Full paper

Taxonomic revision of the Japanese *Tricholoma ustale* and closely related species based on molecular phylogenetic and morphological data

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ABSTRACT

"*Kakishimeji*" identified as *Tricholoma ustale* and belonging to *Tricholoma* sect. *Genuina* is a common poisonous mushroom in Japan. *Kakishimeji* contains the toxic compound ustalic acid and causes digestive trouble. However, this fungus is consumed in some regions of Japan without any digestive issues. We clarified the probable species complex of *Kakishimeji* based on a phylogenetic analysis. We collected 89 basidioma specimens of *Kakishimeji* and related species from various forest sites in Japan and conducted phylogenetic analyses using 7 nuclear and mitochondrial gene sequences. *Kakishimeji* was found to consist of four distinct phylogenetic clades based on all DNA regions tested. Of these, two clades included European *T. stans* and *T. albobrunneum* type specimens. Another two clades consisted of sister clades to *T. pessundatum* and *T. ustaloides*. In addition, all four phylogenetic clades of *Kakishimeji* had different spore and basidium sizes. Therefore, we regarded the latter two clades as two new *Tricholoma* species: *T. kakishimeji* and *T. kakishimejioides*.

Keywords: cryptic species, ectomycorrhizal fungi, poisonous mushrooms

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1. Introduction

"*Kakishimeji*" has long been identified as *Tricholoma ustale* (Fr.) P. Kumm. in Japan and belongs to sect. *Genuina* of the genus *Tricholoma* (Kawamura, 1925, 1929; Imazeki & Hongo, 1987; Heilmann-Clausen, Christensen, Frøslev, & Kjeller, 2017). Sect. *Genuina* is defined by a reddish brown pileus and is commonly known as "burnt knight" in the United Kingdom (Christensen & Heilmann-Clausen, 2013). *Kakishimeji* was first identified as *T. ustale* Fr. in Japan by Kawamura (1925) and was morphologically described as *T. ustale* Fr. (= *T. ustale* (Fr.) P. Kumm.) by Kawamura (1929), based on a specimen collected at the foot of Mt. Iizuna-yama, Nagano Prefecture. Imai (1938) described two similar species in the sect. *Genuina*, i.e., "*Matsushimeji*" identified as *T. albobrunneum* (Pers. ex Fr.) Quél. (= *T. albobrunneum* (Pers.) P. Kumm.) and "*Ookakishimeji*" identified as *T. pessundatum* (Fr.) Quél.; both were based on specimens sampled mostly in Hokkaido, Japan. Imazeki (1942) reported *Matsushimeji* as a poisonous mushroom.

Kakishimeji and *Matsushimeji* were often confused in their identifications and conspecificity due to their close resemblance in morphology and ecology (Imazeki & Hongo, 1957, 1965, 1987). *Ookakishimeji* has limited reports lately (Ito, 1959). Matsuda and Hongo (1962) described *T. maculatipus* with the common name "*Azashimeji*" collected in Sandankyo, Hiroshima Prefecture and Oguni, Yamagata Prefecture. Another three closely related species to *Kakishimeji* in the sect. *Genuina* known from Japan are *Murewashimeji*, identified as *T. populinum* J. E. Lange (Murata, 1978), *Nigashimeji* and *Kakishimeji-modoki*, both identified as *T. ustaloides* Romagn. (Ikeda, 2005; Miyauchi, 2010), and *Matsubarashimeji*, identified as *T. stans* (Fr.) Sacc. (Miyauchi, 2010).

Kakishimeji has long been recognized as a poisonous mushroom in Japan (Ichioka, 1799). Although Kawamura (1925) described *Kakishimeji* as poisonous, Kawamura (1931) described its edibility as uncertain. Hiroe (1949) described *Kakishimeji* as edible, especially in the basidiomata harvested under seaside pine forests, and salting was recommended for safe use because boiling occasionally causes stomach trouble. Imazeki and Hongo (1965) described *Kakishimeji* as slightly poisonous. Later publications regarded *Kakishimeji* as a distinct poisonous mushroom (Imazeki, Hongo, & Tubaki, 1970; Imazeki & Hongo, 1987; Yamashita & Furukawa,

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1993; Hongo, 1994; Ikeda, 2005; Nagasawa, 2009). In Europe, *T. ustale* is described as edible after removing its bitter taste by boiling (Romagnesi, 1971), inedible (Breitenbach & Kränzlin, 1991) or as poisonous (Phillips, 1981). The causal toxic compound of *Kakishimeji*, ustalic acid, was identified (Sano et al., 2002). Ustalic acid inhibits Na⁺/K⁺-ATPase purified from porcine cerebral cortex and its crude enzyme extracted from mouse intestinal mucosal cells (Hayakawa, Watanabe, & Kigoshi, 2008), thereby causing digestive troubles such as stomachache, vomiting, and diarrhea in human. In 2000–2015, 18 incidents of, and 61 persons suffering from, stomach troubles after eating *Kakishimeji* were reported (Ministry of Health, Labor and Welfare; <https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000142705.html>). This fungus causes one third of mushroom poisoning cases in Japan.

Tricholoma ustale (Fr.) P. Kumm. was described as *Agaricus ustalis* Fr. collected near Germany (Fries, 1818). Afterwards, it was moved to genus *Tricholoma* (Kummer, 1871). The morphological characteristics of Japanese *T. ustale* named *Kakishimeji* described by Kawamura (1929) are as follows: pileus 3–8 cm diam, first convex, later plane, reddish brown, pale to margin, glabrous, viscid in moist; flesh white and sinuate; stipe cylindrical to tapering upwards, surface pale reddish brown, hollow to medulla; gill white, frequently brown spot, sinuate; spore 4–6 × 3–4 µm in length, one oily droplet; taste bitter; grows in forest and fruits in autumn. Other Japanese species closely related to *Kakishimeji* in the sect. *Genuina* as mentioned above are characterized comparatively with *Kakishimeji*, because *Kakishimeji* is quite commonly sampled, but the others have a limited record of samples (Table 1).

Genus *Tricholoma* including *Kakishimeji* is an ectomycorrhizal taxon (Trappe, 1962; Miller, Koo, & Molina, 1991; Agerer, 1987–2008). In fact, cultured strain AT610 of *Kakishimeji* (identified as *T. ustale*) reportedly showed *in vitro* ectomycorrhization with *Pinus densiflora* host (Yamada et al., 2010). However, there are limited data on the ectomycorrhizal associations in *Tricholoma*, particularly sect. *Genuina* (Agerer, 1987–2008; Duñabeitia, Hormilla, Salcedo, & Peña, 1996; Yamada, Kanekawa, & Ohmasa, 1999; Yamada, Ogura, & Ohmasa, 2001; Lefevre, 2002; Endo et al., 2015). Further investigation of those ectomycorrhizae, especially in terms of the host relationships, will illuminate the taxonomy of *Kakishimeji* and its closely related species in sect. *Genuina*.

Here, we revised the taxonomic position of *Kakishimeji* and closely related species in sect. *Genuina* sampled in Japan, based on multi-gene molecular phylogenetic analyses, as has been reported for genus *Tricholoma* in diverse geographic regions (e.g., Grubisha, Levens, Olson, & Taylor, 2012; Halama, Wany, Czosnykowska-Lukacka, & Dobosz, 2016; Heilmann-Clausen et al., 2017; Reschke, Popa, Yang, & Kost, 2018). Our hypothesis is that there is taxonomic confusion regarding the poisonous *Kakishimeji* and closely related species, which has long hampered discussion of the edibility and toxicity of these mushroom species.

2. Materials and Methods

2-1. Specimens examined

Eighty-nine basidioma specimens (Table 1) were collected from various forest sites in Japan from 2000 to 2019. They were lyophilized, oven-dried at 60 °C overnight to inactivate DNases and other oxidative enzymes, and stored in the laboratory. Where necessary, specimens were deposited in the National Museum of Nature and Science (TNS). We recorded the macroscopic characteristics, such as shape, size, color and texture, of the specimens. In addition, seven specimens from the Mycological Herbarium, Totto-

ri University (TUMH), nine from Tottori Mycological Institute (TMI), eight from TNS, and eight from Osaka Museum of Natural History (OMNH) were borrowed and studied (Table 1).

Fresh basidiomata sampled in 2018–2019 were tested using guaiac reagent (Cléménçon, 2009); 1 g guaiac resin (Nacalai Tesque, Kyoto) was dissolved in 50 mL 70% ethanol, and the prepared reagent was used within 3 mo. Three to four drops of the reagent were applied to sectioned basidiomata, and the positive (blue color)/negative (brown color) reaction was recorded at 5 min.

2-2. Microscopic observation of selected specimens

Dried material was rehydrated in a drop of 70% ethanol and then in distilled water for 1 h. Fully rehydrated samples were mounted using lactic acid on a glass slide and observed under a differential interference contrast microscope (AXIO Imager A1, Carl Zeiss, Göttingen, Germany). We observed the sizes of the basidiospore, basidium, sterigma, pileipellis, stipeipellis, and trama (Cléménçon, 2012). Melzer's reagent was added to the spores and basidia (Cléménçon, 2012). The Q-value (length/width) of each spore was calculated. Fifty cells in each structure and specimen were measured for length and width. Numerical data were subjected to one-way analysis of variance using R (<https://www.r-project.org/>), and the significance of differences was determined by Tukey's *post hoc* test ($P < 0.05$). For the morphological definition of *Kakishimeji* (= *Tricholoma ustale* sensu Kawamura), we followed Kawamura (1929).

