

COPULATION OF *Opisthogonimus fonsecai* RUIZ & LEÃO, 1942 (TREMATODA, DIGENEA, PLAGIORCHIIDAE), PARASITE OF *Bothrops moojeni* HOGE, 1966 (SERPENTES, VIPERIDAE)

SILVA R. J. (1)

(1) Departament of Parasitology, Institute of Biosciences, Unesp, Botucatu, São Paulo, Brazil

ABSTRACT: Copulation in *Opisthogonimus fonsecai* Ruiz & Leão, 1942 (Trematoda, Digenea, Plagiorchiidae), parasite of *Bothrops moojeni* Hoge, 1966 (Serpentes, Viperiade), was reported. Data on the histology of the cirrus and metraterm of the copulants were presented. Morphology of the cirrus was also examined by scanning electron microscopy.

KEY WORDS: copulation, *Opisthogonimus fonsecai*, Trematoda, histology, SEM.

CORRESPONDENCE TO:

R. J. SILVA, Departamento de Parasitologia, Instituto de Biociências, Unesp, 18618-000, Botucatu, São Paulo, Brasil. Phone: 55 14 3811 6239. Email:

reinaldo@ibb.unesp.br

INTRODUCTION

Most digenean trematode species are hermaphrodites. Literature reports that self-fertilization may happen but it is uncommon, since a number of flukes usually occur in the same host. Reproduction of some of these helminths involves copulation, which happens when the cirrus of one fluke is inserted into the terminal portion of the other fluke's uterus (metraterm) (14).

Studies on the mating behavior of adult digenean trematodes have been carried out with some species of the families Philophthalmidae (5,6,7), Echinostomidae (9,10), and Paramphistomidae (11). In these reports, self and cross-insemination patterns were observed by using transplantation and autoradiography techniques. Besides, Sey *et al.* (13) reported the copulation of *Lasiotocus* sp. by scanning electron microscopy (SEM).

The copulatory organ of most digenean is a cirrus. Frequently, it has circular and longitudinal muscles in its walls and presents spines (4). The metraterm is muscular to varying degrees in different forms and may serve as a vagina (4).

Although some reports about trematodes reproduction are available in literature, histological and SEM data on the copulation of trematode parasites are scarce. Thus, the objective of the present study was to report copulation in *Opisthogonimus fonsecai* (Trematoda, Digenea, Plagiorchiidae).

MATERIAL AND METHODS

The trematodes were collected from the mouth of a *Bothrops moojeni* (Serpentes, Viperidae) specimen from Porto Primavera, São Paulo State, and fixed in cold AFA solution without compression (2). Some trematodes were stained with carmine and mounted in Permount resin. The *O. fonsecai* (Figure 1) species was identified (1,12,15). Among the collected helminths, three pairs of copulants were found (Figure 2A). One of them was studied by SEM, and the other two were analyzed after histology. For the histological study, fixed helminths were embedded in historesin (2-hydroxyethyl methacrylate); 4- μ m transverse serial sections were prepared, stained with hematoxylin-eosin and examined by using a computerized image analysis system (Qwin Lite 2.5 - Leica).

RESULTS

In each of the three cases of copulation observed, only one of the copulants had its cirrus introduced into the metraterm of the other fluke. Trematodes that were receiving the cirrus had their own intromittent organ everted in one case and withdrawn in the other two cases. The trematodes were parallel or diagonal to one another. In two cases, the copulants had similar size, and in the third, they were slightly different (Figure 2A).

