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## **BOJAMYCES REPENS: A NEW GENUS AND SPECIES OF HARPELLALES (TRICHOMYCETES) FROM A LENTIC MAYFLY**

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The order Harpellales (Trichomycetes) consists of two families, the holocarpic, unbranched Harpellaceae, which primarily inhabit the peritrophic membrane of their aquatic hosts (Diptera), and the eucarpic, branched Legeriomycetaceae, which have been reported from the hindguts of nymphs of the insect orders Diptera, Ephemeroptera, and Plecoptera. In both families, the morphology of the trichospore (shape, number of appendages, presence or absence of a collar) has been an important generic characteristic. No genus in either family has been described as having trichospores with a collar but without appendages. Lichtwardt (1986) provided a review of this order of fungi.

During May, 1986, while examining exuviae of the lentic mayfly *Leptophlebia intermedia* (Traver) for zoosporic fungi, I observed pieces of a fungus that formed collared trichospores without appendages. I observed the fungus again in 1987; in 1988, I studied it in more detail. This sparsely branched fungus differed from members of other genera of the Legeriomycetaceae in morphology of both trichospore and thallus. Because of these differences, I am placing this fungus in a new genus.

### **Bojamyces** Longcore, *gen. nov.*

Thallus sparso ramosus, indeterminatus, ad hostis proctodaei cuticulum affixus. Cellulae generativae sparsae in thallo, interspersae cum cellulis vegetativis. Trichosporae elongato-ovoideae cum collo; sine appendiculis. Zygosporae non scitae. TYPUS: *Bojamyces repens*.

### **Bojamyces repens** Longcore, *sp. nov.*

FIGS. 1–8, 10–12

Trichosporae (30–)45(–77) × 6–8 μm, collum 3–5 × 3–5 μm. Thallus irregulare divisus. Habitatum in Ephemeroptis nymphis.

Thallus sparsely branched, indeterminate, attached to cuticle of hindgut of host. Generative cells scattered on thallus, interspersed with vegetative cells. Trichospores elongate-ellipsoidal with collar; lacking appendages. Zygosporae unknown. Trichospores (30–)45(–77) × 6–8 μm, collar 3–5 × 3–5 μm. Thallus disarticulating at irregular intervals. From Ephemeropteran nymphs.

ETYMOLOGY: *Bojamyces* is derived from the Latin *boia* or *boja*, meaning collar, combined with *myces* meaning fungus. The specific epithet

*repens* means sprawling, which describes the growth-form of the thallus.

**HOLOTYPE:** Microscope slide JL01V88 deposited with R. W. Lichtwardt, Department of Botany, University of Kansas, Lawrence, Kansas 66045, prepared from the lining of the hindgut from an exoskeleton of *Leptophlebia intermedia*. *Paramoebidium* sp. thalli also occur on the slide.

**Collections.**—Salmon Pond, Hancock County, Maine [latitude 44°38'; longitude 68°04'; area = 2.4 ha; pH = 6.3 (Hunter *et al.*, 1986)]. From *Leptophlebia intermedia* nymphs collected 2 May 1986, 10 May 1987 and 1 May 1988 (TYPE COLLECTION). Uncertain from *Siphonurus* sp., same site; 7 Sep 1987.

This fungus has not been grown axenically. Attempts to culture it on PmTG (Peptonized milk 0.1%, tryptone 0.1%, glucose 0.5%, agar 1%) resulted in the eventual conversion of the thallus into generative cells. Most observations were made of the fungus growing on the hindgut lining of exuviae rather than from dissected hosts. Mature mayfly nymphs were collected on 1 May 1988, and maintained in the laboratory until ecdysis. Exuviae were examined within minutes to hours of being shed. After initial observation, the fungi on the hindgut cuticle were allowed to develop in a Petri dish containing  $\frac{1}{3}$  strength sterilized lake water. Photomicrographs were made from living material. Trichospores ( $n = 35$ ) were measured from material on the type slide fixed with lactophenol-cotton blue. Trichospore length included the collar; width, which varied little, did not differ between live and fixed material.

