

# Blood protozoans in elasmobranchs of the family Rajidae from Galicia (NW Spain)

W. Aragort<sup>1</sup>, M. F. Alvarez<sup>2</sup>, J. L. Leiro<sup>2</sup>, M. L. Sanmartín<sup>1,2,\*</sup>

<sup>1</sup>Avenida Principal Las Delicias, Unilab, Sanidad Animal, Instituto Nacional de Investigaciones Agrícolas (INIA), Maracay, Estado Aragua, Venezuela

<sup>2</sup>Instituto de Investigación y Análisis Alimentarios (IAAA), Universidad de Santiago de Compostela, C/Constantino Candeira s/n, 15782, Santiago de Compostela, Spain

**ABSTRACT:** Blood smears from 132 skates *Raja* spp. captured on the continental shelf off Galicia (NW Spain) were examined for blood parasites. The skate species were *Raja brachyura* (n = 60), *R. microocellata* (n = 52) and a total of 20 specimens belonging to *R. undulata*, *R. montagui* and another 2 unidentified *Raja* species, all captured between March 1999 and March 2000. Two blood parasite species were found, *Trypanosoma giganteum* and *Haemogregarina delagei*. Of the 132 skates, 16% were infected only by *T. giganteum*, 17% only by *H. delagei*, and 5% by both *T. giganteum* and *H. delagei*. Both parasites showed highest prevalence in *R. brachyura* (22% *T. giganteum* only, 38% *H. delagei* only, 12% *T. giganteum* and *H. delagei*). Mean leucocyte percentages (n = 132 fish) were lymphocytes (43%), eosinophils (35%), neutrophils (20%) and monocytes (2%); basophils were not found. As far as we are aware, this eosinophil percentage is the highest reported to date for elasmobranchs. We did not detect any statistically significant differences in leucocyte percentages between infected and uninfected fish, between male and female fish, among species or among weight groups.

**KEY WORDS:** *Haemogregarina delagei* · *Trypanosoma giganteum* · *Raja* spp. · Blood parasites · Leucocyte counts · Galicia (NW Spain)

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## INTRODUCTION

Relatively little is known about the diseases of elasmobranchs or about possible haematological effects of blood parasites in these fishes. Examination of blood smears allows both detection of blood parasites and characterization of the different types of elasmobranch blood cells, namely erythrocytes, leucocytes and platelets (Kjeldsberg 1994). Leucocytes are conventionally classified on morphological grounds into 5 categories: neutrophils, lymphocytes, monocytes, eosinophils and basophils. Their abundances in blood vary widely, depending on species and physiological condition (Kjeldsberg 1994).

A recent study of a trypanosome-infected population of the red-spotted catshark *Schroederichthys chilensis* (Guichenot, 1848) (infection prevalence 87%) found the highest eosinophil percentage reported to date in fishes (Valenzuela et al. 2003).

In the present study, we examined blood smears from a total of 132 specimens of various *Raja* species

(family Rajidae) captured in the northeast Atlantic off Galicia (NW Spain), with the aim of detecting blood parasites and estimating their prevalences. We also performed leucocyte counts in smears from each fish specimen to assess whether infection by blood parasites affects the composition of the leucocyte population.

## MATERIALS AND METHODS

A total of 132 elasmobranchs were caught between March 1999 and March 2000 on the continental shelf off the estuary of Muros and Noia: 60 *Raja brachyura* Lafont, 1873 (27 males, 23 females, 10 indeterminate); 52 *Raja microocellata* Montagu, 1818 (24 males, 26 females, 2 indeterminate), and a total of 20 specimens belonging to the species *Raja montagui* Fowler, 1910, *Raja undulata* Lacepede, 1802, and 2 unidentified *Raja* species. Since the total of these latter 4 species was only 20 specimens (of which 13 were males,

\*Corresponding author. Email: mpduran@usc.es

5 females and 2 indeterminate), they were considered together as 'other species'. The map coordinates of the capture area were 42° 35' to 42° 41' N, 09° 02' to 09° 10' W; mean capture depth was  $11.64 \pm 4.11$  m. All specimens were captured with local traditional fishing apparatus called 'trasmallos' or 'miños' (Rivas Lago 1996).

