Mycoscience 42: 433–437, 2001

New records of minute litter-decomposing basidiomycetes from Japan. *Actiniceps thwaitesii* (Pterulaceae) new to Japan and *Anastrophella podocarpicola* sp. nov. (Tricholomataceae)

Isshin Tanaka[1] and Tsuguo Hongo[2]

[1] Lead Discovery Research Laboratories, Sankyo Co., Ltd., 33, Miyukigaoka, Tsukuba, Ibaraki 305–0841, Japan
[2] 3–3–7, Oogaya, Otsu, Shiga 520–2144, Japan

Received 16 April 2001
Accepted for publication 23 July 2001

Actiniceps thwaitesii **(Pterulaceae) new to Japan and** ***Anastrophella podocarpicola*** **sp. nov. (Tricholomataceae) are described and illustrated. The former is characterized by forming stipitate-capitate basidiomata under 1 mm tall with encrusted cystidia and acanthophyses. The latter, occurring on a decaying leaf of** ***Podocarpus macrophyllus*****, differs from other taxa of** ***Anastrophella*** **in having undifferentiated hymenial cystidia and smaller basidiospores and basidia. Their cultural features are also presented.**

Key Words——*Actiniceps thwaitesii*; *Anastrophella podocarpicola*; new record; new species.

During recent surveys of litter-decomposing basidiomycetes in Japan, two minute species were collected and their multisporous isolates were obtained. These species are described and illustrated with their cultural features.

Materials and Methods

Descriptions of macroscopic and microscopic features are based on dried specimens. Dried basidiomata were rinsed with 70% ethanol, then transferred to distilled water for rehydration. Rehydrated basidiomata were mounted for micromorphological observation in distilled water, 3% KOH plus Congo red and phloxine, or Melzer's reagent. Color terms and notations are taken from Kornerup and Wanscher (1978). Spore statistics included: $\bar{x}$, the arithmetic mean of the spore length and spore width (±SD) for n spores measured; Q, the quotient of spore length and spore width in any one spore, indicated as a range of variation in n spores measured; and $\bar{Q}$, the mean of Q-values (±SD).

Spore deposits were obtained on potato dextrose agar (PDA; Nissui, Tokyo) and subsequent multisporous cultures were isolated. Cultures were grown on PDA and malt extract agar (MEA) (Nobles, 1965). The Nobles species code (Nobles, 1965) was recorded for 4-wk-old cultures on PDA. Extracellular oxidase reactions were tested on 4-wk-old cultures on PDA according to Desjardin (1990).

Descriptions

Actiniceps thwaitesii Berk. & Broome, J. Linn. Soc. Bot. **15**: 85. 1877.

=*Dimorphocystis capitata* Corner, Ann. Bot. Mem. **1**: 695. 1950. ("capitatus") Figs. 1, 2, 5

Basidiomata 0.3–0.6 mm tall, stipitate-capitate, white. Capitula 0.1–0.2 mm diam, globose at maturity. Stipe 0.2–0.3×0.02–0.05 mm, cylindrical, insititious. Basidiospores 6.6–9.4×3.3–4.6 μm ($\bar{x}$=7.8±0.8×3.9±0.4 μm; Q=1.7–2.8; $\bar{Q}$=2.0±0.3; n=20), oblong to subcylindrical, smooth, hyaline, thin-walled, inamyloid. Basidia 19–30×5–7 μm, narrowly clavate, 2-spored; sterigmata 4.0–4.5 μm long. Cystidia 47–115×7–10 μm, conical, thickly encrusted, projecting up to 40 μm beyond hymenial surface, often secondary septate, thick-walled up to 1.5 μm, hyaline, inamyloid; crystals very slowly soluble in 3% KOH. Acanthophyses 20–44×4–7 μm, narrowly clavate, often slightly encrusted, densely beset with outgrowths, projecting 12–20 μm beyond hymenial surface, thin- to thick-walled up to 1.5 μm, hyaline, inamyloid; outgrowths up to 2.5 μm long, 0.5–2.0 μm wide. Stipe hyphae 2–4 μm diam, glassy, parallel, tubular, smooth, thick-walled up to 1.5 μm, hyaline, irregularly turgescent in 3% KOH, dextrinoid. Caulocystidia absent. Clamp connections absent.

