

Diversity and dynamics in larval digenean assemblages parasitizing *Heleobia parchappii* in a freshwater shallow lake from the Southeastern Pampa plain, Argentina

Research Article

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Abstract

Digeneans have important roles within ecosystems; however, it is estimated that only 14% of the species have been described. Therefore, before being able to detail their role, digenean species' identification and the diversity present in the ecosystems must be known. In this study, the diversity and the temporal-spatial dynamics of larval digeneans in the freshwater snail *Heleobia parchappii* were analysed in a shallow lake. Specimens of *H. parchappii* were collected seasonally at three points during one year and a total of 2871 molluscs were analysed. A total of 23 species of digenea were registered and both the overall prevalence and the composition of the assemblages presented temporal and spatial variations, responding to the differential environmental conditions characteristics (anthropic effect, presence of native forests, and differential use of the habitat by the definitive hosts) of three sampled sites. The assemblages of larval digenean in their first intermediate host support the idea that this area is of great importance in biodiversity, and could be endemic areas of some species of digenean that use reptiles, amphibians and bats as hosts, groups that are at risk of conservation. Protection of these environments is a fundamental pillar in the policies for the conservation of wild flora and fauna.

Introduction

The 'Global parasite project' proposed by Carlson *et al.* (2020a, 2020b) is an internationally coordinated effort to revolutionize the process of cataloguing parasite diversity with an ambitious goal of describing 50% of species in the next decade. Systematically collecting parasite data would help to establish current parasite biodiversity baselines, identify rare species and monitor future changes in parasite biodiversity (Gehman *et al.*, 2019). Estimations suggest a global total of 100 000–350 000 species of helminth endoparasites of vertebrates, of which 85–95% are unknown to science (Carlson *et al.*, 2020b). In the case of digeneans (Platyhelminthes: Trematoda), this estimation is 44 262 species but only 14% have been described (Carlson *et al.*, 2020b). Despite the vast diversity of this group, they are also one of the less protected, even when the global impact of losing some of those parasites could lead to severe consequences to ecosystems. Parasites often play key roles that are usually underestimated for being considered dangerous, when the fact is that parasite diversity benefits ecosystems and decrease community-level disease risk (Lafferty *et al.*, 2008). For example, digeneans have an important role in ecosystems as ecosystem engineers, by contributing to biomass flow, food web connectivity, population control altering predator–prey interactions and driving the evolution of other species (Lafferty *et al.*, 2008; Sato *et al.*, 2011; Dunne *et al.*, 2013; among others). They can also be used as bioindicators of environmental natural fluctuations, abundance and diversity of biotic communities, climate changes, or anthropogenic impact, as they are sensitive to changes in habitat quality and land use (Lafferty, 1997; Huspeni *et al.*, 2005). Monitoring studies have shown that surrogate species can provide good information for the diversity of taxa present in an environment (Lindenmeyer *et al.*, 2015; Moore *et al.*, 2020), even indicating the presence of the more elusive ones as diverse as shorebirds (Byers *et al.*, 2008), terrapins (Byers *et al.*, 2010), small fishes and benthic invertebrates (Hechinger *et al.*, 2007). However, digenean species' identification and their diversity in the ecosystems must be known before being able to reveal their true role.

In studies with mollusc hosts, the temporal and spatial distributions of the hosts involved in the complex life cycles and the characteristics of the host's habitat (including biotic and abiotic factors) produce variations in the diversity and the structure of larval digenean communities (Kuris and Lafferty, 1994; Huspeni *et al.*, 2005; Thieltges *et al.*, 2009). Additionally, habitat features over large and small spatial scales can also directly or indirectly influence transmission dynamics, and thus, the recruitment success of parasites to their hosts (Sousa and Grosholz, 1991). Particularly, local factors play a major role in determining the richness and abundance of larval digeneans in mollusc hosts (Byers *et al.*, 2008).

In the last 20 years, much progress has been made to increase the knowledge of the diversity of digeneans in the lotic and lentic environments of Argentina, as well as their temporal and spatial dynamics in several mollusc hosts (Flores *et al.*, 2010, 2015; Merlo and Etchegoin, 2011; Fernández *et al.*, 2013; Parietti *et al.*, 2013, 2020, 2021; Merlo *et al.*, 2019; among others). However, due to the large number of water bodies that cover the Argentine territory, we are still far away from having adequate knowledge of the diversity of digeneans and the factors that determine their distribution that allows us to perform robust macroecological and biogeographical studies. Therefore, the main objective was to study the diversity of larval digeneans of *Heleobia parchappii* (Mollusca: Cochliopidae) in a small shallow lake (Nahuel Rucá). In addition, given the natural features and low human impact in this environment, the temporal and spatial dynamics of larval digenean assemblage was studied within the lake. These studies are fundamental to reduce the gap in the knowledge of digenean diversity and to establish in the future the true role of this group of parasites in freshwater ecosystems.