When exuviae were shed, most thalli were in an early stage of growth (FIG. 1) consisting of an occasionally-branched hypha with few cross walls attached to an elongate-pointed structure (FIG. 1a, c), probably a sporangiospore that had previously been extruded from a trichospore (Williams, 1983). It appeared that the sporangiospore had attached to the cuticle of the gut by a small, peg-like holdfast (FIG. 1c) and that the thallus grew from this basal holdfast. After two days at 10 C, the thallus increased in length, more branches formed (FIGS. 1, 2) and additional cross walls were evident in areas that were previously aseptate (FIG. 1a, b). Within several days, additional cross walls formed, and trichospores began to develop from generative cells (FIG. 10). Generative cells were interspersed with vegetative cells, often with a repeating series of one or two, occasionally three or more shorter genera-

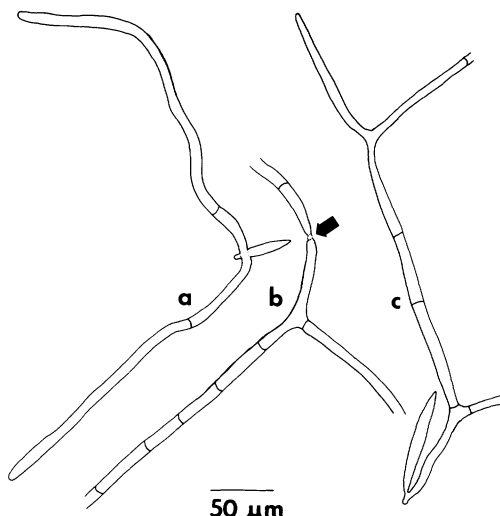
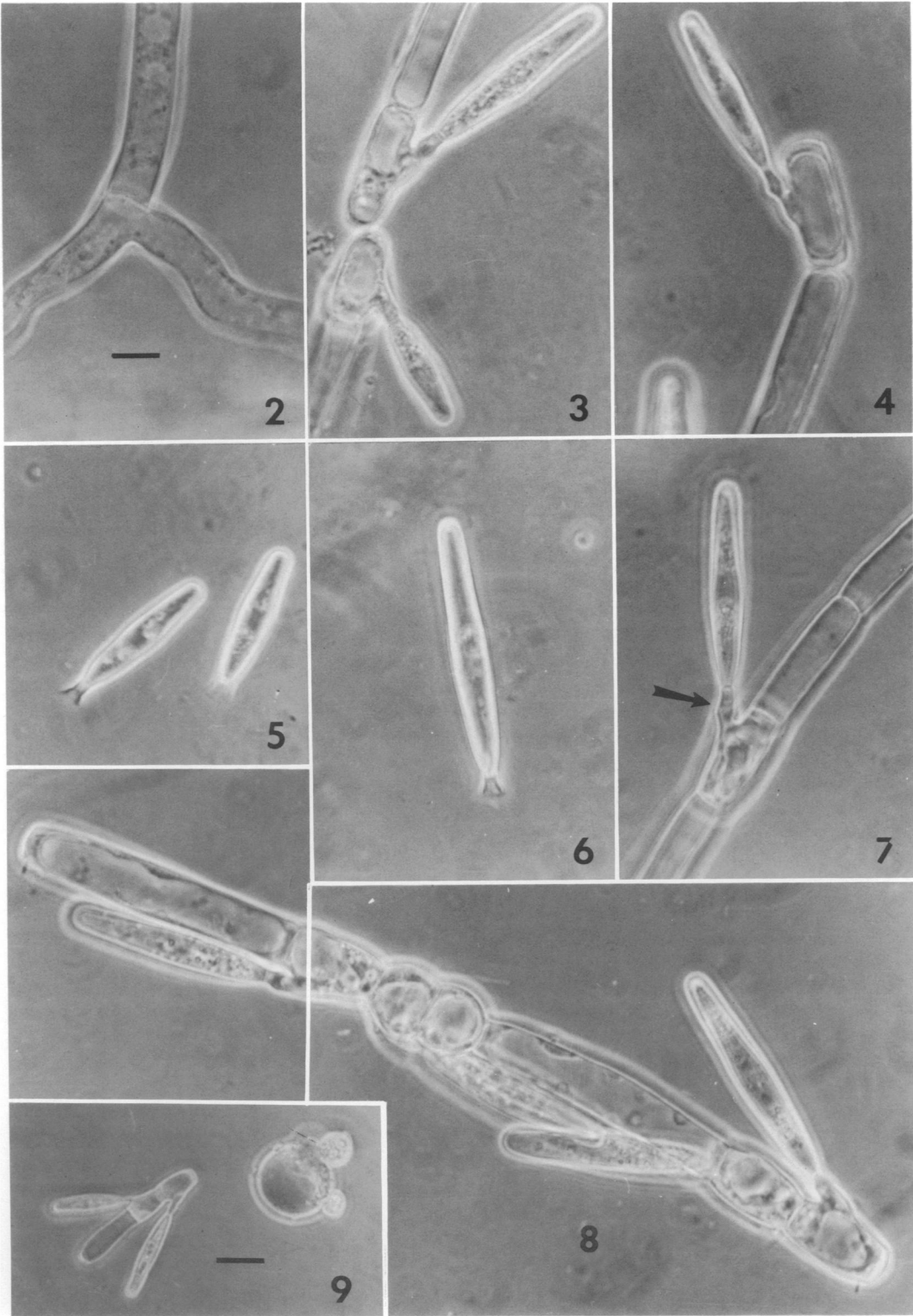


