

## ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

### NEW HOSTS AND DISTRIBUTION RECORDS FOR NEMATODE PARASITES OF FRESHWATER FISHES FROM SÃO PAULO STATE, BRAZIL

### NUEVO HOSPEDADORES Y REGISTROS DE DISTRIBUCIÓN PARA NEMATODOS PARÁSITOS DE PECES DE ÁGUA DULCE DEL ESTADO DE SÃO PAULO, BRASIL

Vanessa Doro Abdallah\*, Rodney Kozłowski de Azevedo, Edmir Daniel Carvalho & Reinaldo José da Silva

UNESP- Univ Estadual Paulista, Campus de Botucatu, Instituto de Biociências,  
Departamento de Parasitologia, Botucatu, São Paulo, Brazil.

\*E-mail: vanessaabdallah@ig.com

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#### 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*; *Cosmoxynema 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.

## Resumen

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), *Dioctophyma renale* (larva), *Philometroides caudata*, *P. (Spirocamallanus) inopinatus*, *P. (Spirocamallanus) neocaballeri* (larva) y *P. (Spirocamallanus) saofranciscensis* en *Acestrorhynchus lacustris*; *Contracaecum* sp. (larva), *Guyanema* sp., *Hysterothylacium* sp. (larva) y *Ichthyouris* sp. en *Cyphocharax modestus*; *Contracaecum* sp. (larva), *Cosmoxynemoides aguirrei* y Pharyngodonidae gen. sp. en *C. nagelii*; *Dioctophyma 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 *Triporthes angulatus* y *Rhabdochona acuminata* en *Triporthes 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 km<sup>2</sup> running north-south (Caramaschi, 1986).

To date, there are no studies on nematode 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 °GL. 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 Helminológica 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 2<sub>A</sub>), *Contracaecum* sp. (larvae) (Figures 2<sub>B-D</sub>), *Cosmoxynema vianai* Travassos, 1948 (Figures 2<sub>E-F</sub>), *Cosmoxynemoides aguirrei* Travassos, 1948 (Figures 3<sub>A-B</sub>), *Diectophyma renale* (Goeze, 1782) (larvae) (Figures 3<sub>C-D</sub>), *Guyanema* sp. (Figures 3<sub>E-F</sub>), *Hysterothylacium* sp. (larvae) (Figures 4<sub>A-B</sub>), *Ichthyouris* sp. (Figures 4<sub>C-D</sub>), Pharyngodonidae gen. sp., *Philometroides caudata* Moravec, Scolz & Vivas-Rodríguez, 1995 (Figures 4<sub>E-F</sub>), *Procamallanus (Spirocamallanus) inopinatus* Travassos, 1929 (Figures 5<sub>A-B</sub>), *P. (Spirocamallanus) neocaballeroii* (Caballero-Deloya, 1977) (Figures 5<sub>C-D</sub>), *P. (Spirocamallanus) rebecca* (Andrade-Salas, Pineda-López & García-Magaña, 1994) (Figures 5<sub>E-F</sub>), *P. (Spirocamallanus) saofranciscensis* (Moreira, Oliveira & Costa, 1994) (Figure 6<sub>A</sub>), *Rhabdochona acuminata* (Molin, 1860) (Figures 6<sub>B-D</sub>), *Rhabdochona* sp., and *Travnema travnema* Pereira, 1938 (Figures 6<sub>E-F</sub>).

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. *Acestrorhynchus 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 Helminológica do Instituto de Biotécnicas de Botucatu; \*new host record.

