

**SPATIAL DISTRIBUTION AND MICROHABITAT SELECTION OF COPEPODS
(COPEPODA, ERGASILIDAE), GILL PARASITES OF *MUGIL PLATANUS*
(PISCES, MUGILIDAE) FROM LAGUNA DE ROCHA, URUGUAY**

Gabriela Failla Siquier

Laboratorio de Zoología de Invertebrados, Departamento de Biología Animal, Facultad de Ciencias, Iguá 4225 Montevideo 11400, Uruguay. gfailla@fcien.edu.uy

ABSTRACT

Mulletts inhabit tropical and warm-temperate estuaries where they play an important ecological role and commonly support artisanal fisheries. This study involved 123 specimens of mullet, *Mugil platanus*, captured in the estuarine lagoon, Laguna de Rocha (Dpto. de Rocha), Uruguay. Three species of ergasilid copepods, *Ergasilus versicolor*, *Ergasilus lizae* and *Therodamas* sp., have been found parasitizing the branchial arches on *M. platanus*. A total of 1787 ectoparasites were removed from the gill filaments and in *E. versicolor* a highly significant preference for the internal hemibranch was observed. Prevalence of infection was high in *Ergasilus* species and very low in *Therodamas* sp. No preference for the right or left side of the fish host was observed. Microhabitat selection for each species was as follows: *E. lizae* on gill rakers of arches 3 and 4; *E. versicolor* and *Therodamas* sp. on distal end of gill filaments of arches 1 and 2, both with different attachment strategies. The data showed a distinct distribution pattern for each parasite with no overlapping and corroborated the pathway of entry of parasites to the host against the water respiratory current. Also a new locality for these parasites is reported.

Key words: *Ergasilus*, *Therodamas*, branchial arches, Laguna de Rocha.

RESUMEN

Distribución espacial y selección del microhábitat de copépodos (Copepoda, Ergasilidae), parásitos branquiales de la "lisa" *Mugil platanus* (Pisces, Mugilidae) de la Laguna de Rocha, Uruguay. Las lisas habitan estuarios tropicales y templados donde juegan un rol ecológico importante y comúnmente están sujetas a la pesca artesanal. Este estudio involucra 123 especímenes de lisas, *Mugil platanus*, capturadas en la Laguna de Rocha, Uruguay. Tres especies de copépodos ergasilidos fueron hallados parasitando los arcos branquiales de *M. platanus*: *Ergasilus versicolor*, *Ergasilus lizae* y *Therodamas* sp. Se retiraron 1787 ectoparásitos de los filamentos branquiales, observándose en *E. versicolor* una preferencia altamente significativa por la hemibranchia interna. La prevalencia fue alta en las especies de *Ergasilus* y muy baja en *Therodamas* sp. No se observó preferencia por el lado derecho o izquierdo del hospedador. La elección del microhábitat de cada especie fue la siguiente: *E. lizae* en las branquiespinas de los arcos 3 y 4; *E. versicolor* y *Therodamas* sp. en el extremo distal de los filamentos branquiales de los arcos 1 y 2, ambos con estrategias de fijación diferentes. Los datos mostraron un patrón de distribución distinto sin superposición y corrobora la vía de entrada de los parásitos en contra de la corriente respiratoria. Se reporta además, una nueva localidad para dichos parásitos.

Palabras clave: *Ergasilus*, *Therodamas*, arcos branquiales, Laguna de Rocha.

INTRODUCTION

Mugilids are pelagic, coastal fishes, occurring in estuaries (even in freshwater) and are of economic importance worldwide. They have been captured in almost all continents between 42°N and 42°S (Rossi *et al.*, 1998). The distribution of the commonly called "lisa" (*Mugil platanus* Günther, 1880) is mainly restricted to coastal estuarine waters of subtropical regions, along the southern coast of Brazil and north of Argentina (Cousseau *et al.*, 2005). Although this species always spawn at sea, these organisms are highly euryhaline and eurythermic (Menezes & Figueiredo, 1985).