In one case histologically examined, the two trematodes had the genital atrium at a similar level; and in the transverse histological sections, it was observed that the cirrus of one specimen was introduced into the metraterm of the other (Figure 2B). The longest cirrus observed measured 636 μm . The extruded extent of the cirrus was 414 μm , but only 121 μm was introduced into the other fluke's metraterm (Figure 2C). The widest cirrus measured 134 μm in diameter; and a constriction, which resulted in a decrease of the cirrus diameter to 42 μm , was observed at the genital pore of the trematode that was receiving the cirrus (Figure 2C). The cirrus presented a canal (ejaculatory duct) with a lumen of 6-7 μm in diameter. This duct was covered with a mucous epithelium of 8-11.5 μm thickness and two layers of smooth musculature: a circular one, with 1-1.5 μm thickness, and a longitudinal one, with 1-3 μm thickness. Externally, the cirrus was covered with a tegument of 9.5-12 μm thickness. Under this tegument, there was a basal lamina of 0.5-1 μm thickness and two layers of smooth musculature: a circular one, with 2.5-3 μm thickness, and a longitudinal one, with 1-2 μm thickness. Internally, there was a parenchyma and also glands. The cirrus had no spines (Figure 2D).

In the other case histologically studied, the cirrus was in a diagonal position; and after the analysis of all histological serial sections, it was observed that only a small portion of the cirrus' extremity was introduced into the metraterm of the other fluke (Figure 2E). In this second case, we obtained a good section of the metraterm with a great amount of spermatozoa in the lumen (Figures 2E-F). This metraterm was surrounded by a large number of glands (Figure 2E-F) and measured 288 μm long and 125.5 μm wide. The lumen of this structure was 58 μm in diameter. There was a mucous epithelium of 8.5-15 μm thickness; a basal lamina of 1-2.5 μm thickness; and

two layers of smooth muscle: a circular one, with strong muscle fibers of 15.5-21 x 1-1.5 μm thickness, and a longitudinal one of 2-5.5 μm thickness (Figure 2F).

SEM examination showed that the cirrus had no spines and was tapered. Next to the metraterm's opening, we could also observe some eggs of this trematode adhered to the cirrus (Figures 2G-H).

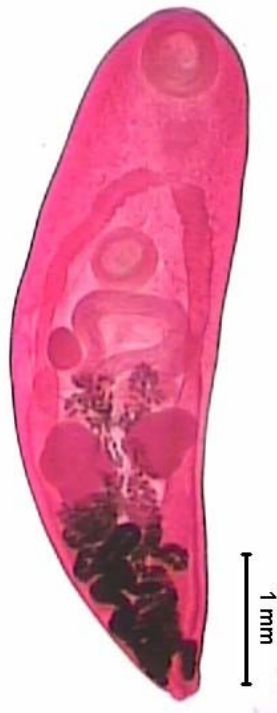


Figure 1. Specimen of *Opisthognimus fonscai* collected from the mouth of a *Bothrops moojeni* snake.