Cultural features. Macromorphology on PDA: Mean radius of colony at 23°C is 20 mm, 1 wk (n=4); 31 mm, 2 wk; 40 mm, 3 wk; over 45 mm, 4 wk. Advancing zone appressed. Aerial mycelium wooly, dense, white. Reverse yellowish white (3A2). Odor indistinct. Macromorphology on MEA: Mean radius of colony at 23°C is 12 mm, 1 wk (n=4); 23 mm, 2 wk; 34 mm, 3 wk; 42 mm, 4 wk. Aerial mycelium wooly, sparse, white. Reverse white. Odor indistinct. Micromorphology on

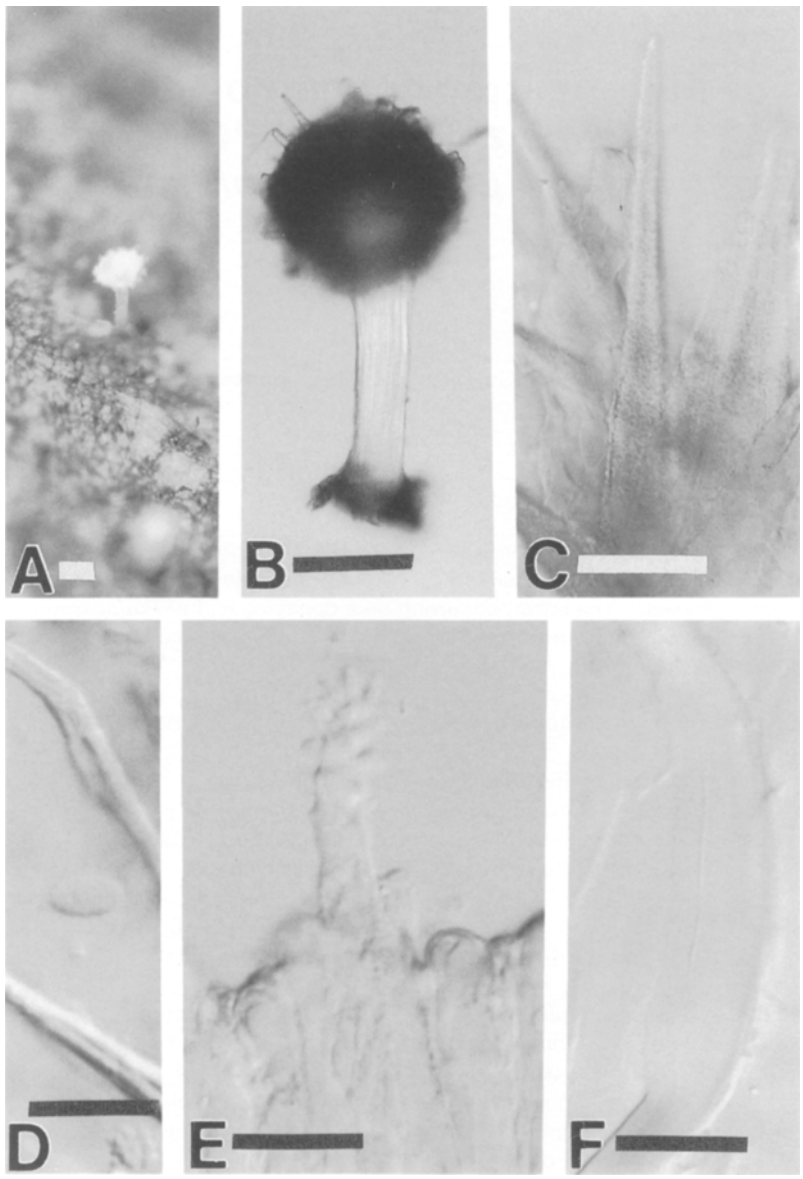

Fig. 1. *Actiniceps thwaitesii*. A, Basidioma on a decaying leaf; B, Basidioma; C, Cystidia; D, Basidiospores; E, Acanthophyses; F, Swollen stipe hypha in 3% KOH. Scales: A, B=0.1 mm; C–F=10 μm.

