

Two new species of *Heleococcum* with *Acremonium* anamorphs

Shun-ichi Udagawa¹⁾, Shigeru Uchiyama²⁾ and Seigo Kamiya²⁾

¹⁾Nodai Research Institute, Tokyo University of Agriculture, 1-1, Sakuragaoka 1-Chome, Setagaya-ku, Tokyo 156, Japan

²⁾New Drug Discovery Research Laboratories, Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., 3 Ookubo, Tsukuba-shi, Ibaraki 300-33, Japan

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Two new species of *Heleococcum* (a cleistothecial nectrioid genus in the Hypocreaceae) are described and illustrated. *Heleococcum alatosporum*, isolated from Indonesian soil, is recognized by the production of salmon-colored ascomata, cylindrical asci, and hyaline, small, bicellular ascospores with walls that are verruculose and ornamented with longitudinal ridges. *Heleococcum inapertum*, isolated from Philippine soil, is characterized by yellow ascomata, clavate asci, and pale yellow, middle-sized, bicellular, verruculose to weakly striate ascospores surrounded with a hyaline sheath. Anamorphs of the new species are included in *Acremonium*. A key to the accepted species of the genus is provided.

Key Words—*Acremonium*; ascomycetes; *Heleococcum*; Hypocreales; soil fungi.

The genus *Heleococcum*, based on *H. aurantiacum* Jørg., was erected by Jørgensen in 1922. The type species was isolated from humid soil in the botanical gardens of the University of Copenhagen (Jørgensen, 1922). It is characterized by non-ostiolate, translucent, areolated ascomata, globose asci, large orange-colored, bicellular ascospores, and no anamorph. *Heleococcum aurantiacum* did not produce its ascomata in culture. In 1960, Dennis found an additional collection of *H. aurantiacum* on mushroom compost in England, but did not mention its cultural details. Dennis (1978) placed this genus in the Eurotiaceae (=Trichocomaceae), and noted its affinity with the Nectriaceae (=Hypocreaceae). Currently, a majority of taxonomists who have dealt with hypocrealean fungi have assigned the genus to the Hypocreaceae (Rogerson, 1970; Cannon et al., 1985; Eriksson and Hawksworth, 1993).

The second species of the genus, *H. japonense* Tubaki, was isolated by Tubaki from a wood panel in the sea in Hokkaido, Japan (Tubaki, 1967). The culture of this fungus has been maintained at the Institute for Fermentation, Osaka (IFO 8643), Japan, and still produces its ascomata and conidia. *Heleococcum japonense* differs from the first species in its non-areolated ascomatal wall, rough-walled ascospores and the presence of an *Acremonium*-like anamorph.

As Malloch (1970) implied, *Heleococcum* appears to be a cleistothecial counterpart of *Nectria* Fr. In a recent molecular systematic study (from Abstracts, Fifth International Mycological Congress, 1994), Rehner and Samuels stated that gene phylogenies for the nuclear ribosomal large subunit RNA and nuclear encoded orotidine-5'

monophosphate decarboxylase are congruent and provide a robust phylogenetic statement of relationships among teleomorph genera of the Hypocreales, including several genera with derived (cleistothecial) ascocarps. In their study, they examined the culture of *H. japonense* as a representative of *Heleococcum*. There seems to be no reason to regard *Heleococcum* as separate from *Nectria*, because recently there has been a tendency to evaluate the presence or absence of an ostiolum as being of minor significance (von Arx, 1973; Cailleux, 1971; Krug et al., 1983). However, the placement of *Heleococcum* species in *Nectria* remains unresolved, since there is no molecular systematic analysis for the type species *H. aurantiacum*.

During an exploratory survey of soil-borne microfungi as producers of secondary metabolites useful to the pharmaceutical industry, two nectrioid ascomycetes were recently isolated from tropical soils in Southeast Asia. These isolates, characterized by light colored, non-ostiolate ascomata, hyaline or pale yellow, bicellular ascospores and hyaline conidia of the *Acremonium*-type, appear to be previously undescribed species in the genus *Heleococcum* and are herein described as new. A key to the known species is presented.