Materials and methods

Study area

Nahuel Rucá shallow lake is located in the Pampean region of Argentina (37°37'S–57°25'W) (Fig. 1). It has a surface area of about 245 ha, and a mean depth of 1.5 ± 0.16 m (Isla and Gaido, 2001), and is 9.6 km from the Argentinian Sea. It has the contribution of a tributary, Dulce Stream, and flows through an artificial channel into the Sotelo Stream (effluent). It has reed beds (*Schoenoplectus californicus*) at the intersection between the streams and the lake, and its borders and along its coastline, which makes this water body important breeding, resting and feeding area for a large number of local and migratory birds species (Josens *et al.*, 2009). In addition, five molluscs were found in this lake *H. parchappii*, *Biomphalaria peregrina*, *Pomacea canaliculata*, *Musculium argentinum* and *Uncancylus concentricus* (Tietze and De Francesco, 2010; Personal observation). However, *H. parchappii* is the only species present throughout the lake and with abundances ranging from 240 to 1478 ind. h^{-1} (Merlo *et al.*, 2016). Nahuel Rucá is a particularly unstable environment, with a varied rainfall (10–268 mm monthly) and air temperature range (2–28°C) (personal data). It also suffered a total drought at the end of 2008 and the beginning of 2010. These abiotic features are particular and common to water bodies of the Pampean region (Sosnovsky and Quirós, 2006).

Three sampling sites were selected from the lake. Site A at the extreme southwest, near the Sotelo stream (effluent), and characterized by having a coastline free of vegetation and with reed beds arranged in a semi-circular shape located 10 m from the water edge, forming a structure similar to a bay. At the central point of the lakeshore is located the site B with an abundant number of reeds arranged parallel to the coastline. And a site C, located in the extreme northwest of the lake near Dulce creek, with similar physiognomy to site A in terms of the disposition of the reeds, and it is an area used by birds as a roost (Josens *et al.*, 2009).

Sample collections

Specimens of *H. parchappii* were collected from one sampling trip per season during autumn, winter and spring 2017 and during summer 2018. The samplings were carried out in the second month of each season during the morning (e.g. May in autumn, August in winter, etc.) and all three sites (A, B and C) were sampled in the same sampling trip. Five replicate samples per trip and site were taken. Snails were collected along a 200 m transect

parallel to the coastline with the aid of sieves (0.1 mm \times 0.1 mm) and placed into plastic containers of 1.5 L capacity for transportation. Water temperature and depth were measured at every trip per season in each site. In the laboratory, the shell length of each mollusc was measured to the nearest 0.05 mm using a Leica DM 500 stereomicroscope. Sixty randomly selected molluscs from each replicate and each season were isolated individually in 45 ml plastic cups and maintained under a 12–12 light-dark photoperiod for 48 h to stimulate shedding of larval digenean (cercariae). Finally, all molluscs were dissected under a stereomicroscope and all organs and tissues were examined to detect the presence of sporocysts, rediae, developing cercariae and metacercariae (Curtis and Hubbard, 1990). Emerged cercariae were studied alive, under a light microscope, and were identified according to Yamaguti (1975), Martorelli (1986a), Martorelli and Etchegoin (1996), Etchegoin (1997), Etchegoin and Martorelli (1998) and Merlo *et al.* (2014).

Statistical analysis

Three indices were used to analyse and compare the temporal and spatial dynamics of the community of larval digeneans in *H. parchappii*: (a) species richness (S) which represents the total number of species in a sample (Magurran, 1988); (b) prevalence by species (number of snails parasitized by that species/number of collected snails \times 100) and (c) overall prevalence (number of parasitized snails/number of collected snails \times 100).

Generalized Linear Models (GLMs) with a Negative Binomial probability distribution model and the function `glm` from the MASS package for R (Venables and Ripley, 2002) were used to explore differences between seasonal and spatial overall prevalence. A total of five variables were considered for model selection in GLM: three continuous and explanatory variables: snail's size, water temperature and water depth; and two variable categories: season and site. Model selection in GLM involved the hypothesis testing (Zuur *et al.*, 2009), starting with all three exploratory variables, and removing the water depth variable in the final model as it was not significant. Post-hoc comparisons were made between the sites and seasons.

A Bray–Curtis similarity matrix was computed at the infra-community level for the parasite assemblage in *H. parchappii* by site and season and was subjected to a permutational multivariate analysis of variance (PERMANOVA), using the function `adonis` from the `vegan` package for R (Oksanen *et al.*, 2016). Due to the large differences in parasite abundance across parasite species, data were square-root transformed prior to multivariate analyses to down-weight the importance of very abundant species, such that less dominant species also played a role in determining similarity among samples. Since PERMANOVA is sensitive to differences in multivariate dispersion between groups (*sensu* homogeneity of variances), the same models were tested for differences in dispersion using multivariate homogeneity of group dispersions PERMDISP. To visualize possible temporal and spatial patterns in the composition of parasite assemblages, non-metric multidimensional scaling (nMDS) of the similarity matrix were performed between all infra-communities. Average values were then visualized in an nMDS using as many dimensions as needed to closely match the original distance matrix (correlation coefficient of $\rho = 0.99$).

