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First report on the presence of *Enterobryus* species (Trichomycetes: Eccrinales) in South Africa and the description of three new species

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ABSTRACT

The occurrence of Trichomycetes in the Republic of South Africa is reported for the first time. Thus far only various juliform millipedes have been examined for the presence of these fungi. Three new species of *Enterobryus* have been found and are described in detail, viz. *E.* **centroboli** Gorter, *E.* **chaleponci** Gorter and *E.* **zinophorae** Gorter.

UITTREKSEL

Die voorkoms van Trichomycetes in die Republiek van Suid-Afrika word vir die eerste keer aangemeld. Tot dusver is slegs verskillende wurmagtige duisendpote vir die aanwesigheid van hierdie swamme ondersoek. Drie nuwe spesies van *Enterobryus* is gevind en word beskryf, nl. *E.* **centroboli** Gorter, *E.* **chaleponci** Gorter en *E.* **zinophorae** Gorter.

INTRODUCTION

Enterobryus species are gut fungi which belong to the class Trichomycetes, lower fungi which are placed in the Subdivision Zygomycotina (Lichtwardt 1986). All Trichomycetes are characterised by the production of sporangiospores and the presence of a secreted holdfast with which the thalli are attached, mostly to the internal cuticular surface of certain Arthropoda. Four orders have been recognised in the Trichomycetes by Manier & Lichtwardt (1968), viz. Harpellales, Asellariales, Eccrinales and Amoebidiales. Sexual reproduction has been observed only in the Harpellales and in one genus (*Enteropogon*) of the Eccrinales (Hawksworth *et al.* 1983). None of these fungi have been grown in axenic culture except a few of the Harpellales (*Smittium* spp. and *Trichozygospora chironomidarum*) and one of the Amoebidiales (*Amoebium parasiticum*) (Lichtwardt 1978). However, this does not imply that they are parasites. Their relationship with their hosts is considered to be commensalistic, the host neither benefitting nor suffering from the association (Moss 1979). They produce one or more types of sporangiospores basipetally. Lichtwardt (1954, 1958) designated these spore types as A, B, C, D, E, F and G. They are attached by a holdfast to the hindgut cuticle of Diplopoda, less often Insecta (Coleoptera) and Crustaceae (Lichtwardt 1986). As noted by Lichtwardt (1978), the Eccrinales is, from a taxonomic viewpoint, the most difficult order of the Trichomycetes to study, probably on account of the unstable morphology of its members and the inability of researchers to culture them.

Work on the Eccrinales has been done principally in Europe, particularly in France (Duboscq *et al.* 1948; Manier 1950, 1954, 1961, 1963, 1964, 1969, 1970; Poisson 1927, 1928, 1929, 1931) and in the United States of America (Leidy 1849a, b; Lichtwardt 1954, 1957a, b, 1958, 1960a, b), but studies have also been done in India (Rajagopalan 1967) and Japan (Lichtwardt *et al.* 1987).

This article records the presence of these fungi for the first time in South Africa.

MATERIALS AND METHODS

Fungi for study were obtained from live Diplopoda. The procedures for dissection of the millipedes and observation for the presence of fungi were similar to those used by Lichtwardt (1954). However, to facilitate the cutting off of the head and anal segment, the live millipedes were first immobilised by placing them in tubes of a suitable dimension and cooling them in a freezer for 10 or more minutes, depending on the size of the millipede. The gut was then removed by grasping the posterior portion of the hindgut with a forceps and gently pulling the gut from the body. Next, the gut was cut open with finely pointed, curved scissors after which the undigested gut content was removed with the aid of a thin jet of water. As the removal of the cuticle with the fungi from the gut was found to be laborious and not always successful, most examinations were made of the fungi *in situ*, either in water or after treatment with lactophenol, with or without cotton blue. Observations on nuclei were made after fixing in Clarke's fluid (Bradbury 1973) and staining with Heidenhain's iron-alum-haematoxylin.

