Abstract

A study on the nematode parasites of nine species of freshwater fishes from Peixe River São Paulo, State, Brazil, was conducted. Fish were collected between February 2010 and March 2011 and the following species were found: *Procamallanus (Spirocamallanus) inopinatus* and *Contracaecum* sp. (larvae) in *Astyanax altiparanae*; *Contracaecum* sp. (larvae), *Dioctophyma renale* (larvae), *Philometroides caudata*, *P. (Spirocamallanus) inopinatus*, *P. (Spirocamallanus) neocaballeroi* (larvae) and *P. (Spirocamallanus) saofranciscensis* in *Acestrorhynchus lacustris*; *Contracaecum* sp. (larvae), *Guyanema* sp., *Hysterothylacium* sp. (larvae) and *Ichthyouris* sp. in *Cyphocharax modestus*; *Contracaecum* sp. (larvae), *Cosmoxynemoides aguirrei* and *Pharyngodonidae* gen. sp. in *C. nagelii*; *Dioctophyma renale* (larvae), *Hysterothylacium* sp. (larvae) and *Rhabdochona* sp. in *Gymnotus sylvius*; *Capillariidae* gen. sp. in *Hoplosternum littorale*; *Cosmoynema vianai*, *Guyanema* sp., *Ichthyouris* sp. and *Travnema travnema* in *Steindachnerina insculpta*; *Contracaecum* sp. (larvae), *Procamallanus (Spirocamallanus) rebecae* (larvae) in *Triportheus angulatus* and *Rhabdochona acuminata* in *Triportheus nematurus*. This is first study of nematode parasites from the Peixe River, therefore all the species found are new geographical records and 19 are new host records.

**Key words:** Acestrorhynchidae – Biodiversity – Callichthyidae – Characidae – Curimatidae – Gymnotidae – Helminthes - Parasites.
Se llevó a cabo un estudio de los nematodos parásitos de nueve especies de peces de agua dulce del Río do Peixe, Estado de São Paulo, Brasil. Los peces fueron colectados entre febrero de 2010 y marzo de 2011 y las especies encontradas fueron: Procamallanus (Spirocamallanus) inopinatus y Contracaecum sp. (larva) en Astyanax altiparanae; Contracaecum sp. (larva), Diocotphyma renale (larva), Philometroides caudata, P. (Spirocamallanus) inopinatus, P. (Spirocamallanus) neocaballeroi (larva) y P. (Spirocamallanus) saofranciscensis en Acestrorhynchus lacustris; Contracaecum sp. (larva), Guyanema sp., Hysterothylacium sp. (larva) y Icthyouris sp. en Cyphocharax modestus; Contracaecum sp. (larva), Cosmoxynemoides aguirrei y Pharyngodonidae, gen. sp. en C. nagelii; Diocotphyma renale (larva), Hysterothylacium sp. (larva) y Rhabdochona sp. en Gymnotus sylvius; Capillariidae gen. sp. en Hoplosternum littorale; Cosmoxynema vianai, Guyanema sp., Ichthyouris sp. y Travnema travnema en Steindachnerina insculpta; Contracaecum sp. (larva), Procamallanus (Spirocamallanus) rebecca (larva) en Triportheus angulatus y Rhabdochona acuminata en Triportheus nematurus. Este es el primer estudio de nemátodos parásitos de Río do Peixe, por lo tanto todas las especies son nuevos registros geográficos y 19 son nuevos registros de hospedadores.

**Palabras clave:** Acestrorhynchidae – Biodiversidad – Callichthyidae – Characidae – Curimatidae - Gymnotidae - helmintos - parásitos.

**INTRODUCTION**

Parasitic nematodes constitute one of the earliest known groups of helminthes in fishes. They infect freshwater, marine and brackish water fish species and sometimes cause substantial damage to the host. Although parasitic nematodes can infect almost all fish organs, the majority of the currently known species has been described from the intestine. Most nematodes infect fish as adults, but a large proportion of them occur as larval stages.