2-3. DNA analysis

DNA was extracted from dried basidioma specimens as described by Gardes and Bruns (1993) with minor modifications. For PCR, we focused on multiple genes: the internal transcribed spacer (ITS) and intergenic spacer 1 (IGS1) regions of the nuclear ribosomal RNA gene (nrDNA), *tef-1*, *gapdh*, *rpb2*, small subunit of the mitochondrial ribosomal RNA gene (mtSSU), and *atp6*. The primers are listed in Table 2. PCR was conducted using the GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA). The 25 µL PCR mixture consisted of 2.5 µL 10× DreamTaq buffer, 2.5 µL 0.2 mM dNTP mixture, 2.5 µL 0.5 µM each primer, 0.125 µL 0.625 U DreamTaq DNA Polymerase (Thermo Fisher Scientific, Waltham, MA) and 0.5 µL extracted DNA solution. The PCR cycle parameters were initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 46–55 °C for 30 s, and extension at 72 °C for 1.5 min, and a final extension step at 72 °C for 10 min. The PCR products were electrophoresed (Mupid[®]-exU; TaKaRa Bio, Kusatsu, Japan) on a 1.5% agarose gel (Agarose for ≥ 1 kb fragments, 01163-76, Nacalai Tesque, Kyoto, Japan) for 30 min, stained with 0.001% ethidium bromide solution, and visualized using an ultraviolet illuminator (NM-15; UVP, Upland, CA). The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Venlo, the Netherlands) and subjected to cycle sequencing. The ITS and IGS1 regions of nrDNA, *gapdh*, and the mtSSU were cloned manually before cycle sequencing using the Mighty TA-Cloning Kit (TaKaRa Bio) and then inserted into competent *Escherichia coli* JM109 cells (TaKaRa Bio).

For cycle sequencing, the 10 µL reaction mixture consisted of 2 µL distilled water, 2 µL 10× buffer, 1 µL 5 mM primer, 1 µL Ready Reaction Mix (BigDye Terminator v. 3.1 Cycle Sequencing Kit, Thermo Fisher Scientific), and 4 µL purified DNA. The PCR conditions for cycle sequencing were initial denaturation at 96 °C for 1 min, followed by 25 cycles of denaturation at 96 °C for 10 s, annealing at 46–55 °C (Table 2) for 5 s, and extension at 60 °C for 4 min.

Table 1 *Tricholoma* specimens referred to *Kakishimeji* and used for this study.

Species	Specimen ID	Sampling data of basidioma specimen			Status of specimen ²
		Date (Y/M/D)	Site (Site, Municipal area, Prefecture)	Canopy tree ¹	
(Kakishimeji-like, section <i>Genuina</i>)					
<i>T. ustale</i> (“Kakishimeji”)	AT612 [*] (=NBRC 33141; TNS-F41488)	1997/11/4	Naka, Hitachi-ohmiya, Ibaraki	Pd	C
<i>T. ustale</i> (“Kakishimeji”)	AT610 [*] (=NBRC 33139, ATCC MYA-922; TNS-F11550)	1997/11/21	Naka, Hitachi-ohmiya, Ibaraki	Pd	C
<i>T. ustale</i> (“Kakishimeji”)	TUA-67, 68	2018/10/8	Mt. Madarao-san, Nakano, Nagano	Fc	D
<i>T. ustale</i> (“Kakishimeji”)	AY 2101103-001 (TUA-19)	2010/11/3	Mt. Reisenji-san, Shinano, Nagano	Qs	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-75	2018/10/17	Mt. Iizuna-yama, Iizuna, Nagano	Pa	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-76, 77	2018/10/17	Mt. Iizuna-yama, Iizuna, Nagano	Qs	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-78	2018/10/17	Mt. Iizuna-yama, Iizuna, Nagano	Pd	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-126	2019/11/8	Mt. Iizuna-yama, Iizuna, Nagano	Qs	D
<i>T. ustale</i> (“Kakishimeji”)	OMNH 2802	1963/10/7	Hasuike Pond, Yamanouchi, Nagano	ND	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-38	2018/9/20	Mt. Norikura-dake, Matsumoto, Nagano	Qc	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-40	2018/9/20	Mt. Norikura-dake, Matsumoto, Nagano	Td	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-58	2018/10/2	Mt. Norikura-dake, Matsumoto, Nagano	Td	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-59–61, 63–65	2018/10/2	Mt. Norikura-dake, Matsumoto, Nagano	Qc	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-62	2018/10/2	Mt. Norikura-dake, Matsumoto, Nagano	Td	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-113, 114	2019/10/10	Mt. Norikura-dake, Matsumoto, Nagano	Td	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-96	2019/9/26	Mt. Tateshina-yama, Saku, Nagano	Td, Av	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-101–105	2019/10/3	Mt. Yatsugatake, Koumi, Nagano	Td	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-35	2018/9/14	Juumonji-touge Pass, Kawakami, Nagano	Td	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-41	2018/9/20	Juumonji-touge Pass, Kawakami, Nagano	Td	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-42–46	2018/9/25	Juumonji-touge Pass, Kawakami, Nagano	Td	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-106, 107	2019/10/3	Juumonji-touge Pass, Kawakami, Nagano	Td	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-71	2018/10/14	Yokokawa, Tatsuno, Nagano	Qs	F+D
<i>T. ustale</i> (“Kakishimeji”)	AY 2001029-001 (TUA-6)	2000/10/29	Orikusa-touge Pass, Nakagawa, Nagano	Qs	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-70	2018/10/10	Orikusa-touge Pass, Nakagawa, Nagano	Qs, Pd	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-74	2018/10/16	Orikusa-touge Pass, Nakagawa, Nagano	Qs	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-118, 119	2019/10/30	Kuwahara, Nakagawa, Nagano	Pd	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-66	2018/10/5	Tajima-zawa, Nakagawa, Nagano	Qa	F, D
<i>T. ustale</i> (“Kakishimeji”)	TUA-72	2018/10/16	Tajima-zawa, Nakagawa, Nagano	Qa	D
<i>T. ustale</i> (“Kakishimeji”)	AY 2001027-001 (TUA-8)	2000/10/27	Koshibu, Matsukawa, Nagano	ND	D
<i>T. ustale</i> (“Kakishimeji”)	AY 2001029-001 (TUA-9)	2000/10/29	Koshibu, Matsukawa, Nagano	ND	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-69	2018/10/10	Shoubu-zawa, Ooshika, Nagano	Ts	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-73	2018/10/16	Shoubu-zawa, Ooshika, Nagano	Ts	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-117	2019/10/30	Shoubu-zawa, Ooshika, Nagano	Ts	D
<i>T. ustale</i> (“Kakishimeji”)	AY 2070706-001 (TUA-4)	2007/6/28	Toyooka, Nagano	Pd	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-79	2018/10/31	Misakubo, Hamamatsu, Shizuoka	Pd	D
<i>T. ustale</i> (“Kakishimeji”)	OMNH 1938	1958/10/24	Ishiyama-sotohata, Otsu, Shiga	ND	D
<i>T. ustale</i> (“Kakishimeji”)	OMNH 1947	1958/10/30	Sato, Otsu, Shiga	ND	D
<i>T. ustale</i> (“Kakishimeji”)	OMNH 3008	1964/10/31	Senchou, Otsu, Shiga	ND	D
<i>T. ustale</i> (“Kakishimeji”)	OMNH 3606	1967/11/9	Otsu, Shiga	ND	D
<i>T. ustale</i> (“Kakishimeji”)	TaY20191104-101	2019/11/4	Kashihara Shrine, Kashihara, Nara	Cc, Qg	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-81	2018/12/26	Wakayama	Pd	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-127	2019/11/13	Tottori Sand Dune, Tottori, Tottori	Pt	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-143	2019/11/14	Tottori Sand Dune, Tottori, Tottori	Pt	D
<i>T. ustale</i> (“Kakishimeji”)	TUMH 62876	2016/11/12	Kokufu, Tottori, Tottori	Qg, Qm	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-134	2019/11/13	Kokufu, Tottori, Tottori	Qg, Qm	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-138, 141, 142	2019/11/13	Kokufu, Tottori, Tottori	Qg	D
<i>T. ustale</i> (“Kakishimeji”)	TUMH 62707	2016/12/16	Hamasaka, Tottori, Tottori	Pt	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-128	2019/11/13	Hamasaka, Tottori, Tottori	Pt	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-129–132	2019/11/13	Hamasaka, Tottori, Tottori	Pt	D
<i>T. ustale</i> (“Kakishimeji”)	TUMH 62228	2015/11/24	Ooike Pond, Tottori, Tottori	Qg, Qm	D
<i>T. ustale</i> (“Kakishimeji”)	TUA-133, 134, 136, 137	2019/11/13	Ooike Pond, Tottori, Tottori	Qg, Qm	F+D
<i>T. ustale</i> (“Kakishimeji”)	TUA-140, 145	2019/11/13	Fuse, Tottori, Tottori	Qa	D
<i>T. ustale</i> (“Kakishimeji”)	TUMH 62875	2016/11/3	Makibara, Tottori, Tottori	F	F+D
<i>T. ustale</i> (“Kakishimeji”)	OMNH 1756	1958/4/13	Hitotsuba, Miyazaki, Miyazaki	ND	D
<i>T. ustale</i> (“Kakishimeji”)	TUMH 61677	2013/11/23	Nishifumoto, Takaharu, Miyazaki	F	D
<i>T. albobrunneum</i> (“Matsushimeji”)	TMI 5588	1938/10/8	Ishikari, Sapporo, Hokkaido	ND	D
<i>T. albobrunneum</i> (“Matsushimeji”)	TMI Fukuti 6	(estimated 1930s)	Ishikari, Hokkaido	ND	D
<i>T. albobrunneum</i> (“Matsushimeji”)	TMI Fukuroda 52	1937/10/11	Fukuroda, Daigo, Ibaraki	ND	D
<i>T. albobrunneum</i> (“Matsushimeji”)	TMI Mizu 5	1940/9/27	Mt. Mizugaki-yama, Hokuto, Yamanashi	ND	D
<i>T. albobrunneum</i> (“Matsushimeji”)	TMI 12A, 12B	1940/10	Narusawa, Yamanashi	ND	D
<i>T. albobrunneum</i> (“Matsushimeji”)	TaY20181117-001	2018/11/17	Tottori Sand Dunes, Tottori, Tottori	Pt	D
<i>T. stans</i> (“Matsubarashimeji”)	TUMH 62879	2016/12/16	Hamasaka, Tottori, Tottori	Pt	D
<i>T. pessundatum</i> (“Ookakishimeji”)	TMI 5615	1934/10/8	Sapporo, Hokkaido (market)	ND	D
<i>T. pessundatum</i> (“Ookakishimeji”)	TMI 5614	1933/10/8	Jozankei, Sapporo, Hokkaido	ND	D

