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Population dynamic of two digenean species parasitizing the grass shrimp *Palaemonetes argentinus* Nobili 1901 (Decapoda: Palaemonidae) in a lentic environment from Argentina

Manuela Parietti*, Matías Javier Merlo and Jorge Alejandro Etchegoin

Laboratorio de Parasitología, Instituto de Investigaciones Marinas y Costeras (IIMyC). Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Funes 3350, 7600 Mar del Plata, Argentina.CONICET

Abstract

The microphallids *Microphallus szidati* and *Levinseniella cruzi* parasitized at least three crustacean species as second intermediate host in Mar Chiquita coastal lagoon: the crabs *Neohelice granulata* and *Cyrtograpsus angulatus* and the grass shrimp *Palaemonetes argentinus*. *Palaemonetes argentinus* acts as host for both metacercariae in brackish and freshwater environments; however the seasonal variations of these digeneans in freshwater habitats remain unexplored. The aim of the present study was to analyze the seasonal dynamic of the larval digenean populations parasitizing *P. argentinus* in a lentic environment. From the total sample of 600 *P. argentinus* collected seasonally, 28.370 and 194 metacercariae of *M. szidati* and *L. cruzi*, respectively, were recovered. *Microphallus szidati* was characterized by high prevalence, 100% in all seasons. In contrast, *L. cruzi* presented low prevalence values, which increased towards summer. The two species of digeneans exhibit seasonal fluctuations in intensity of infection, which was related to host size. Temporal variation of larval digenean assemblage in the crustacean host is probably associated with the presence of the other hosts included in their life cycles. The high values of prevalence found for *M. szidati* in Nahuel Rucá lagoon suggest that *P. argentinus* could favor the contact between the larval digenean and the definitive hosts, establishing the presence of *M. szidati* also in freshwater environments and, thus, increasing the presence of the parasite in different areas of Mar Chiquita coastal lagoon.

Keywords

Crustacean host, larval digeneans, second intermediate host, *Microphallus szidati*, *Levinseniella cruzi*

Introduction

To complete their life cycles, digeneans usually requires three hosts: a definitive host (generally a vertebrate), a first intermediate host (always a mollusc) and a second intermediate host that can be invertebrate or vertebrate (e.g.: molluscs, annelids, crustaceans, fishes, amphibians). The second intermediate host bears the metacercaria stage and could favor the contact with the definitive host and, in consequence, the completion of the life cycle. Metacercariae of Microphallidae exhibit intensive morphogenetic modifications of its internal organ systems that could reduce the number of species used as second intermediate; consequently, the members of this family could exhibit enhanced host specificity (Galaktionov *et al*. 1996; Galaktionov and Drobrovolskij 2003).

In Mar Chiquita coastal lagoon (Argentina), the life cycles of three microphallid species were elucidated: *Microphallus szidati* Martorelli 1986, *Maritrema bonaerensis* Etchegoin and Martorelli 1997, and *Levinseniella cruzi* Travassos 1920. The crabs *Neohelice granulata* and *Cyrtograpsus angulatus* (Crustacea: Decapoda: Varunidae) act as second intermediate host for *M. szidati* and for *M. bonaerensis* in brackish areas of the lagoon (Etchegoin 1997, 2001) and the grass shrimp *Palaemonetes argentinus* (Crustacea: Decapoda: Palaemonidae) was found hosting *L. cruzi* (Martorelli 1988; Martorelli and Schuldt 1990). In freshwater environments related to this lagoon, *P. argentinus* was also indicated as second intermediate host for *M. szidati* and for *L. cruzi* (Martorelli 1988; Martorelli and Schuldt 1990). Bray *et al*. (2008) suggested that Microphal-

*Corresponding author: manuelaparietti@hotmail.com

lidae can develop in brackish, marine and freshwater environments, in decreasing order of frequency. The number of species used as second intermediate hosts by *M. szidati* seems to support the arguments of Galaktionov and Drobrovolskij (2003), who stated that Microphallidae presents a closer relationship with their second intermediate host, using as general, one or two species and rarely three or four.