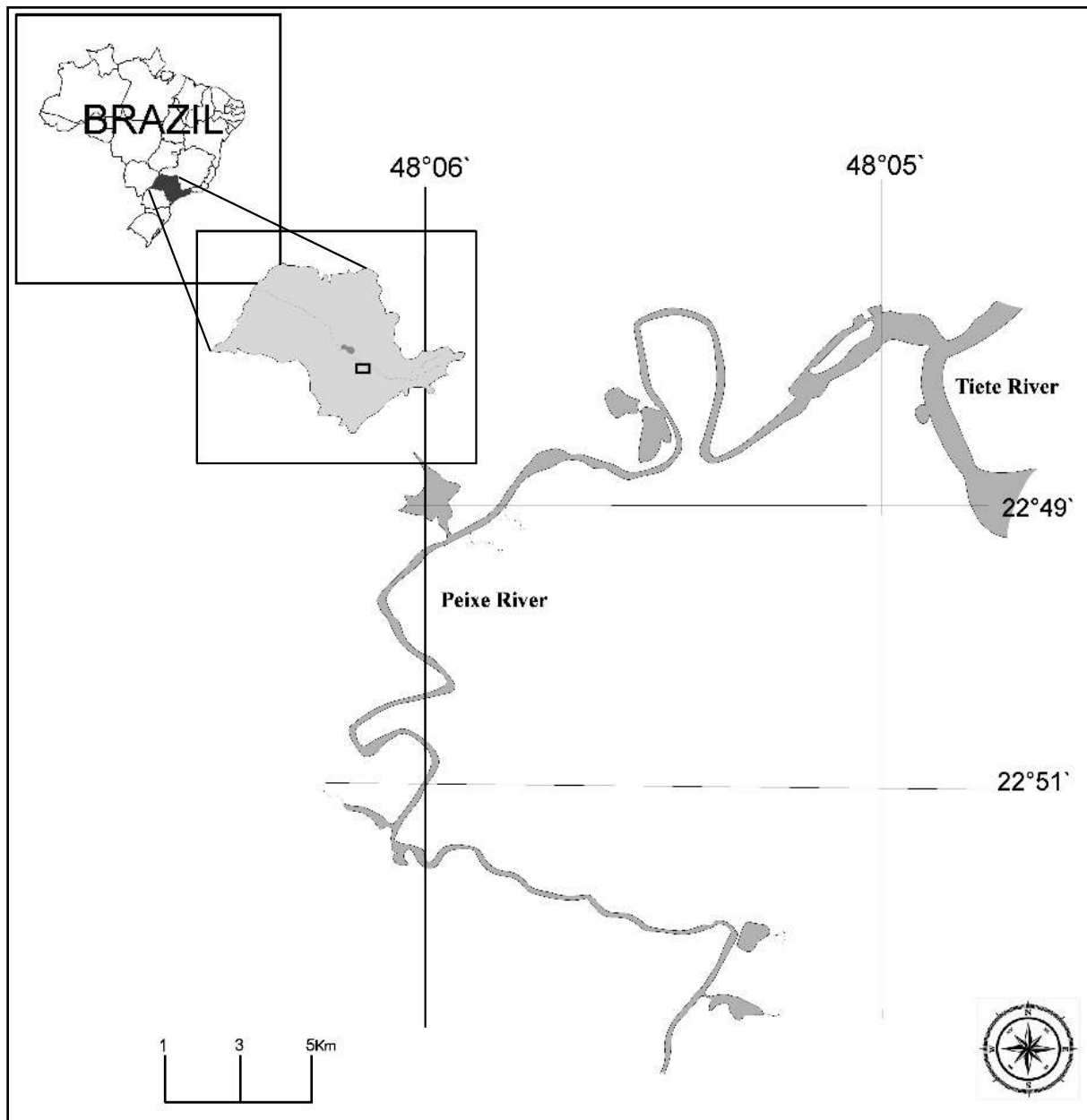
| Host   | N  | Parasite  | SI                           | P    | MI I ± SD   | MA ± SD     |
|--|----|---|------------------------------|------|-------------|-------------|
| <i>Astyanax altiparanae</i><br>Garutti & Britski, 2000 | 82 | <i>Contracaecum</i> sp. (larvae)                              | Intestine                    | 13.3 | 1           | 0.13        |
|  |    | CHIBB 6808  |                              |      |             |             |
| <i>Acestrorhynchus lacustris</i><br>(Lütken, 1875)     | 62 | <i>Procamallanus (Spirocamallanus) inopinatus</i>             | Stomach and intestine        | 73.3 | 1.45 ± 0.07 | 1.06 ± 0.05 |
|  |    | CHIBB 6809  |                              |      |             |             |
|  |    | <i>Contracaecum</i> sp. (larvae)                              | Stomach, liver and intestine | 25   | 2.40 ± 0.33 | 0.60 ± 0.08 |
|  |    | CHIBB 6810  |                              |      |             |             |
|  |    | <i>Diocotophyma renale</i> (larvae)*                          | Intestine                    | 5    | 1           | 0.05        |
|  |    | CHIBB 6811  |                              |      |             |             |
|  |    | <i>Philometroides caudata</i> *                               | Intestine                    | 10   | 1           | 0.10        |
|  |    | CHIBB 6812  |                              |      |             |             |
|  |    | <i>Procamallanus (Spirocamallanus) inopinatus</i> *           | Intestine                    | 10   | 1           | 0.10        |
|  |    | CHIBB 6813  |                              |      |             |             |
| <i>Cyphocharax modestus</i><br>(Fernández-Yépez, 1948) | 58 | <i>Procamallanus (Spirocamallanus) neocaballeri</i> (larvae)* | Intestine                    | 10   | 2           | 0.10        |
|  |    | CHIBB 6814  |                              |      |             |             |
|  |    | <i>Procamallanus (Spirocamallanus) saofranciscensis</i>       | Intestine                    | 10   | 1           | 0.10        |
|  |    | CHIBB 6815  |                              |      |             |             |
|  |    | <i>Contracaecum</i> sp. (larvae)*                             | Liver                        | 10   | 1           | 0.10        |
| <i>Cyphocharax nagelii</i><br>(Steindachner, 1881)     | 56 | CHIBB 6816  |                              |      |             |             |
|  |    | <i>Guyanema</i> sp.*  | Intestine                    | 20   | 1           | 0.20        |
|  |    | CHIBB 6817  |                              |      |             |             |
|  |    | <i>Hysterothylacium</i> sp. (larvae)*                         | Intestine                    | 10   | 1           | 0.10        |
|  |    | CHIBB 6818  |                              |      |             |             |
|  |    | <i>Ichthyouris</i> sp.*                                       | Intestine                    | 20   | 1.50 ± 0.35 | 0.30 ± 0.07 |
| CHIBB 6819   |    |   |                              |      |             |             |
| <i>Cyphocharax nagelii</i><br>(Steindachner, 1881)     | 56 | <i>Contracaecum</i> sp. (larvae)*                             | Liver and intestine          | 20   | 1           | 0.20        |
|  |    | CHIBB 6820  |                              |      |             |             |
|  |    | <i>Cosmoxyneimoides aguirrei</i> *                            | Intestine                    | 23   | 6,17 ± 7,86 | 1,43 ± 4,41 |
|  |    | CHIBB 6821  |                              |      |             |             |
|  |    | Pharyngodonidae gen. sp.*                                     | Intestine                    | 10   | 1           | 0.10        |
| CHIBB 6822   |    |   |                              |      |             |             |