Specimens were transferred to the laboratory in refrigerated containers, and analysed immediately: the heart was dissected out and used to obtain 3 blood smears (2 thin smears, and 1 thick smear). The thin smears were air-dried at ambient temperature, then fixed with methanol and stained with Giemsa, May-Grunwald-Giemsa or Wright's stain. The thick smear was viewed fresh. All smears were examined by light microscopy (40 $\times$ ), noting the presence or absence of blood parasites. The trypanosomes found were measured with a calibrated micrometer, following the procedure described by Lom & Dyková (1992).

Prevalence was calculated according to Bush et al. (1997). *Trypanosoma* abundance, being very scarce, is given as the number of specimens per smear in infected hosts. *Haemogregarina* abundance is given as the mean parasite density and calculated by counting the number of erythrocytes per 100 microscope fields in each infected host, taking note of the number of infected erythrocytes in each host. The percentage (= density) of infected erythrocytes was then calculated for each host, from which the mean density was calculated.

Leucocyte counts were obtained by examining 100 leucocytes in a thin smear, and counting the number of lymphocytes, monocytes, eosinophils, neutrophils and basophils. The percentage of each cell type was thus determined for each individual ray. From these data, the mean and standard deviation for each fish species (*Raja montagui*, *R. microocellata*, 'other species') or sex were calculated.

The data thus obtained for each type of leucocyte did not show a normal distribution in most cases, so non-parametric tests were used for data analysis. Means were compared between male and female fish of each species by the Mann-Whitney *U*-test, and among fish species by the Kruskal-Wallis test followed by Dunn's test for one-to-one comparison of means. The Kruskal-Wallis test was also used to compare means among fish infected with none, 1 or both blood parasite species. To investigate possible influences of host age on infection prevalences, we divided the rays into 4 different groups by weight: (1) up to 500 g, (2) 501 to 1000 g, (3) 1001 to 1500 g, and (4) >1500 g, and determined the prevalence of each parasite species in each group; prevalences were compared between groups by Fisher's exact test. All statistical analyses were performed with the aid of GraphPad InStat version 3.05 (GraphPad Software).

Table 1. Comparative measurements ( $\mu$ m) of *Trypanosoma giganteum* and other elasmobranch trypanosomes from previously published papers

	<i>T. giganteum</i> (This paper) <sup>a</sup>	<i>T. giganteum</i> (Neumann 1909)	<i>T. rajae</i> (Kudo 1923)	<i>T. rajae</i> (Laveran & Mesnil 1902)	<i>T. variabile</i> (Neumann 1909)	<i>T. scylli</i> (Laveran & Mesnil 1902)	<i>T. marplatense</i> (Bacigalupo & de la Plaza 1948) <sup>b</sup>	<i>T. gargantua</i> (Laird 1951)
Length <sup>c</sup>	81 $\pm$ 9 (70–90)	100	30–35	55–60	90–100	54–61	31–46 / 60–65	66.7–131.1
Free flagellum length	10 $\pm$ 1 (10–12)	25–30	6–8	20	25–30	14	up to 15	Absent
Maximum width	9 $\pm$ 2 (6–11)	8	–	6	5	5–6	6.1 / 10–12	4.6–13.7
Nucleus length	4 $\pm$ 1 (3–5)	3–5	3	–	3–4	–	3.6 / 7.2	3.9–9.3
Nucleus width	4 $\pm$ 1 (3–5)	2.5–4	2.2	–	3–4	–	– / 4.2	3.3–6.7
Kinetoplast— posterior end	19 $\pm$ 4 (4–25)	10	6	4–10	10–12	–	–	13–25% body length
Kinetoplast— nucleus	39 $\pm$ 5 (30–45)	–	–	–	–	–	–	–

<sup>a</sup>Mean  $\pm$  standard deviation (minimum–maximum); <sup>b</sup>polymorphism: small specimens/large specimens; <sup>c</sup>lengths do not include the free flagellum, except in the case of *T. variabile*

## RESULTS AND DISCUSSION

Two blood parasites were found, *Trypanosoma giganteum* Laveran & Mesnil, 1902 and *Haemogregarina delagei* Laveran & Mesnil, 1901.

