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NOTE

Stunting of the penis in *Heleobia parchappii* (Mollusca: Cochliopidae) and its relationship with parasitism

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ABSTRACT: Penis anatomy is used to discriminate species of gastropods belonging to the family Cochliopidae; however, this characteristic may be affected by the presence of parasites. To evaluate the possible effect of parasites on penis length and number of papillae in *Heleobia parchappii*, 195 males were collected from the Nahuel Rucá Lagoon, Argentina. Male snails were only infected by trematode digeneans (total prevalence 45.13%). Three out of 9 species of digeneans registered showed prevalence values higher than 10%: *Microphallus szidati*, *M. simillimus*, and Notocotylidae sp. 1. The penis length of non-parasitized males and those parasitized by *M. szidati* and *M. similimus* increased with increased snail length; however, this increase was lower in infected snails. In the case of snails infected with Notocotylidae sp. 1, no relationship between shell length and penis length was apparent. Differences in the life cycles of these 3 digeneans could explain the null or lower penis growth rate in relation to host body growth. In contrast, no change was observed in the number of penial papillae of *H. parchappii* when these snails were infected by larval digeneans compared to those that were not infected. This indicates that penial papillae may be a more stable characteristic than penis length to discriminate between species within the Cochliopidae. The study of penial papillae should be central in the taxonomy and identification of new species within the Cochliopidae, as well as in previously described species.

KEY WORDS: Digenean · Penis morphology · Penial papillae · *Microphallus szidati* · *Microphallus simillimus* · Notocotylidae

INTRODUCTION

Gastropod species belonging to the genus *Heleobia* Stimpson, 1865 are widely distributed in southern South America. They are abundant snails, numerically dominant in many lentic communities, and play an important role in the life cycles of parasites (Etchegoin 1997, Cazzaniga 2011, Merlo & Etchegoin 2011, Merlo et al. 2014).

Shell morphology has been used to distinguish between different species belonging to the genus *Heleobia*; however, this approach is not foolproof, because of the typically minimal variation in shell characters. For this reason, the comparative study of other characters, such as penis and radula morphology, has recently been incorporated in taxonomic studies (Cazzaniga 2011).

However, according to previous studies (Gorbushin 1997, Probst & Kuube 1999) in other host species, all of these morphological characters could be influenced by the presence of parasites. Parasitism by larval digeneans has been related to alterations in

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shell morphology and growth and to changes in the development of reproductive organs. These alterations could be a source of phenotypic variation among hosts (Minchella 1985, Gorbushin 1997, Morley 2006) and could therefore interfere with the correct identification at the genus or species level.

H. parchappii is one of the most representative species of the genus in freshwater bodies (streams, rivers, shallow lakes) in northern and central Argentina, Uruguay, and southern Brazil (Gaillard & Castellanos 1976, Castellanos & Landoni 1995). *H. parchappii* is a gonochoric species and is identified according to the morphology of the copulatory organ. The penis is elongated, moderately curved, and with black pigmentation on its convex side; the proximal portion has a wide base with 5 to 9 papillae in a continuous or interrupted row and with rounded or subquadrate edges. The distal portion narrows to an elongated conical tip, with 1 additional papilla on the convex side (Gaillard & Castellanos 1976). *H. parchappii* has been recorded hosting up to 24 species of digeneans (Merlo 2014, Merlo et al. 2014), and we hypothesized that the copulatory organ could be altered by the presence of these parasites. We therefore examined penis morphology in order to evaluate the possible effect of larval digeneans on this structure and to determine whether parasites could interfere with the correct identification of species.

MATERIALS AND METHODS

Specimens of *Heleobia parchappii* were collected in April (2011) at Nahuel Rucá Lagoon (37° 37′ S, 57° 26′ W), in the province of Buenos Aires (Argentina). Snail identification was based on Gaillard & Castellanos (1976). Snails were collected from the lagoon shoreline with the aid of sieves (0.1 mm × 0.1 mm) and placed into plastic containers of 1.5 l capacity for transportation. In the laboratory they were placed individually in plastic containers until further analysis.

Prior to dissection, a digital photograph was taken of each snail using a digital camera (Leica DFC 295). For the photography, each snail was oriented in the same way, with the shell aperture facing toward the camera lens. The total shell length (the distance from the apex to the anterior margin of the aperture) of each *H. parchappii* was measured to the nearest 0.01 mm using Leica Application Suite v3.6.

Each *H. parchappii* was dissected under a stereomicroscope (Leica DM 500) in order to determine the sex and to detect the presence of sporocysts, rediae, and cercariae. Only males were used for subsequent observations. For all males found after dissection, the total length of the penis was measured using Leica Application Suite v3.6, and the penial papillae were counted. Sporocysts, rediae, and cercariae were identified according to Martorelli (1986), Martorelli & Etchegoin (1996), Etchegoin & Martorelli (1998), and Merlo et al. (2014).