PDA: Advancing zone hyphae 1–4 μm diam, tubular. Aerial hyphae 1.5–3 μm diam, tubular, forming mycelial strands, smooth, hyaline, thin-walled, inamyloid; mycelial strands 20–35 μm diam, abundant. Submerged hyphae 1.5–4 μm diam, tubular, often interlocking. Conidia not observed. Clamp connections not observed. Micromorphology on MEA: Micromorphology was almost the same as that on PDA. Phenoloxidase reactions (PDA): Laccase (−), Tyrosinase (−), Peroxidase (−). Nobles species code (PDA): 1, 6, 16, 32, 36, 38, 44, 55.

Habit, habitat and distribution. Caespitose to solitary, on decaying leaves. Java, Ceylon, Indonesia, Japan.

Specimen examined. Japan: Iriomote island, Taketomi-cho, Okinawa-ken, on a decaying leaf of evergreen tree (remoistened and incubated in the laboratory), 24 Mar. 1999, coll. Issh. Tanaka TRL-MU-1240 (TNS-F-2347) (Culture SANK 10600).

Notes. Three species have been accepted in *Actiniceps* Berk. & Broome. The specimen (TRL-MU-1240) accorded well with *A. thwaitesii* redescribed by Boedijn (1959) in having a combination of small basidiomata under 1 mm tall, oblong to subcylindrical basidiospores measuring 6.6–9.4×3.3–4.6 μm, encrusted cystidia, and in the absence of caulocystidia. The other species of this genus, *A. laevis* (Corner) Boedijn and *A. subcapitata* (Corner) Boedijn, differ from TRL-MU-1240 in the size of basidiomata and basidiospores, and in the presence of caulocystidia.

In *A. thwaitesii*, the number of basidiospores on basidia is a matter of controversy. Boedijn (1959) observed only 2-spored basidia, while Hjortstam et al. (1990) considered this taxon mainly had 4-spored

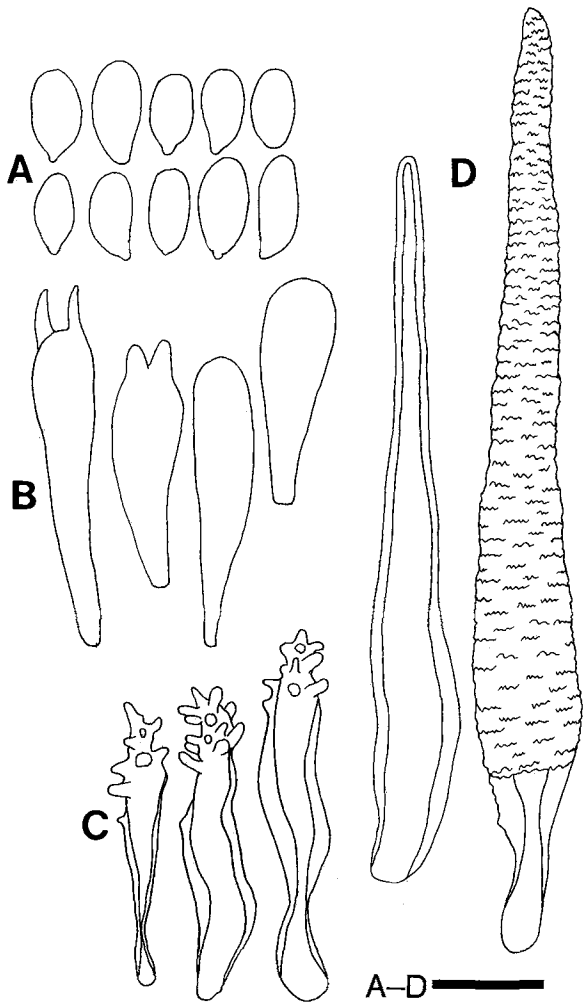

Fig. 2. *Actiniceps thwaitesii*. A, Basidiospores; B, Basdia and basidioles; C, Acanthophyses; D, Cystidia. Scales: A–D = 10 μm.

basidia. In TRL-MU-1240, only 2-spored basidia were observed.