The morphology of *Trypanosoma giganteum* coincides approximately with that reported by Neumann (1909) (measurements in Table 1), though with some minor differences, notably an apparently shorter free flagellum ( $10 \pm 1 \mu\text{m}$ ). This may be a preparation artefact, or may reflect the fact that the flagellum was in most cases coiled freely or around an erythrocyte (Fig. 1), making accurate measurement difficult. Also, the kinetoplast-posterior end distance was slightly longer than that given by Neumann (1909).

*Haemogregarina delagei* (Fig. 2) was observed in various stages. Firstly, inside erythrocytes, 2 types of development stages were observed. Gamonts were banana-shaped (Fig. 2A,D), with measurements ( $11 \pm 1 \mu\text{m} \times 2 \pm 1 \mu\text{m}$ ) very similar to those described by Lom & Dyková (1992) ( $11.3 \times 3.7 \mu\text{m}$ ). With Giemsa staining, they showed grey or light blue cytoplasm; the nucleus was stained dark, like that of host erythrocytes, and had a granular appearance. The second less frequent type was oval in shape, and smaller (length  $6 \mu\text{m}$ ) (Fig. 2E). No differences in size were observed between infected and uninfected erythrocytes, and infected erythrocytes contained only a single parasite. Secondly, in plasma, we observed banana-shaped merozoites similar in shape and size to the large gamonts seen in erythrocytes (Fig. 2B,C); and thirdly, in 1 host, groups of 8 to 12 merozoites were observed free in plasma—these were arranged like the rays of a fan and measured  $18 \times 3 \mu\text{m}$  (Fig. 2F). This might be the final phase of merogony freed from ruptured erythrocytes.

Prevalences of the 2 parasite species are summarized in Table 2. About 5% of fish examined were infected by both species. The host with the highest prevalences of both species was *Raja brachyura*, while *Raja microocellata* showed far lower values, in particular for *Haemogregarina delagei*. Infection by both parasite species together was likewise more common in *R. brachyura*. *Trypanosoma giganteum* was very scarce in the infected hosts, with only 0 to 2 specimens per thin blood smear examined. *H. delagei* was slightly

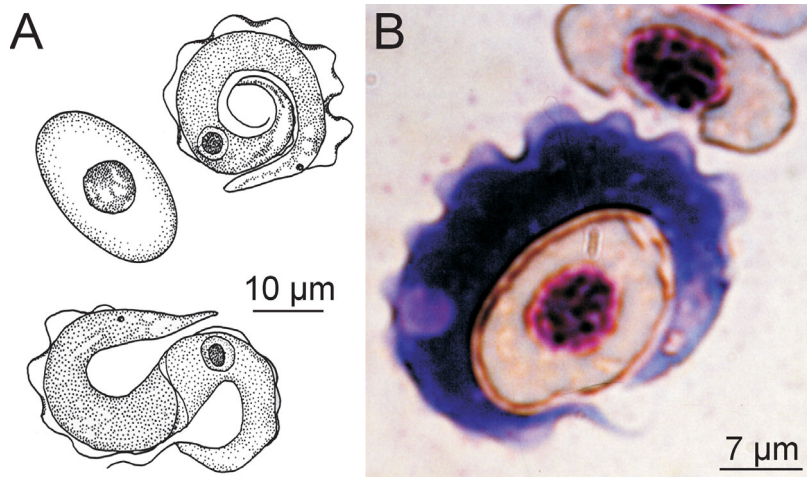


Fig. 1. *Trypanosoma giganteum*. (A) Camera lucida drawing. (B) Giemsa-stained preparation

Table 2. *Trypanosoma giganteum* and *Haemogregarina delagei* from *Raja* spp. Prevalences of the 2 blood parasites in the different *Raja* species. Sum of the single-infection and joint-infection prevalences = total prevalences for each protozoan species

	<i>Raja</i> spp. (%) (n = 132)	<i>R. microocellata</i> (%) (n = 52)	<i>R. brachyura</i> (%) (n = 60)	Other species (%) (n = 20)
<i>Trypanosoma giganteum</i> only	16	13	22	5
<i>Haemogregarina delagei</i> only	17	2	38	0
<i>T. giganteum</i> + <i>H. delagei</i>	5	2	12	0

Table 3. *Trypanosoma giganteum* and *Haemogregarina delagei* from *Raja* spp. Prevalences (%) in fish (all species) grouped by weight. n = number of skates in each weight group

Weight groups (g)	n	<i>T. giganteum</i> (%)	<i>H. delagei</i> (%)
≤500	7	0	0
501 – 1000	49	16.7	12.5
1001 – 1500	57	19.3	21.0
>1500	19	10.5	36.6

more abundant, and was present in 2% of the erythrocytes examined in both *R. brachyura* and *R. microocellata* and also in the 'other species' group.

