

FOUR NEW SPECIES OF *ASPERGILLUS* FROM IVORY COAST SOIL

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Four new species of *Aspergillus*, *A. ivoriensis*, *A. coremiiformis*, *A. anthodesmis* and *A. ochraceo-roseus*, are illustrated and described from cultures obtained from forest soils of the Tai National Park, Ivory Coast.

Within MAB project 1 on tropical rain-forest ecosystems, the Laboratory of Mycology of the University of Rome is carrying out microbiological studies on forest soils of the Tai National Park in the Ivory Coast. A preliminary work (Bartoli *et al.*, 1978) reports the data obtained from the first sample analyses, carried out mainly to identify optimal sampling areas and techniques. In December 1976 the area best suited for microbiological studies was selected and delimited and the first samples were collected from it. Examining soil mycoflora in these samples, we found four species belonging to the genus *Aspergillus*, which we propose to describe as new, namely: *A. ivoriensis*, *A. coremiiformis*, *A. anthodesmis* and *A. ochraceo-roseus*.

MATERIALS AND METHODS

Culture media used were: Czapek-Dox agar Difco, malt agar Difco, soil extract agar, made with Tai forest soil, plus glucose (10%) and yeast extract (1%). Cultures were grown in dishes 8 cm diam and 6 cm high. Cultures were subsequently deposited with CBS and IMI. Colour terminology is according to Ridgway (1912).

***Aspergillus ivoriensis* sp. nov.** (Figs. 1-4)

Coloniae in Agar Czapekii moderate crescentes, 2 cm duabus hebdomadibus, 24-26°, pulvino similes, initio virido-flavae (R., XVI 23' k) deinde oleagineae (R., XVI 19' m); mycelio submerso abundanti flavo (R., XVI 19' -) usque ad luteo-fuscum (R., XV 17' -, 13' i, 15' i), fuscum (R., XV 13' i) aetate una cum reverso; 'Hülle' cellulae productae adherente coloniae margines irregulares. Exudato nullo; odore mucido. Capitula conidica copiosa duobus generibus colore dissimilibus, simul viridia (R., XLVII 33' "b) ac luteo-fusca (R., XVI 21 b, 19' -) usque ad flavum colorem aetate, aequalibus forma dimensioneque. Capitula conidica praecipue radiantia vel divisa in 2-4 columnis divergentibus, rare columnaria. Conidiophorae e substrato crescentes, clara ima cellula deficientes, finem habentes formam carotae. Conidiophorae erectae, 800-2500 × 9-35 µm, constrictae sub vesicula, membrani scissis, granis incrusatis vel

echinulatis, rarissime enodibus, incoloribus vel fusco-flavescentibus (R., XV 17' -, 17' i); vesiculae globosae vel subglobosae plus minusve fuscae (R., XV 13' i), plerumque 45-70 µm in diam, interdum minores (25-30 µm) in tota superficie fertiles. Sterigmata duoseriata, densa, 9-10 × 2.5-3 µm. Conidia viridia in massa in capitulis conidicis viridibus, flava in massa in capitulis flavis, ovata, elliptica vel oblonga, enodia 2.3-6 × 1.5-2.3 µm. Particulosae conidicae fructificationes inaequales forma dimensioneque. 'Hülle' cellulae virido-flavae (R., XXXI 25' b) copiosae in flavas (R., XVI, 21' b) massas concretas, vel in catellis, plerumque subglobosae, interdum elongatae; 11.5-40 µm in diam. magis etiam. 'Hülle' cellulae nullae in Agar maltoso.

E solo silvestri collecto in Tai (Ivory Coast), Horto Botanico Roma 101 S, RO, holotypus.

On Czapek-Dox agar: slow growing at room temperature 24-26°, attaining a diameter 2 cm in 15 days; colony surface somewhat raised, Serpentine Green (Ridgway, XVI) tending with age to Saccardo's Olive (R., XVI) but including both Celandine Green (R., XLVII) and yellow conidial heads. Immersed mycelium extremely abundant, Primuline Yellow (R., XVI) to Yellow Ocher (R., XV), tending with age to Tawny (R., XV), Ochraceous-Tawny (R., XV), giving the same colour to the reverse. With age a continuous Amber Yellow (R., XVI) layer of Hülle cells is formed, giving to the colony an irregular margin. Exudate absent. Slight mouldy odour. Conidial heads abundant, both Celandine Green (R., XLVII) and Amber Yellow (R., XVI) to Primuline Yellow (R., XVI) appearing later; on microscopical examination, however, colour seems to be the only difference between them. Conidial heads mostly radiate, sometimes splitting into 2-4 divergent and compact columns of conidia, rarely columnar. Conidiophores arising from the substratum, without a true foot cell, but narrowing toward the base to a more or less bent carrot-like shape. Conidiophores straight, 800-2500 × 9-35 µm, slightly narrowing toward the vesicle; thick walled; walls rough or echinulate, sometimes

smooth, colourless to Yellow Ocher or Buckthorn Brown (R., XV). *Vesicles* globose to subglobose, light to deep Tawny (R., XV), mostly $45\text{--}70\text{ }\mu\text{m}$ in diam, sometimes smaller, $25\text{--}30\text{ }\mu\text{m}$; fertile over their entire surface. *Sterigmata* biserial, closely packed, $9\text{--}10 \times 2.5\text{--}3\text{ }\mu\text{m}$. *Conidia* greenish in mass on green heads, yellowish on yellow heads; oval to elliptical or oblong, smooth-walled, $2.3\text{--}6 \times 1.5\text{--}2.3\text{ }\mu\text{m}$. Very anomalous conidial

heads may also be present. *Hülle cells* very abundant, in groups or chains, Citron Green (R., XXXI), mostly rounded, similar to those found in *A. versicolor*- and *A. nidulans*-groups, sometimes pyriform or elongate, mostly $11.5\text{--}40\text{ }\mu\text{m}$ in diam, sometimes larger and internally granular. Perfect state unknown.

