

**TRICHARINA STRIISPORA FROM ARGENTINA AND THE FINDING OF ITS ANAMORPH, ASCORHIZOCTONIA**

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**Abstract.** For first time the anamorph of *Tricharina striispora* was obtained in culture from germinated ascospores and was placed in the genus *Ascorhizoctonia* (*Mycelia sterilia*). Macro-micromorphological characters are described, using light and electron microscopy.

**Keywords:** Pezizales, teleomorph-anamorph connection, Scanning and Transmission Microscopy.

The genus *Tricharina* was coined by Eckblad (1968) to replace *Tricharia* Boud. (1885) which is illegitimate as a later homonym of *Tricharia* Fée 1824, a lichenised fungi (Santesson 1952). This genus is close to *Trichophaea* Boud. but it is readily separable on the lack of oil drops and on the type of anamorph produced, being *Dichobotrys* Hennebert for *Trichophaea* species (Hennebert, 1973) and *Ascorhizoctonia* for *Tricharina* species (Yang & Korf, 1985 a).

Yang and Korf (1985 b) in their monograph of *Tricharina*, concluded to split it into two genera: *Tricharina sensu stricto* and *Wilcoxina* Chin S. Yang & Korf based on apothecial structure, on germination of ascospores, on type of anamorph and on mode of nutrition.

During a survey of species belonging to the Pezizales in the Central East of Argentina an interesting collection belonging to *Tricharina* was found with longitudinal striate ornamentation of the ascospore wall. According to Yang and Korf (1985 b) this kind of ornamentation is only found in one species of the genus: *Tricharina striispora* Rifai, Chin S. Yang & Korf. This species was originally described from Australia (Yang & Korf, 1985 b), on the basis of a material collected in 1920 by J. Cleland and first examined by Rifai, though not published.

The objective of this paper is to describe for the first time the anamorph, the *Ascorhizoctonia* state of *Tricharina striispora*, to present the results of the germination of the ascospores and the preliminary

studies of the ascospores with Scanning and Transmission Electron Microscopy (SEM and TEM).

### Materials and Methods:

Fresh collections of apothecia found on decayed wood and soil, were examined in the field, where taking data about substrate, size and color of the ascomata were also taken. The specimens and living culture were deposited at BAFC Herbarium and BAFC culture collections respectively (Holmgren *et al.*, 1990). Parts of the BAFC collections were also kept at CUP and living cultures at CUP and MUCL.

At the laboratory micromorphological observations were made mounting sections of portions of the apothecium in phloxine or lactophenol plus cotton blue for examination in light microscopy.

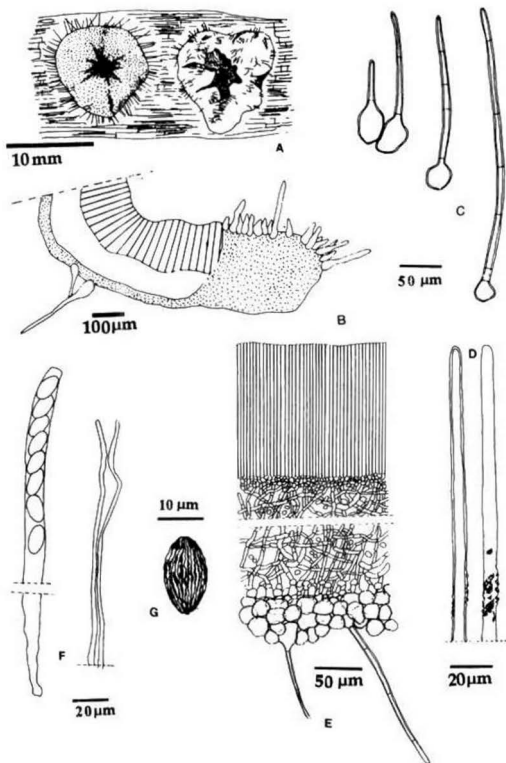
For observations in SEM a small portion of an apothecium was separated with dissecting needles and dehydrated in alcohol 30 %.

For ultrastructural examination the applied techniques are according to Samuelson (1978) except for some modifications: one millimetre squares of apothecia were used and fixed in Millonig's buffer (Millonig, 1961) and 3% glutaraldehyde for 24 hs, postfixed in 1% osmium tetroxide for 2 hs; dehydration in alcohol series and the final rinse with acetone; embedded in Spurr resin.

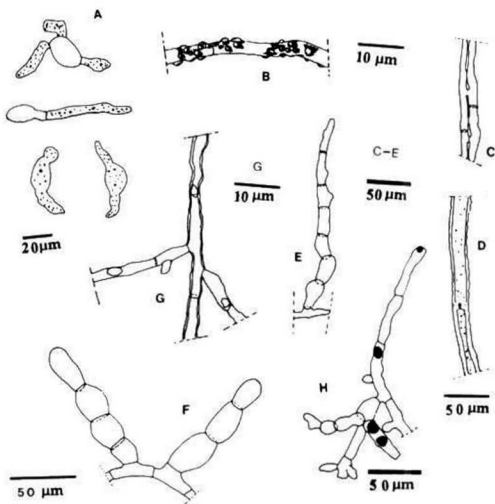
Isolations were made from ascospores obtained by stimulating the "puffing" from an apothecium placed in the cover of an inverted petri dish containing water agar with streptomycin so the hymenium faced the agar surface, illuminating it as explained by Donadini (1984). Once the ascospores germinated, they were transferred onto MA, OA plates (Hawksworth *et al.* 1995) and CM (M-5 Stevens, 1974), and kept at room temperature (23°C).

