# Trichophaeopsis tetraspora, a New Coprophilous Discomycete from Denmark

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Trichophaeopsis tetraspora DISSING & PAULSEN NOV. sp. is described from old cow dung from Denmark, and Trichophaeopsis bicuspis is reported for the first time from Denmark.

#### Trichophaeopsis tetraspora nov. sp.

Carposomata 0.5-1 mm lata, turbinata vel disciformia, regulariter orbicularia, solitaria vel gregaria. Hymenium albidum, planum vel paulum cavum. Margo laevis, elevatus. Superficies exterior carposomatis fuscida, pilis longis rigidis fuscidis obsita, summis simplicibus sursum directis, pilis mediae partis saepe bifidis, ramo breviore deorsum directo.

Excipulum exterius in parte superiore textura angulata, 50-56 µm crassum, in inferiore textura angulata vel globosa, 53-66 µm crassum. Cellulae eius in parte superiore  $8.3-14.5 \times 6.6-9.9 \ \mu m$ , in inferiore  $6.6-16.5 \times 6.6-13.2 \,\mu\text{m}$  magnae, in ipso margine minores, claviformes,  $3.3-8.3 \times$ 5.0-8.3 µm magnae, superficiales totius excipuli in series verticales dispositae (Fig. 1), membranis fuscidis, valde cyanophilis. Pili e cellulis excipuli exterioris orti ad 580 µm longi 23 µm crassi fuscidi acuti septis ad 15 divisi, membranis ad 3.3 µm crassis. Rami inferiores pilorum bifidorum ad 400 µm longi. Pars inferior excipuli pilis flexuosis instructa hyphoidibus hyalinis septatis ad 400 µm longis 3.3-3.5 µm crassis, membranis tenuibus.

Excipulum medullare e cellulis hyalinis membranis tenuibus formatum, aliis hyphoidibus 3.3–6.6  $\mu$ m latis, aliis angulatis 9.9–16.5 × 8.3– 16.5  $\mu$ m magnis. Subhymenium paulum manifestum. Hymenium 170–190  $\mu$ m altum; asci tetraspori 13–14  $\mu$ m crassi cylindrici operculati iodo non mutati, basibus pleurorhynchis; paraphyses supra paulum incrassatae, ad 3  $\mu$ m crassae, rectae vel subcurvae septatae.

Sporae  $18.2-19.5-20.5 \times 10.9-12.2-13.2 \ \mu m$ magnae ovales uniseriatae hyalinae laeves uninucleatae, guttulis nullis, nonnullae vagina gelatinosa indutae, nonnullae unam vel duas bullulas debaryanas exhibentes.

Typus die 28 Sept. anni 1974 in paeninsula Ulfshale insulae danicae Møen cum Cheilymenia stercorea, Zygospermella insigni, aliis fungis, alga chlorococcacea fimi bovini vetusti incola a MAREN D. PAULSEN, NORA TAMS, HENRY DIS-SING lectus, frigore exsiccatus in Museo Botanico Hauniensi (C) depositus, eadem species die 30 Oct. rursus ab iisdem inventa ibidem deposita.

### Trichophaeopsis tetraspora

#### Figs. 1-4.

Fruit bodies 0.5–1 mm broad, turbinate to disc-shaped, regular in outline, solitary or gregarious. Hymenium whitish, flat or slightly concave. Margin even, raised. Outside brownish, with long, stiff, brownish hairs. Uppermost hairs unbranched, pointing upwards, while hairs on the middle part often are bifurcate, with a shorter branch pointing downwards.

Outer excipulum above of textura angulata, 50–56  $\mu$ m thick, below of textura angulata to textura globosa, 53-66 µm thick. Individual cells above  $8.3-14.5 \times 6.6-9.9 \ \mu\text{m}$ , below 6.6- $16.5 \times 6.6 - 13.2 \,\mu\text{m}$ . Margin proper consists of smaller, club-shaped cells  $3.3-8.3 \times 5.0-8.3 \,\mu\text{m}$ . Outermost cells in the outer excipulum arranged in vertical rows (Fig. 1). The walls are brownish and strongly cyanophilic. Hairs originating from cells in the outer excipulum, to 580 µm long and to 23 µm broad, brownish, pointed, with up to 15 septa. Walls to 3.3 µm thick. In the bifurcate hairs the lower pronge is up to 400 µm long. Flexuous hairs from the lower part are hyphae-like, hyaline, thinwalled, septate, to 400 µm long and 3.3-3.5 µm broad.

