

Aspergillus implicatus, a new species isolated from Ivory Coast forest soil

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Aspergillus implicatus sp. nov. is described from tropical forest soil in Tai National Park (Ivory Coast). The species shows an affinity with species of Section *Candidi* and is characterized by the peculiar presence of parallel sterile hyphae surrounding the conidiophore.

During researches on tropical forest soils, within the Tai Project, MAB Programme No. 1, an interesting *Aspergillus* species was repeatedly isolated. It was reported in previous works as *Aspergillus* sp. no. 2 (Rambelli *et al.*, 1983, 1984; Maggi & Persiani, 1992) and it has been widely studied for its

outstanding features. It is morphologically distinct from any known species of *Aspergillus* because of the presence of sterile corticating hyphae surrounding the conidiophore. These hyphae begin to branch at the base of vesicle to form a hyphal tangle around the mature conidial head.

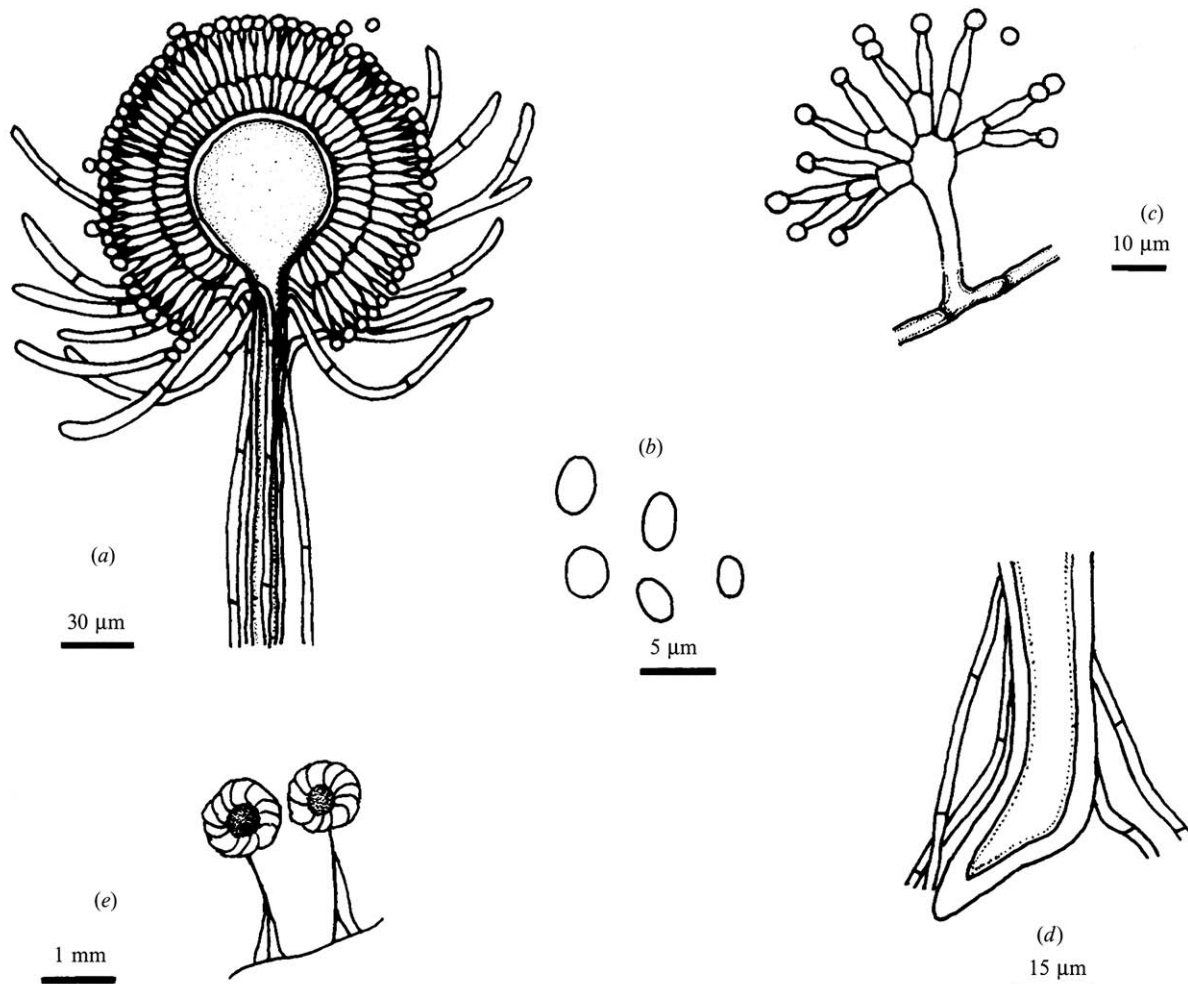
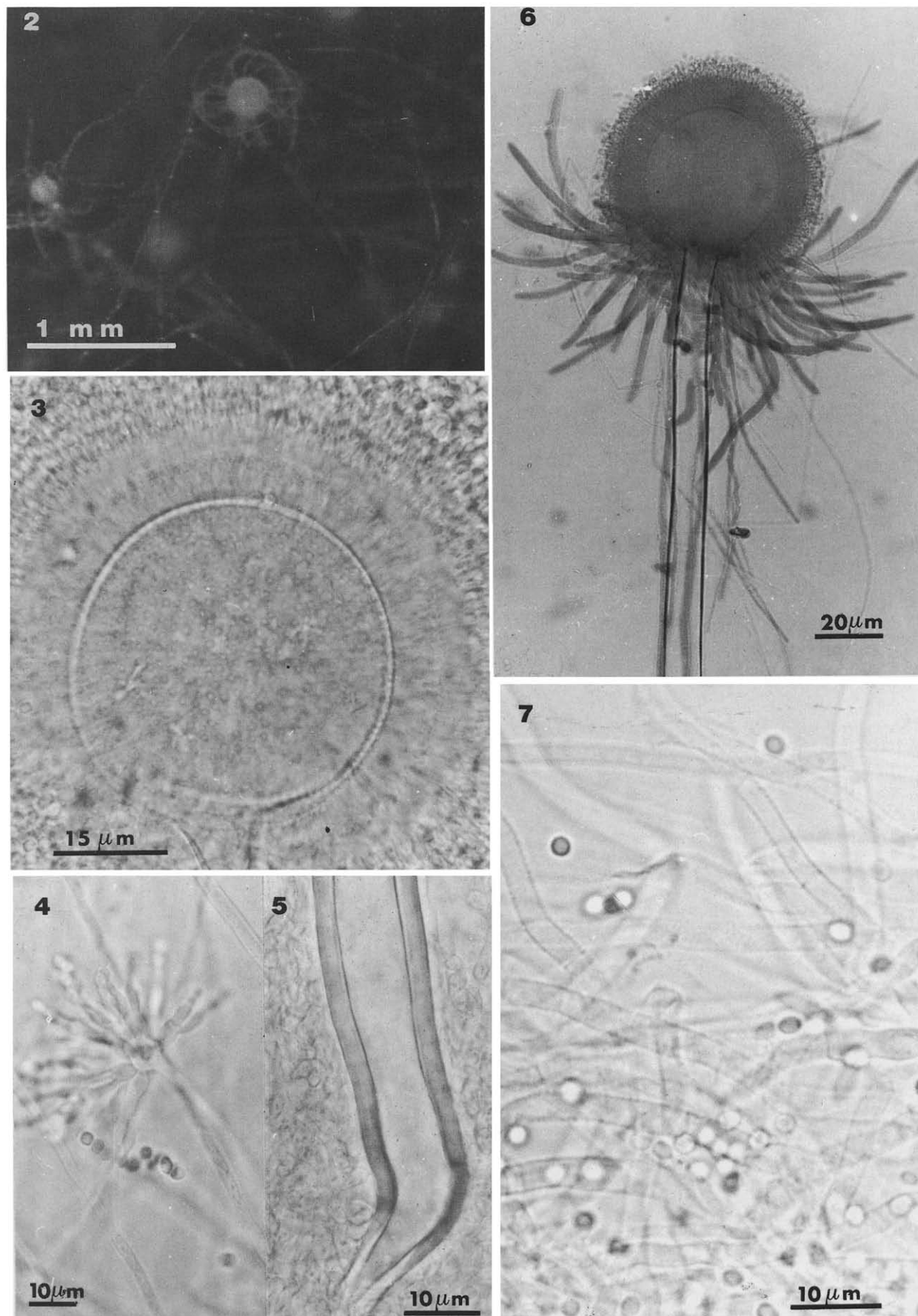


Fig. 1. *Aspergillus implicatus*. a, conidiophore with corticating hyphae; b, conidia; c, diminutive head; d, foot cell bearing young conidiophore; e, habit.



