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Taxonomy of an anamorphic xylariaceous fungus from a termite nest found together with *Xylaria angulosa*

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Abstract Two xylariaceous fungi were isolated from a nest of a termite, *Odontotermes formosanus*, that was incubated in a laboratory after collecting from Iriomote Is., Okinawa Pref., in Japan. One of the two fungi was identified as *Xylaria angulosa* on the basis of the morphology of branched stroma produced on medium, tiny asci, and ascospores having a germ slit. Another fungus is an anamorphic fungus that produces synnemata up to 50mm long from which dendritic conidiophores branch out. Unicellular conidia are holoblastically produced on a sympodially proliferating conidiogenous cell. Such morphological characters resemble those of the genus *Geniculosporium*. However, its distinctive synnema formation and dendritic conidiophores do not assign the fungus to *Geniculosporium* or other known genera and warrant establishment of a new genus. The phylogenetic tree based on the ITS regions of rDNA shows that the fungus is nested in the cluster of the genus *Nemania* (Xylariaceae), whose species have mainly *Geniculosporium*-like anamorphs. We describe here the present anamorphic fungus as *Geniculisynnema termiticola* gen. et sp. nov., and discuss its phylogenetic and ecological relationships to xylariaceous fungi, especially termiticolous species.

Key words *Geniculisynnema termiticola* · New genus · Taxonomy · Termite · Xylariaceae

Introduction

Xylariaceous fungi are known mainly as decomposers of angiosperms, and several species have been reported to be plant pathogens (Petrini and Petrini 1985). Some xylariaceous fungi also have been found to inhabit within plant

tissues and are considered to be important endophytes on palm trees and other tropical plants (Rodrigues and Petrini 1997). Furthermore, nine species, one variety, and one undescribed species of *Xylaria* have been found from termite nests, i.e., *Xylaria arenicola* Welw. et Curr., *X. brasiliensis* (Theiss.) Lloyd, *X. escharoidea* (Berk.) Fr. (= *X. melanaxis* Ces.), *X. furcata* Fr. var. *furcata*, *X. furcata* Fr. var. *hirsuta* J.D. Rogers et Y.-M. Ju, *X. nigripes* (Klotzsch) Cooke, *X. piperiformis* Berk., *X. readeri* F. Muell., *X. rhizomorpha* (Mont.) Mont., *X. tanganyikaensis* (Dennis) D. Hawksw., and *Xylaria* sp. (Dixon 1965; Sabramanian 1972; Rogers et al. 1987, 2005; Ju and Rogers 1999). In addition, *X. angulosa* J.D. Rogers, Callan et Samuels, although it had been isolated from a soil sample, was described as being closely related to the termiticolous species *X. nigripes* and *X. escharoidea*.

In the course of taxonomic and ecological studies on endophytic xylariaceous fungi, we have been surveying xylariaceous fungi of decomposing plant materials and from termite nests to compare with the endophytic isolates. We obtained two xylariaceous fungi from a nest of a termite, *Odontotermes formosanus* (Shiraki), collected from Iriomote Is., Okinawa Pref., Japan. One of the two fungi formed the synnematus anamorphic stage on the nest incubated in a moist chamber and on medium, and its morphological characteristics seemed to assign the fungus to the genus *Geniculosporium* Chesters et Greenh. However, its distinctive morphology, namely, antler-shaped synnemata and dendritic conidiophores, indicated that none of the extant genera can accommodate the present fungus properly. To infer the phylogeny and taxonomic position of the fungus, we carried out phylogenetic analysis based on the ribosome DNA sequences. The other xylariaceous fungus produced stromata followed by formation of the ascigerous stage on oatmeal agar medium, although the fungus did not produce the ascigerous stage on the nest. We investigated the morphology of the fungus for identification and examined the possibility of the teleomorph–anamorph connection between the above two xylariaceous fungi found from a termite nest by morphological and phylogenetic approaches.

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In this article, we describe and discuss the taxonomy and phylogeny of the synnematos anamorphic fungus and the other xylariaceous fungus isolated from the termite nest.

Materials and methods

Isolation

A fresh nest of the termite *Odontotermes formosanus* was found under the roots of a fallen evergreen broadleaf tree in Iriomote Is., Okinawa Pref., Japan (about 24° N, 123° E, subtropical region) on Oct. 18, 2001. Pieces of the nest were taken back to the laboratory and incubated in a moist chamber under ambient temperature, ~20°–25°C. After 2 days incubation, synnematos structures, on which dried and hyaline conidia were produced, protruded from the pieces of the nest. After a few days, other brown-colored stromata that were apparently distinguishable from the previous whitish synnemata appeared from other parts of the nest pieces. Hyphae of the two fungi were picked up from their synnemata and stromata using a fine tungsten needle and inoculated on oatmeal agar (OA) and potato dextrose agar (PDA) media in 90-mm plates to establish pure cultures. These cultures of the synnematos fungus [IOC-1255 (= NBRC 102095)] and the stroma-forming fungus [IOC-1249 (= NBRC 33288)] were incubated at 25°C for the following experiments. Because spore germination of the synnematos fungus was not good, the culture was subcultured by transferring mycelia. These cultures were maintained by freezing in a –80°C freezer and deposited into the NITE Biological Resource Center (NBRC) collection.

Morphological observations

Morphology of the two fungi was observed on OA and PDA. Morphological observations were conducted by light and scanning electron microscopy. Fungal materials, such as synnemata and stromata formed on OA and PDA, were mounted in one drop of lactophenol solution on glass slides for light microscopic observation. For measurement of spore dimension, 50 spores were measured under the light microscope. To prepare the SEM specimens, synnemata were fixed in 1% OsO₄ at 4°C overnight, then dehydrated in an ethanol series, and finally substituted with isoamyl acetate. After critical-point drying and coating with platinum-palladium, the specimens were observed with a JSM 5400 (JEOL) operated at 15 kV.

