Incidence of Digenea larvae in *Heleobia parchappii* (Mollusca, Gastropoda, Cochliopidae)

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Abstract: We scored the presence of Digenea larvae in the gonadal follicles and digestive glands of males and females of a *Heleobia parchappii* (d'Orbigny, 1835) (Mollusca, Cochliopidae) population living in an artificial lake located adjacent to an anthropically impacted area, region of Tigre in the Province of Buenos Aires, Argentina; making seven samplings from May 2005 through May 2006. In the laboratory, we separated subsamples for both histology and parasite-emergence assessment. The former examined the gonadal-development stages in relation to larval presence along with the effect of the larvae on the gonadal tissue and calculated parasite prevalence per age and size. Larval prevalence increased with host length and was greater in post-reproductive-stage individuals, more than 7.5 mm long the parasitism reached 100%. That stage became maximal in August, while juveniles predominated in December and January. The females were predominant almost throughout the year, but were parasitized at the same frequency as the males ($p=0.38$). This year-round *H. parchappii* parasitization indicates that Digenea can complete their life cycle in an anthropically impacted environment, thus affecting the autochthonous fauna, pets, and even humans. Mollusk parasitization by Digenea larvae is used to evaluate anthropic environmental pressure as an indicator of pollution by wastes, chemicals, or other refuse resulting from the human presence. The study site exemplifies the recent spate of urban construction projects characterized by housing developments around modified water bodies**.**

Key words: Cochliopidae; digenea larvae; histology; Buenos Aires; Argentina

Total word: 3335

Heleobia Stimpson 1865, belonging to the family Cochliopidae Tryon 1866 (Mollusca, Gastropoda) (Wilke et al., 2001), contains 101 species with 71 being found in South America (Silva and Veitenheimer-Mendes, 2004). Rumi et al. (2006, 2008) cited 16 species as present in Argentina, 10 of which are endemic. Moreover, in Argentina various Digenea larvae have been reported in *Heleobia*, including cercariae causing human dermatitis (Szidat, 1958; Ostrowski de Núñez, 1978). Etchegoin (2001) described 29 different morphologic types of cercariae parasitizing *H. australis* (d'Orbigny, 1835), *H. conexa*, (Gaillard, 1974) and *H. parchappii* (d'Orbigny, 1835), and compared the prevalence and abundance of digenean larvae in *H. australis* and in *H. conexa*, in the Mar Chiquita Lagoon (Buenos Aires, Argentina). Alda and Martorelli (2014) identified 15 Digenea species parasitizing *H. australis* in an estuary in Bahía Blanca (Buenos Aires Province), while Merlo et al. (2014) studied the composition and seasonal variation in the digenean larval community in *H. parchappii* in the Nahuel Rucá Lagoon (Buenos Aires Province). In the Patagonian region of Argentina, Flores and Brugni (2006) investigated digenean larval forms in *Heleobia hatcheri* (Pilsbry, 1911). In Uruguay, Castro (2000) and Castro et al. (2006) reported four morphologic types of Digenea (oculocercariae, xiphidocercarias, furcocercariae, and magnicaudae) parasitizing *H. australis*; while in Brazil, Thiengo et al. (1998, 2001) found various types of cercariae in different families of freshwater gastropods, and among those of the Cochliopidae in *Heleobia davisi* (Silva and Thomé, 1985).

This form of parasitism can cause damage in the host tissues causing parasitic castration, giantism, and dwarfism (Sorensen and Minchella, 2001). Since one of the mechanisms for determining the nature of these and other pathologic consequences of digenean parasitism is through histology, we accordingly undertook an extensive analysis

of the pathologic alterations produced by the larvae in *H. parchappii,* and in this instance in a population of those gastropods living within an anthropically impacted environment.

Materials and Methods

Study site: The *H. parchappii* material was collected in seven samplings from May, 2005 through May, 2006 in an anthropically impacted environment within the region of Tigre in the Province of Buenos Aires, Argentina (34°26' S and 58°37' W). The gastropods were collected with a sieve of 15 mm diameter and 0.14 mm pore size in the metal grid (Thiengo, 1995; Gutiérrez Gregoric and Núñez, 2010). In the laboratory, we separated subsamples for histological studies and for assessing parasite emergence.

