

***Arthrinium japonicum* Pollack & Benjamin from its
natural habitat in Japan**

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横山 竜夫*・原田 幸雄**：日本の自生地で初めて確認された
Arthrinium japonicum Pollack & Benjamin

Summary

Arthrinium japonicum, a basauxic Hyphomycete originally reported from the United States as an introduced fungus on straw packing from Japan, was found on the dead leaves and culms of *Carex dispalata* in Hirosaki, Aomori Prefecture, Japan. This is the first record of this fungus from its natural habitat. Some cultural characteristics are also described based on the Japanese isolate IFO 30500, and two additional collections in Aomori Prefecture are reported.

Arthrinium japonicum Pollack & Benjamin was first found in the United States on the wet straw packing around a shipment of roses from Japan which had been intercepted by the Plant Quarantine Division, ARS, USDA, at the entry port San Francisco (Pollack and Benjamin, 1969).

However, our efforts to find this fungus in the natural habitat in Japan have been unsuccessful.

In October of 1977, the junior author found an almost same fungus on the dead leaves and culms of *Carex dispalata* Boott which was collected at the Tennozawa Trail, Oinomori, Hirosaki, Aomori Prefecture, Japan. Apparently the same fungus was also found on the same host plant at two additional locations in Aomori Prefecture.

Comparing this fungus with the original description and the type specimen of *A. japonicum* kindly sent us by the National Fungus Collections, together with those of hitherto described related species of the genus *Arthrinium*, it was concluded that the present fungus is conspecific with *A. japonicum*. These three Japanese specimens, however, revealed some differences from the original description in having longer conidiophores and conidia and also thinner conidiophores. In the original description, the conidiophores were reported to be 6 μm wide and less than 90 μm long and the conidia were 38-48(-56) μm long, while the conidiophores of the Japanese specimens are up to

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160 μm and the conidia are mostly 42–56 μm , averaging 48.9 μm long.

Irrespective of the original description, however, direct examination of the type specimen showed that there were no significant differences in these points as well as other characteristics between the Japanese collections and the type specimen.

Since the original description has been given in detail, it is faithfully copied in this paper with a few minor corrections and additions.

***Arthrinium japonicum* Pollack & Benjamin**, *Mycologia* 61: 187. 1969. Figs. 1–3, Pls. 1 & 2.

Colonies amphigenous but mostly hypophyllous, superficial, discrete, pulvinate, circular to elongate, 0.2–0.5 mm in diam or (0.2–)0.5–0.7 \times 0.3–0.5 mm, black, velvety, compact, consisting of densely packed conidiophores with conidia and sterile cells. Superficial mycelium composed of a network of branched and anastomosing, septate,