DNA isolation

The strains NBRC 102095 and NBRC 33288 were incubated for 2–3 weeks at 25°C on OA, and their mycelia were harvested and put into 2-ml plastic tubes using a spatula. DNA was extracted using Nucleon PhytoPure DNA extraction kit

(Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions.

Sequence analysis of rDNA

The internal transcribed spacer (ITS) regions of rDNA were amplified by polymerase chain reaction (PCR) using TaKaRa Taq (TaKaRa Bio, Shiga, Japan) as a single fragment with the standard primer pairs ITS5 (5'-GGAAG TAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCG CTTATTGATATGC-3') (White et al. 1990). Amplification of the desired fragment was performed with a GenAmp PCR System 7000 thermal cycler (Applied Biosystems, Foster City, CA, USA) with the following program: 30 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 55°C, extension for 2 min at 72°C, incubation for 5 min at 72°C, and soaking at 4°C. Amplified DNA was sequenced with the a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in a thermal cycler employing the following ramp: 25 cycles of 15 s at 96°C and 4 min at 55°C, followed by a 4°C soak. Nucleotide sequences were determined in both directions using the primers ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'), ITS3 (5'-GCATC GATGAAGAACGGAGC-3'), ITS4, and ITS5 (White et al. 1990). Sequences were analyzed with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The CLUSTAL X version 1.8 software (Thompson et al. 1997) package was used to generate the evolutionary distances [the K_{nuc} value (Kimura 1980)] and the similarity values and to perform the neighbor-joining (NJ) analysis (Saitou and Nei 1987) from K_{nuc} values and the bootstrap resampling method (Felsenstein 1985) with 1000 replicates for evaluation of the topology of the phylogenetic tree. The NJ plot (Perrière and Gouy 1996) was used for plotting the phylogenetic tree. The alignment was deposited in TreeBASE (<http://www.treebase.org/treebase/index.html>) under the study number of S1699 and matrix number of M3071.

18S rDNA was amplified by PCR using TaKaRa Taq as a single fragment with a pair of primers, 18-F (5'-ATCTG GTTGATCCTGCCAGT-3') and 18-R (5'-GATCCTTC GCAGGTTCCACC-3') designed by Ueda and Mikata (1999). Amplification of the desired fragment was performed as already mentioned. Amplified DNA was sequenced using the same manner and condition employed in ITS sequencing as already described. Nucleotide sequences were determined in both directions using the primers 550-F (5'-GCAGCCGCGGTAATTCCAGC-3'), 950-F (5'-TCAAGAACGAAAGTTAGGGG-3'), 1300-F (5'-TTGGTGGAGTGATTTGTCTG-3'), 550-R (5'-GAA TTACCGCGGCTGCTGGC-3'), 950-R (5'-TCCCCTA ACTTTCGTTCTTG-3'), and 1300-R (5'-AGACAAAT CACTCCACCAAC-3') that were also designed by Ueda and Mikata (1999), in addition to 18-F and 18-R. The sequences of 18S rDNA were analyzed using the same equipment and methods for ITS as previously stated.

The sequence data of ITS regions of known species of xylariaceous fungi deposited in the DDBJ/EMBL/GenBank nucleotide sequence database were employed for phyloge-

Table 1. Morphology of the present anamorphic fungus and related fungi

	The present fungus	<i>Padixonia bispoda</i> ^{bs}	Anamorph of <i>X. melanaxis</i> ^d	Anamorph of <i>X. nigripes</i> ^f	<i>Geniculosporium serpens</i> ^a	<i>G. sporodochiale</i> ^e	<i>Geniculodendron pyriforme</i> ^{g,h}
Conidia (µm)	4-6 × 2-4 (5.1 × 3.2) (number of conidia: 17-20)	Spherical type 10-12 diameter Apiculate type 10-15 long	4-4.5 × 2.5-3	2.5-5 × 1.5-2	3-5 × 2-3	4-6 × 2.5-3	5-7 × 3-5
Conidiogenous cell (µm)	Basal 5-11 × 1.5-3 (7.3 × 2.2) Proliferous 13-35 × 2-4 (23.1 × 3.3)	30-70 long	7-15 × 2.5-3	-	-	-	34-48 × 3.2-4.4
Conidiophores (µm)	167-341 × 4.5-7.0 (242 × 5.7)*	-	-	25 × 1.5-2	Up to 300	30-45 × 3-4	150-450 × 8-12 in above (4-5 in below)
Synnematal structure	+	-	-	-	-	-	-
Location of conidial stage	-	On stroma	On stroma	On stroma	On stroma	On stroma	-
Colony color	White to pale brown	-	Light yellowish brown	-	Grayish to brownish	Olivaceous	White to pale brown
Ascigerous stage	Unknown	<i>Xylaria furcata</i>	<i>X. melanaxis</i>	<i>X. nigripes</i>	<i>Nemania serpens</i>	<i>Anithostomella aquatica</i>	<i>Caloscypha fulgens</i>

* Length of conidiophores radiating out from the main axis of synnema Data are derived from: ^a Chesters and Greenhalgh (1964), ^b Dixon (1965), ^c Hyde and Goh (1998), ^d Ju and Rogers (1999), ^e Rogers et al. (1978), ^f Paden et al. (2005), ^g Sabramanian (1972), ^h Salt (1974)

netic analysis (see Fig. 16). The nucleotide sequence data of 18S and ITS regions of rDNA of fungal strains obtained in this study were deposited into the DDBJ nucleotide sequence database as follows: the synnematus anamorphic fungus NBRC 102095/AB274812 for 18S rDNA and AB274813 for ITS regions; the stroma-forming ascomycetous fungus NBRC 33288/AB274814 for 18S rDNA and AB274815 for ITS regions.

