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The Status of Voucher Specimens of Mushroom Species Thought to Be Extinct from Japan

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Abstract We surveyed some major fungal herbaria in Japan to find collections of "extinct" mushroom species. The presence of voucher collections was confirmed for a total of 21 out of 26 "extinct" mushroom species listed in a Red Data Book of Japan. We then assessed the type status and quality of these specimens based on collection data on collection labels and physical appearance of fruit bodies. The majority of voucher collections of "extinct" species were demonstrated to be the type (holotype, isotype or syntype) with various degrees of quality. Some were heavily contaminated by mold or highly fragmented into small pieces. In future, attempts will be made to obtain high quality DNA sequences from these "extinct" species to clarify their biological entity.

Key words: Agaricomycetes, DNA barcoding, fungi, herbarium, nomenclature, Red Data, taxonomy, type specimen.

Introduction

The Red Data Book of Japan (Ministry of the Environment, 2015) lists over 60 fungal (mostly mushroom) species as threatened (critically endangered, endangered, or vulnerable). Of those, a total of 26 fungal species are listed as "extinct" and one species is categorized in "extinct in the wild." All of such "extinct" species have been recorded more than 70 years ago, and most of them have very limited distribution (e.g., one locality) in Japan. Accordingly, all species, except for three species (*Chlorophyllum agaricoides*, *Ganoderma colossus* and *Lycoperdon henningsii*), are considered endemic to Japan (Hosoya *et al.*, 2016; Hosaka, 2018).

It is noteworthy that a total of 23 out of the 26 "extinct" species are the mushrooms domestically recorded only from Chichijima Island or Hahajima Island, both belonging to the Ogasawara (Bonin) Islands, Japan. These mushroom species were described or recorded in a series of publications during the 1930's to 1940's by Ito and Imai (1937, 1939, 1940) and Kobayasi (1937), and the information of all species are compiled in a check-list by Ito (1955, 1959).

The Ogasawara Islands have been visited by several mycologists since Ito, Imai and Kobayasi. For example, Hongo (1977, 1978, 1980) published a series of papers recording more than 30 species, including 5 taxa new to science, from the islands. Later, Neda and Hattori (1991), Neda

and Sato (2008), Sato *et al.* (2010) and Hosaka (2018) published a list of mushroom species from the islands based on extensive fieldwork. Despite their efforts, however, the "extinct" species have never been re-documented. The remaining three "extinct" species known outside the Ogasawara Islands also have not been recorded since their first discovery in Japan more than 70 years ago.

In this study, voucher specimens of these "extinct" mushroom species were examined. Because all of these specimens are more than 70 years old, physical condition of specimens was expected to be poor, or collections were expected to be lost. In both scenarios, some taxonomic issues regarding the extinct species could not easily be resolved using the voucher specimens. We therefore first surveyed some major fungal herbaria in Japan to locate the collections of "extinct" mushroom species. After collections were found, we then assessed the quality of specimens based on physical appearance of fruit bodies. The aforementioned challenges are further emphasized, and a potential solution for future studies is presented.

Materials and Methods

Herbarium survey

To locate voucher collections of "extinct" mushroom species, we conducted an extensive herbarium survey at two fungal herbaria in Japan, i.e., Hokkaido University Museum, Sapporo, Hokkaido (SAPA) and National Museum of Nature and Science, Tsukuba, Ibaraki (TNS). The SAPA herbarium was chosen for survey mainly because the majority of "extinct" species were described by Seiya Ito and Sanshi Imai, who both worked at Hokkaido University during the 1930's to 1940's. The TNS herbarium was chosen for survey because it contains the largest fungal collections in Japan (ca. 120,000 collections) and the large part of these have already been databased and the data are publicly accessible at http://db.kahaku.go.jp/webmuseum_en/.

The SAPA fungal herbarium was surveyed during November 21 to 24, 2017. All collections housed in the collection room were carefully checked for taxon names, collection date, and locality. There were additional formalin-fixed collections in separate rooms but they could not be accessed due to on-going renovation work in that part of the museum.

The TNS fungal herbarium was surveyed using the public database (http://db.kahaku.go.jp/webmuseum_en/) as well as our in-house database. In cases some of our target species have not been databased, we also physically checked all collections stored in the type collection cabinet. In addition to SAPA and TNS, the OSC herbarium (Oregon State University, Corvallis, Oregon, USA) was also investigated. This is because our preliminary survey indicated that some duplicate collections of Japanese gasteroid fungi were shipped to OSC during 1930's to 1940's.

Validating the type status of collections

Because not all type collections are clearly labeled as "type", we have validated the type status of each collection by cross-checking taxon names, collection date and locality, collector's names, and collection numbers. Specimens were considered as type when all such data were in agreement with the original species description.

Accessing quality of specimens

All specimens of "extinct" mushroom species retrieved for this study, including the collection labels and packets, were photographed. Assessment of the quality of the collections include: 1) presence of mold contamination, 2) presence of insect damage, 3) quantity (the number of fruit bodies), 4) maturity (presence of mature spores), and 5) overall appearance (e.g., fruit bodies intact or fragmented). Based on such criteria, the collections were qualitatively assigned to the categories of "Good", "Moderate" or "Poor".

Sample preparation for molecular studies

Small fragments of clean tissue (e.g., gleba, pileus or stipe tissue) from each specimen were cut using a clean, sterilized razor blade. Contamination of visible soil particles or moldy parts

was carefully avoided. Tissue fragments were soaked in DMSO buffer (Seutin *et al.*, 1991) with an addition of 100 mM Tris-HCl (pH 8.0) and 0.1 M sodium sulfite (Na_2SO_3), and stored under 4°C, following the procedures of Hosaka (2009) and Hosaka and Castellano (2008). This was done to facilitate future molecular works on "extinct" or rare mushroom species in Japan.