These are usually parasites of piscivorous birds, mammals or reptiles, or less frequently of predatory fishes (Molnár et al., 2006). These helminthes cause considerable economic loss in the fishery industries (Thatcher, 2006). Since many nematode larvae are large enough to be seen by the naked eye, their presence can make fish unmarketable (Thatcher, 2006). The damage caused to fish by nematodes varies greatly depending on the species, organ infected and intensity of infection (Thatcher, 2006). Among the nematodes parasitizing fishes, anisakid species causing human infections are well documented (Molnár et al., 2006).

Currently, the biodiversity of freshwater ecosystems of Latin America is threatened, mainly by environmental problems resulting from the degradation of the ecosystems. In this context, parasite biodiversity can be very important because parasitism plays a key role in ecosystems, regulating the abundance or density of host populations, stabilize food webs and structuring animal communities. Thus, knowledge of parasite diversity and its possible decline are crucial for environmental management and conservation (Luque & Poulin, 2007). Therefore, biotic diversity studies are presently conducted with a sense of urgency and they underscore the need for baseline biological inventories and surveys. The faunal survey is critical, because even the most
elementary information is lacking for many groups in many parts of the Neotropics (Moravec, 1998).

Considering the rapid development of marine, brackish-water and freshwater aquaculture in different countries during recent years, the significance of nematodes as important fish pathogens is also increasing. However, the present knowledge of these parasites is very incomplete, particularly in regard to their biology and ecology, but also in taxonomy, geographical distribution and others aspects (Moravec, 1998).

The Peixe River (48°06'38”W, 22°49'53.1”S) (Fig.1) is a tributary on the left bank of the middle Tiete River Basin in the region of Barra Bonita, São Paulo State (SP), Brazil. This river originates in the municipality of Torre de Pedra (SP) in the Basaltic Cuesta Botucatu Environmental Preservation Area and is a drainage basin corresponding to 584 km² running north-south (Caramaschi, 1986).

To date, there are no studies on nematodes parasites in the Peixe River. The aim of this is to report the nematode species associated with fishes from this river.

**MATERIAL AND METHODS**

Four hundred forty-eight fish specimens belonging to nine species from Peixe River, municipality of Anhembi, São Paulo State, Brazil, were collected between February 2010 and March 2011 in order to study of their nematodes. The protocol for collection of fishes and nematodes are in accordance with the protocols recommended by the ethics committee and authorized by the IBAMA/ICMBio (license 15549-1).

Fish were collected using nylon monofilament gill nets with mesh sizes of 3–14 cm from three sites on the Peixe River. Nets were placed at 5:00 p.m. and removed at 7:00 a.m. the following day, total exposure 14 h. Fishes were taken from nets and each specimen was separated in plastic bags. Some fish specimens were maintained in coolers until necropsy that was realized until one hour after the collection and others were frozen and analyzed posteriorly. Organs were removed, washed and sieved in mesh of 75 µm aperture. The nematodes collected were fixed in alcohol 70 ºGL. For the identification the specimens were mounted in lactophenol. The ecological descriptors of parasitism were calculated according Bush et al. (1997).

Voucher specimens were deposited in the Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB), São Paulo State, Brazil.

**RESULTS**

Seventeen taxa of nematodes (Table 1) were collected, as follow: Capillariidae gen. sp. (Figure 2a), Contracaecum sp. (larvae) (Figures 2b–d), Cosmoxynema vianai Travassos, 1948 (Figures 2e–f), Cosmoxynemoides aguirrei Travassos, 1948 (Figures 2g–h), Dioctophyma renale (Goeze, 1782) (larvae) (Figures 3a–d), Guyanema sp. (Figures 3e–f), Hysterohylacium sp. (larvae) (Figures 4a–d), Icthyouris sp. (Figures 4e–f), Pharyngodonidae gen. sp., Philometroides caudata Moravec, Scolz & Vivas-Rodríguez, 1995 (Figures 4g–i), Procamallanus (Spirocamallanus) inopinatus Travassos, 1929 (Figures 5a–b), P. (Spirocamallanus) neocaballeroi (Caballero-Deloya, 1977) (Figures 5c–d), P. (Spirocamallanus) rebecae (Andrade-Salas, Pineda-López & García-Magaña, 1994) (Figures 5e–f), P. (Spirocamallanus) sao franciscensis (Moreira, Oliveira & Costa, 1994) (Figure 6a), Rhabdochona acuminata (Molin, 1860) (Figures 6b–d), Rhabdochona sp., and Travnema travnema Pereira, 1938 (Figures 6e–g).

