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# Morphology and molecular phylogeny of *Tretopileus sphaerophorus*, a synnematous hyphomycete with basidiomycetous affinities*

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***Tretopileus sphaerophorus*, a synnematous hyphomycete with basidiomycetous affinities was newly isolated from the decaying petiole and peduncle of *Cocos nucifera* collected in Depok, Indonesia. The species produced first a bulbil as a propagule on the top of a synnema. After the bulbil had fallen, the synnema proliferated about seven times to produce new bulbils, each time making conspicuous nodes at the upper part. By careful morphological observation, clamp connections were confirmed on the hyphae in the specimens and culture. In culture, each hyphal cell with or without a clamp was found to be dikaryotic by DAPI nuclear staining. Germination of the bulbils occurred first from projecting hyphal tips on their upper surface, which have been treated as germ pores. The inner structure of the bulbils, the hyaline mucus of the bulbils, and conidium-like hyphal fragments were also examined. Phylogenetically, *T. sphaerophorus* was inferred to be related to the Aphyllophorales based on the nuclear encoded small subunit (18S) rDNA using the homology search system (FASTA) and the neighbour-joining method.**

Key Words——Aphyllophorales; ribosomalDNA; synnematous hyphomycete; taxonomy; *Tretopileus sphaerophorus*.

Based on a curious fungus growing on a cactus (*Opuntia ammophila* Small) and allies in USA, Dodge (1946) proposed a new genus *Tretopileus* Dodge typified by *T. opuntiae* Dodge, and provided informative sketches and photographs. Deighton (1960) found that *Monotospora sphaerophora* Berk. & Curt. 1868 collected from Cuba and Sierra Leone (Africa) was the same as *T. opuntiae*, and he proposed a new combination for the fungus, *T. sphaerophorus* (Berk. & Curt.) Hughes & Deighton. Subramanian (1971), Seifert (1990) and Matsushima and Matsushima (1995) reported the species from India, Indonesia, Thailand, Malaysia and Taiwan. Munjal (1962) described a second species from India, *T. indicus* Munjal, in which the bulbils (also cited by some authors as multicellular gemmae/caps/discs/spores) were bigger than those of Dodge's specimens of *T. opuntiae*.

The higher taxonomical rank of the genus *Tretopileus* has long been uncertain. Dodge (1946) speculated that *Tretopileus* might be the sterile form of some ascolichens (*Calicium* Pers.) or gasteromycetes (*Myriostoma* Desv. and *Calvatia* Fr.). Deighton (1960) classified the genus into the Mycelia Sterilia, and Berkeley and Curtis (1868; as *Monotospora* Corda), Munjal (1962) and Subramanian (1971) placed it in the Hyphomycetes. In the Thailand strain of *T. sphaerophorus*, Matsushima and Matsushima (1995) discovered typical clamp connections of the vegetative and synnematous hyphae. They mentioned that *T. sphaerophorus* was a mitotic basidiomycete.

We recently collected *T. sphaerophorus* from dead *Cocos nucifera* L. in Indonesia and isolated a fertile pure culture. Although it is not difficult to isolate this fungus, especially from bulbils (Dodge, 1946), there is unfortunately no strain of *Tretopileus* species available at any of the culture collections listed by the World Federation for Culture Collections (*T. sphaerophorus* IMI 141217 is no longer available). Using our Indonesian specimens and isolate of *T. sphaerophorus*, we observed the morphology and tried to infer its phylogenetic position in the Basidiomycota based on 18S rDNA sequences.

## Materials and Methods

**Strain examined** *Tretopileus sphaerophorus* JCM 10092 (=ANMR 62, =G. Okada OFC 5172) was used for sequencing and morphological observation.

**Morphology** The culture was incubated at 20°C under light for a wk and later in laboratory conditions on Difco potato dextrose agar (PDA). The Methuen "Handbook of colour" (Kornerup and Wanscher, 1978) was cited for the color names of the colonies and microscopic details. For light and scanning electron microscopy, the procedures of Okada (1996) were used.

**Nuclear staining** The hyphae grown on thin PDA film on

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a slide were fixed in 100% methanol for 15 min at 4°C. The nuclei in hyphae were stained with the following fluorescent dye solution for 60 min at 4°C (Hamada and Fujita, 1983): 100 ng/ml 4′,6-diamidino-2-phenylindole (DAPI) in the buffer (10 mM Tris, 10 mM EDTA-2Na, 100 mM NaCl, 10 mM 2-mercapto-ethylamine (pH 7.4)). The same fresh staining solution was dropped again on the hyphae on a slide and covered with a slip sealed by nail enamel. The nuclei were observed under an incident-light fluorescence microscope (Nikon Fluophot) with an UV excitation set (UV 330-380 excitation filter, DM400 dichroic mirror and 420K barrier filter).