Millipedes were obtained from various parts of the country. Studies were confined to the juliform type of millipedes.

RESULTS AND DESCRIPTIONS

During the rainy seasons of 1989/90 and 1990/91, dozens of millipedes of different species including common ones such as *Doratogonus setosus* (Voges), *D. circulus* (Attems) and *Orthoporoides pyrocephalus* (L. Koch), were examined for the presence of Trichomycetes. Every species showed some signs of infestation. However, most of them lacked primary spores whereas the mycelium with secondary spores had traits similar to those described species which produce primary spores. This has made identification impossible. Nevertheless, in different groups of

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millipedes, three fungi were encountered which differed sufficiently from described species to be recognised as new.

Figures illustrating the newly described species serve as holotypes because the slides on which they are based are subject to deterioration.

Enterobryus centroboli *Gorter*, sp. nov.

Hyphae rectae vel undulatae, 3000–3800 × 7.5–12.5 (–15) μm, plerumque hyalinae. Cytoplasma granulosum et obscurum in hyphis laterioribus. Hyphae saepe tumidae prope basim sed angustiores ad retinaculum. *Retinaculi* orbis basalis 10.0–12.5(–15) μm diam., caulis perbrevis, circa 5.0 × 7.5 μm, vel absens. *Sporangiosporae* (typus A) 15.0 × 7.5 μm (uninucleatae) et 40–60 × 7.5 μm (quadrinucleatae). Sporae multinucleatae (typus E) 90–125 × 10–20 μm. Sporae uninucleatae (typus F), 5.0–7.5 × 10 μm.

Habitat affixa in intestino posteriore *Centroboli* spp., Natal, 1991.

TYPUS.—Figura 1A–F nititur laminis vitreis *PREM 50874 (NC 20)*, *PREM 50875 (NC 18)* et *PREM 50876 (NC 43) Centroboli lawrencei* (Schubart).

The fungus develops unbranched thalli which are attached to the anterior part of the hindgut, just below but occasionally also just inside the sphincter muscle (the muscle which divides the fore- and hindgut). Hyphae also develop towards the posterior end of the hindgut. In both cases, the hyphae are fairly straight to undulating, 7.5–12.5 (–15) μm wide, those at the front are up to 3800 μm long and those at the back up to 3000 μm. Most hyphae are hyaline but some of those in front tend to become wider and more opaque as the result of dense granular cytoplasm towards the hyphal apex. The narrower hyaline hyphae produce at their apex short or longer type A sporangiospores. The short ones (15.0 × 7.5 μm) are uninucleate and the longer ones (40–60 × 7.5 μm) are quadrinucleate (Figure 1A, C). The wider, more opaque hyphae produce 3 to 4 apical or sometimes intercalary spores, 90–125 × 10–20 μm (Figure 1F), which appear to be multinucleate and of Lichtwardt's type E.

Holdfasts are produced at the straight basal ends of the hyphae which are often swollen (by 2.5–5.0 μm) towards the holdfast and then narrow towards the point of attachment (Figure 1E). The holdfast has a very short stalk which is often shorter than wide, e.g. 5.0 × 7.5 μm. The diameter of the attachment disc is comparatively small, viz. 10.0–12.5(–15) μm.