According to Rohde (1993), gill ectoparasites exhibit a marked preference for certain host microhabitats within the branquial cavity (e.g. left or right side, gill arches, external or internal gill filaments). Preferences in site selection may vary according to several conditioning variables. Some differences were found depending on the water currents over the four gills, the available area of arches for attachment, fish traits such as immunity, seasonality or geographic distribution; and abiotic environmental conditions such as salinity and temperature (Gutiérrez & Martorelli, 1994; Iannacome & Alvarino, 2009; Alarcós & Etchegoin, 2010). Although the spatial distribution and interactions of gill ectoparasites of fishes has been investigated by several authors (Geets *et al.*, 1997; Gutiérrez & Martorelli, 1999a; Matejusova *et al.*, 2002; Baker *et al.*, 2005; Soyly *et al.*, 2010), most of the studies have been focused on niche preferences and the co-infection of monogeneans. At the present day, there are relatively few published records of teleost fishes specifying the pathway of parasites to the host and its subsequent election and permanence in the infection site (Ben Hassine & Raibaut, 1981; El Hafidi *et al.*, 1998). Also, studies focused on the specific site of infection of branchial ectoparasitic copepods in *M. platanus* are scarce (Ranzani-Paiva & Silva-Souza, 2004).

The present study was conducted to identify the species of copepods infecting the branchial arches of *Mugil platanus*, and to determine both the precise site of infection and the possible manner of penetration of these parasite species.

MATERIAL AND METHODS

A total of 123 individuals of *M. platanus* were collected by fishermen, in the estuarine lagoon Laguna de Rocha (34°33'– 34°41'S and 54°02'– 54°22'W), Rocha, Uruguay, from December 1993 to December 1994. For collection, a net with a mesh size of 5 – 7 cm was used. Fish were dissected in the field and gills arches were immediately removed. Arches were numbered 1 to 4 in an anterior-posterior direction; both hemibranches of each gill were designated external and internal. In order to better understand the microhabitat preferences, each arch was divided into 4 sectors (I – IV in a dorso-ventral direction, Fig. 1). All pieces were fixed separately in 4% formalin.

Each sector was meticulously examined under a stereoscopic microscope for determining the spatial distribution of parasites. For that purpose, the exact position and number of parasites was recorded. Once removed, parasites were fixed in 70° ethanol for a posterior systematic identification (Boxshall & Montú, 1997; El-Rashidy & Boxshall, 2001). Prevalence was used to

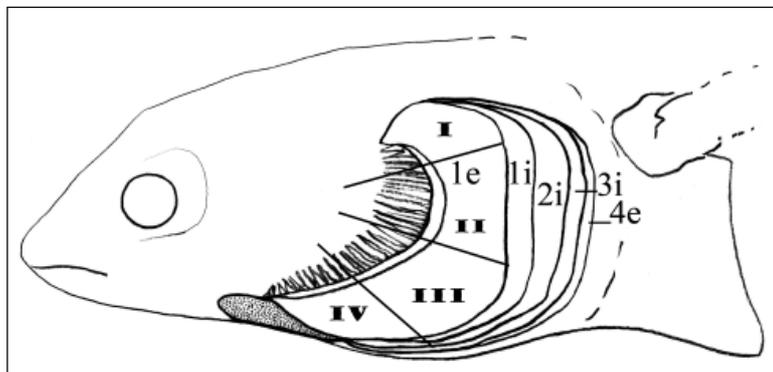


Fig. 1. Diagram showing hemibranches position seen in *Mugil platanus* beneath the operculum (operculum removed). Gill sectors: I-IV; branchial arches: 1-4, e: external hemibranch, i: internal hemibranch.

determine core ($P > 70\%$), secondary ($70\% > P > 5\%$) and satellite ($P < 5\%$) species (Hansky, 1982). Parasitological indexes were used according to Bush *et al.*, (1997): prevalence is defined as the number of individuals of a host species infected with a particular parasite species \div number of hosts examined, mean intensity is the total number of individuals of a particular parasite species in a sample of a host species \div number of infected individuals of the host species in the sample, and mean abundance is the total number of individuals of a particular parasite species in a sample of hosts \div total number of hosts species (infected+uninfected). Relative dominance was calculated as the number of individuals of a parasite species \div the total number of parasites.

The distribution of the parasites was analyzed by nonparametric statistical tests. The Chi square test was used for testing differences between the number of parasites on the left or right side of branchial arches, external or internal hemibranches (of all arches) and site along the branchial arch (hemibranch or gill rakers). To determine the parasite infrapopulation differences between gill arches and sectors, a Kruskal-Wallis ANOVA Test, followed by Mann-Whitney U-Test (with Bonferroni correction), and Friedman ANOVA test (followed by Wilcoxon's matched pair test), were performed respectively. Significance was established at $p < 0.05$, using the statistical software Past v. 2.16 (Hammer *et al.*, 2001) and Statistica 7.0.