**Isolation of DNA, PCR amplification, cloning and sequencing of genomic DNA, and phylogenetic analysis**

The methods detailed in the previous report (Okada et al., 1997) were used for DNA isolation, amplification, cloning, sequencing and phylogenetic analysis. The determined 18S rDNA sequence of *T. sphaerophorus* was deposited in DDBJ under the accession number AB 006005. This sequence was aligned with the following published sequences from the nucleotide sequence databases (GenBank, EMBL and DDBJ): *Agaricostilbum hyphaenes* U40809, *Auricularia auricula* L22254, *Auriscalpium vulgare* U59060, *Bensingtonia yuccicola* U40810, *Boletus santanas* M94337, *Bondarzewia berkeleyi* U59062, *Bulleromyces alba* X60179, *Calocera cornea* L22256, *Coprinus cinereus* M92991, *Cronartium ribicola* M94338, *Dacrymyces chrysospermus* L22257, *Echinodontium tinctorium* U59068, *Erythrobasidium hasegawianum* D12803, *Eurotium rubrum* U00970, *Filobasidiella neoformans* D12804, *Filobasidium floriforme* D13460, *Fistulina hepatica* U59070, *Graphiola cylindrica* D63929, *G. phoenicis* D63928, *Gymnoconia nitens* U41565, *Hericium ramosum* U59073, *Heterogastridium pycnidioideum* U41567, *Heterotextus alpinus* L22259, *Kondoa malvinella* D13776, *Lentinula lateritia* U59075, *Lepiota procera* L36659, *Leucosporidium scottii* X53499, *Mixia osmundae* D14163, *Neurospora crassa* X04971, *Peridermium harknesseii* M94339, *Phanerochaete chrysosporium* U59084, *Polyporus squamosus* U59089, *Pseudohydnum gelatinosum* L22260, *Rhodosporidium toruloides* D12806, *Rhodotorula glutinis* X60180, *Saccharomyces cerevisiae* J01353 (M27607), *Schizophyllum commune* X54865, *Sparassis spathulata* U59096, *Spongipellis unicolor* M59760, *Sporidiobolus johnsonii* L22261, *Sporobolomyces roseus* X60181, *Sympodiomycopsis paphiopedili* D14006, *Taphrina wiesneri* D12531, *Thanatephorus cucumeris* M92990, *Tilletia caries* U00972, *Tremella foliacea* L22262, *T. globospora* U00976, *Trichosporon cutaneum* X60182, *Ustilago hordei* U00973, *U. maydis* X62396, *Xerocomus chrysenteron* M94340. Phylogenetic trees were constructed mainly by the neighbour-joining method based on the comparison of 1408 sites for the partial sequence data set, and the topology of the trees was evaluated by a bootstrap analysis. The aligned sequence data file used in this study is obtainable from the corresponding author.

**Description**

***Tretopileus sphaerophorus*** (Berkeley & Curtis) Hughes & Deighton, Mycol. Pap. **78**: 2. 1960. Figs. 1–37

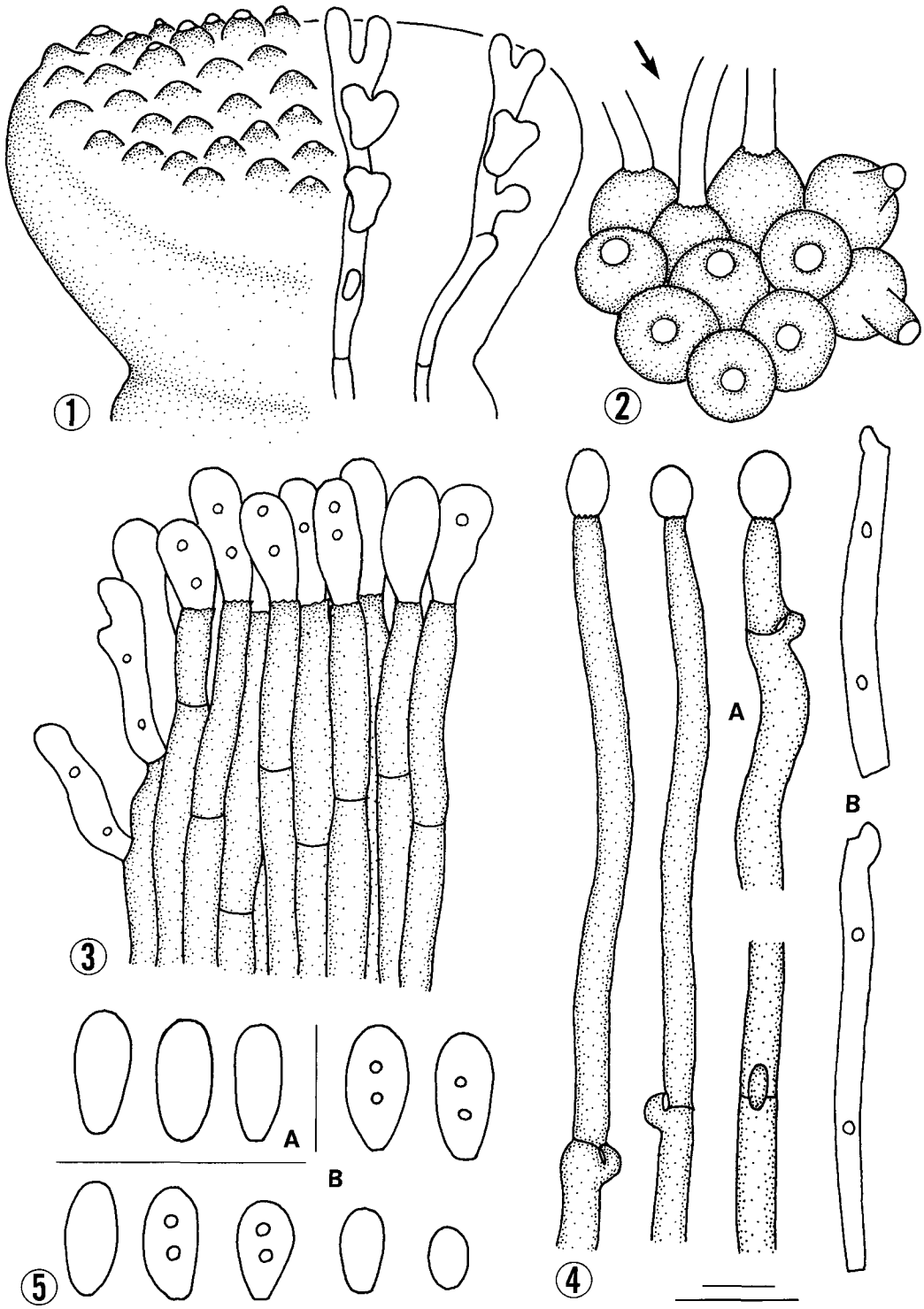
≡*Monotospora sphaerophora* Berkeley & Curtis, J. Linn. Soc., Bot. **10**: 360. 1868.

=*Tretopileus opuntiae* Dodge, Bull. Torrey Bot. Club **73**: 223. 1946.

