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Research Note

FIVE NEW ISOLATES OF THE MOSQUITO PATHOGENIC FUNGUS *LAGENIDIUM GIGANTEUM* (OOMYCETES: LAGENIDIALES) FROM COLOMBIA

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Lagenidium giganteum Couch, a water mold of the Class Oomycetes, is a facultative parasite of mosquito larvae that has been shown to be a promising biological control agent (R. C. Axtell, 1983, *Mosq. News, 43:* 122-125). Recently its status has changed and it is available for mosquito control operations (J. Kerwin et al., 1990, *J. Econ. Entomol., 83:* 374-376).

This pathogenic species has been isolated mainly from North America; however, literature reports indicate that isolations have been performed in other places of the world (J. N. Couch & S. V. Romney, 1973, *Mycologia, 65:* 250-252; L. G. Willoughby, 1969, *Trans. Br. Mycol. Soc., 52:* 393-410; M. S. Goettel et al., 1983, *J. Invertebr. Pahtol., 41:* 1-7; S. P. Frances et al., 1989, *J. Invertebr. Pathol., 54:* 103-111), although some of the cultures were not maintained for further evaluation and comparison. Recently, J. Kerwin & R. Washino (1988, *J. Med. Entomol., 25:* 452-460) isolated another strain from California. In the present study five new isolates of *L. giganteum* from northwestern Colombia are reported as well as their pathogenicity towards mosquito larvae and their zoosporogenesis pattern.

Between 1988 and 1991 five isolates of *L. giganteum* were found in three different mosquito habitats in Colombia; two in the Uraba area (Northwest) CIB 79-MED infecting *Aedes* sp. larvae, and CIB 79-TDT, in *Culex quinquefasciatus* sentinel larvae both in semipermanent pools. In Quibdo, Western Colombia, another strain was isolated by using sentinel cages (CIB 163-PDCH) baited with *Cx. quinquefasciatus*. During the first months of 1991 two more strains were isolated from the Pacific localities of Arusi and Nuqui, CIB 183-ARU from water and detritus collected from bromelia plants, and CIB 183-NUQ from water samples collected in a permanent pond. In both cases, first instar *Ae. aegypti* larvae were exposed to the samples, and the fungi were isolated after two and five days respectively. In all cases the fungi infecting mosquito larvae were morphologically indistinguishable from *L. giganteum*. Infected larvae were placed in plastic cups containing 100 ml of dechlorinated tap water where 15 healthy laboratory-reared 3-day-old *Cx. quinquefasciatus* larvae had been placed previously. Cadavers were washed in sterile tap water, and submerged in a gentamicin solution (4 mg/ml) for 1 min and transferred individually to petri dishes containing (PYG) peptone-yeast-glucose (per liter 1.3 g peptone, 1.3 g yeast extract, 3 g glucose, and 2% agar) as basal medium, and a mixture of penicillin V, 10.000 units, and streptomycin 10 mg/ml to a final concentration of 0.5% of each antibiotic.

Lagenidium giganteum California (CA) was obtained from P. T. Brey, Institut Pasteur, Paris; North Carolina (NC), and Louisiana (LA) isolates were obtained from J. Kerwin, University of California, Davis; the Australian isolate (ARSEF 2532) was obtained from A. W. Sweeney, Army Malaria Research Unit, New South Wales. These isolates and the Colombian strains used in these experiments are maintained on agar sunflower seed extract (SFE) as described by S. T. Jaronski & R. C. Axtell (1984, *Mosq. News 44:* 377-381), and D. R. Guzman & R. C. Axtell (1986, *J. Amer. Mosq. Control Assoc. 2:* 196-200) at room temperature (23 ± 3 °C), and subcultured from dead larvae every 2-3 weeks.