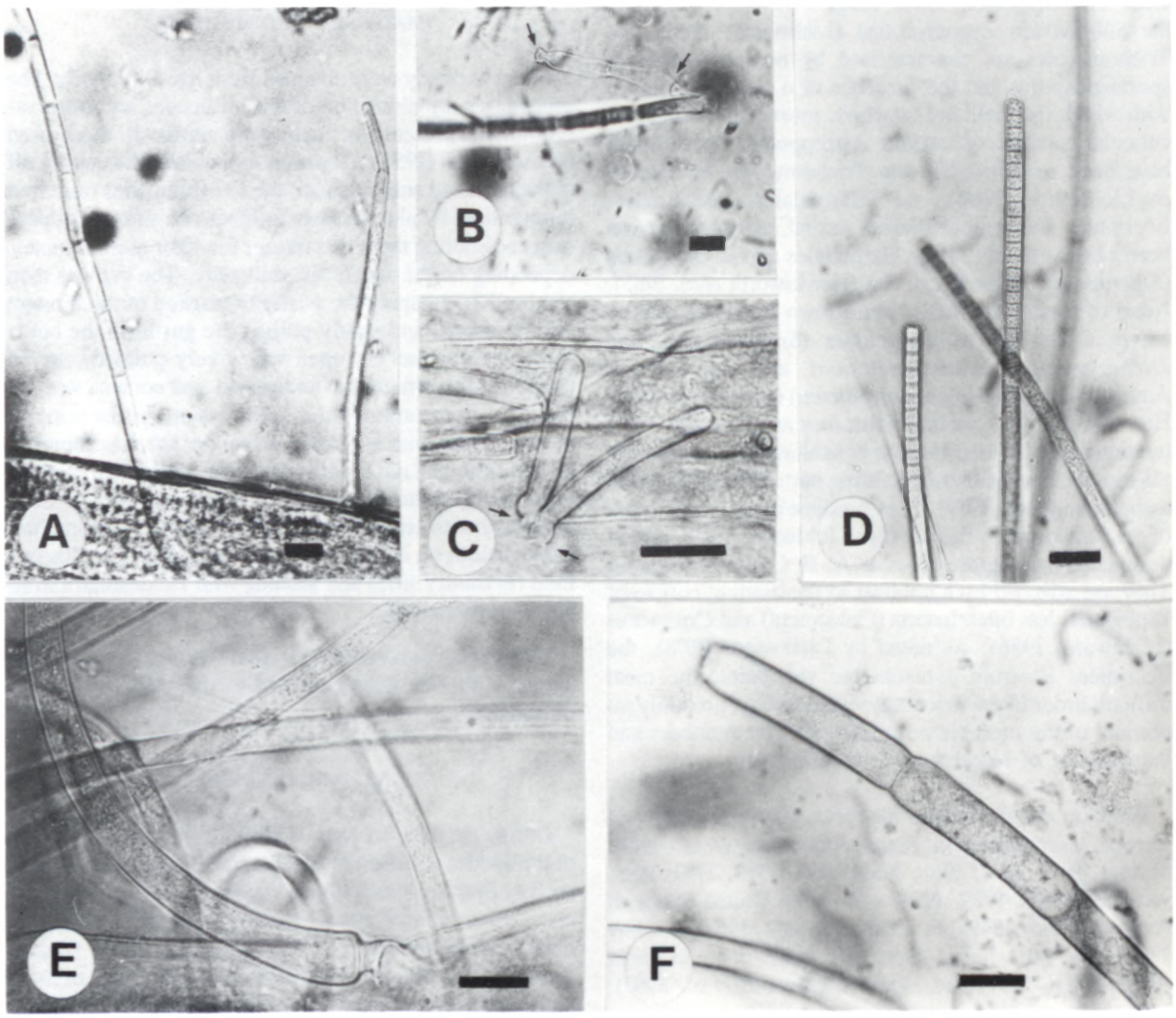

FIGURE 1.—*Enterobryus centroboli* Gorter. A, left, hyphae with 4-nucleate A spores, right, germinated type A spore fastened to substrate; B, empty sporangia (arrowed) from which type A spores have escaped; C, germinated type A spores which immediately develop holdfasts (arrowed); D, stacks of uninucleate primary spores; E, base of hypha which widens towards holdfast; F, type E spores. Scale bars = 25 μm.

