Nematodes in *Trichomycterus nigromaculatus* (Pisces: Trichomycteridae) from the Río Gaira, Sierra Nevada de Santa Marta, Colombia

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ABSTRACT

**Objective.** To determine the prevalence of helminth infection in *Trichomycterus nigromaculatus* captured in the Gaira River, in Minca, Sierra Nevada de Santa Marta, Colombia, and characterization of the physicochemistry of the aquatic environments where it occurs. **Material and Methods.** During both the wet and dry seasons, we collected 476 specimens of *T. nigromaculatus* which were dissected to examine the visceral cavity for the presence of parasites. Isolated parasites were fixed in 10% formalin for subsequent clarification. The specimens were identified by observation of adult stages under a stereoscopic microscope after clarification. **Results.** Only the unidentified nematode worm, *Spirocamallanus* sp. was found. The parasite load ranged from 0% in August to 28.57% in April with an abundance of 1.09 worms/host. Environmental parameters such as dissolved oxygen (mean: 5.65 mg L\(^{-1}\), max: 7.5 mg L\(^{-1}\), min: 4.41 mg L\(^{-1}\)); pH (mean: 7.2, max: 9.3, min: 5.7) and conductivity (mean: 72.82 µs.cm\(^{-1}\), max: 123.7 µs.cm\(^{-1}\), min: 40 µs.cm\(^{-1}\)) had statistically significant differences in this eutrophic river environment. **Conclusions.** The discovery of *Spirocamallanus* sp. in *T. nigromaculatus* from the Gaira River is a new record of this parasite for the Colombian Caribbean and an extension of its geographic distribution.

**Keywords:** Endemism; diversity; parasitology; *Spirocamallanus*; tropical fishes (Sources: CAB, FAO, AGROVOC).

RESUMEN

**Objetivo.** Determinar la prevalencia de infección por helmintos en *Trichomycterus nigromaculatus* capturados en ambientes definidos desde la fisicoquímica del Río Gaira, en Minca, Sierra Nevada de Santa Marta, Colombia. **Material y Métodos.** Se recolectaron 476 ejemplares de *T. nigromaculatus*...
abarcando ambos períodos climáticos de la zona, los cuales fueron disectados para analizar la cavidad visceral. Los parásitos aislados fueron fijados en formol al 10% para su posterior aclaración. Los ejemplares fueron identificados a través de observación de estadios adultos al microscopio estereoscópico posterior a su aclaración. **Resultados.** Se encontraron ejemplares exclusivamente del género *Spirocamallanus* (Nematoda) la especie *Spirocamallanus* sp. La prevalencia parasitaria osciló entre 0% en agosto a 28.57% en abril con una abundancia de 1.09 vermes/hospedador. Parámetros ambientales como oxígeno disuelto (Promedio: 5.65 mg. L\(^{-1}\), Max: 7.5 mg. L\(^{-1}\), Min: 4.41 mg. L\(^{-1}\)); pH (Promedio: 7.2, Max: 9.3, Min: 5.7) y conductividad (Promedio: 72.82 µs.cm\(^{-1}\), Max: 123.7 µs.cm\(^{-1}\), Min: 40 µs.cm\(^{-1}\)) presentaron diferencias estadísticamente significativas, el ambiente se comportó como eutrofizado. **Conclusiones.** En las áreas de presencia de la asociación parasitaria fueron descritos *Spirocamallanus* sp. en *T. nigromaculatus* constituye un nuevo registro para el Caribe colombiano y una ampliación de la distribución geográfica y del rango de hospedadores del nemátodo.

**Palabras clave:** Diversidad; endemismo; peces tropicales; *Procamallanus*; parasitología. *(Fuentes: CAB, FAO, AGROVOC).*

**INTRODUCTION**

One of the most important factors for the management of parasitosis is the study of the environmental conditions in which they occur in natural populations, which allows us to know the variations in their distribution and prevalence in their intermediate and definitive hosts (1). Initial stages of studies of parasitic communities’ ecology consists of the description of their richness in terms of composition, prevalence and parasitic intensity (2), which would with adequate anticipation allow reconstructing life cycles, and inferring mitigation strategies for parasitic infections that influence the density and ethology of host populations (3). Parasites play an important role in the behavior of fish species and their relationship with the environment (4, 5), since depending on the parasite load they affect the behavior, physiology and survival of the hosts (sterility, tumors, lymphocystis, among others) (6,7).