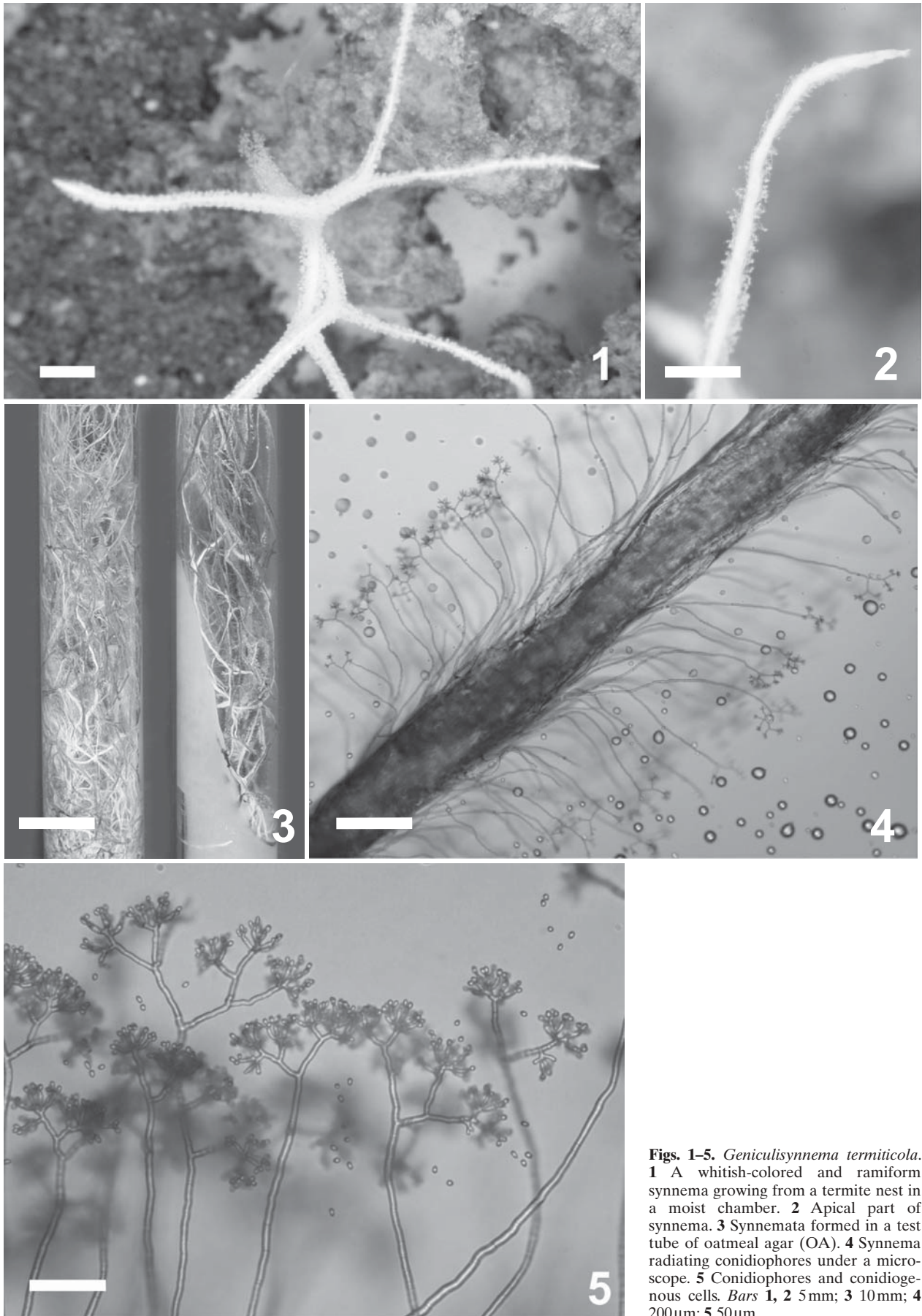
Results

Morphology

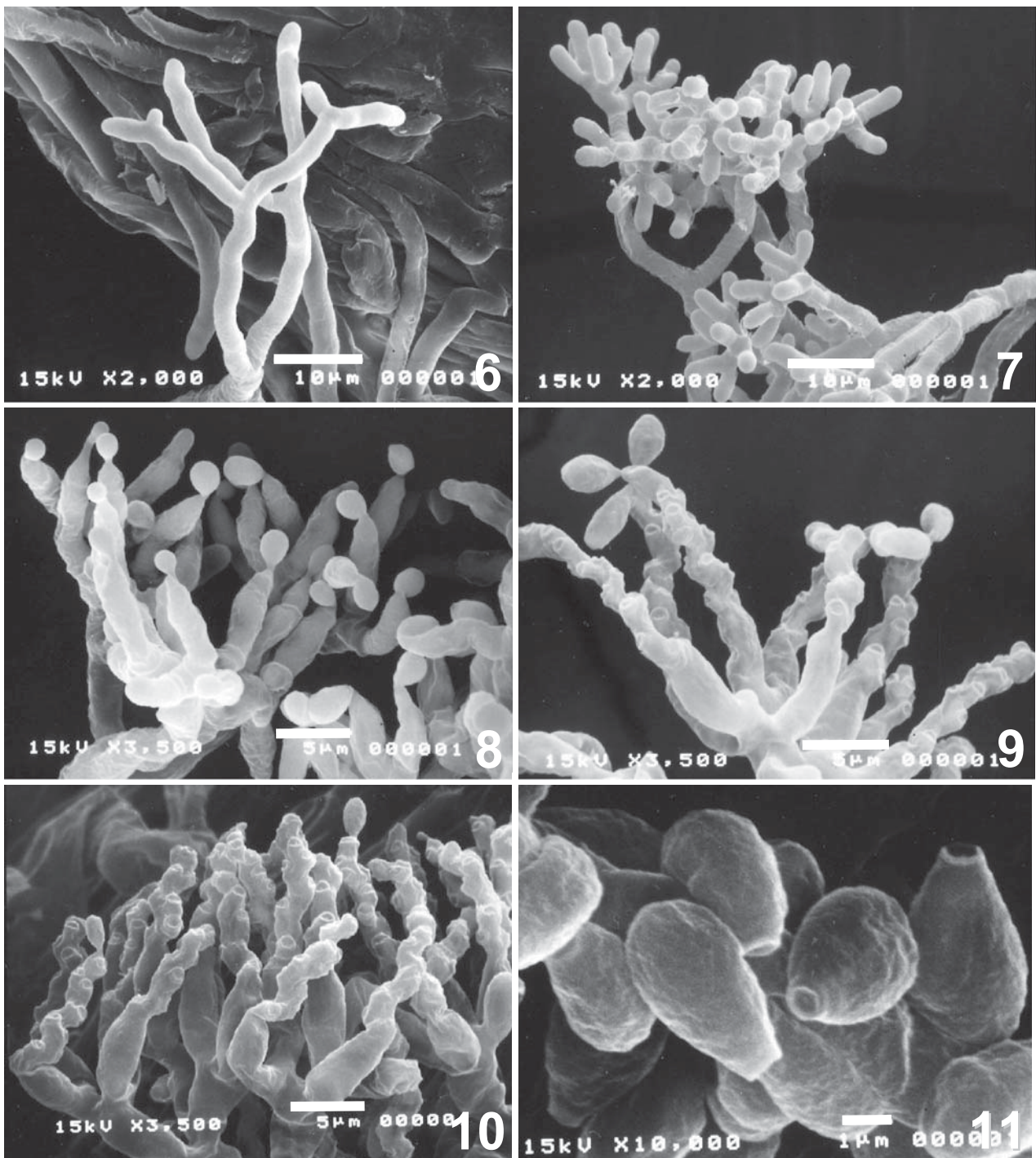
From a piece of a termite nest incubated in a moist chamber, the present anamorphic fungus produced white-colored and antler-shaped synnemata (Figs. 1, 2). In cultivation on OA, the fungus formed hair-like synnemata vigorously, mostly filling a test tube with synnemata in 3 weeks (Fig. 3). Conidiophores radiating from the synnemata usually repeat dichotomous branching three times, and a conidiogenous cell subsequently develops on the apex of the each branch (Figs. 4-8). Conidiogenous cells proliferate and geniculate sympodially (Figs. 9, 10, 12A) to generate unicellular and dry conidia successively in a holoblastic manner (Figs. 8, 9, 11). Scars from which conidia are detached obviously remain on the proliferating conidiogenous cell (Figs. 10, 12B). Thus, the present fungus shows affinity with *Geniculosporium* or other anamorphs of xylariaceous fungi. Table 1 shows the morphological comparison between the anamorphic fungus and related fungi: *Padixonia bispoda* Subram. and the anamorphs of termiticolous *Xylaria*, *Geniculosporium* spp., and *Geniculodendron pyriforme* G.A. Salt. The present fungus cannot be assigned properly to any of these genera.

Another fungus isolated from the same termite nest produced the ascigerous stage on stromata culturing on OA (Fig. 14), whereas it formed only brown-colored and club-shaped sterile stromata on PDA. The stromata were similar to those formed on the termite nest kept in a moist chamber, on which sporulation could not be observed (Fig. 13). Morphological characters of the ascomycetous fungus were as follows: stromata upright on culture, sometimes branched at the upper part of the stromata in mature, dimensions of asci (Fig. 15), 41-51 × 3-5 µm; ascospores, 3.6-5.6 × 1.6-2.8 µm (\bar{x} = 4.5 × 2.3 µm), and having a germ slit on ascospores. Such morphological characters of the fungus revealed it to be identical with *Xylaria angulosa* found from soil in Indonesia (Rogers et al. 1987). The isolate of the fungus was deposited in the NBRC culture collection (*X. angulosa*, NBRC 33288; specimen, NBRC H-12264).

Although the undescribed anamorphic fungus and the ascomycetous fungus identified as *X. angulosa* were isolated from the same termite nest, they were clearly different from each other in their colony appearances and morphology. The synnemata or stromata of the two fungi were likely produced on a termite nest as it became old following the decline of termite activity.