(Continued Table 1)

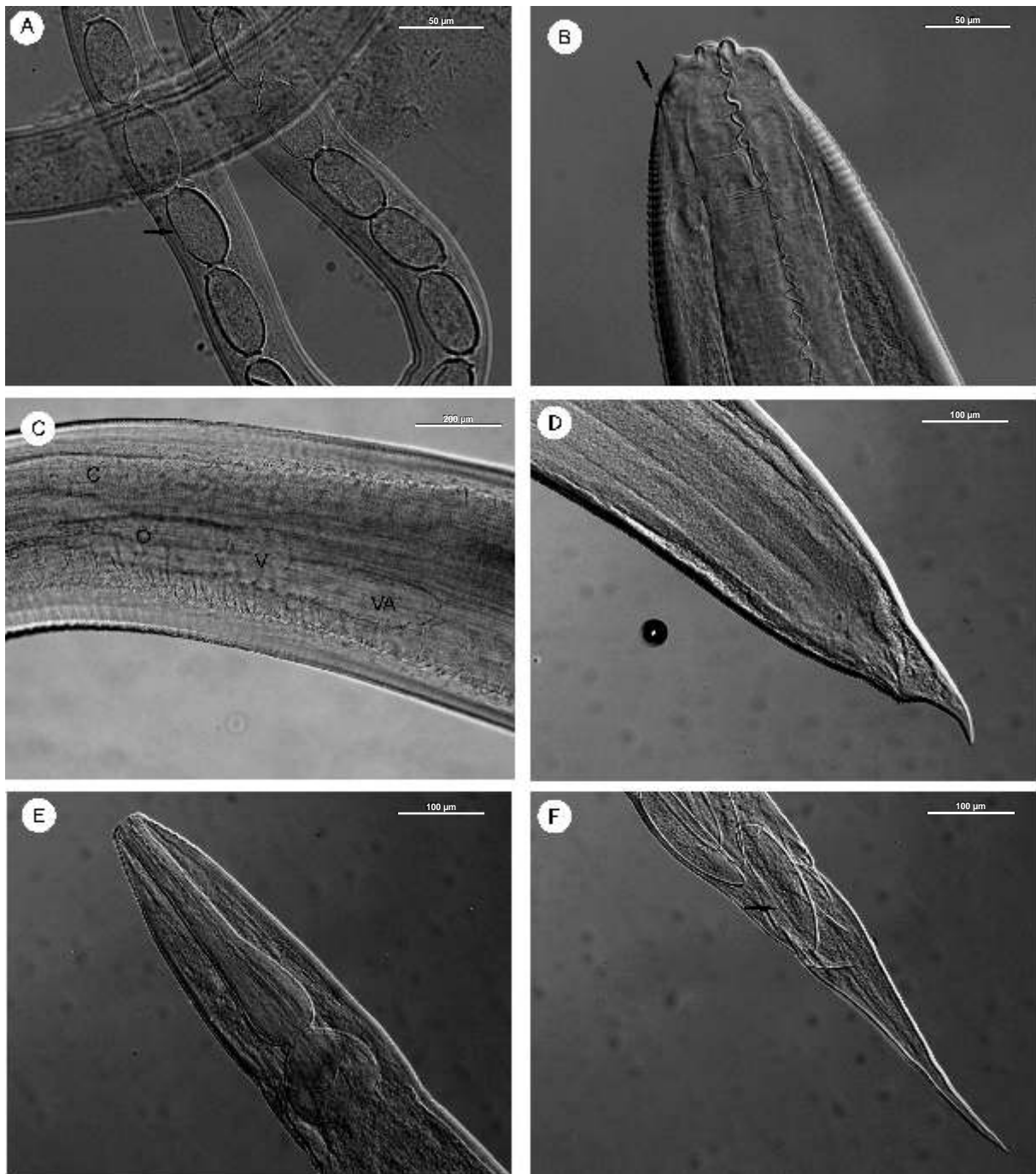
| Host   | N  | Parasite  | SI                                 | P    | MII ± SD    | MA ± SD     |
|--|----|---|------------------------------------|------|-------------|-------------|
| <i>Gymnotus silvius</i><br>Albert & Fernandes-Matioli,<br>1999 | 51 | <i>Diocotylhyma renale</i> (larvae)*<br>CHIBB 6823                      | Intestine                          | 20   | 1.50 ± 0.35 | 0.30 ± 0.07 |
|  |    | <i>Hysterothylacium</i> sp. (larvae)*<br>CHIBB 6824                     | Intestine                          | 10   | 1           | 0.10        |
|  |    | <i>Rhabdochona</i> sp.*<br>CHIBB 6825                                   | Stomach and<br>intestine           | 20   | 6           | 1.20        |
|  |    | <i>Capillariidae</i> gen. sp.<br>CHIBB 6826                             | Stomach,<br>intestine and<br>liver | 19.3 | 2.00 ± 0.21 | 0.40 ± 0.04 |
| <i>Hoplosternum littorale</i><br>(Hancock, 1828)               | 60 | <i>Cosmoxynema vianai</i><br>CHIBB 6827                                 | Intestine                          | 6.6  | 1.00 ± 0.19 | 1.00 ± 0.03 |
|  |    | <i>Guyanema</i> sp.*<br>CHIBB 6828                                      | Intestine                          | 6.6  | 0.40 ± 0.20 | 0.13 ± 0.01 |
|  |    | <i>Ichthyouris</i> sp.*<br>CHIBB 6829                                   | Intestine                          | 6.6  | 1           | 0.06        |
| <i>Triportheus angulatus</i> (Spix<br>& Agassiz, 1829)         | 17 | <i>Travnema travnema</i><br>CHIBB 6830                                  | Intestine                          | 13.3 | 2.75 ± 0.13 | 0.35 ± 0.01 |
|  |    | <i>Contracaecum</i> sp. (larvae)*<br>CHIBB 6831                         | Intestine                          | 17   | 1.00 ± 0.19 | 1.00 ± 0.03 |
| <i>Triportheus nematurus</i><br>(Kner, 1858)                   | 2  | <i>Procammallanus (Spirocamallanus) rebecca</i> (larvae)*<br>CHIBB 6832 | Intestine                          | 20   | 1           | 0.20        |