Dumping of organic and inorganic waste, mining exploitation, and high tourist demand are recurring activities on the banks of the Gaira River, near the town of Minca, a town renowned for its environmental heritage. The river is impacted by human activities that deteriorate aquatic systems, generating changes in water quality; potentially causing a significant decrease in the ichthyofauna and ultimately, directly degrading its biodiversity (8, 9). Significant changes in temperature, concentration of dissolved oxygen, increase in salinity and concentrations of hydrogen ions, due to anthropogenic action, are adjuvants to the presence of parasites, which also contributes to the decrease in the population density of fishes (10,11).

The genus *Trichomycterus* has 54 species in Colombia, distributed in the trans-Andean foothills and mountainous river systems, of which 50 are endemic (12). Despite this, reports of parasites in Trichomycterids for Colombia are limited to those reported for *Trichomycterus chapmani* and *Trichomycterus* sp. in the San Juan River (13), both were reports of *Spirocamallanus*, one of 21 parasitic taxa so far found in *Trichomycterus* in the Neotropical region (14).

*Trichomycterus nigromaculatus* (Boulenger, 1887) is an endemic species, one of the four species recorded from mid-upper part of the Gaira River (15). Despite not being a species of economic interest, information on parasite-host interactions and their distribution is necessary to increase knowledge of the parasite fauna in the Neotropical region.

In this article, the helminth prevalence in *Trichomycterus nigromaculatus*, a definitive host is characterized in physicochemically and ecologically described environments of the Gaira River, Sierra Nevada de Santa Marta, Colombia.

**MATERIALS AND METHODS**

**Study area.** The study was carried out in the Gaira River in Minca township (670 meters above sea level), in the northern part Magdalena department, Colombia (Figure 1). The region has a bimodal rainfall pattern, with high rainfall from May to November and low rainfall from December to April (Figure 2).
Fieldwork phase. Sampling was carried out covering the periods of minimum and maximum precipitation defined for the area based on a ten-year history of the Gaira climatological station. (Where: M1= June, M2= April 2017, M3= June, M4= August, M5= October, M6= December, M7= January, M8= February, M9=April, M10=June, M11= August, M12= October) see Table 1. Fish were captured with two types of fishing gear, seines 2 m long by 0.50 m high and a mesh of 0.5 cm and a cast net of 2 m in diameter with a mesh of 0.50 cm. Sampling was carried out between 8:00 p.m. and 12:00 p.m., sweeps were made with and against the current, and from bank to bank, in order to include all the biotopes of the system (14). For the capture of the specimens, the ethical considerations determined in the national statute for the protection of animals (law 84 of 1989) were taken into account. Collecting was authorized by the license to collect wild specimens within the framework of scientific research, granted to the Universidad del Atlántico through resolution No. 00594 of 2018 (Ministry of the Environment, National Environmental License Authority-ANLA-). The specimens were immediately placed on ice in polystyrene coolers. For each individual, the total weight (g) was evaluated with a scale (CAMRY, model: EHA401 and precision mg), and the total and standard length (mm) to a digital caliper (DISCOVER, precision mm) to hundredths of a millimeter. Subsequently, the specimens were dissected with a uroventral cut to extract gills, stomach, intestine, gonads and observe the coelomic cavity in order to reveal the presence of helminth parasites. Each of these organs and structures were weighed and measured, fixed in 70% ethanol and placed in properly labeled bottles (16). The specimens of _T. nigromaculatus_ were taken to the laboratory located at the Universidad del Atlántico, Barranquilla, Colombia, fixed in 10% formalin and then preserved in 70% alcohol and deposited in the fish collection of the same university (UARC- IC).

At the time of sampling, measurements of in situ variables such as pH, dissolved oxygen (mg L-1), water surface temperature (°C) and conductivity (µs.cm-1) were made with a HANNA® instruments multiparametric meter. Using a PROOF portable weather station model ADC - PRO, altitude (m), ambient temperature (°C) and relative humidity (%) were measured. Water samples were taken in 250 mL bottles, which were stored on ice and transported to the laboratory of the Universidad del Atlántico for analysis of ex situ variables such as total hardness (µg L-1), sulfates (µg L-1), acidity (µg L-1), nitrites [(NO2- N) (µg. L-1)], nitrites [(NO3-N) (µg. L-1)], phosphates (mg L-1), alkalinity (µg L-1), and chlorides (µg L-1) with kits from Hanna Instruments® (Woonsocket, Rhode Island, United States) and ammonium (mg.L-1) through a Milwaukee model MI 405® ammonium meter, according to the methodology proposed by the American Public Health Association (17).