Synnemata determinate, cylindrical to subulate with a hemi-convex head (bulbil), 80–400 μm tall, 15–80 μm wide, black or rarely Brownish Grey (M. 6F8) on the surface; stipes proliferate to produce white/gray nodes and black internodes at least seven times after the dropping of each bulbil, with a tip covered with white powdery crystalline substance, consisting of the core bundle made by parallel hyaline hyphae (1.5–3 μm wide) with/without clamp connections containing two guttules (maybe nuclei) and the cortex made by Dark Brown (M. 7F8) hyphae (2.5–4 μm wide) with/without clamps. Bulbils multicellular, hemi-convex with an upper surface almost flat, more or less circular in face view, broadly flabelliform or obtrapezoid in side view, 80–150 μm in diam, 30–50 μm thick, dark brown to almost black on the surface, covered with a cuticular peridium-like structure, with a powdery white to gray lacuna, very deciduous from vivid white top of the stipe; upper surface almost flat, but possessing some projecting brown hyphae (except hyaline thin-walled tip) mainly at the center that give the surface a brightly spotted/pitted appearance (ca. 1.5–3 μm in diam); lower surface with a lacuna, brightly spotted/pitted appearance on the surface, but with neither prominent projecting hyphae nor holes; inner hyphae branched dichotomously toward the top, slightly projecting from the black cuticular peridium-like structure at the upper surface. Conidium-like structures sometimes produced from the phialide-like hyphal tip at the cut-off end of the synnema after the dropping of bulbils, visible only in the crushed synnemata, oblong, obovoid, hyaline, with two guttules (maybe nuclei), 5–12 × 2.5–6 μm; sporogenesis not detected clearly (looks to be phialidic or blastic).

Colonies on PDA more than 90 mm in diam after 7 d at 20°C, almost flat, producing little aerial hyphae and some Pale Orange (M. 5A3) warty stromata from which synnemata develop in clusters as the culture dries up, with very tough mycelial mat, Pale Orange (M. 5A3) to Greyish Orange (M. 5B4); hyphae dikaryotic, mostly without, but sometimes with clamp connections. Synnemata often derived from a single hypha and bulbils almost the same as those on natural substrates, but very variable in dimension; stipes of synnemata not often proliferated; upper surface of bulbils covered with hyaline mucus when fresh, but easily dried up to become flat and wrinkled in appearance. Conidium-like structures not observed at the cut-off end of the synnema after the dropping of bulbils.

Specimens examined: G. Okada (GO) 1481 and 1482 (originally as Indonesia(I)-24), on a decaying petiole of *Cocos nucifera*, leg. G. Okada, Kukusan beside the University of Indonesia, Depok, Java Isl., Indonesia, 11




Figs. 1–5. *Tretopileus sphaerophorus*.

1. Semi-diagramatic sketch of a bulbil attaching to the top of a synnema (JCM 10092): Outward view (left) showing the pigmented cuticular peridium-like structure with slightly projecting hyphae on the upper flat surface; inside view (right) showing the inner hyphae that branch dichotomously toward the top. 2. Germination from the tips of the projecting hyphae (arrow) on the upper surface of a bulbil (JCM 10092), 10 h after inoculation on PDA. 3. Hyphal regeneration from the cut-off end of a synnema, in which the tips are presumably dikaryotic (shown by two guttules), stained with acid fuchsin in lactic acid (JCM 10092); somewhat similar to certain phialides producing conidia. 4. Regenerating hyphal tips of a synnema with clamp connections (A) or hyaline hyphal fragments of a synnema with clamps and two guttules stained with phloxine in KOH solution (B) (I-24). 5. Conidium-like separated tips from the regenerated top of the synnemata having two guttules stained with phloxine (A, I-24; B, I-28). Short bar=10 μm in Fig. 1; long bar=10 μm in Figs. 2–5.

Jan. 1996. GO 1483 and 1484 (originally as I-28), BO 20737 (=GO 1485; originally as I-28), on a decaying peduncle of *Cocos nucifera*, leg. G. Okada, Kukusan beside the University of Indonesia, Depok, Java Isl., Indonesia, 11 Jan. 1996. TNS-F-182384 and the Herbarium of the University of Indonesia (Depok) 61/HD/FAM/1997 (Curator: Padmi Kramadibrata), a dried PDA culture derived from JCM 10092 [=Asian Network on Microbial Researches (ANMR) 62,=G. Okada OFC 5172], isol. ex GO 1483.

