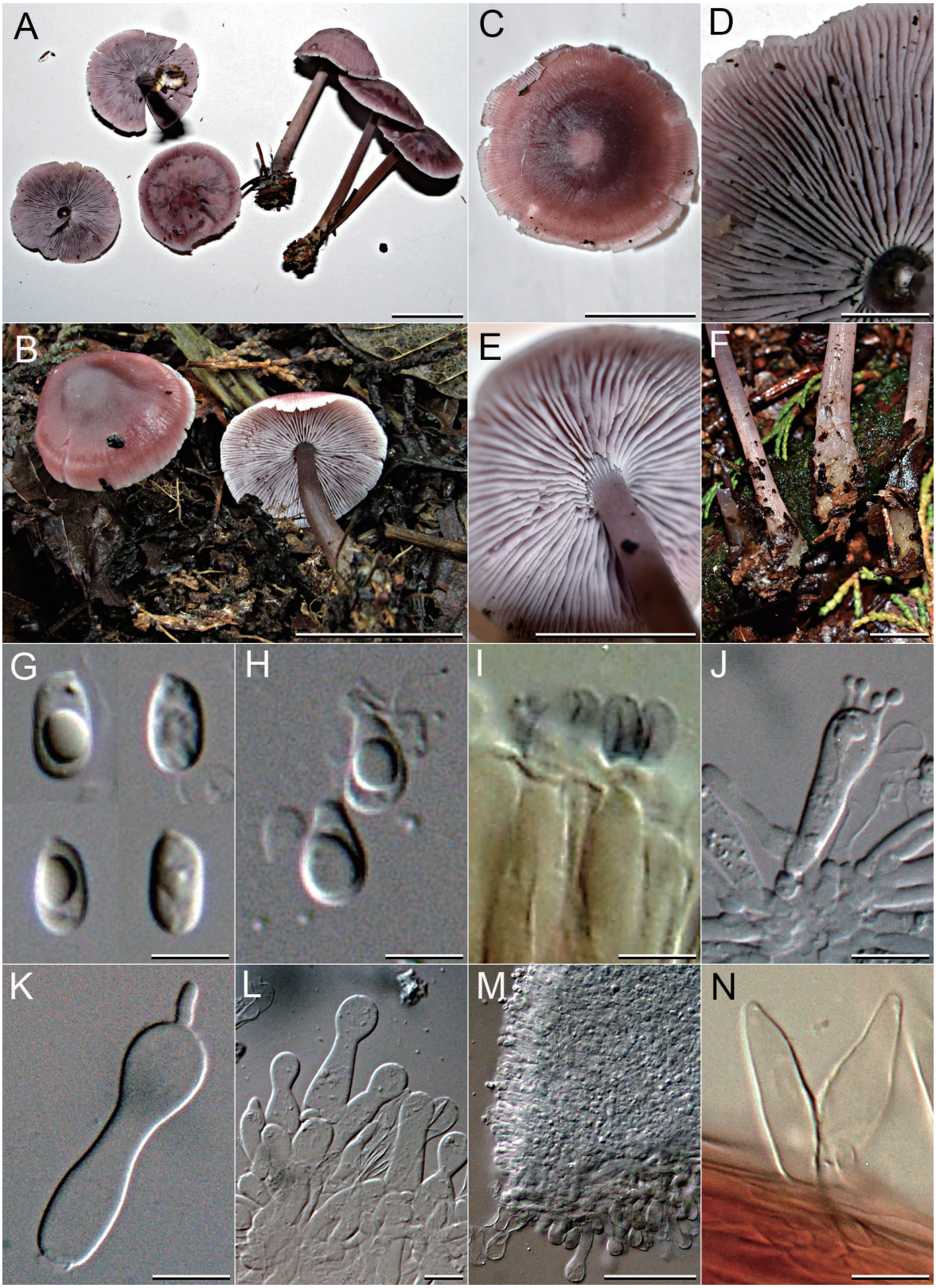

Fig. 4 – *Mycena densilamellata*. Macroscopic (A–F) and microscopic (G–N) images. A, B: Basidiomata (A: TUMH 65481, holotype; B: TUMH 65482), C: Pileus (TUMH 65486), D: Lamellae (TUMH 65482), E: Decurrent lamellae (TUMH 65486), F: Stipes (TUMH 65486). G: Basidiospores (TUMH 65481), H: Pip-shaped basidiospores (TUMH 654826), I: Basidiospores dyeing with Melzer's reagent (TUMH 65481), J: Basidium (TNS-F-75029), K: Cheilocystidium with protruding tip (TUMH 65481). L: Clavate and bowling-pin shaped cheilocystidia (TUMH 65486), M: Hymenium and lamellar edge (TUMH 65482), N: Caulocystidia in Melzer's reagent (TUMH 65486). *Bars*: A, B 20 mm; C, E 10 mm; D, F 5 mm; G–I 5 μm; J–L, N 10 μm; M 50 μm.