Laboratory phase. Identification of the parasites was carried out in the laboratory of the museum of scientific collections of the Universidad del Atlántico, the helminths previously separated and extracted were preserved in 70% alcohol, subsequently they were immersed in 10% formalin for two days for fixation. The clarification process was carried out by immersion in acetic acid for 30 seconds, then in 70% ethanol for 5 minutes and in 70% glycerinated ethanol. Next, they were passed again through 70% ethanol, then through the ethanol battery, first at 95% and then 100%, then absolute butanol, followed...
by absolute toluene for clarification and final preservation in glycerin (18). The specimens were observed, photographed and measured in a stereomicroscope (SteREO Discovery V20, Carl Zeiss AG, Oberkochen, Germany) and identified following (19,20). Later they deposited in the museum of scientific collections of the Universidad del Atlántico (UARC).

**Data analysis.** In order to know the dynamics of the infection in *T. nigromaculatus*, the Prevalence (Clopper-Pearson), average intensity (Bootstrap BCa) and average abundance (Bootstrap BCa) were calculated using Quantitative Parasitology software version 3.0 (20). The condition factor K for host fish was calculated. K measures the degree of development and robustness of fish individuals as a function of allometric increase in relation to size (equation K= Wt/ (LE*b); Wt=total weight of the specimen (g), LE=standard length (mm), b=angular coefficient of the regression between total weight-standard length) (21).

Descriptive statistics were performed for the physicochemical data, weights and lengths of the fish. Subsequently, to determine the distribution of the data, a Shapiro-Wilk normality test was performed (N<30), which indicated non-normal distribution for sexes; and normal for sizes and physicochemical variables (p<0.05). To determine whether there were significant differences among the samples for each variable, Mann-Whitney tests were applied between sexes, t-test between sizes and nested ANOVA between the physicochemical variables, in addition to Tukey’s test to determine where the differences were found (p<0.05 ). All analyses were performed with PAST 4.03 under Windows.

**RESULTS**

476 individuals of *T. nigromaculatus* that were examined for parasites (Figure 3). The standard length of the fish varied from 17.2 to 121.5 mm (83.9 ± 19.2, n=476) with the highest values in samples 10 and 12 (High rains) with 85.8 ± 17.2 and 83.9 ± 19.2 respectively. The weight of the specimens varied from 0.42 to 26.94g (4.76±3.78, n=476) (Table 1) with the highest value in sample 10 with 8.38 ± 4.95. The values of the condition factor K show a high degree of robustness for the species at the sampling times. It was higher for females than for males with the maximum values for females found in samples 2 and 6, and for males in sample 9 (Figure 4), these three correspond to the low rain season.

![Figure 3. Live colors of *Trichomycterus nigromaculatus*, 23.5mm Standard Length (UARC-IC 807) (Foto CG-A).](image1)

![Figure 4. Condition factor K of *Trichomycterus nigromaculatus* from the Gaira River, Minca, Sierra Nevada de Santa Marta.](image2)

Regarding the prevalence, it was observed that 19.1% (90% CI: 16.1%-22.3%) of the individuals were parasitized (n=91), with a minimum in sample 4 (0%) and a maximum in sample 10 (43.48%). Most (99%) of the parasites were found in the stomach and intestine, only one was found in the gonads. The mean intensity was 8.29 (90% CI: 7.58-8.88) and the mean abundance was 1.58 (90% CI: 1.34-1.88). There were no statistically significant differences between the samples, by sex (p=0.8827; p<0.05) or size intervals (p=2.10; p<0.05).

The parasites were identified as *Spirocamallanus* sp.

**Description.** Color reddish in life with a striated cuticle, an orange-colored buccal capsule that is slightly longer than it is wide, a muscular esophagus smaller than the glandular esophagus and a posterior end with a filiform appendage (Figures 5 and 6).
Females (6 specimens) length 7.1-27.5 mm (18.9±7.7), width 0.24-0.61 mm (0.44±0.14), buccal capsule 0.07-0.63 mm (0.17±0.22) in length, width 0.056-0.11 mm (0.07±0.02), muscular esophagus 0.34-1.20 mm (0.60±0.34).

**Figure 5.** *Spirocamallanus* sp. from *Trichomycterus nigromaculatus* collected from the Gaira River, Sierra Nevada de Santa Marta, Colombia. **A:** The arrow at the top indicates the muscular esophagus, while the lower one indicates the glandular esophagus, scale bar 0.2mm; **B:** Anus, scale bar 0.25mm; **C:** Filiform termination of the body of *Spirocamallanus* sp., scale bar 0.25mm.

