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A new *Coelomomyces* pathogenic to mosquitoes in Costa Rica

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Abstract: A new species of fungus belonging to the lethal genus *Coelomomyces*, *C. neotropicus*, is described and illustrated. It was found parasitizing larvae of two species of Culicidae, *Culex pilosus* and *Aedes* sp., in a lowland tropical wet forest swamp in northeast Costa Rica.

Key words: *Coelomomyces*, Costa Rica, fungus, mosquito, pathogen.

The fungal genus *Coelomomyces* Keilin, *emend.* Couch (Blastocladiales, Coelomomycetaceae) consists of about 70 described species and varieties, all of which obligately parasitize aquatic arthropods, primarily larvae of mosquitoes (more rarely chironomids) and their alternate hosts consisting of copepods or ostracods (Couch and Bland 1985). Species of *Coelomomyces* are generally found in moribund or dead mosquito larvae, and are easily recognized by the yellow to golden-brown color of the relatively large resting sporangia which may completely fill the hemocoel and other body parts of the insect host.

The fungal genus has attracted considerable interest because of its potential use in biological control of mosquitoes (Chapman 1985, Laird 1985), which are vectors of important human diseases such as malaria, yellow fever, and dengue. However, attempts in the past to control these diseases by artificial introduction of *Coelomomyces* into mosquito populations met with unexplained inconsistent results until the discovery by Whisler *et al.* (1974, 1975) that species of *Coelomomyces* have an essential alternate host. The posteriorly uniflagellate zoospores that emerge from resistant sporangia produced by sporophytic thalli in the insect host are capable of infecting only particular copepods or ostracods. Gametophytic thalli develop within these small crustaceans and produce uniflagellate male and female gametes. The resulting biflagellate zygotes are capable of infecting the larvae of mosquitoes or chironomids. The crustacean alternate hosts of most species of *Coelomomyces* have not been identified.

Coelomomyces had not been found in Costa Rica prior to our chance discovery in the area of Sarapiquí. The junior author started a systematic search for these fungi in 1964 and examined 17 652 larvae collected from sea-level to alpine bogs, including the tank-bromeliad fauna from which Picado (1913) reported, without detailed description, the coelom fungal infection of a chironomid. Picado could not have known *Coelomomyces*, as the genus was not described by Keilin until 1921.