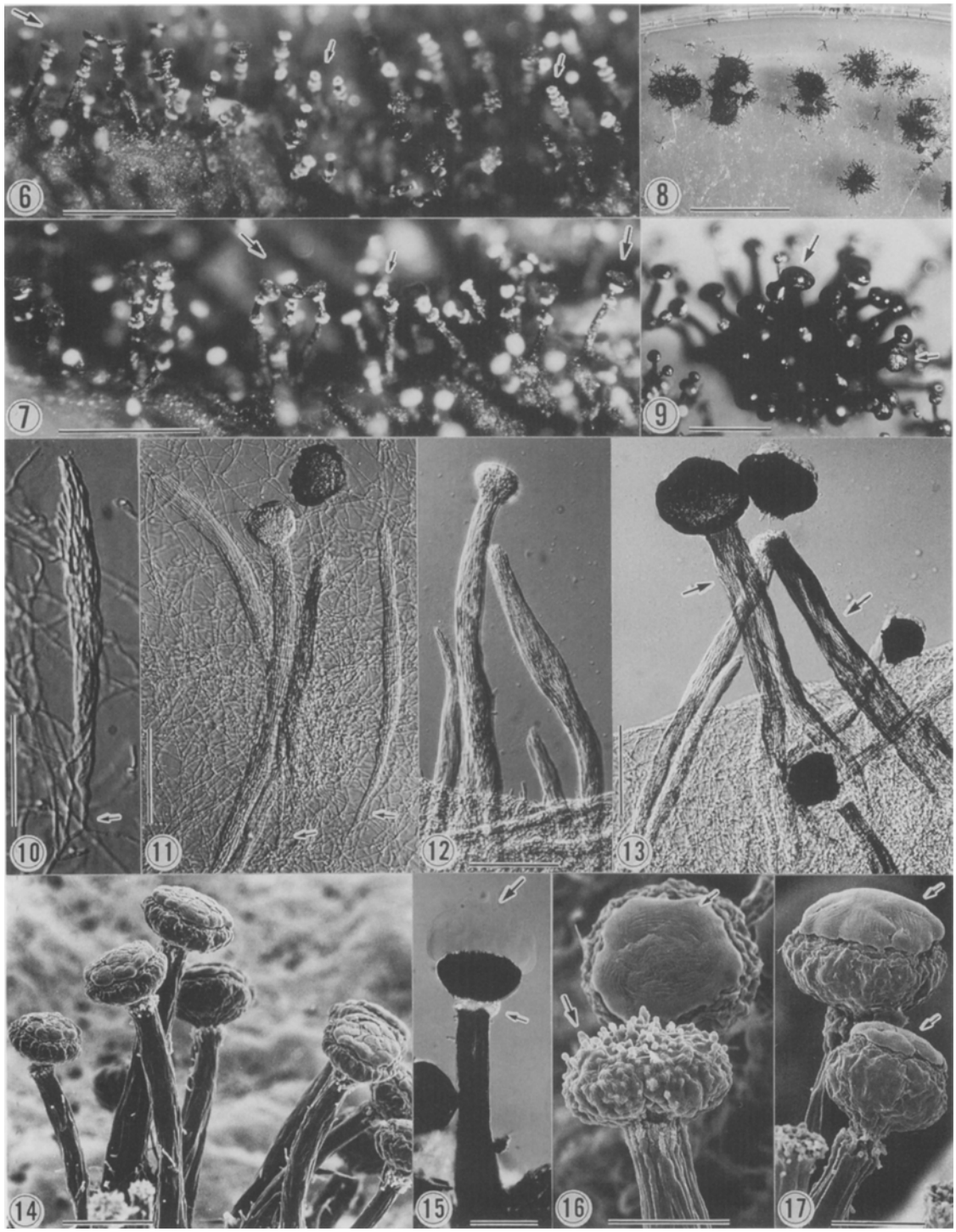
## Results and Discussion

**Morphology** Deighton (1960) classified *T. sphaerophorus* in the Mycelia Sterilia, not in the Stilbellaceae, because it produces no spores. However, we use the term "synnema" in this paper for the fructification of *T. sphaerophorus*. Synnema means a conidioma composed of a more-or-less compacted group of erect and sometimes fused conidiophores bearing conidia (Hawksworth et al., 1995). However, the fructification of *T. sphaerophorus* proliferates (Figs. 6, 7) to produce a single bulbil (strictly not a conidium) at the top. Seifert (1990) and Matsushima and Matsushima (1995) used the term "synnema" for its fructification, while others have preferred other terms (i.e., stalk, stipe and gemmiferous stipe). The bulbils of *T. sphaerophorus* are functionally a kind of multicellular propagule (Figs. 18, 22, 23) that are produced on a bundle of elect conidiophore-like hyphae (or a synnema-like hyphal stalk of the fructification) derived from a single hypha (Figs. 10, 11). In practice, it is very difficult to distinguish this structure from a synnema.

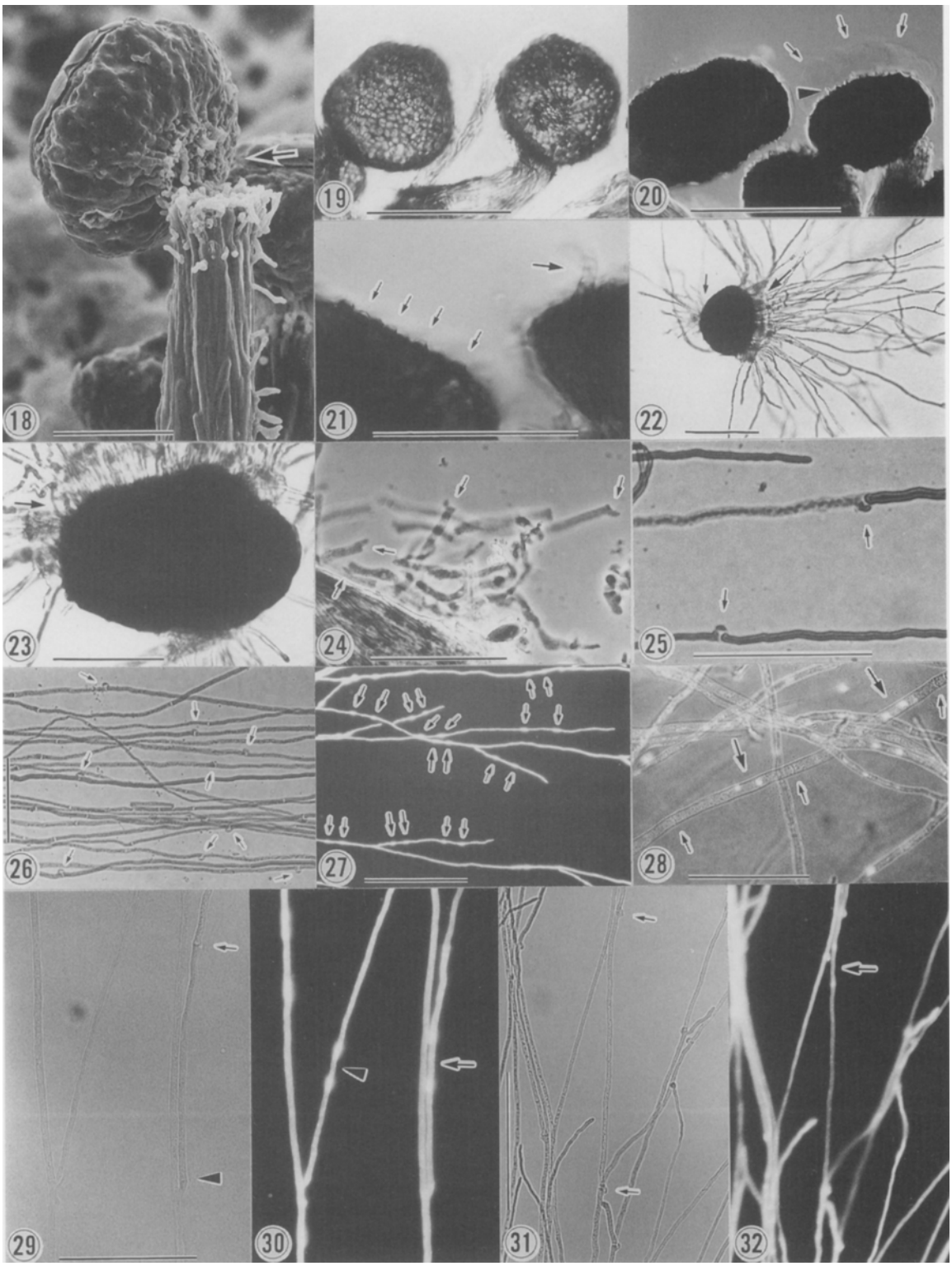
Seifert and Okada (1990) misunderstood the hyphal type of the synnema in *Tretopileus* as monomitic. As stated explicitly or implicitly by Dodge (1946), Deighton (1960), Munjal (1962) and Matsushima and Matsushima (1995), the synnemata are composed of outermost dark brown hyphae and inner hyaline hyphae (Figs. 4, 13, 35): i.e., they fall in the category "corticated with darkened marginal hyphae" (Seifert and Okada, 1990). Immature bulbils and synnemata are wholly hyaline (Figs. 10–12, 33), but the outermost hyphal layer of both becomes a dark brown cuticular sheath (Figs. 1, 3, 4A, 11, 13, 15, 19, 20, 35). On the natural substrate, we also found a few subhyaline immature synnemata, as recorded by Deighton (1960) and Munjal (1962).