For scanning electron microscopy (SEM), *L. giganteum* PDCH infecting *Cx. quinquefasciatus* larvae, and sporangia from a 2-day-old SFE-liquid culture were fixed with phosphate

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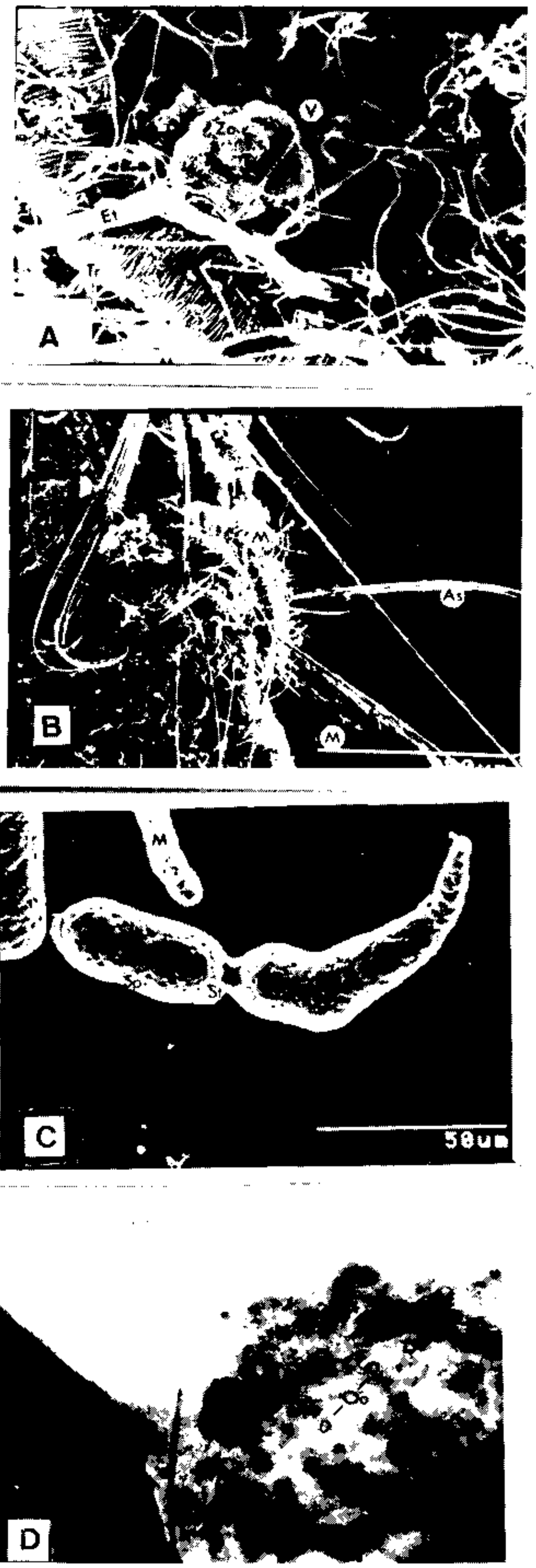

