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Full paper

Athelia termitophila sp. nov. is the teleomorph of the termite ball fungus *Fibularhizoctonia* sp.

Nitaro Maekawa [a, *], Hiroaki Yokoi [a], Kozue Sotome [a], Kenji Matsuura [b], Chihiro Tanaka [b], Naoki Endo [a], Akira Nakagiri [a], Shuji Ushijima [c]

[a] Fungus/Mushroom Resource and Research Center, Faculty of Agriculture, Tottori University, 4-101 Koyama, Tottori, 680-8553, Japan
[b] Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan
[c] The Tottori Mycological Institute, 211 Kokoge, Tottori, 689-1125, Japan

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ABSTRACT

A new species of *Athelia*, *A. termitophila*, from Japan is described and illustrated on the basis of morphological and phylogenetic analyses. Basidiomes of this species are characterized by having hyphae sometimes with clamp connections at the septa, basidia without clamp connections at the basal septa, and ellipsoid to ovoid basidiospores measuring 4.5–6 × 3–4.5 μm. In culture, mycelia produce pale brown, orange-brown to brown, globose sclerotia measuring 0.24–0.41 mm diam. The sclerotia are distinctly different in shape and size from those of other *Athelia* species, and are occasionally found inside the woody substrate beneath basidiomes. They are identical in shape and size to those of *Fibularhizoctonia* sp., also known as termite balls. Phylogenetic analysis using internal transcribed spacer (ITS) sequence data revealed that *A. termitophila* is the teleomorph of *Fibularhizoctonia* sp.
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1. Introduction

The genus *Athelia* Pers. (Atheliales, Agaricomycetes, Basidiomycota), is characterized by producing thin and pellicular (athelioid) basidiomes that are easily separable from their substrates; microscopically, it is characterized by its loosely intertwined subicular hyphae, clavate basidia, and non-amyloid basidiospores. Jülich (1972) described the macro- and micro-morphological features of 21 species of *Athelia* in his comprehensive taxonomic study. Subsequently, several new taxa were added to this genus (Jülich, 1978; Ginns, 1992; Gilbertson & Adaskaveg, 1993; Gilbertson & Hemmes, 1997). According to the Index Fungorum fungal database (http://www.indexfungorum.org/names/names.asp, accessed on Feb 28, 2019), 31 species are treated as members of this genus. Some *Athelia* species have been studied in culture, and *A. arachnoidea* (Berk.) Jülich (Stalpers, 1978), *A. epiphylla* Pers., *A. neuhoffii* (Bres.) Donk (Stalpers, 1978), *A. psychrophila* (Stalpers & R. P. de Vries) P.M. Kirk (as *Fibularhizoctonia psychrophila* Stalpers & R.P. de Vries; de Vries, de Lange, Wösten, & Stalpers, 2008) and *A. rolfsii* (Curzi) C.C. Tu & Kimbr (Goto, 1930). have been reported to produce sclerotia in their cultured mycelia. All *Athelia* species have been described on the basis of morphological studies of basidiomes and/or cultures. However, sequence data for phylogenetic study are available for only a few species.

From Japan, *A. acrospora* Jülich, *A. binucleospora* J. Erikss. & Ryvarden, *A. decipiens* (Höhn. & Litsch.) J. Erikss., *A. epiphylla*, *A. fibulata* M.P. Christ., *A. laxa* (Burt) Jülich, *A. repetobasidiifera* N. Maek., *A. sibirica* (Jülich) J. Erikss. & Ryvarden (Maekawa, 1993a; Maekawa, 1993), and *A. rolfsii* (as *Corticium centrifugum* (Lév.) Bres.; Ito, 1955) have been reported. *Athelia laxa* was transferred to the genus *Lyoathelia* by Hjortstam and Ryvarden (2004).

Matsuura, Tanaka, and Nishida (2000) reported that brown fungal balls of a similar size but different shape to termite eggs were found frequently among eggs of the subterranean termite *Reticulitermes speratus* in Japan (Fig. 1). The balls, called “termite balls”, were identified as sclerotia of a fungus in the genus *Fibularhizoctonia* G.C. Adams & Kropp, and rDNA analysis indicated an association with a teleomorph in the genus *Athelia* (Matsuura et al., 2000). Behavioral assays demonstrated that termite balls use both morphological and chemical camouflage to induce tending by subterranean termites (Matsuura, 2006). To date, termite balls have been found in the egg piles of seven termite species including

* Corresponding author.
E-mail address: kin-maek@tottori-u.ac.jp (N. Maekawa).

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R. amamianus, *R. miyatakei* and *R. kanmonensis* in Japan (Yashiro & Matsuura, 2007; Yashiro, Matsuura, & Tanaka, 2011), *R. flavipes* and *R. virginicus* in the United States (Matsuura, 2005, 2006), *R. labralis* in China (Ye et al., 2019) and *Coptotermes formosanus* in Japan (Matsuura & Yashiro, 2010). Phylogenetic analysis based on the internal transcribed spacer (ITS) sequences showed that all the termite ball fungi isolated from Rhinotermitid termites belong to the same clade of *Fibularhizoctonia* (Matsuura & Yashiro, 2010). However, the teleomorphic and anamorphic stages of the termite ball fungus have yet to be identified to the species level.