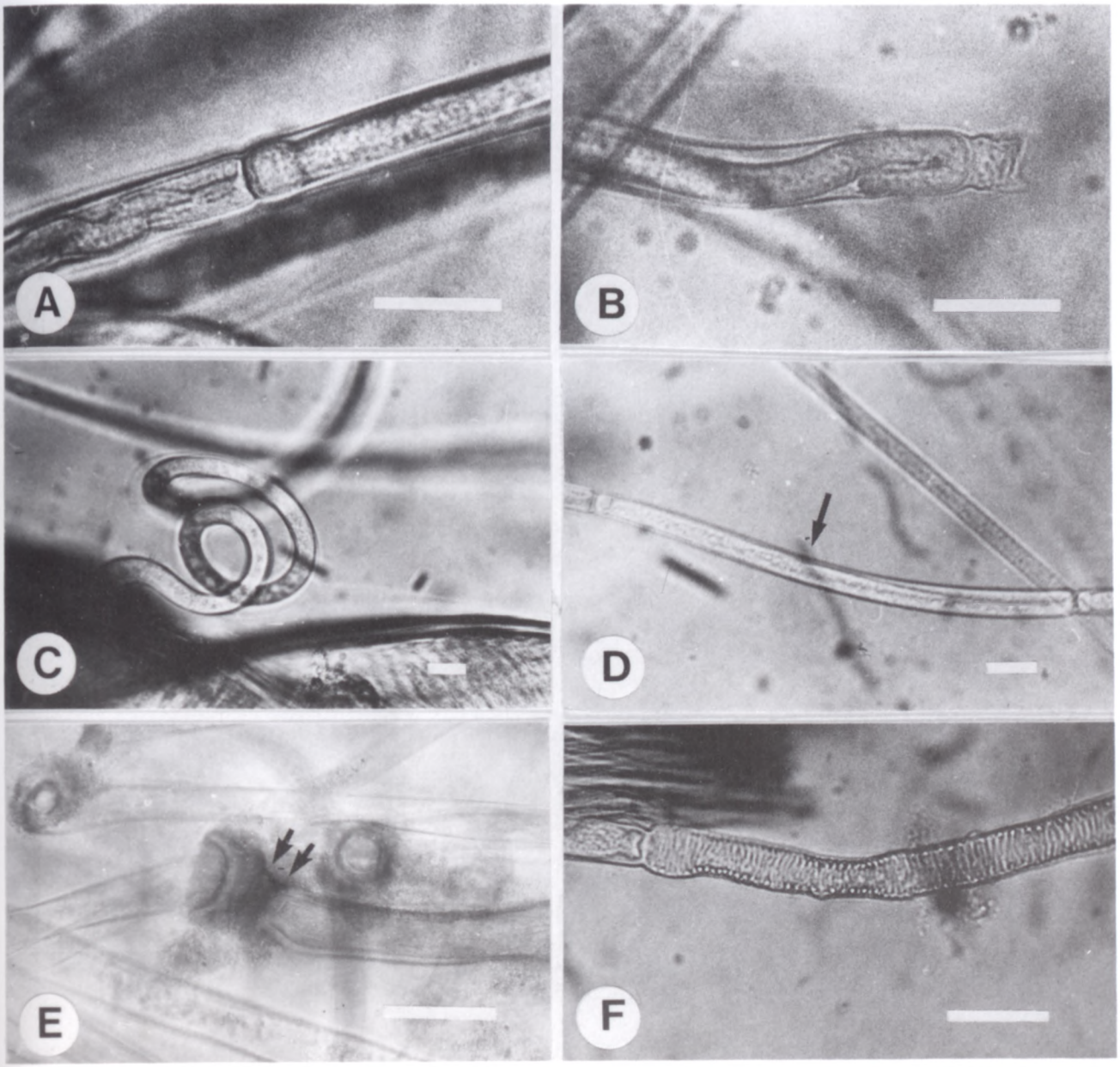

FIGURE 2.—*Enterobryus zinophorae* Gorter. A, rounded base of folded spore inside basal part of sporangium; B, apex of developing folded spore; C, spiral base of hypha devoid of sporangia; D, mature folded spore inside sporangium in straight part of hypha (spore tip arrowed); E, holdfast with short stalk (arrowed) on base of straight hypha; F, empty sporangium cell with transverse folds in wall. Scale bars = 25 μm.