Histological studies: The specimens were measured under a stereoscopic microscope equipped with a micrometer-containing ocular and then relaxed in water laced with menthol tablets for about 12 h before fixation for 48 h in an alcohol-based Bouin's solution in order to decalcify the shells. We then dissected individuals of both sexes and extracted the gonads and digestive glands. The visceral mass including those organs was treated with Paraplax in order to make 10-µm histological sections. The samples were next stained with hematoxylin-eosin and photographed with an Olympus camera mounted on a Leitz binocular microscope equipped with 25X, 40X, and 100X objectives. We examined the histologic stages of gonadal development in relation to the presence of the larvae and monitored the effects that parasitosis caused on the gonadal tissue. On the basis of these data, we calculated the parasitic prevalence with respect to sex and body size. The latter parameter was grouping, according to the developmental stage, in juveniles (length less than 4 mm), adults (between 4 and 6 mm) and post-reproductives (more than 6 mm) (Martín, 2008; Martín and Díaz, 2011). Moreover, we evaluated the possible relationship

between the prevalence of parasitism and the sex of the mollusk host by means of a contingency table (Zar, 1996).

Assessing parasite emergence: For the specimens not analyzed histologically, the prevalence of parasitism was calculated as number of snails from which emerged cercariae in laboratory conditions. To that end, the snails were housed individually in receptacles containing nonchlorinated water and exposed under light and heat. Subsequently they were monitored for three days by stereomicroscopy, at the end of which time all the specimens were dissected to screen for the possible presence of nonemerged larval stages. We classified the cercariae down to the family level following Ostrowski de Nuñez (1992).

Results

The histological analyses revealed that the prevalence of parasitism by Digenea in *H. parchappii* as host increased roughly in proportion to the length of the snail (Fig. 1). This increment plateaued at 100% parasitization for individuals in the postreproductive stage of body lengths ≤ 7.5 mm, which developmental phase was registered at the highest proportion in the winter month of August. In contrast, the proportion of juveniles peaked during the summer months of December and January (Fig. 2). Of the total number of specimens analyzed, almost 64% were females, predominance in general, observed over the entire year. Despite this higher proportion of females, the parasitism by Digenea occurred at an equivalent prevalence for both sexes (p=0.38) over the year as a whole, while the highest percent of the snails parasitized (44.4%) was recorded in the months of August plus the Mays of both years (Fig. 3). Those three months, furthermore, represented an exception to the rest of the year in that during those sampling periods the percent parasitism was more

different between the sexes: the two Mays contained a significantly higher proportion of males parasitized than females, while the reverse obtained for the intervening month of August (Fig. 4).

The adult females (as evidenced by gonadal follicles in a state of maturation or the evacuation of gametes) in the two months of May and the juveniles in January were recorded with peak levels of parasitism (Fig. 5A). The gonadal follicles of the adult males became 100% parasitized by digenean larvae in January and May of 2006, whereas during 2005 the parasitization never reached a level of even 75% (Fig 5B). We observed that the female follicles in a complete state of maturation contained abundant larvae that produced an emptying of the gonadal follicles along with a disordering of the acini of the digestive gland (Fig 5D, F). In some instances, the cell types —*e. g.*, oogonia— were seen to be partially deformed (Fig. 5E).

In contrast, with the specimens exposed to light and heat in the laboratory the prevalence of digenean parasitism reached only 50%, with but marginally higher values occurring in only the two months of May (Fig. 6). The cercariae that emerged belonged to eight known families (plus one not identified; Fig. 7), while sporocysts and rediae were found in the visceral masses of the dissected specimens. Among the emerged cercariae, we noted a marked predominance of the family Notocotylidae

Discussion

Coinciding with the results of Castro et al. (2006) about *H. australis* specimens obtained from the Uruguayan coasts of the Río de la Plata estuary, our findings indicate that the prevalence of digenean-larval parasitism in *H. parchappii* undergoes seasonal variations associated with the host-body size. In those months where we registered a greater number of post reproductive-stage individuals we encountered the highest prevalences, whereas in the months with a predominance of juveniles the prevalence was lower.

Studies on freshwater pulmonates have demonstrated that parasitic infection can reduce or even completely inhibit host reproduction (Minchella et al., 1985). Those observations coincide with the report of Sorensen and Minchella (2001) demonstrating a parasite-induced host-tissue damage that resulted in castration, gigantism, or dwarfism. In the present study, the individuals in the reproductive stage suffered parasitic alterations involving the total or partial destruction of the gonadal follicles, thus preventing normal gamete development. These alterations in *H. parchappii* had also been observed in *Biomphalaria glabrata* (Say, 1818), *Bradybaena similaris* (Férussac, 1821), *Chilina dombeyana* (Bruguière, 1789) and *Hydrobia ventrosa* (Montagu, 1803) (Théron and Gérard, 1994; Paschoal and Amato, 1996; Olmos and George-Nascimento, 1997; Kube et al., 2006).

