Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. *94*(1): 31-35, Jan./Feb. 1999

Some Aspects of the Development of *Lobatostoma jungwirthi* Kritscher, 1974 (Aspidogastrea) in Snails and Cichlid Fishes from Buenos Aires, Argentina

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The molluscs Heleobia castellanosae *and* Ampullaria canaliculata *and cichlid fishes were collected from an artificial pond at Tres de Febrero Park (Buenos Aires city), between June 1994 and May 1995. One hundred and eighty of 2,467* H. castellanosae *examined were infected with pre-adults of* Lobatostoma jungwirthi, *112 of them in concurrent infections with digeneans.* L. jungwirthi *was significantly more prevalent in larger snails, especially those infected with larval digeneans, but the prevalence of infection did not fluctuate significantly throughout the year.* A. canaliculata *and cichlids smaller than 10 cm were not infected, but larger* Cichlasoma facetum *harboured adults of* L. jungwirthi *in their intestines. Experimental infections of* C. facetum *and* Gymnogeophagus meridionalis *with pre-adult aspidogastreans obtained from* H. castellanosae *were successful. The* development *of* L. jungwirthi *in the snail host involves five arbitrary growth phases. Its life-cycle is heteroxenous, similar to that described for* L. manteri, *with* H. castellanosae *as the intermediate host and cichlid fishes as the definitive host in this pond.*

Key words: Aspidogastrea - *Lobatostoma jungwirthi* - *Heleobia castellanosae* - concurrent infections - prevalence of infection - seasonality - Buenos Aires - Argentina

In one of the artificial ponds at Tres de Febrero Park (Buenos Aires city), Szidat (1971) found a species of the genus *Lobatostoma* Eckmann, 1932 (Aspidogastrea) parasitizing the gonad of the prosobranch snail *Heleobia castellanosae* (Gaillard, 1974) Davis, Mazurkiewicz and Mandracchia, 1982 and considered it identical to *L. pacificum* Manter, 1940. Since *L. pacificum* parasitizes marine fishes, its taxonomic status seems to be quite uncertain and needs to be reconsidered. In this work, the species of *Lobatostoma* from the same snail and pond as that found by Szidat is identified as *L. jungwirthi* Kritscher, 1974.

L. jungwirthi was first described parasitizing the freshwater cichlid fish *Geophagus brachyurus* Cope from Rio do Sinos, Brazil. In 1984, Lunaschi redescribed this species in *Cichlasoma facetum* (Jenyns) from Buenos Aires Province. This is the only member in the genus which parasitizes freshwater fishes.

Operculate snail species serve as intermediate hosts not only for digenetic but also for various aspidogastrean trematodes (Rohde 1972, Hendrix et al. 1985, Huehner 1987). Rohde and Sandland (1973) reported *Lobatostoma manteri* Rohde, 1973 to occur more frequently in a prosobranch snail naturally infected with digenean parthenitae. Huehner (1975, 1987) experimentally demonstrated that *Aspidogaster conchicola* von Baer could successfully establish itself and develop in the gastropod *Elimia livescens* with pre-existing digenetic infections, and that these aspidogastrean-digenean double infections could occur seasonally in nature. Prevalences of single and concurrent infections of *L. jungwirthi* in *H. castellanosae* from Buenos Aires city are reported herein.

Since its discovery, the morphology of *L. jungwirthi* has been thoroughly studied, but not so its development and transmission. Aspidogastrean life-cycles have been worked out for *Aspidogaster indicum* Dayal (see Rai 1964), *Multicotyle purvisi* Dawes (see Rohde 1972) and *A. conchicola* (see Huehner & Etges 1972, 1977, Bakker & Davids 1973, Rohde 1994). Among the species of the genus *Lobatostoma*, only the life-cycle of the Australian *L. manteri* from the marine teleost *Trachinotus blochi* (Lacépède) is known (Rohde 1973, 1994). The present study analyzes *L. jungwirthi*'s development in its molluscan host and elucidates its life-cycle.

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Received 20 May 1998
Accepted 22 October 1998

MATERIALS AND METHODS

Monthly samples of *H. castellanosae* (Prosobranchia: Hydrobiidae) were collected using nets from a pond at Tres de Febrero Park between June 1994 and May 1995. *A. canaliculata* (Prosobranchia: Ampullariidae) and the cichlid fishes *C. facetum* and *G. meridionalis* were irregularly captured in the same pond.

The snails were measured and dissected in the laboratory. *Lobatostoma* specimens were collected from the gonads of their molluscan hosts, washed in 0.3‰ NaCl saline solution, fixed with Raillet-Henry's fixative (8‰ NaCl saline solution, formalin, acetic acid), stained with alcoholic-hydrochloric carmine (Langeron 1949), cleared in creosote and mounted in Canada balsam.