As part of a taxonomic survey of the genus *Athelia* in Japan, we collected several specimens of an unidentified fungus that produced small, globose, brown sclerotia in culture. Here, we propose this taxon as a new species of *Athelia*, identical to *Fibularhizoctonia* sp. that produces termite balls.

2. Materials and methods

2.1. Specimens and isolates examined

All of the Japanese specimens examined in this study are deposited at the Tottori University Mycological Herbarium (TUMH) and the isolates are deposited in the fungal culture collection (TUFC) of the Fungus/Mushroom Resource and Research Center, Tottori University, Tottori, Japan.

2.2. Morphological observation

Morphological descriptions were based on observations of dried specimens. For microscopic observations, sections of basidiome were mounted in Melzer's reagent and 5% (w/v) KOH. Measurements, line drawings, and photographs of microscopic elements were made using differential interference contrast (DIC) microscope (Eclipse 80i, Nikon Imaging, Tokyo, Japan) equipped with a microscope with a digital camera (DS-Fi1, Nikon Imaging) and microscope zoom drawing arm. Each morphological element was measured 25–30 times. The descriptions use the following values: D_m, mean sclerotium diameter; L_m, mean basidiospore length; W_m, mean basidiospore width; Q, L_m/W_m; and Q_m, mean Q. We observed each specimen using a stereoscopic microscope (SMZ 1500, Nikon Imaging) to evaluate whether or not it formed sclerotia around the basidiomes and inside the substrate.

Fig. 1. Termite balls in the egg pile of the termite *Reticulitermes flavipes*. Termite eggs are transparent and oval, whereas termite balls are brown and spherical.

2.3. Culture studies

Polysporous isolates TUFC 14530, 14531, 34057, and 34079 were obtained from specimens TUMH 40433, 40434, 61446, and 61447, respectively. Macro- and micro-morphological characteristics of the cultures were observed on 1.25% malt extract agar (MA; Nobles, 1965). For comparison of shape and size of sclerotia produced in culture, we examined three isolates of *Fibularhizoctonia* sp., isolated from termite balls: TUFC 14546 (= KA110728A), TUFC 14548 (= TA110510A), and TUFC 14550 (= TU100904A).

2.4. DNA preparation, PCR, and sequencing

DNA was extracted from cultured mycelia using a Maxwell 16 Tissue DNA Purification Kit (Promega, Madison, WI, USA) with automatic DNA extraction by a Maxwell 16 Instrument (Promega). The complete sequence of ITS region and a partial sequence of the large subunit (LSU) region were amplified and sequenced with primers ITS4/ITS5 for ITS (White, Bruns, Lee, & Taylor, 1990) and LR0R/LR5 for LSU (Hopple & Vilgalys, 1994). PCR for ITS and LSU amplification was carried out in 25-μL reaction mixtures containing 12.5 μL of GoTaq Master Mix (Promega), 0.5 μM each primer, distilled water, and 1 μL of DNA extract. The PCR amplification protocol was as follows: initial denaturation at 95 °C for 2 min; 35 cycles at 94 °C for 45 s, 50 °C for 45 s, and 70 °C for 1 min; and final elongation at 72 °C for 10 min in a thermal cycler (PC818, Astec Co., Ltd., Fukuoka, Japan). LSU region amplification products were cloned by the TA cloning technique in a pGEM-T Easy Vector System (Promega) with ECOS competent *Escherichia coli* JM109 cells (Nippon Gene Co., Ltd., Tokyo, Japan). The DNA of at least five transformed clones from each sample was amplified as described above. PCR products were purified with an ExoSap-IT (GE Healthcare, Tokyo, Japan) or MonoFas DNA purification kit (GL Sciences, Tokyo, Japan) and sequenced by a DNA sequencing service (Fasmac, Atsugi, or Eurofins Genomics, Tokyo, Japan).

2.5. Sequence alignment and phylogenetic analyses

The ITS sequences were aligned in MAFFT v. 7 software by the L-INS-i strategy (Katoh & Standley, 2013). The trailing ends were trimmed and the alignments were manually edited in BioEdit v. 7.2.6.1 software (Hall, 1999). Maximum parsimony (MP) analyses were performed in PAUP* 4.0a166 software (Swofford, 2003), with gaps treated as missing data. The most parsimonious trees were identified using heuristic searches with 1000 random-addition sequence replicates, *maxtrees* set to auto increase, and tree bisection and reconnection branch swapping. Maximum likelihood (ML) trees were drawn in MEGA 7 software (Kumar, Stecher, & Tamura, 2016). The best-fit substitution model for ML analysis was estimated in MEGA 7 (Kumar et al., 2016). Statistical support for the ML and MP trees was assessed with bootstrap values for maximum likelihood analysis (MLBS) and maximum parsimony (MPBS), respectively, from 1000 replicates (Felsenstein, 1985). The alignment datasets and trees have been deposited in TreeBase under accession number S25656. *Anomoporia bombycina* (Fr.) Pouzar and *Amylocorticium subsulphureum* (P. Karst.) Pouzar (Amylocorticiales) were selected as outgroups because they are outside the Atheliales (Binder, Larsson, Matheny, & Hibbett, 2010; Hibbett et al., 2014). These DNA sequences newly determined were deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (DDBJ).