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Fig. 1. Trichophaeopsis tetraspora. – vertical rows of cells in outer excipulum, and hairs. – from Holotype (C).  $\times 325$ .

Medullary excipulum of thin-walled, hyaline cells. Some are hyphoid,  $3.3-6.6 \ \mu m$ broad, others angular,  $9.9-16.5 \times 8.3-16.5 \ \mu m$ . Subhymenium indistinct. Hymenium 170– 190  $\mu m$  high; asci 4-spored,  $13-14 \ \mu m$  broad, cylindrical, operculate, J-, with a pleurorhynchous base; paraphyses above slightly enlarged, to 3  $\mu m$  broad, straight or slightly curved, septate.

Spores 18.2–19.5–20.5  $\times$  10.9–12.2–13.2  $\mu$ m, ovale, uniseriate, hyaline, smooth, uninucleate, without guttules. Some spores with a gelatinous sheath, and some with one or two de Bary bubbles.

Material: Møen, Ulfshale, on old cow dung, together with i.a. *Cheilymenia stercorea* and *Zygospermella insignis*, and a Chlorococcaceous alga, September 28, 1974, leg. MAREN D. PAULSEN, NORA TAMS, and HENRY DISSING (Holotype, freeze-dried, in C); – Ibid. October 30, idem. (several collections, C).

The new species of *Trichophaeopsis* was found on several samples of old cow dung when the



Fig. 2. Trichophaeopsis tetraspora. – to the left, ascus with spores with a gelatinous sheath; note the refringent bodies in the spores,  $\times 400$ . – to the right, fruit bodies, schematic,  $\times 20$ . – from Holotype (C).

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Forest in the northern part of the island of Møen (see p. 199). More than 50 cows are feeding in a fenced common outskirt situated west of the forest, with scattered groups of Juniperus communis and Pinus. Fresh cow dung is normally not especially attractive, but these old samples with numerous fruit bodies of vellowish-orange Cheilymenia stercorea and a reddish colour from populations of a Chlorococcaceous alga were very fascinating. Furthermore a closer examination showed a very rich flora of Pyrenomycetes, i.a. Zygospermella insignis, which is new to Denmark. Fresh material for fixation was obtained on a visit on October 30, 1974. After this visit about 10 samples were examined in detail. Besides T. tetraspora the following coprophilous fungi were found: Cheilymenia stercorea, Coprotus granuliformis, Coprotus sexdecimsporus, Lasiobolus ciliatus, Sporormiella megalospora, Sporormiella octonalis (new to Denmark), Zygospermella insignis (new to Denmark), Coniochaete scatigena, Trichodelischia bisporula and Coprinus pellucidus. Besides the



Fig. 3. Trichophaeopsis tetraspora. - a. spore, SEM photo, b. spores with gelatinous sheath, phase contrast, c. part of spore with transverse wrinkling, in Cotton Blue, Light Microscope, d. ascus with spores, containing refringent bodies, in water, Light Microscope. - a. & c. Ulfshale, October 30, 1974 (C), b. & d. from Holotype (C). a.  $\times 2000$ , b. & d.  $\times 650$ , d.  $\times 1550$ .

a Fig. 4. Trichophaeopsis tetraspora. - a. EM photo

of spore wall, b. detail of the same, arrows indicate the position. - from Holotype (C). a.  $\times$  6000, b.  $\times$  26000. (Method: Freeze dried material fixed for 24 h in 4% glutaraldehyde in 0.1 M cacodylate buffer

at pH 7. Postfixed in 2% OsO4 in the same buffer. Dehydrated in a graded ethanol series and embedded in Spurr's medium. The sections were stained at 30°C in 2% aqueous uranyl acetate for 30 min and subsequently 10 min in Reynold's lead citrate).

above mentioned reddish Chlorococcaceous alga a blue-green alga were seen.

During our studies of the material collected on October 30, we were surprised to find that on some spores, mounted in Cotton Blue, the spore walls showed a very delicate transverse striation (Fig. 3 c). A restudy of spores on slides of the Tian-Shan material of T. bicuspis (see DISSING & RAITVIIR, 1973) and of Danish material collected during the above mentioned congress revealed the same very delicate striation. With the Light Microscope it was not possible to get an impression of the true nature of the striation, but the observations re-

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called the studies by RIFAI (1968) and ERB (1972) on spore material of the genus *Rhizoblepharia*. This genus was erected (RIFAI, l.c.) because the transverse wrinkling was considered to be "unique among the *Pezizineae*". For a number of reasons this genus is considered to be closely related to the genus *Trichophaeopsis* (KORF & ERB, 1972). Spores from fresh material were then studied in the Scanning Electron Microscope. The wall was completely smooth (Fig. 3 a). This needs not, however, be in discordance with ERB's (l.c.) observations on *Rhizoblepharia*, since it is here the inner spore wall which is transversally wrinkled.