Species	Specimen ID	Sampling data of basidioma specimen			Status of specimen ²
		Date (Y/M/D)	Site (Site, Municipal area, Prefecture)	Canopy tree ¹	
<i>T. pessundatum</i> (“Ookakishimeji”)	TMI 25	1940/10/4	Saiko Lake, Fuji-kawaguchiko, Yamanashi	ND	D
<i>T. pessundatum</i> (“Ookakishimeji”)	TUMH 63117	2017/11/16	Ooike Pond, Tottori, Tottori	Qg, Qm	D
<i>T. ustaloides</i> (“Nigashimeji”)	TNS-F18995	1975/10/14	Kashiwagi, Noto, Ishikawa	Qs	D
<i>T. ustaloides</i> (“Nigashimeji”)	TNS-F18996	1978/10/14	Kunishige, Noto, Ishikawa	Pd, F	D
<i>T. ustaloides</i> (“Nigashimeji”)	TNS-F18997	1985/10/19	Akiyoshi, Noto, Ishikawa	Pd	D
<i>T. ustaloides</i> (“Nigashimeji”)	TNS-F18999	1994/10/9	Matsunami, Noto, Ishikawa	Pd, F	D
<i>T. ustaloides</i> (“Nigashimeji”)	TNS-F18998	1986/10/10	Ohara, Kanazawa, Ishikawa	Pd, F	D
<i>T. ustaloides</i> (“Nigashimeji”)	TNS-F19000	2002/10/27	Tatsunokuchi, Nomi, Ishikawa	Pd, F	D
<i>T. ustaloides</i> (“Nigashimeji”)	TNS-F38557	2008/10/11	Kamiwasumi, Noto, Ishikawa	Pd, F	D
<i>T. ustaloides</i> (“Nigashimeji”)	TNS-F38558	2010/10/24	Shichikuro, Tsubata, Ishikawa	Pd, F	D
<i>T. maculatipus</i> (“Azashimeji”)	OMNH 2392	1961/10/22	Awanoyu-onsen, Oguni, Yamagata	Fc	D
<i>T. maculatipus</i> (“Azashimeji”)	OMNH 3162	1965/10/2	Yonezawa, Yamagata	ND	D
<i>Tricholoma</i> sp. (“Kakishimeji-like”)	AY2080904-001, 002 (TUA-1, 2)	2008/9/4	Mt. Rishiri-dake, Rishiri, Hokkaido	Pp	D
<i>Tricholoma</i> sp. (“Kakishimeji-like”)	AY2161105-001 (TUA-18)	2016/11/5	Shimokita-hantou Peninsula, Mutsu, Aomori	Pd	D
<i>Tricholoma</i> sp. (“Kakishimeji-like”)	AY2111014-001 (TUA-17)	2011/10/14	Asahi, Matsumoto, Nagano	Pa	D
<i>Tricholoma</i> sp. (“Kakishimeji-like”)	TUA-125	2019/11/5	Asahi, Matsumoto, Nagano	Pd	F+D
<i>Tricholoma</i> sp. (“Kakishimeji-like”)	TUA-95	2019/9/20	Orikusa-touge Pass, Nakagawa, Nagano	Fj	D
<i>Tricholoma</i> sp. (“Kakishimeji-like”)	TUA-48	2018/9/26	Tajima-zawa, Nakagawa, Nagano	Qs, Qa	D
(not <i>Kakishimeji</i> -like, section <i>Genuina</i>)					
<i>T. japonicum</i>	AY2101020-001, 002 (TUA-20, 21)	2010/10/20	Kuwahara, Nakagawa, Nagano	Qs, Qa, Pd	D
<i>T. japonicum</i>	AY2001029-001 (TUA-10)	2000/10/29	Koshibu, Nakagawa, Nagano	ND	D
<i>T. japonicum</i>	AY2001102-001 (TUA-11)	2000/11/2	Koshibu, Matsukawa, Nagano	ND	D
<i>T. imbricatum</i>	AY2011020-001 (TUA-5)	2001/10/20	Kuwahara, Nakagawa, Nagano	Pd	D
<i>T. aff. acerbum</i>	AY2000925-001 (TUA-14)	2000/9/25	Ibaraki	ND	D
(Section <i>Caligata</i>)					
<i>T. matsutake</i>	TUA-84	2018/10/10	Sekizawa, Shiojiri, Nagano	Ts	D
(Section <i>Tricholoma</i>)					
<i>T. flavovirens</i>	AY1970920-001 (TUA-15)	1997/9/20	Naka, Hitachi-ohmiya, Ibaraki	Pd	D

¹ Cultures isolate established from basidioma tissue.

² Pd: *Pinus densiflora*, Pp: *P. pumila*, Pt: *P. thunbergii*, Pa: *Picea abies*, C: *Pinaceae*, F: *Fagaceae*, Fc: *Fagus crenata*, Fj: *F. japonica*, Qa: *Quercus acutissima*, Qc: *Q. crispula*, Qg: *Q. glauca*, Qm: *Q. myrsinifolia*, Qs: *Q. serrata*, Cc: *Castanopsis sieboldii*, Td: *Tsuga diversifolia*, Ts: *T. sieboldii* Av: *Abies veitchii*, ND: No description.

³ D: Dried basidioma specimen was observed, F+D: Both fresh and dried basidioma specimens were observed, C: Cultured mycelium was observed.

Table 2 PCR primers used in the present study.

Targeted region	Name	Sequence (5'→3')	Tm value (°C)
ITS of nrDNA	ITS1F (F) ¹	CTTGGTCATTTAGAGGAAGTAA	55
	ITS4 (R) ²	TCCTCCGCTTATTGATATGC	60
IGS1 of nrDNA	CNL12 (F) ³	CTGAACGCCTCTAAGTCAG	56
	5S-Anderson (R) ⁴	CAGAGTCTATGGCCGTGGAT	66
<i>rpb2</i>	rpb2_tu_f1 (F) ⁵	CTGTCCGGYTCYTATTCTGC	53
	rpb2_tu_r1 (R) ⁵	GCTRGATGAATCTCACAAATG	52
<i>gapdh</i>	gpd_tu_f1 (F) ⁵	ATGTTGTGTGTTGACGCTA	49
	gpd_tu_r1 (R) ⁵	AGGTCTACATGTTAARTACG	52
<i>tef-1</i>	tef_tu_f1 (F) ⁵	GTGAACAAGATGGACACYAC	53
	tef_tu_r1 (R) ⁵	GACAACGYGGYTTCAAC	51
SSU of mtrDNA	MS1 (F) ⁶	CAGCAGTCAAGAATATTAGTCAATG	52
	MS2 (R) ⁶	GCGGATTATCGAATTAATAAC	48
<i>atp6</i>	ATP-3f (F) ⁶	TCTCCTTTAGAACAAATTTGA	46
	ATP-6r (R) ⁷	AACTAATARAGGAAGCTAAAGCTA	48

¹ Gardes and Bruns (1993), ² White et al. (1990), ³ Anderson and Stasovski (1992),

⁴ Henrion et al. (1992), ⁵ Designed in this study, ⁶ Kretzer and Bruns (1999), ⁷ Binder and Hibbett (2003; http://www2.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.pdf).

Samples were sequenced using the Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems).

The sequences were aligned using ClustalW (Larkin et al., 2007) and MEGA7 (Kumar, Stecher, & Tamura, 2016), and the consensus sequences in each region were established and deposited in DDBJ (Supplementary Table S1). Several sequences were downloaded from GenBank and UNITE (Supplementary Table S2). In the phy-

logenetic analysis of each DNA region, alignment gaps were treated as missing data, and ambiguous positions were excluded from the analysis. Datasets for ITS (ITS1-5.8S-ITS2; 632 bp), *rpb2* (472 bp), *gapdh* (646 bp), *atp6* (373 bp), *tef-1* (517 bp), mtSSU (599 bp) and IGS1 (490 bp) were prepared. Maximum likelihood (ML) and Bayesian inference analyses were conducted to clarify the phylogenetic relationships among specimens. ML trees were constructed using MEGA7 with 1000 bootstrap replicates, which followed a Tamura three-parameter for ITS and mtSSU, Kimura two-parameter for *atp6*, *tef1*, *rpb2*, and *gapdh*, or Tamura Nei model for IGS1 with a gamma distribution. For Bayesian inference analysis, the SYM model of nucleotide substitution with a discrete gamma distribution (+G) was performed using MrBayes 3.2.1 (Ronquist et al., 2012). Two runs with four chains of Markov Chain Monte Carlo iterations were performed for 1,000,000 generations when the average standard deviation of split frequencies was below 0.01 (the first 25% of generations were treated as burn-in). Trees were kept for every 100 generations, and the remaining 75% of trees were used to calculate the 50% majority-rule consensus topology and to determine the posterior probabilities for individual branches.

3. Results

3-1. Phylogenetic analysis of specimens based on multi-gene data

The phylogenetic tree of the ITS region of nrDNA (Fig. 1) showed that *Kakishimeji* and closely related species sampled from Japan comprised five clades with strong bootstrap support values.

Fig. 1 – ML phylogenetic tree of the ITS1-5.8S-ITS2 region of nrDNA of *Kakishimeji* and closely related species. Several known *Tricholoma* species within and outside sect. *Genuina* were analyzed as outgroup species. Bootstrap (BS) values > 60% from ML (left) and Bayesian posterior probabilities (PP) > 0.60 (right) are shown near the nodes. Thick nodes indicate significant support by both BS (> 85%) and PP (> 1.0) values. Taxon names in bold indicate the specimens examined in this study.

ITS Clade-1 was positioned as the sister clade of European *T. pessundatum* (JV04-482; epitype specimen). Clade-2 and -3 included European specimens of *T. stans* (MC98-018 and MC95-145; epitype) and *T. albobrunneum* (MC99-060 and AT2003102), respectively. Subclade-A and -B in Clade-3 showed independent branch with moderate bootstrap probability (BP) and high posterior probability values. Clade-4 was positioned as the sister clade to European *T. ustalooides* (TU106243, MC99-067, and MC99-047). Interestingly, European *T. ustale* clade (JHC92-299 and MEN95213) was

distinct from all five phylogenetic clades of *Kakishimeji* and closely related species from Japan.