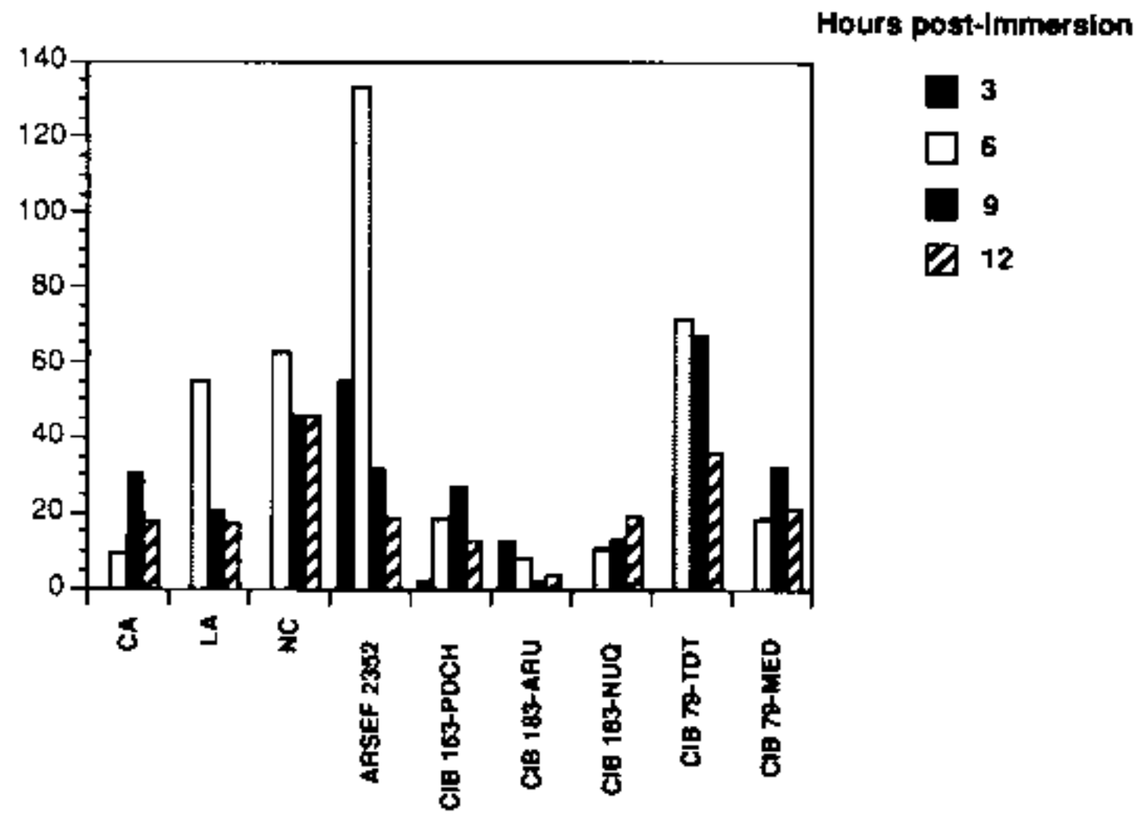

Fig. 2: comparison of vesicle formation during zoosporogenesis of *Lagenidium giganteum* isolates from Australia, United States and Colombia. CA, California; LA Louisiana; NC, North Carolina; ARSEF, Agriculture Research Service; PDCH, Quibdo; MED, Medio; TDT, Temporal dos; ARU, Arusi; NUQ, Nuqui.

buffer (pH 7.2) and 2.5% glutaraldehyde for 2hr. The fixed specimens were rinsed in the above mentioned buffer and samples oxidized by exposition to osmiun tetroxide vapors, and dehydrated with a series of ethanol solutions (30-100%) followed by critical point drying. Samples were coated with a 15 nm layer of gold-palladium and examined with a Hitachi S-510 scanning electron microscope.

Pathogenicity of each of the nine strains of *L. giganteum* was evaluated by challenging twenty *Cx. quinquefasciatus* 3-day-old larvae with 1 to 5 20 mm^2 mycelial discs of SFE-agar of each isolate of *L. giganteum,* not more than a week old, in 100 ml of dechlorinated tap water. Larval mortality was scored 48 hr after treatment.

By duplicate a 20 mm^2 mycelia disc was set in a petri dish with 15 ml of destilled water. The number of formed vesicles was recorded every 3 hr during the first 12 hr after immersion of the mycelia discs; readings are the sum of five microscope fields (x100).

All the Colombian isolates of *L. giganteum* infecting laboratory reared *Cx. quinquefasciatus* larvae showed mycelia growing and branching, and becoming septated. Each segment in the septated mycelia becomes an irregularly rounded sporangia, that when mature, forms an exit tube towards the external part of the larval cadaver, through which cytoplasm is extruded and wher zoospores mature (Fig. 1A), and after 5-15 min are released to the environ-

Fig. 1-A: *Lagenidium giganteum* zoospores formed in the vesicle outside larval body. Et, exit tube; Zo, zoospores; V, vesicle; M mycelia. Note bacterial and fungal contaminants on the vesicle surface. Bar 50 µm, 1500x. Fig. B: *L. giganteum* mycelia (M) coming out of a dead *Culex quinquefasciatus* larva through the abdominal region. As, abdominal setae. Bar 200 µm, 200x. Fig. C: *L. giganteum* sporangium formed (Sp) with septum (St) from liquid SFE culture. Bar 50 µm, 800x. Fig. D: *Cx. quinquefasciatus* larva showing *L. giganteum* oospores (Oo) in the thoracic area. 100x.