Analyses were done using the statistical programming language R, version 2.15.2. (R Development Core Team, 2018).

Results

Between autumn 2017 and summer 2018, a total of 2871 individuals of *H. parchappii* were analysed and 23 species of larval

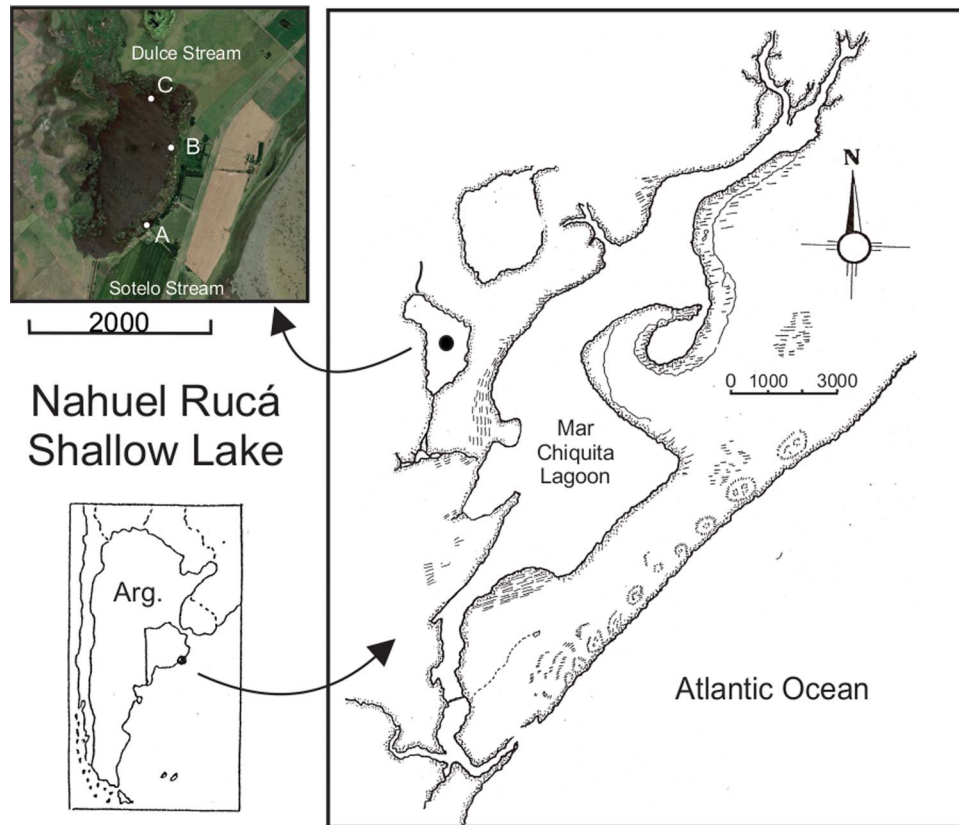


Fig. 1. Map showing locations of the Nahuel Rucá Shallow Lake in closeness to Mar Chiquita Coastal Lagoon in Argentina. In the satellite photograph, the three sampling sites are indicated.

digenea belonging to 12 families were recorded (Table 1). The greatest species richness was found in summer for site B and winter for site C (both $S = 13$), and the lowest species richness was found in summer for site C ($S = 6$) (Table 1). Of the total species types of digenea, only three were found during the four seasons and at the three sites: Notocotylidae sp. 1 (Notocotylidae), *Levinseniella cruzi* (Microphallidae) and *Microphallus similimus* (Microphallidae). On the contrary, six were registered in one season and one site. Cercaria Haploporidae sp. 2 (Haploporidae) was registered in the winter of site A. In site B, Xiphidiocercaria sp. 2 (Ochetosomatidae) was registered in autumn, and cercariae [Xiphidiocercaria sp. 3 and Xiphidiocercaria sp. 4 (Plagiorchiidae)] in summer. In site C cercariae Schistosomatidae sp. 1 (Schistosomatidae), Furcocercaria sp. 2 (Schistosomatidae) and Pleurolophocercaria IV aff. *Pygidicpsis crassus* (Heterophyidae) were registered in winter.