The solubility of crystals on cystidia and swollen stipe hypha in 3% KOH, and the dextrinoidity of stipe hypha observed in TRL-MU-1240 were not mentioned in previous descriptions of *A. thwaitesii* (Boedijn, 1959; Corner, 1950).

Actiniceps thwaitesii is the type species of the genus *Actiniceps*. Berkeley and Broome (1877) described only the morphology of sporophores, spicules and spores of *A. thwaitesii*, leaving its taxonomic position uncertain. Boedijn (1959) observed basidia and basidiospores in this taxon and placed it in basidiomycetes. Boedijn (1959) also reduced the genus *Dimorphocystis* Corner, previously described as a minute basidiomycete with two kinds of projecting strile cells, to a synonym under *Actiniceps*. Later, Corner (1961) pointed out that *Actiniceps* might be an anamorph of *Dimorphocystis*, because basidia and basidiospores were not observed in the type specimen of this taxon. Our study, showing that conidia were observed neither on TRL-MU-1240 nor on culture, might support Boedijn's reduction of *Dimorphocystis*.

Anastrophella podocarpicola Issh. Tanaka, sp. nov.
Figs. 3–5

Basidiomata 0.3–0.6 mm alta, brebissime stipitata, sicco brunnea. Pilei usque ad 0.6 mm diam, disciformes. Hymenia laevia, epigena. Stipites usque ad 0.2 mm longi, centrali, cylindracei, insititii. Basidiosporae 5.4–7.7 × 2.6–3.4 μm, subcylindraceae, hyalinae, leaves, inamyloideae. Basidia 17–30 × 5–6.5 μm, 4-spora, clavata. Cystidia hymenii nulla. Pileipellis hymeniformes, ex cellulis vesiculosis vel clavatis 9–26 × 4.5–20 μm compositae. Pileocystidia 25–55 × 5–10(–13) μm, fusiformia, apice subcapitata et exsudato resinoso adhaerenti praedita. Caulocystidia variabilia. Hyphae fibulatae.

Basidiomata 0.3–0.6 mm tall, stipitate-discoid, brown when dry. Pileus up to 0.6 mm diam, upper surface fertile and smooth. Under a lens, magnification of × 25, resinous exudates observed on the apex of pileocystidia. Stipe up to 0.2 mm long, 0.04–0.1 mm wide, often indistinct, central, cylindrical, insititious. Basidiospores 5.4–7.7 × 2.6–3.4 μm ($\bar{x}$ = 6.4 ± 0.6 × 2.9 ± 0.2 μm; Q = 1.9–2.7; $\bar{Q}$ = 2.2 ± 0.2; n = 23), subcylindrical, smooth, hyaline, thin-walled, inamyloid. Basidia 17–30 × 5–6.5 μm, clavate, 4-spored; sterigmata 3 μm long. Hymenial cystidia absent. Pileipellis hymeniform, composed of vesiculate to clavate cells 9–26 × 4.5–20 μm, thin- to thick-walled. Pileocystidia 25–55 × 5–10(–13) μm, narrowly fusiform to fusiform, subcapitate at the apex, projecting up to 30 μm beyond pileipellis, thin- to thick-walled, with resinous exudates adherent to the apex; resinous exudates persist after drying, soluble in Melzer's reagent and in 3% KOH. Caulocystidia variable in shape, narrowly fusiform with subcapitate apex like pileocystidia or narrowly conical to cylindrical. Stipe tissue monomitic; cortical hyphae 2–3.5 μm diam, parallel, tubular, smooth, hyaline, thin-walled, inamyloid; medullary hyphae 2–8 μm diam, inflated, otherwise similar to cortical hyphae. Clamp connections present.