In addition to parasitic castration, the larvae can produce modifications in the digestive gland of a gastropod host (in both adults and juveniles), as reported by Rees (1936) in *Littorina littorea* (Linné, 1758) and *Littorina saxatilis* (Olivi, 1792). In the present work, we observed that the digestive acini underwent a markedly pronounced disordering to the point that, in certain specimens, a nearly complete absence of secretory and excretory cells from the interior of the follicles was evident. Cheng (1971) suggested

that trematode larvae could elevate the calcium-ion levels of the digestive gland in both freshwater and marine snails by transporting those ions to the epithelia of the mantle, where the presence of the calcium would then enlarge the shell. Since our studies did not include that type of investigation, we can offer no comparison with respect to these *H. parchappii* specimens.

The 60% prevalence of parasitism overall for both males and females of *H. parchappii* observed in this work is substantially greater than the figures registered in *H. australis* by Castro et al. (2006) and Etchegoin (2001) of 22.6% and 10.4%, respectively. The number of digenean-larval families that emerged and were characterized in this investigation with *H. parchappii* were considerably fewer than those identified by Alda and Martorelli (2014) in *H. australis* and Merlo et al. (2014) in *H. parchappii* and *H. australis* in freshwater environments and estuaries of the Buenos Aires Province.

The study of digenean-larval communities in mollusks has been used to evaluate anthropic pressure on the environment from the release of domestic refuse, industrial wastes, and chemical discharges or other disturbances resulting from the presence of man (Etchegoin and Merlo, 2011). In recent years the area containing the study site has experienced the generation of a number of urban projects involving housing developments typically constructed around modified bodies of water; the vast majority of the latter having originally been marshes or low-lying regions that were then transformed into "lagoons". Most of these artificial lakes therefore have no influent or effluent streams and thus lack internal circulation. Because of this stagnant condition, the water in these lakes is characterized by algal blooms that render it impotable and even unfit for recreational activities. The presence of Digenea larvae in *H. parchappii* throughout the year in the body of water investigated demonstrate that these trematodes can indeed complete their life cycle

in an anthropically compromised environment and thus impact on the associated autochthonous fauna, pets, and even humans present.

In conclusion, coinciding in some aspects with Sorensen and Minchella (2001) the results obtained in this study allow to inferring that alteration have been found in gonadal follicles from *H. parchappii* by the presence of larvae to trematoda, which have caused cytological modifications in these follicles.

Acknowledgements

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References

Alda P, Martorelli S, 2014. Larval trematodes infecting the South-American intertidal mud snail *Heleobia australis* (Rissooidea: Cochliopidae). Acta Parasitologica 59: 50–67.

Castro D, 2000. *La comunidad de trematodos larvales del caracol Heleobia australis como un potencial indicador del grado de contaminación de la costa de Montevideo.* Jornadas sobre Animales Silvestres, desarrollo Sustentable y Medio Ambiente (pp. 34-35). Montevideo, Uruguay. (In Spanish).

Castro D, Carnevaia D, Perretta A, Venzal JM, 2006. Comunidad componente de trematodos larvales de *Heleobia australis* (Mollusca, Cochliopidae) en la costa uruguaya del Río de la Plata. *Vida Silvestre: Bases para la conservación y el manejo de la costa uruguaya,* Uruguay, Pp: 421-425. (In Spanish).

Cheng TC, 1971. Enhanced growth as a manifectation of parasitism and shell deposition in parasitized molluscs. In: Cheng TC ed, Aspects of the biology of symbiosis. University Park Press, Baltimore, 103-137.

Etchegoin J, 2001. Dinámica de los sistemas parasitarios. In: Iribarne O ed. Reserva de Biosfera Mar Chiquita: Características físicas, biológicas y ecológicas. Editorial Martín, Mar del Plata, 171-185. (In Spanish)

Etchegoin J, Merlo MJ, 2011. *Heleobia conexa* y *H. australis* como bioindicadores de fauna y de fluctuaciones ambientales en la laguna Mar Chiquita (Buenos Aires, Argentina). Amici Molluscarum, Número especial, 33-35. (In Spanish)

Flores V, Brugni N. 2006, *Catatropis hatcheri* n. sp. (Digenea: Notocotylidae) from *Heleobia hatcheri* (Prosobranchia: Hydrobiidae) and notes on its life-cycle in Patagonia, Argentina. Systematic Parasitology 63: 109-116.