Liu et al. (2022) did not specify the subsection of *M. subulata* based on the amyloid basidiospores and lack of pleurocystidia. On the other hand, all five of our Japanese specimens demonstrated inamyloid basidiospores, indicating their similarity to the species of subsect. *Violacellae*. Harder et al. (2012) suggested the phylogenetic position of subsect. *Violacellae* in the ITS region by designating the epitype of *M. pearsoniana* Dennis ex Singer. *Mycena subulata* showed a phylogenetic position close to *M. pearsoniana* (Liu et al., 2022); our results support that (Fig. 1). Moreover, *M. pearsoniana* has inamyloid to weakly amyloid basidiospores, suggesting that amyloidity is not a diagnostic feature of species in *M. pearsoniana* (Harder et al., 2012). *Mycena subulata* and *M. pearsoniana* form a highly supported clade (MLBS/BPP = 96/1), which includes the *M. shengshanensis* described by Liu et al. (2022); a similar pattern was also found in the present study (MLBS/BPP = 100/1) (Fig. 1). *Mycena subulata* possesses both inamyloid and weakly amyloid basidiospores within the species as *M. pearsoniana*. Based on these results, we propose that *M. subulata* should be assigned to subsect. *Violacellae*. Although the morphological characteristics of subsect. *Generosae* coincide with those of *M. subulata* described by Liu et al. (2022), the phylogenetic position of subsect. *Generosae* has not been clarified. Hence, it is necessary to investigate the phylogenetic position of *M. generosa*, the type species of subsect. *Generosae* and the phylogenetic relationship between this subsection and *M. subulata*.

We assigned the Japanese name "*Mitsuhida-sakura-take*" to *M. densilamellata* consistent with Kigawa (2017). This species differs from other species in sect. *Calodontes* based on the formation of close lamellae, lack of pleurocystidia, and presence of bowling pin-shaped cheilocystidia and amyloid basidiospores. A few specimens had protruding tips of cheilocystidia, the most of which had broadly rounded apices (Fig. 4). These characteristics indicate that *M. densilamellata* belongs to subsect. *Purae* according to the definition by Maas Geesteranus and de Meijer (1997). *Mycena diosma* Krieglst. & Schwöbel from the subsect. *Purae* resembles *M. densilamellata* in terms of its pileus color (Harder et al., 2010; Krieglsteiner & Schwöbel, 1982). Despite the lack of significant statistical support and topological incongruence between datasets, our three-loci-concatenated and TEF1 trees suggested a potential sister relationship between the two species (Fig. 1; Supplementary Fig. S3). *Mycena diosma* has close lamellae (with 24–32 reaching the stipe) (Krieglsteiner & Schwöbel, 1982), whereas *M. densilamellata* exhibits crowding (with 32–44 reaching the stipe). In addition, *M. diosma* has few to no pleurocystidia depending on the specimen (Harder et al., 2010; Krieglsteiner & Schwöbel, 1982), whereas pleurocystidia are entirely lacking in *M. densilamellata*. *Mycena densilamellata* resembles *M. yuezhuoi* in terms of amyloid basidiospores and lack of pleurocystidia. However, *M. yuezhuoi* has slightly closer lamellae (with 21–26 reaching the stipe) emarginated to a stipe (Liu et al., 2021), whereas *M. densilamellata* exhibits crowded, adnate to decurrent lamellae (with 32–44 reaching the stipe). Furthermore, these two species are categorized in different clades according to the phylogenetic analysis (Fig. 1; Supplementary Figs. S1–S4). *Mycena kuehneriana* A.H. Sm. resembles *M. densilamellata* in terms of its amyloid basidiospores and lack of pleurocystidia. However, *M. kuehneriana* has subfusoid to nearly cylindric cheilocystidia and scattered to rare cheilocystidia (Smith, 1947). *Mycena densilamellata* is distinguishable from *M. kuehneriana* by its abundant bowling pin-shaped cheilocystidia. According to Maas Geesteranus (1989), 20 lamellae of *M. kuehneriana* reached the stipe, with scattered pleurocystidia, whereas *M. densilamellata* produced 32–44 stipe-reaching lamella without pleurocystidia. Although the crowding of lamellae is a characteristic feature of *M. densilamellata*, *M. rosea*, *M. sororia* Perr.-Bertr., Boissel. & Lambourd. and *M. vinacea* Cleland also have similar characteristics [with 40, 38–42, and 32–40 lamellae reaching the stipe, respectively (Grgurinovic, 1997; Maas Geesteranus, 1989; Perreau-Bertrand et al., 1996)]. However, *M. densilamellata* lacks pleurocystidia, whereas the other three have pleurocystidia. In addition, *M. densilamellata* is distinguishable from the other three based on cheilocystidia shape: those of *M. densilamellata* are bowling pin-shape, whereas they are fusiform, clavate, or subcylindrical in *M. rosea* (Maas Geesteranus, 1989); clavate, subcylindrical, or fusiform in *M. sororia* (Perreau-Bertrand et al., 1996); and cylindrical, cylindro-ventricose, or clavate in *M. vinacea* (Grgurinovic, 1997). Further detailed comparisons with species, including the remaining sect. *Calodontes* species, are presented in Supplementary Table S1. The original description of *M. sororia* is available, but the lack of type specimen and molecular information make it difficult to verify the taxonomic status of this species (Harder et al., 2010). For future revision of the whole sect. *Calodontes* species, there is an urgent need to redesignate the type specimen to allow molecular phylogenetic analyses of such species.