On soil extract agar: *colony* surface yellow green, reverse reddish yellow to reddish brown; mycelium

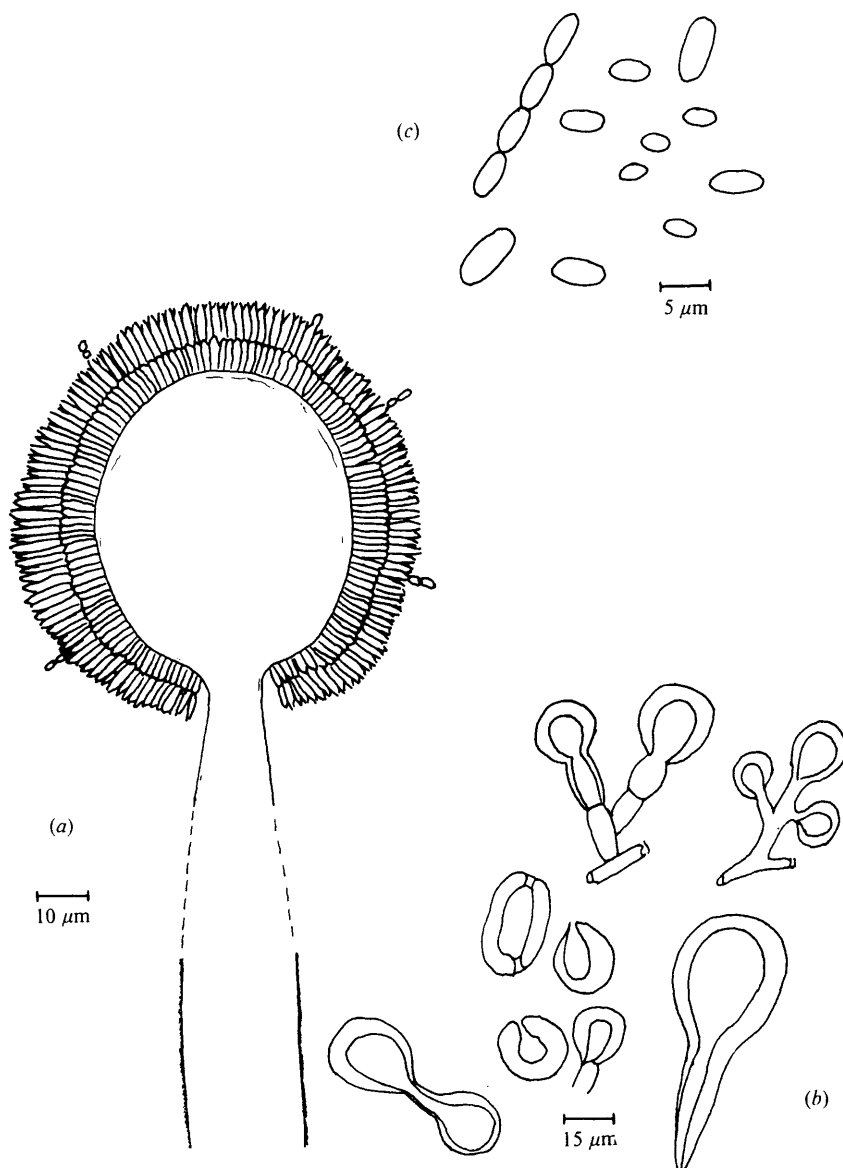


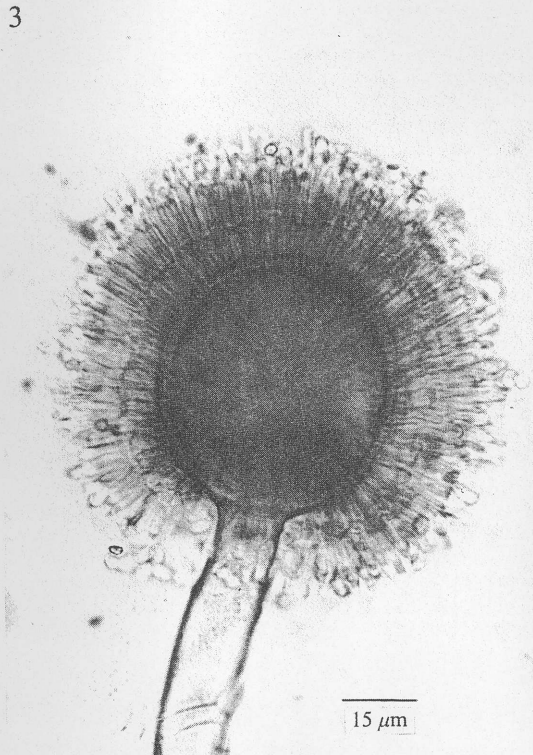
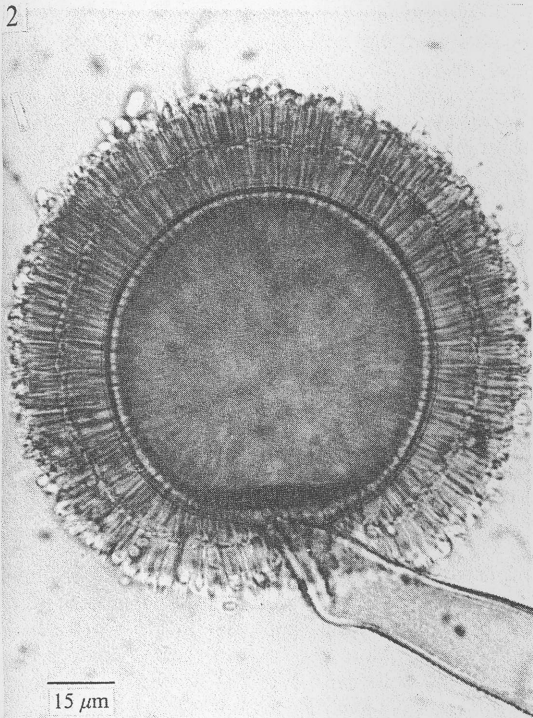
Fig. 1. *Aspergillus ivoriensis*. (a) Conidial head; (b) Hülle cells; (c) conidia.

mostly immersed; margin irregular. *Conidial heads* radiate; *conidiophores* up to 2 mm long, rough-walled, lighter in colour than on Czapek-Dox agar; *conidia* mostly oblong; *Hülle cells* absent.