RESULTS

Three species of copepods belonging to the family Ergasilidae have been recorded: *Ergasilus versicolor* Wilson, 1911; *Ergasilus lizae* Krøyer, 1863 and *Therodamas* sp. Krøyer, 1864. Only adult females of copepods belonging to this family are parasitic.

Ninety six out of 123 fish collected were found to be infected with 1787 parasites, reaching an overall prevalence of 78%. Mean intensity and mean abundance of infection was detected as 18.6 ± 23.0 and 14.5 ± 21.8 , respectively. The distribution of copepods was considered as contagious after calculation of: $S^2/x = 32.59$.

While none of the parasites exceeded a prevalence of 70%, *E. versicolor* could be considered as core species showing a 67% prevalence (and 0.7 relative dominance), *E. lizae* fit as a secondary species with a 58 % prevalence (and 0.3 relative dominance). *Therodamas* sp. showed a 9% prevalence and was also considered as a secondary species (although narrowly exceeds the 5% prevalence and its relative dominance is almost zero). Both prevalence and mean abundance of co-infections were lower than those of each parasite itself. Mean intensity of infection was higher in those individuals parasitized with two or three species of ergasilids. A summary of the parasitological descriptors for these species is presented in Table 1.

Site of infection per species

In this study there were no significant differences in infection between both sides of the fish, however differences in spatial distribution along the hemibranches were found to be significant (Table 2).

Table 1. Prevalence (P as porcentaje), intensity (I), mean intensity (MI), standard deviation (SD) (number of infected fish indicated between parentheses), mean abundance (MA), relative dominance (RD) and co-infection by copepods parasitizing *Mugil platanus*, (n=123). *Ev*: *Ergasilus versicolor*, *El*: *Ergasilus lizae*, *Therod*: *Therodamas* sp.

Parasites	P (%)	I	MI ± SD	MA ± SD	RD
<i>E. versicolor</i>	67	1-108	14.9 ± 21.0 (83)	10.1 ± 18.6	0.69
<i>E. lizae</i>	58	1-35	7.3 ± 7.5 (71)	4.2 ± 6.8	0.29
<i>Therodamas</i> sp.	9	1-15	2.7 ± 4.1 (11)	0.2 ± 1.4	0.02
<i>Ev + El</i>	32	2-84	22.4 ± 19.5 (39)	7.1 ± 15.1	0.98
<i>Ev + Therod</i>	9	2-109	27.3 ± 38.2 (11)	2.4 ± 13.4	0.71
<i>El + Therod</i>	8	2-28	9.4 ± 8.6 (8)	0.6 ± 3.1	0.31
<i>Ev + El + Therod</i>	8	3-111	31.3 ± 35.5 (8)	2.0 ± 11.5	1

Table 2. χ^2 test results of comparisons of parasite frequencies in the branchial cavity of *Mugil platanus*.

Parasite	left vs. right side	external vs. internal hemibranches	hemibranches vs. gill rakers
<i>E. versicolor</i>	$\chi^2 = 0.007$, df=1 p < 0.93	$\chi^2 = 422.32$, df=1 p < 0.00	$\chi^2 = 12.80$, df = 1 p < 0.00
<i>E. lizae</i>	$\chi^2 = 0.93$, df = 1 p < 0.33	$\chi^2 = 12.07$, df = 1 p < 0.00	$\chi^2 = 14.63$, df = 1 p < 0.00
<i>Therodamas</i> sp.	$\chi^2 = 3.33$, df = 1 p < 0.07	$\chi^2 = 1.68$, df = 1 p < 0.19	*

* no infection recorded in gill rakers.

Table 3. *Mugil platanus* (N=83) infected by *Ergasilus versicolor*. Number of parasites (n) and mean intensity (MI) of copepods by sectors of each branchial arch.