Palaemonetes argentinus is one of the most common species inhabiting lentic and brackish environments in north and center of Argentina, Uruguay and south of Brazil (Spivak 1997; Collins *et al.* 2004). Due to its very high nutritional quality (70% of dry weight is protein content) *P. argentinus* plays an important ecological role in trophic chains, being one of the principal items in several fishes and birds diets and also a potential influence in the nutrient cycle (González Sagrario 2004; Collins *et al.* 2006). Studies involving larval digeneans in *P. argentinus* are generally focused in the descriptions of new species and the elucidation of digenean life cycles (Martorelli 1988; Martorelli and Schuldt 1990). However, the seasonal fluctuations in abundance of these digeneans and their effects on this intermediate host in freshwater environments remain unexplored. For this reason, the aim of the present study was to analyze the seasonal dynamic of the larval digenean populations parasitizing *P. argentinus* in a lentic environment in Argentina**.**

Materials and Methods

Sampling procedures

The study was conducted in the Nahuel Rucá lagoon (37°37´S–57°25´W), a shallow water body located in a large wetland area belonging to the Mar Chiquita coastal lagoon basin (UNESCO Man and the Biosphere Reserve since 1996) (Fig. 1). Individuals of *P. argentinus* were collected seasonally, using dip nets, from autumn (2012) to summer (2013) and in each season 5 samples were taken. Shrimps were transported to the laboratory and maintained in aerated water. Tetra Min Pro Tropical Crisps was provided as food.

In the laboratory, each specimen of *P. argentinus* was measured with a Vernier caliper (precision: 0.1 mm) from the

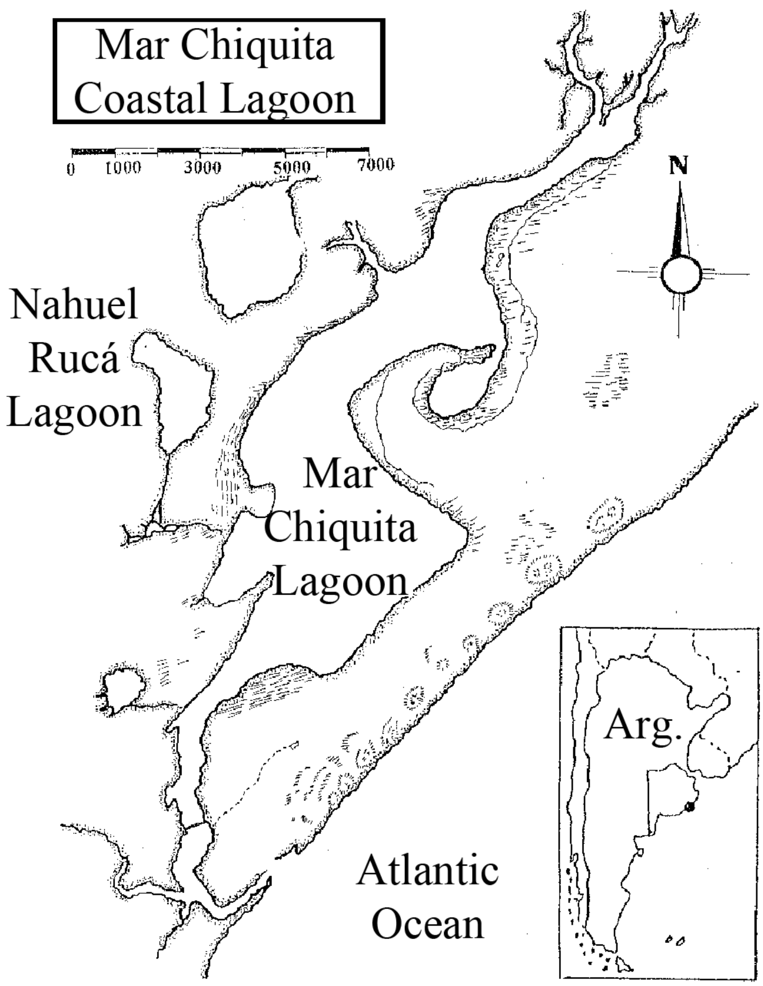

Fig. 1. Map showing Nahuel Rucá lagoon and its connection with Mar Chiquita coastal lagoon