Cultural features. Macromorphology on PDA: Mean radius of colony at 23°C is 10 mm, 1 wk (n = 4); 16 mm, 2 wk; 23 mm, 3 wk; 33 mm, 4 wk. Advancing zone appressed, radially sulcate, often felty. Aerial mycelium cottony, white to light brown (5C6). Reverse brown (7D6). Brown pigment diffused in the agar. Exudate abundant, hyaline to brown. Odor indistinct. Macromorphology on MEA: Mean radius of colony at 23°C is 9 mm, 1 wk (n = 4); 19 mm, 2 wk; 29 mm, 3 wk; 40 mm, 4 wk. Advancing zone appressed. Aerial mycelium cottony, white to yellowish white (3A2). Reverse yellowish white (3A2) to pale yellow (3A3). Odor indistinct. Micromorphology on PDA: Advancing zone hyphae 1.5–3.5 μm diam, tubular. Aerial hyphae 1.5–4 μm diam, tubular, smooth, hyaline to light brown, thin-walled to slightly thick-walled up to 0.5 μm diam, with terminal swellings at the center of colony, inamyloid; terminal swellings narrowly clavate, thin-walled to slightly thick-walled, with clamp connections at the base. Submerged hyphae 1.5–3.5 μm diam, often interlocking, with hyphal