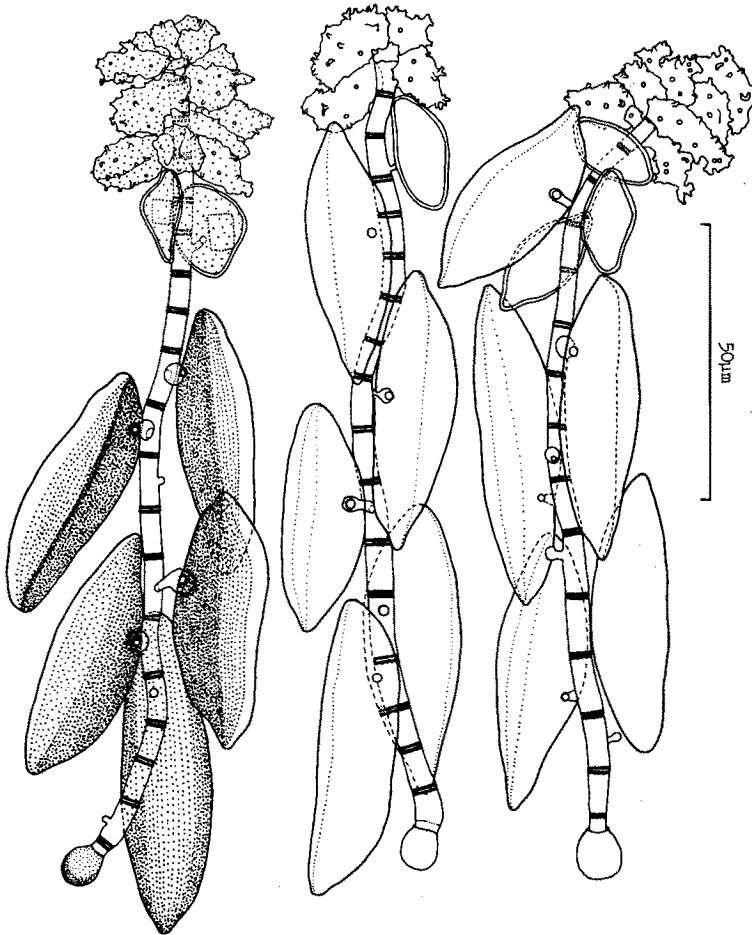


Fig. 1. *Arthrinium japonicum*. Conidial structures.

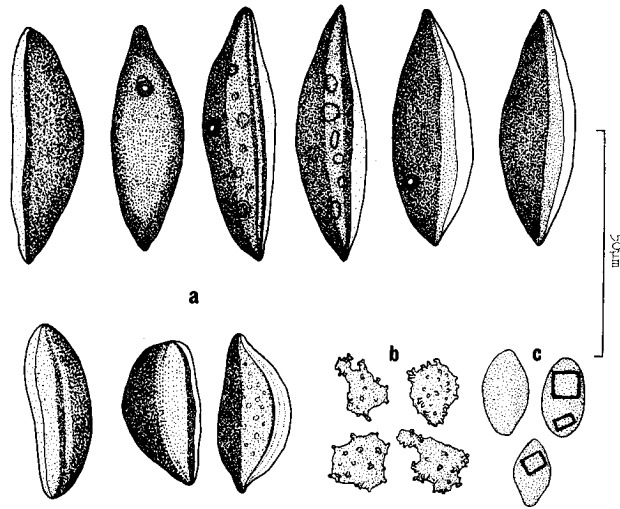


Fig. 2. *Arthrimum japonicum*. Conidia (a), papillate sterile cells (b) and smooth sterile cells (c).

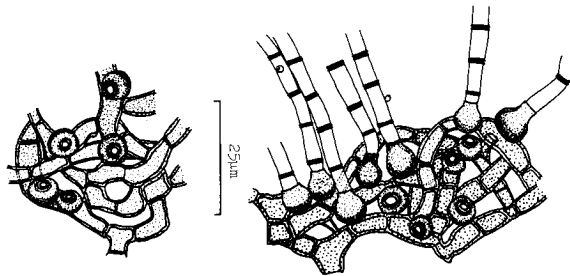


Fig. 3. *Arthrimum japonicum*. Superficial mycelial network and conidiophore mother cells.

smooth, thick-walled, brown to dark brown, $8-14 \times 2-4 \mu\text{m}$ hyphae. Conidiophore mother cells subspherical to lageniform, $6-8(-10) \times 5-7 \mu\text{m}$, subhyaline to pale brown, darker and thick-walled at the base, paler above. Conidiophores arising from the conidiophore mother cells, of basauxic type, erect or ascending, simple, flexuous or nearly straight, cylindrical, thin- and smooth-walled, hyaline, with very thick and brown transverse septa at $8-14 \mu\text{m}$ intervals, $60-150(-160) \times 3-4 \mu\text{m}$, bearing conidia laterally and clusters of sterile cells terminally or subterminally. Conidia normally fusiform in face view and navicular in side view, but often variable in shape and size, $(40-48-56(-60) \times 14-16(-18) \mu\text{m}$, with convex side dark brown, with concave side commonly swollen and pale brown, often with a distinct hyaline rim, with an attachment pore of $1.5-1.8 \mu\text{m}$ diam on convex side connected by short hyaline pegs of $3-4 \times 2-3 \mu\text{m}$. Sterile cells pale olivaceous brown, ellipsoid, thin- and smooth-walled, $16-24 \times 10-14 \mu\text{m}$, but very often irregularly lobed with distinct papillae or branched projections, $14-20 \times 8-12 \mu\text{m}$, clustered near the apex of the conidiophores forming a shield over the conidial structures,

often containing one or more highly refractive cubical bodies.