Figs. 1-5. *Geniculisynnema termiticola*.
 1 A whitish-colored and ramiform synnema growing from a termite nest in a moist chamber. 2 Apical part of synnema. 3 Synnemata formed in a test tube of oatmeal agar (OA). 4 Synnema radiating conidiophores under a microscope. 5 Conidiophores and conidiogenous cells. Bars 1, 2 5mm; 3 10mm; 4 200 μ m; 5 50 μ m



Figs. 6–11. *Geniculisynecha termiticola* under SEM. **6** A conidiophore branching in dichotomous manner. **7** Apical parts of a conidiophore branching. **8** Conidiogenous cells forming conidia on the tips. **9, 10**

Conidiogenous cells sympodially proliferated and geniculated. **11** Conidia. Bars **6, 7** 10 μ m; **8–10** 5 μ m; **11** 1 μ m

Phylogenetic analysis

Sequence analysis based on the partial sequence of 18S rDNA (about 1700 bases) revealed that both the anamorphic fungus NBRC 102095 and *X. angulosa* NBRC 33288 from a termite nest were included in a large cluster consisting of the species belonging to Xylariaceae (data not shown). The two fungi are nested in a single cluster in the 18S rDNA

tree, while their sequence similarity was only 98% (1737/1757 bp). The tree showed no phylogenetically close relationship between the two fungi and certain genera of Xylariaceae supported with high bootstrap value.

In the analysis based on the sequence data of ITS regions of rDNA, the anamorphic fungus NBRC 102095 and *X. angulosa* NBRC 33288 were connected to each other and nested in a cluster consisting of *Nemania*

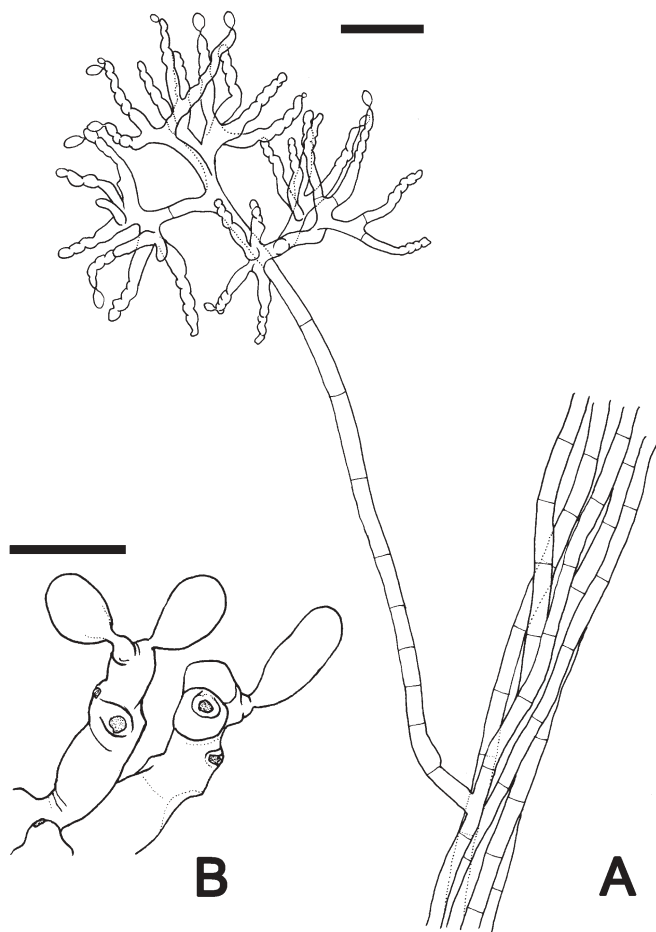


Fig. 12. *Geniculisynnema termiticola*. **A** A conidiophore radiating from a synnema. **B** Conidiogenous cells. Bars **A** 20 μm ; **B** 5 μm

spp. (Fig. 16). However, the anamorphic fungus is clearly distinguishable from *X. angulosa* NBRC 33288 by their sequence difference (85% similarity) and also in morphology, as described.

Although the phylogenetic tree indicates the anamorphic fungus and *X. angulosa* NBRC 33288 have affinity to the species of *Nemania*, the low bootstrap values on branches of the tree may suggest that further studies are required to examine whether these species belong to the genus *Nemania* or *Xylaria*. Thus, we retain the original taxonomic status of *X. angulosa* NBRC 33288 in the present study. Because morphological and phylogenetic studies show that no extant xylariaceous anamorphic fungi can accommodate properly the present anamorphic fungus, we establish a new anamorphic genus. The description of the new fungus is as follows.

Taxonomy

Geniculisynnema Okane et Nakagiri, gen. nov.

Coloniae effusae. Mycelium immersum vel superficiale, septatum. Synnemata erecta, longa, crassa. Conidiophora

macronematica, radiata ad rhacosynnema, dichotome ramosa. Cellulae conidiogenae polyblasticae, sympodialiter proliferantae, geniculatae, cicatricibus claris. Conidia holoblastica, solitaria, sicca, hyalina, eseptata, basi truncata.

Species typica: *Geniculisynnema termiticola* Okane et Nakagiri.

Etymology: *Geniculum* = genicule, *synnema* = synnema; referring to the shape of conidiogenous cells and synnema formation.

Colonies effuse. Mycelium immersed and superficial, septate. Synnemata erect, long, stout. Conidiophores macronematous, synnematos. Conidiophores radiated out from main axis of synnema, branched in dichotomous manner. Conidiogenous cells, polyblastic, sympodially proliferated and geniculated, remaining obvious scars. Conidia, holoblastic, solitary, dry, hyaline, aseptate, with truncate base.

Type species: *Geniculisynnema termiticola* Okane et Nakagiri.

Geniculisynnema termiticola Okane et Nakagiri, sp. nov.