The following maturation stages were arbitrarily established for the analysis of the aspidogastreans in the snail host, according to their size and morphology. *Class I*: very small, up to 0.8 mm long; forebody (body region anterior to ventral sucker) short, without head lobes surrounding mouth; adhesive ventral sucker subcylindrical and not yet alveolated; sexual organs and vitelline glands inconspicuous (Fig. 1A). *Class II*: small, not exceeding 1 mm long; forebody short, still lacking head lobes; ventral sucker subcylindrical, with developing marginal alveoli; testis and ovary absent or starting to develop, vitelline follicles still inconspicuous (Fig. 1B). *Class III*: medium-sized, up to 1.25 mm long; forebody of medium size, with head lobes still absent or starting to develop; ventral sucker oval, with fully-formed marginal alveoli and developing median alveoli; longitudinal median ridge appears dividing median alveoli; sexual organs and vitelline follicles rudimentary (Fig. 1C). *Class IV* (pre-adults): large, (1-3) mm long; forebody large, with head lobes completely developed; ventral disc oval, with complete alveolation; genitalia and vitelline follicles fully developed (Fig. 1D). *Class V* (pre-adults): as class IV, but with presence of 1-8 eggs (Fig. 1E).

The fishes *C. facetum* and *G. meridionalis*, captured in a pond where *H. castellanosae* was absent, were experimentally infected several times with aspidogastrean pre-adults. They where manually fed with the dissected bodies of the parasitized snails.

A chi-square test including Yate's correction with a two-tailed P value was carried out to analyze differences between prevalence of infection of *L. jungwirthi* in singly and concurrently infected *H. castellanosae*. Kruskal-Wallis non-parametric ANOVA tests were employed to evaluate the statistical significance of the associations between prevalence of infection, snail size and month, both for single and double infections. The level of significance was set at $P<0.05$ (Rohlf & Sokal 1981). Unless otherwise stated, all measurements are given in millimetres. We used "prevalence of infection" as defined by Bush et al. (1997).

RESULTS

The parasites obtained from *H. castellanosae* and from experimentally infected *C. facetum* were identified as *L. jungwirthi* Kritscher, 1974, as redescribed by Lunaschi (1984). *L. jungwirthi* can be distinguished by the absence of a tail overlapping the ventral disc posteriorly, the testis located in the median third of the body, a small, ovoid cirrus-pouch and the presence of 30-40 marginal alveoli on the ventral disc.

Of 2,467 *H. castellanosae* examined, 180 (7.3%) had their gonads infected with one, rarely two or more, immature specimens of *L. jungwirthi*. Prevalence of infection varied significantly, depending on snail size: *L. jungwirthi* was more prevalent in larger snails (K-W=21.389, $P<0.0001$) (Fig. 2). Although it showed a peak in the month of June (23.1%) (Fig. 3), the prevalence did not display any significant changes throughout the year.

L. jungwirthi exhibited significant variations in prevalence of infection between singly and concurrently infected snails: it co-occurred with digeneans to produce 112 (4.5%) double infections, while 68 snails lacked digenean larval stages (2.8%) (c^2=12.519, P=0.0004). Aspidogastrean-digenean concurrent infections were significantly more common in larger snails (K-W=18.628, $P<0.0001$) (Fig. 2), but they did not vary significantly throughout the year (Fig. 3).

Worm body lengths ranged from 0.63 to 2.95, with mean values ranging between 1.32 in March 1995 and 2.32 in July 1994 (Table). The smallest individuals (class I) were observed irregularly between May and October, while those belonging to class II appeared from October to April. Class III was observed in April, June, September and December, whereas the largest individuals (class IV) were always prevalent (Table). Only four specimens belonging to class V were found, one carrying a single egg (April), another with two eggs (September) and two with one and eight eggs within the uterus (November) (Table).

Eighteen *A. canaliculata* plus 33 *G. meridionalis* and *C. facetum* (<10 cm long) captured in the pond were not infected with *L. jungwirthi*. Two *C. facetum* (11 and 12.5 cm long) harboured in their intestines ten and one specimens, respectively; the latter being mature and of great size (3 mm).