As shown in Table 3, neither parasite was found in fish weighing less than 500 g, although this might be due to the small number of skates in this group. In the remaining 3 weight groups, no clear trend



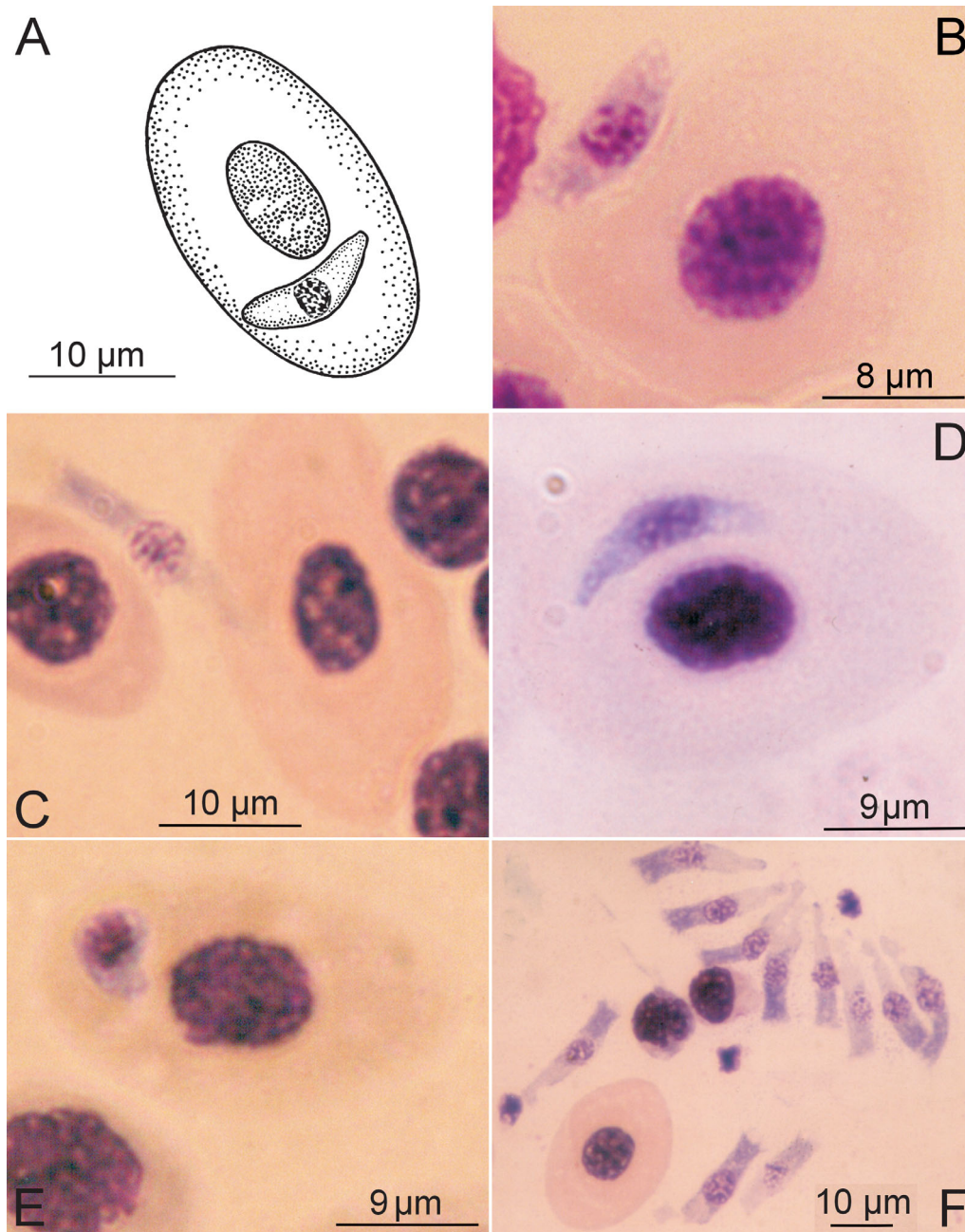


Fig. 2. *Haemogregarina delagei*. (A) Camera lucida drawing of gamont within erythrocyte. (B) Merozoite free in blood. (C) Merozoite penetrating erythrocyte. (D) Banana-shaped gamont within erythrocyte. (E) Small ovaloid specimen within erythrocyte. (F) Final phase of merogony free in plasma

was observed for *Trypanosoma giganteum*, nor were between-group differences statistically significant. Prevalence of *Haemogregarina delagei* increased with weight, but the between-group differences were not statistically significant.

Table 4 shows prevalences in male and female fish. In *Raja microocellata*, both parasite species showed

higher prevalence in males than in females, while in *Raja brachyura* both parasite species showed higher prevalence in females; however, none of these between-sex differences were statistically significant.

Numerous species of the genus *Trypanosoma* have been described from elasmobranchs. *Trypanosoma giganteum* was described by Neumann (1909) in *Raja*

*oxyrhynchus* from European waters. We are not aware of any other reports of this species. Other trypanosome species that have been reported from *Raja* spp. (see Table 1) include *T. rajae* Neumann, 1909 in *R. punctata*, *R. clavata* and *R. radiata* (Lom & Dyková 1992); *T. gargantua* Laird, 1951 in *R. nasuta* from New Zealand (Laird 1951); and *T. variabile* Neumann, 1909

(now considered a synonym of *T. rajae*) in *R. punctata*. The species *T. scylli* and *T. rajae* have been found in the anterior intestine of elasmobranchs (Lom & Dyková 1992). *T. humboldti* Morillas, Nascimento & Khan, 1987, has been reported from the red-spotted catshark *Schroederichthys chilensis* (Guichenot, 1848) by Valenzuela et al. (2003).