Although several researchers observed the specimens and isolates of *T. sphaerophorus*, Matsushima and Matsushima (1995) were the first to find the clamp connections. They mentioned that only one isolate (Thailand strain) produced clamps on the vegetative hyphae and the outermost brown hyphae of synnemata. We confirmed the presence of clamps on some vegetative hyphae of the isolate (Figs. 29, 31) and some of the outermost brown (Fig. 4A) or inner hyaline (Figs. 4B, 24) hyphae of the synnemata on the natural substrate. Clamp connections were especially abundant in the germinating hyphae from the bulbil (Figs. 25, 26). In old cultures, most hyphae were septate and without clamps. By staining with DAPI, however, we found many paired nuclei in the vegetative hyphae (Fig. 27) and some dikaryons in the hyphae without clamp connections (Fig. 28) as well as in the clamped hyphae (Figs. 29–32). The reason why Matsushima and Matsushima (1995) found two different types of strains (i.e., one with and the other without clamp connections) is unknown, but it is likely that both strains are dikaryotic based on our observation of nuclei in the hyphae without clamps. In this respect, *T. sphaerophorus* might differ from *Antromycopsis broussonetiae* Pat. & Trabut, the synnematous anamorph of *Pleurotus cystidiosus* Miller, in which monokaryotic and dikaryotic synnematous anamorphs exist in the life cycle (Moore, 1985).

The multicellular propagules of *Tretopileus*/*Monotospora* have been referred to as spores (Berkeley and Curtis, 1868), caps (Dodge, 1946), gemmae (Deighton, 1960; Subramanian, 1971; Matsushima and Matsushima, 1995), discs (Munjal, 1962) or bulbils (Carmichael et al., 1980; Seifert, 1990). According to Hawksworth et al. (1995), the most suitable term for the propagules of *Tretopileus* is "bulbils" because of their multicellular nature. Young bulbils were hyaline (Figs. 11, 12) and their inner structures were visible (Figs. 1, 33), as mentioned by Deighton (1960). Staining young bulbils with acid fuchsin produced further good results. Dodge (1946) and Deighton (1960) both reported the inner structures of the bulbils by sectioning. As shown in Fig. 2C of Deighton (1960), a synnema was slightly constricted just below the bulbil. In our Indonesian samples, however, the hyphae were continuous between the synnema and bulbil (Figs. 1, 33). Hyphae branched maybe dichotomously to expand the hyphal mass toward the top of the bulbils (cf., Fig. 2B of Deighton, 1960). The general morphology of the bulbils of *T. sphaerophorus* is similar to that of some aero-aquatic fungi (e.g., *Aegerita* Pers.: Fr.). The young hyphal branch of *T. sphaerophorus* was presumably embedded in a hyaline substance (Fig. 33) without a distinct peridium-like structure. The dichotomous hyphal tips projected slightly from the hyaline substance in young hyaline bulbils (Fig. 33), which may finally turn black. Therefore, we think that the black cuticular peridium-like structure in the mature bulbils is the dried up substance, not a structure of fungal origin. The generic name is etymologically derived from the pores or thin places (Fig. 19) in the bulbils (Dodge, 1946). Although Seifert (1990) reported that the face of bulbils was punctate with numerous depressions (2–10 $\mu$m wide), the pellucid spot-like structures (ca. 1.5–3 $\mu$m in diam) are in fact brown thick-walled hyphae with hyaline thin-walled tips (Figs. 1, 2, 21) projecting through the outermost cuticle cover of bulbils (Figs. 1, 16, 20). Under certain optical conditions, these structures give the surface of the bulbil a brightly spotted/pitted appearance. The upper surface of bulbils was found here to be covered with hyaline mucus, especially in fresh materials (Figs. 9, 14–18, 20; Pl. 103, P-040 in Matsushima and Matsushima, 1995). The hyaline mucus was sticky when fresh, but easily dried up (Fig. 9). It is likely that the bulbils are distributed by insects and