Arch		1	2	3	4	n/Sector	Arch		1	2	3	4	MI/Sector
Sector	I	104	105	42	10	261	Sector	I	2.4	2.8	2.0	1.4	2.4
	II	129	236	65	14	444		II	3.1	4.4	2.2	1.3	3.3
	III	165	178	54	27	424		III	3.4	4.2	2.1	2.1	3.3
	IV	40	40	13	17	110		IV	2.2	2.0	1.9	1.9	2.0
n/Arch		438	559	174	68	1239	MI/Arch		2.9	3.7	2.1	1.7	14.9

Table 4. Wilcoxon's matched pairs results corresponding to the distribution of *E. versicolor*, comparing between sectors for each arch.

<i>Ergasilus versicolor</i> p-value	Sector I-II	Sector I-III	Sector I-IV	Sector II-III	Sector II-IV	Sector III-IV
Arch 1	0.40	0.01	0.00	0.27	0.00	0.00
Arch 2	0.00	0.03	0.01	0.04	0.00	0.00
Arch 3	0.02	0.72	0.00	0.13	0.00	0.00
Arch 4	0.34	0.06	0.24	0.51	0.93	0.31

1. *Ergasilus versicolor*

The copepod *E. versicolor* showed highly significant differences both in the preference of arches (Kruskal-Wallis ANOVA, $H = 78.83$, $p = 7.098E-16$, $n = 1239$) and in the gill sector (Friedman test, $\chi^2 = 88.22$, $p < 0.0000$). The total number and mean intensity per arch and sector of gill parasites in 83 specimens of infected *Mugil platanus* are summarized in Table 3. The analysis of individuals' distribution between pairing sectors in each branchial arch is given in Table 4.

Branchial arch 1: highly significant (Friedman test = 34.61, $p < 0.000$), recorded between the pairs: I - III; I - IV; II - IV and III - IV.

Branchial arch 2: highly significant (Friedman test = 41.82, $p < 0.000$), as well as all pairs of sectors analyzed.

Branchial arch 3: highly significant (Friedman test = 28.57, $p < 0.000$), showing significant differences between pairs of sectors I-II I-IV, II-IV and III-IV.

Branchial arch 4: the distribution of *E. versicolor* in the four sectors of this arch were not significant (Friedman test = 4.20, $p < 0.24$)

2. *Ergasilus lizae*

No significant differences were found in the preference of infection between the internal or external side of gill rakers ($\chi^2=2.382$, $df=1$, $p < 0.122$). Only 11% of individuals ($n = 511$) were

found attached on the basal portion of gill filaments.

The analysis showed that this parasite presents highly significant differences in the preference of gill arches (Kruskal-Wallis ANOVA $H = 43.29$ $p = 1.558E-08$), due to the following pairs of arches: 1-3; 1-4; 2-3 and 2-4. When comparisons among gill sectors were made, significant differences were observed due to the pair I-III (Friedman test, $\chi^2 = 9.17$, $p < 0.03$). The total number and mean intensity per arch and sector of gill parasites in 71 specimens of *Mugil platanus* infected are summarized in Table 5. Comparisons of parasite distribution among sectors of each branchial arch are showed in Table 6.

Branchial arch 1: no significant differences have been recorded (Friedman test $\chi^2 = 3.02$, $p < 0.39$).

Branchial arch 2: the distribution of *Ergasilus lizae* was highly significant (Friedman test, $\chi^2 = 17.48$, $p < 0.0005$), given by the pairs of sectors I-III I-IV, II-III and II-IV.

Branchial arch 3: no significant differences have been recorded (Friedman test $\chi^2 = 1.48$, $p < 0.69$).

Branchial arch 4: there were significant differences between the following pairs of sectors analyzed: I-III; II-IV and III-IV.

When considering the distribution in each gill arch, sector I and II of arch 2 were significantly preferred; on arch 4, the most highly parasitized, the preferred site of infection were sectors I and IV.

Table 5. *Mugil platanus* (N=71) infected with *Ergasilus lizae*. Number of parasites (n) and mean intensity (MI) of copepods by sectors of each branchial arch.

Arch	1	2	3	4	n/Sector	Arch	1	2	3	4	MI/Sector		
Sector	I	17	24	50	73	164	Sector	I	1.5	1.6	1.4	2.4	1.7
	II	8	18	49	48	123		II	1.0	1.3	2.0	2.3	1.8
	III	5	4	56	38	103		III	1.0	1.3	2.2	1.8	1.9
	IV	10	3	43	72	128		IV	1.3	1.0	2.0	2.9	2.2
n/Arch		40	49	198	231	518	MI/Arch		1.3	1.4	2.0	2.4	7.3

Table 6. Wilcoxon's matched pairs results corresponding to the distribution of *E. lizae*, comparing between sectors for each arch.