Habitat: On leaves and culms of *Carex* spp.

Distribution: Japan (Honshu).

Specimens examined: *Arthrinium japonicum*, on straw packing (?*Carex* sp.) introduced from Japan, San Francisco, 23 January 1968, P. R. Frick (BPI 11769); on dead leaves of *Carex dispalata* Boott ("Kasasuge"), Aomori Pref., Hirosaki, Oinomori, Tennozawa Trail, 28 October 1977, Y. Harada (IFO H-11799=IFO 30500); Nishi-tsugaru-gun, Fukaura-machi, Kodojigawa Trail, 23 April 1978, Y. Harada (IFO H-11800); Kita-tsugaru-gun, Shiura-mura, Sannosawa Trail, 8 April 1979, Y. Harada No. 10027 (IFO H-11802=IFO 30798).

Other specimen examined: *Arthrinium caricicola* Kunze ex Ficinus & Schubert, on *Carex ericetorum* Poll., Norway, Asker, Langåra, Norway Akershus Co., 24 May 1964, H. B. Gjaerum.

According to the original description given by Pollack and Benjamin, this fungus most closely resembles *A. caricicola* Kunze ex Ficinus & Schubert (Cooke, 1954; Ellis, 1965, 1971; Gjaerum, 1966), but differs from the latter in having wider conidia with tapering ends and irregular-shaped papillate and lobed sterile cells.

Cultural characteristics

Germination of conidia

Conidia of this fungus easily germinated within 1–2 days after seeding on MYA (malt extract 5 g, yeast extract 0.5 g, agar 20 g, distilled water 1,000 ml) at 20 C under 12 hr-photoperiod with a fluorescent lamp (daylight type, 500 lux). Germination of conidia may occur bipolarly or laterally.

Culture on agar media

Colonies on PSA (potato 200 g, sucrose 20 g, agar 20 g, distilled water 1,000 ml) growing very slowly at first, but becoming faster as the colonies develop, very thin and sparse, almost colorless, then grayish sepia to vinaceous fawn; reverse vinaceous brown; aerial mycelium thin-walled, less branched, hyaline, septate, slender, 2–5 μm thick, becoming pale olivaceous brown; immersed mycelium thick-walled, much branched, often swollen, torulose or nodulose, reaching up to 15 μm thick, mixed with thin-walled, less branched hyphae, sometimes intermingled with clavate to elongate, often subspherical, olivaceous, verrucose or papillate cells resembling the sterile cells formed on the apex of the conidiophores.

Colonies on MA (malt extract 20 g, glucose 20 g, peptone 1 g, agar 20 g, distilled water 1,000 ml) growing very slowly at first, but later more or less rapidly; aerial mycelium more abundant than on PSA, subhyaline to vinaceous umber; reverse concolor.

Colonies on OA (oatmeal 50 g, agar 20 g, distilled water 1,000 ml) growing moder-

ately; aerial mycelium white to rosy buff, finally pale vinaceous to vinaceous brick; reverse pale vinaceous to vinaceous gray. No growth was observed on CMA.

Conidial structures or conidia have not been observed on either of the media tested.

The optimum growth temperature

A 5 mm-square of mycelial block cut from the edge of 1 month-old colonies of *A. japonicum* (IFO 30500) on PSA plate was inoculated on the following agar slants with five replicates; PSA, MA, OA and CMA. Incubation was made at temperatures ranging from 3 to 37 C. After 21 days of incubation, radial growth at each temperature was recorded (Table 1).

On PSA, MA and OA, the mycelium of *A. japonicum* grew at a wide range of temperatures from 15 to 32 C and showed the best growth at 24 C both on PSA and MA. The optimum temperature for the mycelial growth of this fungus was not affected with the culture media tested. On CMA, no growth was observed at any temperature tested so far.

Table 1. Growth of *Arthrinium japonicum* on various media at different temperatures

Medium	Temperature (C)							
	3	8	15	20	24	28	32	37
PSA	—	±	+	++	##	++	+	—
MA	—	±	+	++	##	++	+	—
OA	—	±	+	++	++	++	+	—
CMA	—	—	—	—	—	—	—	—

PSA: potato sucrose agar, MA: malt extract agar, OA: oatmeal agar, CMA: cornmeal agar.
##, best growth; ++, good growth; +, poor growth; ±, doubtful growth; —, no growth.