Figs 2–7. Light micrographs of *Aspergillus implicatus*. **Fig. 2.** Habit. **Fig. 3.** Typical globose conidial head. **Fig. 4.** Diminutive head. **Fig. 5.** Foot cell of the conidiophore. **Fig. 6.** Typical conidiophore with corticating hyphae. **Fig. 7.** Conidia.

It is described below as a new species on the basis of light and electron microscopic observations.

Aspergillus implicatus A. M. Persiani & O. Maggi sp. nov.
(Figs 1–12)

Etym.: *L. implicatus* – entangled; in reference to the conidial head appearance.

Coloniae in Agar Czapekii plus Extractum Fermenti 2.6–2.8 cm diam. septem diebus, 25 °C crescentes, margine regulare, planae, textura floccosa, mycelio continue albo, claro, pauco exudato producto; reverso pallide flavo (Kornerup & Wanscher, 1978, 4-A3), aetate rubro-luteo (Kornerup & Wanscher, 1978, 7-B7) usque and brunneum-rubrum (Kornerup & Wanscher, 1978, 10-D7). *Capitula conidica* tarde (25–30 diebus) atque laxe evoluta, in margine coloniae posita, pauca, alba, globosa usque ad radiantia, duoseriata, vesicula, metulis, phialidibus, conidiis atque hypharum glomerulo composita; mensura variabili, plerumque 60–100 µm. *Conidiophora* sicut rami perpendiculares ex hyphis vegetativis orientia, vero pede praedita, aseptata, sine colore, parietibus crassis, 1.3–1.5 µm, aequali crassitudine, levia, 13–18 µm diam., 2–3 mm longitudine; parallelis atque sterilibus hyphis e mycelio orientibus, simul concretescentibus usque ad turgescens vesiculam, circumdata; deinde ipsis hyphis augescentibus saepius ramosis, leviter echinulatis, circum capitula conidica implicata. *Vesiculae* primum subglobosae, globosae maturitate, circa 30–50 µm, plerumque 40 µm diam., parietibus aequaliter 2 µm crassis, concolores conidiophoro, fertiles in tota superficie; ferentes metulas atque phialides in duplice serie; saepe ferentes paucas metulas atque phialides ut videantur quasi penicillatae. *Metulae* sine colore, densae, dimensione variabiles, circa 7–9 µm × 2–2.5 µm. *Phialides* sine colore lageniformes, maxime colligatae, circa 10–15 µm × 1.9–2.2 µm, brevi collo praeditae. *Conidia* copiosa, adherentia capitulis, levia, hyalina, pallide subflava in massa, 0-septata, in massa mucosa, globosa usque and ovalia, 2.2–3.8 µm × 2.5–3.4 µm.

Habitat: e solo silvestri, Tai, Ora Eboris.

Holotypus: ROHB 110 S.

Colonies on CYA reaching 2.6–2.8 cm in diam. in 7 d at 25°, with regular margin, plane, surface texture floccose, mycelium persistently white with small amounts of clear exudate produced; reverse pale yellow (Kornerup & Wanscher, 1978, 4-A3), reddish orange in age (Kornerup & Wanscher, 1978, 7-B7) to brownish red (Kornerup & Wanscher, 1978, 10-D7). *Conidial heads* late (25–30 d) and sparsely produced on the marginal areas, few, white, globose to radiate, biseriata, consisting of vesicle, metulae, phialides and conidia, all surrounded by a spherical hyphal tangle, variable in size, mostly 60–100 µm diam. *Conidiophores* arising perpendicularly from the substrate, with a true foot in the basal part, aseptate, colourless, thickening, 1.3–1.5 µm fairly uniform throughout the length of the conidiophore, smooth, 13–18 µm in diam., 2–3 mm in length; surrounded by parallel sterile hyphae, slightly echinulate, originating from the base growing up to the vesicle and later then branching to build the hyphal tangle. *Vesicles* at first subglobose, at maturity globose, mostly 40 µm but ranging from 30 µm to 50 µm, with wall uniformly 2 µm thick, coloured like the conidiophore, typically fertile over the whole surface, bearing a series of metulae and a series of phialides; small vesicles often bearing only a limited number of metulae and phialides and appearing almost penicillate, are