<i>Ergasilus lizae</i> p-value	Sector I-II	Sector I-III	Sector I-IV	Sector II-III	Sector II-IV	Sector III-IV
Arch 1	0.11	0.05	0.39	0.42	0.67	0.28
Arch 2	0.60	0.01	0.01	0.02	0.01	0.75
Arch 3	0.99	0.57	0.62	0.73	0.50	0.33
Arch 4	0.18	0.04	0.66	0.48	0.04	0.02

3. *Therodamas* sp.

While the distribution across all branchial arches showed no significant differences (Kruskal-Wallis ANOVA, $H=0.62$, $p=0.92$, $n=30$), the site of infection corresponding to sector IV showed significant differences (Friedman test, $\chi^2 = 8.10$, $p<0.040$).

The statistical analyses of the distribution of *Therodamas* sp. on each arch were only performed in arches 1 and 2 owing to the low number of registered individuals. No statistical differences were found among sites: Friedman test, $\chi^2 = 0.333$, $p=0.95$; Friedman test, $\chi^2 = 5.40$; $p=0.14$, for arch 1 and 2 respectively. Both total number and mean intensity of *Therodamas* sp. were much lower than the rest of the copepods (Table 7).

Table 7. *Mugil platanus* (N=11) infected with *Therodamas* sp. Number of parasites (n) and mean intensity (MI) of copepods by sectors of each branchial arch.

Arch		1	2	3	4	n/Sector	Arch		1	2	3	4	MI/Sector
Sector	I	3	1	0	2	6	Sector	I	1.5	1	0	1	1.2
	II	2	4	4	1	11		II	1	1	1	1	1
	III	5	2	2	1	10		III	5	2	1	1	2
	IV	2	1	0	0	3		IV	1	1	0	0	1
n/Arch		12	8	6	4	30	MI/Arch		1.7	1.1	1	1	2.7

All species of parasites were found deeply embedded in the distal end of gill filaments, therefore only the posterior end of the cephalosoma and its long ovisacs were visible. Copepods have been observed on both external and internal hemibranches of all branchial arches, decreasing in number in an antero-posterior direction.

DISCUSSION AND CONCLUSIONS

Previous studies, addressing quantitative information and infestation dynamics of copepods on gill arches filaments are scarce (Ben Hassine & Raibaut, 1981). Experimental work related with the speed of water flow through the gill cavity of teleost fishes, showed that in arches 1 and 4 the stream flow is minimal and is highest in arches 2 and 3 (Paling, 1968; Gutiérrez & Martorelli, 1999a). These measurements suggest the regions with lower and higher dissolved oxygen, where the higher speed of water flow increases oxygen. Furthermore, arches 2 and 3 correspond to the most turbulent regions, where parasites must use their attachment strategies to avoid being dragged away (Gutiérrez & Martorelli, 1999b).

In the present study no preference for lateral side (left or right) of the host was recorded. However, the distribution of *E. versicolor*, *E. lizae* and *Therodamas* sp., found in the branchial cavity of *M. platanus* showed unique patterns of infection preference across gill arches and filaments:

1. *Ergasilus lizae*

According to experimental studies performed by Ben Hassine & Raibaut (1981) in *Liza aurata*, the parasite *E. lizae* would enter the branchial cavity through the operculum of the fish against the stream, entering the gill. After its fixation on the external hemibranch by the antennae, the parasite would move along the gill filament from the distal end to the basal region and finally reach the gill rakers. The site of infection recorded for *E. lizae* on *M. platanus* agreed with these observations (gill rakers of arches 3 and 4) and a marked preference for sectors I and IV of arch 4. One possible mechanism could be that copepods enter the fish through the dorsal and ventral sides of the operculum, given that the arch 4 could allow a better access due to the reduced flow of water and then migrate along the gill filament to the proximal region of the hemibranch. Once parasites reach the gill rakers, they could either stay on that site of infection or migrate to the gill rakers of arch 3. Because the water flow in arches 2 and 3 is maximal, both arches may present some difficulties for parasites to entering and reaching gill rakers.