rostrum tip to the end of the cephalotorax (carapace), and from the rostrum tip to the telson end. Sex was determined by the presence or absence of the appendix masculina on their second pair of pleopods (Capitulo Rodriguez and Freyre 1989). Later, shrimps were dissected under stereo-microscope in order to detect the presence of metacercarial cysts. All parts of the body were searched for parasites, including the appendages. The number and species of metacercariae *per host* was recorded for each shrimp. Metacercariae were collected and placed in Petri dishes containing physiological solution and traces of musculature of the host. Some metacercariae released spontaneously from their cysts while others were released with the help of dissecting needles. Larval digeneans were identified according to Martorelli (1986); Martorelli (1988) and Martorelli and Schuldt (1990).

Data analysis

The following quantitative descriptors of parasite populations were used: prevalence, intensity (of infection) and mean intensity (Bush *et al.* 1997). Differences in prevalence and mean intensity were evaluated separately using Two-way ANCOVAs, with season of the year (autumn, winter, spring and summer) and digenean species (*M. szidati* and *L. cruzi*) as the main factors and shrimp size as covariable. To analyze whether males and females of *P. argentinus* have different mean intensities of infection, a Two-way ANCOVA was used separately for both *M. szidati* and *L. cruzi*. The main factors were season of the year (autumn, winter, spring and summer) and sex of *P. argentinus* (male or female), and again shrimp size were used as covariable. Finally, to establish a possible relationship between mean intensity values of both metacercariae species (*M. szidati* and *L. cruzi*) and the body part of the shrimp (cephalotorax and pleon) a two-way ANCOVA was applied, using shrimp size as covariable. In all performed tests, a Tukey test was used for *post hoc* comparisons (Zar 2009). All the assumptions for these tests were evaluated according to Balzarini *et al.* (2008) and Di Rienzo *et al.* (2008). In the cases in which the covariate (host size) had a significant effect on the study variable (prevalence or intensity), values were corrected prior to making comparisons between the main factors. These corrections and all analyzes were done using the statistical Program InfoStat/L (Balzarini *et al.* 2008; Di Rienzo *et al.* 2008).

Results

During the study, a total of 600 *P. argentinus* were examined (150 per season) and 28,564 metacercariae belonging to two species of the family Microphallidae were collected: 28.370 metacercariae of *M. szidati* and 194 of *L. cruzi*.

Prevalence analysis revealed a significant interaction between seasons and digenean species (*M. szidati* and *L. cruzi*) ($F_{3;31}$ = 471; P = 0.008), but no effect of the covariable (host size) was observed acting over the main factors ($F_{1;31}$ = 0.52; P = 0.474). Prevalence of *M. szidati* were significantly higher in all seasons compared with the prevalence of *L. cruzi* (P<0.05; in all cases). The seasonal prevalence of both digenean species presented different temporal dynamics. Prevalence of *L. cruzi* varied along the year of study, increasing towards summer, with significant differences found between autumn-spring and autumn-summer (Tukey test: P<0.05; in both cases). In contrast, prevalence for *M. szidati* showed no temporal heterogeneity, remaining constant throughout the year, reaching in all seasons a value of 100% (Fig. 2).