|  |    | <i>Rhabdochona acuminata</i> *<br>CHIBB 6833                            | Intestine                          | 50   | 6.00 ± 4.24 | 3.00 ± 2.12 |

Table 2. Parasitological records in host studied in others Hydrographic Basins.

| Hosts   | Nematodes  | Localities  | References  |
|---|--|---|---|
| <i>Astyanax altiparanae</i><br>Garutti & Britski, 2000      | <i>Contraecaecum</i> sp.<br><i>Procamallanus (Spirocamallanus) caballeroi</i><br><i>Procamallanus (Spirocamallanus) inopinatus</i><br><i>Spiroxys</i> sp.  | Paraná River<br>Pond in Aguai, São Paulo<br>State                                       | Takemoto <i>et al.</i> (2009)<br>Saraiva <i>et al.</i> (2006)   |
| <i>Acestrorhynchus lacustris</i><br>(Lütken, 1875)          | <i>Contraecaecum</i> sp.<br><i>Contraecaecum</i> Type 1<br><i>Contraecaecum</i> Type 2<br><i>Eustrongylides</i> sp.<br><i>Heterotyphlum</i> sp.<br>Philometridae<br><i>Procamallanus</i> sp.<br><i>Procamallanus (Spirocamallanus) paraensis</i><br><i>Spirocamallanus saofranciscensis</i><br><i>Travassosnema</i><br><i>travassosi paranaensis</i><br><i>Travassosnema travassosi travassosi</i> | Amazon Basin<br>Mogi Guaçu River<br>Paraná River<br>São Francisco River<br>Tibagi River | Buhrnheim (1976)<br>Carvalho <i>et al.</i> (2003)<br>Costa <i>et al.</i> (1991)<br>Moravec <i>et al.</i> (1993)<br>Moreira <i>et al.</i> (1994)<br>Silva-Souza & Saraiva (2002)<br>Takemoto <i>et al.</i> (2009)<br>Thatcher (2006) |
| <i>Hoplosternum littorale</i><br>(Hancock, 1828)            | Capillariidae gen. sp.<br><i>Goezia</i> sp.  | Guandu River  | Abdallah <i>et al.</i> (2006)   |
| <i>Steindachnerina insculpta</i><br>(Fernández-Yépez, 1948) | <i>Cosmoxynema vianai</i><br><i>Travnema travnema</i>  | Paraná River  | Takemoto <i>et al.</i> (2009)   |
| <i>Triportheus angulatus</i><br>(Spix & Agassiz, 1829)      | <i>Procamallanus (Spirocamallanus) inopinatus</i>  | Laguna de Yarinacocha,<br>Ucayali-Peru  | Iannacone <i>et al.</i> (2000)  |