MATERIAL AND METHODS

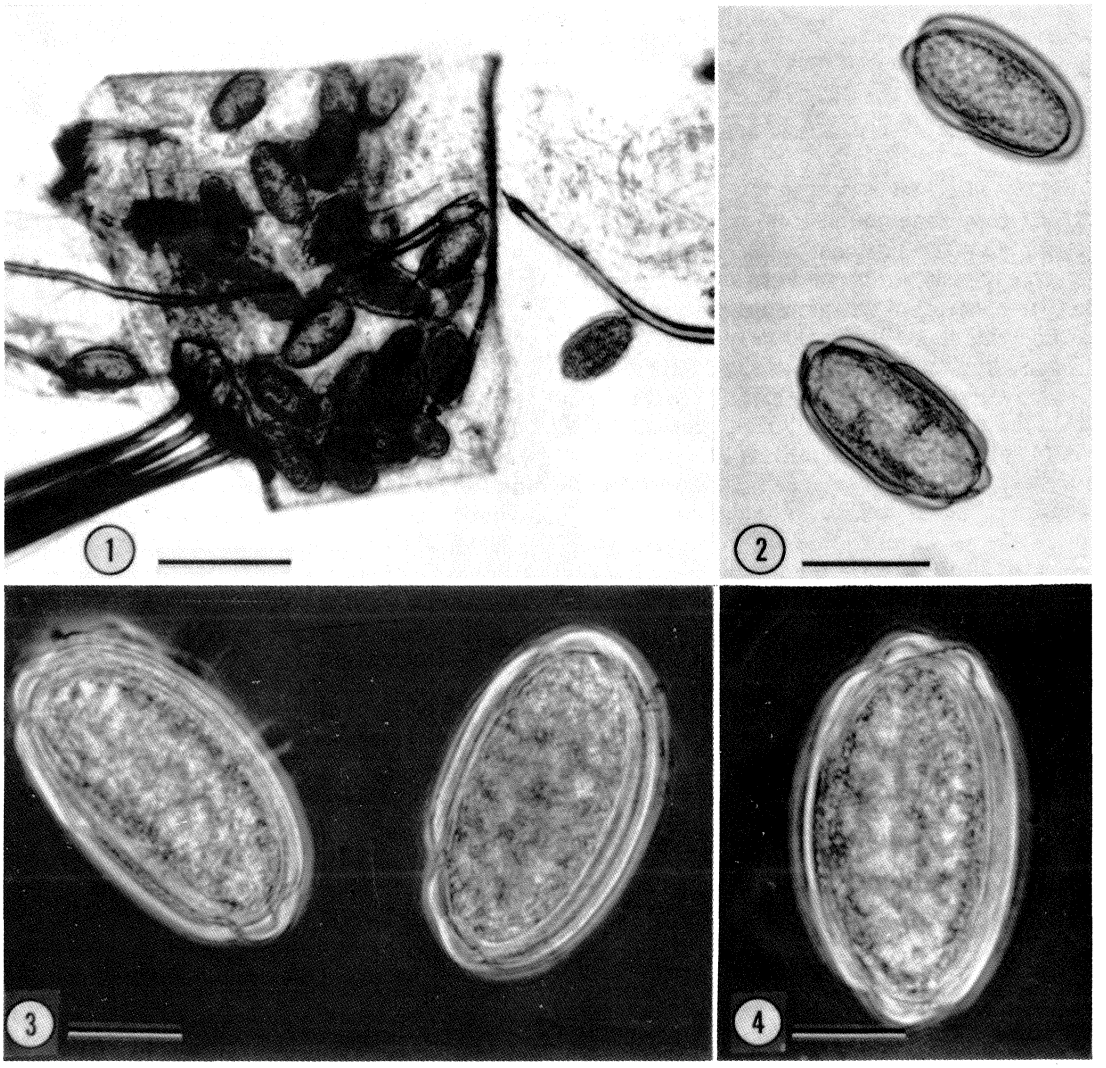
Mosquito larvae infected with *Coelomomyces* were collected on the 3rd and 4th of June 1988 in a swamp near Camino Experimental Sur located about 200 m from the beginning of the trail, at the Organization for Tropical Studies' La Selva Biological Station near Puerto Viejo de Sarapiquí, Costa Rica. Both living and dead larvae were dissected, and resting sporangia and body parts in water-mount slides were examined using brightfield and phase-contrast microscopy, and photographed on Kodachrome 64 transparency film. Microscope slides were subsequently infiltrated with lactophenol-cotton blue for preservation, and sealed with clear fingernail polish. Larvae and larval parts were preserved in 70% ethanol.

Scanning electron micrographs were made of resting sporangia removed from the hemocoel of a mosquito larva that had been preserved in 70% ethanol (specimen CR-169-33). The sporangia were collected on an 8 μm Nuclepore polycarbonate capillary pore disk membrane, washed with 70% ethanol, and further dehydrated in 90% and 100% ethanol. The sporangia on the membrane were kept in a silica gel desiccator for four days, then coated with 300 Å gold-palladium and studied in a Hitachi 570 LaB_6 scanning electron microscope using an accelerating voltage of 10 kV.