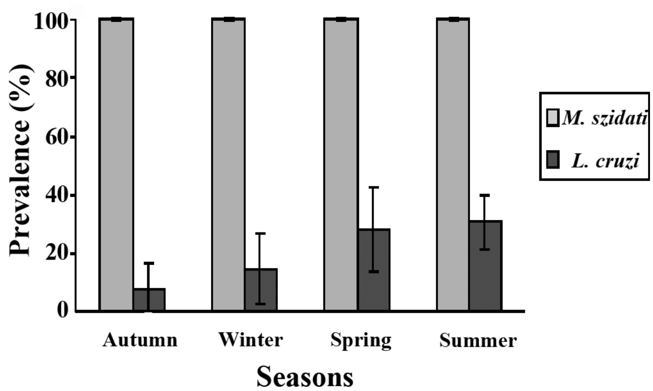

Fig. 2. Seasonal variation in the overall prevalence (mean ± S.D) of *Microphallus szidati* and *Levinseniella cruzi* parasitizing *Palaemonetes argentinus*

Intensity ranged from 1 to 239 for *M. szidati* and from 1 to 15 for *L. cruzi* (mean intensity are shown in Table I). In the statistical analysis, mean intensity revealed interaction between the main factors (digenean species and seasons of the year) ($F_{3;1.145}$ = 59.62, P<0.001) and varied with shrimp size ($F_{1;1.145}$ = 136.37; P<0.001). Mean intensity of *M. szidati* were higher than those of *L. cruzi* for all seasons of the year (Tukey

Table I. Seasonal values of mean intensity for *Microphallus szidati* and *Levinseniella cruzi* parasitizing *Palaemonetes argentinus* (mean ± S.D.)

Seasons	*M. szidati*	*L. cruzi*
Autumn	33.12 ± 22.93	1.36 ± 0.67
Winter	31.25 ± 30.25	1.41 ± 0.80
Spring	75.99 ± 47.62	1.64 ± 0.88
Summer	53.78 ± 30.14	1.72 ± 2.16

test, P<0.05; in all cases). However, each digenean species presented a different temporal dynamics. *Levinseniella cruzi* did not present differences along the year of study (P>0.05; in all cases), while mean intensity of *M. szidati* showed temporal heterogeneity, increasing from autumn to summer, with significant differences between autumn-spring, autumn-summer, winter-spring, winter-summer and spring-summer (P<0.05, in all cases). There was no significant effect of the host's sex on the seasonal mean intensity of *L. cruzi* ($F_{1;568}$ = 0.24; P = 0.623), and *M. szidati* ($F_{1;568}$ = 0.2; P = 0.652).

Metacercariae of both species were observed in the cephalotorax and pleon of *P. argentinus*. However, an interaction between the body parts and digenean species was observed ($F_{1;2.303}$ = 619.76; P<0.001) and an effect of the covariable (host size) ($F_{1;2.303}$ = 292.55; P<0.01). The two species showed the same pattern of preference of infection, being the host's pleon the body part with higher mean intensity (Tukey test: P <0.05; for all cases). *Microphallus szidati* presented a mean intensity greater than *L. cruzi* for each part of the body (Tukey test: P<0.05; in all cases) (Table II).

Table II. Mean intensity for *Microphallus szidati* and *Levinseniella cruzi* in cephalotorax and pleon of *Palaemonetes argentinus* (mean ± S.D.)

Body part	*M. szidati*	*L. cruzi*
Cephalotorax	7.75 ± 7.89	1.38 ± 0.66
Pleon	41.42 ± 33.73	1.96 ± 2.82

Discussion

During the study, *M. szidati* presented higher values of mean intensity and prevalence than those of *L. cruzi*. Nevertheless, seasonal fluctuations were observed for *L. cruzi* in prevalence values. The two species of digeneans exhibited seasonal fluctuations in intensity of infection, which were related to host size. Furthermore, differences were found between regions of the body used by metacercariae. The two species showed the same pattern of preference of infection, being the pleon the body part with higher intensities.

The marked differences between intensity and prevalence values observed for both metacercariae may be due to the fact that *P. argentinus* is probably included in the life cycle of *L. cruzi* only as a paratenic host (Schuldt *et al.* 1972). Another plausible explanation could be an inter-specific competition between both metacercariae. Sharing an intermediate host can result in intra- and inter-specific competition among parasites for host resources and this interaction within the intermediate host can be costly to one or both parasite species (Dezfuli *et al.* 2001; Fredensborg and Poulin 2005).