Table 4. *Trypanosoma giganteum* and *Haemogregarina delagei* from *Raja* spp. Prevalences of the 2 blood parasites in male and female fish

	<i>T. giganteum</i> (%)		<i>H. delagei</i> (%)	
	Males	Females	Males	Females
<i>R. microocellata</i>	20.8	8.0	4.2	0
<i>R. brachyura</i>	14.8	30.4	40.7	47.8
Other species	7.7	0	0	0

The type species of the genus *Haemogregarina* is *H. stepanowi* Danilewky, 1885, a parasite of *Emys orbicularis* Linnaeus, 1758. Numerous species have been described from reptiles, amphibians and fishes, over a very wide geographic range (Daves & Johnston 2000). These parasites infect both red and white blood cells (Cheung 1992).

*Haemogregarina delagei* is one of a group of species lacking a polar cap (Lom & Dyková 1992); this group also includes *H. platessae* Lebailly, 1904 (gamonts  $7.7 \times 1.4 \mu\text{m}$ ), *H. nothotheniae* Barber, Mills, Westermann & Storoz, 1987 (gamonts 15 to 22  $\mu\text{m}$ ) and *H. myoxocephali* Fantham, Porter & Richardson, 1942 (gamonts  $7.8 \times 2.4 \mu\text{m}$ ). *H. delagei* is common in *Raja erinacea* Mitchell, 1825 (Siddall & Desser 2001) and in *Squalus acanthias* Linnaeus, 1758 (Lom & Dyková 1992). This is the first report of *H. delagei* in *R. microocellata* and *R. brachyura*. Our morphometric data (gamonts  $11 \pm 1 \times 2 \pm 1 \mu\text{m}$ ) coincide with those reported previously by Lom & Dyková (1992), i.e.  $11.3 \times 3.7 \mu\text{m}$ .

In diverse marine fishes in the northwest Atlantic, Khan et al. (1980) found *Haemogregarina* spp. to be present with a high prevalence (36%). This value is similar to that which we obtained in *Raja brachyura* (38%), while the values for the other *Raja* species were much lower (Table 2). Siddall & Desser (2001) found *H. delagei* in *R. erinacea* with prevalences of between 77 and 91%.