Results

We found voucher collections of 21 species out of 26 "extinct" species from Japan. Collections of five species (*Chlorophyllum agaricoides*, *Ganoderma colossus*, *Lactarius ogasawarashimensis*, *Lentinus lamelliporus*, and *Russula boninensis*) could not be located (Table 1). A total of 17 collections were in the SAPA herbarium, whereas three collections were found in the TNS herbarium. One collection (*Circulocolumella hahashimensis*, isotype) was found from the OSC herbarium (Table 1, Fig. 2D–F). Most of them were type collections (holotype, isotype or syntype) with the only exception being *Lycoperdon henningsii* (Table 1, Fig. 5A–B). Below is a list of 26 "extinct" species, with comments on nomenclature, quality of collections, and distribution.

1. ***Agaricus hahashimensis*** S.Ito & S.Imai, Trans. Sapporo Nat. Hist. Soc. 16: 51 (1940) [Fig. 1A]

Collection examined: SAPA10000004, syntype.

Comments. The collection was considered in "poor" condition because it only has a single fruit body, which is heavily contaminated by mold on the hymenial surface. The collection label indicates its syntype status, collected on November 20, 1936 from Hahajima Island, the Ogasawara Islands, Japan. However, the original description by Ito and Imai (1940) indicated it was collected on "Nov. 20, 1939". We believe it is a typographical error in the description because all the other species described in the same paper were collected in November 1936.

2. ***Albatrellus cantharellus*** (Lloyd) Pouzar, Česká Mykol. 26: 196 (1972) [Fig. 1B–E]

Basionym: *Polyporus cantharellus* Lloyd, Mycol. Writ. 4(Letter 54): 5 (1915).

Collection examined: TNS-F-201306, isotype.

Comments. The collection was considered in "good" condition because it contains multiple (more than 20) fruit bodies of various developmental stages, without any obvious mold contamination. Although the collection was labeled as "CO-TYPUS" (Fig. 1B), it should clearly be considered an isotype. In his original description of the species (found at https://biodiversitylibrary.org/page/17882210), Lloyd mentioned "Specimen (259) from A. Yasuda, collected at Sendai, Japan." which is in good agreement with the collection label of TNS material (Fig. 1C).

3. ***Camarophyllus microbicolor*** S. Ito, Mycol. Fl. Japan 2(5): 71 (1959) [Fig. 2A]

Synonym: *Hygrophorus bicolor* S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 14 (1939), nom. illegit.; non Berk. & Broome, J. Linn. Soc., Bot. 11: 565 (1871).

Collections examined: SAPA10000041, syntype; SAPA10000040, syntype.

Comments. The collection (SAPA10000041, from Hahajima Island) was considered in "moderate" condition. Although it contains only one fruit body, the hymenium is well-preserved without obvious mold contamination or insect damage. The other syntype collection (SAPA-10000040, from Chichijima Island) contained only a small volume of fragmented tissue (photo not provided). We could only locate these two collections, but the original description by Ito and Imai (1939) indicated there should be two more specimens collected from Chichijima Island, the Ogasawara Islands, Japan. The original taxon name by Ito and Imai (1939), *Hygrophorus bicolor*, is illegitimate because it is a later homonym of *Hygrophorus bicolor* Berk. & Broome.

Table 1. A list of "extinct" mushroom specimens with distribution and collection information

Nos.	Taxon[*1]	Distribution[*2]	Year collected in Japan	Herbarium[*4]	Type status	Quality[*7]
1	*Agaricus hahashimensis*	H	1936	SAPA	Syntype	Poor
2	*Albatrellus cantharellus*	S	1914	TNS	Isotype[*6]	Good
3	*Camarophyllus microbicolor*	C, H	1936	SAPA	Syntype	Moderate
4	*Campanella boninensis*	C	1936	SAPA	Holotype	Good
5	*Chlorophyllum agaricoides*	O	1936	—[*5]	—	—
6	*Circulocolumella hahashimensis*	H	1936	OSC	Isotype	Good
7	*Clitocybe castaneofloccosa*	C	1936	SAPA	Syntype	Moderate
8	*Collybia matris*	C, H	1936	SAPA	Syntype	Moderate
9	*Coprinus boninensis*	C	1936	SAPA	Holotype	Poor
10	*Crepidotus subpurpureus*	H	1936	SAPA	Holotype	Good
11	*Cyathus badius*	C, H[*3]	1936, 2015[*3]	TNS	Holotype	Good
12	*Cyathus boninensis*	C	1936	SAPA	Holotype	Good
13	*Entoloma japonense*	C	1936	SAPA	Holotype	Mederate
14	*Ganoderma colossus*	M	1947	—[*5]	—	—
15	*Gymnopilus noviholocirrhus*	H	1936	SAPA	Holotype	Good
16	*Hygrocybe macrospora*	C	1936	SAPA	Holotype	Moderate
17	*Hygrocybe miniatostriata*	C	1936	SAPA	Holotype	Poor
18	*Lactarius ogasawarashimensis*	C	1936	—[*5]	—	—
19	*Lentinus lamelliporus*	T	1902	—[*5]	—	—
20	*Lepiota boninensis*	C	1930	SAPA	Holotype	Moderate
21	*Lycoperdon henningsii*	C	1936	TNS	non type	Moderate
22	*Pleurotus cyatheae*	C	1936	SAPA	Syntype	Moderate
23	*Pluteus daidoi*	H	1936	SAPA	Holotype	Moderate
24	*Pluteus horridilamellus*	H	1936	SAPA	Holotype	Moderata
25	*Psathyrella boninensis*	H	1936	SAPA	Holotype	Poor
26	*Russula boninensis*	C	1936	—[*5]	—	—

[*1] The current names of each taxon, according to Index Fungorum (http://www.indexfungorum.org/), are shown.
[*2] The known distribution in Japan based on the Ministry of Environment (2015) is shown. C = Chichijima Island, The Ogasawara (Bonin) Islands, Japan; H = Hahajima Island, The Ogasawara (Bonin) Islands, Japan; S = Sendai City, Miyagi, Japan; M = Miyazaki, Japan; T = Tokyo, Japan; O = Otaru City, Hokkaido, Japan.
[*3] Collection data newly recorded by Cruz *et al.* (2018).
[*4] Herbarium abbreviations are according to the Index Herbarium (http://sweetgum.nybg.org/science/ih/)
[*5] Specimens which could not be located during this study.
[*6] The original collection label indicated it is a "Co-type".
[*7] Based on the visual inspection of fruit body surface by naked eyes and stereo microscope. More details are discussed in the text.