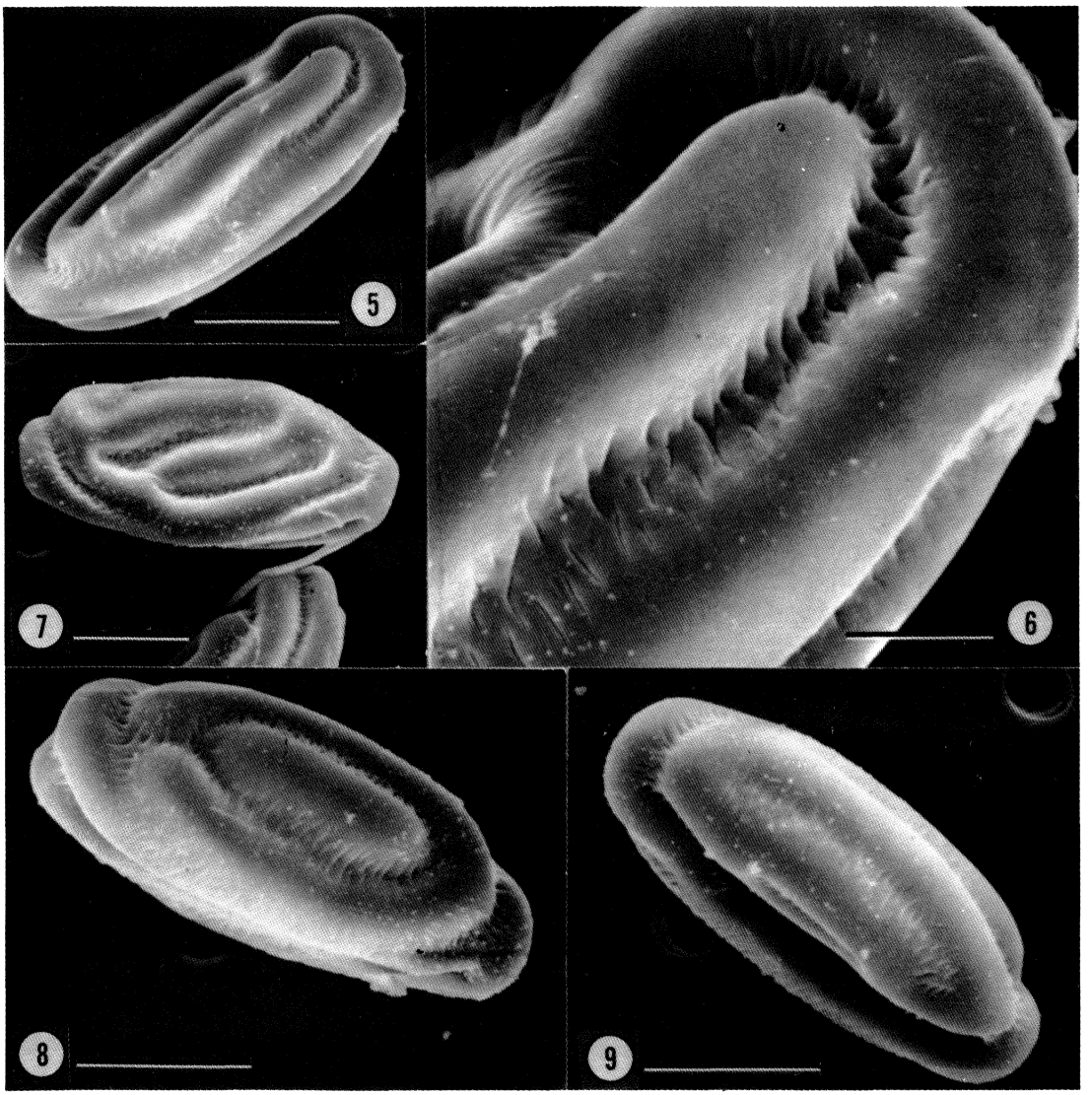
Measurements provided in the description are based on photomicrographs of 52 resting sporangia either unfixed in water mounts, fixed in 70% ethanol, or from SEM-prepared specimens. No differences in sporangial dimensions were detected that could be attributed to fixation treatments.