Figs. 6–17. *Tretopileus sphaerophorus*.
6,7. Proliferated synnemata with (big arrows) or without (small arrows) a bulbil, on a dead palm (I-28). 8. Synnemata in a cluster developing from warty stromata (JCM 10092), on PDA. 9. Grouped synnemata producing bulbils of which the upper surface was covered with hyaline mucus (big arrow), which easily dried up (small arrow), on PDA. 10–12. Almost white, but partly brown young synnemata derived from a single hypha (arrows) that branched repeatedly to produce a synnematous stipe, on PDA; DIC. 13. Immature synnemata corticated with darkened marginal hyphae (arrows), on PDA; DIC. 14. Mature synnemata producing a bulbil, on PDA; SEM. 15. A mature black synnema with a white node (small arrow) between the synnematous stipe and bulbil, on which hyaline mucus (big arrow) was accumulated, on PDA; DIC. 16, 17. Bulbils bearing the projecting hyphae (big arrow) on the upper surface covered with mucus (small arrows) in a moist condition, on PDA; SEM. Bar=0.5 mm in Figs. 6, 7, 9; 5 mm in Fig. 8; 50 $\mu$m in Figs. 10, 16, 17; 100 $\mu$m in Figs. 11–15.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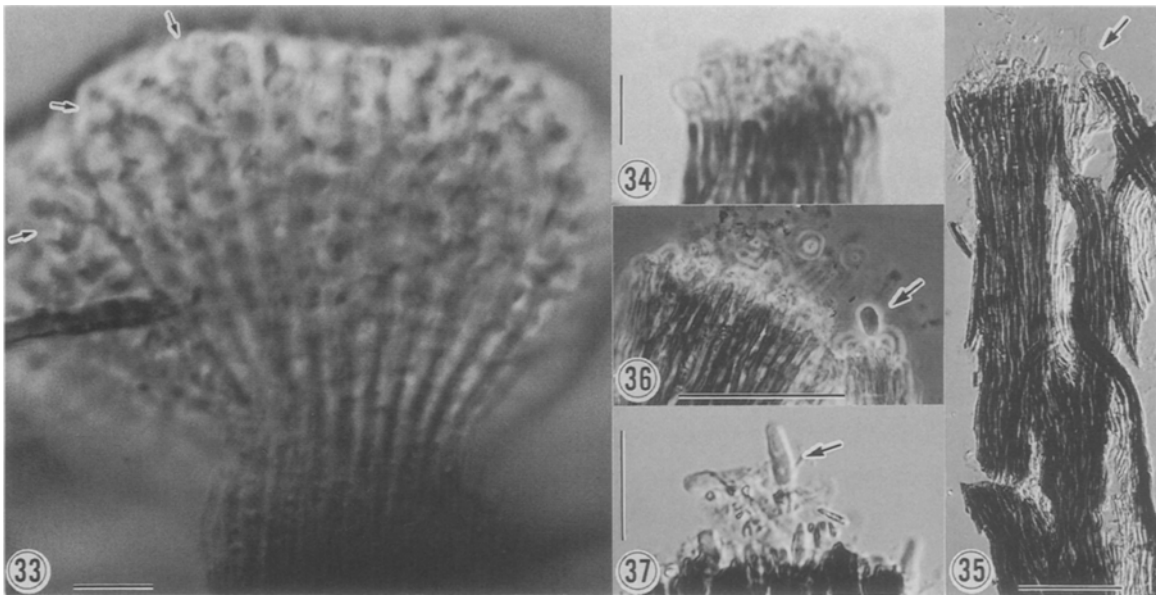


Figs. 33–37. *Tretopileus sphaerophorus*.
33. A young white bulbil stained with Acid Fuchsin in which the inner hyphae branched dichotomously (arrows) toward the top and were embedded wholly in a hyaline substance (JCM 10092), on PDA; DIC. 34. Regenerating bright-colored tip of a synnema, on PDA. 35. Crushed synnema, consisting of brown outermost and hyaline inner hyphae, with a conidium-like structure (arrow) at the regenerating tip, I-24; DIC. 36. Conidium-like structure (arrow) at the regenerating tip of a synnema, I-24; phase contrast. 37. Conidium-like separated tip of the regenerated hypha that contains two guttules (arrow), I-28; DIC. Bar=10 μm in Figs. 33, 34; 20 μm in Fig. 37; 50 μm in Figs. 35, 36.

similar vectors in wet conditions by virtue of the mucus, and by wind in dry conditions. Confirming the speculation of Dodge (1946) and Deighton (1960), we observed germination from the pellucid spots (in fact, the projecting hyphal tips; Figs. 2, 21–23), as well as from the cut-off hyphae at the lacuna (Figs. 22, 23) of the bulbils in *T. sphaerophorus*. Munjal (1962), however, did not observe the germination from the poroid markings of the bulbils in *T. indicus*.

Besides the bulbils, Dodge (1946) speculated that minute spores would finally develop inside the bulbil (cap) under certain conditions in nature and be distributed through the pores of the bulbils. He also mentioned that a few brown and two-celled spores were associated with coarse hyphae around the base of the synnema. In our Indonesian specimens and the isolate, we could not observe these spores (cf., Munjal (1962) for *T. indicus*). However, we sometimes observed hyaline, non-septate and subcylindrical conidium-like structures (Figs. 5, 35–37) produced from the phialide-like hyphal tip (Figs. 3, 4A) at the cut-off end of the synnema. These structures were not observed in normal conditions because the cut-off end of the synnema was often covered with white powdery crystalline (Figs. 6, 7, 15) and regenerated hyphae (Fig. 34), but they were visible when the synnemata on the natural substrates were crushed gently on a slide (Figs. 35–37). On the other hand, it is conceivable that these conidium-like structures were just separated fragments of the regenerated hyphal tips (Figs. 3, 4A). Staining with Phloxine/Acid Fuchsin revealed conidium-like structures containing two guttules that might be nuclei (Figs. 5, 37). Sporogenesis was not clearly detected, but appeared similar to the phialidic or blastic mode (Figs. 3, 4A). Further studies are required on this sporogenesis to confirm the germination from the conidium-like structures.