One of seven experimentally infected *G. meridionalis* (4.5 cm long) carried six *L. jungwirthi* in the rectum, one of them showing incipient egg

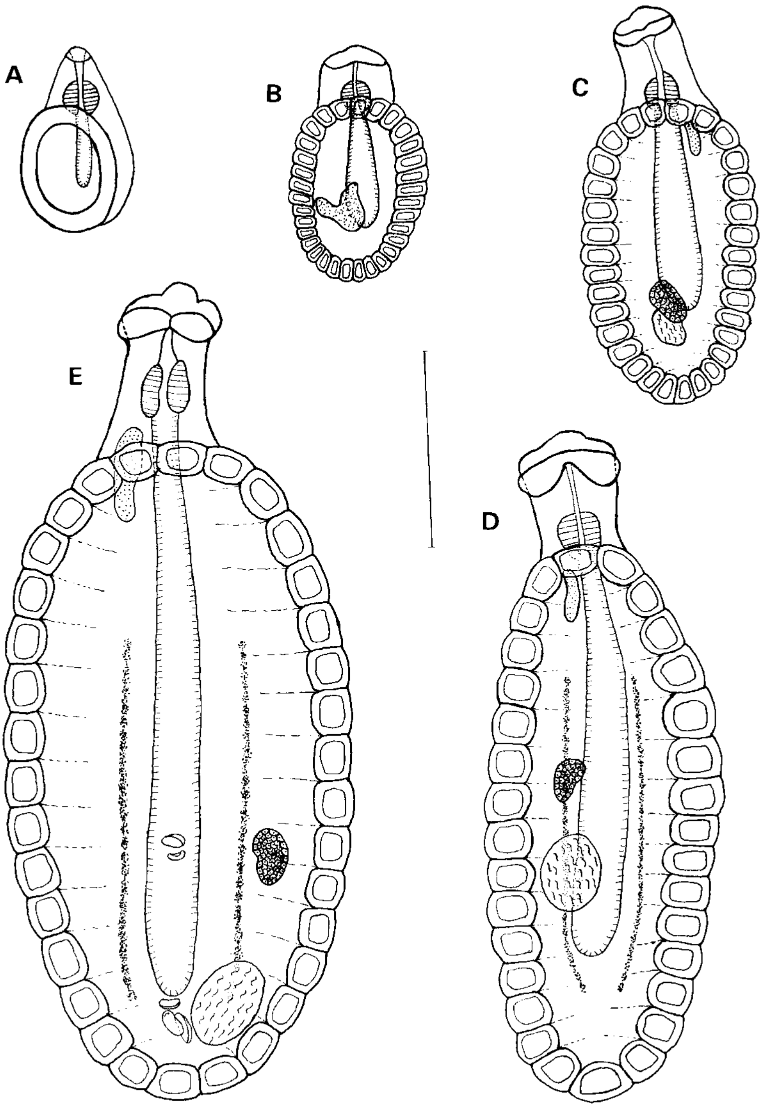

Fig. 1: maturation stages of *Lobatostoma jungwirthi*: A: class I; B: class II; C: class III; D: class IV; E: class V. Bar = 500 µm.

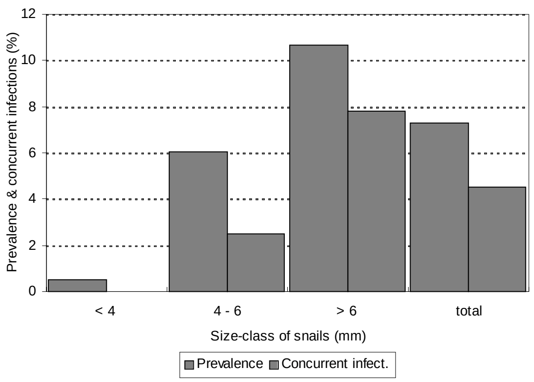

Fig. 2: *Lobatostoma jungwirthi* and aspidogastrean-digenean concurrent infections in *Heleobia castellanosae* in different host size-classes: prevalence of infection.

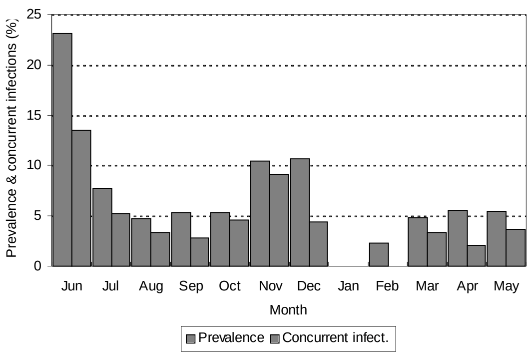

Fig. 3: *Lobatostoma jungwirthi* and aspidogastrean-digenean concurrent infections in *Heleobia castellanosae*: monthly prevalence of infection 1994-1995.