By identifying the larval stages at the family level and based on published life cycle data, it was possible to establish the possible definitive hosts that would contribute to forming the assemblages of larval digenea of *H. parchappii* in the Nahuel Rucá Lake. Birds and mammals, with a 66.6% contribution, are the main definitive hosts of the digenea that parasitize *H. parchappii*, followed by fishes (18.8%), and to a lesser extent, 8.7% use reptiles, and 1.5% amphibians as definitive hosts. Only one species [*Genarchella genarchella* (Hemiuridae)] has all monoxene life cycle in the mollusc host.

Significant effects of the water temperature and host's size on the prevalence, and interaction between sites and seasons were found (water temperature: $P = 0.004$, host's size: $P = 0.03$, interaction: $P < 0.00005$, Fig. 2). Between sites, the prevalence of spring was lower at A than C ($P = 0.004$). In contrast, the prevalence in summer was higher at A than C ($P = 0.006$). No differences were

found in the overall prevalence between sites for autumn and winter, and between sites A and B in spring and summer ($P > 0.05$, for all cases). The *post hoc* analysis within each site yielded differences between the site A prevalence in spring compared to autumn ($P = 0.0001$), winter ($P = 0.004$) and summer ($P = 0.000009$). However, at sites B and C, no seasonal differences were observed ($P > 0.05$, for all cases).

The results of PERMANOVA showed an effect of sites and seasons on the parasite community structure [season \times site: $F = 1.94$, P (perm) = 0.0013]. Pairwise tests agreed in general with nMDS ordinations (Fig. 3). In the analysis of pairs between sites by season, differences were observed between autumn of sites A, B and C [A vs B: P (perm) = 0.0053; A vs C: P (perm) = 0.0047; B vs C: P (perm) = 0.006], between winter and spring of sites B and C [winter B vs C: P (perm) = 0.023; spring B vs C: P (perm) = 0.026], and between summer of sites A and C [P (perm) = 0.03]. While in the seasonal analysis within each site, different patterns were observed. Site A presented differences between autumn and the other all seasons [autumn vs winter: P (perm) = 0.017; autumn vs spring: P (perm) = 0.015; autumn vs summer: P (perm) = 0.008], and winter was different from summer [P (perm) = 0.0073]. In site B, autumn presented differences from spring and summer [autumn vs spring: P (perm) = 0.02; autumn vs summer: P (perm) = 0.034]. Site C presented differences between almost all seasons [autumn vs winter: P (perm) = 0.012; autumn vs spring: P (perm) = 0.018; spring vs winter: P (perm) = 0.022; spring vs summer: P (perm) = 0.009; summer vs winter: P (perm) = 0.047], only summer was similar to autumn [P (perm) = 0.062]. A proportion of these differences can be attributed to differences in the dispersions of parasite communities in the lake [PERMIDISP: $F = 5.99$, P (perm) = 0.0007].

Table 1. List of species or morphological type of larval digeneans that parasitize *Heleobia parchappii* in Nahuel Rucá shallow lake