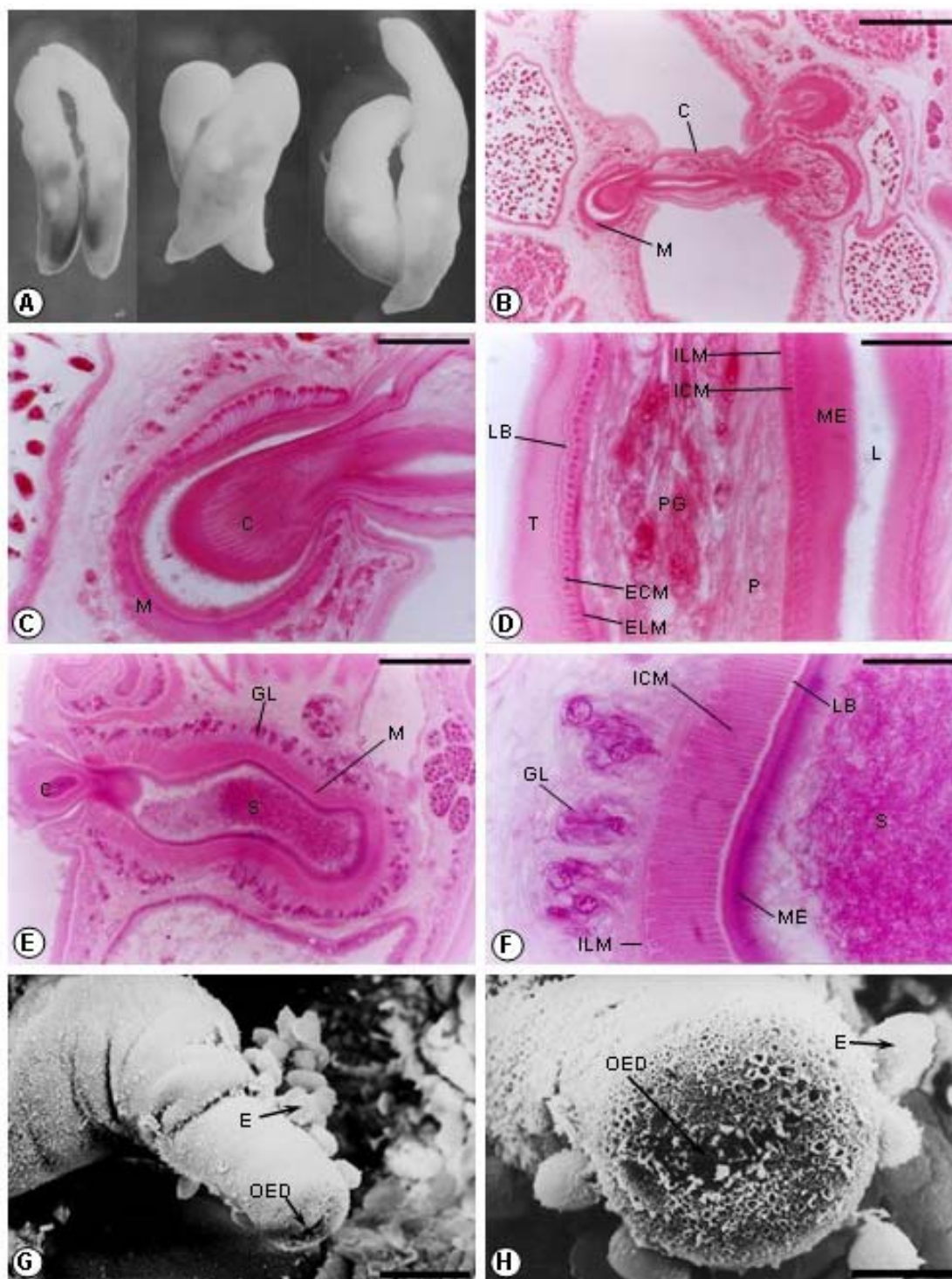


Figure 2. Three cases of copulation in *O. fonsecai*.

- A) general view of the co-copulants;
- B) histological view of the two individuals – note the cirrus (C) of one specimen being introduced into the metraterm (M) of the other (scale bar = 200 μ m);
- C) detail of the metraterm receiving the cirrus (scale bar = 50 μ m);

- D) detail of the cirrus: ejaculatory duct: lumen (L); mucous epithelium (ME), and internal circular (ICM) and longitudinal (ILM) musculatures; parenchyma (P); gland cells (GC); and tegument (T) with basal lamina (BL) and external circular (ECM) and longitudinal (ELM) musculatures (scale bar = 20 μm);
- E) histological view of the cirrus partially introduced into the metraterm, lumen of the metraterm with large amount of spermatozoa (S) and gland cells surrounding the metraterm (scale bar = 100 μm);
- F) detail of the metraterm: mucous epithelium, basal lamina, internal circular and longitudinal musculatures, spermatozoa in the lumen, and glands surrounding the metraterm (scale bar = 20 μm);
- G-H) scanning electron microscopy of the cirrus:
- G) cirrus projected outside showing a distal region less dilated than the proximal region (scale bar = 50 μm);
- H) detail of the unarmed cirrus showing the opening of the ejaculatory duct (OED). Some eggs (E) were observed adhering to the cirrus (scale bar = 20 μm).

DISCUSSION

The male and female reproductive systems of *O. fonsecai* were described by Ruiz & Leão (12). *Bothrops moojeni* infection by this species was previously reported by Correa (3). However, there are no reports on the copulation of this trematode.