DESCRIPTION

***Coelomomyces neotropicus* Lichtwardt & Gómez (Figs. 1-9)**



Figs. 1-4. *Coelomomyces neotropicus* in light microscopy. 1. Dissected anal segment of mosquito larva with resting sporangia occupying dorsal saddle region, one sporangium (left) within an anal papilla. 2-4. Resting sporangial wall ornamentation as seen with phase-contrast optics. Scale bars: Fig. 1 = 100 μm; Fig. 2 = 40 μm; Figs. 3, 4 = 20 μm.



Figs. 5-9. Resting sporangia of *Coelomomyces neotropicus* in scanning electron microscopy, showing irregular patterns of ridges and uneven transverse striae and fine slits (Fig. 6) between ridges. Scale bars = 20 μm, except Fig. 6 = 5 μm.

Sporangia perennantia in Culicidarum larvis ellipsoidea, (26-)36(-46) x (54-)66(-80) μm, nonnulla leviter applanata. Superficies liris latis longitudinalibus vel obliquis interterstis polos involventibus ornata. Inter liras striae inaequales transversae et incisurae subtiles efformatae.

Resting sporangia in Culicidae larvae ellipsoid, (26-)36(-46) x (54-)66(-80) μm, some slightly flattened. Surface ornamented with broad longitudinal to oblique interconnecting ridges that wrap around the poles. Numerous uneven transverse striae and fine slits located between ridges. Dehiscence slit inconspicuous. Thin-walled sporangia not observed. Alternate host(s) not known.

Holotype: Specimen CR-169-15, consisting of a dissected larva of *Culex (Melanoconion) pilosus* (Dyar & Knab) (Diptera, Culicidae) infected with *C. neotropicus*, preserved in 70% ethanol. Collected 3-VI-88 from a swamp next to Camino Experimental Sur near 200 m marker, La Selva Biological Station, near Puerto

Viejo de Sarapiquí, Heredia, Costa Rica. Deposited with R.W. Lichtwardt, University of Kansas.

Other infected specimens: Four larvae of *Aedes (Ochlerotatus)* sp. and one unidentified mosquito larva collected from the same swamp site on 3-VI-88 and 4-VI-88.

DISCUSSION

Uninfected specimens of mosquito larvae from the swamp included *Aedes (Ochlerotatus) dupreei* (Coquillett) group, *Aedes (Ochlerotatus) serratus* (Theobald) group, *Culex (Melanoconion)* sp., and *Psorophora (Janthinosoma) ferox* (Humboldt). Collections of larvae were limited in number because of the need for minimal disturbance of the site, which was being used for other ongoing biological experiments and observations. Some larvae of *Aedes (Ochlerotatus)* sp., not infected with *Coelomomyces*, were infested with the commensalistic hindgut fungus *Smittium culisetae* Lichtwardt (Trichomycetes, Harpellales) (Lichtwardt 1986). The hindgut and midgut of several species of larval midges (Chironomidae) were infested with other currently undescribed Trichomycetes belonging to the genera *Smittium* Poisson and *Stachylina* Léger & Gauthier, but none of the many midges examined was infected with *Coelomomyces*.

Coelomomyces neotropicus differs from other described species and varieties in both the pattern of wall ornamentation, as revealed by SEM micrographs (Couch and Bland 1985), and the size range of the resting sporangia. The species of mosquitoes infected with *C. neotropicus* have not been reported previously in the literature as hosts of *Coelomomyces* spp.

The genus is essentially worldwide. Although many species of *Coelomomyces* have been reported from the Paleotropics, those found in the Neotropics are few. These include: *C. lacunosus* var. *lacunosus* Couch & Sousa in *Trichoprosopon longipes* (Fab.) from Panama; *C. reticulatus* var. *parvus* Couch, Farr & Mora in *Aedomyia squamipennis* (L.-A.) from Colombia; and *C. seriostriatus* Couch & Davies in *Culex portesi* S. & A. from Trinidad. It seems likely that other neotropical species of *Coelomomyces* remain to be discovered.

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