| Family | Species | Second intermediate host | Definitive host | Site A | Site B | Site C | Ref. |
|------------------|---|--|--|-------------|-------------|-------------|--|
| Notocotylidae | Notocotylidae sp. 1 | Absent | Birds and mammals | A, W, Sp, S | A, W, Sp, S | A, W, Sp, S | Etchegoin and Martorelli (1998) |
| Heterophyidae | Pleurolophocercaria VI | Fishes | Birds and mammals | A, W, Sp, S | S | W, Sp | Martorelli and Etchegoin (1996) |
| | Pleurolophocercaria III | Fishes | Birds and mammals | A, W, Sp | W, Sp, S | A | Martorelli and Etchegoin (1996) |
| | Pleurolophocercaria II aff. <i>Ascocotyle (Leighia) hadra</i> | <i>Cnesterodon decemmaculatus</i> and <i>Jenynsia lineata</i> (fishes) | Birds and mammals Experimental in <i>Gallus gallus</i> and <i>Mus musculus</i> . Natural host unknown | A | – | A, Sp | Etchegoin (1997), Ostrowsky de Nuñez (1992) |
| | Pleurolophocercaria IV aff. <i>Pygiodicpsis crassus</i> | <i>Cnesterodon decemmaculatus</i> and <i>Jenynsia lineata</i> (fishes) | Birds and mammals Experimental in <i>Gallus gallus</i> and <i>Mus musculus</i> . Natural host unknown | – | – | A | Martorelli and Etchegoin (1996), Ostrowsky de Nuñez (1995) |
| | Cercaria Heterophyidae sp. 8 | Fishes | Birds and mammals | A, W, Sp, S | W, Sp, S | A, W, Sp, S | Merlo et al. (2014) |
| Acanthostomidae | Cercaria Acanthostomidae sp. 1 | Fishes and amphibians | Fishes, amphibians and reptiles | S | Sp | Sp | Merlo et al. (2014) |
| Ochetosomatidae | Xiphidiocercaria sp. 2 | Amphibians | Reptiles | – | A | – | Etchegoin and Martorelli (1998) |
| Echinostomatidae | Echinocercaria sp 1 aff. <i>Echinocasmus talaensis</i> | <i>Cnesterodon decemmaculatus</i> (fishes) | Birds, mammals and fishes. Known host: <i>Pitangus sulphuratus</i> (birds) | S | S | Sp | Etchegoin and Martorelli (1998), Martorelli (1986c) |
| Haploporidae | Cercaria Haploporidae sp. 1 | Absent | Fishes | – | Sp | – | Etchegoin and Martorelli (1998) |
| | Cercaria Haploporidae sp. 2 | Absent | Fishes | W | – | – | Etchegoin and Martorelli (1998) |
| | Cercaria Haploporidae sp. 3 | Absent | Fishes | A, W | W, Sp, S | W, Sp, S | Merlo et al. (2014) |
| Microphallidae | <i>Levinseniella cruzi</i> | <i>Palaemonetes argentinus</i> (crustacea) | Birds and mammals Known host: <i>Hymantopus melanurus</i> , <i>Rollandia rolland chilensis</i> (birds) | A, W, Sp, S | A, W, Sp, S | A, W, Sp, S | Martorelli (1988), Parietti et al. (2014) |
| | <i>Microphallus simillimus</i> | Absent | Birds and mammals Known host: <i>Hymantopus melanurus</i> (birds) | A, W, Sp, S | A, W, Sp, S | A, W, Sp, S | Etchegoin (1997) |
| | <i>Microphallus szidati</i> | <i>Palaemonetes argentinus</i> and <i>Cyrtograpsus angulatus</i> (crustacea) | Birds and mammals Known host: <i>Hymantopus melanurus</i> and <i>Rallus sanguinolentus sanguinolentus</i> (birds) | W, Sp | A, W, Sp, S | A, W, Sp | Parietti et al. (2014), Martorelli (1986a, 1986b) |
| Psilostomidae | <i>Psilochasmus oxyurus</i> | Absent | Birds and mammals Known host: <i>Larus maculipennis</i> (birds) | A, W, S | A, S | A, W, Sp, S | Etchegoin and Martorelli (1998), Labriola and Suriano (2001) |
| Hemiuridae | Cercaria Hemiuridae sp. 1 | Molluscs | Fishes Known host: <i>Pimelodus sp.</i> , <i>Astyanax sp.</i> , <i>Cynopotamus sp.</i> , <i>Moenklausia doceana</i> and <i>Roeboides sp.</i> (fishes) | A | A | W | Martorelli (1989), Merlo (2014) |
| | <i>Genarchella genarchella</i> | Absent | Absent | W | A, W, Sp, S | – | Szidat (1957), Martorelli (1989), Merlo (2014) |

| | | | | | | |
|-------------------|----------------------------------|-----------------------------|-----------------------------|------|----------|----------------------------|
| Schistosomatidae | Cercaria Schistosomatidae sp. 1 | Absent | Birds and mammals | - | W | Merlo <i>et al.</i> (2014) |
| Lecithodendriidae | Furcocercaria sp. 2 | Absent | Birds and mammals | - | W | Merlo (2014) |
| Plagiorchiidae | Cercaria Lecithodendriidae sp. 1 | Odonata larvae (arthropoda) | Mammals | A, S | A, W, Sp | Merlo (2014) |
| | Xiphidocercaria sp. 3 | Amphibians | Reptiles, birds and mammals | - | S | Merlo <i>et al.</i> (2014) |
| | Xiphidocercaria sp. 4 | Amphibians | Reptiles, birds and mammals | - | W | Merlo <i>et al.</i> (2014) |

The second intermediate host and definitive host according to bibliography available are also given. Ref. A, autumn; W, winter; Sp, spring; S, summer.

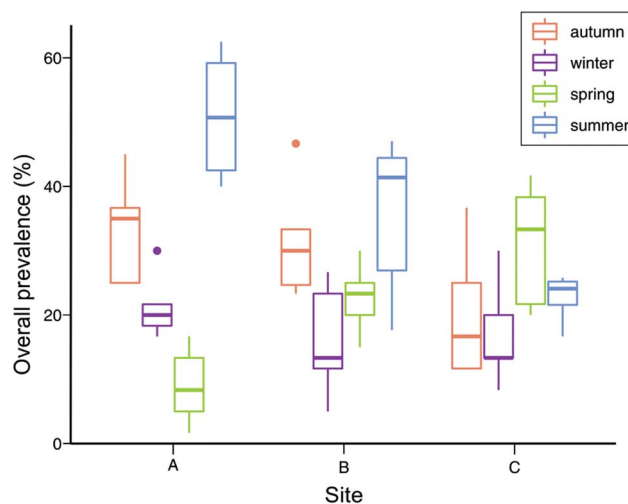


Fig. 2. Seasonal and spatial variation of the overall prevalence of larval digeneans that parasitize *Heleobia parchappii*. Boxes represent means and vertical bars denote maximum and minimum values.