3. Results

3.1. Taxonomy

Athelia termitophila N. Maek., Yokoi & Sotome, sp. nov. Figs. 2 and 3.

MycoBank no.: MB 836440.

Diagnosis: Basidiomes pellicular, thin; hymenium smooth, surface white to pale cream to grayish white. Hyphae 2.5–5.5 μm diam, simple-septate, but occasionally with clamp connections at the septa. Basidia clavate, 10.5–17.5 × 4–6.5 μm, 4-sterigmate, simple-septate at the base. Basidiospores ellipsoid to ovoid, 4.5–6 × 3–4.5 μm. Sclerotia globose, initially white, and then becoming pale brown, orange-brown to brown in maturity, 0.24–0.41 mm diam, produced in culture and sometimes inside substrate.

Holotype: JAPAN, Tottori Pref., Tottori City, Katsurami, on dead branch of a broad-leaved tree, collected by H. Yokoi on Apr 13, 2011, TUMH 40433.

Gene sequence ex-holotype: LC516620 (ITS), LC516626 (LSU)

Etymology: *termitophila*, referring to the association with termites.

Basidiomes resupinate, loosely adnate, effused, thin, athelioid, fragile; hymenial surface white, grayish white to pale cream, in the herbarium turning to pale ochre to ochre, smooth, reticulate or continuous, sometimes cracked when dried; margin white to grayish white when fresh, indeterminate. In vertical section subhyaline, pellicular (athelioid). Hyphal system monomitic; hyphae 2.5–5.5 μm diam (D_m = 3.8 μm), smooth, thin-to slightly thick-walled (up to 0.5 μm), simple-septate, but sometimes with a clamp connection at the septum in the subiculum, partly encrusted with crystal-like materials. Leptocystidia rarely present at the hymenium, obclavate to lageniform, 15–26 × 3–4 μm (L_m = 19.0 μm, W_m = 3.7 μm), thin-walled, without a basal clamp. Basidia clavate, 10.5–17.5 × 4–6.5 μm (L_m = 14.2 μm, W_m = 5.4 μm), thin-walled, simple-septate at the base, producing 4 sterigmata. Basidiospores ellipsoid to ovoid, 4.5–6 × 3–4.5 μm (L_m = 5.4 μm, W_m = 3.4 μm, Q = 1.2–2, Q_m = 1.6), smooth, thin-walled, non-amyloid.

Specimens examined: TMI 6747 on shiitake bedlog of *Quercus serrata* Thunb. ex Murray, Kokoge, Tottori City, Tottori Pref., Apr 27, 1981; TMI 7568 on dead branch of *Quercus* sp., Yazu-cho, Yazu-gun, Tottori Pref., May 8, 1982; TUMH 40428 on dead bark of *Pinus densiflora* Sieb. & Zucc., Kokoge, Tottori City, Tottori Pref., Oct 30, 1982; TUMH 40429 on shiitake bedlog of *Q. serrata*, Kokoge, Tottori City, Tottori Pref., Mar 2, 1983; TUMH 40430 on shiitake bedlog of *Q. serrata*, Tottori City, Tottori Pref., Mar 13, 1986; TUMH 40431 on shiitake bedlog of *Q. serrata*, Niida, Sukagawa City, Fukushima Pref., Nov 2, 1986; TUMH 40432 on shiitake bedlog of *Q. serrata*, Nishikimura, Senpoku-gun, Akita Pref., Nov 6, 1986; TUMH 61445, TUMH 61446, and TUMH 61447 on dead wood of broad-leaved trees, Mt. Chokaisan, Nikaho City, Akita Pref., May 19, 1999; TUMH 40433 (holotype) and TUMH 40434 on dead wood of a broad-leaved tree, Katsurami, Tottori City, Tottori Pref., May 19, 2011, collected by H. Yokoi.

3.2. Sclerotia formed in substrates of basidiomes

Sclerotia were not observed around basidiomes in any of the 12 specimens examined in this study, but were observed inside the substrate directly beneath basidiomes of 3 specimens (TUMH 40431, 40432, and 61445). The sclerotia were detected in gaps where *Lentinula edodes* (Berk.) Pegler had decomposed the substrate in TUMH 40431 and 40432, and in insect galleries in the substrate of TUMH 61445 (Fig. 2C and D). The sclerotia were pale brown to brown and globose, measuring 0.22–0.32 mm diam (D_m = 0.28 mm).

