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Tolypocladium bacillisporum (Ophiocordycipitaceae): A new parasite of *Elaphomyces* from Japan

国内から採集されたツチダンゴ属生の新種シロアシヒメハナヤスリタケ *Tolypocladium bacillisporum*（オフィオコルディセプス科）

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Abstract

The genus *Tolypocladium* (Ophiocordycipitaceae) includes species that are parasitic on the truffle-like ascomycete *Elaphomyces*. In addition to the 16 known species of mycoparasitic *Tolypocladium* from Japan, at least 19 undescribed species have been recorded from there. One of these species, called in Japan as "*Shiroashi-hime-hanayasuri-take*", is a parasite of the *Elaphomyces* sect. *Ceratogaster*, and has been collected from Hokkaido Pref., Japan. We found a new locality for this species in Miyagi Pref., and conducted morphological observations, isolation, and molecular phylogenetic analyses of the specimens from Japan. The part-spore shape of this species was different from that of *T. jezoense*, a macromorphologically similar species and the phylogenies strongly indicated that "*Shiroashi-hime-hanayasuri-take*" is an independent species. Accordingly, this undescribed species is herein proposed as a new species, *T. bacillisporum*.

要旨

トリポクラジウム属（オフィオコルディセプス科）には、トリュフ型の子嚢菌類であるツチダンゴ属に寄生する種が含まれる。日本国内からは 16 既知種に加え、少なくとも 19 種の未記載種が報告されている。その内の一種であるシロアシヒメハナヤスリタケは、ツチダンゴ属 *Ceratogaster* 節の一種を宿主とし、北海道から報告されている。我々は宮城県内より本種の新産地を見出し、日本産標本について形態観察、分離培養、分子系統解析を行った。その結果、本種は子実体の外観が類似するエゾハナヤスリタケとは部分胞子の形が異なり、系統学的に独立種であることが強く支持された。以上の結果に基づき、本種を新種 *Tolypocladium bacillisporum* として記載した。

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Introduction

The family Ophiocordycipitaceae (Hypocreales) described by Sung et al. (2007), includes not only numerous arthropod pathogens but also mycoparasites of the truffle-like ectomycorrhizal ascomycete *Elaphomyces* (Elaphomycetaceae, Eurotiales). Those mycoparasites were formerly known as *Elaphocordyceps* spp., but the genus was recently synonymized into *Tolypocladium* (Quandt et al., 2014). Besides mycoparasites of *Elaphomyces*, *Tolypocladium* also includes insect pathogens, soil inhabitants, and endophytes (Gazis et al., 2014; Quandt et al., 2014). Since Nikoh & Fukatsu (2000) suggested that the common ancestor of extant cicada pathogenic and mycoparasitic *Tolypocladium* spp. was likely to be a cicada pathogenic fungus, inter-kingdom host jumping within the genus has been receiving increased attention (Quandt et al., 2016, 2018).

So far, there are 24 described species of *Tolypocladium* parasitizing *Elaphomyces* (Index Fungorum [http://www.indexfungorum.org/], accessed on 10 Feb. 2022), and at least 16 known species have been reported from Japan (Kobayasi & Shimizu, 1960; Shimizu, 1997). Furthermore, Shimizu (1997) and Japanese Society of Cordyceps Research (2014) suggested that 19 taxonomically unsettled species were present in Japan. One of these *Tolypocladium* species with the Japanese name "*Shiroashi-hime-hanayasuri-take*" (from the Japanese *shiroashi* = whitish stipe, *hime* = small, and *hanayasuri-take* = *T. ophioglossoides* (J.F. Gmel.) C.A. Quandt, Kepler & Spatafora; hereafter referred to as "*Shiroashi*") was illustrated in a book (Japanese Society of Cordyceps Research, 2014). This species is parasitic on a species of sect. *Ceratogaster*, which was tentatively identified as *Elaphomyces* cf. *anthracinus* Vittad., and known only from Hokkaido Pref., Japan (Japanese Society of Cordyceps Research, 2014), but was recently also found in Miyagi Pref., Japan by the second author. Here, we critically examined the morphology and phylogenetic position of this species from Hokkaido Pref. and Miyagi Pref., and provide a taxonomic treatment.

