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NEW BOOK

M. L. Sood: Fish nematodes from South Asia. Second revised and enlarged edition

Kalyani Publishes, Ludhiana, New Delhi etc., India, 2017, 1039 pp., including 752 plates of illustrations.

South Asia, representing the main part of the zoogeographical Oriental Region, is a remarkable area the fauna of which is noted for its high degree of diversity and species-richness. This also concerns fishes and their helminth parasites including nematodes, in both freshwater and marine environments. Nematodes are common parasites of fishes, in which they attack practically all body organs, parasitizing them as adults or as larvae. Fish nematodes, as one of the most significant groups of fish parasites in tropical and subtropical countries, are not only a theoretical interest, but some of them are known to be the agents of serious diseases of fishes, domestic animals and man. The significance of nematodes as important fish pathogens is also increasing with the rapid development of marine, brackish-water and freshwater aquaculture in different countries during recent years.

Regardless of the large number of mostly taxonomic and faunistic papers treating fish nematodes in South Asian countries, knowledge of the fauna of these parasites in the region in question is still scarce. Nearly twenty years ago, the much needed compilation of this extensive literature was initiated by Prof. M. L. Sood of the Punjab Agricultural University in Ludhiana, a well-known Indian helminthologist, when his comprehensive book "Fish Nematodes of South Asia" (1989) appeared, being followed by his subsequent monographs "Amphibian Nematodes of South Asia" (1990), "Reptilian Nematodes of South Asia" (1999) and "Nematode Parasites of Birds (Including Poultry) from South Asia" (2006).

During two decades since the first of the above-mentioned Sood's books was published, more than 200 additional forms of fish nematodes from this region appeared in the literature. So there was a need for the author to undertake the very difficult work to compile all the extensive materials for the second revised and enlarged edition of "Fish Nematodes of South Asia". He coped this task very well and produced the comprehensive book, which will undoubtedly serve for years as an indispensible tool for studies of fish parasites in the region.

The text of the volume is divided into 5 chapters. After the short Introduction (3 pages), the most extensive part of the book is the text entitled Description of Species (847 pages), dealing with representatives of the individual nematode taxa of the subclasses Adenophorea and Secernentea, being followed by Systematic Position of Fish Nematodes from South Asia (26 pages), Host-Parasite List (41 pages) and Literature Cited (49 pages). Included is also Appendix (29 pages), compiling mostly more recently published data on fish nematodes, followed by Author Index, Parasite Index and Host Index. In contrast to the first edition of the book, now the illustrations are placed directly in the respective texts, which makes the use of the book for readers easier. Like in the author's earlier volumes, the description, hosts, localization, distribution and relevant notes are given for each species. The majority of species are also illustrated, with line drawings and few SEM micrographs taken from the original papers.

The survey contains almost all nematode species described to date from fishes of this geographical region, including those which have already been synonymized with others (this being mentioned in accompanying remarks) or the forms (e.g. *Cylicostrongylus*, *Furconema*, *Ichthyostrongylus*, *Neocylicostrongylus*, *Neoichthyostrongylus*, *Premana*, *Pseudomazzia*, *Rastellascaris*, *Spirocotyle*) that are evidently doubtful. On the other hand, representatives of *Hakynema*, *Ichthyouris*, *Neosynodontisia* and *Royandersonia* (all Oxyuroidea) and *Orientatractis* (Cosmocercoidea), as well as *Camallanus hampalae*, *Oceanicucullanus chitwoodae*, *Procamallanus punctatus* or *Rhabdochona equispiculata*, reported from fishes in Thailand, Malaysia and Vietnam, are missing.

Although the author of this monograph has stated that the nematode fauna of fishes in South Asia is "fairly well known", this survey again documents the unsatisfactory situation in the taxonomy of these parasites in this region where, usually without a critical evaluation of previous data, a number of additional, mostly poorly described species are newly established, resulting in an apparent inflation of species. For example, Kakar *et al*. (2011) reported 17 (!) poorly described species of *Rhabdochona* from three species of fishes of Balochistan, Pakistan, although representatives of this genus are known to exhibit a rather high degree of host specificity. Therefore, future detailed taxonomic revisions in individual groups of these nematodes will undoubtedly reduce considerably the number of species of fish nematodes in this region.

Nevertheless, this monograph represents one of the fundamental ichthyoparasitological works in the countries of South Asia and may become a basis for subsequent revisions of taxonomy as well as studies on the biology, ecology and zoogeography of fish nematode parasites in this region. It will serve as an important source of information for parasitologists, biologists, veterinarians, workers in fisheries and university students, although it will also be of interest to ichthyologists, museum curators and those engaged in nature conservation.

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Biomolecular changes and cortical neurodegenerative lesions in *Trichinella spiralis* infected BALB/c mice: a preliminary study elucidating a potential relationship between systemic helminthic infections and idiopathic Parkinson's