Figs. 1–15

Coloniae effusae, albae vel albido-brunneae. Mycelium immersum vel superficiale, septatum, hyalinum vel albido brunneum. Stromata nulla. Setae et hyphopodia absentia. Synnemata erecta, corniformia, longa, crassa, alba, ad maturitatem albido-brunnea. Conidiophora macronematica, a rhacosynnema radiata, dichotomice circa tres ramosa, 167–341 \times 4.5–7.0 μm (\bar{x} = 242 \times 5.7). Cellulae conidiogenae polyblasticae, sympodialiter proliferantia, geniculatae, 5–11 \times 1.5–3 μm (\bar{x} = 7.3 \times 2.2) in parte basali, 13–35 \times 2–4 μm in parte proliferationis, cicatricibus claris. Conidia successive efferentia, holoblastica, solitaria, sicca, obovata, hyalina, eseptata, basi truncata, 4–6 \times 2–4 μm (\bar{x} = 5.1 \times 3.2), in mediis usque ad 20 formantia.

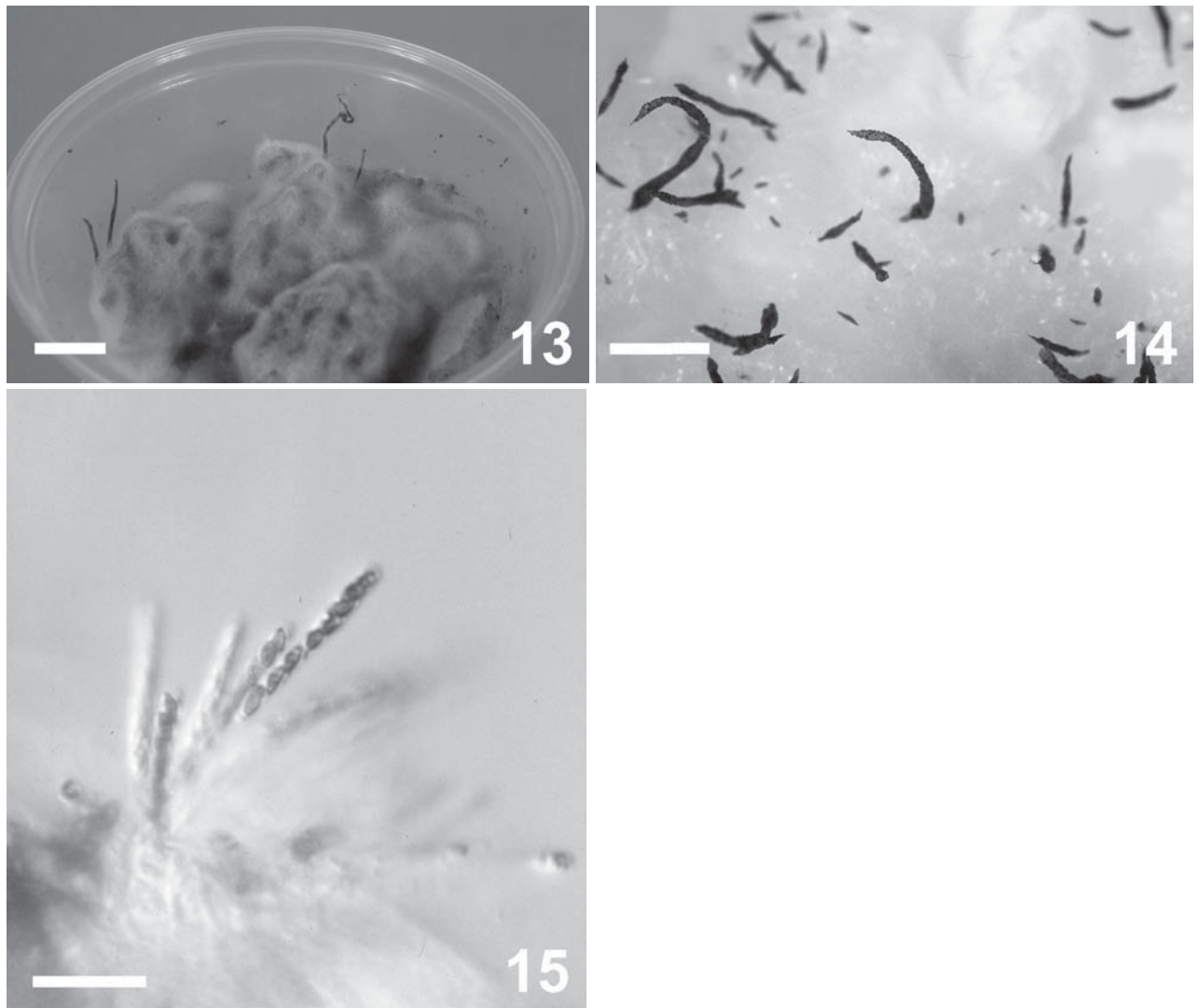
Habitatio: In nidus *Odontotermis formosani*

Holotypus: NBRC H-12554, colonia exsiccata in cultura ex nido *Odontotermis formosani*, a Ins Iriomote, Okinawa Pref., Japonia, a I. Okane isolata, in Herbario NBRC conservata.

Colonies effuse, white to whitish brown. Mycelium immersed and superficial, septate, hyaline to whitish brown. Stroma none. Setae and hyphopodia absent. Synnemata, erect, long, stout, branched like antler, white, pale brown in mature. Conidiophores macronematous radiated out from main axis of synnema, branched approximately three times in dichotomous manner, 167–341 \times 4.5–7.0 μm (\bar{x} = 242 \times 5.7). Conidiogenous cells subsequently developed on the tip of conidiophores, polyblastic, sympodially proliferated and geniculated, 5–11 \times 1.5–3 μm (\bar{x} = 7.3 \times 2.2) in basal, 13–35 \times 2–4 μm (\bar{x} = 23.1 \times 3.3) in proliferated part, remaining obvious scars. Conidia successively produced in holoblastic manner, solitary, dry, obovate-shape, hyaline, aseptate, with truncate base, 4–6 \times 2–4 μm (\bar{x} = 5.1 \times 3.2), produced up to 20 approximately on media.