Taxonomy

Heleococcum alatosporum Udagawa, Uchiyama et Kamiya, sp. nov. Figs. 1, 2

Coloniae in agar farina avenae mixto paulo effusae, plus minusve floccosae, radiatim sulcatae, ex mycelio basali coacto tenuiter constantes, ascomatibus

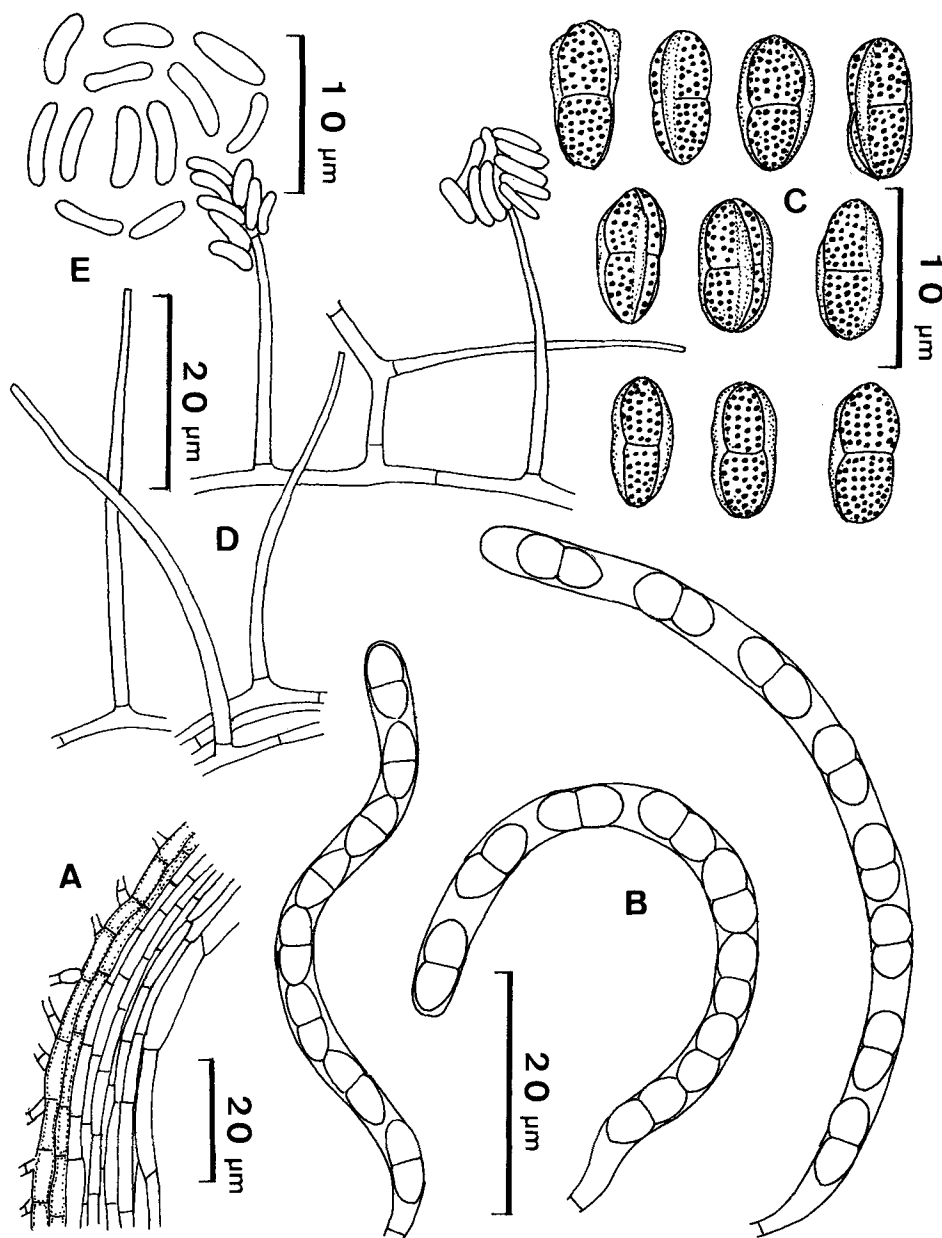


Fig. 1. *Heleococcum alatosporum*, BF 47300.