4. ***Campanella boninensis*** (S. Ito & S. Imai) Parmasto, Nova Hedwigia 34: 438 (1981) [Fig. 2B]

Basionym: *Dictyolus boninensis* S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 20 (1939).

Collection examined: No. 19 (SAPA), holotype.

Comments. The collection was considered in "good" condition because it contains multiple (more than 10) fruit bodies without any obvious mold contamination. The species is known only from one locality on Chichijima Island, the Ogasawara Islands, Japan (Ito and Imai, 1939).

5. ***Chlorophyllum agaricoides*** (Czern.) Vellinga, Mycotaxon 83: 416 (2002)

Synonyms: *Endoptychum agaricoides* Czern., Bull. Soc. Imp. Nat. Moscou 18(2, III): 148 (1845); *Secotium agaricoides* (Czern.) Hollós, Term. Füz. 25: 93 (1902).

Comments. The collection from Japan could not be located during this study. It may be possible that the collection is housed at Tottori Mycological Institute (TMI) (E. Nagasawa, pers. comm.). Although the species has been collected only once in Japan (Sept. 7, 1930, Otaru City, Hokkaido), it is also known from North America, Europe, Australia, Mongolia and China (Imai, 1936; Dörfelt and Gube, 2007; Ministry of the Environment, 2015).

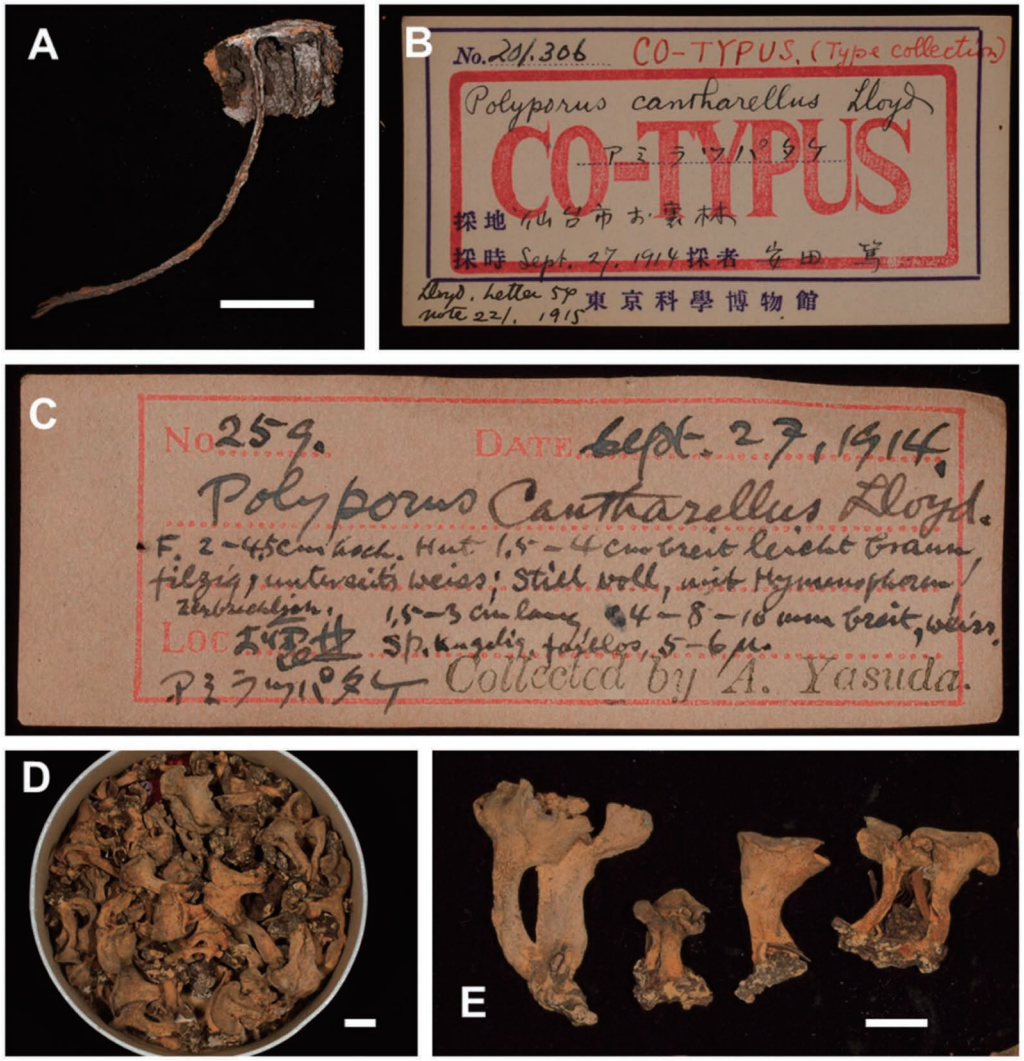

Fig. 1. Specimens of "extinct" mushroom species in Japan (1). A. *Agaricus hahashimensis* (SAPA10000004, Syntype). Bar = 1 cm. B–E. *Albatrellus cantharellus* (TNS-F-201306, Isotype). B. Collection label indicating "CO-TYPUS" status of the specimen. C. Additional label presumably written by the collector (A. Yasuda). D. Numerous fruit bodies in collection box. Bar = 1 cm. E. Fruit bodies. Bar = 1 cm.