Culture on sterilized natural substrates

In order to induce the formation of the conidial structures on the artificial media, attempts were made to evaluate the effect of the natural substrates on the sporulation of this fungus. For this purpose, in addition to the culms of *Carex dispalata*, wheat and rice straws were used.

Several pieces of the straws or the culms including the sheaths, cut into a 6–7 cm long, were put into a 100 ml Erlenmeyer flask together with 25 ml of 2% agar or distilled water, followed by sterilizing in an autoclave (121 C, 20 min). Incubation was made with a single conidial isolate of this fungus by placing the mycelial patches on the straws or culms in the media. After about one month incubation at 20 C under 12-photoperiod with a fluorescent lamp, it was found that the conidial structures appeared both on the wheat and sedge straws or culms as black, velvety pustules. The pustules thus formed on the sterilized substrates differed in shape and size from those on the natural host, *Carex dispalata*; they were usually irregular in size ranging from 0.1–0.2 mm in diam to

2 mm long and 2–3 mm wide (Pl. 1, D). No conidial structure was observed on the sterilized rice straws.

Microscopic examination clearly showed that both the conidiophores and sterile cells were formed normally on the two substrates above mentioned. The conidia produced in these cultures, however, were more or less irregular in shape as compared with those on the natural hosts, varying from fusiform to triangular. Furthermore, the conidia were $33\text{--}47 \times 14\text{--}19 \mu\text{m}$ in size and therefore it was found that the size of conidia on the sterilized straws or culms was smaller than that on the natural hosts.

The superficial mycelium composed of a network of the branched and anastomosing hyphae and the conidiophore mother cells on it were easily observed on the sterilized substrates as has already been pointed out by Tubaki (1971).

The conidia produced on these substrates did not germinate immediately after formation and it was necessary to age them for at least two weeks or more to germinate. This indicates that the conidia of *A. japonicum* may require a given after-ripening period for germination.

Yokoyama and Tubaki (1971) have reported that at least four species of *Arthrimum* were found in Japan: *Arthrimum phaeospermum* (Corda) M. B. Ellis, *A. sphaerospermum* Fuckel, *A. sacchari* (Speg.) M. B. Ellis and *Arthrimum* state of *Apiospora montagnei* Sacc. In addition, unpublished data by the senior author suggest that additional species, for examples, *A. urticae* M. B. Ellis, together with *Pteroconium pterospermum* (Cooke & Masee) Grove and *Pteroconium* state of *Apiospora camptospora* Penz. & Sacc. are widely distributed in Japan. In this respect, *A. japonicum* is the fifth published species in Japan and is considered to be associated with the species of *Carex* preferably in the northern Japan.

The original description of *A. japonicum* did not indicate the scientific name of the host plant on which the holotype fungus was found. However, on examining a part of the type material, the junior author has decided that it is no doubt a species of the genus *Carex*.

A. caricicola, the type species of the genus, has the conidia of apparently similar shape to the present fungus and is distributed in the cool temperate and subarctic zones in North Europe with its host plants, *Carex* spp. Therefore, it is concluded that *A. japonicum* is closely related to *A. caricicola*, not only morphologically, but also ecologically.

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摘 要

青森県下でカサスゲ *Carex dispalata* 上に不完全菌類の1種 *Arthrinium japonicum* が採集された。本種は最初サンフランシスコ港で日本からの輸入バラの梱包材として用いられていたスゲ属植物らしい枯茎葉上に発見され、1969年に新種として記載されたものである。しかし、これまで日本国内での発生が確認できず、本種の分布と生態については不明であった。今回の発見により *A. japonicum* はスゲ属を寄主として日本に自生することが明らかになった。北歐に分布する *A. caricicola* は本種同様にスゲ属上に生じ、形態的にも生態的にも近縁の種類と考えられる。日本産菌の培養上の諸性質についても記述した。

