Akenomyces costatus, an interesting basidiomycetous anamorph with unknown affinities

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Abstract: The Austrian find of the rare basidiomycetous anamorph genus *Akenomyces* with its only known species, *Akenomyces costatus* is described and illustrated. Its systematic position is within the basidiomycetes; however, its closer relatives are unknown, as up to now only the anamorph is known which has an isolated position within the basidiomycetes. The lenticular propagules of *A. costatus* resemble sclerotia with peculiar morphology: The fertile, hyaline hyphae in the center capable of germination are enclosed by a one-celled layer of dark and thick-walled palisade hyphae arranged parallely in vertical direction; these are further loosely enclosed by hyaline encrusted hyphae growing vertically upwards from the base of the sclerotium, enclosing the sclerotium like a basket. These morphological features characteristic for a member of the ecological group of the aero-aquatic fungi are suggested to play an important role in dispersal, as the sclerotia are the propagules dispersed at the water-air interface, their good buoyancy resulting from the inclusion of air and their hydrophobic nature. Thus, the Austrian and French finds seem to be clearly water-dispersed which can be concluded both from habitat and morphology of sclerotia. However, the English records indicate that *A. costatus* may also be common on suitable terrestrial sites.

During a thesis on aero-aquatic fungi in Austria, a peculiar fungus with basidiomycetous affinities was encountered growing on submerged leaves of *Cladium mariscus* (L.) POHL. Further investigation showed that it represented the rare *Akenomyces costatus* HORNBY. *A. costatus* was originally described from France by ARNAUD (1954); however, as his description does not have a Latin diagnosis, the name was invalid until it was validated by HORNBY (1984) using material collected in England.

Material and methods

Collecting and culturing: Leaves and stems of *Cladium mariscus* submerged in a slow flowing ditch were taken out of the water, put into nylon bags and transferred to the laboratory. The leaves were then spread out in Petri dishes lined with moistened filter paper and incubated for several days under light, checking the material for the presence of propagules of aero-aquatic fungi from time to time. After the detection of sclerotia of *Akenomyces* these were transferred on 0.1% MEA plates where they germinated easily.

For the determination of growth characteristics, agar blocks approximately 5 x 5 mm in size were cut out of the edges of actively growing cultures and transferred to 0.1 and 2 % MEA plates, respec-

tively. The agar plates were incubated in a Conviron growth chamber under illumination at 20 °C for 7 days, then cultural characteristics were recorded.

Microscopy: Light microscopy was done using a Reichert Diavar microscope; illustrations were drawn using a drawing tube. Hand sections of the sclerotia were made with the help of a sharp razor blade.

For SEM well developed sclerotia were airdried, mounted on Cambridge stubs, sputter coated with gold and examined in a Jeol T 300 scanning electron microscope at 10 kV.

Specimen examined: Austria, Lower Austria, district Wien Umgebung, community Mitterndorf/Fischa, 180 m s. m., map grid 7964/4, dried sporulating cultures on 2% MEA ex leaves and stems of *Cladium mariscus* submerged in a small rivulet located in a calcareous mesotrophic mire, leg. 4. 11. 1993 H. VOGLMAYR & I. KRISAI-GREILHUBER (WU).

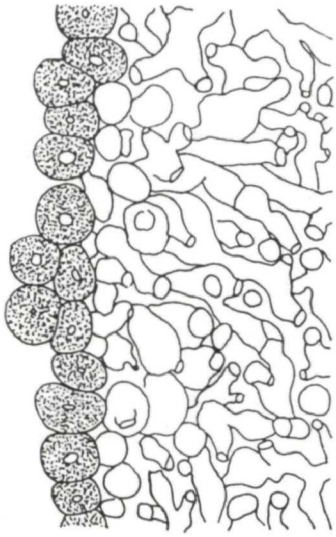
Results

Description: Colonies growing very rapidly and easily in MEA, on 0.1% MEA reaching 25 mm, on 2% MEA 35 mm diameter in seven days at 20 °C, whitish to pale yellow, reverse of the same color. Mycelium mostly immersed, composed of hyaline hyphae 1.5-5 µm thick, with clamp connexions at the septa. Chlamydospores absent. Sclerotia produced superficially, formed abundantly in culture, elliptical-lenticular, 230-350 µm long, (140-)170-280 µm broad, laterally compressed, brown, consisting of a complex structure: vertically arranged hyaline hyphae being 2.5-3 µm thick and incrusted by needle-shaped crystals which loosely enclose the whole sclerotium externally; a one-layered dark brown cortex consisting of vertically and parallely arranged, unbranched, brown, thick-walled, septate palisade hyphae 4-5 µm thick, which are locally interrupted by groups of parallelly arranged tubercles; and inside the cortex a tightly interwoven mass of hyaline, thin-walled, much branched hyphae being 1.8-3.7 µm thick and capable of germination (Figs. 1, 2).