A. Part of lateral ascomatal wall. B. Asci. C. Ascospores. D. Conidiogenous cells and conidia. E. Conidia.

salmoneis abundantibus formantes, cum hyphis aeriis albis et conidiis laxè obtectae; conidiogenesis conspicua; reversum cremeum vel bubalinum. Coloniae in agarò cum decocto tuberorum et carotarum (PCA) paulo effusae, floccosae, planae, tenues, ex mycelio vegetativo submerso constantes, albae, ad centrum ascomatibus persicinis abundantibus formantes, ex hyphis aeriis et conidiis laxè obtectae; reversum persicinum vel salmoneum.

Ascomata non stromatica, superficialia, dispersa vel gregaria, salmonea vel carnea, non ostiolata, globosa vel subglobosa, 250–450 µm diam, cum hyphis hyalinis sinusis septatis levibus vel grosse asperatis 1.5–2.5 µm diam dense obtectae; peridium 15–20 µm crassum, membranaceum, ex “textura intricata” vel “textura angularis”

compositum. Asci irregulariter dispositi, saepe tortuosi, 8-sporei, cylindracei, 70–90 × 3.5–4 µm, brevi-stipitati, sine apparatus apicali, evanescentes; paraphyses nullae. Ascosporeae uniseriatae, bicellulares, hyalinae, ellipsoideae, (6–)7–8 × 3–3.5 µm, aequè uniseptatae, ad septum parum constrictae, verruculosae, cum duabus vel tribus alis longitrorsum ornatae. Status anamorphus: *Acremonium* sp.

Holotypus BF 47300, colonia exsiccata in cultura ex solo sativa, Jogjakarta, in Java, Indonesia, 14.iii.1994, a S. Uchiyama et S. Kamiya isolata et ea collectione fungorum, Musei et Instituti Historiae Naturalis Chiba (CBM) conservata.

Etymology: from Latin, *alatus*=winged, *sporus*=seed, referring to the winged ascospores.