Phylogenetic trees of *rpb2* (Fig. 2), *gapdh* (Supplementary Fig. S1), *atp6* (Supplementary Fig. S2), *tef-1* (Supplementary Fig. S3), and the IGS1 region of nrDNA (Supplementary Fig. S4) showed similar topology to that of the ITS region and comprised four distinct clades of *Kakishimeji* and closely related species. However, each Clade-3 corresponded to the compiled Clade-3A and -3B of the ITS phylogenetic tree. The mtSSU (Supplementary Fig. S5)

Specimens in each clade in each phylogenetic tree did not show nested relationships among phylogenetic trees, suggesting that the four clades in the ITS phylogenetic tree, i.e., Clades-1, -2, -4, and combined -3A and -3B, had similar numbers of species (Supplementary Table S1). However, only specimen TUA-19 showed equivocal phylogenetic positions, i.e., Clade-1 in the ITS, *rpb2*, *gapdh*, and mtSSU trees and Clade-2 in the *tefl*, *IGS1*, and *atn6* trees. Sev-

Based on the confirmed consensus that *Kakishimeji* and closely related species from Japan are divided into four clades (Supplementary Table S1), we characterized the specimens in each phylogenetic clade morphologically. We regarded Clade-3A and Clade-3B in the ITS phylogenetic tree as having an intraspecific relationship and treated them as Clade-3 in the analysis. We did not use TUA-19 in the analysis due to the equivocal phylogenetic posi-

tion, as described above.

3-2. Morphological comparison of basidiomata in the phylogenetic clades of *Kakishimeji*

The pileal surface of basidiomata specimens in all four phylogenetic clades was dark reddish brown to reddish brown and viscid when moist (Figs. 3A–C, 4A, B, 5A, B, 6A). Specimens in ITS Clade-1 showed blackish spot-like scales on the pileal surface (Fig. 3C). Specimens in ITS Clade-2 frequently showed a squamous-like fine split on top of the stipe surface (Fig. 4D). Specimens in the other clades showed this character infrequently. Specimens in ITS Clade-3 showed a striate-like structure at the pileal margin (Fig. 5C). Specimens in the other clades sometimes showed this character, albeit indistinctly. Specimens in ITS Clade-4 showed a squamous texture on the pileal surface when matured under dried conditions (Fig. 6A, B). Specimens in ITS Clades-1 and -2 had a positive (blue staining) guaiac test result (Figs. 3B, 4E), whereas those in ITS Clades-3 and -4 had a negative result (Figs. 5D, 6A).

Basidiospores were commonly ellipsoidal to subglobose and contained a large oily droplet in fresh specimens (Figs. 3D, 4F, 5E, 6D). Spore length and width were significantly different among clades (Table 3), and the spore Q-value was significantly lower in Clade-4.

Basidia generally developed four sterigmata and contained a few oily droplets in fresh specimens (Figs. 3E, 4G, 5F, 6E). Basidia

were significantly shorter in Clade-1 than in the other three clades (Table 3). The mean basidium width differed among the clades. Sterigmata were significantly shorter in Clade-1 than in the other three clades (Table 3). The mean sterigma width differed among the clades (Table 3).

Pileipellis hyphae were cylindrical, without a clamp connection, and brown in color due to intracellular pigment (Figs. 3F, 4H, 5G, 6F). Subpellis consisted of ixocutis or ixotrichodermoid tissue (Figs. 3G, 4I, 5H, 6G). The mean pileipellis hyphal length was significantly shorter in Clades-1 and -2 than in Clade-3 (Table 3), while the value in Clade-4 was intermediate. The pileipellis hyphal width was significantly greater in Clades-1 and -3 than in Clades-2 and -4 (Table 3).

Four phylogenetic clades of *Kakishimeji* could be distinguished morphologically (Table 4). Therefore, we assigned those clades as two new species and two new records in Japan, as below.

4. Taxonomy

Tricholoma kakishimeji W. Aoki & A. Yamada, sp. nov. Fig. 3.
MycoBank no.: MB 837875.

Diagnosis: *Tricholoma kakishimeji* can be distinguished phylogenetically from closely resembled European *T. pessundatum*. They are also distinguishable by the pileipellis hyphal width.

Type: JAPAN, Nagano Prefecture, Shinano Town, at the foot of

Fig. 3 – Morphological characteristics of phylogenetic ITS Clade-1 specimens (*Tricholoma kakishimeji*). A: External morphology of basidiomata in the field (TNS-F82064 [TUA-75]); B: external morphology of basidiomata and guaiac test (TUA-67); C: pileal surface texture showing blackish spot-like small scales (arrowhead) (TNS-F82064 [TUA-75]); D: basidiospore; E: basidium; F: pileipellis hyphae; G: texture of pileipellis and subpellis; H: cystidium. Bars: D, E, H 5 μ m; F 10 μ m; G 20 μ m.

Fig. 4 – Morphological characteristics of phylogenetic ITS Clade-2 specimens (*Tricholoma stans*). A: External morphology of basidiomata in the field (TUA-45); B: external morphology of basidiomata and positive guaiac test (TUA-101); C: gills; D: upper part of stipe; E: positive guaiac test; F: basidiospore; G: basidium; H: pileipellis hyphae; I: pileipellis and subpellis; J: cystidium. Bars: F, G, J 5 μ m; H 10 μ m; I 20 μ m.

Fig. 5 – Morphological characteristics of phylogenetic ITS Clade-3 specimens (*Tricholoma albobrunneum*). A: External morphology of basidiomata in the field (TUA-127); B: external morphology of basidiomata (TUA-118); C: pileal margin; D: negative guaiac test; E: basidiospore; F: basidium; G: pileipellis hyphae; H: pileipellis and subpellis; I: cystidium. Bars: E, F, I 5 μ m; G 10 μ m; H 20 μ m.

Fig. 6 – Morphological characteristics of ITS Clade-4 specimens (*Tricholoma kakishimejioides*). A: External morphology of basidiomata (TNS-F82065 [TaY20191104-101]) and a weakly positive guaiac test; B: pileal margin texture; C: gills; D: basidiospore; E: basidium; F: pileipellis hyphae; G: pileipellis and subpellis; H: cystidium. Bars: D, E, H 5 μ m; F 10 μ m; G 20 μ m.

Table 3 Microscopic comparison of four *Tricholoma* species in “*Kakishimeji*” complex distinguished by the phylogenetic analyses.

Measured parameters		Clade-1 <i>T. kakishimeji</i>			Clade-2 <i>T. stans</i>			Clade-3 <i>T. albobrunneum</i>			Clade-4 <i>T. kakishimejioides</i>		
		N ²	Min–Ave–Max ³	S ⁴	N	Min–Ave–Max	S	N	Min–Ave–Max	S	N	Min–Ave–Max	S
Basidio-spore	L ¹	14	4.1–4.6–5.0	C	27	4.9–5.5–6.2	A	14	4.5–4.9–5.7	B	4	5.4–5.8–6.6	A
	W ¹		3.0–3.3–3.9	C		3.5–3.9–4.3	B		3.1–3.5–3.9	C		4.2–4.5–5.1	A
	Q ¹		1.3–1.4–1.6	A		1.3–1.4–1.5	A		1.3–1.4–1.5	A		1.2–1.3–1.4	B
Basidium	L	12	20.6–23.9–27.0	C	21	23.3–27.9–31.5	A	13	23.1–26.0–27.9	B	3	25.6–27.5–29.1	A
	W		4.7–5.2–6.2	C		3.9–5.9–6.8	AB		4.9–5.5–6.3	B		6.1–6.8–8.0	A
Sterigma	L	12	1.4–2.0–3.1	B	21	1.5–3.0–4.2	A	12	2.4–3.1–3.8	A	3	3.3–3.6–3.8	A
	W		1.0–1.3–1.5	AB		0.9–1.2–1.5	B		0.9–1.3–1.5	AB		1.2–1.6–2.3	A
Pileipellis	L	8	41.6–50.6–56.2	B	13	41.8–52.6–68.9	B	10	49.4–61.3–72.9	A	3	54.4–56.3–58.0	AB
	W		4.0–5.1–7.0	A		3.2–3.9–4.9	B		4.6–5.2–5.8	A		3.7–3.9–4.2	B

¹ L: Length (μ m), W: Width (μ m), Q: Q-value (length/width).

² N: number of specimens measured for the size.

³ The indicated numerical data are based on mean values in each specimen, which were used for the statistical analysis.

⁴ S: Different alphabets indicate significant difference of means between species at $P < 0.05$.

Table 4 Morphological comparison between *T. kakishimeji* and related species

	ITS Clade-1 <i>T. kakishimeji</i>	ITS Clade-2 <i>T. stans</i>	ITS Clade-3 <i>T. albobrunneum</i>	ITS Clade-4 <i>T. kakishimejioides</i>	European <i>T. ustale</i>
Shape and surface	First convex and later flat, often with dark brown to blackish spot-like small scales	First semispherical to convex, later low convex or flat, rarely with dark brown to blackish spot-like small scales	First convex, later flat, margin striate, viscid when moist	First convex, later flat, squamous-like under dry weather condition	First hemisphere to bell-shaped, later convex to flattened with low
Pileus color	Center dark brown to brown, reddish brown or pale brown in the margin	Center dark reddish brown, reddish brown pale to margin	Reddish brown, dark brown in center, and paler color in the margin	Center dark reddish brown to reddish brown, reddish brown to pale brown in the margin	Central part yellowish brown, dark brick to fuscous or umber, towards margin paler
Shape of gills	Crowded	Crowded	Close	Close	Medium space to crowded
Color and shape of stipe	White to brown, hollow	White, later pale brown, top surface is distinctly squamous	White or brownish, powdery at the top surface	Pale brown to brown	Whitish to pale chrome at top
Spore size	4.1–4.6–5.0 × 3.0–3.3–3.9 μm, Q = 1.3–1.4–1.6	4.9–5.5–6.2 × 3.5–3.9–4.3 μm, Q = 1.3–1.4–1.5	4.5–4.9–5.7 × 3.1–3.5–3.9 μm, Q = 1.3–1.4–1.5	5.4–5.8–6.6 × 4.2–4.5–5.1 μm, Q = 1.2–1.3–1.4	6.1–6.9 × 5.1–5.7 μm, Q = 1.18–1.32
Basidium	20.6–23.9–27.0 × 4.7–5.2–6.2 μm	23.3–27.9–31.5 × 3.9–5.9–6.8 μm	23.1–26.0–27.9 × 4.9–5.5–6.3 μm	25.6–27.5–29.1 × 6.1–6.8–8.0 μm	35–50 × 7.0–9.0 μm
Sterigma	1.4–2.0–3.1 × 1.0–1.3–1.5 μm	1.5–3.0–4.2 × 0.9–1.2–1.5 μm	2.4–3.1–3.8 × 0.9–1.3–1.5 μm	3.3–3.6–3.8 × 1.2–1.6–2.3 μm	—
Pileipellis	41.6–50.6–56.2 × 4.0–5.1–7.0 μm	41.8–52.6–68.9 × 3.2–3.9–4.9 μm	49.4–61.3–72.9 × 4.6–5.2–5.8 μm	54.4–56.3–58.0 × 3.7–3.9–4.2 μm	2.5–5.0 μm
Canopy vegetation	Fagaceae, Pinaceae	Fagaceae, Pinaceae	Pinus	Fagaceae	Fagus
Climate of habitat	Temperate	Temperate to subalpine	Temperate to alpine	Temperate	Temperate