**Figure 6.** *Spirocamallanus* sp. **A-B:** Posterior tip of *Spirocamallanus* sp., scale bar = 0.2mm **C:** Striated cuticle, scale bar = 0.2mm **D-E:** Posterior tip of *Spirocamallanus* sp., scale bar = 0.1mm **F:** Buccal capsule in dorsal-ventral view, scale bar = 0.1mm.

**Table 1.** Monthly averages of the morphometric variables and infection parameters of the analyzed specimens of *T. nigromaculatus* from the Gaira River, SNSM analyzed.

<table>
<thead>
<tr>
<th>Month</th>
<th>Sample</th>
<th>PT/PP</th>
<th>Weight (g)</th>
<th>Standard length (cm)</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2016</td>
<td>M1*</td>
<td>54/13</td>
<td>4.49 ± 3.18</td>
<td>64.94 ± 16.68</td>
<td>24.07</td>
</tr>
<tr>
<td>April 2017</td>
<td>M2**</td>
<td>39/2</td>
<td>2.56 ± 3.33</td>
<td>48.83 ± 19.83</td>
<td>5.13</td>
</tr>
<tr>
<td>June 2017</td>
<td>M3*</td>
<td>49/2</td>
<td>2.06 ± 2.68</td>
<td>62.35 ± 28.68</td>
<td>4.08</td>
</tr>
<tr>
<td>August 2017</td>
<td>M4*</td>
<td>29/0</td>
<td>3.65 ± 5.12</td>
<td>55.50 ± 14.87</td>
<td>0.00</td>
</tr>
<tr>
<td>October 2017</td>
<td>M5*</td>
<td>32/2</td>
<td>2.99 ± 2.60</td>
<td>61.03 ± 23.57</td>
<td>6.25</td>
</tr>
<tr>
<td>December 2017</td>
<td>M6**</td>
<td>20/1</td>
<td>3.64 ± 3.03</td>
<td>59.67 ± 16.54</td>
<td>5.00</td>
</tr>
<tr>
<td>January 2018</td>
<td>M7**</td>
<td>21/6</td>
<td>5.14 ± 4.85</td>
<td>67.19 ± 23.05</td>
<td>28.57</td>
</tr>
<tr>
<td>February 2018</td>
<td>M8**</td>
<td>50/9</td>
<td>3.83 ± 2.60</td>
<td>59.79 ± 16.08</td>
<td>18.00</td>
</tr>
<tr>
<td>April 2018</td>
<td>M9**</td>
<td>48/5</td>
<td>5.21 ± 3.76</td>
<td>70.95 ± 18.93</td>
<td>10.42</td>
</tr>
<tr>
<td>June 2019</td>
<td>M10*</td>
<td>46/20</td>
<td>8.38 ± 4.95</td>
<td>85.78 ± 17.15</td>
<td>43.48</td>
</tr>
<tr>
<td>August 2019</td>
<td>M11*</td>
<td>42/16</td>
<td>6.75 ± 4.31</td>
<td>75.83 ± 18.96</td>
<td>38.10</td>
</tr>
<tr>
<td>October 2019</td>
<td>M12*</td>
<td>46/15</td>
<td>8.38 ± 4.95</td>
<td>83.91 ± 19.21</td>
<td>32.61</td>
</tr>
</tbody>
</table>

*High rains; **Low rains; PT= total fish, PP= parasitized fish.
The average dissolved oxygen average value was 5.65 mg. L⁻¹ (ranged between 4.41 mg. L⁻¹ in the October 2019 sample and 7.5 mg. L⁻¹ in December; high and low rainfall respectively), conductivity had an average of 72.82 µs. cm⁻¹ (40 µs. cm⁻¹ in sample 2 in April in the low rain season and 123.7 µs. cm⁻¹ in sample 12 in October in high rain) and pH had an average of 7.2 (5.7 in sample 4 in August, high rain season, and 9.3 in sample 9 in April, low rain season). Dissolved oxygen p=0.002681, conductivity p=1.842-11 and pH p=0.006812 had statistically significant differences. The ex situ variables also had statistically significant differences. High concentrations of nitrites (0.15 µg. L⁻¹), sulfates (900 µg. L⁻¹) and chlorides (1800 µg. L⁻¹) were found (Table 2), which exceeded the permissible limits established by the Ministry of Environment in Colombia for the conservation of flora and fauna.