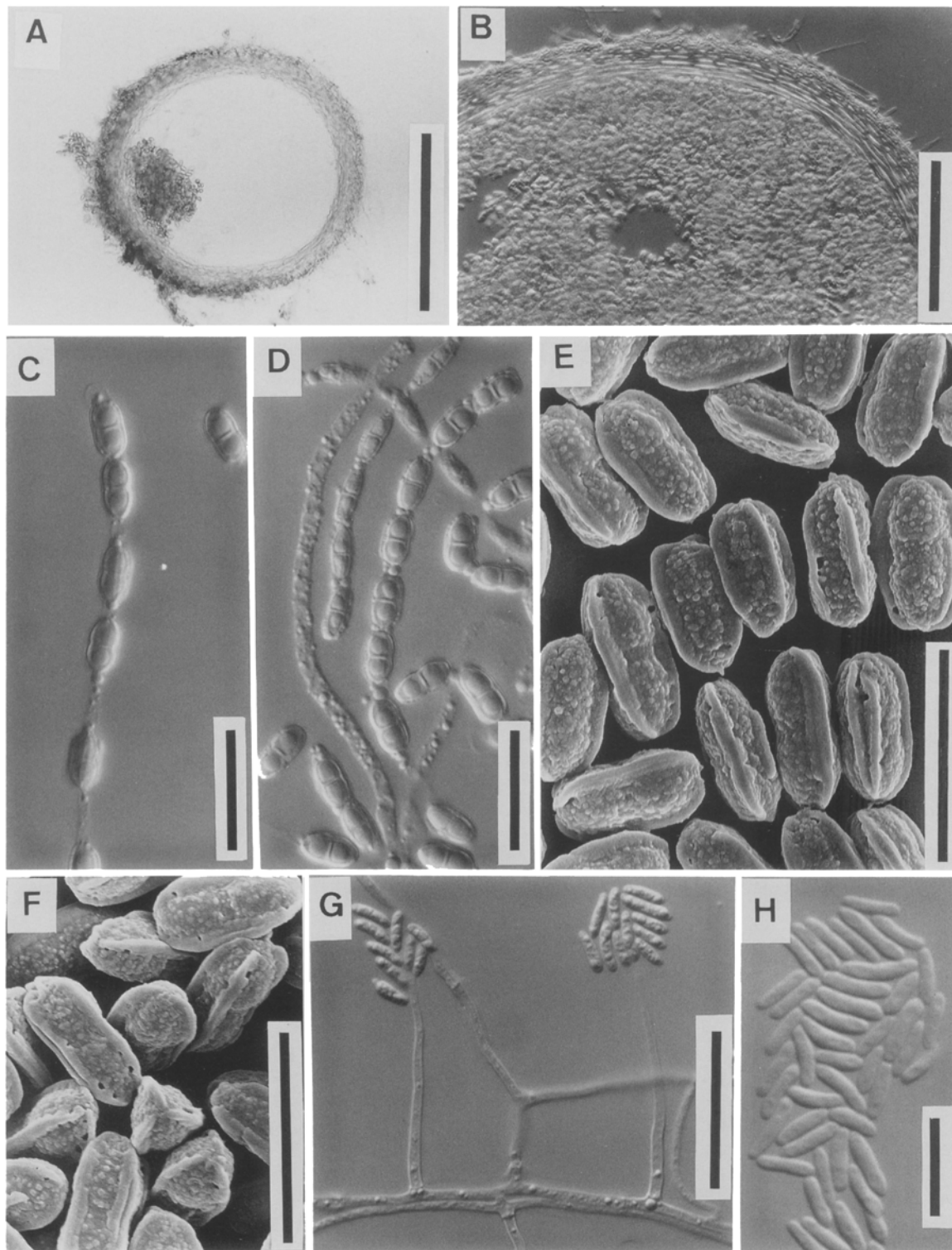


Fig. 2. *Heleococcum alatosporum*, BF 47300.

A. Section through a mature ascoma. B. Section through lateral ascomatal wall. C, D. Asci. E, F. Ascospores (SEM). G. Conidiogenous cells and conidia. H. Conidia. Scale bars: A=200 μm ; B=50 μm ; C-F, H=10 μm ; G=20 μm .

Anamorphosis: *Acremonium* sp.

Mycelium ex hyphis hyalinis ramosis levibus septatis 1-2.5 μm diam compositum, saepe aggregatum in fasciculis. Conidiophora semi-macronemata vel micronema-

ta, mononemata, vulgo simplicia, hyalina, levia, 1.5-2 μm diam, basi septata. Cellulae conidiogenae (monophialides) hyalinae, subulatae, rectae vel plus minusve sinuatae, 25-60 \times 1-1.5 μm , leves, collari minu-