Source: Nest of a termite, *Odontotermes formosanus*.

Holotypus: NBRC H-12554, dried colony on oatmeal agar of IOC-1255 (= NBRC 102095) isolated from termite



Figs. 13–15. *Xylaria angulosa* NBRC 33288. **13** Stromata growing from a termite nest in a moist chamber. **14** Ascomata on OA plate. **15** Asci and ascospores. Bars **13, 14** 10mm; **15** 20 μ m

nest collected in Iriomote Is., Okinawa Pref., Oct. 18, 2001, collected and isolated by I. Okane; deposited in the herbarium of NBRC.

Strain examined: IOC-1255 (= NBRC 102095), AN-1697, AN-1698 (= NBRC 102681).

Etymology: *termitus* = termite, *cola* = inhabiting; referring to fungus inhabiting in a termite nest.

Discussion

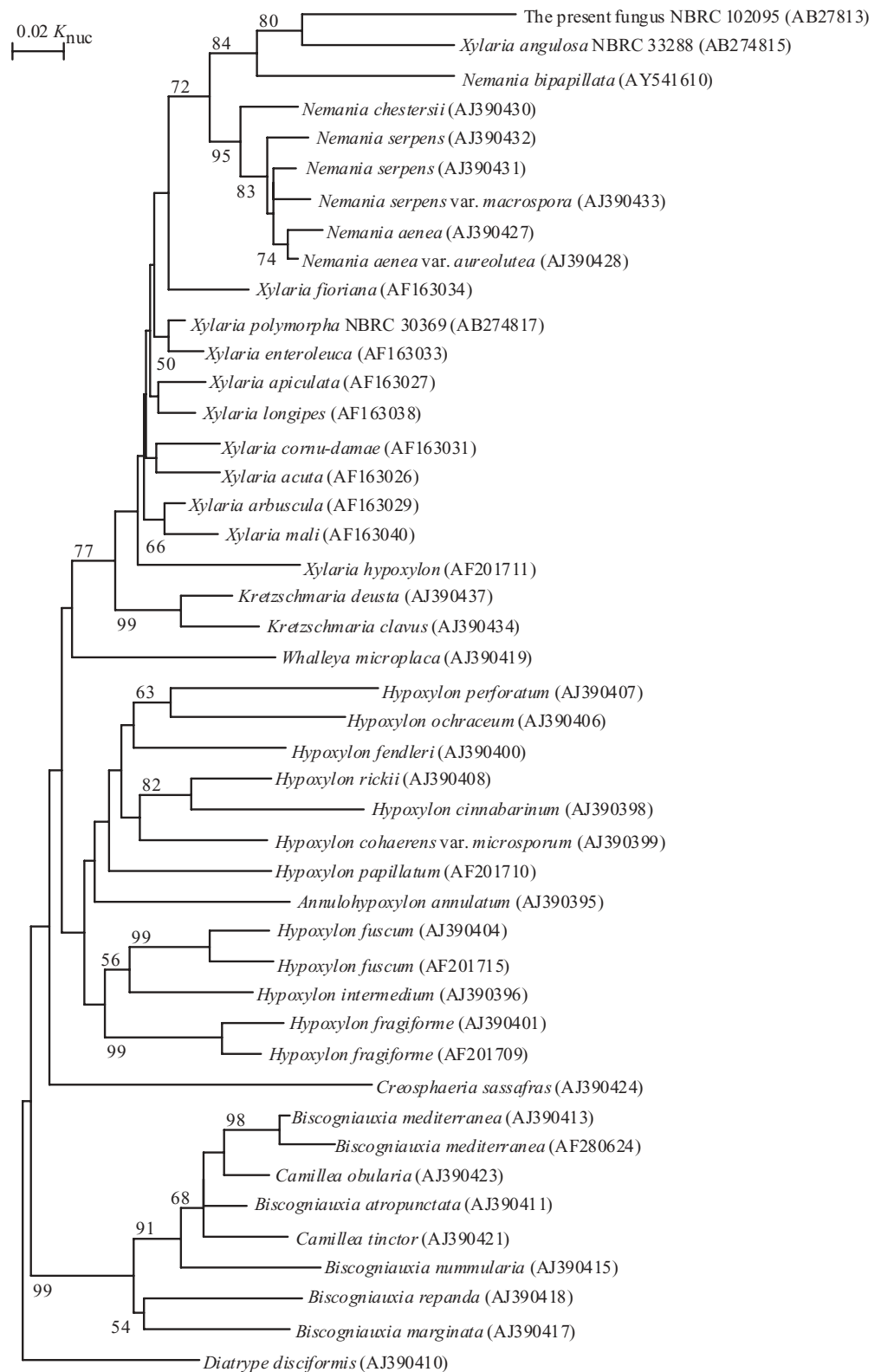
Among anamorphic xylariaceous fungi, *Geniculosporium* species seemed to have affinity with the present new fungus. The genus *Geniculosporium* was described with *Geniculosporium serpens* Chesters et Greenh. as the type species (Chesters and Greenhalgh 1964). In the genus *Geniculosporium*, *G. corticioides* (Ferraris et Sacc.) de Hoog, *G. densissimum* (Schwein.) de Hoog, *G. serpens* [teleomorph: *Nemania serpens* (Pers.: Fr.) Gray var. *serpens*], and *G.*

sporodochiale K.D. Hyde et Goh have been described. *Geniculosporium sporodochiale* was described as the anamorph of *Anthostomella aquatica* K.D. Hyde et Goh (Hyde and Goh 1998). In addition, many unnamed *Geniculosporium* anamorphs relating to *Nemania* or other genera of Xylariaceae have been reported (Whalley et al. 1983; Petrini and Petrini 1985; Petrini and Rogers 1986; Rogers et al. 1987). Thus, *Geniculosporium* are known to have affinity with several xylariaceous teleomorphs.