Five out of 17 species of nematodes were larvae. The nematode species with lower specificity was the Contracaecum sp. (larvae), which was reported in five host species. Acesthorhynchus lacustris (Lütken, 1875) presented the greatest richness of nematodes, with six nematode species found. The main infection site was the intestine, which was parasitized by all parasite species.
Table 1. Nematode parasites of fishes from Peixe river, São Paulo State, Brazil. N – number of fish; SI – site of infection; P – prevalence (%); MII – mean intensity of infection; MA – mean abundance; SD standard deviation; CHIBB – Coleção Helmintológica do Instituto de Biociências de Botucatu; * new host record.

<table>
<thead>
<tr>
<th>Host</th>
<th>N</th>
<th>Parasite</th>
<th>SI</th>
<th>P</th>
<th>MII ± SD</th>
<th>MA ± SD</th>
</tr>
</thead>
</table>
| Astyanax altiparanae  
Garutti & Britski, 2000 | 82  | Contracaecum sp. (larvae)  
CHIBB 6808  
Procamallanus (Spirocamallanus) inopinatus  
CHIBB 6809 | Intestine  
Stomach and intestine | 13.3 | 1 | 0.13 | |
|  |  |  |  |  | 1.45 ± 0.07 | 1.06 ± 0.05 |
| Acestro rhynchus lacustris  
(Lütken, 1875) | 62  | Contracaecum sp. (larvae)  
CHIBB 6810  
Dioctophyma renale (larvae)*  
CHIBB 6811  
Philometroides caudata*  
CHIBB 6812  
Procamallanus (Spirocamallanus) inopinatus*  
CHIBB 6813  
Procamallanus (Spirocamallanus) neocaballeroi (larvae)*  
CHIBB 6814  
Procamallanus (Spirocamallanus) saofranciscensis  
CHIBB 6815 | Stomach, liver and intestine  
Intestine | 25  | 2.40 ± 0.33 | 0.60 ± 0.08 |
|  |  |  |  |  |  | |
| Cyphocharax modestus  
(Fernández-Yépez, 1948) | 58  | Contracaecum sp. (larvae)*  
CHIBB 6816  
Guyanema sp.*  
CHIBB 6817  
Hysterothylacium sp. (larvae)*  
CHIBB 6818  
Icthyouris sp.*  
CHIBB 6819 | Liver  
Intestine | 10  | 1 | 0.10 | |
|  |  |  |  |  | 1.50 ± 0.35 | 0.30 ± 0.07 |
| Cyphocharax nagelii  
(Steindachner, 1881) | 56  | Contracaecum sp. (larvae)*  
CHIBB 6820  
Cosmoxynemoides aguirrei*  
CHIBB 6821  
Pharyngodonidae gen. sp.*  
CHIBB 6822 | Liver and intestine  
Intestine | 20  | 1 | 0.20 | |
<p>|  |  |  |  |  | 6.17 ± 7.86 | 1.43 ± 4.41 |
|  |  |  |  |  |  | 0.10 |</p>
<table>
<thead>
<tr>
<th>Host</th>
<th>N</th>
<th>Parasite</th>
<th>SI</th>
<th>P</th>
<th>MII ± SD</th>
<th>MA ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gymnotus silvyus Albert &amp; Fernandes-Matioli, 1999</td>
<td>51</td>
<td><em>Diocotophymina renale</em> (larvae)* CHIBB 6823</td>
<td>Intestine</td>
<td>20</td>
<td>1.50 ± 0.35</td>
<td>0.30 ± 0.07</td>
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<tr>
<td></td>
<td></td>
<td><em>Hysterothylacium</em> sp. (larvae)* CHIBB 6824</td>
<td>Intestine</td>
<td>10</td>
<td>1</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rhabdochona</em> sp.* CHIBB 6825</td>
<td>Stomach and intestine</td>
<td>20</td>
<td>6</td>
<td>1.20</td>
</tr>
<tr>
<td>Hoplosternum littorale (Hancock, 1828)</td>
<td>60</td>
<td>Capillariidae gen. sp. CHIBB 6826</td>
<td>Stomach, intestine and liver</td>
<td>19.3</td>
<td>2.00 ± 0.21</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td>Steindachnerina insculpta (Fernández-Yépez, 1948)</td>
<td>62</td>
<td><em>Cosmoxynema</em> vianai CHIBB 6827</td>
<td>Intestine</td>
<td>6.6</td>
<td>1.00 ± 0.19</td>
<td>1.00 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Guyanema</em> sp.* CHIBB 6828</td>
<td>Intestine</td>
<td>6.6</td>
<td>0.40 ± 0.20</td>
<td>0.13 ± 0.01</td>
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<tr>
<td></td>
<td></td>
<td><em>Ichthyuris</em> sp.* CHIBB 6829</td>
<td>Intestine</td>
<td>6.6</td>
<td>1</td>
<td>0.06</td>
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<tr>
<td></td>
<td></td>
<td><em>Travnema travnema</em> CHIBB 6830</td>
<td>Intestine</td>
<td>13.3</td>
<td>2.75 ± 0.13</td>
<td>0.35 ± 0.01</td>
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<td>Triportheus angulatus (Spix &amp; Agassiz, 1829)</td>
<td>17</td>
<td><em>Contracaecum</em> sp. (larvae)* CHIBB 6831</td>
<td>Intestine</td>
<td>17</td>
<td>1.00 ± 0.19</td>
<td>1.00 ± 0.03</td>
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<td></td>
<td></td>
<td><em>Procamallanus (Spirocamallanus) rebecae</em> (larvae)* CHIBB 6832</td>
<td>Intestine</td>
<td>20</td>
<td>1</td>
<td>0.20</td>
</tr>
<tr>
<td>Triportheus nematus (Kner, 1858)</td>
<td>2</td>
<td><em>Rhabdochona acuminata</em> CHIBB 6833</td>
<td>Intestine</td>
<td>50</td>
<td>6.00 ± 4.24</td>
<td>3.00 ± 2.12</td>
</tr>
</tbody>
</table>
Table 2. Parasitological records in host studied in others Hidrographic Basins.