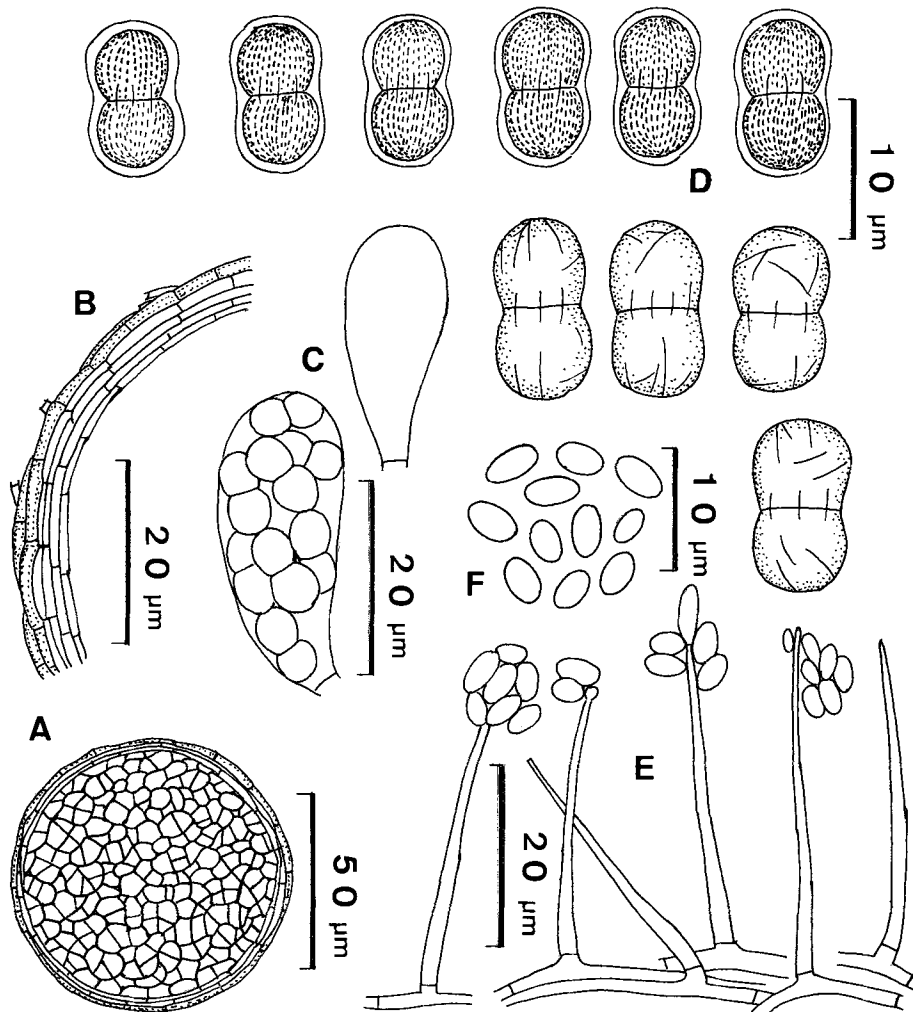


Fig. 3. *Heleococcum inapertum*, BF 42524.

A. Ascoma. B. Part of lateral ascomatal wall. C. Ascus. D. Ascospores. E. Conidiogenous cells and conidia. F. Conidia.

to terminatae. Conidia in capitulis mucidis connexa, hyalina, cylindracea vel allantoidea, (4-)5-8 × 1-1.5 µm, levia, in uno apice vel utrinque inconspicue apiculata. Status teleomorphus: *Heleococcum alatosporum*.

Holotypus BF 47300, loc. cit.

Colonies on oatmeal agar growing rather rapidly, attaining a diameter of 26-28 mm in 14 days at 25°C, more or less floccose, radially sulcate, consisting of a thin basal felt, producing abundant ascomata on the felt, Light Orange (M. 6A4, after Kornerup and Wanscher, 1978) or Salmon (Rayner, 1970), loosely covered by white aerial hyphae and conidial heads; margins thin, entire; conidiogenesis conspicuous; exudate clear, often in large droplets; odor moldy; reverse Pale Yellow (M. 4A3) or Buff (R). Colonies on PCA growing rather rapidly, attaining a diameter of 28-30 mm in 14 days at 25°C, floccose, plane, thin, vegetative mycelium submerged, white, centrally Pastel Red (M. 7A4) or Peach (R) due to the development of abundant ascomata, covered by a loose network of aerial hyphae and conidial heads; exudate limited at the center, clear; odor indistinct; reverse Pastel Red (M. 7A4) or Salmon (R).

Ascomata non-stromatic, superficial, scattered or gregarious in small clusters, salmon to flesh, not changing color in 3% KOH and lactic acid, non-ostiolate, globose to subglobose, 250-450 µm in diam, glabrous, densely covered with hyaline, sinuous, septate, smooth or coarsely roughened, 1.5-2.5 µm wide hyphae; peridium 15-20 µm thick, membranaceous, textura intricata and textura angularis, salmon or pale red; outer layer consisting of thick-walled, pale colored, hyphal cells; inner layer of hyaline, rather angular, thin-walled, flattened cells measuring 10-40 × 6-20 µm. Asci irregularly and often tortuously arranged in the cavity, 8-spored, cylindrical, mostly curved, 70-90 × 3.5-4 µm, rounded above, short-stalked, thin-walled, without apical apparatus, evanescent; paraphyses lacking. Ascospores uniseriate in the ascus, two-celled, hyaline, ellipsoidal, (6-)7-8 × 3-3.5 µm, equally uniseptate, slightly constricted at the septum, with verruculose surface, striate with two or three longitudinal wing-like ridges, without sheath.