3.3. Characteristics in culture

3.3.1. Athelia termitophila

The optimum growth temperature for the polysporous isolates TUFC 14530, 14531, 34057, and 34079 was 20 °C–25 °C. Isolates could grow between 5 °C and 30 °C, but no visible growth was observed at 35 °C or 40 °C. Growth on MA was 18–23 mm in radius

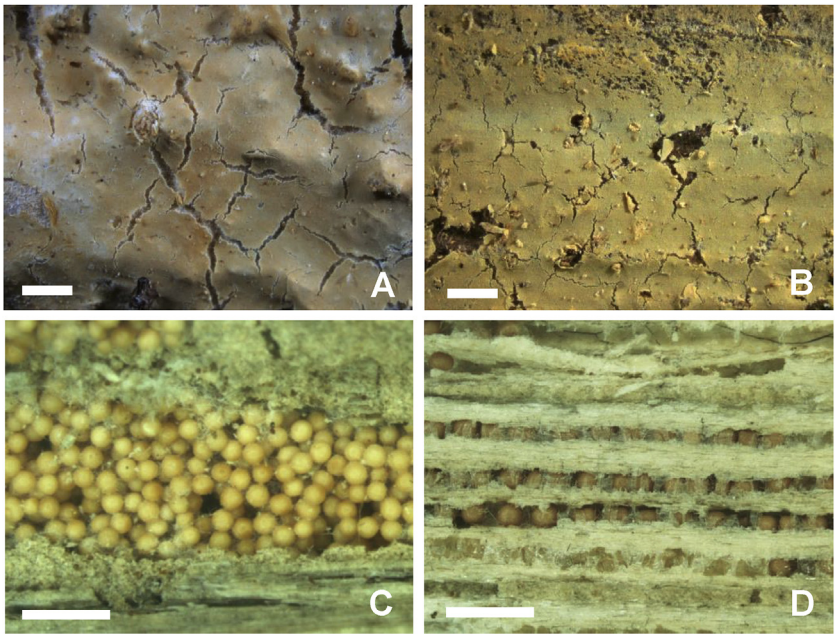

Fig. 2. *Athelia termitophila*. A, B: Basidiomes (A, TUMH 40433, holotype; B, TUMH 40431). C: Sclerotia formed inside an unknown insect gallery of the substrate directly beneath basidiome (TUMH 61445). D: Sclerotia formed inside vessels of substrate directly beneath basidiome (TUMH 40431). *Bars*: A, B 1 cm; C, D 1 mm.

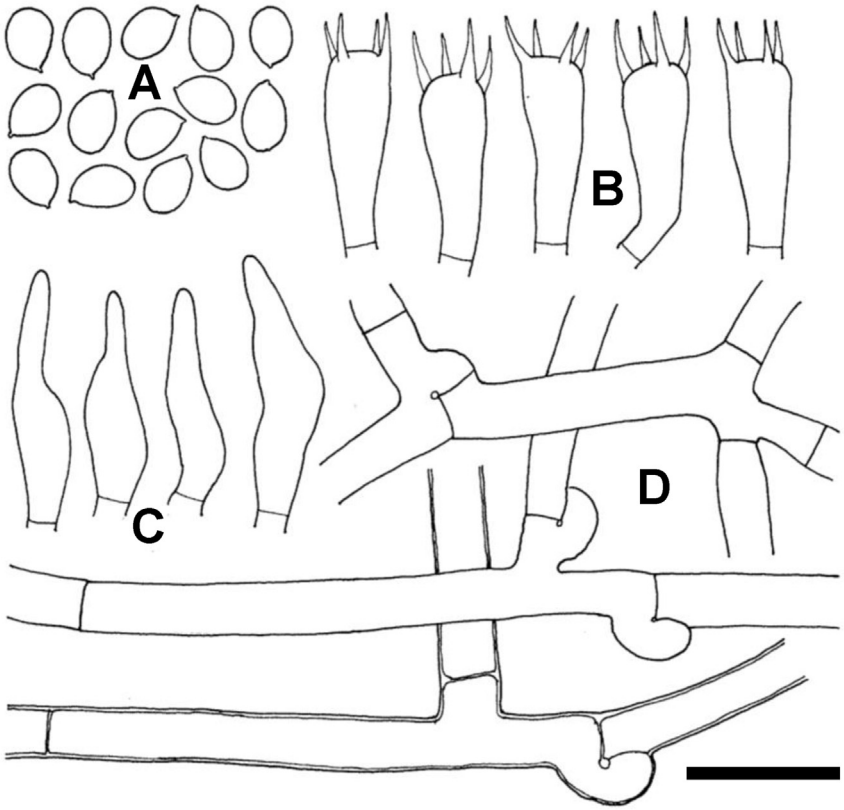

Fig. 3. Micro-morphological features of *Athelia termitophila* (TUMH 40433, holotype). A: Basidiospores. B: Basidia. C: Leptocystidia. D: Hyphae. *Bar*: 10 μm.