Materials and methods

Morphological observations

Morphological observations were conducted based on fresh and freeze-dried specimens of "*Shiroashi*" following the methods of Yamamoto et al. (2020). Hand-cut sections of ascomata and ascospores discharged from perithecia were mounted in lactoglycerol and observed under a light microscope (BX40; Olympus, Tokyo, Japan). All measurements were made using PhotoRuler version 1.1.3 (http://inocybe.info/). After observation and isolation, fresh specimens were freeze-dried and oven-dried at 60°C. The specimens were deposited in the herbarium of Tochigi Prefectural Museum (TPM) and the National Museum of Nature and Science, Tokyo (TNS) in Japan under TPM-M-9859, 9860, 9861 and TNS-F-82223, respectively.

Isolation and observation of mycelial growth

Ascospores discharged from mature perithecia onto potato dextrose agar (PDA; Nissui, Tokyo, Japan) were used to obtain cultures (Uchiyama, 1999). Plates were incubated at 20°C in the dark until germination. Germinated ascospores were transferred to another plate. The established isolates, C23 from TPM-M-9859, C53 from TPM-M-9860 and 202009-1 from TPM-M-9861, were deposited in the Medical Mycology Research Center, Chiba University (Chiba, Japan) under accession numbers IFM 67757 (for C23), IFM 67758 (C53), and IFM 67759 (202009-1). Mycelial growth on PDA was measured based on Yamamoto et al. (2020).

DNA sequencing and phylogenetic analyses

DNA was extracted from cultured mycelia on PDA, as described by Izumitsu et al. (2012), or CTAB method (Ishida et al., 1999). PCR was performed using KOD FX Neo (Toyobo, Osaka, Japan) as described by Yamamoto et al. (2021) or using KOD FX (Toyobo) in a total volume of 25 μL containing 5 μL dNTPs (0.4 mM each), 0.75 μL of each primer (0.3 μM each), 12.5 μL 2× PCR buffer for KOD FX (Toyobo), 0.5 μL 1.0 U μL^{-1} KOD FX, 1.5 μL template DNA, and 4 μL sterile distilled water. For C23 and C53, amplification of (1) the small subunit (18S)-internal transcribed spacer (ITS)-large subunit (28S) of nuclear rDNA operon and (2) the translation elongation factor 1-alpha (*tef1*) was performed using the primer pairs NS1 (White et al., 1990) and LR5 (Vilgalys & Hester, 1990), and 983F and 2218R (Rehner & Buckley, 2005), respectively. For 202009-1, amplification of (3) the ITS was performed using ITS5 and ITS4 (White et al., 1990). The PCR conditions were as follows: (1) 94°C for 2 min and 40 cycles of 98°C for 10 s, 51°C for 30 s, and 68°C for 3.5 min; (2) 94°C for 2 min, 10 cycles of 98°C for 10 s, 60°C for 30 s, and 68°C for 1 min 45 s, followed by 30 cycles of 98°C for 10 s, 55°C for 30 s, and 68°C for 1 min 45 s; (3) 94°C for 5 min and 10 cycles of 98°C for 10 s, 55°C for 30 s (0.5°C decrease per cycle), and 68°C for 1 min, followed by 25 cycles of 98°C for 10 s, 50°C for 30 s, and 68°C for 1 min. The PCR products were purified using Illustra™ ExoProStar™ (GE Healthcare, Amersham, UK) or Applied Biosystems™ ExoSAP-IT™ (Thermo Fisher Scientific, Waltham, MA, USA) and sent to Macrogen Japan (Tokyo, Japan) or FASMAC (Kanagawa, Japan) for sequencing, using the same primers for PCR [except for NS1, NS5, NS6, ITS3, ITS4, ITS1 (White et al., 1990), and LR5 for (1)]. The resulting bidirectional sequences were edited using 4Peaks 1.8 (http://nucleobytes.com/4peaks) and assembled using MEGA 11 (Tamura et al., 2021). Newly generated

sequences were deposited in the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp) under LC684522–LC684526.