Mycelium consisting of hyaline, branched, smooth-walled, septate, 1-2.5 µm wide hyphae, often forming bundles. Conidiophores semi-macronematous or

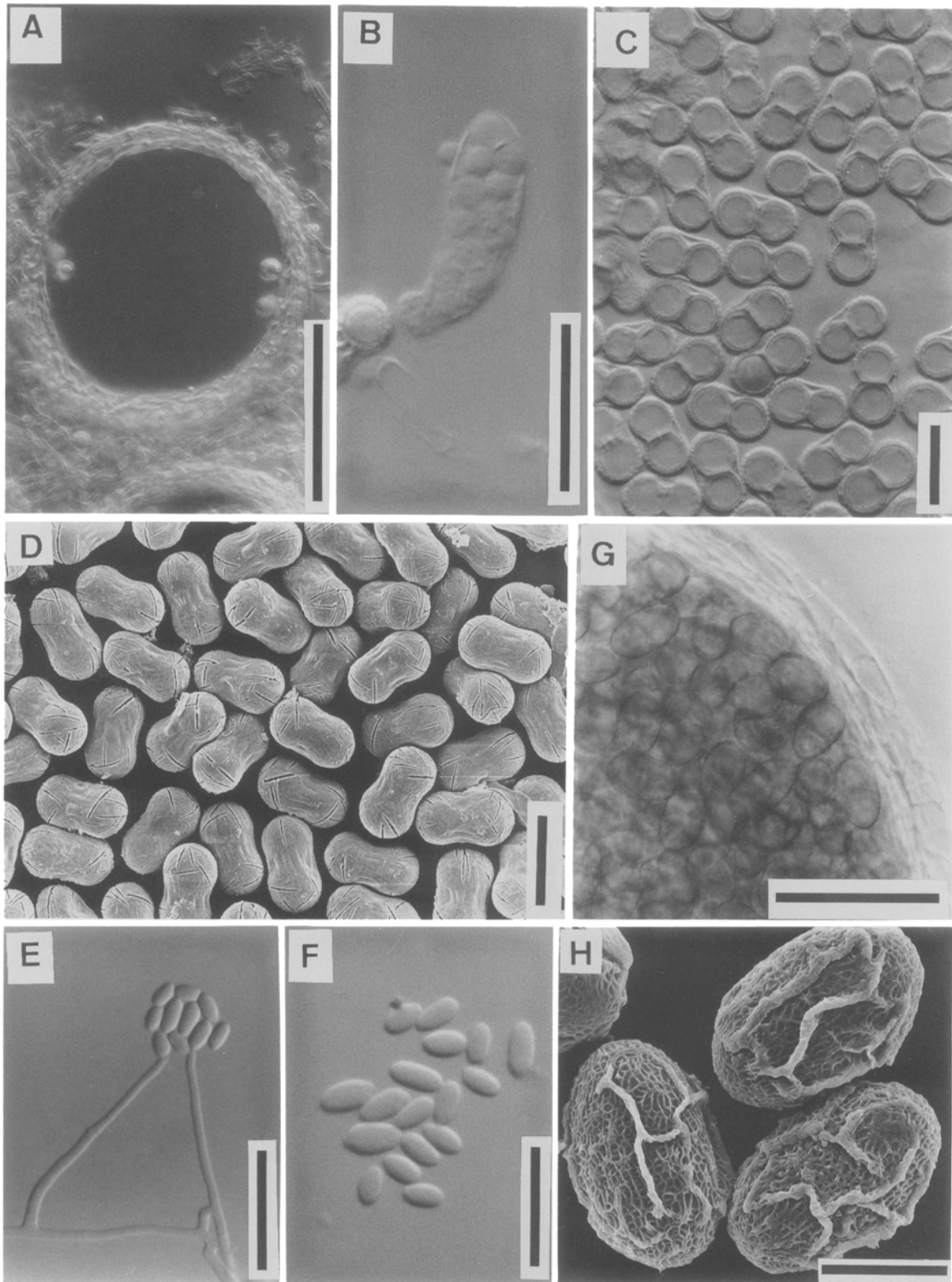


Fig. 4. A-F. *Heleococcum inapertum*, BF 42524; G, H. *Heleococcum japonense*, IFO 8643. A. Ascoma. B. Ascus. C. Ascospores. D. Ascospores (SEM). E. Conidiogenous cells and conidia. F. Conidia. G. Section through lateral ascomatal wall. H. Ascospores (SEM). Scale bars: A=50 μm ; B, E=20 μm ; C, D, F=10 μm ; G=50 μm ; H=10 μm .

micronematous, mononematous, usually unbranched, hyaline, smooth-walled, 1.5–2 μm wide, basally septate, integrated into conidiogenous cells (phialides). Phialides hyaline, subulate, straight or slightly sinuous, 25–60 \times 1–1.5 μm , smooth-walled, collarettes small, not flared, peridinal thickening not obvious. Conidia aggregated in slimy heads measuring 10–16 μm in diam, hyaline, cylindrical to allantoid, (4–)5–8 \times 1–1.5 μm , smooth-walled, subapiculate at one or both ends.