Prevalence for each digenean species was calculated as: (number of male snails infected with species *i*/ total number of male snails examined) × 100, and total prevalence was calculated as: (number of parasitized snails/number of collected snails) × 100.

Statistical analyses were performed only for digenean species with prevalences higher than 10%; by this criterion, 3 out 9 digenean species identified were suitable for analysis (see 'Results'). Two analyses of covariance (ANCOVA) were used to test the effect of digenean species on penis length and number of penial papillae. Each digenean species and non-parasitized *H. parchappii* were used as categorical factors. *H. parchappii* penis length and the number of penial papillae were used as dependent variables, and *H. parchappii* body length was used as a continuously controlled covariable. When the slopes were not equal (heterogeneous), *a posteriori* Tukey tests were used for further comparisons between digenean species and non-parasitized snails (Zar 2009). *H. parchappii* shell length, penis length, and number of papillae were transformed according to the formula ln (x + 1) to meet the assumptions of each analysis. All analyses was carried out using 'R.'

RESULTS

In total, 700 specimens of *Heleobia parchappii* were collected (505 females and 195 males). In male specimens, 9 species of digeneans were found, with a total prevalence in males of 45.13%. Each *H. parchappii* host was parasitized by only 1 species of digenean (no double infections were found). Only 3 out 9 digenenan species (*Microphallus szidati*, *M. simillimus*, and Notocotylidae sp. 1) showed prevalences higher than 10%. Individual prevalences of the remaining species did not exceed 3% (Table 1).

For statistical analysis, only 4 groups were considered: (1) males without parasites (Nop), (2) males infected with *M. simillimus* (Ms), (3) males infected with *M. szidati* (Msz), and (4) males infected with Notocotylidae sp. 1 (Nsp).

Table 1. Prevalence (Prev.) and detailed list of families and species/morphological type of larval digeneans parasitizing male *Heleobia parchappii*

Family	Species/morphological type	Prev. (%)	Reference
Heterophyidae	Pleurolophocercaria III	1.03	Martorelli & Etchegoin (1996)
	Cercaria, Heterophyidae sp. 8	2.56	Merlo et al. (2014)
Hemiuridae	Cercaria, Hemiuridae sp.1	1.54	Merlo (2014)
Plagiorchiidae	Xiphidiocercaria sp. 3	1.03	Merlo et al. (2014)
Psilostomatidae	Cercaria, aff. *Psilochasmus*	1.03	Etchegoin & Martorelli (1998)
Haploporidae	Cercaria, Haploporidae sp. 3	0.51	Merlo et al. (2014)
Microphallidae	*Microphallus simillimus* (Travassos, 1920)	10.26	Etchegoin (1997)
	Microphallus szidati Martorelli, 1986	11.79	Martorelli (1986), Etchegoin (1997)
Notocotylidae	Notocotylidae sp. 1	12.82	Etchegoin & Martorelli (1998)
	Redia + Sporocyst	2.56	
Overall prevalence		45.13	

The groups Nop, Ms, and Msz showed a significant positive linear relationship between shell length and penis length (Fig. 1). ANCOVA revealed an interaction between the covariable and the categorical factor ($F_{2,46} = 7.10$; $p < 0.01$); thus, the slopes of the 3 groups were statistically different. The slope of Nop was higher than that of Ms and Msz (Tukey test, $p < 0.01$, in all cases), while no significant difference was observed between Ms and Mz (Tukey test, $p = 0.36$). Conversely, the group Nsp did not show a significant relationship between shell length and penis length. Finally, none of the groups (Nop, Ms, Msz, and Nsp) showed a significant relationship between the number of penial papillae and shell length, for which ANCOVA to compare linear regressions was not performed.