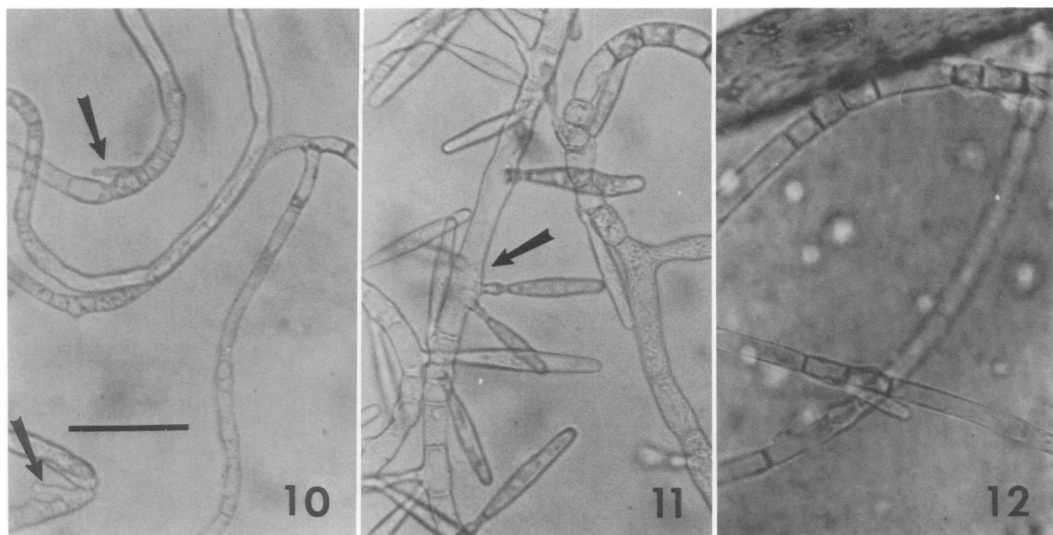
FIG. 1. Camera lucida drawings of immature *Bojamyces repens* thalli. a. Probable sporangiospore with thallus growing in two directions. b. Same thallus as a. after 48 h; arrow points to site of sporangiospore, which was in a different plane. Note the new branch and septation of a previously aseptate area of thallus. c. Thallus arising from holdfast area at base of sporangiospore. Note small, basal, peg-like holdfast.

tive cells followed by a vegetative cell. Thalli that matured on nutrient agar formed primarily generative cells (FIG. 11), whereas hyphae that matured within exuviae formed long, vegetative cells interspersed with shorter, generative cells (FIG. 12). The orientation of the trichospores on the thallus was non-polar, with adjacent spores frequently pointing in opposite directions (FIGS. 3, 8).

Separation points occurred between any cells of the thallus, leaving sections of thallus of various sizes (FIGS. 3, 4, 8). One piece of thallus that matured undisturbed inside the insect exoskeleton was 1.5 mm long and consisted of 22 vegetative cells and 37 generative cells. This unbranched section of thallus contained at least 3 sites of disarticulation. Dissection or agitation of the exuviae caused thalli to separate into sections; normal spore development followed on each separate section. Mature spores subsequently separated from generative cells, each retaining a piece of generative stalk to form the collar (FIGS. 5–7). Zygospores were not seen.