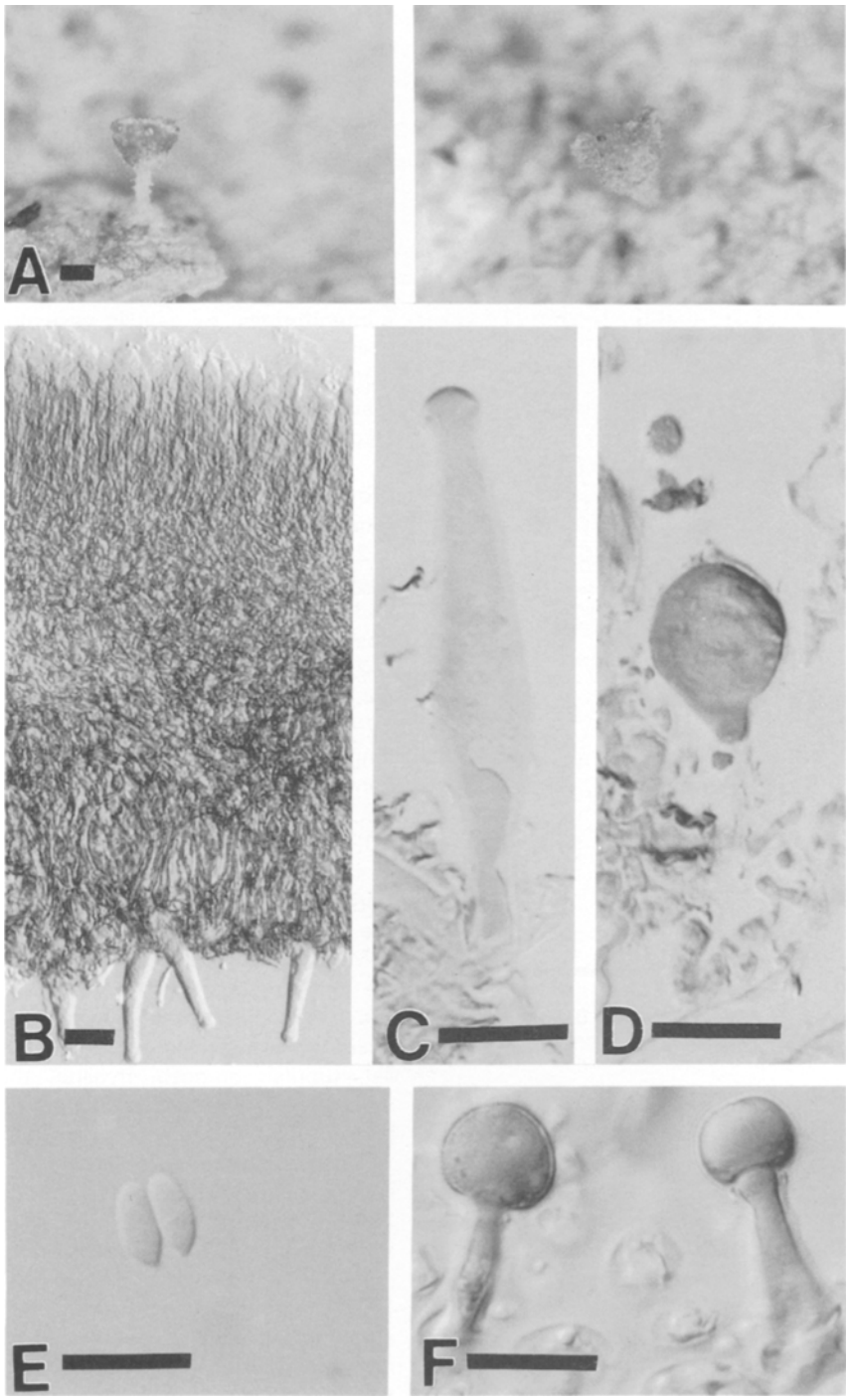

Fig. 3. *Anastrophella podocarpicola*. A, Basidiomata; B, Section of pileus; C, Pileocystidium; D, Vesiculate cell in pileipellis; E, Basidiospores; F, Pileocystidia with resinous exudates adherent to the apex in water. Scales: A=0.1 mm; B–F=10 μm.

swelling up to 4 μm diam. Conidia not observed. Clamp connections rare and only observed at the center of colony. Micromorphology on MEA: Micromorphology was almost the same as that on PDA. Phenoloxidase reactions (PDA): Laccase (+), Tyrosinase (−), Peroxidase (−). Nobles species code (PDA): 2, 3, (13), 26, 32, 37, 39, 46, 55.

Habit, habitat and distribution. Solitary on decaying leaves. Japan.

Holotypus: On decaying leaves of *Podocarpus macrophyllus* var. *maki*, Ootawara-shi, Tochigi-ken, Japan, 6 Jun. 1997, coll. Issh. Tanaka TRL-MU-541 (TNS-F-2348) (Culture SANK 39800).

Notes. *Anastrophella podocarpicola* is characterized by its stipitate-discoid basidiomata, subcapitate pileocystidia with resinous exudates adherent to the

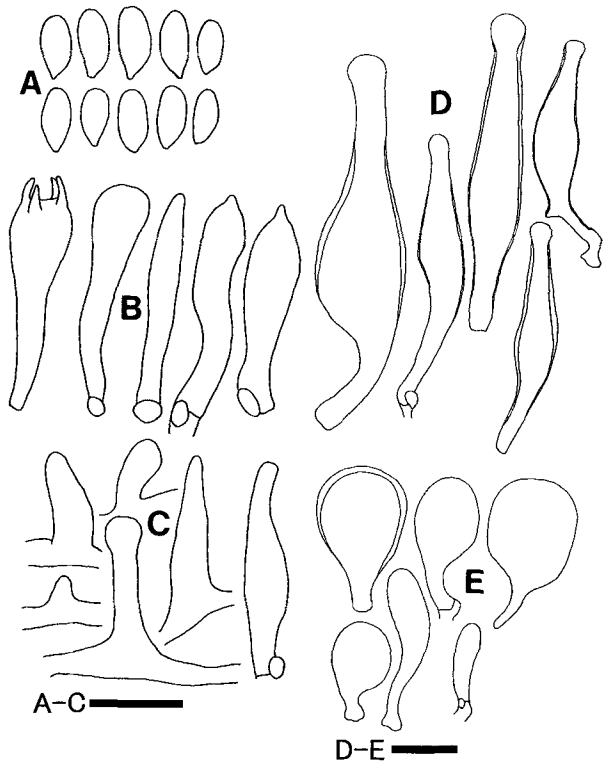

Fig. 4. *Anastrophella podocarpicola*. A, Basidiospores; B, Basidium and basidioles; C, Caulocystidia; D, Pileocystidia; E, Cells in pileipellis. Scales: A–E = 10 μm.