On malt agar: *colony* similar but more restricted than on Czapek-Dox agar. Colony centre consisting of whitish mycelium and small, anomalous

conidial heads. *Conidial heads* mostly greenish. *Hülle cells* absent.

Conidial structures of this species might be related to *Aspergillus panamensis* Raper & Thom; it seems difficult however to include our isolate in the *A. ustus*-group. The *Hülle* cell shape, although larger, might suggest inclusion in the *A. versicolor*- or *A. nidulans*-group but the general



Figs. 2-4. *Aspergillus ivoriensis*. Figs. 2-3: typical globose conidial heads. Fig. 4: *Hülle* cells irregular in shape and dimensions.

pattern of conidial structures however exclude this possibility. Therefore, while the morphological structures of our isolate indicate a new species, its inclusion in any of the *Aspergillus* groups proposed by Raper & Fennell (1965) seems to us rather uncertain.

***Aspergillus coremiiformis* sp. nov.** (Figs. 5-10)

Coloniae in Agar Czapeckii luxuriantes: 4 cm in diam septem diebus, 24-26° crescentes, tegentes totam superficiem substrati duabus hebdomadibus; superficies primum alba et floccosa producens flavas conicas fructificationes deinde omnino flava (R., IV 21-), circiter fusca (R., XXX 19" i) aetate; post quindecim, viginti dies producentes coremia flava celeriter adsurgentes usque 4-5 cm. Exudato non manifesto oculis sed ex microscopicis sine colore guttulis constituto. Odore gravi. Verso primum albo-flavo (R., XXX 19" d) deinde fusco (R., XL 13" -; 17" -). Mycelio vegetativo sine colore 8 µm in diam. Capitula conidica virido-fusca (R., XXX (21"-), radiantia; in centrosa colonia minima atque interdum inaequalia, sterigmatibus uniseriatis. Conidiophorae ex hyphis vegetativis orientes angulo acuto; singulae vel parallelae confluentes in coremia. Conidiophorae singulae, erectae 1500 × 20-45 µm; maximo modulo in 2/3 superiore parte, attenuatae sub vesicula; membranis crassis granis incrustatis vel echinulatis, rarissime enodibus, sine colore vel flavo-fuscis (R., XV 17'-; 17' i); vesiculae plus minusve fuscae (R., XV 13' i), subglobosae vel elongatae, fertiles in tota superficie 60-50 × 60-45 µm. Sterigmata duoseriata, densa, 15-18 × 4-4.5 µm. Conidia flava in massa, globosa, ovata, oblonga vel cylindracea, plus minusve granis incrustata plerumque 6.9-9 µm in diam etiam 2.5-12 × 2.5-6.9 µm. Conidiophorae in coremiis similes singulis, usque ad 4-5 cm × 70 µm in diam; ab axe coremii divergentes ultima parte. Vesiculae obclavatae membranis corrugatis ante sterigmata constituta deinde spatulatae 90-150 × 85-100 µm; sterigmata duoseriata, densa, primaria 35-50 × 4.5-5.5 µm; secundaria 10-25 × 5-5.5 µm mucronata. Conidia sicut in capitulis singulis. Nulla coremia producta in Agar maltoso.

E solo silvestri collecto in Tai (Ivory Coast), Horto Botanico Roma 102 S, RO, holotypus.

On Czapek-Dox agar: colony rapidly growing, at room temperature 24-26°, attaining a diameter of 4 cm in one week, covering the entire agar surface in 15-20 days; first floccose and white with yellow conidial heads intermixed, then Lemon Chrome (R., IV) to Isabella Colour (R., XXX) with age. After 15-20 days, from the basal felt arise true yellow coremia, rapidly attaining 4-5 cm in height. Exudate absent, however, at low magnification small colourless droplets may be seen. Odour pronounced. Reverse from Cream-Buff (R., XXX) to Fawn Colour, deep Wood Brown (R., XL). Mycelium composed of hyaline hyphae about 8 µm wide. Conidial heads Olive-Ocher (R., XXX), radiate, smaller at colony

centre, where uniseriate anomalous conidial heads may be also present. *Conidiophores* arising from vegetative hyphae at acute angles. They are simple or in coremia, in which each conidiophore grows parallel to the main axis, tending to diverge in its terminal portion. *Simple conidiophores* straight, up to 1500 µm long by 20-45 µm wide, attaining maximum diameter in the upper two-thirds, and then narrowing towards the vesicle; thick-walled; walls roughened to echinulate, rarely smooth, hyaline to Yellow Ocher or Buckthorn Brown (R., XV). *Vesicles* light to deep Tawny (R., XV), subglobose to elongate, fertile over almost the entire surface, 60-50 × 60-45 µm. *Sterigmata* biseriata, each series composed of closely packed sterigmata, 15-18 × 4-4.5 µm. *Conidia* yellowish in mass, very variable in shape, from globose to oval, oblong or cylindrical, more or less encrusted, mostly 6.9-9 µm but ranging from 2.5-12 × 2.5-6.9 µm. *Coremial conidiophores* similar to simple ones, but up to 4-5 cm long by 70 µm. *Vesicles* obclavate and wrinkled before sterigmata production, then spatulate, 90-150 × 85-100 µm. *Sterigmata* biseriata, closely packed, primaries 35-50 × 4.5-5.5 µm, secondaries mucronate, 10-25 × 5-5.5 µm. *Conidia* as in simple conidiophores. Perfect state unknown.

On malt agar and soil extract agar: mycelial mat less abundant and in a lighter shade of yellow than on Czapek-Dox agar, white floccose aerial hyphae persisting on the surface. True coremia lacking.

The presence of coremia-like formations is reported only in *A. clavato-flavus* Raper & Fennell. The presence of true coremia and the variability in shape and dimensions of conidial structures in our isolate, induces us to propose a new species but, at the same time does not allow us to fit it into any of the *Aspergillus* groups (Raper & Fennell, 1965).