Discussion

This study examined temporal and spatial changes in composition and prevalence in larval digenea assemblages of *H. parchappii*. Our results indicate a high diverse parasite fauna with 23 species. The species richness remains constant throughout the lake with 16–17 species per site, but temporal and spatial differences were observed in the species composition. Also, the overall prevalence showed spatial variation between two of three sites and temporal variation only was observed at one site.

The larval digenean assemblage of *H. parchappii* has been studied in other shallow lakes, urban and peri-urban streams in Argentina, and 12–13 digenean species were found. The low specific richness found in those environments was mainly associated with heavily human-impact ecosystems due to recreation and agriculture (Merlo *et al.*, 2019; Parietti *et al.*, 2020). The shallow lake studied in this work is a very unstable environment, but with a lower human impact over it. A native *Celtis tala* forest is located on its southeast coast, and a little area of the southwest coast is used for small-scale cattle grazing. Site A is the site with the most human impact because it is close to the house and stable of a small family and a couple of workers that live there. Also, this lake is located in the vicinity of Mar Chiquita Coastal Lagoon Basin, a biosphere reserve under the UNESCO Man and Biosphere Program since 1996. The low human disturbance in this environment may explain the larger diversity registered in this lake, in contrast with the other environments already studied in the region (Merlo *et al.*, 2019; Parietti *et al.*, 2020).

Similar parasite diversity was registered in other molluscs species of the same genus, such as 22 species in *Heleobia conexa* in Mar Chiquita lagoon (Etchegoin, 1997; Merlo and Etchegoin, 2011) and 15 species (Alda and Martorelli, 2014) and 22 species (Parietti *et al.*, 2013) in *Heleobia australis* from estuarial habitats. Both the Nahuel Rucá lake, the Mar Chiquita lagoon and the estuarial habitats studied by these authors are sites extremely connected with the marine environment, wetter, greener and more productive, which leads to the great biodiversity of possible definitive hosts and consequently a high diversity of parasites.

All digenea life cycle types are present in the digenean assemblage studied (three-host life cycle, two-host life cycle and monoxene life cycle). Thirteen species presented a typical three-host cycle (57%), 8 out of 23 species (35%) a two-host life cycle and even a monoxene life cycle was registered in Nahuel Rucá Lake.

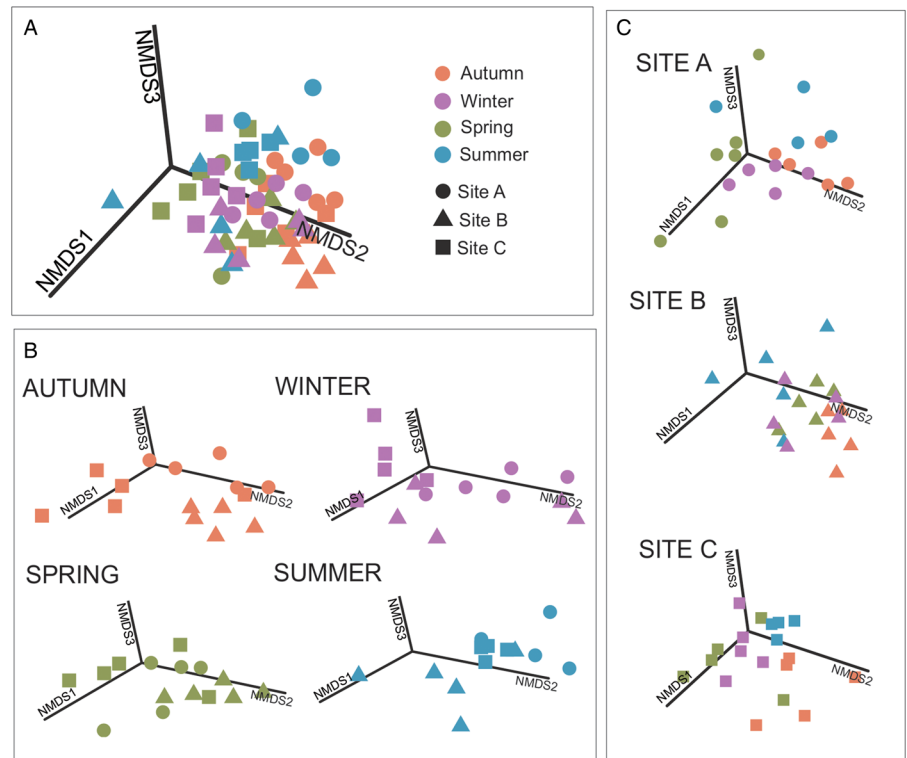


Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plot based on Bray-Curtis similarity on parasite prevalence. (A) By seasons and sites. (B) By season. (C) By sites.