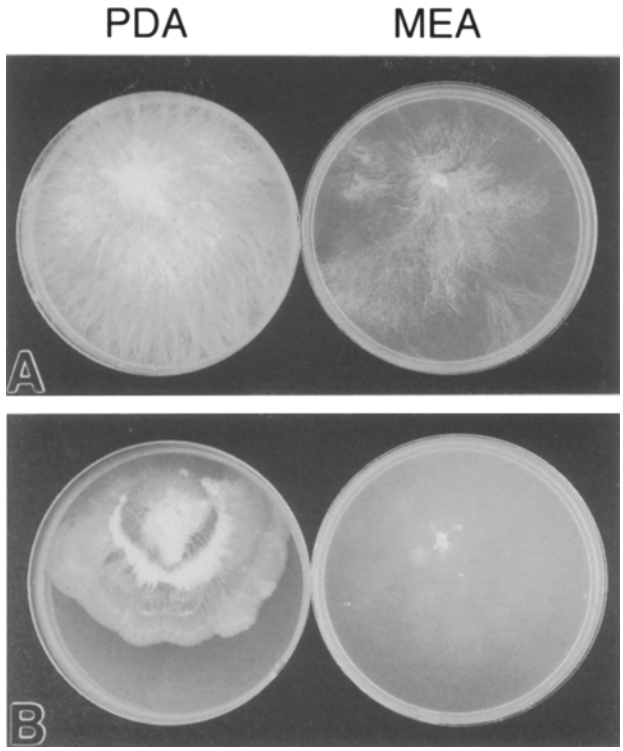

Fig. 5. Cultures of *Actiniceps thwaitesii* and *Anastrophella podocarpicola*. A, *Actiniceps thwaitesii*; B, *Anastrophella podocarpicola*. All cultures were 6 wk old.

apex, and undifferentiated hymenial cystidia. These morphological features suggest affinity with *A. subpeltata* (Redhead) E. Horak & Desjardin (Redhead, 1979; Horak and Desjardin, 1994). However, *A. subpeltata* is different from *A. podocarpicola* in its larger basidiospores (10–12.5 × 4.5–5 μm) and basidia (32–33 × 9–9.5 μm).

Sterile or abnormal basidioma formation of *A. subpeltata* was observed in two different cultures (Redhead, 1979). However, *A. podocarpicola* formed no basidiomata or primordia in the present study.

Acknowledgements——We wish to thank Dr. T. Hosoya for his helpful suggestions.

Literature cited

Berkeley, M. J. and Broome, C. E. 1877. Supplement to the enumeration of fungi of Ceylon. J. Linn. Soc. Bot. **15**: 82–86.

Boedijn, K. B. 1959. The genus *Actiniceps* Berk. & Br. Persoonia **1**: 11–14.

Corner, E. J. H. 1950. A monograph of *Clavaria* and allied genera. Ann. Bot. Mem. **1**: 1–740.

Corner, E. J. H. 1961. A note on *Wiesnerina* (Cyphellaceae). Trans. Br. Mycol. Soc. **44**: 230–232.

Desjardin, D. E. 1990. Culture morphology of *Marasmius* species. Sydowia **42**: 17–87.

Hjortstam, K., Spooner, B. M. and Oldridge, S. G. 1990. Some Aphyllophorales and Heterobasidiomycetes from Sabah, Malaysia. Kew Bull. **45**: 303–322.

Horak, E. and Desjardin, D. E. 1994. Reduced marasmioid and mycenoid agarics from Australasia. Aust. Syst. Bot. **7**: 153–170.

Kornerup, A. and Wanscher, J. H. 1978. Methuen handbook of colour. 3rd ed. Eyre Methuen, London.

Nobles, M. K. 1965. Identification of culture of wood-inhabiting Hymenomycetes. Can. J. Bot. **43**: 1097–1139.

Redhead, S. A. 1979. *Physalacria subpeltata* sp. nov. from Hawaii. Mycotaxon **10**: 46–48.