***Aspergillus anthodesmis* sp. nov.** (Figs. 11-14)
(Etym. Gk, *anthos*, flower; *desmis*, bunch)

Coloniae in Agar Czapeckii moderate crescentes 3.5 cm duabus hebdomadibus 24-26°; mycelio hyalino perfecte submerso, vix discreto a substrato, saepe torulideo, 1.8-4.5 µm diam fructificationes conicas erectas ferente. Exudato nullo. Odore levi. Capitula conidica primum globosa deinde plus minusve radiantia vel divisa in duabus vel pluribus columnis. Conidiophorae erectae in fasciculis orientes, membranis crassis, enodibus atque fuscis, 235-750-1300 × 10-15 µm basi conidiophorae, maximo modulo media parte, attenuatae sub vesicula (6-9 µm). Ima cellula conspicua, forma varia. Vesiculae sine colore usque ad brunneum coloreum (R., III 17 k), globosae, fertiles in tota superficie 25-45 µm in diam. Sterigmata duoseriata, densa, primaria 4.5-5 × 2.5-3 µm, secundaria

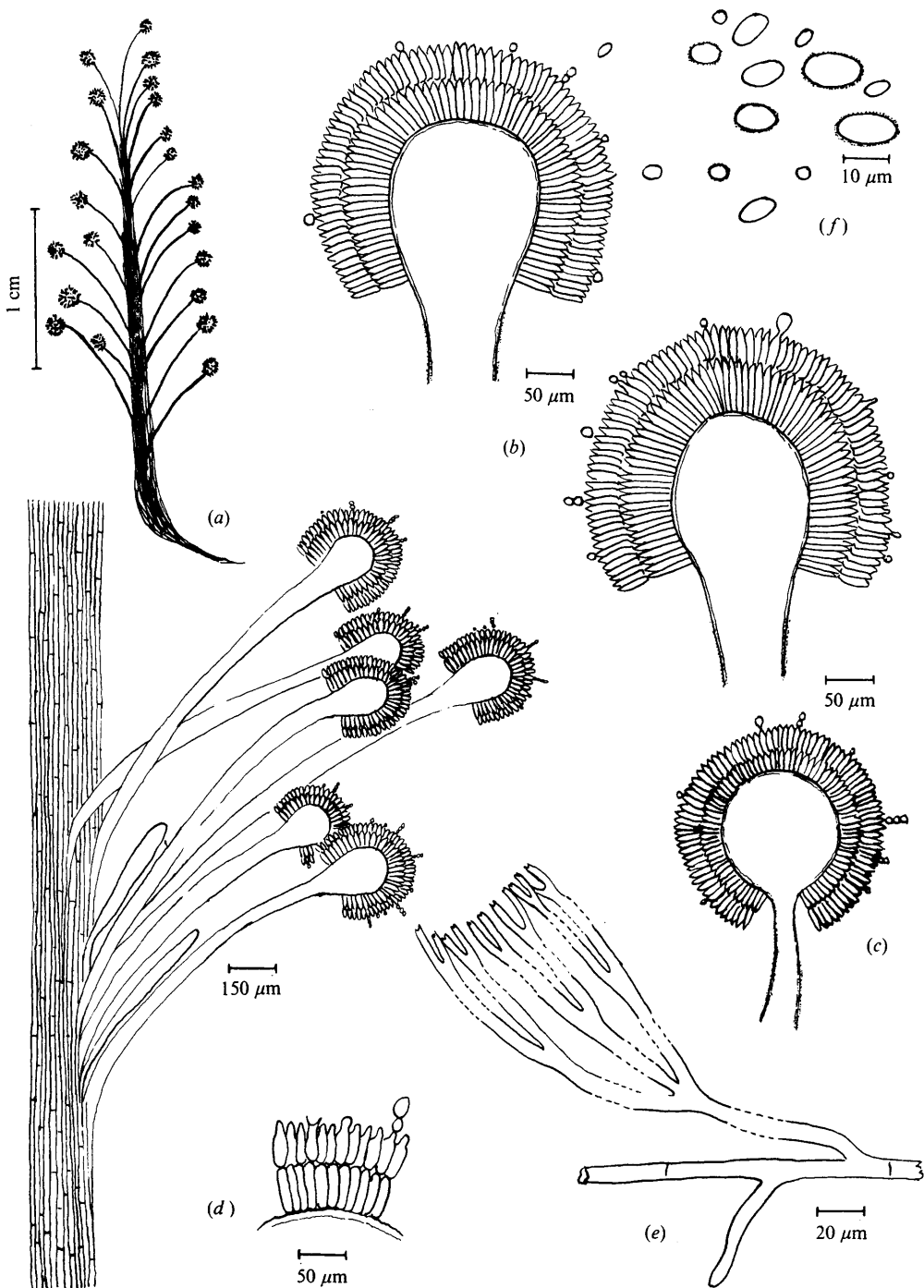


Fig. 5. *Aspergillus coremiiformis*. (a) Coremium; (b) coremial conidial heads; (c) conidial head of a simple conidiophore; (d) biseriate sterigmata; (e) typical origin of the coremium; (f) conidia.