Gutiérrez Gregoric DE, Núñez V, 2010. Método de colección de moluscos: gasterópodos continentales. División Zoología Invertebrados, Museo de La Plata. FCNyM. UNLP Serie Didactica 1 : 1-9.

Kube S. Kube J, Bick A, 2006. A loss fecundity in a population of mudsnails *Hydrobia ventrosa* caused by larval trematodes does not measurably affect host population equilibrium level. Parasitology 132: 725-732.

Martín SM. 2008. Individual growth of *Heleobia piscium* in natural populations (Gastropoda: Cochliopidae) from the Multiple Use Natural Reserve Isla Martin Garcia, Buenos Aires, Argentina. Brazilian Journal of Biology 68: 617-62.

Martín SM, Díaz AC. 2011. *Ciclo reproductor de* Heleobia piscium *(Caenogastropoda. Cochliopidae) en la Reserva de Usos Múltiples" Isla Martín García", Buenos Aires,*

Argentina. VIII Congreso Latinoamericano de Malacología (p. 196). Puerto Madryn, Argentina. (In Spanish).

Merlo MJ, Parietti M, Etchegoin JA, 2014. Digeneos larvales de *Heleobia parchappii* y *Heleobia australis* en ambientes dulceacuícolas y estuariales de la provincia de Buenos Aires (Argentina). Revista Argentina de Parasitología 2: 14 -21. (In Spanish).

Minchella DJ, Leathers BK, Brown KM, McNair JN, 1985. Host and parasite counter adaptations: an example from a freshwater snail. American Naturalist 126: 843-854.

Olmos V, George-Nascimento M, 1997. El gremio de larvas de Digenea en el caracol del sur de Chile *Chilina dombeyana*: ¿Qué indica la tasa metabólica de los hospedadores parasitados? Revista Chilena de Historia Natural 70: 109-118. (In Spanish).

Ostrowski de Núñez M, 1978. Fauna de agua dulce de la República Argentina, VII: cercarias de la familia Schistosomatidae (Trematoda: Digenea). Revista Museo Argentino Ciencias Naturales Bernardino Rivadavia, Parasitología 2(3): 65–76. (In Spanish).

Ostrowski de Núñez M, 1992. Trematoda. Familias Strigeidae, Diplostomidae, Clinostomidae, Schistosomatidae, Spirorchiidae y Bucephalidae. Fauna de agua dulce de la República Argentina 9: 5-55 (In Spanish).

Paschoal S, Amato SB, 1996. *Eurytrema coelomaticum* (Giard et Billet)(Digenea, Dicrocoeliidae) em *Bradybaena similaris* (Férussac)(Gastropoda, Xanthonychidae): alteracoes nos dapósitos de calcio e na funcao reprodutiva do primeiro hospedeiro intermediário. Revista Brasileira de Zoologia 13: 411-418. (In Portuguese).

Rees WJ, 1936. The effect of parasitism by larval trematodes on the tissues of *Littorina littorea* (Linné). Proceedings of the Zoology Society of London 2: 357- 368.

Rumi A, Gutiérrez Gregoric DE, Núñez V, César II, Roche MA, Tassara MP, Martín SM, Armengol MF, 2006. Freshwater Gastropoda from Argentina: species richness, distribution patterns, and an evaluation of endangered species. Malacologia 49: 189-208.

Rumi A, Gutiérez Gregoric DE, Núñez V, Darrigran GA, 2008. Malacología Latinoamericana: Moluscos de agua dulce de Argentina. Revista de Biología Tropical 56: 77-111. (In Spanish).

Silva MC Pons da, Veitenheimer-Mendes IL, 2004. New species of *Heleobia* (Rissooidea, Hydrobiidae) from south plain coast of Brazil. Iheringia 94(1): 89-94.

Sorensen RF, Minchella DJ, 2001. Snail-trematode life history interactions: past trends and future directions. Parasitology 123(7): 3-18.

Szidat L, 1958. Investigaciones sobre *Cercaria chascomusi* n. sp. Agente causal de una nueva enfermedad humana en la Argentina: "la dermatitis de los bañistas de la laguna Chascomús". Boletín del Museo Argentino de Ciencias Naturales Bernardino Rivadavia 18: 13. (In Spanish).