According to Chew et al. (2014) and Harder et al. (2012), the species of subsect. *Marginatae* and subsect. *Violacellae* formed independent monophyly, whereas those of subsect. *Purae* were separated into multiple clades. The phylogenetic trees constructed by Liu et al. (2021, 2022) based on the three loci (ITS, TEF1, and RPB1) exhibited polyphyly of subsect. *Purae*. In our phylogeny based on the three loci, monophyly of subsect. *Marginatae* was observed; however, subsect. *Purae* was also polyphyletic. *M. pearsoniana* and *M. subulata* had a similar morphological appearance to subsect. *Violacellae*, forming a robust monophyletic clade. However, *M. shengshanensis*, which cannot be assigned to any subsection due to its morphological characteristics (Liu et al., 2022), occupies an intermediate position between *M. pearsoniana* and *M. subulata* (Fig. 1), thereby necessitating the revision of subsect. *Violacellae* after consideration of the accuracy of the morphological description of *M. shengshanensis* by Liu et al. (2022). However, we found potential inaccuracies in the original description of *M. subulata*.

Currently, there is insufficient DNA data to determine the phylogenetic relationships among infrageneric taxa. To date, ITS sequences of 20 species belonging to sect. *Calodontes* have been registered in the GenBank database, which is < 50% of the known species. Among them, only 15 species, including the novel species, have records of authentic sequences obtained from the type materials. (Chew et al., 2014; Cooper, 2018; Harder et al., 2012, 2013; Liu et al. 2021, 2022; Olariaga et al., 2015). Moreover, the number of available sequences of TEF1 and RPB1 genes is even more limited (Harder et al., 2013; Liu et al., 2021, 2022). The phylogenetic position of subsection *Generosae* has not been confirmed. Thus, the unavailability of DNA information makes it challenging to determine whether or not the characteristic morphological features of each subsection reflect their phyletic relationship. Harder et al. (2013) suggested that TEF1 may be a more reliable marker for species identification in sect. *Calodontes* compared to ITS and RPB1. Indeed, only TEF1 indicated potential separation between the Japanese and Chinese populations of *M. subulata*, although this lacked statistical support (Supplementary Figs. S2–S4). In addition, TEF1 alone suggested a potential sister group relation between *M. densilamellata* and *M. diosma*, but this association also lacked statistical support (Supplementary Fig. S3). In future studies, it will be necessary to collect specimens from various parts of the world and obtain additional DNA sequences. This should include not only ITS, TEF1, and RPB1, but also other frequently used regions, such as RPB2, ATP6, etc., along with morphological characteristics.

These combined efforts will contribute to the construction of a robust infrageneric classification system accurately reflecting the phylogenetic relations and help to determine the most effective DNA marker for species identification in sect. *Calodontes*.

The fruiting bodies of *M. subulata* and *M. densilamellata* predominantly emerged on litter within the broadleaved forests dominated by oak and beech trees at altitudes exceeding 450 m above sea level across the Japanese mainland. Specimens of *M. subulata* were collected from Eastern (Saitama Pref.) and Western (Tottori Pref.) regions of Japan. Similarly, specimens of *M. densilamellata* were collected from Northern (Hokkaido) and Western (Tottori Pref.) regions of Japan. Thus, both species are widely distributed in broadleaved forests in Japan. *Mycena* species in sect. *Calodontes* have been well described in Europe since the mid-18th century, whereas few taxonomic studies have been conducted in Asia (Aravindakshan & Manimohan, 2015; Chew et al., 2014; Hennings 1900; Liu et al., 2021, 2022). Further studies on the taxonomy and ecology of *Mycena* species in Asia are required to gain a thorough understanding of the distribution of these species. On the cultured mycelia of *M. subulata* and *M. densilamellata*, we observed acanthocyte-like structures similar to those described by Yang et al. (2021) (Supplementary Figs. S6, S7). On the other hand, Chew et al. (2014) reported inamyloid crystals on mycelia of *M. cahaya* cultured on MEA. Because we could not verify whether the acanthocyte-like structures covered with glassy substance found in the two *Mycena* species are identical with one of the previously reported structures, further studies are required to observe their detailed structure and development process. It is also essential to consider their taxonomic importance and ecological function by culture studies of various fungal species of sect. *Calodontes*.

Disclosure

The authors have no conflicts of interest to declare. All experiments undertaken in this study complied with the current laws of the country where they were performed.

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