Mt. Iizuna-yama, 36°44'48.20" N, 138°11'24.32" E, alt 842 m, in a small man-made stand of *Picea abies* surrounded by a mixed forest of *Quercus serrata*, *Q. crispula*, and *Pinus densiflora*, 17 Oct 2018, leg. W. Aoki (holotype, TNS-F82064 [TUA-75]).

Gene sequences ex-holotype: LC589085 (nrDNA ITS; Clade-1 in Fig. 1), LC587217 (nrDNA IGS), LC587129 (*tef-1*), LC586915 (*gapdh*), LC586873 (*rpb2*), LC587164 (mtSSU), LC587087 (*atp6*).

Etymology: *Kakishimeji*, common name of this fungus in Japan.

Description: *Pileus* 6–8 cm diam, center dark brown to brown, reddish brown or pale brown in the margin (Fig. 3A, B), first convex and later flat, margin involved and later flat, surface innately fibrillose and viscid when moist, often with a few or many dark brown to blackish spot-like small scales (Fig. 3C). *Stipe* white to brown, hollow, cylindrical or tapering upwards. *Gills* white, cloudy, sinuate, later with frequent brown stains. *Flesh* white, guaiac test positive (Fig. 3B), smell moderate to strong farinaceous, taste bitter in the pileal surface, faintly bitter or mild in the inner tissue. *Basidiospores* 4.1–4.6–5.0 × 3.0–3.3–3.9 μm (minimum–average–maximum values of length × minimum–average–maximum values of width), Q (length/width) = 1.3–1.4–1.6, ellipsoidal, non-amyloid, single intracellular large transparent oily droplet in fresh materials (Fig. 3D). *Basidia* 20.6–23.9–27.0 × 4.7–5.2–6.2 μm, clavate to subcylindrical, four-spored, several intracellular transparent oily droplets in fresh materials, clamp connections not observed on the basal septum (Fig. 3E). *Sterigmata* 1.4–2.0–3.1 × 1.0–1.3–1.5 μm (Fig. 3E). *Pileipellis* ixocutis, hyphae 41.6–50.6–56.2 × 4.0–5.1–7.0 μm, cylindrical, intracellular brown pigment (Fig. 3F, G), no clamp connections. *Stipitipellis* 19.5–38.7–63.6 × 3.6–5.8–8.8 μm, colorless to pale brown, smooth, cylindrical, no clamp connections, occasionally cystidia-like structures were observed on top surface of the top of stipe, clavate to cylindrical, colorless, no clamp connections (Fig. 3H).

Specimens examined other than holotype: JAPAN: Aomori Prefecture: TUA-18 (A. Yamada); Nagano Prefecture: TUA-6 (A. Yamada), TUA-17 (N. Endo), TUA-19 (A. Yamada), TUA-58 (W. Aoki), TUA-67 (W. Aoki), TUA-75 (W. Aoki), TUA-117 (W. Aoki), TUA-125 (A. Yamada); Tottori Prefecture: TUA-133 (W. Aoki), TUMH 62228 (N. Endo); Hokkaido: TMI 5614 (S. Imai); Yamanashi Prefecture: TMI Mizu5 (S. Imai).

GenBank accession number of DNA sequences: see Supplement-

tary Table S1 (nrDNA ITS Clade-1).

Ecology: *Tricholoma kakishimeji* is distributed under temperate zone, in *Fagaceae* and *Pinaceae* forests, fruiting during Oct–Nov.

Comments: *Tricholoma kakishimeji* is microscopically similar to Japanese *T. ustale* reported by Kawamura (1929) and *T. albobrunneum* reported by Imai (1938), but basidium size is smaller in *T. kakishimeji* than *T. albobrunneum* sensu Imai. Macroscopically, pileal surface of *T. kakishimeji* has few to many small dark-brown spot-like scales, similar to *T. pessundatum* (Fr.: Fr.) Quélet (Christensen & Heilmann-Clausen, 2013). However, as this character was sometimes observed even in Japanese *T. stans* specimens, it cannot be used to distinguish *T. kakishimeji* and *T. stans*. Pileipellis hyphal width of *T. pessundatum* is 2.0–4.5 μm, thinner than that of *T. kakishimeji*. The ecology of *T. kakishimeji* and *T. pessundatum* is largely overlapped, i.e., Fagaceous tree associations. *T. kakishimeji* prominently contains a toxic compound, ustalic acid, but the other closely resembled Japanese *Tricholoma* species distinguished in this study harbor this compound at negligible levels (Ito et al., 2021). The *T. pessundatum* TMI 5614 specimen sampled in Hokkaido in 1933 and used for the description of this species as new to Japan under the common name “*Ookakishimeji*” (Imai, 1938) was synonymized with *T. kakishimeji* based on its phylogenetic position (Fig. 2) and morphological characteristics.

Tricholoma kakishimejioides W. Aoki & A. Yamada, sp. nov. Fig. 6. MycoBank no.: MB 837876.

Diagnosis: *Tricholoma kakishimejioides* can be distinguished phylogenetically from closely related European *T. ustaloides*. They are distinguishable by the pileipellis hyphal width.

Type: JAPAN, Nara Prefecture, Kashihara City, Kashihara-jingu Shrine, 34°29'11" N, 135°47'26" E, alt 72 m, in a broad-leaved forest dominated by *Castanopsis sieboldii* and *Quercus glauca* with sporadic *Abies firma*, 4 Nov 2019, leg. N. Endo (holotype, TNS-F82065 [TaY20191104-101]).

Gene sequences ex-holotype: LC574895 (nrDNA ITS; Clade-4 in Fig. 1), LC587186 (nrDNA IGS), LC587109 (*tef-1*), LC586899 (*gapdh*), LC586850 (*rpb2*), LC587180 (mtSSU), LC587069 (*atp6*).

Etymology: Look-alike species to *Tricholoma kakishimeji*.

Description: *Pileus* 3–6 cm diam, center dark reddish brown to

reddish brown, reddish brown to pale brown in the margin, firstly convex, later flat, margin involved, surface innately fibrillose, squamous-like in dry weather (Fig. 6A, B), viscid when moist. **Stipe** pale brown to brown, hollow, cylindrical or tapered slightly toward the base. **Gills** white, close, sinuate, later with frequent brown stains (Fig. 6C). **Flesh** white, thick, guaiac test largely negative but faintly positive (Fig. 6A), smell strongly farinaceous, taste bitter in the pileal surface, faintly bitter or mild in the inner tissue. **Basidiospores** $5.4\text{--}5.8\text{--}6.6 \times 4.2\text{--}4.5\text{--}5.1 \mu\text{m}$, $Q = 1.2\text{--}1.3\text{--}1.4$, subglabrous to glabrous, non-amyloid, single intracellular large transparent oily droplet in fresh material (Fig. 6D). **Basidia** $25.6\text{--}27.5\text{--}29.1 \times 6.1\text{--}6.8\text{--}7.9 \mu\text{m}$, clavate to cylindrical, four-spored, several intracellular large transparent oily droplets in fresh material (Fig. 6E), non-amyloid, clamp connections not observed on the basal septum. **Sterigmata** $3.3\text{--}3.6\text{--}3.8 \times 1.2\text{--}1.6\text{--}2.3 \mu\text{m}$ (Fig. 6E). **Pileipellis** ixocutis to ixotrichoderm, smooth, hyphae $54.4\text{--}56.3\text{--}58.0 \times 3.7\text{--}3.9\text{--}4.2 \mu\text{m}$, cylindrical, intracellular brown pigment (Fig. 6F, G). **Stipitipellis** $30.3\text{--}51.1\text{--}85.7 \times 2.3\text{--}3.9\text{--}6.2 \mu\text{m}$, colorless to pale brown, smooth, cylindrical, no clamp connections, occasionally cystidium-like structures are observed on the surface of the top of stipe, clavate to cylindrical, colorless, no clamp connections (Fig. 6H).

Specimens examined other than holotype: JAPAN: Nagano Prefecture: TUA-48 (W. Aoki), TUA-95 (W. Aoki); Tottori Prefecture: TUA-140 (W. Aoki), TUMH 62875 (N. Endo); Nara Prefecture: TaY20191104-101 (N. Endo); Ishikawa Prefecture: TNS-F38558 (Y. Ikeda).

GenBank accession numbers of DNA sequences: see Supplementary Table S1 (nrDNA ITS Clade-4).

Ecology: *Tricholoma kakishimejioides* is distributed in *Fagaceae* forests in warm temperate zones, fruiting during Sep–Nov.