6. ***Circulocolumella hahashimensis*** (S. Ito & S. Imai) S. Ito & S. Imai in Imai, Sci. Rep. Yokohama Natl. Univ., Sect. 2, 6: 3 (1957) [Fig. 2C–F]
Synonyms: *Hysterangium hahashimense* S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 15: 10 (1937); *Gelopellis hahashimensis* (S. Ito & S. Imai) Zeller, Mycologia 39 (3): 284 (1947); *Stalactocolumella hahashimensis* (S. Ito & S. Imai) S. Imai, Bot. Mag., Tokyo 63: 28 (1950), nom. inval.

Collection examined: leg. S. Imai (OSC, without number), isotype.

Comments. The collection was considered in "good" condition because glebal structure is fairly well preserved. However, the material has been fixed in formalin since the 1930's and DNA is probably highly fragmented. Although we could not find the collection of *Circulocolumella* in SAPA herbarium during this study, a fair number of uninvestigated liquid collections were

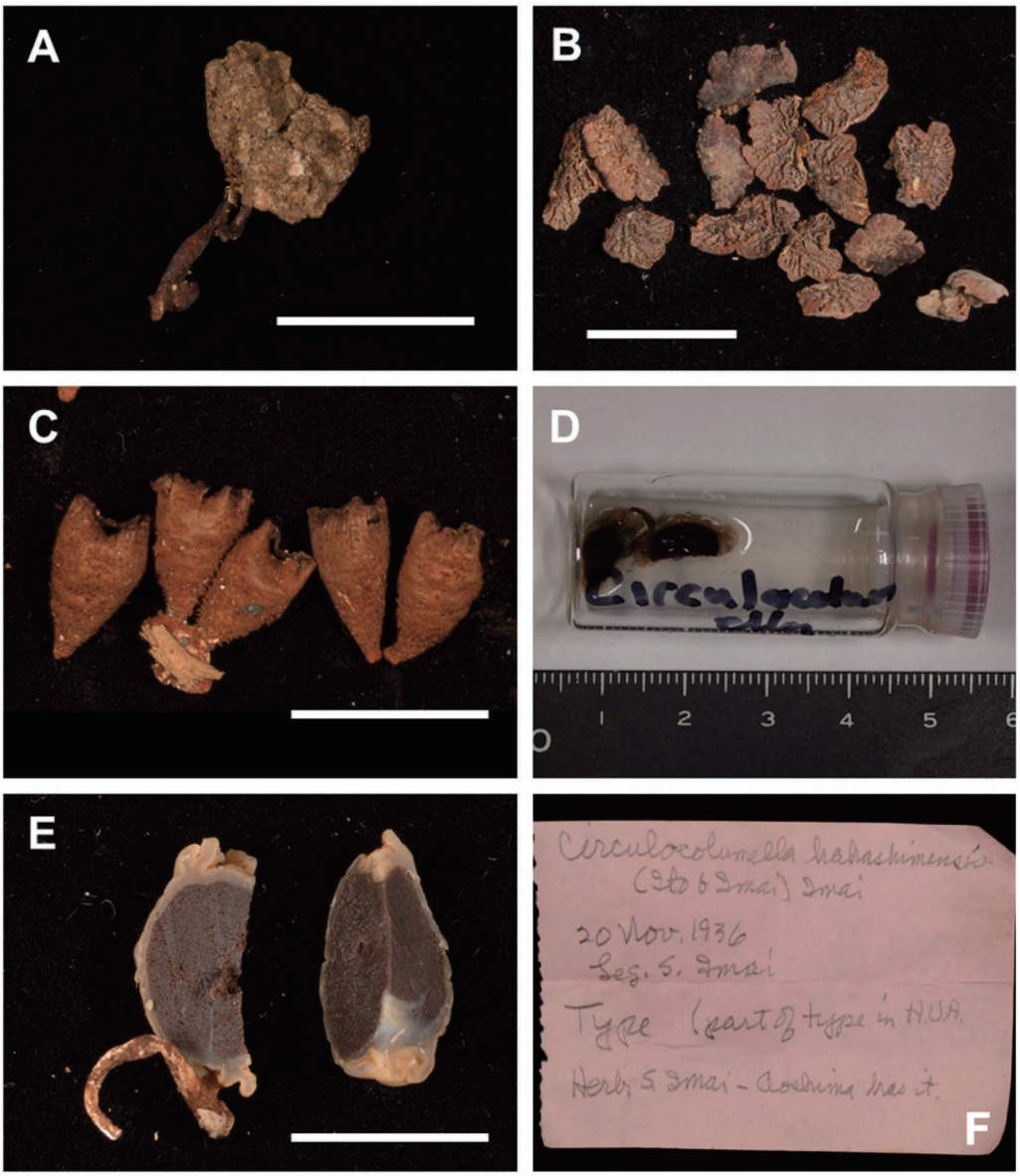

Fig. 2. Specimens of "extinct" mushroom species in Japan (2). A. *Camarophyllus microbicolor* (SAPA10000040, Syntype). Bar = 1 cm. B. *Campanella boninensis* (No. 19, SAPA, Holotype). Bar = 1 cm. C. *Cyathus badius* (TNS-F-172699, Holotype). Bar = 1 cm. D–F. *Circulocolumella hahashimensis* (OSC without number, collected on November 20, 1936, Isotype). D. Fruit bodies in formalin. E. Longitudinal sections of fruit bodies. Bar = 1 cm. F. Collection label written by Dr. James M. Trappe, indicating the type status of the specimen.

found recently (T. Kobayashi, data not shown). Some missing collections of "extinct" species, including *Circulocolumella*, may be included there. The isotype collection at OSC was probably sent from S. Imai to S. M. Zeller, who proposed a new combination, *Gelopellis hahashimensis*, based on the isotype material (Zeller, 1947). The isotype collection contains only one half of a fruit body (Fig. 2E). The original description (Ito and Imai, 1937; Imai, 1957), however, clearly illustrated the presence of more than 5 fruit bodies. The collections of the remain-