TABLE

Variation in body length and in percentage of each maturation stage of *Lobatostoma jungwirthi* in *Heleobia castellanosae* during the period of study

Month/ year	Length range (mm)	Mean length (mm)	Maturation stages (%)				
			I	II	III	IV	V
Jun/94	0.70-2.66	1.91	2.8	0	2.8	94.4	0
Jul/94	0.63-2.95	2.32	8.3	0	0	91.7	0
Aug/94	1.16-2.14	1.77	0	0	0	100	0
Sep/94	0.98-1.86	1.41	0	0	12.5	81.3	6.3
Oct/94	0.63-2.59	1.73	15.4	7.7	0	76.9	0
Nov/94	0.70-1.75	1.43	0	20	0	40	40
Dec/94	0.84-2.28	1.53	0	12.5	4.2	83.3	0
Feb/95	1.33-2.07	1.71	0	0	0	100	0
Mar/95	0.81-1.82	1.32	0	14.3	0	85.7	0
Apr/95	0.67-2.56	1.57	0	10	10	70	10
May/95	0.63-2.21	1.64	15.4	0	0	84.6	0

production 24 hr after the last infection. Six of eight experimentally infected *C. facetum* (8- 9.5 cm long) yielded 16 specimens of *L. jungwirthi*, three of them with eggs after 96 and 72 hr following the last infection.

DISCUSSION

At present, nine species are considered as valid members of the genus *Lobatostoma*: *L. ringens* (Linton, 1905); *L. kemostoma* (MacCallum & MacCallum, 1913); *L. pacificum* Manter, 1940; *L. manteri* Rohde, 1973; *L. jungwirthi* Kritscher, 1974; *L. platense* Mañé-Garzón & Holcman-Spector, 1976; *L. hanumanthai* Narashimhulu & Madhavi, 1980; *L. anisotremum* Oliva & Carbajal, 1984, and *L. veranoi* Oliva & Luque, 1989 as cited by Oliva and Luque (1989) and Rohde (1994). *L. ringens*, *L. pacificum*, *L. jungwirthi*, *L. platense*, *L. anisotremum* and *L. veranoi* are South American species. Only *L. jungwirthi* parasitizes freshwater, rather than marine fishes. Except for the Australian *L. manteri*, whose intermediate host is a mollusc (Rohde 1973), the life cycles of the rest of the species remain unknown.

The positive correlation between larger sizes of *H. castellanosae* and a higher probability of trematode infection may simply result from the fact that older and larger snails have a greater exposure to the parasite's eggs because they eat and are more mobile than smaller individuals, as observed by Huehner (1987) in the case of the host of *A. conchicola*.

The lack of seasonality in terms of prevalence may imply that cycles other than seasonal ones may be involved, since individuals belonging to all maturation stages, except class V, appear throughout the year. The level of aspidogastrean-digenean concurrent infection appears to follow the aspidogastrean prevalence pattern with regard to snail size and seasonality.

Rohde and Sandland (1973) reported a significant tendency for *L. manteri* to occur more frequently in snails with digenean infections and suggested that a reduced resistance to a second infection may be responsible for this pattern. Huehner (1987) reported the same tendency for *A. conchicola* in the gastropod *E. livescens* and suggested that some digeneans may produce beneficial effects on concurrent aspidogastrean infections. These results are consistent with the present results for *L. jungwirthi* in snails with digenean infections. According to Huehner (1987), some findings demonstrate that digenean infections significantly increase the probability of aspidogastrean infection and vice versa. The mechanisms underlying this relationship, either physiological (reduced resistance) or behavioural (i.e., increased feeding or exposure to parasite eggs or miracidia), remain unknown.

Rohde (1972, 1994) had emphasised the low degree of host specificity of aspidogastreans, both for the mollusc and the vertebrate hosts, with possible exceptions. For example, three snail species serve as hosts for *L. manteri* at the Great Barrier Reef, Australia. In the study area, *L. jungwirthi* was exclusively found in the mollusc *H. castellanosae*.

No definite conclusion on host specificity in vertebrates can be formulated, since only a few specimens of all of the potential hosts from one area have been examined. Infection experiments are also inconclusive, because unnatural conditions may influence the results (abnormal diet, stress, etc.). According to Rohde (1973), experimental data should be used with caution when formulating statements on the host specificity of parasites.

The life-cycle of *L. jungwirthi* is heteroxenous, very similar to that described by Rohde (1973, 1994) for *L. manteri* which, according to the author, is the simplest obligatory two-host life-cycle known for trematodes. In his study, Rohde showed, for the first time for the Aspidogastrea, that a vertebrate has become an obligatory host in which sexual maturity is reached: *L. manteri* is not capable of completing its cycle in snails. *L. jungwirthi* also needs a vertebrate host to complete its life-cycle, at least in the study area and for the examined hosts.

ACKNOWLEDGEMENTS

To Lic. Alicia A Gil de Pertierra for helpful comments on this manuscript.

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