Both species showed temporal variation associated with host size while sex of the host had no effect on intensity values. A positive relationship between host size and parasite intensity is generally expected, as the parasites simply accumulate in the host over time (Hudson and Dobson 1995; Pung *et al.* 2002). Nevertheless, even with the size effect was corrected, seasonal fluctuations persisted. This change of larval digenean intensity in the crustacean host is probably associated with the presence of the other hosts included in their life cycles. The diversity and abundance of metacercariae in the second intermediate host may be, in part, influenced by the diversity and abundance of the miracidia and cercariae stages in the environment. The potential definitive host that were found for *L. cruzi* were *Rollandia rolland chilensis* (Podicipediade) and *Himantopus melanurus* (Recurvirostridae) and for *M. szidati* were *Rallus sanguinolentus sanguinolentus* (Rallidae), *H. melanurus* and *Larus atlanticus* (Laridae) (Martorelli 1986, 1988; Etchegoin *et al.* 1996). In Nahuel Rucá lagoon, Josens (2011) found higher richness and abundance of these bird species during the summer months, which match up with the increased of the intensity and prevalence values for both digenean species. Therefore, the presence of birds in the lagoon could determine an increment in the digenean prevalence (primary in the mollusc host and secondary in the shrimp host). In the same way, the maximum values of prevalence and intensity for *L. cruzi* and *M. szidati* in *P. argentinus* were consistent with the prevalence values found in the mollusc host *Heleobia parchappii* for both digenean species (Merlo 2014). So, intensity values in this system are closely related to previous and next host, which are found in warmer months.

Both parasites exhibit some degree of habitat selection inside the host, since both preferred tail region instead of the cephalotorax. These differences observed between regions of the body could be associated with a greater proporti on of muscle and space in the pleon and/or a migration of metacercariae from cephalotorax to pleon. This migration could be due to a raise in the digenean size from the cercariae to the metacercariae stage, which led cercariae to search for more space to develop the cyst (Galaktionov *et al.* 1996; Fredensborg *et al.* 2004). Elaborated migrations and species-specific localization in crustaceans might have originated as an adaptation caused by interspecific competition for the habitats in the body of the second host (Galaktionov 1993). The presence of a large numbers of metacercariae in the pleon could be considered as another adaptation of microphallids, since the metacercariae makes probably the shrimp more visible for the predators (white cyst visible through the cuticle) and thus increases their chances of completing their life cycle (Kunz and Pung 2004).

Microphallids have an ability to develop and complete their life cycle in brackish and freshwater environments, using different species of crustacean as second intermediate host according to their availability (Galaktionov 1993). An inevitable decrease of the probability of infection transmission associated with a narrow specificity seems to be compensated by the fact that this family uses all available crustacean species in the environment as their second intermediate hosts, which matches with a large proportion in the food composition of the definitive hosts, the birds (Galaktionov and Drobrovolskij 2003). In the brackish areas or the lagoon, the digenean *M.*

szidati uses the crabs *N. granulata* and *C. angulatus* as second intermediate hosts (Etchegoin 1997, 2001). The high values of prevalence and intensity of infection found for *M. szidati* in Nahuel Rucá lagoon suggest that *P. argentinus* could favor the contact between the larval digenean and the definitive hosts, establishing the presence of *M. szidati* also in freshwater environments and, thus, increasing the presence of the parasite in different areas of Mar Chiquita coastal lagoon. Nevertheless another microphallid species, *Maritrema bonaerensis*, was found parasitizing only the crabs *N. granulata* and *C. angulatus* in the brackish areas of Mar Chiquita coastal lagoon (Etchegoin and Martorelli 1997). Probably, the absence of *M. bonaerensis* in freshwater environments could be attributed to an ecological barrier imposed by the levels of salinity. Future studies including all the crustacean hosts for these digenean species in the lagoon, will shed light on their interspecific relationships, on the factors determining their distribution and on the strategies of transmission displayed in their life cycles.

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