Fig. 3 shows the different types of leucocytes found in the present study. No basophils were found. We did not detect significant differences in leucocyte counts between fish infected with *Trypanosoma giganteum*, fish infected with *Haemogregarina delagei*, fish infected with both species, or fish not infected with either. Likewise, we did not detect significant differ-

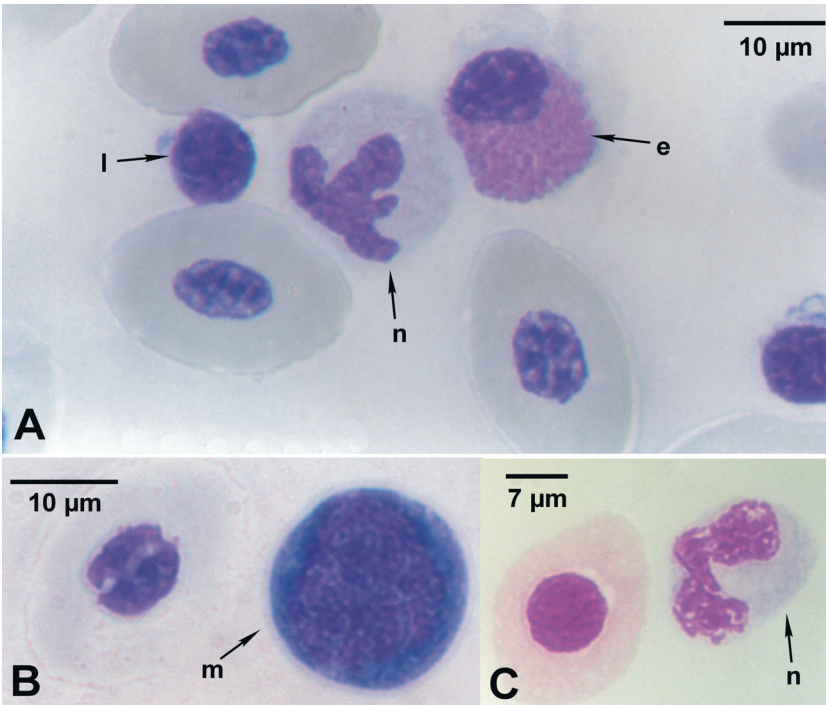


Fig. 3. Leucocyte types in *Raja* spp. (A) Lymphocyte (l), neutrophil (n) and eosinophil (e). (B) Monocyte (m). (C) Neutrophil (n)

Table 5. Leucocyte count percentages in the different *Raja* species. Mean percentages  $\pm$  standard deviation (minimum–maximum)

	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Neutrophils (%)
<i>R. microocellata</i>	43 $\pm$ 14 (12–80)	3 $\pm$ 6 (0–24)	34 $\pm$ 12 (12–68)	20 $\pm$ 11 (0–44)
<i>R. brachyura</i>	44 $\pm$ 15 (12–72)	2 $\pm$ 5 (0–32)	36 $\pm$ 15 (8–84)	18 $\pm$ 10 (0–44)
Other species	43 $\pm$ 14 (12–80)	2 $\pm$ 5 (0–32)	35 $\pm$ 14 (8–84)	20 $\pm$ 10 (0–44)

ences between male and female fish of each species, or among fish species. Similarly, Siddall & Desser (2001) did not detect any relationship between leucocyte counts and host sex, or presence/absence of *H. delagei*, while Valenzuela et al. (2003) likewise did not detect any relationship between leucocyte counts and host sex, or presence/absence of *T. humboldti*.

Total leucocyte counts in the present study were similar to those reported previously for elasmobranchs (Stoskopf 1992), but leucocyte percentages showed some differences: notably, eosinophil counts (Table 5) were markedly higher than in previous studies (Stoskopf 1992, Valenzuela et al. 2003). The eosinophil percentage obtained by Valenzuela et al. (2003) in *Schroederichthys chilensis* infected (87% prevalence) with *Trypanosoma humboldti* was lower than in the present study (15 to 25% vs. about 35%) but nevertheless high, and these authors suggested that this eosinophilia might be physiologically normal for *S. chilensis*, and not attributable to the presence of the trypanosome. Our results with *Raja* species and *T. giganteum* support this hypothesis.

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