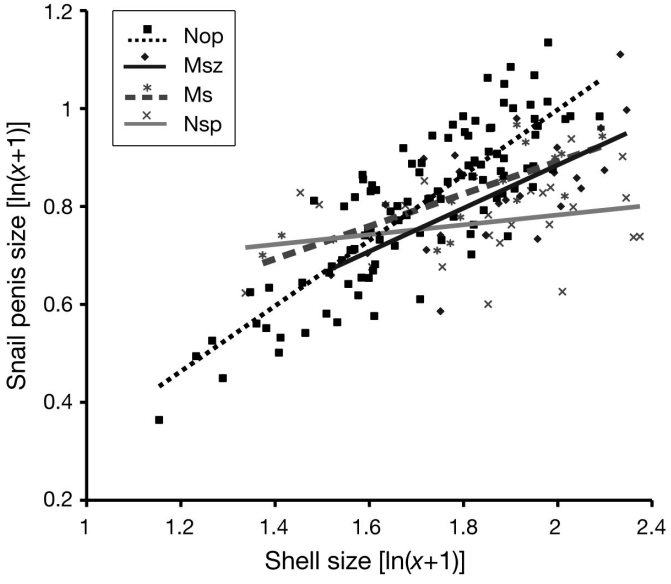

Fig. 1. Comparison of penis length between uninfected *Heleobia parchappii* (Nop) and *H. parchappii* infected with *Microphallus szidati* (Msz), *M. simillimus* (Ms), and Notocotylidae sp. 1 (Nsp), with *H. parachappii* shell length as a covariable

DISCUSSION

Males of *Heleobia parchappii* were parasitized by 9 species of digeneans, with a total prevalence of about 45%. Regarding the phenotypic characteristics of the penis (length and number of papillae), we observed that penis length increased with increased snail length in snails that were not parasitized as well as in those that were parasitized by *Microphallus szidati* and *M. similimus*. However, this increase was lower in *H. parchappii* infected with *M. szidati* and *M. similimus*. Conversely, the lack of correlation between shell length and penis length in snails infected with Notocotylidae sp. 1 could be interpreted as a cessation of growth of the penis. Regarding the number of penial papillae, we found no relationship between the number of papillae and shell length in any of the groups studied.

Infection by parasites has been shown to influence many aspects of host phenotype, including physiology (Thompson 1997), life history (Minchella 1985), behavior (Moore 2002), sexually selected traits (Zuk 1992), and morphology (McCarthy et al. 2004). These modifications of the phenotypic characteristics are merely by-products of infection or adaptations for the parasites to complete their life cycles (Minchella 1985, McCarthy et al. 2004). The digeneans typically have complex life cycles with different larval stages that parasitize a first intermediate host (generally mollusks), a second intermediate host (invertebrates

or vertebrates), and then finally maturing as adults in the definitive host (vertebrates). Host specificity, in the first intermediate host, is one of the characteristic features of larval digenean–mollusk associations, with most digenean species able to develop in a single family or species of molluscan hosts (Galaktionov & Dobrovolskij 2013).

As mentioned above, penis growth stopped in *H. parchappii* infected with Notocotylidae sp. 1, while snails infected with *M. similimus* and *M. szidati* had a lower penis growth rate in relation to non-parasitized snails. Differences in growth rate and reduced penis length associated with larval digeneans belonging to the families Notocotylidae and Microphallidae were also observed in *Hydrobia ulvae* (Gorbushin 1997), and members of the families Notocotylidae and Microphallidae have been observed causing castration in *H. ulvae* (Gorbushin 1997). Digenean species differ in the intra-molluscan stages of their life cycles; Notocotylidae present rediae and cercariae, while digeneans belonging to the Microphallidae present the sporocyst stage. The most relevant difference between rediae and sporocysts is their means of nutrient acquisition. Rediae have a digestive system (mouth, pharynx, and short saccular gut) and actively feed on the cell tissue of Cochliopidae, whereas sporocysts absorb all nutrients directly through the tegument. An additional difference is the limited mobility of sporocysts within the host. Consequently, although both stages cause damage to host organs, rediae can cause greater damage or harm within a shorter infection time (Sousa & Gleason 1989, Probst & Kube 1999, Sorensen & Minchella 2001). In the case of *H. parchappii*, the 3 species of digeneans considered in this study invade the gonad and digestive gland of the snail (Martorelli 1986, Etchegoin 1997, Etchegoin & Martorelli 1998), and thus are a potential cause of castration. All of these combined factors could explain the null or lower rate of penis growth in relation to body growth of the host.

The lack of relationship between the number of penial papillae and shell length of *H. parchappii* coincides with that observed by Cazzaniga (1982), who estimated that the number of penis papillae stabilizes at a snail body length of 3 mm. Given that snails below 4 mm in body length are rarely infected by larval digeneans (Merlo 2014), the number of papillae would not be affected by parasites.

Since shell morphology is an ambiguous approach to discriminate between species in the genus *Heleobia* (Cazzaniga 2011), other characters like morphology of the penis are used to advance our knowledge of the systematics of this group, although this organ presents intraspecific variation (Gaillard & Castellanos 1976, Cazzaniga 1982). According to our results, the number of penial papilla of *H. parchappii* is a more stable character for species identification, as it appears uninfluenced by infection with larval digeneans. Therefore, further studies on the development and intra-specific variation in the number of penial papilla should be central in the diagnosis and taxonomic identification of new species within the family Cochliopidae as well as in previously described species.

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