***Tricharina strilspora*** Rifai, Chin S. Yang & Korf. Mycotaxon 24: 509. 1985. (Figs. 1 and 2, Pl. I)

Apothecia small, discoid, sessile, 0,5-1,7 cm diam., fleshy when fresh; hymenium creamy white, externally with brown hairs; marginal hairs simple, septate, with bulbous base, 200-350 µm long, 5-6 µm diam at apex and 10-15 µm at base, walls thick, smooth or ornamented with warts; cortical excipulum "textura globulosa", 25-40 µm thick, 17-30 µm diam cells; medullary excipulum "textura intricata", 75-120 µm thick; asci cylindrical, 8-spored, inamiloid, 175-185 x 9-12 µm; ascospores 1-celled, ellipsoid, longitudinally striate, with warts over the striae, 15-18 x 8-9 µm; paraphyses filiform, flexuous, simple, septate, 2-3 µm broad, enlarged near apex to 4-5 µm. **Habitat:** On decayed wood; on soil beneath a fallen trunk.

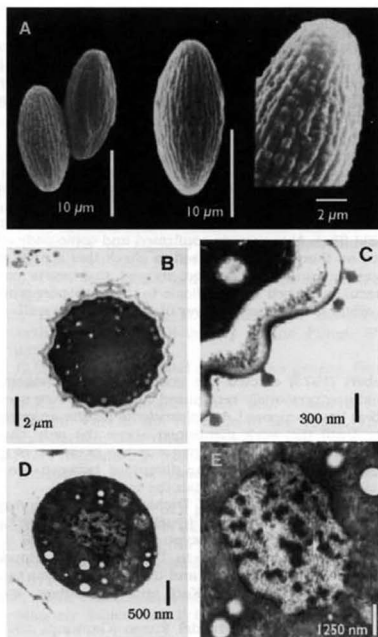


**Fig. 1.** *Tricharina striospora* Rifai, Yang & Korf. A, apothecia; B, longitudinal section; C, hairs with bulbous base; D, detail of apical portion of hairs; E, details of excipulum; F, asci and paraphyses; G, ascospore. (BAFC 34500, mounted in phloxine)



**Fig. 2.** *Tricharina striispora* Rifai, Yang & Korf. A, germinated ascospores in ME; B, detail of hyphal ornamentation; C, hyphal fusions; D, hyphal septum; E and F, monilioid cells; G and H, mycelium growing in ME. (BAFC cult. 202, mounted in cotton blue.)

**Anamorph: *Ascorhizoctonia*-state:** Colonies slow-growing, covering 90 mm petri plates in 10 days on ME at room temperature (23°C), forming concentric inconspicuous bands in ME. Mycelium superficial and colorless, becoming immersed and dark brown at maturity, aerial hyphae also dark brown at maturity. Hyphae cylindrical, 6.5-8 µm diam, brownish, smooth or densely incrustated, sometimes constricted at septum. Hyphae can be parallel and anastomosing forming bridges; others branch at acute angles, with hyphal loops formed by coiling. Monilioid cells aggregated, 14-19-(21-24) x (17-23)-30-32 µm, formed in



**Pl. I.** *Tricharina striispora* Rifai, Yang & Korf. A, ascospores in SEM; B-E, ascospores in TEM; B, transversal section of an ascospore; C, ascospore wall in detail; D, young ascospore in transversal section; E, nuclei of an ascospore. (BAFC 34500)

the agar and on the aerial hyphae, cells containing abundant oil guttules.

Yang & Korf (1985) place *Ascorhizoctonia* in *Mycelia Sterilia*.

**Studied materials:** Argentina, Buenos Aires, Lomas de Zamora, Santa Catalina, leg. Romero *et* Barrera, 10-V-1996, on fallen bark and decayed wood, BAFC 34500 (part of this collection CUP 63644); idem, 4-V-97, on soil, BAFC 34916; idem, IV-1997, BAFC cult. 202. An isolation from BAFC 34500 is deposited at MUCL 41297.

#### Notes:

The ascospores germinated with up to three germ tubes after 48 hrs resting on water agar with streptomycin.

Under light microscopy is hard to observe the longitudinal striae, but in the SEM (Pl. I, A) they were confirmed and some pedicellate warts were observed on them. TEM was used to check this structure (Pl. I, B-E). Ascospores in transverse section showed that warts consist of a dense corpuscle attached by a pedicel to the ascospore wall (Pl. I, C). Probably its origin is the external layer of the ascospore wall.

#### Discussion:

Hennebert (1973) erected the generic name *Dichobotrys* for the anamorphic states previously recognized for *Trichophaea* species. Yang & Korf (1985 a) have proposed *Ascorhizoctonia* for the anamorphic state of *Tricharina*. Until the work mentioned above the only character for separating *Tricharina* from *Trichophaea* was the presence of guttules in the ascospores of the latter, but the difference between the correlated anamorphs clearly helps to distinguish the two genera.

There were two collections of *Trichophaea* cited for Argentina by Gamundi: *Trichophaea fimbriata* (Quél.) Gamundi (1966) and *T. eguttulispora* Gamundi (1975) (Holotypus! LPS 36891, LPS 36889, LPS 36901). The former was transferred to *Tricharina gilva* (Boud. in Cke.) Eckblad by Yang and Korf (1985 b), and the latter was also transferred to *Trichophaeopsis bicuspis* (Boud.) Korf and Erb subsp. *eguttulispora* (Gamundi) Korf (1977).

So far *T. gilva*, from Tierra del Fuego (Gamundi 1975) and, *T. striispora* from Buenos Aires province, are the only two species of this genus present in Argentina.

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