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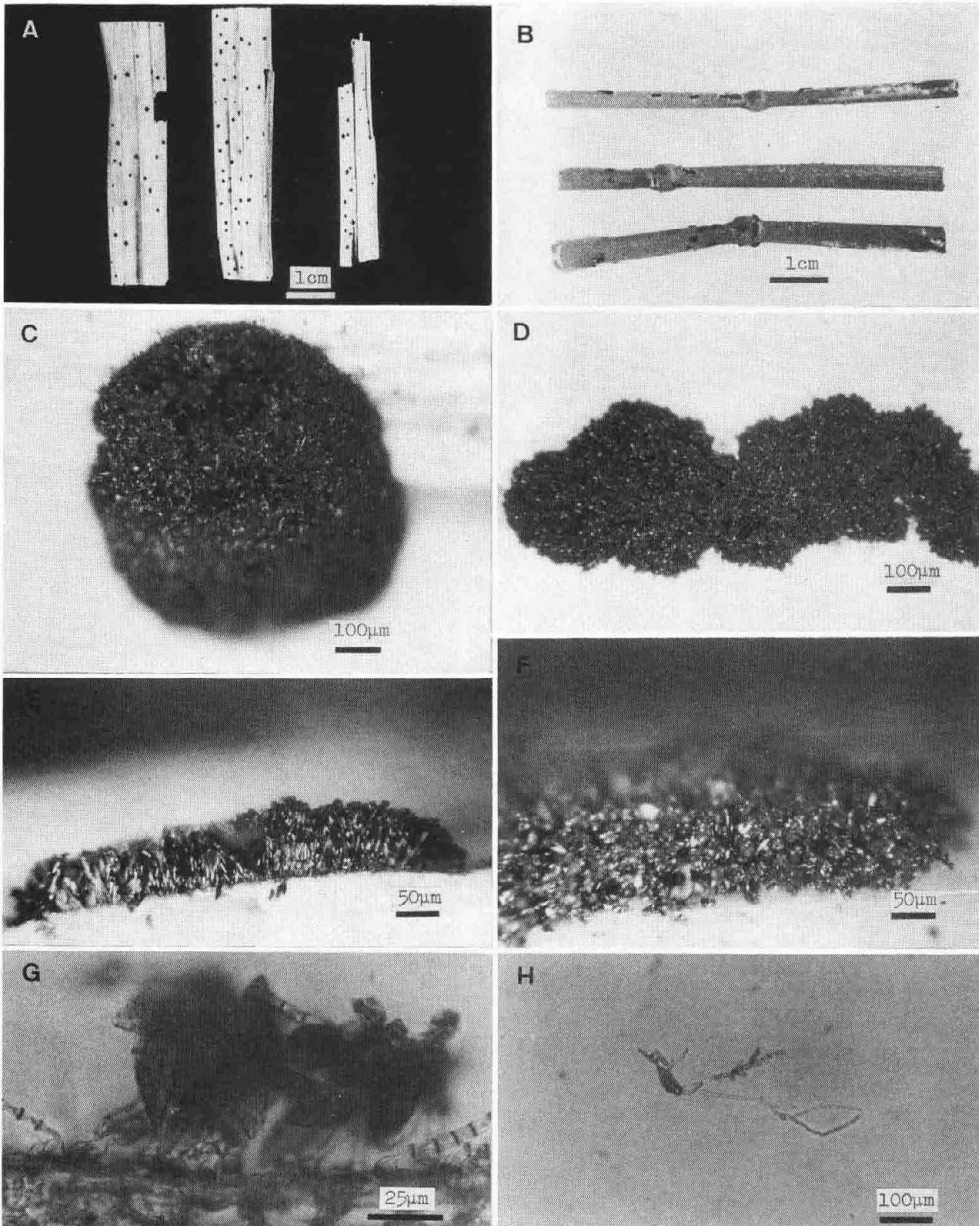


Plate 1. A-H. *Arthrinium japonicum*. A. Natural habit, left: Japanese collection on *Carex dispalata* (upper side); middle: Ditto (reverse side); right: BPI 11679 on *Carex* sp. B. Pustules formed on the sterilized wheat straws. C. Pustule on the natural host *Carex dispalata*. D. Pustule on the sterilized wheat straws. E. Side view of a longitudinal section of the pustule on the natural host. F. Enlarged surface view of the pustule on the sterilized wheat straw. G. Enlarged side view of a section of the pustule on the natural host. H. Germination of conidium.