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Nematodes</th>
<th>Localities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Procamallanus</em> <em>(Spirocamallanus) inopinatus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Spinboxys</em> sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acestrorhynchus lacustris</em> (Lütken, 1875)</td>
<td><em>Contracaecum</em> sp.</td>
<td>Amazon Basin</td>
<td>Buhrnheim (1976)</td>
</tr>
<tr>
<td></td>
<td><em>Contracaecum</em> Type 1</td>
<td>Mogi Guçu River</td>
<td>Carvalho <em>et al.</em> (2003)</td>
</tr>
<tr>
<td></td>
<td><em>Contracaecum</em> Type 2</td>
<td>Paraná River</td>
<td>Costa <em>et al.</em> (1991)</td>
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<td><em>Eustrongylides</em> sp.</td>
<td>São Francisco River</td>
<td>Moravec <em>et al.</em> (1993)</td>
</tr>
<tr>
<td></td>
<td><em>Heterotyphlum</em> sp.</td>
<td>Tibagi River</td>
<td>Moreira <em>et al.</em> (1994)</td>
</tr>
<tr>
<td></td>
<td><em>Philometridae</em></td>
<td></td>
<td>Silva-Souza &amp; Saraiva (2002)</td>
</tr>
<tr>
<td></td>
<td><em>Procamallanu</em> sp.</td>
<td></td>
<td>Takemoto <em>et al.</em> (2009)</td>
</tr>
<tr>
<td></td>
<td><em>Procamallanus</em> <em>(Spirocamallanus) paraensis</em></td>
<td></td>
<td>Thatcher (2006)</td>
</tr>
<tr>
<td></td>
<td><em>Spinocamallanus saofranciscensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Travassosnema</em> Travassosi paranaensis</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><em>Travassosnema</em> travassosi travassosi</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Goezia</em> sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Travnema travnema</em></td>
<td></td>
<td></td>
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</tbody>
</table>
Figure 1. Map of the study area, showing details of river and their geographic coordinates.
Figure 2. A. Detail of eggs (arrow) of Capillaridae gen. sp.; B. Cephalic extremity of *Contracaecum* sp., showing the excretory pore (arrow); C. Region of ventriculus of *Contracaecum* sp., showing (C=caecum, O=oesophagus, V=ventriculus and VA=ventricular appendix); D. Posterior extremity of *Contracaecum* sp. showing the absence of mucrons; E. Anterior end of body of *Cosmoxynema vianai*; F. Tail of *Cosmoxynema vianai* showing the eggs (arrow).
Figure 3. A. Anterior end of body of Cosmoxynemoides aguirei; B Head end of Cosmoxynemoides aguirei; C. Anterior end of body of Dioctophyma renale showing the junction of oesophagus and intestine (arrow); D. Posterior end of male larva of Dioctophyma renale showing the genital bursa (arrow); E. Anterior end of body of Guyanema sp.; F. Gravid specimen of Guyanema sp., showing the uterus with larvae (arrow).
**Figure 4.** A. Tail of *Hysterothylacium* sp. showing the mucrons; B. Cephalic extremity of *Hysterothylacium* sp.; C. Cephalic extremity of *Icthyouris* sp.; D. Tail of *Icthyouris* sp. showing the lateral alae; E. Cephalic extremity of *Philometroides caudate*, showing the ring of slightly elevated cuticle; F. Tail of *Philometroides caudata*. 
Figure 5. A. Buccal capsule of *Procamallanus* (*Spirocamallanus*) *inopinatus*; B. Posterior end of male of *Procamallanus* (*Spirocamallanus*) *inopinatus*, showing the spicules almost equal in length (arrow); C. Buccal capsule of *Procamallanus* (*Spirocamallanus*) *neocaballeroi*; D. Posterior end of *Procamallanus* (*Spirocamallanus*) *neocaballeroi*, showing the tip of tail (arrow); E. Buccal capsule of *Procamallanus* (*Spirocamallanus*) *rebecae*; F. Posterior end of *Procamallanus* (*Spirocamallanus*) *rebecae*, showing the tip of tail (arrow).
Figure 6. A. Buccal capsule of Procamallanus (Spirocamallan) sao franciscensis; B. Anterior end of body of Rhabdochona acuminata; C. Vulva of Rhabdochona acuminata (arrow); D. Posterior end of male, showing the small and larger spicules of Rhabdochona acuminata (arrow); E. Anterior end of body of Travnema travnema showing the buccal capsule without teeth (arrow); F. Posterior end of body of Travnema travnema showing the eggs (arrow).
DISCUSSION