Fewer than half of the exuviae that were examined contained thalli of *B. repens*. However, all exuviae contained thalli of *Paramoebidium* sp. Also, I have observed a chytrid parasite (pos-





FIGS. 10–12. *Bojamyces repens*. Bar = 50  $\mu\text{m}$ . 10. Immature thallus on agar; note branch (at right) and incipient trichospores (arrows). 11. Thallus matured on agar; note trichospore formation to the exclusion of vegetative cells and formation of several trichospores on one cell (arrow). 12. Thallus inside exoskeleton.

sibly a *Rhizophydium* sp.) on the *Paramoebidium* cysts (FIG. 9) in each of the three years of the study.

*Bojamyces repens* is unique in the Harpellales because it consistently forms collared trichospores without appendages. In the branched Legeriomycetaceae, *Smittium* and *Trichozygospora* have collared trichospores but these trichospores also have appendages (Lichtwardt, 1986). The branched genus, *Zygopolaris* has trichospores that lack appendages and, in *Z. borealis* Lichtwardt and Williams, occasionally these trichospores have a very short collar (Lichtwardt and Williams, 1984). *Zygopolaris*, however, differs from *B. repens* in the morphology of the thallus.

The thallus of *B. repens* is branched but has a sprawling (FIG. 10) rather than arborescent form of growth. Branches occur occasionally and only one branch per site and each branch appears to be indeterminate in length. Other members of the Legeriomycetaceae branch more frequently (Lichtwardt, 1986) and have branches of a finite length giving the thalli a shrubby form.

Generative cells are interspersed with vegetative cells in *B. repens*, a characteristic that distinguishes it from species in both families. Thalli of the Harpellaceae are holocarpic and thus every cell is generative [except for *Stachylina reflexa*, which has a small, sterile, basal cell (Lichtwardt and Williams, 1988)]. Members of the Legeriomycetaceae have vegetative cells near the base of the thallus and usually a characteristic number of generative terminal cells. Development of all generative cells and no vegetative cells when *B. repens* matured on nutrient agar (FIGS. 10, 11) leads me to speculate that it is potentially holocarpic, with the ratio of generative to vegetative cells influenced by the nutritional state. Length of the trichospore seems related to diameter of the thallus and thus also may vary depending on nutritional state.

Cells of *B. repens* may separate from each other and mature trichospores also separate from the generative cell. Separation of the thallus has not been reported in other members of the Legeriomycetaceae, but in the Harpellaceae, *Caroux-*

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FIGS. 2–9. *Bojamyces repens*. 2–8. Bar = 10  $\mu\text{m}$ . 2. Branching of thallus. 3. Disarticulation between two generative cells with immature trichospores. 4. Generative cell separating from vegetative cell. 5, 6. Mature trichospores. 7. Mature trichospore attached to the generative cell; arrow where trichospore will separate. 8. Disarticulated section of thallus; note adjacent trichospores oriented in opposite directions. 9. *Rhizophydium*-like parasite on *Paramoebidium* sp. cysts and section of *B. repens*. Bar = 20  $\mu\text{m}$ .

*ella scalaris* Manier, Rioux & Whisler *ex* Manier & Lichtwardt and *Stachylina reflexa* Lichtwardt & Williams have disarticulating generative cells; however, in these species mature trichospores remain attached to their generative cells (Lichtwardt and Williams, 1988).

Lichtwardt (1986) has noted that trichospore appendages allow entanglement with detritus and may prevent spore drift in flowing waters. *Bojamyces repens* occurs in lentic waters and, therefore, does not risk loss of propagules by downstream drift, even though the trichospores lack appendages. In the laboratory, trichospores from *B. repens* thalli that develop undisturbed, lie free in the lumen of exuviae. In a lake, trichospores may remain with exuviae and be ingested by mayfly nymphs along with other detritus.

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Key Words: gut fungus, Trichomycetes, Harpellales, *Bojamyces*, Ephemeroptera.

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## ENDOPHALLUS, A NEW GENUS IN THE PHALLACEAE FROM CHINA

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The Phallaceae is a well-defined family in the Phallales (Berkeley, 1860; Zeller, 1948; Burk, 1980). The various genera of this family share a combination of characters, including a peridium of two or three layers, at first enclosing the receptacle and gleba, finally rupturing from the apex downwards and remaining at the base of the re-

ceptacle as the volva (Fischer, 1900; Teng, 1939; Cunningham, 1979). The mature volva in the Phallaceae usually appears cup-like, with the lower part of the universal veil around the base of the mature receptacle. No taxa have been described with the peridium rupturing from the receptacle base, so the mature fruitbody is with-