produced. *Metulae* colourless, rather variable in size, ranging from 7–9 µm × 2–2.5 µm. *Phialides* colourless, monophialidic, lageniform, closely packed, ranging from 10–15 µm × 1.9–2.2 µm, with a short neck. *Conidia* very abundant, remaining on the conidial head, smooth, hyaline, slightly yellow in mass, 0-septate, produced in mucous mass, from globose to oval, 2.2–3.8 µm × 2.5–3.4 µm. Sclerotia are not produced. No growth at 5° or 37° on CYA.

On Czapek agar colonies growing rather poorly, thin, with submerged mycelium, surface growth consisting of diminutive heads appearing almost penicillate, white becoming orange white (Kornerup & Wanscher, 1978, 5-A2), reverse yellowish white (Kornerup & Wanscher, 1978, 4-A2), conidiophores absent after 25–30 d.

On Malt Extract Agar (MEA) colonies 1.5–1.6 cm in diam. in 7 d at 25°, submerged mycelium, dense, white, velutinous, irregular margin, reverse steady pale yellow (Kornerup & Wanscher, 1978, 3-A3), conidiophores absent after 25–30 d.

On Soil Extract Agar plus coconut milk (SEA + CM) colonies plane, white, floccose, reverse steady pompeian yellow (Kornerup & Wanscher, 1978, 5-C6); conidiophores after 25–30 d. This medium favours the presence of complex conidial heads.

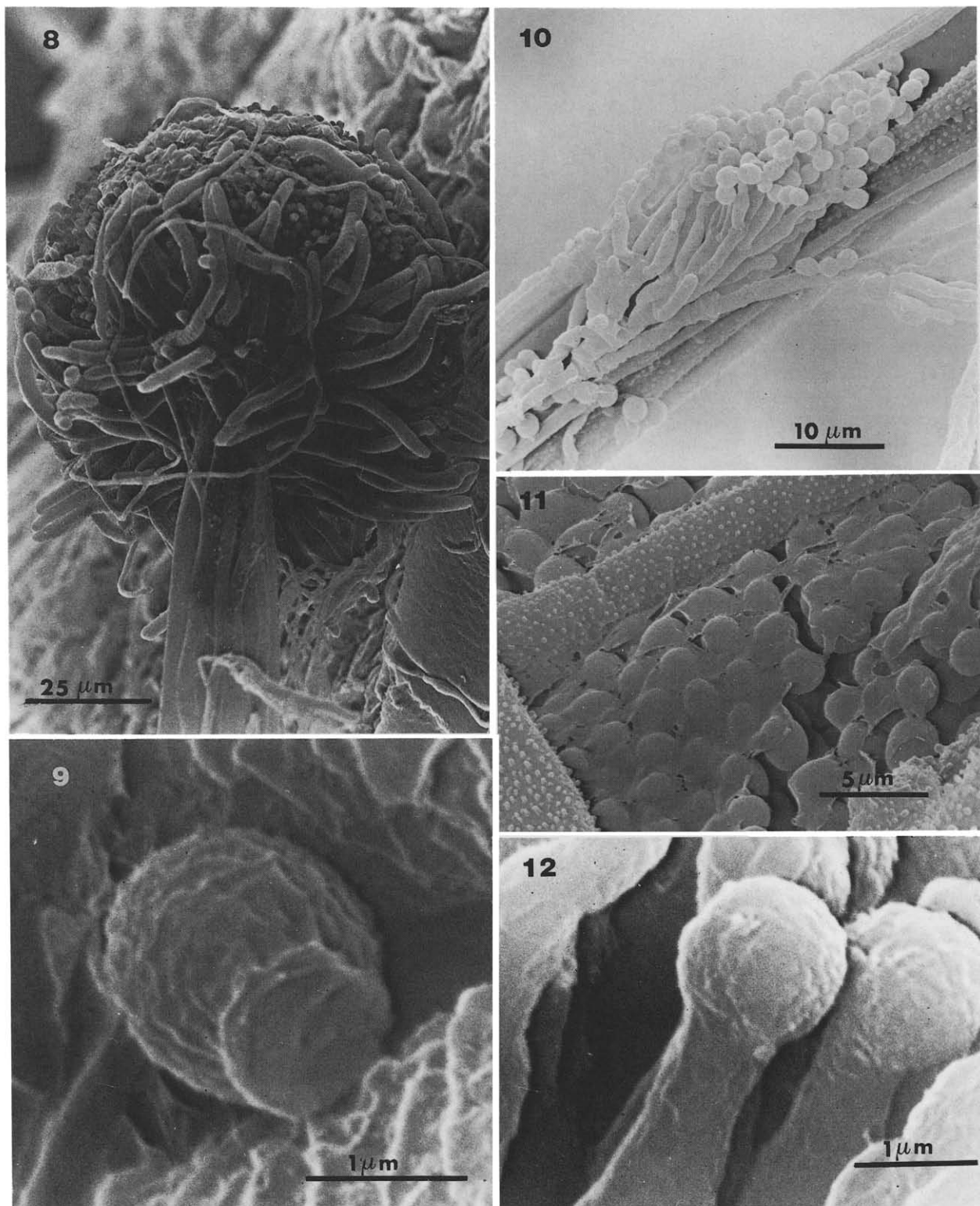
For the peculiarity of its morphology and development this species has been investigated by scanning electron microscopy (SEM). The conidiophore grows to its maximum length, surrounded by adherent, parallel and encrusted corticating hyphae; then it inflates at the apex to form the vesicle and the corticating hyphae produce echinulate branches to form a close net around the mature conidial head. The conidiogenous cells are monophialidic, lageniform and the early phialidic development occurs synchronously as does the formation of primary phialoconidia. Some conidia show an annular scar at the base, a structure possibly limited to the spore produced first from the conidiogenous cell; the presence of a septal pore is also evident. Kozakiewicz (1978) has observed in *Aspergillus* spp. the presence of an orifice in the septum which delimits adjacent conidia and which could provide a passageway for nutrients.