At 37°C, growth is reduced and ascomata are not produced.

Specimen examined: BF 47300 (holotype), in dried culture isolated from soil in paddy field near Borobudur, Jogjakarta, Central Java, Indonesia, 14 March 1994. The holotype has been deposited with the Natural History Museum and Institute, Chiba, Japan (CBM).

In the formation of long cylindrical asci and small striate ascospores, *H. alatosporum* has a closer affinity with members of *Nectria* than with other known species of *Heleococcum*. There are several species of *Nectria* which are characterized by hyaline, small (less than 10 μm in length), striate ascospores and *Acremonium* or *Acremonium*-like anamorphs (Samuels et al., 1990, 1991), but *H. alatosporum* is readily distinguished from similar species of *Nectria* by its non-ostiolate ascomata, irregularly arranged asci and the unique ascospore ornamentation that is composed of two or three wing-like ridges.

Heleococcum inapertum Udagawa, Uchiyama et Kamiya, sp. nov. Figs. 3, 4

Coloniae in agar cum decocto tuberorum (PDA) effusae, funiculosae, ad centrum rugosae et radiatim sulcatae, ex mycelio basali coacto compacto constantes, ascomatibus abundantibus formantes, ex hyphis aeriis albis vel flavis obtectae, dilute aurantiacae vel roseo-bubalinae; conidiogenesis profuse formans; reversum incoloratum vel cremeum. Coloniae in PCA effusae, primo madidae, planae, tenues, ex mycelio vegetativo submerso constantes, deinde funiculosae, albae, ad centrum ascomatibus tarde formantes; conidiogenesis abundans; reversum incoloratum vel flavo-album.

Ascomata non stromatica, semiimmersa vel immersa, dispersa, flava vel cinereo-flava, non ostiolata, globosa vel subglobosa, 50–115 μm diam, glabra; peridium tenue, 6–10 μm crassum, hyalinum, semitranslucidum, membranaceum, ex "textura angularis" compositum. Asci irregulariter dispositi, 8-sporei, clavati vel late clavati, 20–32 \times 10–14 μm , brevi-stipitati, sine apparatus apicali, evanescentes; paraphyses nullae. Ascosporae oblique uniseriatae vel biseriatae, bicellulares, dilute flavae, ellipsoideae, 10–11 \times 5–6 μm , aequae uniseptatae, ad septum constrictae, incrassatae, verruculosae vel aliquantum striatae, in vagina gelatinosa inclusa. Status anamorphus: *Acremonium* sp.

Holotypus BF 42524, colonia exsiccata in cultura ex solo sylvae, Laguna, Los Banos, in Philippinis, 23.ii.1993, a S. Uchiyama et S. Kamiya isolata et ea collectione fungorum, Musei et Instituti Historiae Naturalis Chiba (CBM) conservata.

Etymology: from Latin, *inapertus* = not open, referring to the cleistothecial ascomata.

Anamorphosis: *Acremonium* sp.

Mycelium ex hyphis hyalinis ramosis levibus septatis 1–3 μm diam compositum, saepe aggregatum in fasciculis. Conidiophora semimacronemata vel micronemata, mononemata, simplicia, hyalina, levia, 1.5–2 μm diam, basi septata. Cellulae conidiogenae (monophialides) hyalinae, cylindraceae vel subulatae, rectae vel plus minusve arcuatae, 20–80 \times 1.5–2 μm , leves, collari minuto terminatae. Conidia hyalina, ovoidea vel ellipsoidea, 3–4.5(–6.5) \times 1.5–2.5 μm , levia, in uno apice inconspicue apiculata, in capitulis mucidis connexa. Status teleomorphus: *Heleococcum inapertum*.

Holotypus BF 42524, loc. cit.