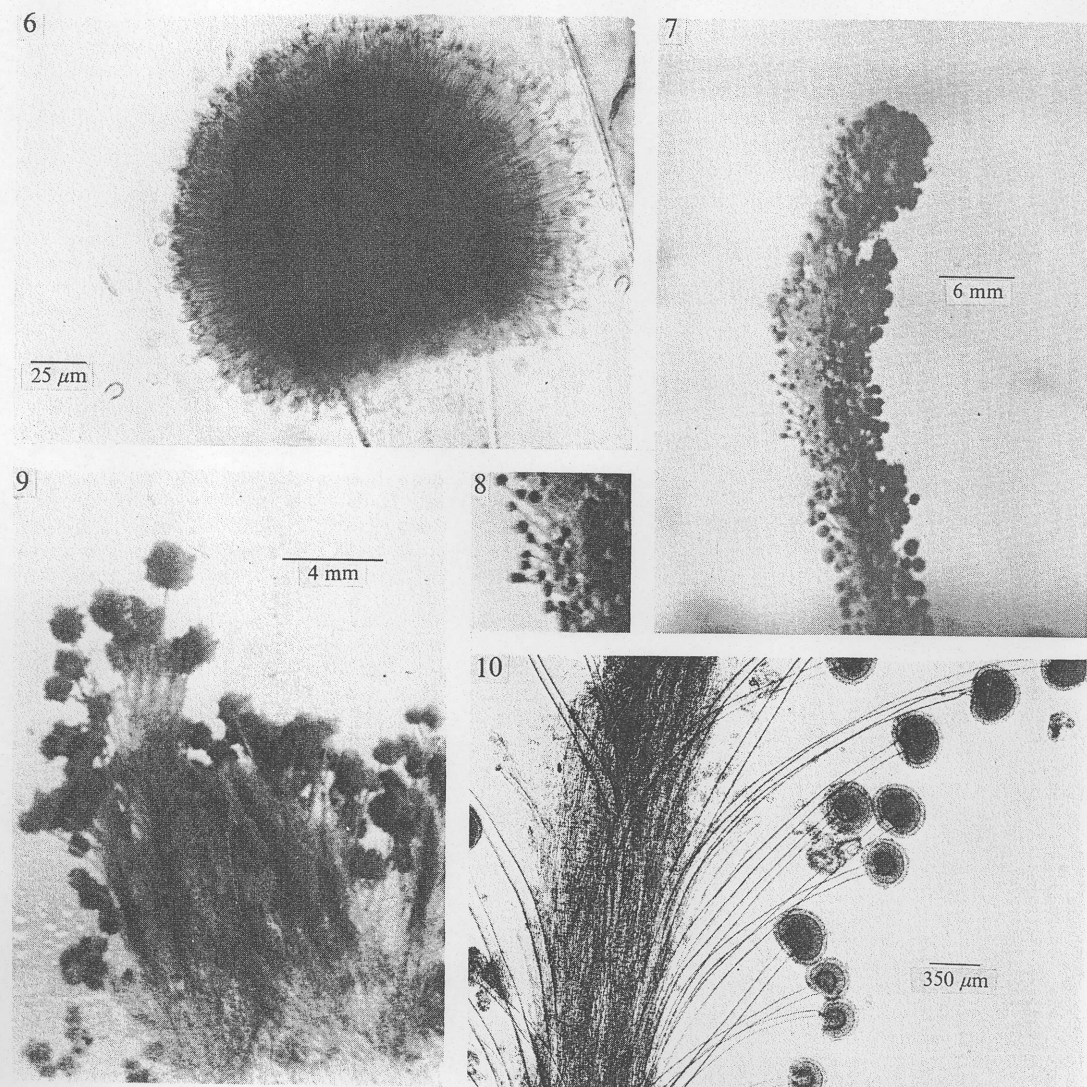
$5-6 \times 2-2.3 \mu\text{m}$. Conidia copiosa adhaerentia capitulis, fusca (R., III 17 k) in massa, globosa vel subglobosa aequalia forma dimensioneque, $2.5 \mu\text{m}$ in diam; membranis crassis, enodibus vel leviter granis incrustatis.

In Agar maltoso, mycelium floccosum, conspicuum, albo-griseum, fuscum in maturitate. Reverso fusco (R., XIV 9' i; II' i); agar sine colore.

E solo silvestri collecto in Tai (Ivory Coast), Horto Botanico Roma 103 S, RO, holotypus.

Colonies on Czapek-Dox agar at room temperature ($24-26^\circ$) growing moderately 3.5 cm in 15

days, consisting of a colourless completely immersed mycelium, hardly distinguishable from substratum, often toruloid, $1.8-4.5 \mu\text{m}$ in diam, giving rise to scattered conidial structures characterized by Chaetura Drab (R., XLVI) heads. Exudate lacking. Odour indistinct. Young conidial heads typically globose, becoming more or less radiate in age and splitting into two or more columns. *Conidiophores* scattered in tufts from substratum, light brown, thick and smooth-walled $235-750-1300 \times 10-15 \mu\text{m}$ at the base,



Figs. 6-10. *Aspergillus coremiiformis*. Fig. 6: coremial conidial head showing typical spathulate vesicle. Fig. 7: well developed coremium. Fig. 8: detail of the coremium. Fig. 9: initials of coremia. Fig. 10: Upper portion of the coremium.