Discussion

Ecology: There seems to be some uncertainty about the ecological niche of *Akenomyces costatus*. In England it has been found abundantly on dead decaying wheat roots in a field without excessive humidity (HORNBY 1984). Subsequently, according to the CBS (1994) it has also been isolated from wheat debris in Switzerland and Finland, indicating that it may be uncommon but widespread in corn fields. In this context it should be noted that, according to the experiments performed by HORNBY (1984), it has no pathogenic abilities, being therefore purely saprotrophic.

Fig. 1. *Akenomyces costatus*. *a* Sclerotium in side view; the left half showing the dark-walled, parallel and tightly appressed palisade hyphae of the cortex and the enclosing hyaline hyphae encrusted by needle-shaped crystals, the right half showing a longitudinal section with the tightly interwoven mass of hyaline, thin-walled, much-branched hyphae inside the sclerotium and the one-layered cortex. *b* Part of a transverse section of a sclerotium showing cortex consisting of dark, thick-walled parallel hyphae and the tightly interwoven hyaline hyphae in the interior. *c* Part of the cortex in side view showing three thick-walled parallel cortical hyphae. *d* Enclosing hyaline hyphae showing the needle-shaped crystals. *e* Hyaline hyphae of the interior. *f* Substrate hyphae with clamp connexions. Bars: *a* 50 µm, *b-f* 10 µm.



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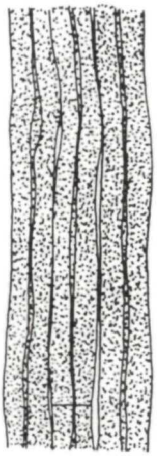

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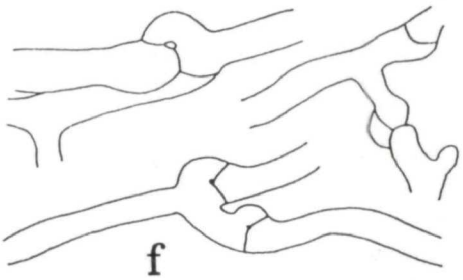

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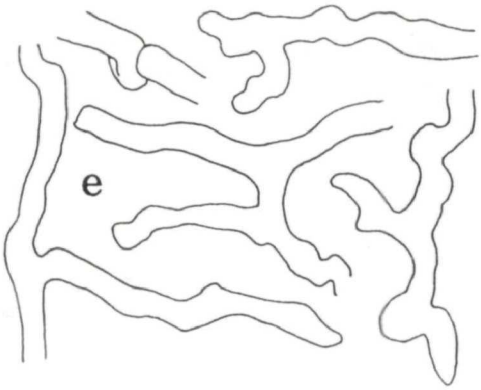

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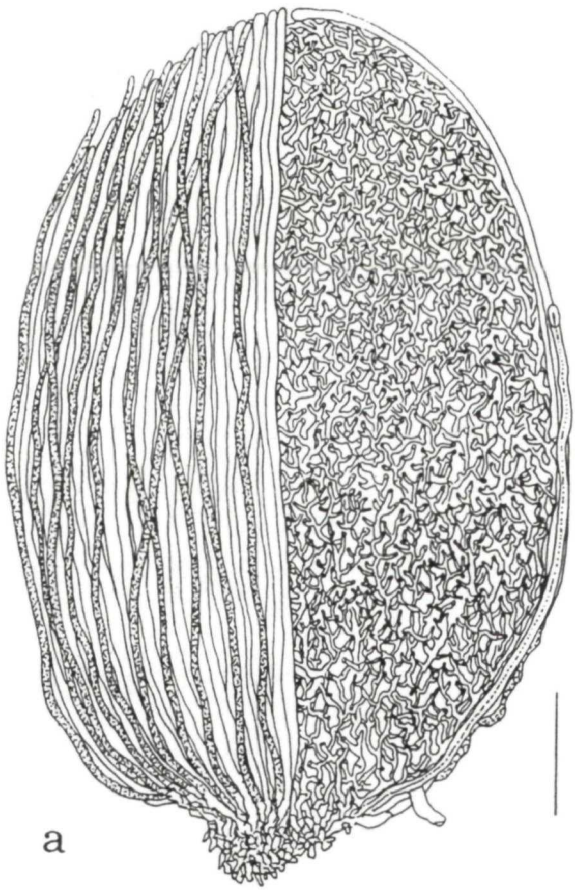