TABLE

Forty-eight hours mortality of *Culex quinquefasciatus* 3 day-old larvae when treated with different concentrations and strains of *Lagenidium giganteum* from Australia, United States and Colombia

Strain	Percent larval mortality					
	Number of mycelia discs					
	0	1	2	3	4	5
	USA					
California	0	100	100	100	100	100
North Carolina	0	40	87.5	100	97.5	87.5
Louisiana	0	0	0	100	100	100
	Australia					
ARSEF 2532	0	0	0	0	0	0
	Colombia					
CIB 163-PDCH	0	0	0	0	0	0
CIB 79-MED	0	12.5	87.5	87.5	100	97.5
CIB 79-TDT	0	30	30	37.5	77.5	100
CIB 183-ARU	0	0	0	0	0	0
CIB 183-NUQ	0	97.5	100	100	100	100

Mean values are the result of two tests, with two replicates per test.
CIB, Corporación para Investigaciones Biológicas; PDCH, Quibdo; MED, Medio; TDT, Temporal dos ; ARU, Arusi; NUQ, Nuqui.

ment. In infected larvae, exit tubes can be observed coming out of the cadaver through the abdomen (Fig. 1B). In SFE liquid culture without antibiotics, mycelia had a width of 14.7 ± 4.2 µm, sporangia are 24.7 ± 7.7 x 79.5 ± 26.5 µm, (Fig. 1C) and size of mature released zoospores was 11.5 ± 1.7 x 12.5 ± 1.7 µm. Sexual phase of the fungal life cycle was also observed in dead larvae, where mature oospores were seen in the thoracic cavity, 48 h after exposing living larvae to a CIB 79-MED culture grown in SFE-agar (Fig. 1D).

Vesicles formation in isolates also followed a characteristic pattern. Isolates with null pathogenicity towards mosquito larvae (ARSEF 2532, CIB 163-PDCH, and CIB 183-ARU) produced vesicles during the first 3 hr, whereas in isolates which display some degree of pathogenicity, vesicle formation was noticed in the reading 6 hr after immersion (Fig. 2).

Isolates ARSEF 2532, CIB 183-ARU, and CIB 163-PDCH did not cause mortality at any of the dosages used when *Cx. quinquefasciatus* larvae were challenged. Isolates CIB 79-MED and CIB 79-TDT showed lower toxicity compared to the North American isolates CA, NC, and LA at the lower dose rates, but similar at higher doses. Isolate CIB 183-NUQ caused comparable mortality to the isolate CA at all dosages tested.

Although S. P. Frances et al. (1989, *J. Invertebr. Pathol., 54:* 103-111) did not report pathogenicity data for the ARSEF 2532 isolate, lack of the pathogenicity could be due to the transportation stress when shipped from Australia to our laboratory in Medellin. Since its arrival, this isolate has demonstrated very low pathogenicity, despite the frequent passages through mosquito larvae. Isolate CIB 163-PDCH lost its pathogenicity soon after its isolation, as was also the case of isolate CIB 183-ARU. Isolates ARSEF 2532 and CIB 183-ARU were isolated from larvae and water samples collected in leaf axils of *Colocasia macrorrhiza* and bromelia plants respectively. Pathogenicity of isolates CIB 79-TDT and CIB 79-MED has been variable. Frequent passages of these two isolates through mosquito larvae and liquid SFE prepared with soil extracts restored almost their initial pathogenicity potential. On the other hand, isolate CIB 183-NUQ isolated from a pond retained its full pathogenicity. The isolates from Colombia reported here represent 50% of the isolates known for the world.

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