The two-host life cycles are not very frequent in marine trematodes given that extra intermediate hosts enhance the dispersion of the larval stages and, consequently, the number of possible definitive hosts (Galaktionov and Dobrovolskij, 2003), but represented an important proportion in this assemblage in *H. parchappii*.

Particularly, three of the species identified in this work are well adapted to varied environmental conditions. Notocotylidae sp. 1 (Notocotylidae), *M. simillimus* (Microphallidae) and *Levinseniella cruzi* (Microphallidae) were registered in all seasons and all sites, the two first have a two-host life cycle, and the third a typically three-host life cycle. Also, these three species were recorded in the same host in other freshwater ecosystems (Martorelli, 1986a, 1986b, 1986c; Merlo et al., 2019; Parietti et al., 2020), in other molluscs species of the same genus, as *H. conxa* in Mar Chiquita lagoon (Martorelli, 1986a, 1986b, 1986c; Etchegoin and Martorelli, 1998, Merlo and Etchegoin, 2011) and *H. australis* in estuarial habitats (Alda and Martorelli, 2014; Parietti et al., 2013), and even in other mollusc genus, as *B. peregrina* in a temporary pond near to Mar Chiquita lagoon (Parietti et al., 2021). In Notocotylidae sp. 1 (Notocotylidae) the cercariae are expelled from the snail host and encyst upon aquatic plants, shells and other objects, then definitive hosts can get infected by feeding on plants that carry the metacercariae (Galaktionov and Dobrovolskij, 2003). The life cycle characteristics of this species could facilitate habitat use with different abiotic conditions and it presented probably low host-specificity (Parietti et al., 2021). *Levinseniella cruzi* and *M. simillimus* belong to the Microphallidae family, a trematode group originally from coastal environments. However, a successive transitional series from a typical (marine) three-host life cycle to a two-host one (freshwater) can be observed when the mollusc acts as the first and second intermediate host (Deblock, 1977).

Prevalence and species composition in the lake showed temporal-spatial dynamics similar to that found in the assemblage that parasitizes *H. australis* on a small scale in Mar Chiquita coastal lagoon (Parietti et al., 2013). This variability could be

the result of the habitat used by the definitive host, mainly birds. More than half of the digenean species registered used birds as definitive hosts in Nahuel Rucá. Several bird species are registered in the literature as definitive hosts of the digenean species registered in this lake (see Table 1). Also, an important increase of migratory birds can be observed during spring and summer in Mar Chiquita coastal lagoon (Ferrero, 2001) and due to the proximity between the lake and the lagoon, migratory birds, together with local breeding species of shorebirds, use the lake as a resting and feeding area, contributing to a temporal increase in the influx of digenean-infective stages, similar to what was observed in a pond also close to the Mar Chiquita lagoon (Parietti et al., 2021). In particular, birds used the reed beds in spring as nesting areas near site C, which is coincident with the higher overall prevalence values registered in this site. Lately, in summer, birds moved south into the lake to feed and perching near site A, coincident with the rise in overall prevalence there (Josens et al., 2009). For their part, fishes, being the 18.8% of digenean hosts, could be homogenizing the internal variances in the lake. Species from Heterophyidae and Echinostomatidae family used small fishes as the second intermediate host, with *Cnesterodon decemmaculatus* and *Jenynsia lineata* cited in the literature (Rauque et al., 2018; Taglioretti et al., 2018; among others and see Table 1). Also, species from Acanthostomidae, Haploporidae and Hemiuridae have fishes as the definitive host, with *Astyanax fasciatus*, *Pimelodus* sp., *Astyanax* sp., *Cynopotamus* sp., *Moenklausia doceana* and *Roeboides* sp. cited in the literature (Ostrowsky de Nuñez et al., 2017 and see Table 1). All these fish species are registered in Nahuel Rucá shallow lake, in addition to *Rhamdia quelen*, species without parasitological studies so far.

It is interesting to mention that one digenean species registered in this lake completed its life cycle in reptiles, as the Ochetsomatidae family (*Xiphidiocercaria* sp. 2), and three others in reptiles and other vertebrate groups, as the Acanthostomidae family (*Cercaria Acanthostomidae* sp. 1) and the Plagiorchiidae family (*Xiphidiocercaria* sp. 3 and *Xiphidiocercaria* sp. 4). All four species were presented mainly in spring and summer.