Théron A, Gérard C, 1994. Development of accessory sexual organs in *Biomphalaria glabrata* (Planorbidae) in relation to timing of infection by *Schistosoma mansoni*: consequences for energy utilization patterns by the parasite. Journal of Molluscan Studies 60(1): 25-31.

Thiengo SC, 1995. Técnicas Malacológicas. In: S. Barbosa dos Santos (ed.), Tópicos em Malacologia Médica (pp. 255-260). Fiocruz, Rio de Janeiro. (In Portuguese).

Thiengo SC, Fernandez MA, Boaventura MF, Stortti MA, 1998. A survey of freshwater gastropods in the Microrregião Serrana of the state of Rio de Janeiro, Brazil. Memórias do Instituto Oswaldo Cruz 93(1): 233-234.

Thiengo SC, Fernandez MA, Boaventura MF, Grault CE, Silva HF, Mattos AC, Santos SB. 2001, Freshwater snails and schistosomiasis mansoni in the state of Rio de Janeiro, Brazil: I-Metropolitan Mesoregion. Memórias do Instituto Oswaldo Cruz 96: 177-184.

Wilke T, Davis GM, Falniowski A, Giusti F, Bodon M, Szarowska M, 2001. Molecular systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. Proceedings of the Academy of Natural Sciences of Philadelphia 151: 1-21.

Zar JH, 1996. Biostatistical Analysis (3rd edition). Prentice-Hall, New Jersey, 920 p.

Legends to The Figures

Fig. 1. Prevalence of Digenea parasitism in the host *Heleobia parchappii* as evidenced by histological analysis. Abscissa: length of *H. parchappii* (mm); ordinate: percentage of snails parasitized (black line), number of snails analyzed (N, gray line)

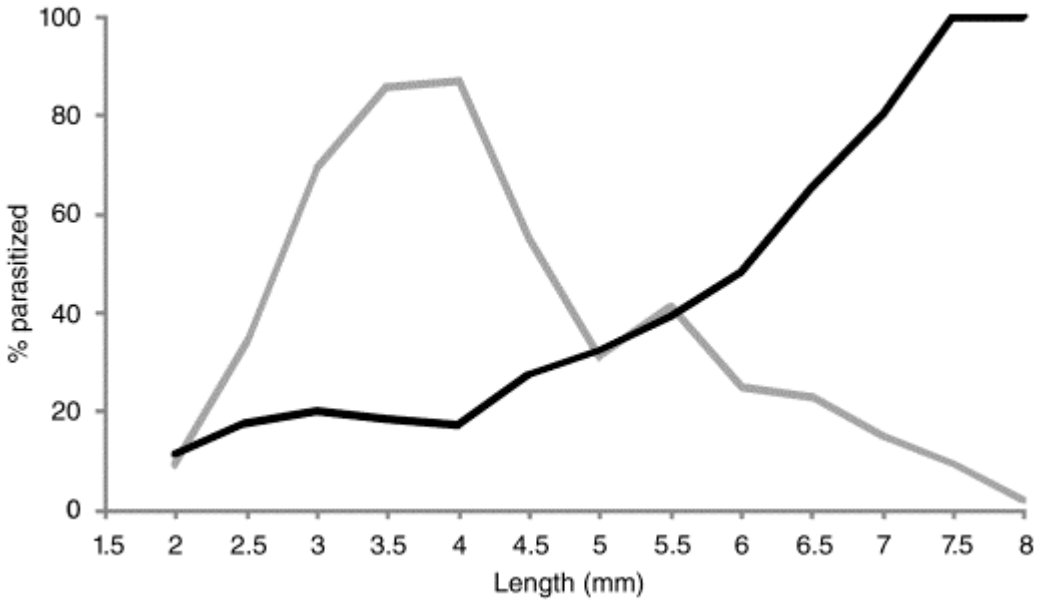

Fig. 2. Fractional length classes of *Heleobia parchappii* per sampling date. The total height of the bars represents 100%, while the fraction of each bar in a given tone indicates the proportion of that developmental stage within the total number of individuals analyzed. Postreproductive snails: upper black; adults: middle gray; juveniles: lower white zone. The sampling dates are listed on the *abscissa*.