The conidia are enveloped by a layer of mucilage which disintegrates leaving the conidia free, although residual mucilage remains on them.

Moreover, the conidia exhibit, during ontogenetic development, remarkable changes in external morphology proceeding from an initially smooth condition to one ultimately roughened. Such variability in the ontogenetic development of conidia was also observed by SEM and described by Kozakiewicz (1985) for some *Aspergillus* spp.

The presence of sterile hyphae surrounding the conidiophore is reported only in *A. dybowskii* (Pat.) Samson & Seifert, a synnematus fungus recently included in *Aspergillus* (Samson & Seifert, 1985). In this species these hyphae form a sheath but cover only the conidiophore.

The general appearance of the conidiophore of *A. implicatus* resembles that of the genus *Heterocephalum* Thaxt. This affinity is confirmed by SEM. However, the arrangement of phialides places this species in *Aspergillus*. *Heterocephalum*, in contrast, shows four or five orders of branches in the conidiophore (Onofri *et al.*, 1986).



Figs 8–12. SEM micrographs of *Aspergillus implicatus*. **Fig. 8.** Young mature conidiogenous head showing the corticating hyphae. **Fig. 9.** Conidium. **Fig. 10.** Diminutive heads growing up the conidiophore and echinulations of corticating hyphae. **Fig. 11.** Mucilaginous envelope of the conidia and echinulations of corticating hyphae. **Fig. 12.** Conidiogenous cells.

The white, globose, biseriolate conidial heads and the vesicles, typically globose and fertile over the entire surface, indicate an affinity with species in the Section *Candidi* (Gams *et al.*, 1985). Raper & Fennell (1965), according to other authors (Thom & Raper, 1945; Nehira, 1949), limited the group to *A. candidus* proposing a broad concept of *A. candidus*.

The presence of small vesicles bearing few metulae and appearing almost penicillate, is frequent in isolates of *A. implicatus*; diminutive heads are also described in some strains of *A. candidus* but *A. implicatus* differs from *A. candidus* in the size of vesicles and conidia.

A. implicatus has been maintained in culture for many years and has never produced a teleomorphic state.

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