Hyphae which produce primary sporangiospores are rare or absent. These hyphae are found at the posterior end of the hindgut where they produce stacks of up to 85 uninucleate spores towards their apex (type F) which are slightly shorter than wide, viz. 5.0–7.5 × 10 μm (Figure 1D). Here, too, the holdfast has a short stalk, up to 5 μm, with a small disc diameter of 10.0–12.5 μm.

Habitat: attached to the hindgut lining of *Centrobolus* spp. from Natal (Cedara and Mtunzini), 1991.

TYPE.—Figure 1A–F, based on slides *PREM 50874 (author's collection number NC 20), PREM 50875 (NC 18) & PREM 50876 (NC 43)* all of *Centrobolus lawrencei* (Schubart).

Enterobryus zinophorae *Gorter*, sp. nov.

Hyphae rectae, flexae vel undulatae, 2000–3000 × (7.5–)10.0–12.5(–15) μm, raro incurvatae prope basim. Cytoplasma hyalinum. Hyphae leviter dilatatae ad basim. *Retinaculum* cum caule brevissimo, breviore quam lato, haud longiore quam 6 μm; orbis basalis 12.5–20.0 μm diam. Nonnullae hyphae 1–6 cellulas sporangiales 175–300 × 10 μm ad apicem producentes. *Sporangia* matura sporam unam longissimam replicatamque continentia. *Sporae* basi tumida et rotunda sine palliolo apicali. Post liberationem sporarum parietes sporangiales contrahuntur fiuntque multirugosi. Sporae liberatae, 250–350 × 7.5–10 μm, a fundo germinantes, hyphas breves cum retinaculo producentes. Formas alias sporarum non vidi.

Habitat affixa intra sphincterem intestini *Zinophorae* spp.

TYPUS.—Figura 2A–F nititur laminis vitreis *PREM 50877 (MP 4)* et *PREM 50878 (MP 7) Zinophorae diplodontae* (Attems), Pretoria, Transvaal, Dec. 1990.

The thalli are inside the sphincter muscle. The hyphae are hyaline, straight, bent or undulating towards their apex but occasionally hooped near their base (Figure 2C), 2000–3000 × (7.5–)10.0–12.5(–15) μm. Hyphae have rounded ends and may widen slightly towards their base where they secrete a holdfast with a short stalk not longer than 6 μm and usually shorter than wide (Figure 2E). Some hyphae produce a number of cells 175–300 μm long and usually 10 μm wide towards their apex. These are sporangia which, when mature, contain long, folded spores (Figure 2B, D) with a slightly swollen obtuse base (Figure 2A) and a pointed apex without a protecting cap as illustrated by Manier *et al.* (1974: fig. 1F). These spores are 250–350 × 7.5–10.0 μm. The sporangia, from which they escape, contract to become empty cells with many folds (Figure 2F). The escaped spores straighten out, immediately develop a hypha from the rounded base and attach themselves to the sphincter lining. The hypha keeps on growing after which the spore presumably degenerates (empty filaments at the end of comparatively short hyphae have been noted). No other type of spore has been observed.

Habitat: attached to the sphincter muscle lining of *Zinophora* spp.

TYPE.—Figure 2A–F, based on slides *PREM 50877 (author's collection number MP 4) & PREM 50878 (MP 7)* of *Zinophora diplodonta* (Attems), Pretoria, Transvaal, Dec. 1990.

Enterobryus chaleponci *Gorter*, sp. nov.