For phylogenetic analyses of the combined dataset of 18S, ITS, 28S and *tef1*, all *Tolypocladium* species parasitic on *Elaphomyces* for which sequences were available, i.e. *T. capitatum* (Holmsk.) C.A. Quandt, Kepler & Spatafora, *T. cucullae* Y.P. Xiao & T.C. Wen, *T. flavonigrum* Noisrip., Tasan., Khons. & Luangsa-ard, *T. fractum* (Mains) C.A. Quandt, Kepler & Spatafora, *T. guangdongense* (T.H. Li, Q.Y. Lin & B. Song) V. Papp, *T. inusitaticapitatum* F.M. Yu, Q. Zhao & K.D. Hyde, *T. japonicum* (Lloyd) C.A. Quandt, Kepler & Spatafora, *T. jezoense* (S. Imai) C.A. Quandt, Kepler & Spatafora, *T. ophioglossoides*, and *T. valliforme* (Mains) C.A. Quandt, Kepler & Spatafora, were retrieved from the International Nucleotide Sequence Databases and included. We also included several insect pathogenic *Tolypocladium* spp., i.e. *T. dujiaolongae* Y.P. Cao & C.R. Li, *T. fumosum* Ruszk.-Mich., J. Pawłowska & Wrzosek, *T. inegoense* (Kobayasi) C.A. Quandt, Kepler & Spatafora, and *T. paradoxum* (Kobayasi) C.A. Quandt, Kepler & Spatafora, which were suggested to be close to "*Shiroashi*" based on the results of National Center for Biotechnology Information nucleotide BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and an endophytic species, *T. amazonense* Gazis, Skaltsas & P. Chaverri, which is distantly related to the above-mentioned species parasitic on *Elaphomyces* and insects (Gazis et al., 2014). *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (Ophiocordycipitaceae) was selected as an outgroup. The sequences were aligned according to Yamamoto et al. (2020). Topological conflicts with significant support among the four trees (18S, 933 bp; ITS, 522 bp; 28S, 859 bp; *tef1*, 1,006 bp) were checked directly by topological comparison of the preliminary maximum likelihood (ML) trees. Next, the four datasets were combined into a single 3,320 bp dataset and deposited in TreeBASE (accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S29449). ML analysis of the combined dataset was conducted based on Yamamoto et al. (2020) using raxmlGUI 2.0 (Edler et al., 2020) under a general time reversible model of nucleotide substitution, with a discrete gamma distribution (+G) and invariant sites (+I), as selected by MEGA 11. A maximum parsimony (MP) analysis was conducted based on Yamamoto et al. (2020).

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Results and Discussion

The stromata of "*Shiroashi*" (Fig. 1A–B) were characterized by the aboveground clavate part, which was olive-colored at maturity and yellowish-green when young (Fig. 1C), and underground white rhizomorphs. This species was often found near broad-leaved trees (Fig. 1D), e.g. *Quercus* and *Carpinus* known to form ectomycorrhizal associations with *Elaphomyces* (Paz et al. 2017). The host was *Elaphomyces* cf. *anthracinus* (Fig. 1E). The perithecia were vertically immersed in the fertile head (Fig. 1F–G). Mycoparasitic *Tolypocladium* spp., such as *T. guangdongense* (Lin et al., 2008), *T. japonicum*, *T. jezoense*, *T. ophioglossoides*, *T. tenuisporum* (Mains) C.A. Quandt, Kepler & Spatafora, and *Elaphocordyceps ×jezoensoides* (Kobayasi) Hirok. Sato, S. Ban, Masuya & Hosoya are characterized by the clavate stromata (Mains 1957; Kobayasi & Shimizu, 1960). Among them, only *T. jezoense* parasitizes species of sect. *Ceratogaster* including *E. anthracinus* (Kobayasi & Shimizu, 1960), and *T. jezoense* is macromorphologically very similar to "*Shiroashi*." However, the latter formed smaller perithecia (700–720 × 200–250 μm in *T. jezoense* vs. 480–590 × 195–235 μm in "*Shiroashi*") (Kobayasi & Shimizu, 1960; Fig. 1H), shorter and narrower asci (450–500 × 13–19 μm vs. 220–370 × 8–12 μm) (Kobayasi & Shimizu, 1960; Fig. 1I–J), and ascospores with a large number of septa (15 vs. 31 or 63) (Japanese Society of Cordyceps Research, 2014; Fig. 1K). Furthermore, there was almost no overlap between the length of the part-spore of "*Shiroashi*" (3.5–12.3 μm; Fig. 1L–M) and those in all of the above-mentioned species (≥10 μm long) except *T. ophioglossoides* and *T. tenuisporum*. These two species clearly differ from "*Shiroashi*" in the length of their perithecia (≥600 μm) (Mains 1957; Kobayasi & Shimizu, 1960). The ascospores of "*Shiroashi*" germinated on PDA within 1 week and formed white colonies within a month (Fig. 1N–O).