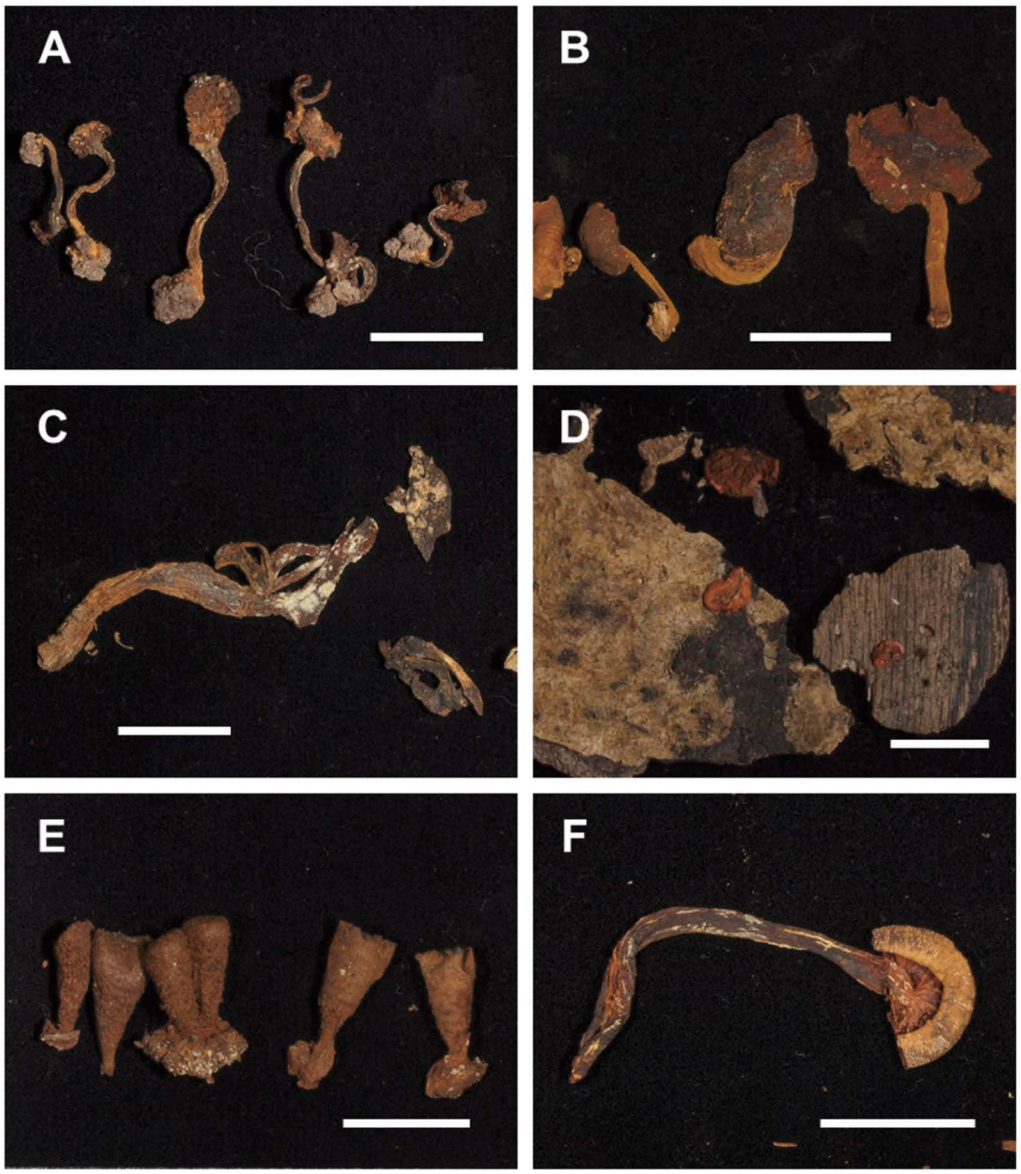

Fig. 3. Specimens of "extinct" mushroom species in Japan (3). A. *Clitocybe castaneofloccosa* (SAPA10000020, Syntype). Bar = 1 cm. B. *Collybia matris* (SAPA10000022, Syntype). Bar = 1 cm. C. *Coprinus boninensis* (SAPA10000024, Holotype). Bar = 1 cm. D. *Crepidotus subpurpureus* (SAPA10000027, Holotype). Bar = 0.5 cm. E. *Cyathus boninensis* (SAPA without number, collected on November 4, 1936, Holotype). Bar = 1 cm. F. *Entoloma japonense* (No. 33, SAPA, Holotype). Bar = 1 cm.

ing fruit bodies may be present elsewhere in Japanese herbaria.

7. ***Clitocybe castaneofloccosa*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 12 (1939) [Fig. 3A]

Collection examined: SAPA10000020, syntype.

Comments. The collection was considered in

"moderate" condition. It consists of multiple (8) fruit bodies with slight mold contamination. Although we have located only one collection at SAPA herbarium, the original description by Ito and Imai (1939) indicated there should be two more specimens collected from Chichijima Island, the Ogasawara Islands, Japan.

8. ***Collybia matris*** S. Ito in S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 16 (1939) [Fig. 3B]

Collections examined: SAPA10000022, syntype; SAPA10000023, syntype.

Comments. The collections were considered in "moderate" condition. It consists of multiple fruit bodies although there is a slight indication of mold contamination. This species is often cited as "*Collybia matris* S. Ito & S. Imai" (e.g., Ito, 1959; Ministry of the Environment, 2015). However, the original description (Ito and Imai, 1939) listed it as "*Collybia matris* S. Ito, sp. nov."

9. ***Coprinus boninensis*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 54 (1940) [Fig. 3C]

Collection examined: SAPA10000024, holotype.