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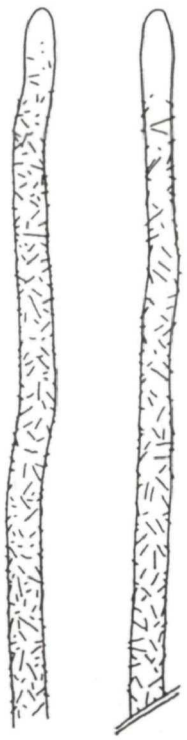

d

Nevertheless, the collections made by ARNAUD were growing on aerial parts, mainly dead leaves, of *Carex riparia* CURT. collected in the vicinity of Versailles (HORNBY 1984). As *C. riparia* is a characteristic plant of very moist to wet conditions (like lake shores, inundated ditches and swampy forests; e.g., ADLER & al. 1994, OBERDORFER 1994), this indicates that *Akenomyces costatus* may be especially adapted to aquatic habitats. Indeed, the habitat from where ARNAUD collected his material was a temporary pond (HORNBY 1984). Similarly, the Austrian collection was growing on submerged leaves and stems of a cyperaceous host, *Cladium mariscus*, indicating a specialisation on dead monocotyledonous host tissue exposed to very wet conditions.

In summary, with the present knowledge it is tempting to suggest that *Akenomyces costatus* is confined to dead monocotyledonous remains; it has, up to now, only been found on wheat debris and on dead leaves and stems of hosts belonging to the *Cyperaceae* (*Carex*, *Cladium*). This is the more astonishing as *A. costatus* does not seem to have special requirements concerning the substrate in culture; it is fast-growing and produces sclerotia profusely on MEA. However, due to the few records it cannot be definitely concluded that it is really confined to monocotyledonous substrate.

When the characteristics of the sclerotia are considered, it is further tempting to suggest a special adaptation to aquatic habitats; they have many features typical of the ecological group of the aero-aquatic fungi. These can be best defined as "...indwelling organisms characterized by the production of purely vegetative mycelium in substrata under water and by the formation of conidia with a special flotation device, formed only when the substrate on which the fungus is growing is exposed to a moist atmosphere" (FISHER 1977), and they have many different strategies to form well buoyant propagules (see, e.g., MICHAELIDES & KENDRICK 1982, FISHER & WEBSTER 1981, WEBSTER & DESCALS 1981, VOGLMAYR 1994). The sclerotia of *Akenomyces costatus* are well buoyant, because air is enclosed in the inner parts of the sclerotia which cannot be expelled due to the dense ensheathing wall consisting of dark, thick-walled, parallel hyphae. The hydrophobic nature is even strengthened by the hyaline, encrusted hyphae enclosing the sclerotium. Therefore, dispersal is likely to take place mainly on an air-water interface in moist habitats. In addition, the sclerotia have an astonishing similarity to the conidia of *Cancellidium applanatum* TUBAKI, an aero-aquatic hyphomycete characterized by irregularly lenticular, partly hollow conidia composed of a one-layered wall of tightly appressed, vertical, parallel, dark-walled hyphae (TUBAKI 1975, WEBSTER & DAVEY 1980); a structure almost identical to the dark-walled one-layered cortex of *Akenomyces*. Similarly, also the interior of the conidia of *Cancellidium applanatum* is partly filled with cells capable of germination; however, these are not much-branched, closely intermingled thin hyphae but unbranched chains of large monilioid globose cells which are oriented parallelly to the hyphae of the cortex. In addition, the ontogeny is completely different, as the conidia of *Cancellidium* are formed by a single conidiogenous cell, whereas the sclerotia of *Akenomyces* are produced by a sclerotial initial consisting of tightly interwoven hyphae. The propagules of *Akenomyces* and *Cancellidium* therefore represent an interesting example of convergent evolution of a similar structure in completely unrelated taxa, which may be caused by the same selection pressure towards buoyancy.