18S-ITS-28S rDNA operon (3,395 bp and 2,853 bp) and *tef1* (952 bp and 979 bp) sequences were obtained from the isolates C23 and C53, respectively. An ITS sequence (498 bp) was obtained from the isolates 202009-1. Sequences of each locus were identical except for the ITS region: a single nucleotide deletion was present in 202009-1. The BLAST search showed that ITS sequences from Japanese specimens were most similar to sequences of *Tolypocladium* spp., but the homology was less than 96%. Fig. 2 shows the ML phylogeny of the combined rDNA and *tef1* dataset (ln L = −8869.48349). The MP statistics were as follows: tree length = 806, consistency index = 0.65, retention index = 0.697, and composite index = 0.453. The sequences from isolates of "*Shiroashi*" and the three abovementioned clavate, mycoparasitic species of *Tolypocladium* formed a well-supported clade (bootstrap support: 94/79). "*Shiroashi*" is a unique species in this clade because it has much smaller part-spores than the other closely related species.

Morphological comparisons and phylogenetic analyses strongly indicated that "*Shiroashi*" is an undescribed species. Here, we propose a new name, *T. bacillisporum* for "*Shiroashi*" and provide its taxonomic description.

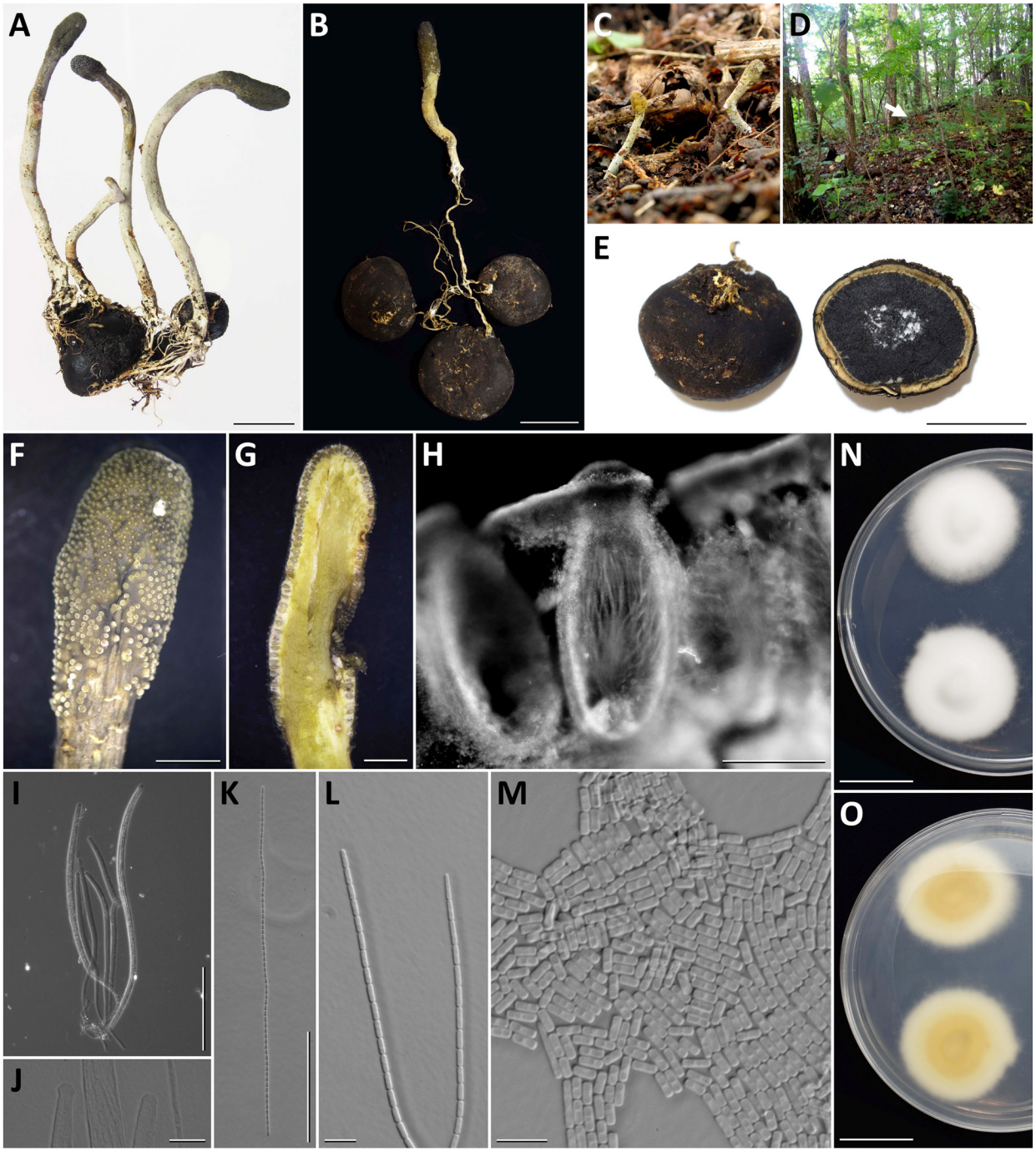