Hyphae hyalinae, satis rectae sed plerumque circulares prope basim, circa 2500 × (10.0–)12.5–15.0(–17.5) μm, in circulis plerumque latiores quam alibi; latitudo

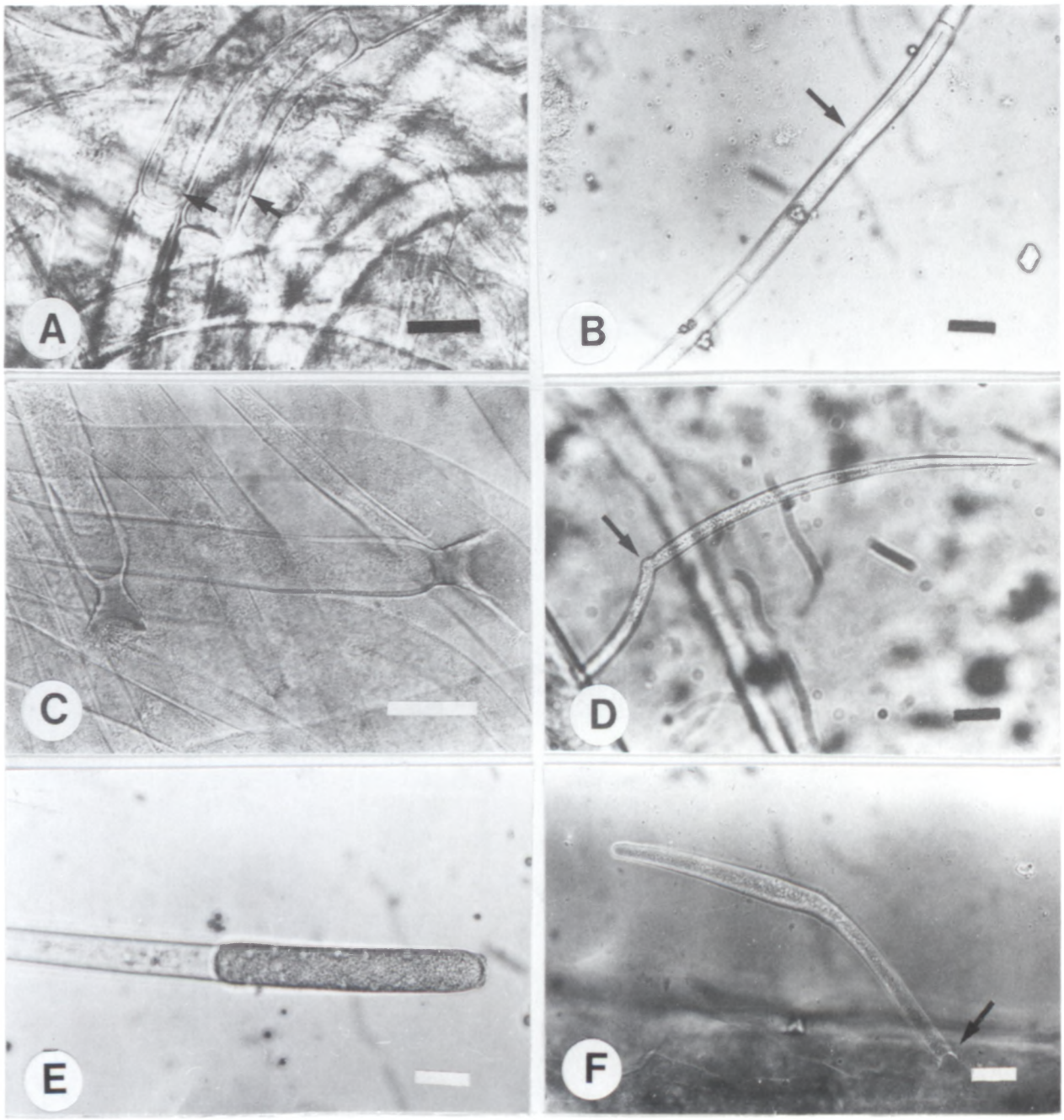

FIGURE 3.—*Enterobryus chaleponci* Gorter. A, short sporangia with folded spores (tips arrowed) inside hoops of swollen hyphae; B, mature spore inside sporangium in straight part of hypha (spore tip arrowed); C, holdfasts without stalks on straight basal end of hyphae; D, germinated, straightened-out acicular spore (base arrowed); E, multinucleate spore of type E; F, germinated multinucleate spore attached to substrate (holdfast arrowed). Scale bars = 25 μm.