Spores from freeze-dried material were then studied with EM. All layers in the wall were seen to be smooth (Fig. 4).

A satisfactory explanation for the curious striation in *T. bicuspis* and *T. tetraspora* was thus not found. SEM and EM studies provided no support for this as a fact.

Presence or not of guttules in the spores of *T. bicuspis* has been much disputed (for a review of the literature on the subject, see KORF & ERB, 1972 and DISSING & RAITVIIR, 1973). In 1974 we had the opportunity to study, at the same time, fresh material of *T. bicuspis* and *T. tetraspora*. No guttules, droplets or granules were seen in spores mounted in water. For *T. tetraspora* this is no surprise, since, to our knowledge, no coprophilous discomycetes have spores with guttules.

A refringent body (Figs. 2 & 3 d) could be seen in the spores of *T. tetraspora*. The position of this, and its size, were very similar to that of the nucleus.

Trichophaeopsis bicuspis has never been described from Denmark. It has now been found on three localities and a description is provided below.

## Trichophaeopsis bicuspis (BOUD.) KORF & ERB

## Syn.: Trichophaea bicuspis (BOUD.) BOUD.

Fruit bodies 2–3 mm broad, turbinate to discshaped, regular in outline, solitary or gregarious. Hymenium whitish, flat. Margin even, raised. Outside brownish, with long stiff brown hairs, some of which are bifurcate, with a shorter branch pointing downwards.

Outer excipulum above of textura angulata, 39-43 µm thick, below of textura angulata to textura globosa, 50-95 µm thick. Individual cells above  $7.3-13.3 \times 10.6-15.5 \ \mu\text{m}$ , below  $16-20 \times 16-23$  µm. Cells in the margin are smaller, club-shaped,  $5.6-9.9 \times 5.0-6.6$  µm. Outermost cells in outer excipulum are arranged in vertical rows (as in T. tetraspora, see Fig. 1). They are with brownish walls, and strongly cyanophilic. The hairs originate from cells in outer excipulum, they are up to 770 µm long, 14.9-19.8 µm broad, pointed, with up to 15 septa, the walls up to 5.8  $\mu$ m thick. In the bifurcate hairs the lower branch is up to 380 µm long. Flexuous hairs from the lower part are hyphae-like, hyaline, thin-walled, septate, to 660 µm long and 3-4 µm broad. Medullary excipulum of thin-walled cells. Some are hyphae-like, 3-4 µm broad, others angular,  $6.6-9.9 \times 6.6-13.2$  µm. Subhymenium indistinct. Hymenium 215-240 µm high; asci 8spored, 13-14 µm broad, cylindrical, operculate, J-, with a pleurorhynchous base. Paraphyses above slightly enlarged, 2-3 µm broad, septate. Spores 15.2-15.8-16.8 × 9.3-9.5-9.8 µm, ovale, uniseriate, hyaline, smooth, without guttules. Some spores with one or two de Bary bubbles.

Material: Island of Samsø, near Tranebjerg, on moist soil and decaying leaves, under a dense cover of *Ranunculus repens*, July 31, 1968, leg. L. DøssiNG (DøssiNG, private herb.); - ibid., August 1968 (C); - Jylland, Ringelmose Skov, under *Populus tremula*, September 21, 1970, leg. H. DissiNG (C); - Møen, Ulfshale Skov, on small twigs of *Populus* sp., September 28, 1974, leg. L. DøssiNG (C).

Trichophaeopsis bicuspis is new to Denmark. On Samsø it has been found several times in the same locality (Døssing, unpublished). In most characters it is much alike T. tetraspora. The distinguishing characters may be summarized as in the following scheme.

	T. tetraspora	T. bicuspis
habitus	cow-dung	soil and de- caying leaves or twigs
size of fruit body thickness of walls	0.5–1 mm	2–3 mm
in hairs length of ascus number of spores	3.3 μm 170–190 μm 4	5.8 μm 215–240 μm 8
size of spores	18.2–20.5 × 10.9–13.2 μm	15.2–16.8 × 9.3–9.8 μm

GAMUNDI (1973) described *Trichophaea* eguttulispora from Argentina. No material was seen by us, but the description and drawings clearly indicate a species congeneric with *T. bicuspis* and *T. tetraspora*. It is separated from both on its ochroleucous to pallid avellaneus hymenium.

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