Fig. 1. *Tolypocladium bacillisporum* collected in Hokkaido Pref. (A, C–G: TPM-M-9860) and Miyagi Pref. (B, H–M: TPM-M-9861), and ex-type culture C23 (N, O). (A–B) Ascomata. (C) Young stromata in natural habitat. (D) Habitat. Arrow indicates the position of ascomata. (E) Host ascoma showing surface (left) and gleba (right). (F) Fertile head. (G) Longitudinal section of fertile head. (H) Perithecia. (I) Asci. (J) Apical cap of two asci. (K) Ascospore with 63 septa. (L) Tips of two ascospores. (M) Part-spores. (N–O) Upper (N) and reverse (O) sides of colonies on PDA after 30 d. Bars: A–B, E = 1 cm; F–G = 2 mm; H = 200 µm; I, K = 100 µm; J, L–M = 10 µm; N–O = 2 cm.

Taxonomy

***Tolypocladium bacillisporum* Koh. Yamam., Sugawa & K. Takeda, sp. nov.**

[MycoBank ID: MB 843120]

Fig. 1.

Diagnosis: This species is most similar to *T. jezoense*. The stroma is macromorphologically similar, and the hosts, *Elaphomyces anthracinus* and its relatives, are common between the species. But the part-spores are much smaller than the latter.

Etymology: From the Latin *bacilli* = bacillus and *sporum* = spore, referring to the bacilliform part-spore of this species.

Type: JAPAN, Hokkaido Pref., Tohma-cho, Kaimei-ni-ku, *ca.* 220 m above sea level, on ascomata of *E.* cf. *anthracinus* hypogeous under a secondary forest dominated by *Quercus dentata* Thunb., 20 Sep.