Patterns of sexual reproduction in platyhelminths were discussed by Nollen (8). According to this author, digeneans have potential for both cross and self-insemination, since they are almost exclusively hermaphrodite and, in most of them, the male and female systems share a common genital atrium or pore. In addition, he cited other papers in which insemination through Laurer's canal in the species *Diphterostomum brusinae* and *Haploporus benedeni*; mating via metraterm in *Podoclype fractum*; reciprocal copulation in *Fasciola hepatica*; and self-insemination in *Leucochloridiomorpha constantinae* were observed. For many digeneans, cross-fertilization is usual. On the other hand, at least in two trematodes (*Liolope copulans* and *Collyriclum faba*), Laurer's canal was used for the reception of the other individual's cirrus during copulation and for the conveyance of the spermatozoa received during this act to the seminal receptacle (4).

In the two specimens histologically examined in this report, the cirrus was introduced only into the distal portion of the metraterm. For trematodes, it is known that copulants start to separate when removed from the host or fixed. This have happened to the pairs of copulants observed in this report and may explain the presence of the cirrus only in the distal portion of the metraterm or even the lack of reciprocal copulation. However, we believe that, during copulation, the full extent of the metraterm is occupied by the cirrus.

REFERENCES

- 1 ARTIGAS PT., PEREZ MD. Sistemática dos Opisthgonimidae (Trematoda, Plagiorchoidea). Criação da Família Bieriidae N. Fam. *Mem. Inst. Butantan*, 1969, 34, 97-110.
- 2 BARRELLA TH., SILVA RJ. Infection by digenetic trematodes in a *Bothrops moojeni* population (Serpentes: Viperidae) from a fauna rescue in Porto Primavera region, São Paulo State, Brazil. *Arq. Bras. Med. Vet. Zootec.*, 2003, 55, 243-5.
- 3 CORRÊA AAS. *Fauna de trematóides parasitos de ofídios da área geográfica brasileira*. São Paulo: Universidade de São Paulo, Instituto de Ciências Biológicas, 1990. 188p. [Tese - Doutorado]
- 4 DAWES B. *The trematoda*. Cambridge: Cambridge University Press, 1968. 644p.
- 5 MOSELEY C., NOLLEN PM. Autoradiographical studies on the reproductive system of *Philophtalmus hegneri* Penner and Fried, 1963. *J. Parasitol.*, 1973, 59, 650-4.
- 6 NOLLEN PM. Autoradiographical studies on reproduction in *Philophtalmus megalurus* (Cort. 1914) (Trematoda). *J. Parasitol.*, 1968, 54, 43-8.
- 7 NOLLEN PM. Autoradiographical studies on the reproductive system of *Philophtalmus grali* using techniques of transplantation and autoradiography. *J. Parasitol.*, 1978, 64, 613-6.
- 8 NOLLEN PM. Pattern of sexual reproduction among parasitic platyhelminths. *Parasitology*, 1983, 86, 99-120.

- 9 NOLLEN PM. *Echinostoma caproni*: mating behavior and the timing of development and movement of reproductive cells. *J. Parasitol.*, 1990, 76, 784-9.
- 10 NOLLEN PM. *Echinostoma trivolvis*: mating behavior of adults grown in hamsters. *Parasitol. Res.*, 1993, 79, 130-2.
- 11 NOLLEN PM., PYNE JL. Observations on spermatogenesis and inseminative behavior of *Megalodiscus temperatus* adults in frogs. *J. Parasitol.*, 1979, 65, 35-7.
- 12 RUIZ JM., LEÃO AT. Três novas espécies de *Opisthgonimus* parasitas de ofídeos brasileiros (Trematoda: Plagiorchiidae). *Mem. Inst. Butantan*, 1942, 16, 171-81.
- 13 SEY O., AL-GHAITH L., NAHHAS FM. Scanning electron microscopy of a copulating Monorchiid (Trematoda: Digenea). *J. Helminthol.*, 1998, 65, 243-5.
- 14 SMYTH JD. *The physiology of trematodes*. San Francisco: WH Freeman and Company, 1966. 256p.
- 15 TRAVASSOS L., FREITAS JFT., KOHN A. Trematódeos do Brasil. *Mem. Inst. Oswaldo Cruz*, 1969, 67, 1-886.