at 25 °C after 9 d in the dark. Mycelial mats at that time were white, thin, downy, partly cottony, wooly, occasionally plumose (Fig. 4A); reverse side of mycelial mats not discolored; agar not bleached; odor not noticeable. Aerial hyphae 1.5–5.0 μm diam, subhyaline, thin-walled, sometimes nodose-septate, sparsely encrusted, moderately branched (Fig. 4G). Marginal hyphae 1.5–4.5 μm diam, subhyaline, thin-walled, simple-septate, moderately branched (Fig. 4I). Submerged hyphae dense, 1.5–5.5 μm diam, subhyaline, thin-walled, sometimes with a clamp connection at the septum, partly encrusted (Fig. 4H). Conidia and cystidium-like cells not observed. After 6 wk of incubation at 25 °C, all isolates produced solitary, sometimes gregarious sclerotia in their mycelia (Fig. 4C). Sclerotia globose, initially white, becoming pale brown, orange-brown to brown in maturity, 0.24–0.41 mm diam ($D_m = 0.32$ mm, $Q = 1–1.25$, $Q_m = 1.07$), smooth; inner tissue white, uniform, pseudoparenchymatous, consisting of thin-to thick-walled hyphae with granular contents (Fig. 4B, D, E). Mature sclerotia were produced between 15 °C and 30 °C, but mature sclerotia were not observed at 5 °C, 10 °C, 35 °C, or 40 °C after 6 wk of incubation. Sclerotia germinated between 10 °C and 30 °C on MA.

Species codes (Stalpers, 1978): 8, (12), 17, (21), (22), 30, (39), 44, 52, 53, (54), (57), 66, (82).

3.3.2. Fibularhizoctonia *sp.*

The optimum growth temperature for the polysporous isolates TUFC 14546, 14548, and 14550 was 20 °C–25 °C. These isolates could grow at between 5 °C and 30 °C, but no visible growth was observed at 35 °C. Growth on MA was 15–22 mm in radius at 25 °C after 9 d in the dark. Mycelial mats at that time were white, thin, downy, partly cottony, wooly, occasionally plumose (Fig. 4F); reverse side of mycelial mats not discolored; agar not bleached; odor not noticeable. Aerial hyphae 1.5–5 μm diam, subhyaline, thin-walled, sometimes nodose-septate, sparsely encrusted, moderately branched. Marginal hyphae 1.5–4 μm diam, subhyaline, thin-walled, simple-septate, moderately branched. Submerged hyphae dense, 2–5 μm diam, subhyaline, thin-walled, sometimes with a clamp connection at the septum, partly encrusted. Conidia and cystidium-like cells not observed. After 2 wk of incubation at 25 °C, all isolates produced solitary, sometimes gregarious sclerotia in their mycelia. Sclerotia globose, initially white, becoming orange-brown to brown in maturity, 0.27–0.38 mm diam ($D_m = 0.32$ mm, $Q = 1–1.09$, $Q_m = 1.02$), smooth; inner tissue white, uniform, pseudoparenchymatous, consisting of thin-to thick-walled hyphae with granular contents. Mature sclerotia were produced between 15 °C and 30 °C.

Species codes (Stalpers, 1978): 8, (12), 17, (21), (22), 30, (39), 44, 52, 53, (57), 66, (82).

3.4. Phylogenetic analyses

In total, 14 sequences of the ITS and LSU regions were generated and submitted to the DDBJ database (Table 1; accession nos. LC516617–LC516630). Additionally, 12 sequences of the ITS region were obtained from publicly accessible databases (Table 1) and used in constructing the alignment datasets. The complete ITS dataset included 609 sites with 138 parsimony-informative characters. The MP analysis of the ITS dataset generated 24 equally parsimonious trees of 414 steps ($CI = 0.40$, $RI = 0.76$). The ML tree based on the ITS dataset generated using the Tamura 3-parameter model with a gamma distribution (T92+G) was selected as the best-fit model. ML analysis of the ITS dataset resulted in a best-scoring tree with a likelihood of $\ln L = -2414.29$. The MP and ML trees showed no inconsistency in any supported clades (bootstrap values $\geq$ 50%) (Fig. 5).