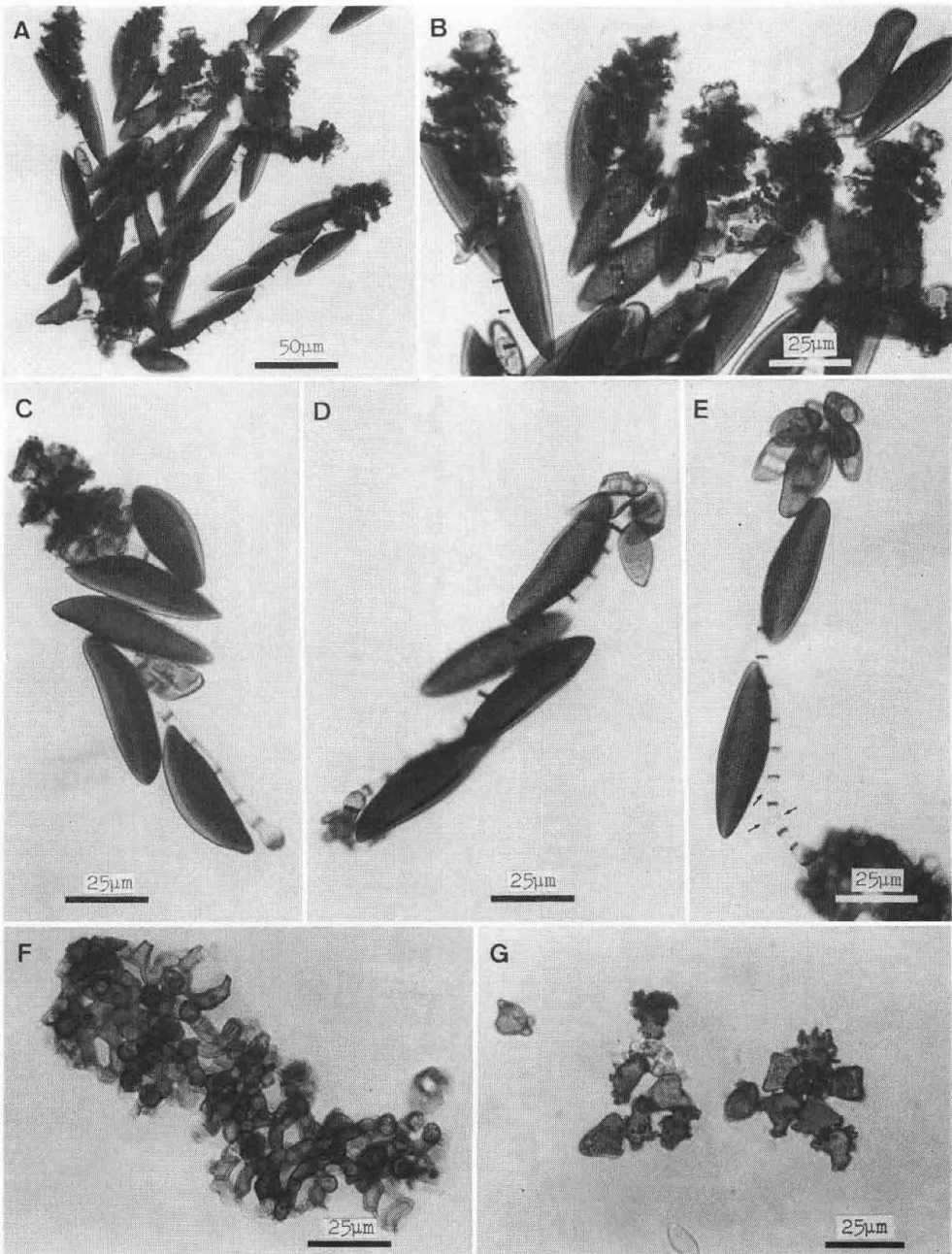


Plate 2. A-G. *Arthrinium japonicum*. A. Conidial structures. B. Clusters of the sterile cells on the conidiophores. C-E. Conidiophores, conidiophore mother cells, conidia and sterile cells (arrows indicate a short hyaline peg). F. Superficial mycelial network and conidiophore mother cells. G. Lobed and papillate sterile cells.