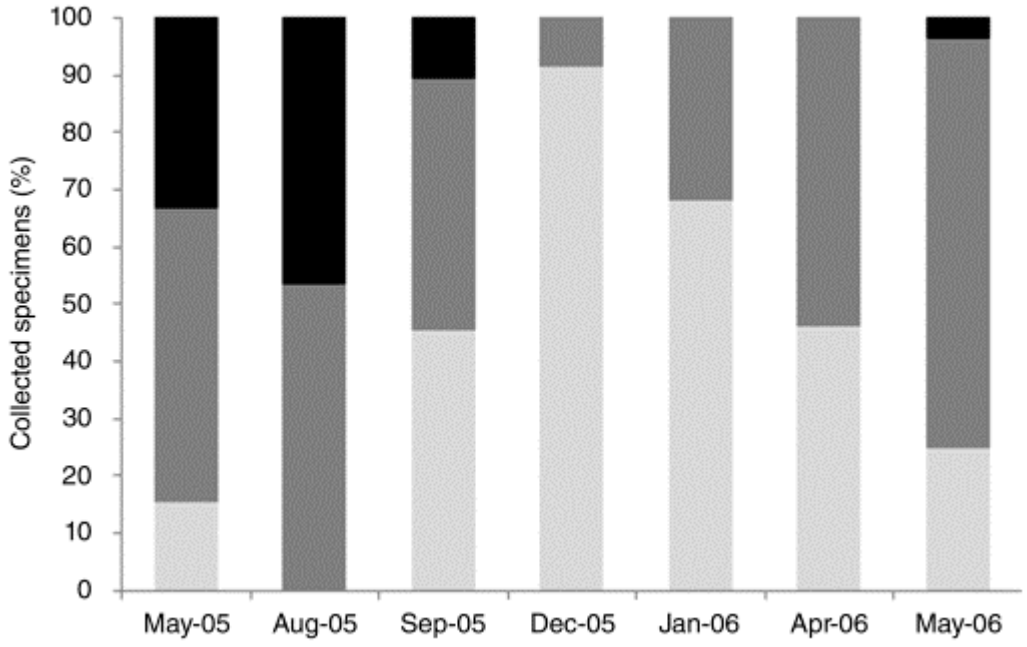

Fig. 3. Proportion of male and female *Heleobia parchappii* parasitized by Digenea throughout the year-long sampling period. The figure shows the percentage of the snails (ordinate) parasitized (solid-black lower bar) and nonparisitized (hatched upper bar) in each sampling month listed on the abscissa. For each month, the left bar represents the females and the right bar the males, as indicated.

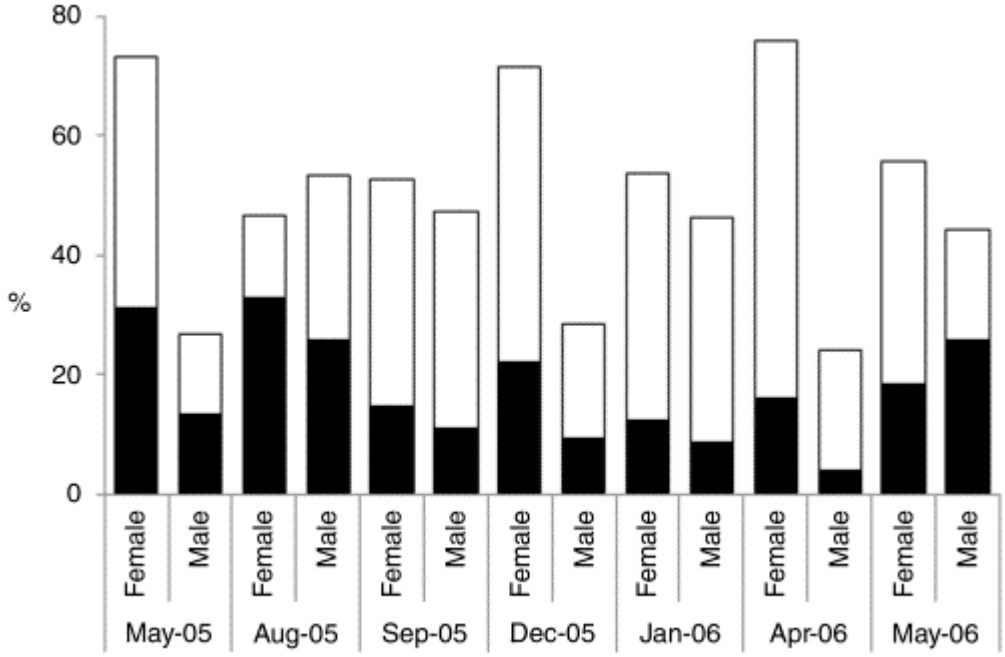

Fig. 4. Prevalence of parasitism by Digenea in males and females of *Heleobia parchappii* as evidenced by histological analysis. In the figure, the percent parasitism is plotted on the ordinate versus the sampling date on the abscissa. Solid-black line: total parasitized; solid-gray line: males parasitized; dotted line: females parasitized