Comments: *Tricholoma kakishimejioides* can be distinguish from *T. stans*, *T. albobrunneum*, and *T. kakishimeji* by the spore shape. *T. ustaloides* Romagn., sister clade to *T. kakishimejioides* (Fig. 1), has a larger basidium, i.e., $30\text{--}50 \mu\text{m}$ in length according to Christensen and Heilmann-Clausen (2013) or $29.5\text{--}37.5 \mu\text{m}$ according to Halama et al. (2016). Although several reports of *T. ustaloides* are known from Japan with the common name of *Nigashimeji*, most of those descriptions are confused with closely related species. In fact, *T. ustaloides* TNS-F18996 used for the description of *Nigashimeji* (Ikeda, 2005) was identified as *T. albobrunneum* (Fig. 2). Therefore, we rejected the description of *Nigashimeji*. The ecology of *T. kakishimejioides* and *T. ustaloides* is shared, i.e., *Quercus* associations, which was first emphasized in the report of *Kakishimeji-modoki* (look-alike species to *Kakishimeji*) by Miyauchi (1989). Therefore, we adopted the common name to the epithet.

Tricholoma stans (Fr.) Sacc. Sylloge fungorum, Sylloge Hymenomycetum, Vol. 1. Agaricineae, 5: 94, 1887. Fig. 4.

= *Tricholoma maculatipus* Hongo & Matsuda, Journal of Japanese Botany, 37: 369, 1962.

= *Tricholoma maculatipes* Hongo & Matsuda, Memoirs of the Faculty of Education, Shiga University, Natural Science, 33: 38, 1983.

Description: **Pileus** $6\text{--}13 \text{ cm}$ diam, first semispherical to convex, later low convex or flat (Fig. 4A, B), center dark reddish brown, reddish brown pale to margin, surface innately fibrillose or unclearly fibrillose, viscous when moist, rarely with dark brown to small blackish spot-like scales. **Stipe** white, later pale brown, cylindrical to robust, hollow to medulla, top surface is distinctly squamous (Fig. 4C, D). **Gills** white, cloudy, thick, sinuate, later brown stained (Fig. 4C). **Flesh** white, guaiac test is positive (Fig. 4B, E),

slightly bitter in the pileal surface, mild in other parts, smell farinaceous. **Basidiospores** $4.9\text{--}5.5\text{--}6.2 \times 3.5\text{--}3.9\text{--}4.3 \mu\text{m}$, $Q = 1.3\text{--}1.4\text{--}1.5$, ellipsoidal to predominantly ellipsoidal, single intracellular achromatic oily droplet in fresh material (Fig. 4F), non-amyloid. **Basidia** $23.3\text{--}27.9\text{--}31.5 \times 3.9\text{--}5.9\text{--}6.8 \mu\text{m}$, clavate to cylindrical, four-spored, several intracellular achromatic oily droplets in fresh material (Fig. 4G), non-amyloid, clamp connections not observed on the basal septum of a basidium. **Sterigmata** $1.5\text{--}3.0\text{--}4.2 \times 0.9\text{--}1.2\text{--}1.5 \mu\text{m}$ (Fig. 4G). **Pileipellis** ixocutis to ixotrichoderm, hyphae $41.8\text{--}52.6\text{--}68.9 \times 3.2\text{--}3.9\text{--}4.9 \mu\text{m}$, cylindrical, straight, intracellular brown pigment, no clamp connections (Fig. 4H, I). **Stipitipellis** cuitis, hyphae $22.8\text{--}46.1\text{--}91.7 \times 2.9\text{--}4.6\text{--}7.6 \mu\text{m}$, colorless to pale-brown, smooth, cylindrical, no clamp connections, occasionally cystidium-like structures were observed on the surface of the top of stipe, clavate to cylindrical, colorless, no clamp connections (Fig. 4J).

Specimens examined: JAPAN, Nagano Prefecture: TUA-9 (A. Yamada), TUA-102 (W. Aoki), TUA-8 (W. Aoki), TUA-73 (W. Aoki), TUA-96 (A. Yamada), TUA-35 (W. Aoki), TUA-74 (W. Aoki); Shizuoka Prefecture: TUA-79 (A. Yamada); Tottori Prefecture: TUMH 62876 (N. Endo), TUA-134 (W. Aoki); Yamanashi Prefecture: TMI 12A (S. Imai); Yamagata Prefecture: OMNH 2392 (T. Hongo); OMNH 3162 (T. Hongo).

GenBank accession numbers of DNA sequences: see Supplementary Table S1 (nrDNA ITS Clade-2).

Ecology: Japanese *Tricholoma stans* is distributed in warm temperate to subalpine zones on Honshu and Hokkaido Islands under various *Fagaceae* and *Pinaceae* trees, especially under *Abies veitchii* and *Tsuga diversifolia* in subalpine zone on Honshu Island, fruiting Sep–Nov.

Japanese common name: “*Azashimeji*” named by Matsuda and Hongo (1962).

Comment: *Tricholoma stans* has relatively large spores compared with *T. kakishimeji* and *T. albobrunneum* (see below) and larger Q -value compared with *T. kakishimejioides* (see above). In addition, pileipellis hyphae of *T. stans* are thinner than those of *T. kakishimeji* and *T. albobrunneum*. These *T. stans* characteristics suggest similarity to *Ookakishimeji* identified as *T. pessundatum* (Fr.) Quél. by Imai (1938) and Ito (1959). However, no *T. pessundatum* specimens identified by S. Imai matched the *T. stans* clade (Clade-2) in the phylogenetic analysis (Fig. 2). Although *T. stans* was once reported from Niigata Prefecture, Japan with the common name *Matsubarashimeji* (Miyuchi, 2010), this fungus has a distinctly squamous pileal surface, suggesting membership in the *T. imbricatum* group. We conducted DNA analysis of *atp6* in two *Azashimeji* specimens, OMNH 2392 and OMNH 3162, and found that they belonged to *T. stans* (Clade-2) (Supplementary Fig. S2). However, *Azashimeji* and *T. stans* descriptions showed slight morphological differences, i.e., pileal center is squamulose (“centro minute squamuloso”) in *Azashimeji* (Matsuda & Hongo, 1962). As a new species, the name *T. maculatipus* was used for *Azashimeji* (Matsuda & Hongo, 1962). Subsequently, Hongo (1983) reported this species as being new to Shiga Prefecture, but misspelled the fungus as “*T. maculatipes*”. Recently, Katsumoto compiled references for this fungus, but unfortunately also used the wrong spelling, *T. maculatipes*. We checked the spelling on the labels of the specimens used by Matsuda & Hongo (1962) and Hongo (1983) and all were labelled *T. maculatipus*. Therefore, we consider *T. maculatipus* as the correct spelling and reject *T. maculatipes*.

All gene regions tested, except for *atp6* in *Azashimeji* specimens, failed sequencing due to degradation of basidioma tissue. Based on the accumulated data for *Azashimeji*, i.e., phylogenetic, morphological, and ecological characteristics, we synonymized *T. macu-*

latipus to *T. stans*. The geographic distribution of *T. stans* in Europe is limited to boreal Fennoscandia and Denmark (Christensen & Heilmann-Clausen, 2013), but Japanese *T. stans* is distributed from subalpine to warm temperate zones in *Quercus* and *Fagus* forests. Therefore, Japanese *T. stans* populations include greater genetic variation compared with European populations.

Tricholoma albobrunneum (Pers.) P. Kumm., Der Führer in die Pilzkunde; 129, 1871. Fig. 5.

Description: **Pileus** firstly convex and later flat, margin involved and flat when matured, reddish brown, dark brown in center, and paler color in the margin, surface innately fibrillose, margin fibrillose and striate, viscid when moist (Fig. 5A–C). **Stipe** white or brownish, cylindrical to tapering slightly toward the base, hollow or medulla, powdery at the top surface (Fig. 5C). **Gills** white, close, later brownish stains occurred (Fig. 5B–D). **Flesh** white, thick, guaiac test negative (Fig. 5D), smell farinaceous, taste slightly bitter in pileal surface. **Basidiospores** 4.5–4.9–5.7 × 3.1–3.5–3.9 μm, Q = 1.3–1.4–1.5, ellipsoidal to predominantly ellipsoidal, single intracellular large achromatic oily droplet in fresh material (Fig. 5E). **Basidia** 23.1–26.0–27.9 × 4.9–5.5–6.3 μm, clavate to near cylindrical, four-spored, several intracellular transparent oily droplets when fresh materials were observed, non-amyloid, clamp connections not observed on the basal septum of a basidium (Fig. 5F). **Sterigmata** 2.4–3.1–3.8 × 0.9–1.3–1.5 μm (Fig. 5F). **Pileipellis** ixocutis to ixotrichoderm, hyphae, 49.4–61.3–72.9 × 4.6–5.2–5.8 μm, cylindrical, intracellular brown pigment, no clamp connections (Fig. 5G, H). **Stipitipellis** 31.3–53.6–82.5 × 5.0–6.7–9.6 μm (TUA-127), cutis, hyaline to pale brown, smooth, cylindrical, no clamp connections, occasionally cystidium-like structures were observed on the surface of top of stem, clavate to cylindrical, colorless, no clamp connections (Fig. 5I).

Specimens examined: JAPAN: Hokkaido: TUA-1 (A. Yamada), TUA-2 (A. Yamada), TMI 5615 (S. Imai); Nagano Prefecture: TUA-4 (A. Yamada), TUA-78 (W. Aoki), TUA-118 (W. Aoki), TUA-119 (W. Aoki), OMNH 2802 (*T. Hongo*); Tottori Prefecture: TUA-127 (W. Aoki), TUA-143 (W. Aoki), TaY20181117-001 (N. Endo); Ishikawa Prefecture: TNS-F18996 (Y. Ikeda); Ibaraki Prefecture: TMI Fukuroda 52 (S. Imai); Shiga Prefecture: OMNH 1756 (*T. Hongo*), OMNH 1938 (*T. Hongo*), OMNH 1947 (*T. Hongo*), OMNH 3008 (*T. Hongo*), OMNH 3606 (*T. Hongo*).

GenBank accession number of DNA sequences: see Supplementary Table S1 (nrDNA ITS Clade-3A, -3B).

Ecology: Japanese *Tricholoma albobrunneum* is specifically found in two-needle pine stands, i.e., *Pinus densiflora* and *P. thunbergii*, established in coastal and inland mountain regions in the temperate zone on Honshu Island; and in five-needle pine shrubs, i.e., *P. pumila*, established in mountain regions in the alpine zone on Mt. Rishiri-dake, Hokkaido. Other five-needle pines, e.g., *P. parviflora* and *P. koraiensis*, are also potential hosts. Fruiting in Sep–Dec, rarely in Jun.