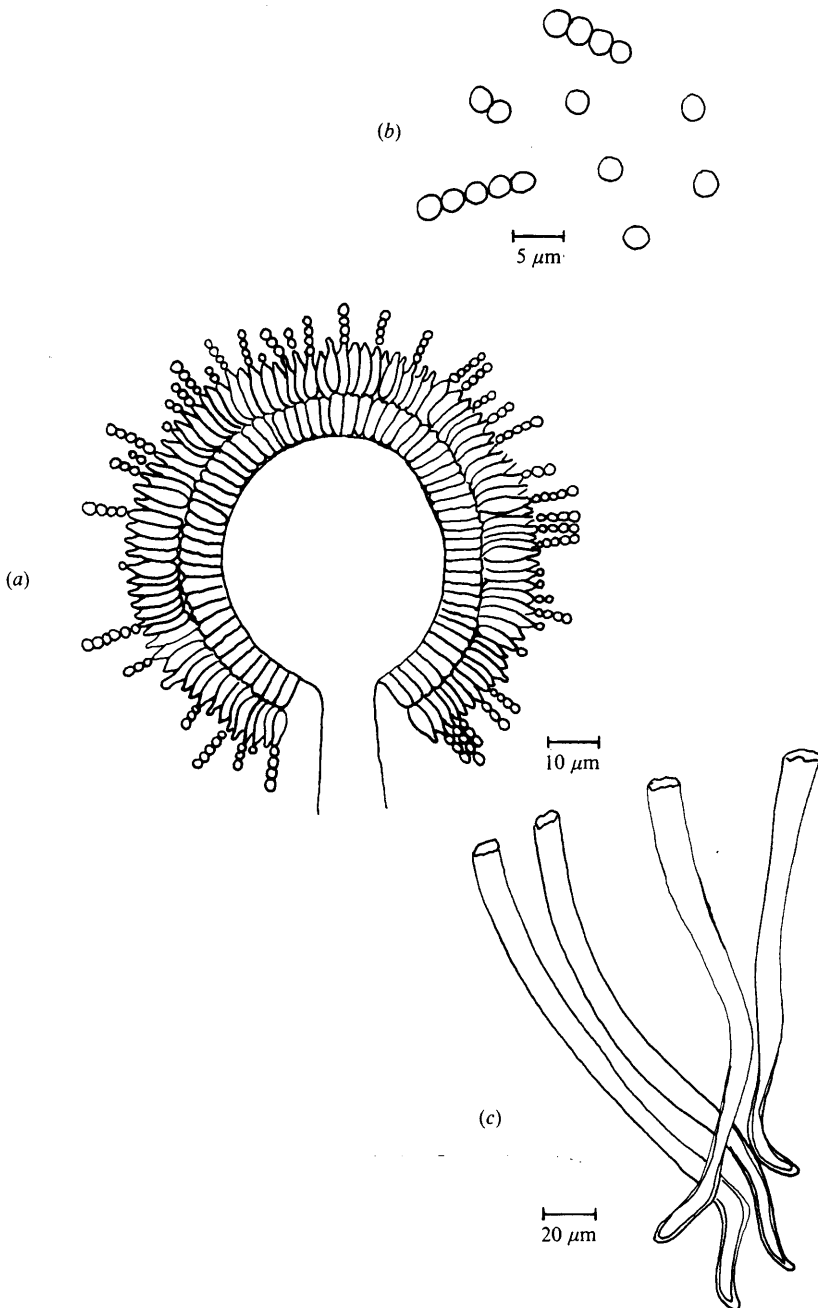
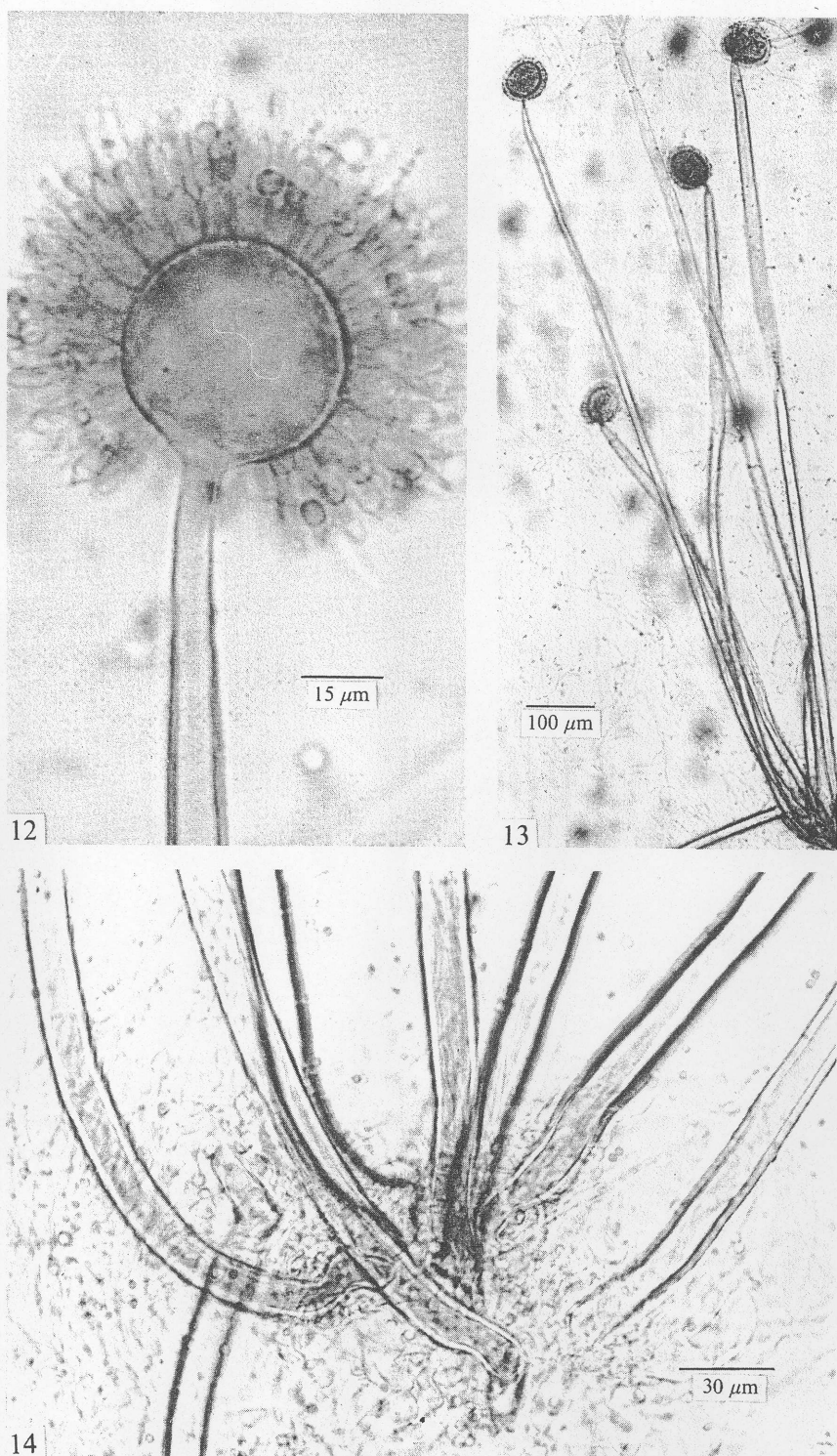


Fig. 11. *Aspergillus anthodesmis*. (a) Conidial head; (b) conidia; (c) bases of conidiophores in tuft.



Figs. 12-14. *Aspergillus anthodesmis*. Fig. 12: conidial head showing globose vesicle. Fig. 13: tuft of conidiophores. Fig. 14: basal portion of a tuft of conidiophores.