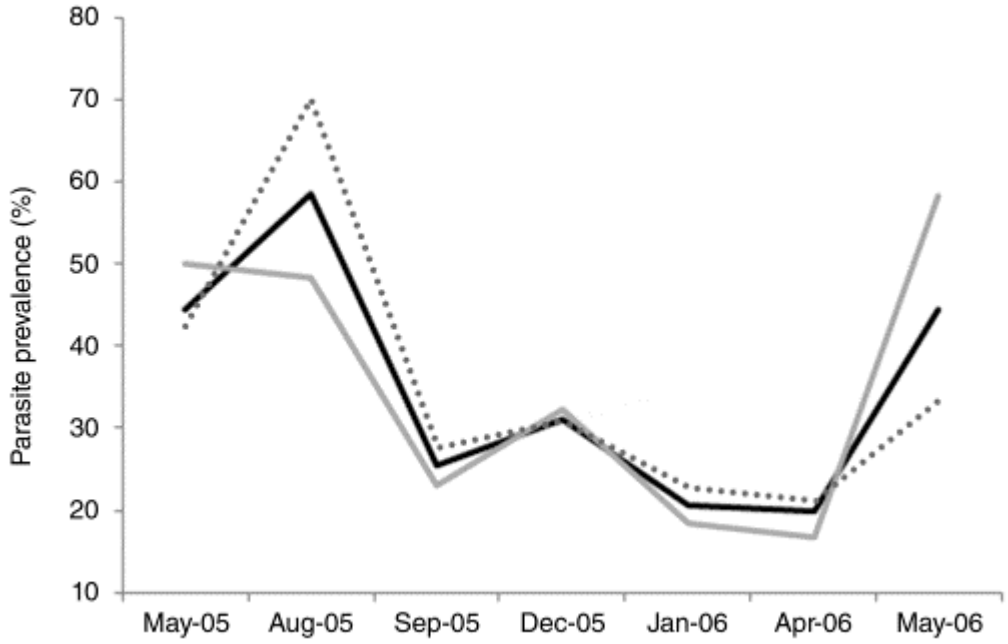

Fig. 5 A: Micrograph shows cercariae infecting *Heleobia parchappii*. (400X). **B:** Cercariae infecting a male. (400X).**C-D:** Fully developed male gametes lie between the cercariae and the digestive gland. (100X). **E:** Female gonads are almost completely absent. (100X). **F:** Cercariae infecting female gonadal maturation. (400X).