**DISCUSSION**

The nematodes collected were identified as *Spirocamallanus* sp., which according to the review of the literature constitutes the first report of parasites of this genus in fish from rivers draining the Sierra Nevada de Santa Marta.

The presence of helminths of the genus *Spirocamallanus* brings with it pathological changes such as swelling, glomeruli, degenerative changes in the tubules and Bowman’s capsule (22), in addition to causing lesions by adhering to the walls of the intestine, while feeding on blood, causing anemia and hemorrhages (8).

*Spirocamallanus* nematodes have been found in the family Trichomycteridae, genus *Trichomycterus* in Peru, Colombia and Argentina (23, 24) in the latter with prevalences of 97% and 7.5% respectively. The results indicate that *T. nigromaculatus* represents a new host for this species of nematode, with a prevalence of 19%. This expands the geographical distribution of *Spirocamallanus* and adds one new host for this genus.

More than half (56%) of the parasitized fish were captured in the last three samplings, corresponding to the season of high rainfall, during which low values of dissolved oxygen and pH were reported, which favors the development and survival of pathogenic organisms (25). This rivers has deteriorated due to the increase in tourism, sewage effluents, pollution, harmful agricultural practices, food preparation and toxic discharges into the river (15, 26), which agrees with Marinho et al (27), Dias et al (7), Silva et al (28), who documented increased parasite prevalence associated with inadequate sanitary conditions.

Parasites of the genus *Spirocamallanus* were found mainly in the intestine (89%), the structure where they reside in their definitive host (23, 28, 29, 30), since the final stage of development of this nematode occurs in this organ. The stages found in the stomach correspond to a larval phase of development; the presence of a verme outside the usual niches for this parasite (gonad) could be due to the fact that when the host was removed from its habitat, the nematodes migrated ectopically.

Although parasites of the genus *Spirocamallanus* cause damage to the host (8), the condition factor K (Figure 4) did not show a relationship between the presence of the parasite and a decrease in the degree of robustness of the captured specimens. Additional histological studies are needed to determine how observed lesions affect fish condition.

In the present study, the values of the ex situ physicochemical variables and the presence of parasites were not found to be statistically related (p>0.05), however, high values of nitrites (0.15 µg. L⁻¹) (Table 1), associated with industrial or municipal wastewater, septic tanks, animal

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Table 2. Physical-chemical variables *ex situ* taken during the last four months of sampling. SD= standard deviation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hardness (µg. L⁻¹)</td>
<td>80</td>
<td>200</td>
<td>160</td>
<td>7</td>
</tr>
<tr>
<td>Nitrites (µg. L⁻¹)</td>
<td>0</td>
<td>15</td>
<td>50.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Nitrites (µg. L⁻¹)</td>
<td>10</td>
<td>35</td>
<td>16.2</td>
<td>12.5</td>
</tr>
<tr>
<td>Sulfates (µg. L⁻¹)</td>
<td>35</td>
<td>900</td>
<td>307.5</td>
<td>4</td>
</tr>
<tr>
<td>Acidity (µg. L⁻¹)</td>
<td>85</td>
<td>500</td>
<td>240</td>
<td>1.8</td>
</tr>
<tr>
<td>Total alkalinity (µg. L⁻¹)</td>
<td>42</td>
<td>150</td>
<td>96.7</td>
<td>61.5</td>
</tr>
<tr>
<td>Chlorides (µg. L⁻¹)</td>
<td>0</td>
<td>1800</td>
<td>502.5</td>
<td>8.6</td>
</tr>
<tr>
<td>Ammonium (µg. L⁻¹)</td>
<td>0</td>
<td>0.1</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>Phosphate (µg. L⁻¹)</td>
<td>0.02</td>
<td>0.13</td>
<td>0.05</td>
<td>0.1</td>
</tr>
</tbody>
</table>
waste and vehicle gas emission discharges (31), sulfates (900 µg. L-1) and chlorides (1800 µg. L-1), influenced by effluents of sewage to the river (32), were found above the maximum values allowed by resolution 2115 of 2007 of the Ministry of the Environment of 0.1 µg. L-1, 250 µg. L-1 and 250 µg. L-1 respectively. The waters from the Gaira River are used to supply water to Minca and neighborhoods of Santa Marta, which raises the need for its monitoring in order to guarantee public health (33, 34).

Conflict of interest

The authors of this article declare that no conflicts of interest exist.

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