Figs. 18–32. *Tretopileus sphaerophorus*.
18. A dropping bulbil with a lacuna (arrow) (JCM 10092), on PDA; SEM. 19. Face view (left) or bottom view (right) of the young bulbils with brightly spotted/pitted appearance, on PDA. 20. Side view of mature bulbils with projecting hyphae (arrowhead) and hyaline mucus (arrows) on the upper surface, on PDA; DIC. 21. Side view of the upper surface of bulbils possessing projecting brown hyphae with a hyaline thin-walled tip (small arrows) and a germinating hypha from the projection (big arrow), on PDA; DIC. 22. Side view of a bulbil germinating from the projecting hyphae on the upper surface (big arrow) and from the bottom (small arrow), on PDA. 23. A bulbil germinating from the projecting hyphae (arrow), on PDA. 24. Hyaline hyphal fragments of a synnema with clamp connections (arrows), I-24; phase contrast. 25, 26. Hyphae growing from a bulbil with (arrows) or without clamps. 27. Hyphae of the isolate with paired nuclei (paired arrows) stained with DAPI, on PDA; FM. 28. Paired nuclei (big arrows) stained with DAPI in a septate (small arrows) hypha, on PDA; FM. 29, 30. Clamped (arrow in Fig. 29) and septate (arrowhead in Fig. 29) hyphal cell containing two nuclei (arrow in Fig. 30), and paired nuclei (arrowhead in Fig. 30), on PDA; FM. 31, 32. Clamped (small arrows in Fig. 31) hypha containing two nuclei (big arrow in Fig. 32), on PDA; FM. Bar=50 μm in Figs. 18, 21, 23–26, 28, 29; 100 μm in Figs. 19, 20, 22, 27, 31.

The molecular data shown below indicate that *T. sphaerophorus* is closely related to the Aphyllophorales. Like the members of the Aphylloporales in general, its colony is very tough and difficult to cut with a hook needle. Although Stalpers (1978) summarized the wood-inhabiting Aphyllophorales in pure culture, *Tretopileus*-like anamorphs were not mentioned in his review.

**Host and distribution** *Tretopileus* species were collected mainly from the dead parts of various plants in tropical/subtropical countries (Table 1). Although *T. sphaerophorus* IMI 141217 could not be revived from the culture in mineral oil (personal communication from N. Smith), the strain was also isolated from the same plant material (i.e., decaying inflorescence of *Cocos nucifera* in Thailand) as in our Indonesian collections. All the isolates grew very well on usual agar media (Dodge, 1946; Munjal, 1962; Matsushima and Matsushima, 1995; this paper). As mentioned by Dodge (1946) and Munjal (1962), we also think that *Tretopileus* species are saprophytic or very weakly parasitic, not strongly parasitic on the plants. Therefore, the distribution of the *Tretopileus* species may be pantropic (Deighton, 1960; Munjal, 1962; Seifert, 1990).

***Tretopileus sphaerophorus* vs. *T. indicus*** Based on the bigger bulbils and stipes, Munjal (1962) established the second species of *Tretopileus*, *T. indicus*, comparing the dimensions of these structures with those in *T. sphaerophorus* reported by Dodge (1946). On *T. sphaerophorus*, later researchers (Deighton, 1960; Subramanian, 1971; Seifert, 1990; Matsushima and Matsushima, 1995; this paper) reported much wider variation in these dimensions, as well as in the color of the fructification, on different plant substrates (Table 1). In particular, Deighton (1960) stated that *T. sphaerophorus* varied somewhat in morphology according to the substratum (host plant) and climatic differences. The Indonesian isolate of *T. sphaerophorus* (JCM 10092) produced on the colony surface many warty stromata from which the synnemata developed (Fig. 8; see also Dodge (1946) and Matsushima and Matsushima (1995)), and Munjal (1962) observed similar stromata in *T. indicus* using an isolate from *Thevetia neriifolia* Juss. ex Steudel. Although we have not yet observed the type (or ex-type culture) of *T. indicus*, it seems that *T. indicus* is a morphological variation of *T. sphaerophorus*. The name might be treated as a synonym of *T. sphaerophorus* in future.

**Molecular phylogeny based on 18S rDNA sequences** Using the determined 18S rDNA sequence (AB006005, 1755 bp), the homology search system in DDBJ, FASTA

Table 1. Comparison of selected characters of *Tretopileus sphaerophorus* and *T. indicus* on natural substrates.

| | Synnema[a] | | Bulbil[a] | | Substrate | Locality |
|---|---|---|---|---|---|---|
| | Length | Width[b] | Diam | Thickness | | |
| *Tretopileus sphaerophorus* | | | | | | |
| This paper | 80–400 | 15–80 | 80–150 | 30–50 | Decaying petiole and peduncle of *Cocos nucifera* | Indonesia |
| Berkeley and Curtis (1868) | — | — | ca. 100[c] | — | Dead stems of *Cajanus cajan* (Congo bean) | Cuba |
| Dodge (1946) | 200–500 | 20–40 | 60–80 | 20–30 | Pads of *Opuntia ammophila* and related species | USA |
| Deighton (1960)[d] | 80–750 | 20–80 | 53–137 | 35–60 | Dead twigs of *Cajanus cajan*, *Citrus limon*, and *Thevetia neriifolia*; shrivelled stem of *Hibiscus esculentus* | Cuba, Sierra Leone |
| Subramanian (1971) | 80–750 | 20–70 | 60–130 | 35–60 | Dead twigs of *Thevetia peruviana* (*T. neriifolia*), *Cajanus cajan*, *Lantana* sp., *Tecoma* sp. | Cuba, India, Sierra Leone, USA |
| Seifert (1990) | 75–800 | 20–80 | 50–140 | 20–60 | Twigs of *Cajanus cajan*, *Citrus* sp., *Hibiscus* sp., *Inga* sp., *Lochnera rosea*, *Opuntia* sp., *Thevetia* sp. | Sierra Leone, Indonesia, Bahamas, Cuba, Paraguay, USA |
| Matsushima and Matsushima (1995) | Variable in size, but agreeable to Deighton (1960) | | | | Decaying leaves of a broad-leaved tree; decaying twigs of broad-leaved trees | Thailand, Malaysia, Taiwan |
| *Tretopileus indicus* | | | | | | |
| Munjal (1962) | 400–700 | 35–75 | 130–175 | 40–80 | Dead or dying twigs of *Thevetia neriifolia* | India |

a) μm. b) Width at the top or base. c) 0.004 inch. d) Data on the dimensions of synnemata and bulbils represent the combined measurements made by Deighton (1969) on specimens from Cuba and Sierra Leone.