Cercaria Acanthostomidae sp. 1 is present in all three sites because, probably, used also fishes as the definitive host, and, as we mentioned, fishes unify the presence and abundance of the digenean in the lake. Site B is the only one with the presence of the four digenean species that used reptiles as definitive hosts. This place is located 200 m from the *C. tala* forest, a native American forest, on its southern edge of distribution. Reptiles probably used the *C. tala* forest as a refuge. Non-native plant species might generate habitat modifications that increase the predation pressure for reptiles (more prey visibilities, fewer refuges, more vantage points to potential predators). Contrary, predation risk is lower in native forests in lizards from avian predators (Stellatelli *et al.*, 2015). Two freshwater turtle species were registered associated with this forest in the region, *Hydromedusa tectifera* and *Phrynops hilarii*, and a brown lizard species, *Tupinambis merianae* (Haene, 2006). The proximity of the forest to the body of water in B could favour the contact between definitive and intermediate hosts allowing the digenean species to complete its life cycle. There is no record of reptile parasites in the bibliography for the area. Trematodes may be usefully employed as ecological indicators even in little studied environments since the taxonomic identification to family level is usually sufficient to identify both types of second intermediate and definitive hosts (Huspeni *et al.*, 2005; Hechinger *et al.*, 2007; Duan *et al.*, 2021). Consequently, these digenean families registered in Nahuel Rucá may indirectly indicate the presence of reptiles using the lake. Taking into account that an important part of the reptile species is at conservation risk (Prado *et al.*, 2012), our work provides an idea of the possible hosts that may habitat in the area.

Another aspect to highlight is the register of the *Cercaria* Lecithodendriidae sp. 1 that belongs to a Lecithodendriidae family that was rarely mentioned in Argentina and are parasites of bats and occasionally of birds (Milano, 2016). Adults of Lecithodendriidae were identified in bats in the Pampean province (Lunaschi, 2002) and dragon-fly naiads (Arthropoda: Odonata) are mentioned as the second intermediate host (Hall, 1958). It is estimated that 8 bat species present a distribution that overlaps with the study region but only 2 species were observed in the study area (Barquez *et al.*, 2020). In Argentina, 67 species of bats have been registered and bats are particularly susceptible to anthropogenic changes because of their low reproductive rate, longevity and high metabolic rates, and out of 1001 species of bats, almost a quarter are globally threatened and listed by the IUCN (Voigt and Kingston, 2016). Given the few records of bats in Nahuel Rucá lake, and that this digenean species has not been found in other water bodies of the region, we suggest an interesting possible host-parasite association to be considered and developed in future studies. However, further studies are needed to determine whether this species could use other definitive hosts.

Multi-host parasite abundance can act as a surrogate for trophic complexity in structured habitats for the presence of their hosts, as is the case of digenean species. Their life cycle incorporates multiple invertebrate and vertebrate taxa, mainly due to the trophic transmission to the vertebrate definitive host, although there are many transmission variants. Except for the direct infection of definitive hosts by the cercariae (e.g. in bird schistosomes) or the monoxene life-cycles [e.g. *G. genarchella* (Hemiuridae)], all transmission strategies registered in this assemblage in Nahuel Rucá (three and two-host cycles) involve the trophic transmission to the definitive host, and thus provide information on trophic interactions and energy flow within the ecosystem. Also, the large number of transmission events from aquatic organisms to birds indicates a substantial energy flow from freshwater to terrestrial systems *via* these predation events. Parasites are usually not directly visible in an ecosystem, while

their hosts are considered naturally regarded as the biome that inhabits an ecosystem. For this reason, parasites have traditionally been omitted from the majority of ecological studies (Poulin, 2007). As mentioned previously, parasites are deeply embedded in larger food webs and active elements of the ecological processes that shape and structure ecological communities, energy flow and the biodiversity of complex ecosystems (Lafferty *et al.*, 2008; Sato *et al.*, 2011; Dunne *et al.*, 2013; among others). This local trematode diversity could reveal information on definitive host occurrence, host's habitat use and trophic interactions within ecosystems, and is also an excellent ecosystem health indicator (Huspeni *et al.*, 2005; Hechinger *et al.*, 2007). Conservation politics for parasites and their hosts are extremely necessary for this environment, due to its pristine characteristics and its closeness to a UNESCO reserve.

A recent legal ordinance enables the construction of an Estate project in the middle of the Mar Chiquita natural reserve that, according to experts and environmentalists, would generate very serious ecological consequences, such as a possible 'increased risk of flooding due to disturbance of wetlands', the 'elimination, reduction and fragmentation of the Pampean grassland', as well as an 'alteration of the quality and quantity of water, both underground and surface'. This could have a serious impact on the biodiversity of the Mar Chiquita lagoon, and due to its proximity, over the Nahuel Rucá lake diversity. Our work supports the idea that this area is of great importance in biodiversity, and could be endemic areas of some species of digenean that use reptiles, amphibians and bats as hosts, groups that are at risk of conservation. The protection of these environments, and even the expansion of their geographic range, are fundamental pillars in the policies for the conservation of wild flora and fauna.

Author contributions.

All authors equally contributed to the work of conceiving and designing the study, conducting data gathering, performing statistical analyses, and writing the article.

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