Comments. The collection was considered in "poor" condition because it is heavily contaminated by mold, including the hymenial surface. The species is known only from one locality on Chichijima Island, the Ogasawara Islands, Japan (Ito and Imai, 1940).

10. ***Crepidotus subpurpureus*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 50 (1940) [Fig. 3D]

Synonym: *Pyrrhoglossum subpurpureum* (S. Ito & S. Imai) E. Horak & Desjardin, Mem. N. Y. Bot. Gdn. 89: 62 (2004), nom. inval.

Collection examined: SAPA10000027, holotype.

Comments. The collection was considered in "good" condition. It consists of multiple (4) fruit bodies on woody substrate. However, dried fruit bodies are less than 5 mm wide, and material for future molecular work is very limited. The species is known only from one locality on Hahajima Island, the Ogasawara Islands, Japan (Ito and Imai, 1940).

11. ***Cyathus badius*** Kobayasi, Bot. Mag., Tokyo 51: 755 (1937) [Fig. 2C]

Collection examined: TNS-F-172699, holotype.

Comments. The collection was considered in "good" condition because it consists of multiple (8) fruit bodies each with abundant peridioles. The species has been known only from Chichijima Island until recently, but new material from Hahajima Island has been reported (Cruz *et al.*, 2018).

12. ***Cyathus boninensis*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 15: 8 (1937) [Fig. 3E]

Collection examined: leg. S. Ito, S. Imai & K. Hino on Nov. 4, 1936 (SAPA without number), holotype.

Comments. The collection was considered in "good" condition because it consists of multiple fruit bodies each with abundant peridioles. No collection numbers were provided on collection labels or in the original description by Ito and Imai (1937), but the collection we found is clearly a holotype because all collection data in the description (locality, collection date, and illustrations) perfectly match the collection label.

13. ***Entoloma japonense*** Blanco-Dios, Tarrelos, Revista da Federation Galera de Micoloxia 17: 35 (2015) [Fig. 3F]

Synonyms: *Leptonia brunneola* S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 48 (1940); *Rhodophyllus brunneolus* (S. Ito & S. Imai) S. Ito, Mycol. Fl. Japan 2 (5): 437 (1959).

Collection examined: No. 33 (SAPA), holotype.

Comments. The collection was considered in "moderate" condition. It only has a single fruit body with slight mold contamination, but the shape of fruit body is intact and the gills are well-preserved. The epithet *brunneola* (*-lum*) was replaced because there is an earlier hom-

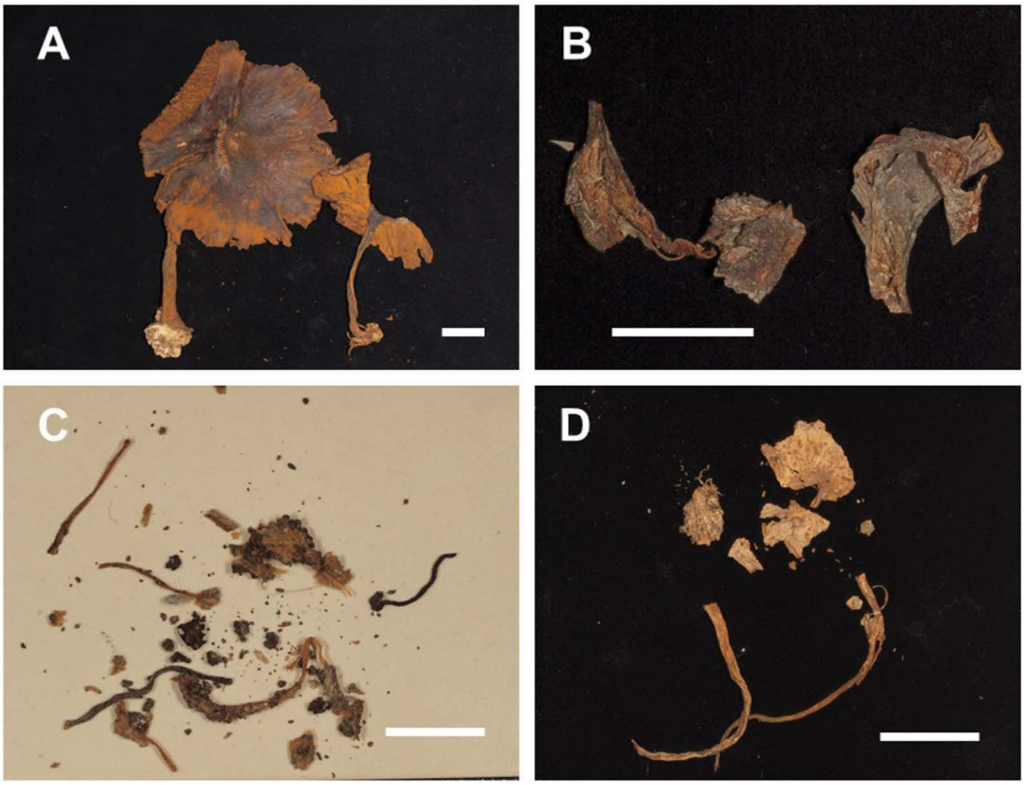

Fig. 4. Specimens of "extinct" mushroom species in Japan (4). A. *Gymnopilus noviholocirrhus* (SAPA10000030, Holotype). Bar = 1 cm. B. *Hygrocybe macrospora* (SAPA10000038, Holotype). Bar = 1 cm. C. *Hygrocybe miniatostriata* (SAPA10000042, Holotype). Bar = 1 cm. D. *Lepiota boninensis* (SAPA1096, Holotype). Bar = 1 cm.

onym, *Entoloma brunneolum* Hesler. The species is known only from one locality on Chichijima Island, the Ogasawara Islands, Japan.