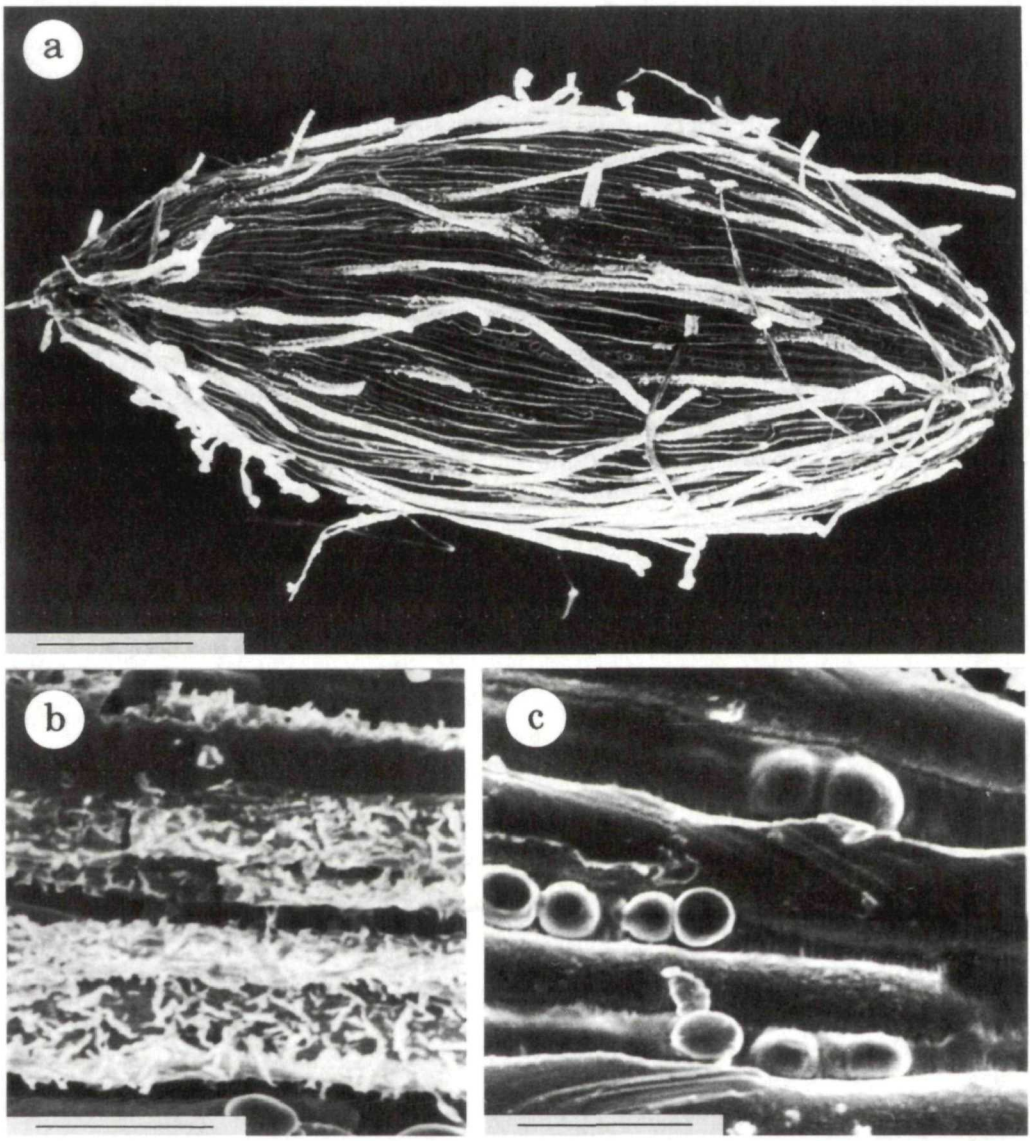

Fig. 2. SEM of *Akenomyces costatus*. *a* Whole sclerotium. *b* Detail showing enclosing hyaline hyphae densely encrusted with needle-shaped crystals. *c* Detail showing parallel hyphae of the cortex with some tubercles. Bars: *a* 50 µm; *b*, *c* 5 µm.

Taxonomy: *Akenomyces costatus* has been divided by HORNBY (1984) into two varieties which are distinguished by the size of the sclerotia: var. *costatus* with smaller sclerotia [(97-)246(-493) x (58-)103(-154) µm] and var. *enigmaticus* ARNAUD ex HORNBY with sclerotia larger than 300 x 170 µm. The sclerotia of the Austrian collection, however, are somewhat intermediate, tending more towards var. *enigmaticus* as its sclerotia are much too broad [(140-)170-280 µm] for var. *costatus*. This is also interesting concerning the ecology, as var. *costatus* has been isolated from wheat fields, whereas isolates attributable to var. *enigmaticus* have always been found on remains of *Cyperaceae* located at the margins of ponds and ditches. However, whether *A. costatus* really consists of two taxa separable by morphology and ecology must remain doubtful as var. *costatus* has been studied from just one locality, making a thorough comparison impossible (The isolates from CBS listed under var. *costatus* have not been available for this study). In addition, the two varieties have not been compared under identical conditions, as var. *enigmaticus* was only examined as herbarium material (HORNBY 1984). Taking into account that the Austrian material is intermediate and that the collections isolated are very few, it seems preferable not to distinguish infraspecific taxa until enough isolates are available for studies on a broader scale.

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