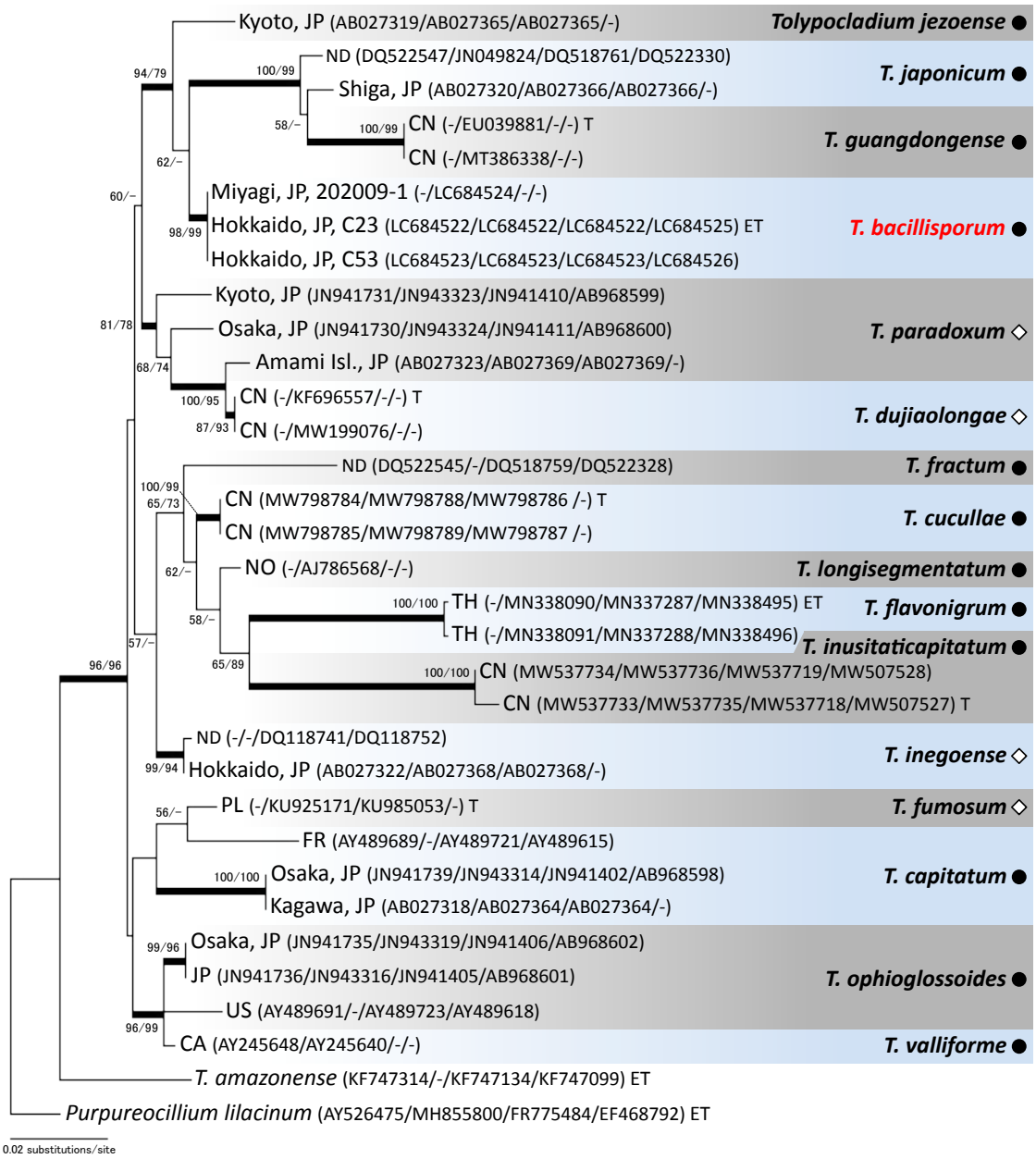

Fig. 2. Maximum likelihood (ML) phylogenetic tree of the combined dataset of 18S, ITS, 28S, and *tef1* sequences of *Tolypocladium* spp. parasitic on *Elaphomyces* (black circle) and their relatives on insects (open rhombus). *Purpureocillium lilacinum* was used as an outgroup. GenBank accession numbers for 18S, ITS, 28S, and *tef1* are shown in parentheses from left to right. Bootstrap (BS) values (1,000 replicates) ≥ 50% from ML (left) and maximum parsimony (MP) (right) analyses are shown near the nodes. Branches supported by BS values ≥ 70% from both ML and MP analyses are depicted as thick lines. CA = Canada; CN = China; FR = France; JP = Japan; ND = no data; NO = Norway; PL = Poland; TH = Thailand; US = USA. T = type specimen; ET = ex-type culture.