14. ***Ganoderma colossus*** (Fr.) C.F. Baker, Broteria, Sér. Bot. 18 (no. 2): 37 (1920)
Synonyms: *Polyporus colossus* Fr., Nova Acta R. Soc. Scient. Upsal., Ser. 3, 1 (1): 56 (1851); *Tomophagus colossus* (Fr.) Murrill, Torreya 5: 197 (1905); *Dendrophagus colossus* (Fr.) Murrill, Bull. Torrey Bot. Club 32 (9): 473 (1905); *Polyporus hollandii* Massee, Bull. Misc. Inf., Kew: 163 (1901); *Thermophymatospora fibuligera* Udagawa, Awao & Abdullah, Mycotaxon 27: 100 (1986).

Comments. The collection from Japan could not be located during this study. It is indicated that the collection is housed at the herbarium of Forestry and Forest Products Research Institute (TFM or TF) in Tsukuba, Ibaraki, Japan (Ministry of the Environment, 2015). Although the species has been collected only once in Japan at Miyazaki Prefecture, it is known to have a pantropical distribution (Ministry of the Environment, 2015).

15. ***Gymnopilus noviholocirrhus*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 49 (1940) [Fig. 4A]

Collection examined: SAPA10000030, holotype.

Comments. The collection was considered in "good" condition because two fruit bodies are available and gill tissue is well-preserved with abundant mature basidiospores. The species is known only from one locality on Hahajima

Island, the Ogasawara Islands, Japan, growing on *Celtis boninensis* Koidz. (Cannabaceae) (Ito and Imai, 1940).

16. ***Hygrocybe macrospora*** (S. Ito & S. Imai) S. Ito, Mycol. Fl. Japan, Basidiomycetes 2 (5): 75 (1959) [Fig. 4B]
Basionym: *Hygrophorus macrosporus* S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 14 (1939).

Collection examined: SAPA10000038, holotype.

Comments. The collection was considered in "moderate" condition. Although it is slightly moldy on the fruit body surface, it contains multiple fruit bodies with relatively well-preserved hymenial tissue. The species is known only from one locality on Chichijima Island, the Ogasawara Islands, Japan (Ito and Imai, 1939).

17. ***Hygrocybe miniatostriata*** (S. Ito & S. Imai) S. Ito, Mycol. Fl. Japan, Basidiomycetes 2 (5): 85 (1959) [Fig. 4C]
Basionym: *Hygrophorus miniatostriatus* S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 15 (1939).

Collection examined: SAPA10000042, holotype.

Comments. The collection was considered in "poor" condition. It only has a highly fragmented fruit body and the hymenial tissue is barely preserved. The species is known only from one locality on Chichijima Island, the Ogasawara Islands, Japan (Ito and Imai, 1939).

18. ***Lactarius ogasawarashimensis*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 55 (1940)

Comments. Voucher collection could not be located during this study. Although the species is known only from Chichijima Island, the Ogasawara Islands, it has an ectomycorrhizal association with *Pinus liuchuensis* Mayr. (Pinaceae), which was artificially introduced by humans from Okinawa region. Because there are no known native ectomycorrhizal plants on the Ogasawara Islands, the fungal partner was most likely introduced to the Ogasawara Islands with *Pinus*, as indicated by Hosaka (2018).

19. ***Lentinus lamelliporus*** Har. & Pat., Bull. Mus. Hist. Nat., Paris 8: 131 (1902)

Comments. The collection (in 1902 from Tokyo) could not be located during this study, but the presence of type collection elsewhere has been confirmed by a previous study (Ministry of the Environment, 2015).

20. ***Lepiota boninensis*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 10 (1939) [Fig. 4D]

Collection examined: SAPA1096, holotype.

Comments. The collection was considered in "moderate" condition. Two fruit bodies are slightly fragmented, but no apparent mold contamination is visible and hymenial tissue is well-preserved. The species is known only from one locality on Chichijima Island, the Ogasawara Islands, Japan.

21. ***Lycoperdon henningsii*** Sacc. & P. Syd., Syll. Fung. (Abellini) 16: 242 (1902) [Fig. 5A–B]

Collection examined: TNS-F-24845.

Comments. The collection was considered in "moderate" condition. It has a single, minute fruit body with well-preserved peridial structure. However, it has limited availability of spores. The species is known only from Chichijima Island in Japan, but it is also reported from Java, Indonesia and Africa (Kobayasi, 1937).

22. ***Pleurotus cyatheae*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 13 (1939) [Fig. 5C]

Collections examined: Nov. 12, 1936, No. 31 (SAPA), syntype; Nov. 15, 1936, No. 31 (SAPA), syntype.

Comments. The collections were considered in "moderate" condition. It has multiple, minute fruit bodies, growing on trunks of tree ferns. Microscopic observation by Neda (2004) demonstrated that it has a dimitic hyphal system with conspicuous skeletal hyphae. The species is known from two localities on Chichijima Island,

Fig. 5. Specimens of "extinct" mushroom species in Japan (5). A–B. *Lycoperdon henningsii* (TNS-F-24845). A. Collection label indicating the substrate (*Schima mertensiana*, Theaceae) in Japanese. B. A single fruit body on *Schima mertensiana*. Bar = 0.5 cm. C. *Pleurotus cyatheae* (No. 31, SAPA, Syntype). Bar = 1 cm. D. *Pluteus daidoi* (No. 66, SAPA, Holotype). Bar = 1 cm. E. *Pluteus horridilamellus* (No. 56, SAPA, Holotype). Bar = 1 cm. F. *Psathyrella boninensis* (SAPA10000046, Holotype). Bar = 1 cm.

the Ogasawara Islands, Japan (Ito and Imai, 1939).

23. ***Pluteus daidoi*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 47 (1940) [Fig. 5D]

Collection examined: No. 66 (SAPA), holotype.