Seventeen nematode taxa were here reported in fishes from Peixe River, São Paulo State, Brazil. All helminthes collected represent new geographic distribution. Nineteen new host records are reported. In addition, this is the first record of nematodes in C. modestus, C. nagelii, G. silvyus and T. nematurus.

The larva that showed low specificity was Contracaecum sp. This species belongs to the family Anisakidae and has great importance in public health due its high zoonotic potential. Anisakids are important to humans on a number of counts, such as economically, politically and mainly pathologically (Takimoto et al., 2009).

Anisakiasis in humans can occur by eating the flesh of the fish raw or insufficiently heat-treated, salted or smoked, containing larvae of the third or fourth stage, in which case the man acts as an accidental host and the larvae do not complete their development. These parasites can penetrate the human digestive tract and invade the organs causing a series of attachment pathological effects (Lymbery & Cheah, 2007). However, the results presented in this paper indicate that no problem can be related to this finding since all anisakid larvae found were encysted or free in the stomach, intestine and liver, which are not used for human consumption.

In relation to nematodes, it is known that fishes can act as intermediate or definitive host. In present paper the majority of fishes species studied were parasitized by adult forms, indicating that they occupy a final position in the food web.

We found higher diversity of nematodes in this study when compared with works performed in others water systems (Table 2). The comparative qualitative analysis of nematode fauna between the fishes of this study in relation to the same species from other localities revealed similarity only four nematode species: Contracaecum sp. and P. (Spirocamallanus) inopinatus in A. altiparanae; Contracaecum sp. in A. lacustris; C. vianai and T. travnema in S. insculpta. This result is possibly due to the fact that some stretches of the Peixe River are still well preserved and have suffered little human action.

This work expanded the geographic distribution and registered new host for some nematode species, contributing to the knowledge of the biodiversity of parasites nematodes of fishes in the São Paulo State.

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