Japanese common name: *Matsushimeji* adopted by S. Imai (1938) from the old name of this mushroom.

Comment: Japanese *T. albobrunneum* showed a smaller trend in basidium size compared with European *T. albobrunneum*, which was regarded as intraspecific variation in the present study, because other morphological characteristics and phylogenetic data support the conspecificity. Pileipellis hyphae of Japanese *T. albobrunneum* tended to be longer compared with those of *T. stans*, *T. kakishimeji*, and *T. kakishimejioides*. The striate character on the pileal margin is unique to *T. albobrunneum* compared with these other three species, despite the variation in each specimen of this

species and *T. stans*. This species has been confused with these three species since Imazeki (1942). Descriptions of Japanese *T. albobrunneum* by Imazeki (1942) and Imazeki and Hongo (1957) were regarded to be *T. kakishimeji* in the present study based on their macroscopic features and spore size. In contrast, *T. ustale* sensu Imazeki and Hongo (1965, 1987) were regarded to be *T. albobrunneum* in the present study. In addition, the *T. pessundatum* TMI 5615 specimen used for the species description as new to Japan as well as TMI 5614 under the common name *Ookakishimeji* (Imai, 1938) was identified as *T. albobrunneum* based on the *rpb2* phylogeny (Fig. 2). Two *T. albobrunneum* specimens identified by S. Imai (TMI Mizu5 and TMI 12A) were unfortunately included in the *T. kakishimeji* and *T. stans* clades, respectively, in the *rpb2* phylogeny (Fig. 2). At present, no *T. albobrunneum* specimens identified by S. Imai as the species are available to confirm the validity of the phylogenetic analyses. However, because the *T. albobrunneum* TMI Fukuroda 52 specimen sampled in Ibaraki Prefecture and used for the species description as new to Japan (Imai, 1938) was confirmed to have the species microscopic features, we adopted the common name *Matsushimeji* (i.e., pine-associated shimeji mushroom in Japanese) for *T. albobrunneum*. In addition, the habitat properties of *T. albobrunneum* basidioma, including “especially under *Pinus*”, reported by Imai (1938) support the validity of his identification. A survey of older scientific records of *T. albobrunneum* in Japan dating back to 1917 showed that K. Minakata recorded a drawing of “*Matsushimeji*” as *T. cryptica* (Hagiwara, 2007), based on the striate at the pileal margin and other external macroscopic features of *T. albobrunneum*. Ikeda (2005) identified *Matsushimeji* as *T. cf. striatum* (Schaeff.) Quél. However, we regarded those descriptions as inappropriate for defining Japanese *Matsushimeji*. The habitats of Japanese *T. albobrunneum* populations are more diverse than those of European populations (Christensen & Heilmann-Clausen, 2013), despite the shared host, *Pinus*. We examined specimens of *T. ustale* sensu Hongo (OMNH 1756, OMNH 1938, OMNH 1947, OMNH 3008, and OMNH 3606) and found that spore size and the presence of striate at the pileal margin matched the characters of *T. albobrunneum*.

5. Discussion

Kawamura (1925) first identified *Kakishimeji* as *Tricholoma ustale* Fr. Since then, *Kakishimeji* was consistently identified as *T. ustale* (Fr.) P. Kumm. (Ito, 1959; Imazeki & Hongo, 1965, 1987; Katsumoto, 2010), and its closely related species, such as *Matsushimeji*, have been controversial. In this study, we conducted phylogenetic analyses of Japanese *Tricholoma* species and revealed four phylogenetic clades, which were morphologically identified at the species level. Of them, two are known species, and the other two are new species. Interestingly, true *T. ustale* was not found, suggesting that *T. ustale* is rare or absent in Japan.

Although *T. albobrunneum* specimens TUA-1 and TUA-2, both collected under *P. pumila* in northern Hokkaido, were regarded as the sister subclade to the other Japanese *T. albobrunneum* specimens based on Bayesian posterior probability in the ITS phylogeny (Fig. 1), they were positioned in the *T. albobrunneum* clade in the other five phylogenetic trees (including *tef-1*, *atp6*, and *rpb2*). Therefore, we regarded these two specimens as *T. albobrunneum* and the variation as infraspecific. However, Reschke et al. (2018) reported that Chinese *T. albobrunneum* is distinct from European *T. albobrunneum* based on the ITS phylogeny. We suggest that although species in the sect. *Genuina* in *Tricholoma* can be distinguished by ITS phylogeny, further phylogenetic analyses are needed to interpret the relationships with look-alike species complexes.

Further investigations are necessary to clarify whether Japanese *T. albobrunneum* and Chinese *T. albobrunneum* reported by Reschke et al. (2018) are conspecific.

The newly described *T. kakishimeji*, a sister clade to *T. pessundatum*, has a wide range of host associations, i.e., both *Pinaceae* and *Fagaceae*. The confused identifications of *Kakishimeji* and *Matsushimeji* in Japan (Imazeki, 1942; Imazeki & Hongo, 1957, 1965, 1987; Ikeda, 2005) were caused in part by this fungal ecology. European *T. pessundatum* is limited to conifers, such as *Picea*, *Pinus*, and *Abies* (Christensen & Heilmann-Clausen, 2013). However, because the conifer-associates of *T. kakishimeji* were mostly sampled in man-made stands in a forest site (TUA-75) or urban area (TUA-17, TUA-125), these preferred hosts might be *Fagaceae*. Bessette, Bessette, Roody, & Trudell (2013) reported that North American *T. pessundatum* comprises two different groups distinguishable by spore size, i.e., $5.7\text{--}6.7 \times 3.8\text{--}4.8\text{ }\mu\text{m}$ around the Great Lakes and $4.8\text{--}5.7 \times 2.9\text{--}3.8\text{ }\mu\text{m}$ elsewhere, the latter matched European *T. pessundatum*. Japanese *T. kakishimeji* shows a similar spore size to that of European *T. pessundatum*. The *T. pessundatum* specimen collected in Yunnan Province in China (MB305071) reported by Reschke et al. (2018) positioned in the *T. kakishimeji* clade (Fig. 1).

The newly described *T. kakishimejioides*, sister clade to *T. ustaloides* Romagn., fruits under fagaceous trees. Japanese *T. ustaloides* was reported by Miyauchi (1989, 2010) as *Kakishimeji-modoki* and described by Ikeda (2005) as *Nigashimeji*. *Nigashimeji* has smaller spores than those of *T. kakishimejioides* and is much smaller compared with European *T. ustaloides* Romagn. In addition, *Nigashimeji* is distributed in mixed forests of pine and oak, or oak forests, unlike *T. kakishimejioides* and *T. ustaloides* in *Quercus* forests, suggesting that *Nigashimeji* includes at least two species: *T. kakishimejioides* and *T. albobrunneum*. In fact, the *Nigashimeji* specimens identified by Y. Ikeda included those two species (Fig. 2). To date, no *T. kakishimejioides* sequence has been reported from China (Reschke et al., 2018). However, we suggest that *T. kakishimejioides* is probably present in China based on the geographic distribution of this fungal host. Recently, *T. orientifulvum* in the section *Genuina* was newly described from China (Xu, Cui & Yang, 2020). This fungus is related to *T. fulvum*, *T. ustaloides*, *T. kakishimejioides*, and *T. ustale*. As it is macroscopically similar to *T. fulvum*, i.e., distinct yellowish gills, it is easily distinguished from *T. kakishimejioides*.

T. kakishimeji specimens TUA-19, TUA-67, and TUA-75 (TNS-F82064) contained ustalic acid ($41.9\text{--}155.7\text{ }\mu\text{g/g}$ in dry material) according to liquid chromatography-tandem mass spectrometry (LC-MS) analysis (Ito et al., 2021), while *T. albobrunneum* and *T. ustaloides* never contain ustalic acid and *T. stans* contains negligible levels (unpublished data). Therefore, stomach problems reportedly caused by *T. ustale* in Japan are in fact probably caused by *T. kakishimeji*.

Disclosure

The authors declare no conflicts of interest. All of the experiments in this study were performed in compliance with the current laws of Japan.