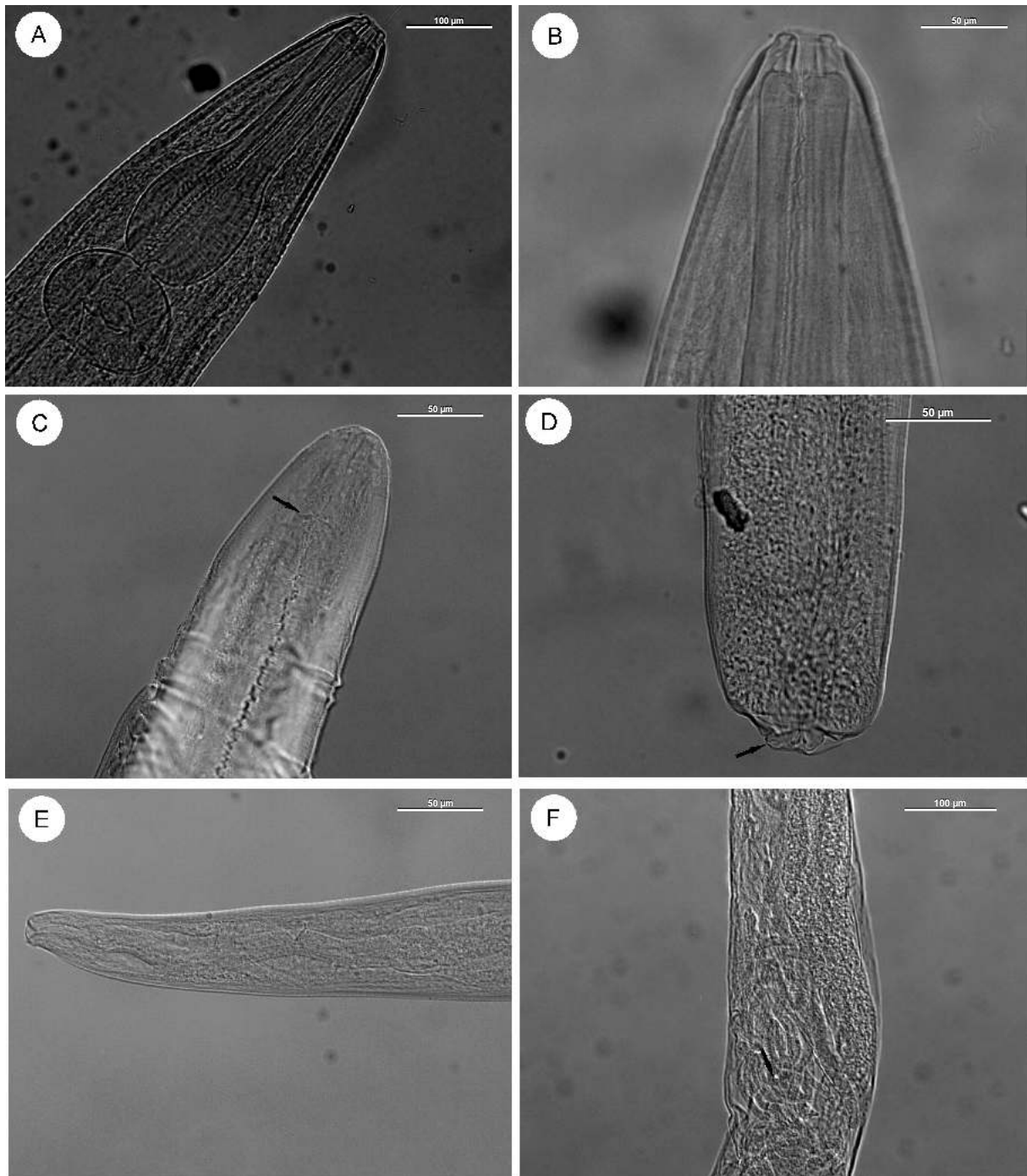


**Figure 1.** Map of the study area, showing details of river and their geographic coordinates.

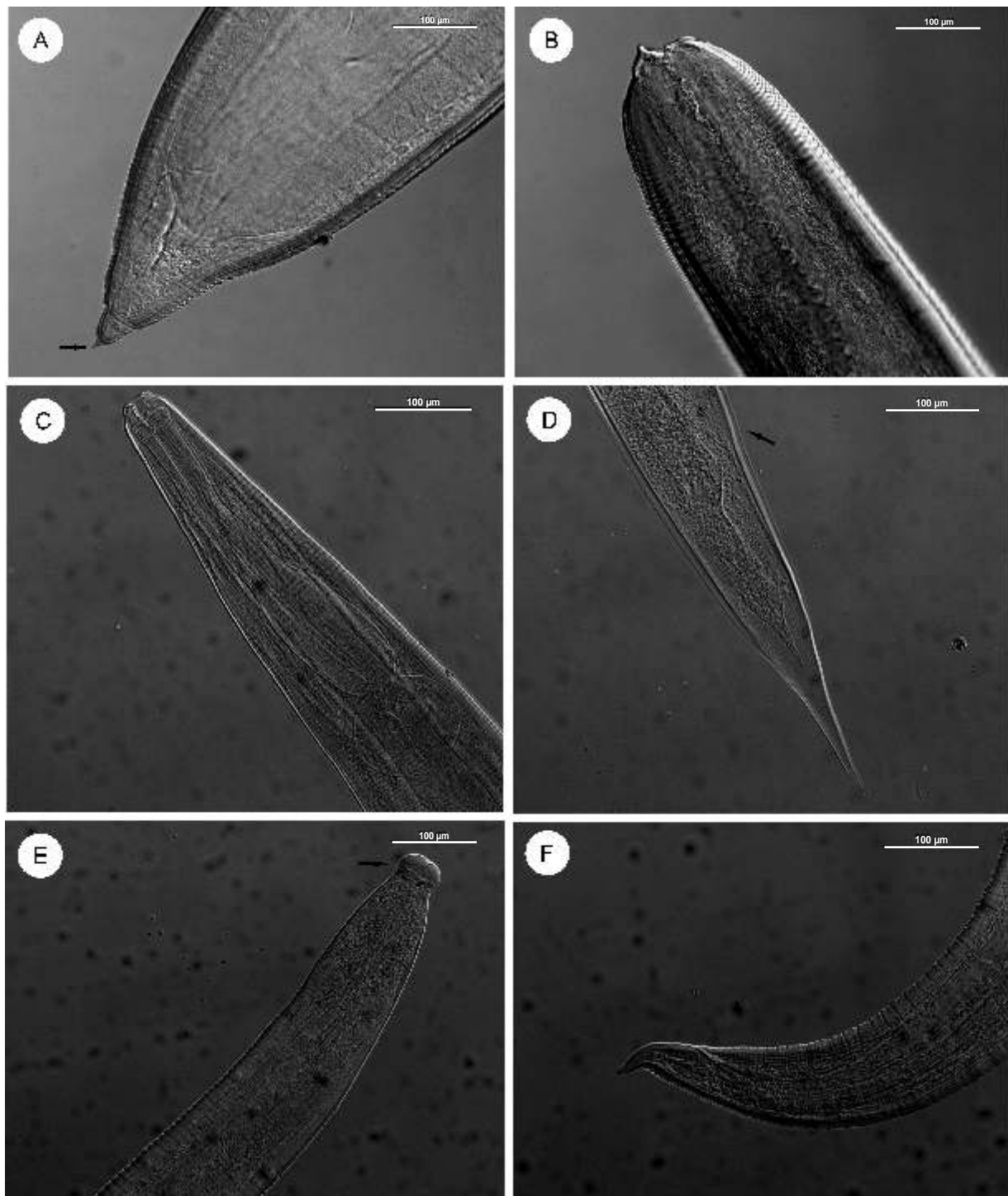


**Figure 2.** A. Detail of eggs (arrow) of Capillaridae gen. sp.; B. Cephalic extremity of *Contracaecum* sp., showing the excretory pore (arrow); C. Region of ventriculus of *Contracaecum* sp., showing (C=caecum, O=oesophagus, V=ventriculus and VA=ventricular appendix); D. Posterior extremity of *Contracaecum* sp. showing the absence of mucrons; E. Anterior end of body of *Cosmoxynema vianai*; F. Tail of *Cosmoxynema vianai* showing the eggs (arrow).