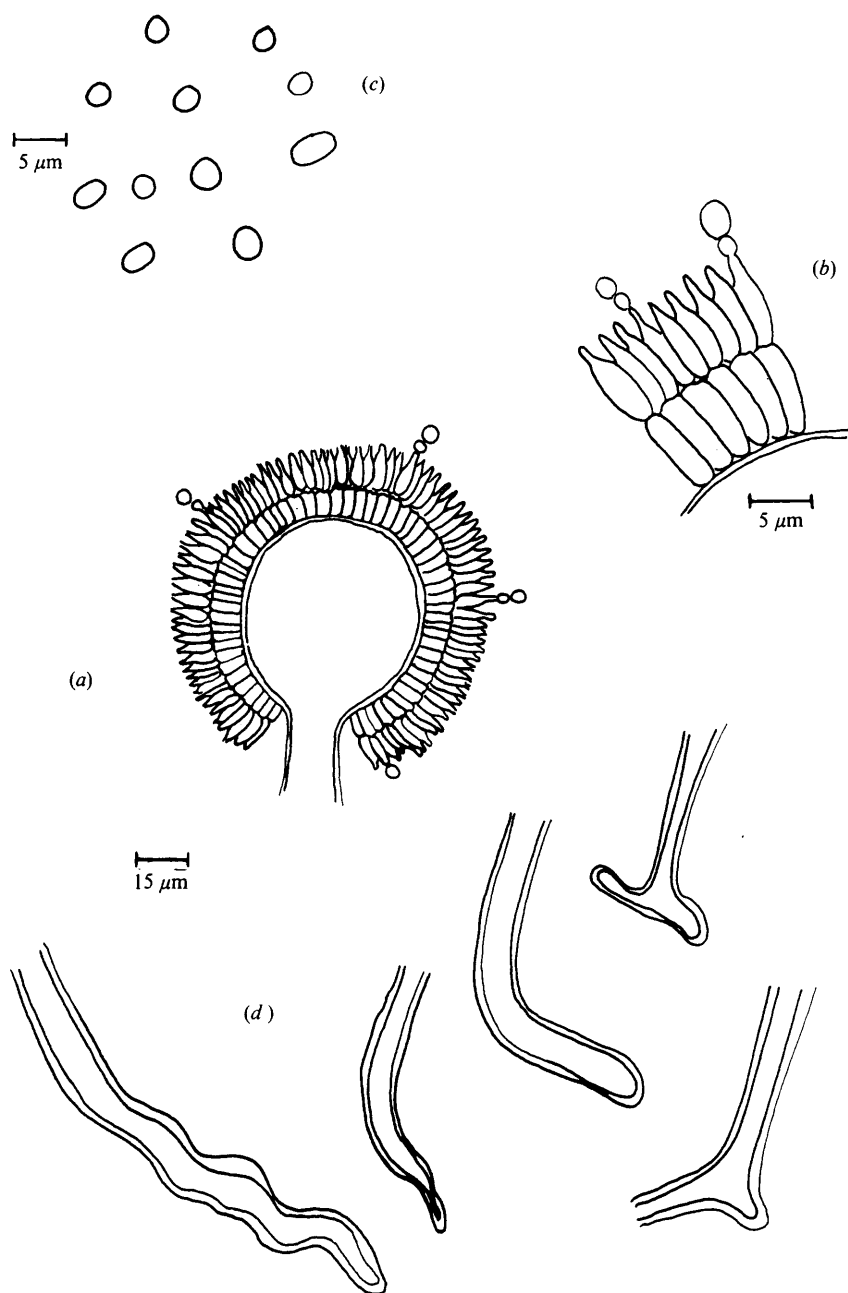
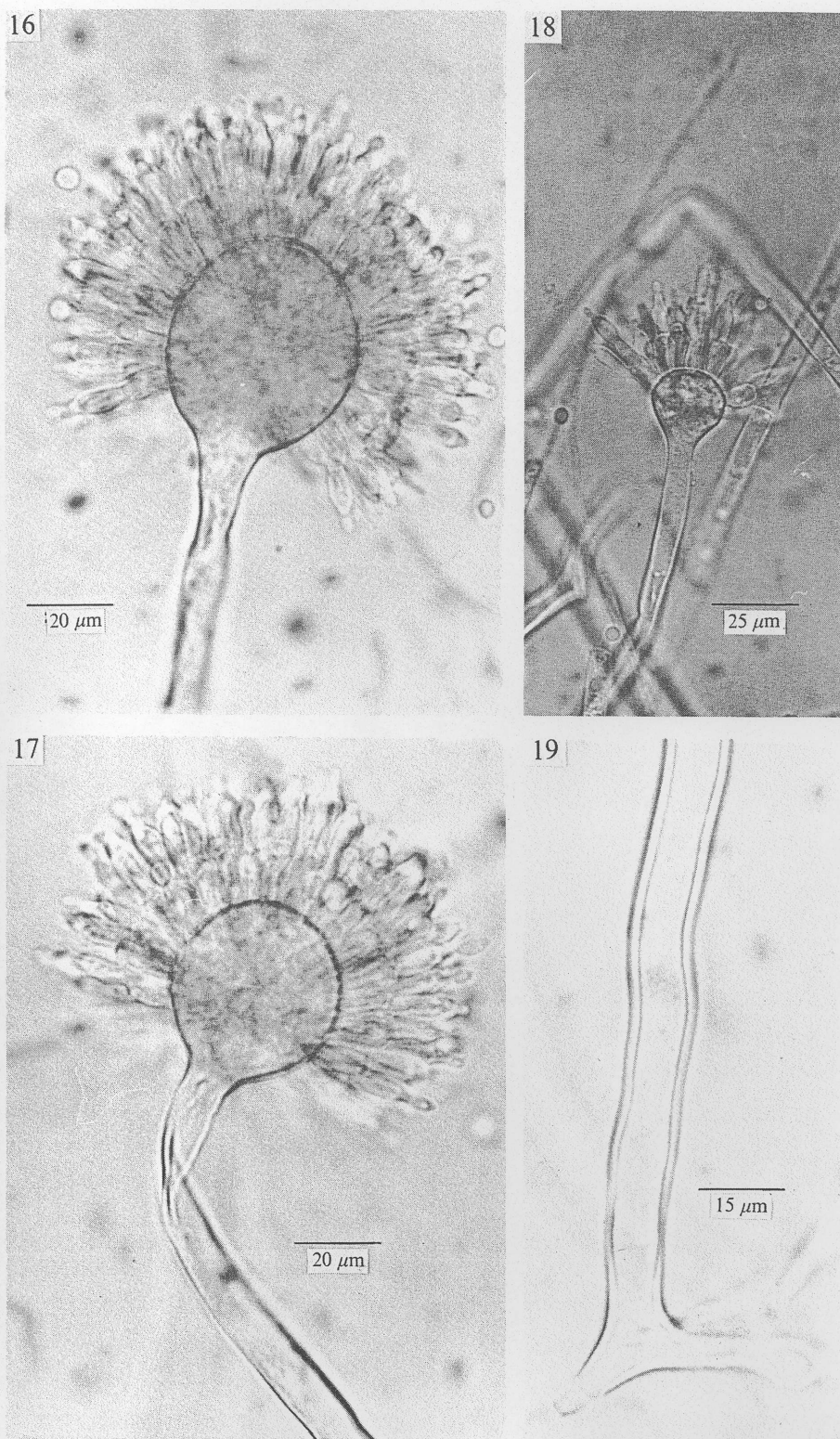


Fig. 15. *Aspergillus ochraceoroseus*. (a) Conidial head; (b) biserial sterigmata; (c) conidia; (d) foot cells.