Colonies on PDA growing rapidly, attaining a diameter of 30–35 mm in 14 days at 25°C, funiculose, centrally wrinkled, radially sulcate, consisting of a tough basal felt, producing abundant ascomata into the basal mycelium or the substratum, overgrown by a loose network of white to yellowish aerial hyphae, Pale Orange (M. 6A3) or Rosy Buff (R) in color; conidiogenesis very profuse; exudate clear, often collecting in large droplets at the center; odor lacking; reverse uncolored to Pale Yellow (M. 4A3). Colonies on PCA growing rapidly, attaining a diameter of 36–40 mm in 14 days at 25°C, wettish in appearance, plane, thin, vegetative mycelium largely submerged, with a limited development of funiculose hyphae, white in color; ascomata slowly produced into the substratum in central area; conidiogenesis abundant but not affecting the colony appearance; exudate clear, small; reverse uncolored or Yellowish White (M. 4A2).

Ascomata non-stromatic, semi-immersed to immersed, scattered, Pastel Yellow to Greyish Yellow (M. 2A4–2B4), not changing color in 3% KOH and lactic acid, non-ostiolate, globose to subglobose, 50–115 μm in diam, glabrous, loosely covered with aerial hyphae; peridium thin, 6–10 μm thick, hyaline, semitransparent, membranaceous, textura angularis, 3–5-layered, consisting of thin-walled, flattened, angular cells measuring 4–10 μm in diam. Asci irregularly disposed in the cavity, 8-spored, clavate to broadly clavate, 20–32 \times 10–14 μm , short-stalked, thin-walled, without apical apparatus, evanescent; paraphyses lacking. Ascospores obliquely uniseriate to biseriate in the ascus, two-celled, pale yellow, ellipsoidal, 10–11 \times 5–6 μm , equally uniseptate, constricted at the septum, thick-walled, verruculose to weakly striate with short ridges that are usually longitudinal in arrangement, surrounded by a 0.5 μm thick almost smooth sheath which is often cracked.

Mycelium consisting of hyaline, branched, smooth-walled, septate, 1–3 μm wide hyphae, often forming bundles. Conidiophores semi-macronematous or micronematous, mononematous, unbranched, hyaline, smooth-walled, 1.5–2 μm wide, basally septate, integrated into conidiogenous cells (phialides). Phialides hyaline, cylindrical to subulate, straight or slightly curved, 20–80 \times 1.5–2 μm , smooth-walled, collarettes small, not flared, peridinal thickening not obvious. Conidia aggregated in slimy heads up to 10 μm in diam, hyaline,

ovoid to ellipsoidal, $3-4.5(-6.5) \times 1.5-2.5 \mu\text{m}$, smooth-walled, with a faintly apiculate base.

At 37°C , growth is reduced and ascomata are not produced.

Specimen examined: BF 42524 (holotype), in dried culture isolated from rhizosphere soil in forest near the campus of the University of the Philippines at Los Banos, the Philippines, 23 February 1993. The holotype has been deposited with the Natural History Museum and Institute, Chiba, Japan (CBM).

Heleococcum inapertum differs from *H. japonense* (Fig. 2, G-H) in producing yellowish ascomata and smaller ascospores that are constricted at the septum and with weakly striate wall instead of finely reticulate one in the latter species.

Key to species of *Heleococcum*

1. Ascospores exceeding $15 \mu\text{m}$ in length 2
Ascospores not exceeding $15 \mu\text{m}$ in length 3
2. Ascomata light orange, areolate; asci globose; ascospores hyaline to pale orange, $25-30 \times 10-15 \mu\text{m}$, not constricted at the septum, smooth-walled; anamorph lacking *H. aurantiacum* Jørgensen
Ascomata pale orange-buff to orange; asci subglobose to ovoid; ascospores hyaline, $18-21 \times 10-13 \mu\text{m}$, not constricted at the septum, with finely reticulate surface; anamorph *Acremonium*-like
..... *H. japonense* Tubaki
3. Ascomata salmon to flesh; asci cylindrical; ascospores hyaline, $6-8 \times 3-3.5 \mu\text{m}$, slightly constricted at the septum, with verruculose surface and with 2-3 longitudinal wing-like ridges; anamorph *Acremonium* *H. alatosporum* Udagawa et al.
Ascomata yellow; asci clavate; ascospores pale yellow, $10-11 \times 5-6 \mu\text{m}$, constricted at the septum, with verruculose to weakly striate surface, surrounded by sheath; anamorph *Acremonium*
..... *H. inapertum* Udagawa et al.

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