2010, *K. Yamamoto* (holotype, TPM-M-9859; isotype, TNS-F-82223; ex-type culture C23 [IFM 67757]).

Gene sequences from ex-type culture: LC684522 (18S), LC684522 (ITS), LC684522 (28S), LC684525 (*tef1*).

Description: Stromata arise from 1–3 hosts, often solitary, sometimes caespitose; epigeous part clavate, not branched, 30–65 mm long; underground part rhizomorphic, 8–75 mm long. Fertile head yellowish-green when young, turning olive-green as it matures, ellipsoidal to oblong, 5–16 mm long, 3–4.5 mm wide; inside yellow, solid; ostioles up to 15 per 1 mm^2. Stipe grayish-white with greenish or yellowish tinge, cylindrical, surface smooth, narrower than fertile head, 1.8–2.7 mm wide; striae sometimes present at the border of the head. Rhizomorphic part white, partially yellowish, frequently branched, fragile, up to 1 mm wide. Perithecia ovoid, vertically immersed, ostiole protuberant, 480–590 × 195–235 µm (mean 528.1 × 218.1 µm, n = 6); wall 12–17 µm thick, composed of 6–7 layers of textura angularis, cells up to 18 µm wide. Asci hyaline, cylindrical, 8-spored, 220–370 × 8–12 µm (mean 326.4 × 9.16 µm, n = 21); apical

cap prominent, 2.5–4 μm high, 5–6 μm wide (mean 3.28 × 5.1 μm, n = 18). Ascospores hyaline, filiform, slightly acute at both ends, 270–355 μm long (mean 313.7 μm, n = 20); septa often 63, rarely 31, fragmenting into part-spores; part-spores cylindrical, bacilliform, 3.5–12.3 × 1.5–2.5 μm (mean ± SD: 5.6 ± 1.63 × 1.93 ± 0.16 μm, n = 50). Colonies on PDA growing 11–13 mm within 30 d at 25°C in the dark; white, surface floccose with aerial hyphae; reverse white to pale yellow.

Distribution, habitat, and fruiting season: On hypogeous ascoma of *Elaphomyces* sect. *Ceratogaster* in broadleaved tree-dominated forests in the temperate zone of Honshu or subarctic zone of Hokkaido Pref., Japan. Found in autumn.

Other specimens examined: JAPAN, Hokkaido Pref., Tohma-cho, same locality as holotype specimen, on hypogeous ascomata of *E.* cf. *anthracinus*, 11 Sep. 2011, *K. Yamamoto* (TPM-M-9860; living culture, C53 [IFM 67758]). Miyagi Pref., Sendai-shi, Taihaku-ku, Kagitori, *ca*. 136 m above sea level, on hypogeous ascomata of *E.* cf. *anthracinus* under a *Carpinus* standing by a stream in mixed forest dominated by *Abies firma* Siebold & Zucc., 27 Sep. 2020, *G. Sugawa* (TPM-M-9861; living culture, 202009-1 [IFM 67759]).

Note: Ascoma of TPM-M-9860 and microstructures of TPM-M-9859 are illustrated as "*Shiroashi-hime-hanayasuri-take*" in the page 259 of Japanese Society of Cordyceps Research (2014).

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References

Edler D., Klein J., Antonelli A., Silvestro D. (2020) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. Methods in Ecology and Evolution 12: 373–377.

Gazis R., Skaltsas D., Chaverri P. (2014) Novel endophytic lineages of *Tolypocladium* provide new insights into the ecology and evolution of *Cordyceps*-like fungi. Mycologia 106: 1090–1105.