aliquando etiam variat in partibus rectis. *Retinaculum* sine caule, vel cum caule perbrevi; orbis basalis (12.5–)15–20 μm diam. *Sporae* longissimae, 125–230 μm, sine palliolo apicali, replicatae, in sporangiis intra circulos 70–85 × 15 μm, in partibus rectis 105–150 × 12.5 μm. Fundus basalis sporae truncatus cum levi depressione in centro. Cytoplasma hypharum latiorum dense granulosum prope apicem. Hyphae latae aliquando cellulas longas (ad 125 μm) producentes endosporium singulum multinuclearem continentes.

Habitat affixa intra sphincterem intestini *Chaleponci polechanci* Kraus, Baltimore, Transvaal, Mai. 1991.

TYPUS. — Figura 3A–F nititur laminis vitreis *PREM 50879 (TB 5)* et *PREM 50880 (TB 30)*.

The thalli are inside the sphincter muscle. The hyphae are hyaline, fairly straight but strongly hooped lower down, ± 2500 × (10–)12.5–15.0(–17.5) μm. The hoops are often wider than the straight parts of the hyphae, 15–20 μm. The latter are of variable width, widening by 2 μm and then narrowing again. A holdfast (Figure 3C) without a stalk or with a very short one, e.g. 2.5 μm, develops at the base of a hypha. The disc diameter varies from (12.5–)15–20 μm. The thicker hyphae and those that widen from bottom to top, have a dense granular cytoplasm and often become slightly brown. Folded spores have been observed in the hooped as well as the straight part of hyphae (Figure 3A, B). In the first, sporangia are 70–85 × 15 μm; in the latter, 105–150 × 12.5 μm. The spores are 125–230 × 10.0–12.5 μm and have a truncated basal end with a slight depression in the centre. After escaping from the sporangia, the folded spores straighten, germinate basally and soon attach themselves to the inside of the sphincter muscle (Figure 3D). At the apex of a hypha with dense cytoplasm, a long cylindrical sporangium (up to 125 μm) is often formed (Figure 3E). These sporangia are slightly swollen in comparison with the rest of the hyphae and each contains a multinucleate endospore.

Habitat: attached to the sphincter muscle lining in the gut of *Chaleponcus polechancus* (Kraus), Baltimore, Transvaal, May 1991.

TYPE. — Figure 3A–F, based on slides *PREM 50879 (author's collection number TB 5) & PREM 50880 (TB 30)*.

DISCUSSION

Apart from reporting the occurrence of Trichomycetes for the first time in South Africa, a most interesting result of this investigation was the discovery of sporangia containing long, folded, pointed spores in some of these fungi. These have hitherto been found only in millipedes from Central Africa by Manier *et al.* (1974/75). They described a number of species in which acicular, folded spores are formed but noted that they develop only in the narrow hyphae. This led Lichtwardt (1986) to pose the question of whether the thalli with long, folded spores perhaps belong to one fungal species, whereas the wider thalli with different spores belong to different fungi. The latter supposition is apparently supported by our finding that, in *E. chaleponci*, the two types of spores present mostly occurred alone in different specimens of a batch of 36 millipedes of this species from one locality. However, the fact that these spore types were found together, albeit infrequently, and the fact that narrow hyphae could enlarge to become wider, point to a close relationship.

The suggestion that hyphae with long, folded spores all belong to one species is probably not valid because I found clear differences in size between the folded spores of *E. chaleponci* and *E. zinophorae*. The shape of their basal ends also differed slightly while they develop at a different locus. In *E. zinophorae* they develop only in the straight part of hyphae, while in *E. chaleponci* also within the hooped part of hyphae. Furthermore, Manier *et al.* (1974/75) observed that in some Eccrinales species the apex of the folded spore is protected by a solid point devoid of protoplasm, whereas in other species this protection is absent. These differences all indicate that different species are involved.

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