Because *Geniculisynnema termiticola* was nested in a cluster consisting of *Nemania* spp. in the ITS phylogenetic tree (Fig. 16), we first considered accommodating it in the genus *Geniculosporium*. However, its distinctive morphology, namely, forming finely antler-shaped synnemata on the termite nest (see Fig. 1) and dichotomously branching conidiophores (see Fig. 5), have not been found in species of *Geniculosporium*.

Although *Geniculodendron (Gd.) pyriforme* has been known to be anamorphic with *Caloscypha fulgens* (Pers.) Boudier (Pezizales), this fungus was previously considered

Fig. 16. Neighbor-joining tree derived from internal transcribed spacer (ITS) region sequences of isolates obtained and strains of known species deposited in the DDBJ/EMBL/GenBank nucleotide sequence database. *Diatrype disciformis* (AJ390410) was specified as the outgroup. Bootstrap values $\geq 50\%$ are shown above branches. Bar 0.02 K_{nuc} in nucleotide sequences



to be taxonomically close to *Geniculosporium* (Salt 1974; Paden et al. 1978). Salt (1974) described that, although *Gd. pyriforme* resembled *Geniculosporium* in lacking lateral and apical swellings on the conidiogenous cells found in *Nodulisporium*, *Gd. pyriforme* was dissimilar in having

conidiophores with a well-defined main axis and distinct dendritic branching. Such morphological characteristics were the reasons why Salt (1974) established *Geniculodendron* as a novel genus to accommodate *Gd. pyriforme*. *Geniculisynnema termiticola* also forms such dendritic

branched conidiophores as *Gd. pyriforme*, but the former species is apparently different from the latter species in producing approximately three times as much dichotomous branching of conidiophores (see Fig. 5) and its antler-shaped synnema formation (see Fig. 1).

Dematophora R. Hartig anamorphs of *Rosellinia* De Not. are known to produce synnemata. However, *G. termiticola* cannot properly be assigned to the genus *Dematophora* because the synnemata of any species of *Dematophora* are apparently different from those of *G. termiticola*, especially in the claviform shape and synnematal size (Sivanesan and Holliday 1972a–d).

The assignment of the present fungus into a separate genus from *Geniculosporium* and other genera based on such morphological characteristics as antler-shaped synnemata and dichotomously branching conidiophores also avoids leading to taxonomic confusion in the present systematics of xylariaceous anamorphs.

Occurrence of both *G. termiticola* and *X. angulosa* from the same termite nest led us to consider that these two fungi might be conspecific. However, cultural examination and molecular analyses revealed that *G. termiticola* is clearly distinguishable from *X. angulosa*.

Molecular analyses have been performed to survey the phylogeny of the Xylariaceae (Ju and Rogers 1996; Sánchez-Ballesteros et al. 2000; Hsieh et al. 2005; Triebel et al. 2005). Ju and Rogers (1996) and Sánchez-Ballesteros et al. (2000) reported that the family was subdivided into at least two major groups based on the molecular phylogeny and anamorphs: one group having *Nodulisporium* or *Nodulisporium*-like anamorphs clustering with *Hypoxyton* and the other having *Geniculosporium* or *Geniculosporium*-like anamorphs clustering into another group including *Nemania*. The teleomorphic stage of *G. termiticola*, if it possesses one, is supposed to belong to the latter group.

According to the phylogenetic tree in this study, it can be assumed that its teleomorph should be a member of the genus *Nemania*, which is known to produce effused-pulvinate to discoid ascostromata on wood. On the other hand, because *G. termiticola* and *X. angulosa* are not so far apart from each other phylogenetically, the possibility that *G. termiticola* might produce erect and sometimes branched stromata as does the genus *Xylaria* is still expected. Because of the low bootstrap values on branches of the tree, we cannot certainly predict the possible teleomorphic type of *G. termiticola*.

Although *G. termiticola* has never formed its teleomorph on agar media, this fungus needs to be examined for the teleomorph–anamorph connection with teleomorphs already described, including species of the genus *Xylaria*, which have been known to have morphologically varied anamorphs (Rogers 1985). Then, further molecular studies over the wide range of species may reveal the details of the teleomorph–anamorph connections of Xylariaceae including *G. termiticola*.

Rogers et al. (1987) mentioned that the brittle stromata of *X. angulosa* with dark flesh, tiny asci, and long rooting bases clearly indicate a taxonomic affinity with *X. nigripes* and *X. melanaxis* (current name, *X. escharoidea*) found

from termite nests. Although *X. angulosa* was described to be terricolous in Indonesia by Rogers et al. (1987), we found this species from a termite nest in this study. Therefore, this fungus may also have ecologically close relationship to termites, as do other termiticolous species including *X. nigripes* and *X. melanaxis*. Although *X. angulosa* NBRC 33288 is a different species from *G. termiticola*, these two fungi was found to be more or less phylogenetically close to each other according to phylogenetic analysis based on ITS sequences in this study. Phylogenetic and ecological relationships between *X. nigripes*, *X. melanaxis*, and other termiticolous xylariaceous fungi may be an interesting research project. Further studies based on molecular phylogeny are necessary to clarify the phylogenetic relationships of the xylariaceous fungi associated with termite nests.

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