Comments. The collection was considered in "moderate" condition. A single fruit body has a moldy appearance on pileus surface, but the overall shape is well-preserved. However, an extensive microscopic study on type material by

Kobayashi (2002) demonstrated that some important structures, such as basidia and cheilocystidia, were not preserved. The original description indicates it grows on the ground (Ito and Imai, 1940), but this feature is unusual for the genus. The species is known only from one locality on Hahajima Island, the Ogasawara Islands (Ito and Imai, 1940).

24. ***Pluteus horridilamellus*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 46 (1940) [Fig. 5E]

Collection examined: No. 56 (SAPA), holotype.

Comments. The collection was considered in "moderate" condition. A single fruit body has a slightly moldy appearance on the surface, but the overall shape is well-preserved. Microscopic observation by Kobayashi (2002) indicated that most of the key microscopic features, including basidiospores, basidia and various types of cystidia, were well-preserved. The species is known only from one locality on Hahajima Island, the Ogasawara Islands, Japan (Ito and Imai, 1940).

25. ***Psathyrella boninensis*** (S. Ito & S. Imai) S. Ito, Mycol. Fl. Japan 2 (5): 307 (1959) [Fig. 5F]

Basionym: *Hypholoma boninense* S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 52 (1940).

Collection examined: SAPA10000046, holotype.

Comments. The collection was considered in "poor" condition. It is heavily contaminated by mold on the hymenial tissue. The species is known only from one locality on Hahajima Island, the Ogasawara Islands, Japan.

26. ***Russula boninensis*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 56 (1940)

Comments. Voucher collection could not be located during this study. Although this species is known only from Chichijima Island, the Ogasawara Islands, it has an ectomycorrhizal association with *Pinus liuchuensis*, which was artificially introduced by humans from Okinawa region. Because there are not known native ectomycorrhizal plants on the Ogasawara Islands, the fungal partner was most likely introduced to the Ogasawara Islands with *Pinus liuchuensis*, as indicated by Hosaka (2018).

Discussion

Our herbarium survey found a total of 21 out of 26 collections of "extinct" mushroom species from Japan. Although the collections of the remaining five species are still missing, it is still possible that we could retrieve them either from other major fungal herbaria in Japan (e.g., TMI and TFM) or overseas herbaria.

The majority of these "extinct" species are known only from the Ogasawara (Bonin) Islands. However, the missing species mentioned above are not the case. For example, *Chlorophyllum agaricoides* has been recorded only from Hokkaido in Japan, but it is also known from North America, Europe, Australia, Mongolia and China (Dörfelt and Gube, 2007; Imai, 1936; Ministry of the Environment, 2015). Likewise, *Ganoderma colossus* is only known from Miyazaki Prefecture in Japan, but has a pantropical distribution (Ministry of the Environment, 2015). The biological status of such missing species in Japan remains unclear, but their worldwide distribution indicates they have not gone extinct across their range.

The two remaining species, i.e., *Lactarius ogasawarashimensis* and *Russula boninensis*, have been recorded only from the Ogasawara Islands, and their voucher collections were not found during this study. However, they have an ectomycorrhizal association with introduced pine species (*Pinus liuchuensis* from Okinawa), and their original distribution is assumed to be in Okinawa region (Hosaka, 2018). They have not been recorded from Okinawa yet, but continuous field survey and taxonomic study may solve this issue.

The collections retrieved during this study showed various levels of quality in physical appearance. Some specimens are heavily con-

taminated by mold (e.g., Fig. 1A, 3C) or almost completely fragmented (Fig. 4C). Sometimes, the specimen with a reasonable appearance (e.g., Fig. 5D) was demonstrated to be in poor quality in terms of microscopic features (Kobayashi, 2002). Regardless, a care must be taken to avoid further degradation of voucher specimens.

Currently, an initial attempt is being made to extract, PCR amplify and sequence the DNA from voucher collections of "extinct" mushroom species. However, for dealing with the 50-year-old or older collections, we are facing a lot of challenges. In general, DNA degrades and becomes fragmented with time (Willerslev and Cooper, 2005). In addition, many herbaria in Japan, including TNS, have been fumigated with various fumigants that effectively degrade DNA molecules (Kigawa *et al.*, 2003). Previous studies clearly demonstrated that old specimens in TNS herbarium contain highly fragmented DNA (150 bp. or shorter) (Hosaka and Uno, 2011, 2013).

In addition, old collections tend to have fungal contamination (Brock *et al.*, 2008; Hosaka, 2017). We also demonstrate that old collections are heavily (e.g., Fig. 1A, 3C) or slightly (e.g., Fig. 3F, 5D) contaminated by mold. When dealing with such collections, visual inspection of specimens by stereo microscope and PCR amplification using taxon specific primers can minimize the risk of contamination, but we always need to recognize that old specimens are always contaminated by other (micro-) organisms to a certain extent.

It is, however, important to recognize that even the specimens with "good" appearance do not necessarily contain high quality DNA. For example, Hosaka (2017) demonstrated that specimens with no obvious mold growth often produced highly contaminated DNA. Similarly, plant specimens of good appearance (greener, for example) often produce the DNA of inferior quality (Erkens *et al.*, 2008). Therefore, specimens categorized as "poor" in this study (Table 1) may produce higher quality DNA than "good" specimens (and vice versa).

A continuous field survey may re-discover some "extinct" mushroom species, as is the case of *Cyathus badius* (Cruz *et al.*, 2018). However, most of such "extinct" species may only rarely fruit in nature. Finding such rarely fruiting species is, therefore, challenging. Because most of such species have been collected only once, mostly more than 70 years ago, only limited information is available to solve some taxonomic issues. Further attempts should be made to obtain "authentic" sequence data from most, if not all, type specimens. However, if no such attempts are successful or type specimens are apparently absent, epitypification from more recently collected specimens (if available) accompanied with good sequence data should be conducted, as seen in the examples by Hyde and Zhang (2008), Alfredo *et al.* (2015), and Cruz *et al.* (2018).

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