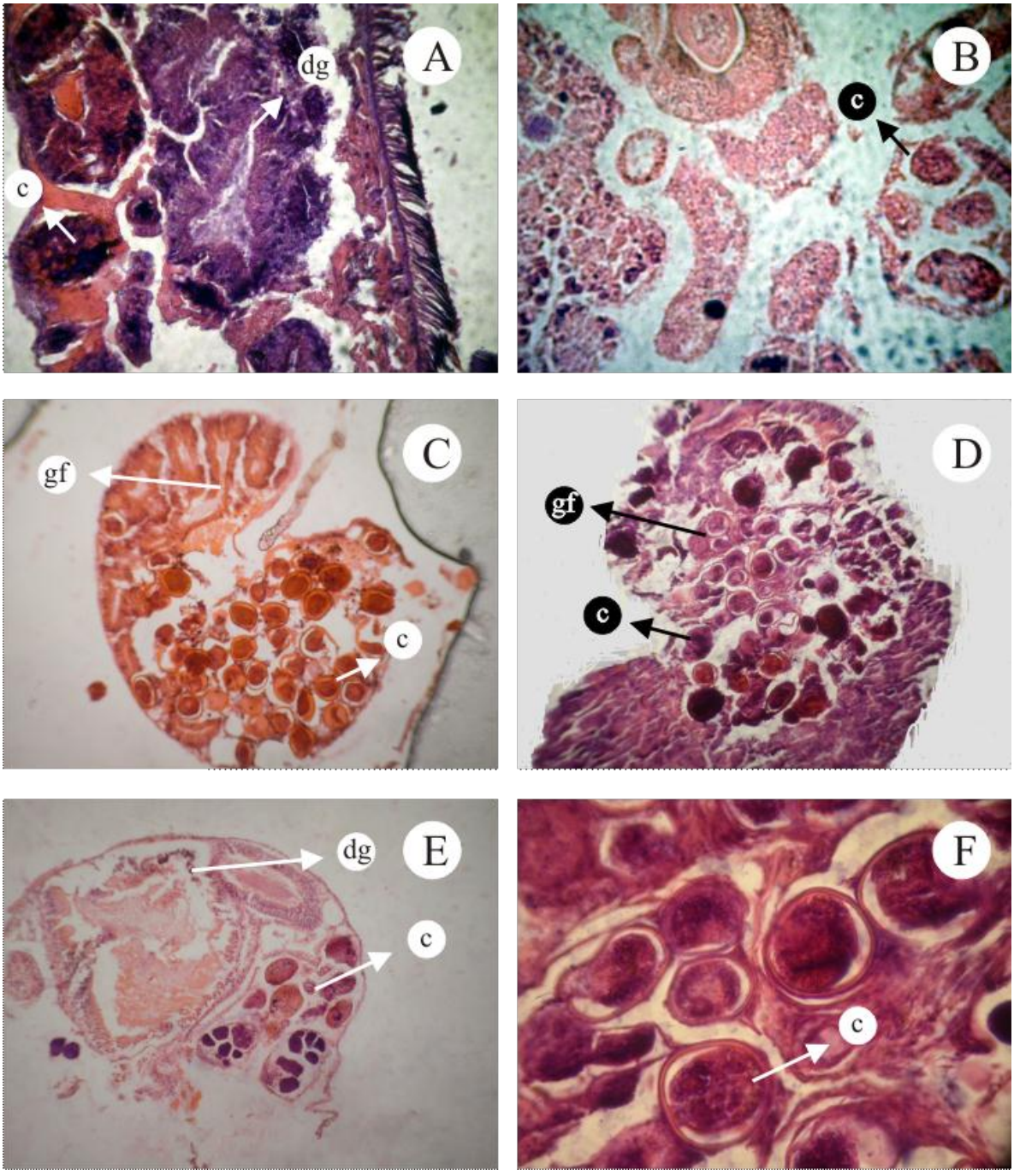
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Fig. 6. Digenea parasitic prevalence in *Heleobia parchappii* specimens exposed to light and heat. Left ordinate: number of snails (solid bars); right ordinate: percent parasitism (black line with triangular points); abscissa: sampling date.

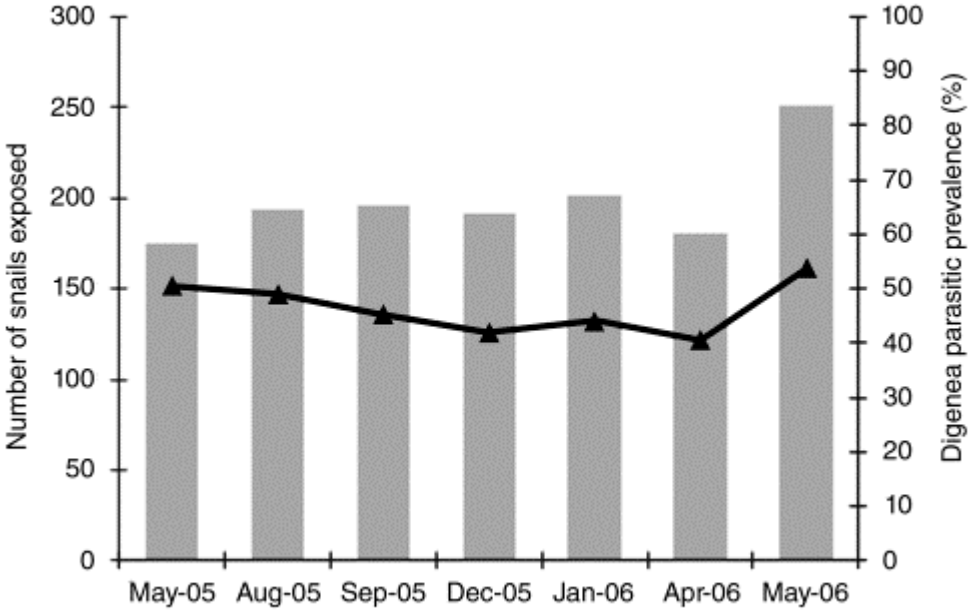

Fig.7. Families of the Digenean cercariae emerging from the *Heleobia parchappii* specimens exposed to light and heat. The ordinate is the percent representation of each family indicated on the abscissa among the total number of the families.