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Reference

- Agerer, R. (ed). (1987–2008). *Colour atlas of ectomycorrhizae, 1st-14th delivery*. Schwäbisch Gmünd: Einhorn-Verlag.
- Anderson, J. B., & Stasovski, E. (1992). Molecular phylogeny of northern hemisphere species of *Armillaria*. *Mycologia*, 84, 505–516. <https://doi.org/10.1080/00275514.1992.12026170>
- Bessette, A. E., Bessette, A. E., Roody, C. W., & Trudell, S. A. (2013). *Tricholoma of North America: A mushroom field guide*. Austin: University of Texas Press.
- Bidartondo, M. I., & Bruns, T. D. (2002). Fine-level mycorrhizal specificity in the Monotropoideae (Ericaceae): specificity for fungal species groups. *Molecular Ecology*, 11, 557–569. <https://doi.org/10.1046/j.0962-1083.2001.01443.x>
- Breitenbach, J., & Kränzlin, F. (1991). *Fungi of Switzerland*, vol. 3. Lucerne: Mykologia.
- Christensen, M., & Heilmann-Clausen, J. (2013). *The genus Tricholoma - Fungi of Northern Europe - vol. 4*. Tilst, Denmark: Svampetryk.
- Cléménçon, H. (2009). *Methods for working with macrofungi: laboratory cultivation and preparation of larger fungi for light microscopy*. Berlin: IHW Verlag.
- Cléménçon, H. (2012). *Cytology and plectology of the Hymenomycetes*, 2nd edn. Stuttgart: J. Cramer.
- Duñabeitia, M. K., Hormilla, S., Salcedo, I., & Peña, J. I. (1996). Ectomycorrhizae synthesized between *Pinus radiata* and eight fungi associated with *Pinus* spp. *Mycologia*, 88, 897–908. <https://doi.org/10.1080/00275514.1996.12026730>
- Endo, N., Dokmai, P., Suwannasai, N., Phosri, C., Horimai, Y., Hirai, N., et al. (2015). Ectomycorrhization of *Tricholoma matsutake* with *Abies veitchii* and *Tsuga diversifolia* in the subalpine forests of Japan. *Mycoscience*, 56, 402–412. <https://doi.org/10.1016/j.myc.2014.12.004>
- Fries, E. M. (1818). *Observationes mycologicae*. Havniae: sumptibus G. Bonnier. <https://doi.org/10.5962/bhl.title.112534>
- Gardes, M., & Bruns, T. D. (1993). ITS primer with enhanced of specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2, 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Grubisha, L. C., Levens, N., Olson, M. S., & Taylor, D. L. (2012). Intercontinental divergence in the *Populus*-associated ectomycorrhizal fungus, *Tricholoma populinum*. *New Phytologist*, 194, 548–560. <https://doi.org/10.1111/j.1469-8137.2012.04066.x>
- Hagiwara, H. (2007). *Color illustration of fungi described by Kumagusu Minakata (In Japanese)*. Tokyo: Shinchosha.
- Halama, M., Ważny, R., Czosnykowska-Lukacka, M., & Dobosz, T. (2016). *Tricholoma ustaloides* (Agaricales, Basidiomycota) in Poland. *Polish Botanical Journal*, 61, 173–180. <https://doi.org/10.1515/pbj-2016-0007>
- Hayakawa, I., Watanabe, H., & Kigoshi, H. (2008). Synthesis of ustalic acid, an inhibitor of Na^+ , K^+ -ATPase. *Tetrahedron*, 64, 5873–5877. <https://doi.org/10.1016/j.tet.2008.04.051>
- Heilmann-Clausen, J., Christensen, M., Frøslev, T. G., & Kjeller, R. (2017). Taxonomy of *Tricholoma* in northern Europe based on ITS sequence data and morphological characters. *Persoonia*, 38, 38–57. <http://dx.doi.org/10.3767/003158517X693174>
- Henrion, B., Le Tacon, F., & Martin, F. (1992). Rapid identification of genetic variation of ectomycorrhizal fungi by amplification of ribosomal RNA genes. *New Phytologist*, 122, 289–298.
- Hiroe, I. (1949). *Science of fungi* (In Japanese). Osaka, Fuminsha.
- Hofstetter, V., Cléménçon, H., Vilgalys, R., & Moncalvo, J. (2002). Phylogenetic analyses of the Lyophylleae (Agaricales, Basidiomycota) based on nuclear and mitochondrial rDNA sequences. *Mycological Research*, 106, 1043–1059.
- Hongo, T. (1983). *Notulae Mycologicae* (18), *Memoirs of the Faculty of Education, Shiga University, Natural Science*, 11, 37–41. https://shiga-u.repo.nii.ac.jp/?action=pages_view_main&active_action=repository_view_main_item_detail&item_id=1590&item_no=1&page_id=13&block_id=21
- Hongo, T. (ed.) (1994). *Yama-kei field book on mushrooms* (in Japanese). Tokyo: Yama-kei Publishers.
- Ichioaka, T. (1979). *Shin-you-kin-pu (Colored illustration of mushrooms in the southern part of Shinshu area)* (In Japanese). Own publication.
- Ikeda, Y. (2005). *Mushrooms and toadstools pictured book of Hokuriku* (In Japanese). Kanazawa, Japan: Kakubundo.
- Imai, S. (1938). Studies on the Agaricaceae of Hokkaido. I, *Journal of the Faculty of Agriculture, Hokkaido Imperial University*, 43, 1–178.
- Imazeki, R. (1942). *Colored illustrations of mushroom: How to collect and identification of mushrooms* (In Japanese). Tokyo: Sanseido.
- Imazeki, R., & Hongo, T. (1957). *Colored illustrations of fungi of Japan* (In Japanese). Osaka: Hoikusha.
- Imazeki, R., & Hongo, T. (1965). *Colored illustrations of fungi of Japan vol. II* (In

- Japanese). Osaka: Hoikusha.
- Imazeki, R., & Hongo, T. (1987). *Illustrations of mushrooms of Japan vol. I* (In Japanese). Osaka: Hoikusha.
- Imazeki, R., Hongo, T., & Tubaki, K. (1970). *Common fungi of Japan in color* (In Japanese). Osaka: Hoikusha.
- Ito, S. (1959). *Mycological flora of Japan, Vol. 2, issue 5* (In Japanese). Sendai: Yoken-do.
- Ito, T., Nagai, H., Aoki, W., Yamada, A., Kawagishi, H., Fukaya, M and Konishi, H. (2021). Quantification of ustalic acid, a chemotaxonomic marker, in *Tricholoma ustale* using liquid chromatography–mass spectrometry. *Journal of Natural Medicines*, 75, 688–691. <https://doi.org/10.1007/s11418-021-01496-z>
- Katsumoto, K. (2010). *List of fungi recorded in Japan* (In Japanese). Tokyo: The Kanto Branch of the Mycological Society of Japan.
- Kawamura, S. (1925). *Illustrated Japanese fungi, 5th delivery* (In Japanese). Tokyo: The Forest Experiment Station of the Department of Agriculture and Forestry.
- Kawamura, S. (1929). *The Japanese fungi* (In Japanese). Tokyo: Daichi-shoin.
- Kawamura, S. (1931). *Edible fungi and poisonous fungi* (In Japanese). Tokyo: Iwanami Shoten.
- Kluting, K. L., Baroni, T. J., & Bergemann, S. E. (2014). Toward a stable classification of genera within the Entolomataceae: a phylogenetic re-evaluation of the *Rhodocybe-Clitopilus* clade. *Mycologia*, 106, 1127–1142. <https://doi.org/10.3852/13-270>
- Kretzer, A. M., & Bruns, T. D. (1999). Use of *atp6* in fungal phylogenetics: an example from the Boletales. *Molecular Phylogenetics and Evolution*, 13, 483–492. <https://doi.org/10.1006/mpev.1999.0680>
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Kummer, P. (1871). Der Führer in die Pilzkunde: Anleitung zum methodischen, leichten und sichern Bestimmen der in Deutschland vorkommenden Pilze: mit Ausnahme der Schimmel- und allzu winzigen Schleim- und Kern-Pilzchen. Zerbst: Verlag von E. Luppe's Buchhandlung.
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2007). ClustalW and ClustalX version 2.0. *Bioinformatics Applications Note*, 23, 2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
- Lefevre, C. K. (2002). *Host associations of Tricholoma magnivelare, the American matsutake*. PhD thesis, Oregon State University.
- Matsuda, I., & Hongo, T. (1962). Larger fungi in the lower temperate deciduous forest at the northern foot of Mt. Iide. *The Journal of Japanese Botany*, 37, 367–373. http://www.jjbotany.com/pdf/JJB_037_367-373.pdf
- Miller S. L., Koo C. D., & Molina R. (1991). Characterization of red alder ectomycorrhizae: a preface to monitoring belowground ecological responses. *Canadian Journal of Botany*, 69, 516–531. <https://doi.org/10.1139/b91-071>
- Miyauchi, S. (1989). *Mushrooms, and its culture* (In Japanese). Mitsuke, Japan: Bunka Publisher.
- Miyauchi, S. (2010). *Mushrooms of Niigata Prefecture* (In Japanese). Niigata: Niigata Nippou Jigyousha.
- Murata, Y. (1978). New records of gill fungi from Hokkaido (1). *Transactions of the Mycological Society of Japan*, 19, 241–247.
- Nagasawa, E. (2009). *Poisonous fungi in Japan* (In Japanese). Tokyo: Gakken.
- Phillips, R. (1981). *Mushrooms and other fungi of Great Britain and Europe*. London: Pan Books.
- Reschke, K., Popa, F., Yang, Z. L., & Kost, G. (2018). Diversity and taxonomy of *Tricholoma* species from Yunnan, China, and notes on species from Europe and North America. *Mycologia*, 110, 1081–1109. <https://doi.org/10.1080/00275514.2018.1512295>
- Romagnesi, H. (1971). *Petit atlas des champignons tome 2*. Paris: Bordas.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., et al. (2012). 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>
- Sano, Y., Sayama, K., Arimoto, Y., Inakuma, T., Kobayashi, K., Koshino, H., et al. (2002). Ustalic acid as a toxin and related compounds from the mushroom *Tricholoma ustale*. *Chemical Communication*, 13, 1384–1385. <https://doi.org/10.1039/b202607d>
- Trappe J.M. (1962). Fungus associates of ectotrophic mycorrhizae. *The Botanical Review*, 28, 538–606. <https://doi.org/10.1007/BF02868758>
- White, T. J., Bruns, T., Lee, S., & Taylor, J. (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* (pp. 315–322). San Diego: Academic Press.
- Xu, X., Cui, Y., Yang, Z. L. (2020). Two new species of *Tricholoma* sect. *Genuina* (Agaricales) from China. *Phytotaxa*, 443, 155–166. <https://doi.org/10.11646/phytotaxa.443.2.3>
- Yamada, A., Kanekawa, S., & Ohmasa, M. (1999). Ectomycorrhiza formation of *Tricholoma matsutake* on *Pinus densiflora*. *Mycoscience*, 40, 193–198. <https://doi.org/10.1007/BF02464298>
- Yamada, A., Ogura, T., & Ohmasa, M. (2001). Cultivation of mushrooms of edible ectomycorrhizal fungi associated with *Pinus densiflora* by in vitro mycorrhizal synthesis. II. Morphology of ectomycorrhizas in open-pot soil. *Mycorrhiza*, 11, 67–81. <https://doi.org/10.1007/s005720000093>
- Yamada A., Kobayashi, H., Murata, H., Kalmış E., Kalyoncu, F., & Fukuda, M. (2010). In vitro ectomycorrhizal specificity between the Asian red pine *Pinus densiflora* and *Tricholoma matsutake* and allied species from worldwide Pinaceae and Fagaceae forests. *Mycorrhiza*, 20, 333–339. <https://doi.org/10.1007/s00572-009-0286-6>
- Yamashita, M., & Furukawa, H. (1993). *Mushroom poisoning* (In Japanese). Tokyo: Kyoritsu Shuppan.