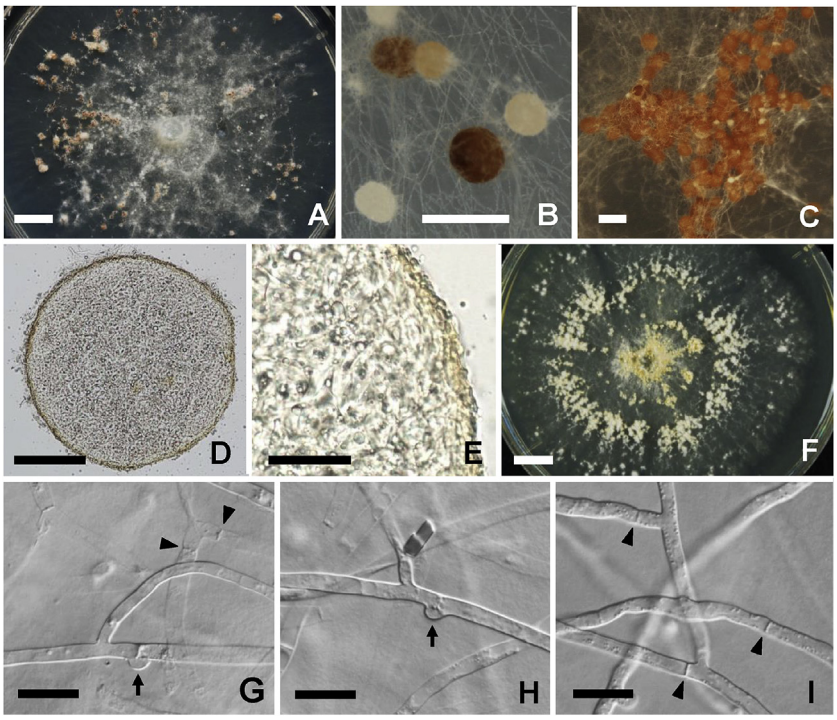

Fig. 4. *Athelia termitophila* (A–E, G–I: TUFC 14531, from TUMH 40434) and *Fibularhizoctonia* sp. (F: TUFC 14548) on 1.5% MA medium after 2 wk of incubation at 25 °C. A: Mycelial colony. B: Mature and immature sclerotia. C: Gregarious sclerotia. D: Cross section of a sclerotium. E: Partially magnified view of the sclerotium in D. F: Colony of *Fibularhizoctonia* sp. G: Aerial hyphae showing simple septa (arrowheads) and a clamped septum (arrow). H: Submerged hyphae showing a clamped septum (arrow). I: Marginal hyphae showing simple septa (arrowheads). *Bars*: A–C, F 1 cm; D, E 1 mm; G–I 10 μm.

In all phylogenetic trees, *A. termitophila* and *Fibularhizoctonia* sp. formed a distinct clade with strong support (MPBS/MLBS = 96/91). Moreover, this clade in combination with a moderately supported clade including four *Athelia* species (MPBS/MLBS = 51/74) formed a single strongly supported clade, labeled the "Athelia clade" (MPBS/MLBS = 100/99).

4. Discussion

Athelia termitophila is morphologically characterized by resupinate, loosely adnate, effused, thin basidiomes, simple-septate hyphae sometimes with a clamp connection at the septum in the subiculum, and ellipsoid to ovoid basidiospores measuring 4.5–6 × 3–4.5 μm. The obclavate to lageniform leptocystidia were

Table 1
Vouchers and GenBank accession numbers of the species used in molecular phylogenetic analyses.

Species	Strain nos. (Specimen nos.)	Locality	DDBJ/ENA/GenBank accession nos.	
			ITS	LSU
Amylocorticium subsulphureum	CFMR: HHB-13817	USA, Alaska	GU187506	–
Anomoporia bombycina	CFMR: L-6240	USA, Colorado	GU187508	–
Athelia acrospora	(UC2022957)	USA, Oregon	KP814332	–
Athelia arachnoidea	CBS: 418.72	Netherlands	GU187504	–
Athelia decipiens	FCUG 1762	Unknown	U85797	–
Athelia decipiens	TUFC 14532	Japan, Tottori	LC516617[b]	–
Athelia epiphylla	CBS 117.71; CBS 419.72	Europe	U85793	–
Athelia epiphylla	TUFC 33567	Japan, Nagano	LC516618[b]	–
Athelia epiphylla	TUFC 33568	Japan, Nagano	LC516619[b]	–
Athelia termitophila	TUFC 14530[a]	Japan, Tottori	LC516620[b]	LC516626[a]
Athelia termitophila	TUFC 31133	Japan, Tottori	LC516621[b]	LC516627[b]
Athelia termitophila	TUFC 34057	Japan, Akita	LC516622[b]	LC516628[b] LC516629[b]
Athelia termitophila	TUFC 34079	Japan, Akita	LC516623[b]	LC516630[b]
Fibulorhizoctonia sp.	LA082103L	USA, Louisiana	AY854062	–
Fibulorhizoctonia sp.	A1RF	USA, Massachusetts	DQ276871	–
Fibulorhizoctonia sp.	Akita1	Japan, Akita	DQ493749	–
Fibulorhizoctonia sp.	Amami 1	Japan, Kagoshima, Amami Is.	DQ493750	–
Fibulorhizoctonia sp.	TUFC 14549; TA110510B	Japan, Okayama	LC516624[b]	–
Fibulorhizoctonia sp.	TUFC 14550; TU100904A	Japan, Ibaraki	LC516625[b]	–
Leptosporomyces raunkiaeri	CFMR: HHB-7628	USA, Michigan	GU187528	–
Piloderma fallax	CFMR: S-12	Finland	GU187535	–

[a] Ex-type strains.
[b] Newly generated sequences.