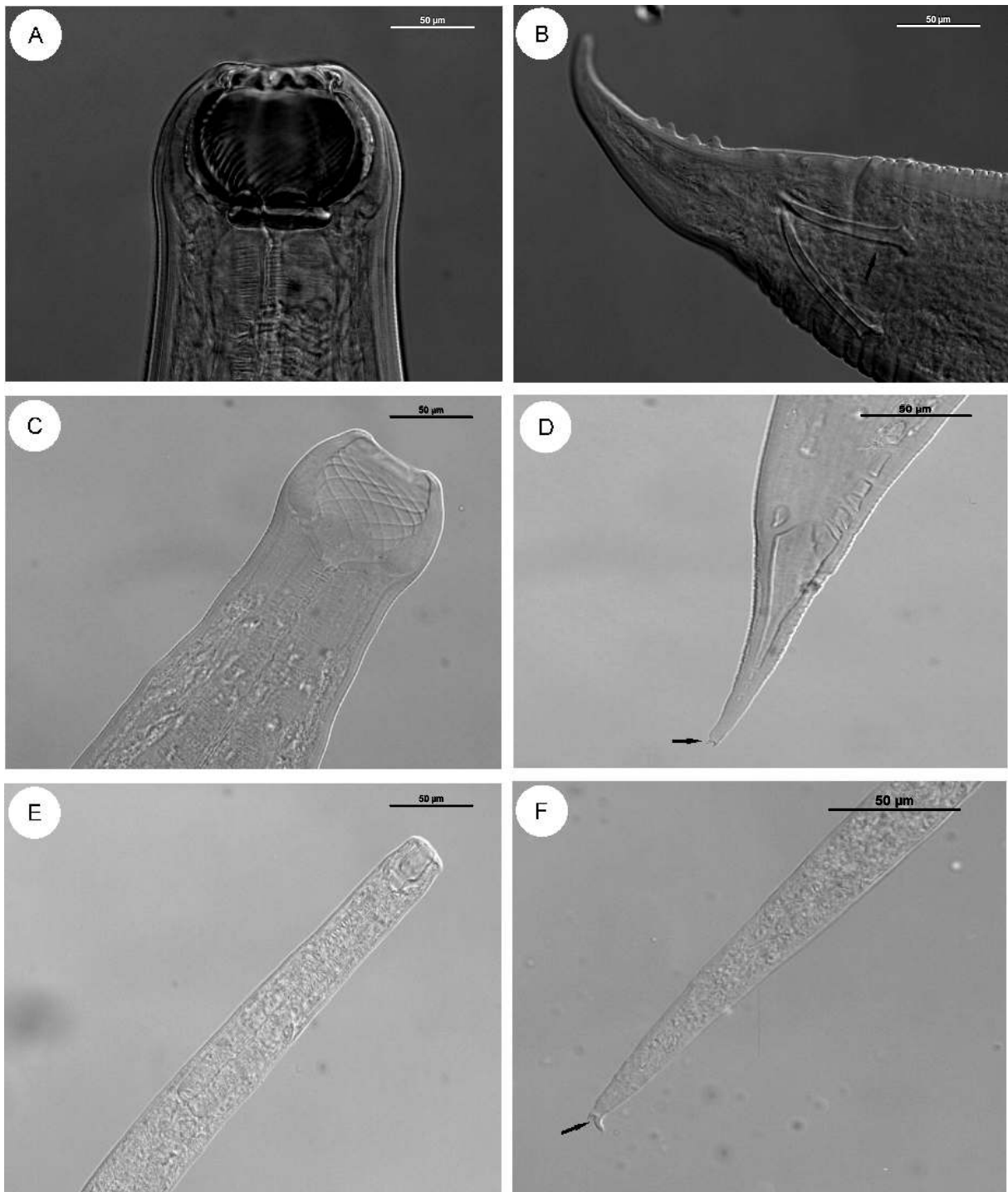




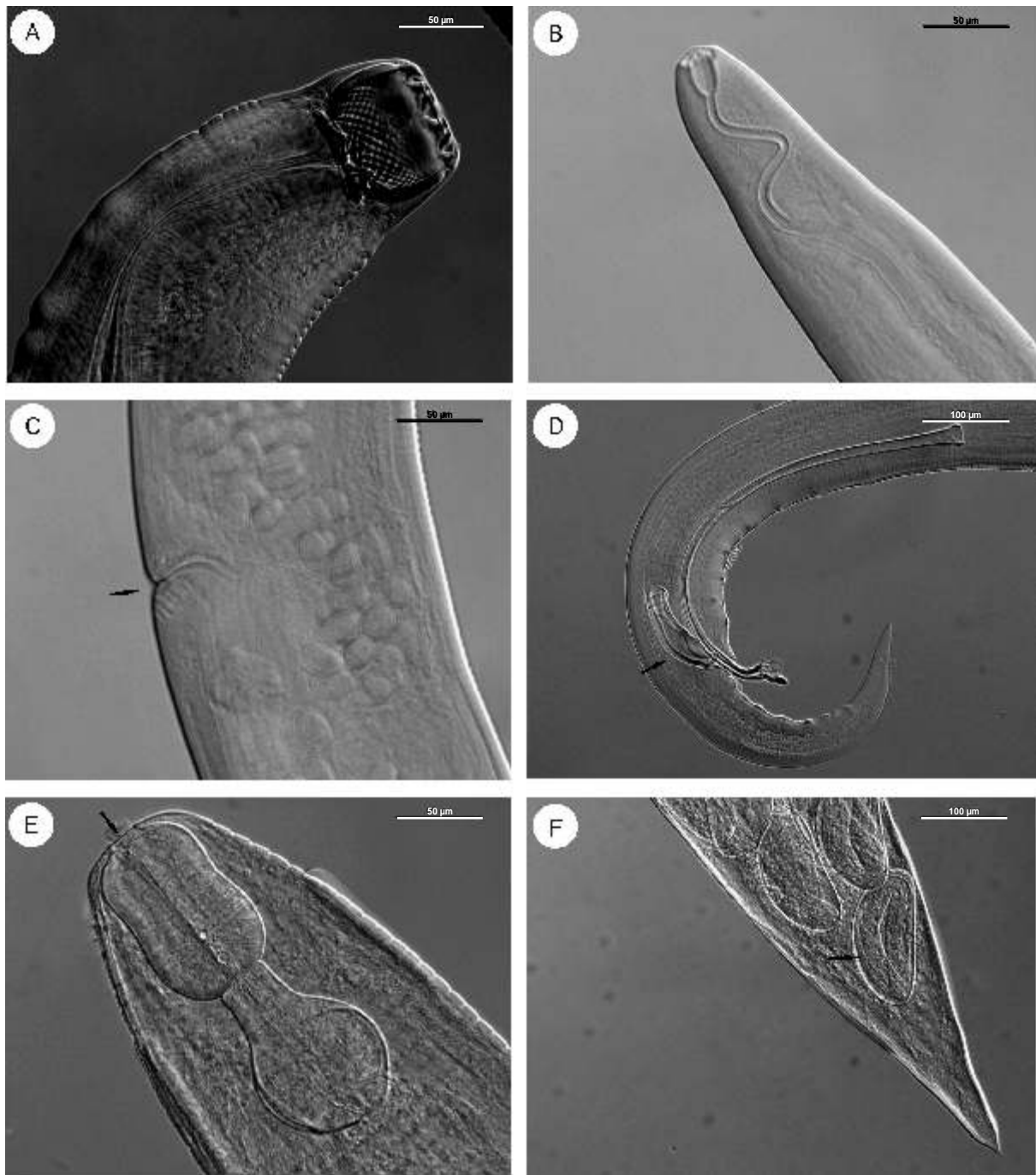
**Figure 3.** A. Anterior end of body of *Cosmoxynemoides aguirei*; B Head end of *Cosmoxynemoides aguirei*; C. Anterior end of body of *Diectophyma renale* showing the junction of oesophagus and intestine (arrow); D. Posterior end of male larva of *Diectophyma renale* showing the genital bursa (arrow); 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 *Ichthyouris* sp.; D. Tail of *Ichthyouris* sp. showing the lateral alae; E. Cephalic extremity of *Philometroides caudate*, showing the ring of slightly elevated cuticle; F. Tail of *Philometroides caudate*.



**Figure 5.** A. Buccal capsule of *Procamlanus (Spirocamallanus) inopinatus*; B. Posterior end of male of *Procamlanus (Spirocamallanus) inopinatus*, showing the spicules almost equal in length (arrow); C. Buccal capsule of *Procamlanus (Spirocamallanus) neocaballeri*; D. Posterior end of *Procamlanus (Spirocamallanus) neocaballeri*, showing the tip of tail (arrow); E. Buccal capsule of *Procamlanus (Spirocamallanus) rebecae*; F. Posterior end of *Procamlanus (Spirocamallanus) rebecae*, showing the tip of tail (arrow).



**Figure 6.** A. Buccal capsule of *Procamallanus (Spirocamallanus) saofranciscensis*; 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 (Takemoto *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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\*Author for correspondence / Autor para correspondencia:

Vanessa Doro Abdallah  
UNESP- Univ Estadual Paulista, Campus de Botucatu, Instituto de Biociências, Departamento de Parasitologia, Botucatu, São Paulo, Brazil.

E-mail/correo electrónico:  
vanessaabdallah@ig.com.br