Ishida K., Green B.R., Cavalier-Smith T. (1999) Diversification of a chimaeric algal group, the Chlorarachniophytes: Phylogeny of nuclear and nucleomorph small-subunit rRNA genes. Molecular Biology and Evolution 16: 321–331.

Izumitsu K., Hatoh K., Sumita T., Kitade Y., Morita A., Gafur A., Ohta A., Kawai M., Yamanaka T., Neda H., Ota Y., Tanaka C. (2012) Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. Mycoscience 53: 396–401.

Japanese Society of Cordyceps Research (2014) An illustrated guide to ecology of Japanese *Cordyceps*. Seibundo Shinkosha, Tokyo. (in Japanese)

Kobayasi Y., Shimizu D. (1960) Monographic studies of *Cordyceps*. 1. Group parasitic on *Elaphomyces*. Bulletin of the National Science Museum, Tokyo 5: 69–85.

Lin Q.Y., Li T.H., Song B. (2008) *Cordyceps guangdongensis* sp. nov. from China. Mycoaxon 103: 371–376.

Mains E.B. (1957) Species of *Cordyceps* parasitic on *Elaphomyces*. Bulletin of the Torrey Botanical Club 84: 243–251.

Nikoh N., Fukatsu T. (2000) Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. Molecular Biology and Evolution 17: 629–638.

Paz A., Bellanger J.-M., Lavoise C., Molia A., Ławrynowicz M., Larsson E., Ibarguren I.O., Jeppson M., Læssøe T., Sauve M., Richard F., Moreau P.-A. (2017) The genus *Elaphomyces* (Ascomycota, Eurotiales): a ribosomal DNA-based phylogeny and revised systematics of European 'deer truffles'. Persoonia 38: 197–239.

Quandt C.A., Di Y., Elser J., Jaiswal P., Spatafora J.W. (2016) Differential expression of genes involved in host recognition, attachment, and degradation in the mycoparasite *Tolypocladium ophioglossoides*. G3 Genes Genomes Genetics 6: 731–741.

Quandt C.A., Kepler R.M., Gams W., Araújo J.P.M., Ban S., Evans H.C., Hughes D., Humber R., Hywel-Jones N., Li Z., Luangsa-ard J.J., Rehner S.A., Sanjuan T., Sato H., Shrestha B., Sung G.-H., Yao Y.-J., Zare R., Spatafora J.W. (2014) Phylogenetic-based nomenclatural proposals for Ophiocordycipitaceae (Hypocreales) with new combinations in *Tolypocladium*. IMA Fungus 5: 121–134.

Quandt C.A., Patterson W., Spatafora J.W. (2018) Harnessing the power of phylogenomics to disentangle the directionality and signatures of interkingdom host jumping in the parasitic fungal genus *Tolypocladium*. Mycologia 110: 104–117.

Rehner S.A., Buckley E. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97: 84–98.

Shimizu D. (1997) Illustrated vegetable wasps and plant worms in colour. IE-NO-HIKARI Association, Tokyo. (in Japanese)

Sung G.-H., Hywel-Jones N.L., Sung J.-M., Luangsa-ard J. J., Shrestha B., Spatafora J.W. (2007) Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Studies in Mycology 57: 5–59.

Tamura K., Stecher G., Kumar S. (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38: 3022–3027.

Uchiyama S. (1999) Methods for collection, detection and isolation: *Cordyceps*. Transactions of the Mycological Society of Japan 40: 173–179. (in Japanese)

Vilgalys R., Hester M. (1990) Rapid genetic identification and mapping

of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246.

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. Academic Press, USA, pp. 315–322.

Yamamoto K., Ohmae M., Orihara T. (2020) *Metarhizium brachyspermum* sp. nov. (Clavicipitaceae), a new species parasitic on Elateridae from Japan. Mycoscience 61: 37–42.

Yamamoto K., Endo N., Ohmae M., Orihara T. (2021) *Balsamia oblonga* (Helvellaceae), a new species from a subalpine forest in Japan. Truffology 4: 1–7.