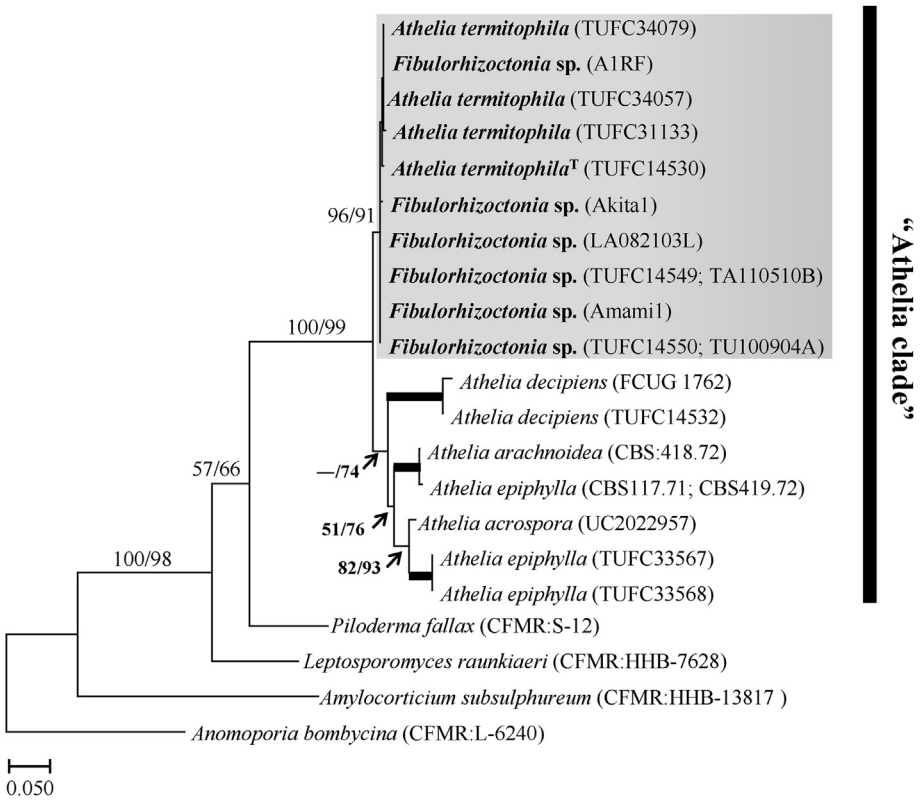

Fig. 5. Maximum likelihood tree generated using Tamura 3-parameter model together with gamma distribution (T92+G) for ITS dataset. Bootstrap values for maximum parsimony (MPBS) and maximum likelihood analysis (MLBS) greater than 50% are indicated above the branches. Bold branches show where nodes supported with 100% MPBS and MLBS. ^{T} = holotype.

easily observed at the hymenium in the holotype (TUMH 40433), but were difficult to detect in the other specimens (TUMH 61445, 61446, and 61447). Thus, the leptocystidia cannot be used as a stable characteristic for this species because their presence is widely variable between specimens. *Athelia termitophila* is similar to *A. decipiens* in the shape and size of basidiospores, but differs in that it sometimes produces clamp connections in the subicular hyphae whereas those of *A. decipiens* are completely simple-septate (Table 2). *Athelia nivea* Jülich and *A. salicum* Pers. resemble *A. termitophila* in basidial and hyphal features, but basidiospores of the former two species are distinctly larger than those of *A. termitophila*. *Athelia rolfsii* is also similar to *A. termitophila* in basidiospore and hyphal features, but *A. rolfsii* sometimes produces clamp connections at the bases of basidia and is parasitic on various seed plants (Tu & Kimbrough, 1978). In contrast, *A. termitophila* lacks clamp connections at the basal septa of basidia and is saprophytic on woody substrates. Furthermore, ITS sequence data showed that *A. termitophila* is phylogenetically distinct from *A. decipiens* and *A. epiphylla* (Fig. 4). In cultured mycelia, *A. termitophila* produced brown, orange-brown to brown, globose, small sclerotia. Production of sclerotia by cultured mycelia has been reported in *A. arachnoidea* (Stalpers, 1978), *A. epiphylla* (Stalpers, 1978), *A. neuhoffii* (Stalpers, 1978), *A. psychrophila* (as *F. psychrophila*; de Vries et al., 2008), and *A. rolfsii* (Goto, 1930), but these sclerotia differ from those of *A. termitophila* in shape, size, or both (Table 3). Sclerotia of *A. termitophila* are globose, measuring 0.24–0.41 μm diam, whereas those of the former five species are irregular-shaped or nearly globose and distinctly larger. Thus, *A. termitophila* can be distinguished morphologically and phylogenetically from other taxa in the genus.

Matsuura et al. (2000) described the termite balls produced by *Fibularhizoctonia* sp. as spherical or nearly spherical (0.33–0.34 mm diam) and ranging from light brown to brown on potato dextrose agar. Here, we observed that *Fibularhizoctonia* sp. grown on MA developed similar colonies (Fig. 4F) and produced sclerotia of the same shape and size (0.27–0.38 mm diam). Its sclerotia were identical to those produced by *A. termitophila* in culture, i.e., globose, measuring 0.24–0.41 mm diam (D_m = 0.32 mm), orange-brown to brown in maturity. Furthermore, the sclerotia of *A. termitophila* were identical to those of *Fibularhizoctonia* sp. in the structure of their inner tissue. Also in the other characteristics in culture, *A. termitephila* is much similar to *Fibularhizoctonia* sp.