Figs. 16-19. *Aspergillus ochraceoroseus*. Figs. 16-17: conidial heads. Fig. 18: anomalous conidial head showing longer and sterile secondary sterigmata. Fig. 19: foot cell detail.

increasing at the centre then narrowing towards the vesicles, up to 6–9 μm diam. Foot cells well-developed, variable in shape. *Vesicles* from hyaline to Antique Brown (R., III), globose, fertile over their entire surface, 25–45 μm diam. *Sterigmata* biserial, closely packed; primaries 4–4.5 $\times$ 2.5–3 μm , secondaries 5–6 $\times$ 2–2.3 μm . *Conidia* very abundant, remaining on the conidial heads, Antique Brown in mass, globose or subglobose, regular in shape and dimension, about 2.5 μm diam, thick-walled from smooth to slightly roughened. Perfect state unknown.

On malt agar: colonies differ greatly from those on Czapek-Dox agar owing to the presence of a whitish-grey mycelial mat from which arise abundant, compact conidial heads. After 15 days colony surface appears Benzo Brown (R., XLVI). Reverse ferruginous Cinnamon-Rufous (R., XIV). Pigment not diffusing into surrounding agar. Microscopic structures are closely similar to those in Czapek.

This species may be included tentatively in the *A. sparsus* group. It lacks however 'feeder hyphae' surrounding the foot cells. It differs from *A. sparsus* Raper & Thom by good sporulation on Czapek, by conidiophore walls which are smooth and smaller and by the regular shape of the conidial structures.

***Aspergillus ochraceoroseus* sp. nov.** Figs. 15–19)

Coloniae in Agar Czapekii lente crescentes: 2 cm in diam duabus hebdomadibus, 24–26°; mycelio primum tenui, aetate floccoso, albo; mycelio submerso conspicuo, adferente coloniae margines irregulares. Superficies primum albo-flava (R., XXX 19" d), rosea (R., XIV 9' d; XXIX 15" b) in maturitate, in senectute flavo-fusca (R., IV 21 d); reverso primum roseo (R., XIV 7' d) deinde colore ferrugineo (R., XIV 9' i) inducente colorem vinaceum (R., XXVII I" d) agar circumstante; exudato nullo; odore levi vel incerto. Capitula conidica flavo fusca (R., IV 21 d), radiantia, aetate divisa in columnis divergentibus. Conidiophorae erectae orientes e substrato imis cellulis conspicuis 800–1500 $\times$ 10–12.5 μm gradibus constrictae ad vesiculam, interdum sinuosae plerumque septatae, membranis crassis, enodibus, sine colore vel leviter coloratis. Vesiculae globosae, 35–50 μm , coloratae sicut conidiophorae vel sufflavae, fertiles in tota superficie. Sterigmata duoseriata, primaria 7–9 $\times$ 4–4.5 μm , secundaria 7–9 $\times$ 2.5–3 μm . Conidia hyalina, subflava in massa, globosa vel ovata, interdum apiculata, membranis enodibus 2.5–4.5 μm . Capitula conidica interdum inaequalia forma dimensioneque vel secundariis sterigmatibus elongatis atque sterilibus.

E solo silvestri collecto in Taï (Ivory Coast), Horto Botanico Roma 104 S, RO, holotypus.

Colonies on Czapek-Dox agar, at room temperature, 24–26°, growing rather slowly reaching a diam of 2 cm in 2 weeks. Immersed mycelium abundant, giving to the colony an irregular margin; colony surface Cream-Buff (R., XXX), becoming Pale Salmon Colour (R., XIV), Light Pinkish Cinnamon (R., XXIX), Buff Yellow (R., IV) in age. Reverse at first Flesh Colour (R., XIV), Ferruginous (R., XIV) in age, with surrounding agar assuming a definite Pale Vinaceous (R., XXVII) shade. Exudate lacking; odour slight or indefinite. *Conidial heads* Buff Yellow, loosely radiate, splitting into several more or less divergent columns in age. *Conidiophores* arising from the substratum with well developed foot-cells; from 800–1500 $\times$ 10–12.5 μm gradually narrowing towards the vesicle; sometimes sinuous, thick and smooth-walled, from almost hyaline to light brown; commonly repeatedly septate. *Vesicles* globose up to 35–50 μm diam, of the same colour as conidiophores or yellowish fertile over their entire surface; *sterigmata* in two series, primaries 7–9 $\times$ 4–4.5 μm , secondaries 7–9 $\times$ 2.5–3 μm . *Conidia* hyaline, yellowish in mass, very variable in shape, form globose, subglobose to oval, sometimes lightly apiculate, smooth-walled, 2.5–4.5 μm . Anomalous conidial heads may be present; sometimes they are small and irregular, otherwise regular in shape and dimensions, bearing longer and sterile sterigmata in secondary series. Perfect state unknown.

On malt agar: colony and microscopic structures closely similar to those on Czapek-Dox agar, except for the lighter colour of reverse, and the surrounding agar remaining hyaline.

Our isolate undoubtedly belongs to the *A. ochraceus* group and is very similar to *A. petrakii* Vörös, but differs in having smooth conidiophores and globose vesicles and in lacking exudate. Besides, *A. petrakii*, *A. ochraceus* Wilhelm and *A. sulphureus* (Fres.) Thom & Church have a white, more or less floccose basal mat, lacking in our strain.

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