The entering through the operculum and migration of the parasite along the gill filaments, and the fact that only 56 out of 511 (11%) of the copepods were found near the base of the branchial filaments (none at the distal end), were quite remarkable with this topic and are concordant with observations of the authors mentioned above (Ben Hassine & Raibaut, 1981). The present study revealed that *E. lizae* is mainly distributed in the gill rakers of the posterior arches (3 and 4), mainly in sectors I and IV of arch 4. These last were the sites of infection with the highest number of parasites.

2. *Ergasilus versicolor*

This copepod was considered as the core species. Its abundance was significantly higher compared with *E. lizae* and *Therodamas* sp., and also showed the highest prevalence and relative dominance among copepods. The pattern of spatial preference was on gill arches 1 and 2, with higher abundance in the second arch, abruptly decreasing in arches 3 and 4. *E. versicolor* individuals were found strongly attached by the antennae, in a parallel position with respect to gill filaments, and the cephalosoma directed towards the base of the gill arch. This orientation was coincident with that found by Fernández-Bargiela (1987), though she did not mention the distribution pattern between arches. The pathway by which copepods enter the host has not been observed. However, assuming that *E. versicolor* uses the same route as *E. lizae*, this consideration would be consistent with the site of infection observed in this study: the distal end of the internal hemibranches on sectors II and III of arches 1-2. This pattern may indicate that parasites could struggle their way against the flow and quickly attach themselves to the longest gill filaments available (in *M. platanus* corresponding to the internal hemibranch of the anterior arches, see Fig.1) just below the operculum. The observed preference for the most turbulent sectors (II and III) by *E. versicolor* could be explained by the parasites firmly attaching themselves to the gill filaments with their second pair of antennae to prevent them from being washed away. This property allows to this species to occupy the largest, most oxygenated and mucus-rich areas. According to this study, *E. versicolor* is mainly distributed in the distal end of the internal hemibranch, on the central sector of gill arches 1 and 2. It is noteworthy that, taking into account all sectors of arch 4, both abundance and mean intensity of parasites remain very low compared to the anterior gill arches.

3. *Therodamas* sp.

This copepod was recorded as a secondary species (prevalence 9%) and was found at the same site of infection as *E. versicolor*. Nevertheless, there were not observed significant arch preferences, though this fact should be taken with caution due to the low relative dominance in the sample. A decreasing trend in an antero-posterior direction was also observed. It's remarkably noticed that none of the fishes presented monoinfection by this parasite. On individuals infected by both *E. versicolor* and *Therodamas* sp., there was almost no coinfection in the same arch, and when it occurred, they were not observed in the same sector. Moreover, in fishes heavily parasitized by *E. versicolor*, *Therodamas* sp. was found in the less infected sectors and viceversa. There are no records for this species regarding the pathway of entry into the host. As Fernando & Hanek (1976) pointed out in *Lernaeocera* sp. Blainville, 1822, a closely related copepod species, the parasite also enters the fish against the water current and remains embedded in the gill filaments. As previously mentioned for *E. versicolor*, the site of infection of *Therodamas* sp. may be given by the longest filaments available for attachment once parasites have entered into the branchial cavity against the water flow. Parasite attachment strategies were quite different. For example, *Therodamas* sp. was found embedded in the distal end of gill filaments, while *E. versicolor* and *E. lizae* were observed embracing gill filaments and rakers respectively by means of the well developed antennae. On the other hand, whereas *Therodamas* sp. remains in place, *E. versicolor* can crawl to another site of infection, thus avoiding overlapping.

All parasites were fecundated females, showing a clear spatial segregation in relation to their microhabitats and, as almost in all species of branchial ectoparasites, no preference for the right or left side of the host was registered. There was also no observed overlapping among species in the use of the microhabitats, even under extreme conditions as high intensity of co-infection. The site of infection of the copepods was consistent with the pathway of entry to the host being through the gill operculum, against the water flow and towards the available hemibranchial area for infection (Ben Hassine & Raibaut, 1981). As gill sectors did not have exactly the same size, differences in the distribution of parasites within each arch may be attributed to this cause. However, a trend of occupation of sectors within the arches, and a strong preference for gill arches in both *Ergasilus* species was observed. Thus, *E. versicolor* preferred the central sector of anterior arches (1 and 2) and *E. lizae* the posterior ones (3 and 4), with a marked infection in sectors I and IV of arch 4. A new locality, Laguna Rocha (Uruguay), is reported for the three species of parasites studied.

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