All of the specimens of *A. termitophila* examined in this study were collected from Honshu Island (Akita, Fukushima, and Tottori prefectures) in spring (Mar–May) and autumn (Oct–Nov). The average monthly temperature at these collection sites was 3.9 °C–17.5 °C when the specimens were collected. On the other hand, optimum temperature for mycelial growth of the isolates obtained from the basidiomes is 20 °C–25 °C on MA. These results indicate that the temperature suitable for basidiome formation in this species is lower than the optimum temperature for mycelial growth, like some other basidiomycetous species, e.g., *Agaricus bisporus* (J.E. Lange) Imbach, *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo (as *Coprinus cinereus* (Schaeff.) Gray), *Flammulina velutipes* (Curtis) Singer, *Pholiota nameko* (T. Itô) S. Ito & S. Imai, and *Pleurotus ostreatus* (Jacq.) P. Kumm. (Chang & Hayes, 1978; Flegg & Wood, 1985; Kües & Liu, 2000).

Matsuura (2005) found termite balls in piles of *R. speratus* eggs from Honshu, Shikoku, and Kyushu islands from Jun to Oct in 1998 and 1999. He suggested that the termite ball-producing fungus (*Fibularhizoctonia* sp.) might have another life stage independent of termites, because it never produces spores in the laboratory and

Table 2
Microscopic comparison of *Athelia termitophila* with the related species.

Species	Basidiospores		Clamp connection		Reference
	Shape	Size (μm)	Hyphal septa	Basal septa of basidia	
A. termitophila	Ellipsoid to ovoid	4.5–6 × 3–4.5	Sometimes present	Absent	This study
A. bombacina	Ellipsoid	4.5–6 × 2.5–3.5	Always present	Always present	Jülich (1972)
A. decipiens	Ellipsoid to ovoid	4–5.5 × 2.5–3.5	Absent	Absent	Jülich (1972)
A. epiphylla	Cylindrical	(5.5–)6–7.5 (–8) × 2.8–3.2	Sometimes present	Absent	Jülich (1972)
A. nivea	Subcylindrical to ellipsoid	6.5–8 × 4.3–5.2	Mostly present	Absent	Jülich (1972)
A. rolfsii	Globose to pyriform	4.5–6.5 × 3.5–4.5	Sometimes present	Sometimes present	Tu and Kimbrough (1978)
A. salicum	Ellipsoid	(5.5–)6–7.5 (–8) × 3.5–4.5	Sometimes present	Absent	Jülich (1972)

Table 3
Comparison of sclerotia produced in culture between *Athelia termitophila* and other *Athelia* species.

Species	Sclerotia in culture		Reference
	Shape	Diameter (mm)	
A. termitophila	Spherical	0.24–0.41	This study
A. arachnoidea	Irregular	1–2	Stalpers (1978)
A. epiphylla	Irregular	1–2	Stalpers (1978)
A. neuhoffii	Irregular	≤1	Stalpers (1978)
A. psychrophila	Semiglobose	≤5	de Vries et al. (2008)
A. rolfsii	Irregular	0.6–1.5	Goto (1930)
Fibularhizoctonia sp.	Spherical	0.27–0.38	This study

termite balls have never been found in rotted wood that has not been colonized by termites in the field (Matsuura, 2005). Also in the present study, we recognized that *A. termitephila* and *Fibularhizoctionia* sp. did not produce any anamorphic spores in culture of all the isolates examined. *Athelia termitophila*, here shown to be the teleomorphic stage of *Fibularhizoctonia* sp., has also been found on Honshu. Furthermore, we found sclerotia inside the substrate directly beneath the basidiomes of 3 of the 12 specimens we examined. These results indicate that the distributions of *A. termitophila* and *Fibularhizoctonia* sp. overlap. In addition, the termite balls might originate from basidiomes of *A. termitophila* on the substrate or from the sclerotia inside the substrate (wood).

Basidiomes of *A. termitophila* have been observed in the field in seasons with lower temperatures (3.9 °C–17.5 °C), whereas sclerotia were produced at 15 °C–30 °C on MA. Thus, the sclerotium production of *A. termitophila* are maintained even at a temperature range higher than that for basidiome formation. Generally, sclerotia help fungi to survive challenging conditions such as high temperature, freezing, desiccation, microbial attack, or the absence of a host (Rotem & Aust, 1991; Smith, Henkel, & Rollins, 2014). We consider that sclerotia produced inside the substrate play an important role in the survival strategy of *A. termitophila*, during relatively high temperature season (summer season) when the fungus is not able to form basidiomes. Furthermore, we speculate that the low rate (25%) of formation of sclerotia associated with basidiomes in the field is due to the difference in the temperatures suitable for formation of sclerotia and formation of basidiomes. To clarify the relationship between *A. termitophila* and termites, it is necessary to investigate the formation of basidiomes and sclerotia in the life cycle of *A. termitophila* throughout the year in the field.

Disclosure

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

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