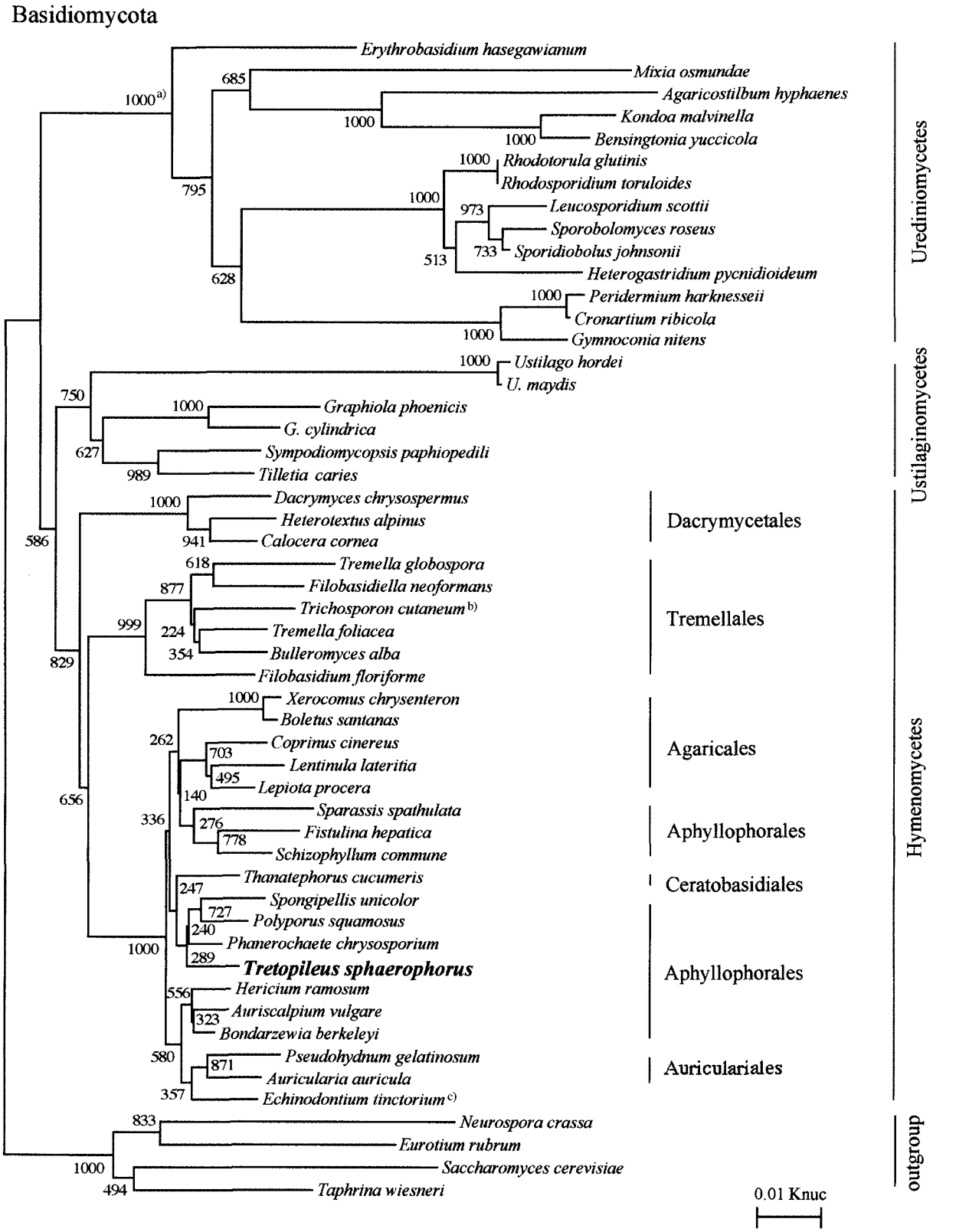


Fig. 38. NJ tree of the Basidiomycota inferred from 18S rDNA sequences, showing the disposition of *Tretopileus sphaerophorus*. The NCBI/GenBank classification system was used. a) Bootstrap values were calculated from 1,000 replications. b) Mitosporic species. c) Order not yet decided in the system.

ver 3.0, was used to search for the closest relative of *T. sphaerophorus*. FASTA showed that *T. sphaerophorus* was closely related to *Phanerochaete chrysosporium* Burdsall U59084 (Anamorph, *Sporotrichum pruinosum* Gilman & Abbott; Stalpers, 1984), classified in the Corticiaceae (Aphyllophorales) in the NCBI/GenBank classification system or in the Meruliaceae (Stereales) in the Dictionary (Hawksworth et al., 1995). Other relatives were *Daedalea quercina* L.: Fr. U59067 (Polyporaceae (Aphyllophorales) in the former; Coriolaceae (Poriales) in the latter), *Fomes fomentarius* (L.: Fr.) Fr. U59069 (Polyporaceae (Aphyllophorales) in the former; Coriolaceae (Poriales) in the latter) and *Panus rudis* Fr. U59086 (a synonym of *Lentinus strigosus* (Schwein.) Fr.; Polyporaceae (Aphyllophorales) in the former; Lentinaceae (Poriales) in the latter), and all are aphyllophoralean fungi.

We then created a NJ tree using the partial sequence data set (1408 sites) of *T. sphaerophorus* and various members of the Basdiomycota to infer the phylogenetic relationships of *T. sphaerophorus* (Fig. 38). Using the DNAML program in the Phylip package ver. 3.51c, we also analyzed the same data set by the maximum likelihood method. Although a trial ML tree is not shown in this paper, both NJ and ML phylogenetic trees led to the same conclusion that *T. sphaerophorus* was included to the Aphyllophorales (Fig. 38). However, the members of the Aphyllophorales were divided into two groups and the bootstrap values were very low in the cluster where *T. sphaerophorus* was included. Basic topology in the NJ tree showing the relationships between the Hymenomycetes, Ustilaginomycetes and Urediniomycetes was almost the same as that of Swann and Taylor (1995).

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