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Summary

Idiopathic Parkinson's (IP) is a neurodegenerative disease that is suspected to be due to exposure to infections during early life. Toxoplasmosishas been the only suspected parasitic infection in IP (Celik et al., 2010). Recently, some non-central nervous system bacterial and viral infections have been incriminated in IP (Çamcı & Oğuz, 2016). So in the current study, we tried to explore if the systemic inflammatory reactions triggered by some helminths like *Trichinella spiralis* can induce Parkinsonian lesions in the brain, especially that the cerebral complications have been reported in 10-20% of *Trichinella spiralis* infected patients . An experimental study was designed to assess the neurodegenerative and biomolecular changes that may occur in *Trichinella spiralis* infected BALB/C mice in comparison to rotenone induced PD model and apparently healthy ones. The motor affection was significantly lesser in the *Trichinella* infected mice than the Parkinson's model, but when the catalepsy score was calculated (through the grid and bar tests) it was found to be significantly higher in the infected mice than in the healthy ones. A significant increase in the blood advanced oxidative protein products (AOPP), IFN-γ, TGF-β, and brain DNA fragmentation was also detected in the *Trichinella spiralis* infected mice. After histopathological examination, a significant increase in the cortical apoptotic neurons and Lewy's body were observed in the *Trichinella* infected and the rotenone induced Parkinson's model sections. A significant decrease in the immunohistochemical expression of the tyrosine hydroxylase expression in the brain sections and the ELISA measured dopamine level in the brain homogenate was also reported in the infected mice group. This study findings may collectively suggest that the systemic inflammatory reactions and the oxidative stresses associated with some systemic helminthic infections like trichinellosis are possible to precipitate neurodegenerative lesions and biomolecular changes in the brain , and manifest with IPD later in life.
Keywords: *Trichinella spiralis*, Idiopathic Parkinson's,Lewy's bodies, Tyrosine hydroxylase, DNA fragmentation, proinflammatory cytokines IFN-γ & TGF-β

Introduction

The brain is considered the most defended organ in the human body. However, this doesn't prevent the continuous trials of some parasites to invade it or to initiate a status of cerebro-vascular and meningeal irritation (Filiano *et al*., 2015). *Trichinella spiralis* (*T. spiralis*) is a nematodal helminth that can cause systemic inflammatory manifestations all over the body before habituating their final destination in the striated muscles. Infection with *T. spiralis* basically starts with ingestion of the encysted larvae in the infected undercooked pork meat. Afterward, the larvae mature into adult worm in the small intestine, and then fertilization takes place while the worms are stitched to the intestinal mucosa (Dupouy-Camet, 2000). Such location allows the newborn larvae to be spelled out

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directly into the circulation to initiate the migratory phase. The larval migratory phase is considered the most dangerous phase of infection. During such phase there is a generalized intense immune inflammatory reaction due to the high antigenicity of larvae (Pozio, 2007). Cardiac and cerebral affections are of the most dangerous complications in this phase (Hall *et al*., 2012). Within three to four weeks, most of the larvae in the circulation finally habituate the moderately active striated muscles all over the body where the chronic phase of infection will start (Gottstein *et al*., 2005).

Parkinson's disease (PD) is considered the second most frequent neurodegenerative cerebral disease all over the world (Tabrez *et al*., 2012). It gradually occurs after the death of the dopaminergic neurons, especially those located in the substantia nigra (Braak *et al*., 2007). Manifestations of PD are mainly motoric e.g. akinesia, muscular rigidity, resting tremors, motion slowness and walking difficulties. Psychological problems, autonomic dysfunction, and dementia may also occur but to a less extent (Branchi *et al*., 2008; Jillinger, 2009). A considerable number of PD patients fall under the category of idiopathic Parkinson's (IP) in which there is no clear reason for the death of the dopaminergic neurons (Barbe *et al*., 2017). There is growing evidence that exposure to high levels of injurious agents like the proinflammatory cytokines and oxidative stress product from the circulation may end with dopaminergic neurons apoptosis (Kang *et al*., 2012). Hence, early exposure to systemic pathogens and injurious environmental agents like insecticides has been incriminated in precipitating IP (Liu *et al*., 2003).

The dopaminergic neurons death results in a marked decrease in the brain dopamine level, which is one of the major catecholamine neurotransmitters. Moreover, any dysfunction in the tyrosine hydroxylase (TH) enzyme, which is responsible for the first step of dopamine synthesis, also results in a decrease in the brain dopamine level and the development of IP. Hence, TH expression and dopamine level in the brain are considered the most important diagnostic markers for PD (Daubner *et al*., 2011).

Unexplained mitochondrial dysfunction and active oxidative stress reactions products are frequently recorded before the death of dopaminergic neurons in IPD (Perfeito *et al*., 2013; Medeiros *et al*., 2016). Moreover, advanced oxidation protein products (AOPPs) are considered of the novel oxidative stress inflammatory biomarkers that enjoy high stability in the circulation after release. They are easily detected in the circulation. Hence, and can be good indicator for the incidence of oxidative stress reactions (Gonzalez *et al*., 2015; Qian *et al*., 2015).

Since *T. spiralis* triggers an aggressive inflammatory reaction with the release of a massive amount of pro-inflammatory cytokines and oxidative stress products, and has been also reported as a cerebro-vascular disease in 10 – 20 % of patients; we tried to explore if *T. spiralis* infection can precipitate Parkinsonian like lesions in the brain. For this purpose, an experimental study was designed using BALB/c mice to explore if any motor dysfunction and/or brain biomolecular and histopathological changes may occur after the migratory phase of trichinellosis.

The rotenone induced Parkinson's disease model was chosen as a reference for the motor dysfunction and the brain changes in BALB/c mice. Rotenone is a highly lipophilic insecticide that is famous for its high accessibility through the blood brain barrier,andits accumulation in the brain mitochondria create a marked oxidative stress condition (Sherer *et al*., 2003; Tansey *et al*., 2010; Pringsheim *et al*., 2014).

Material and Methods

Animals and Grouping

Thirty laboratory bred BALB/C male mice (about 8weeks old and 25 ± 5 grams) were brought from the University Laboratory Animal House. The mice were housed in polypropylene cages with wire-mesh floors (ten per cage). Temperature was maintained at 25°C, with a standard 12-hour light/dark cycle. The animals were allowed to access food and water ad libitum, and the cages were cleaned daily.

After one week of acclimatization; the mice were randomly and equally divided into three groups. Ten mice were left to represent the apparently healthy control group I. Another ten mice received the rotenone drug regimen to induce the Parkinson's disease model (group II). The remaining ten mice were prepared to receive a moderate dose of the infective *T. spiralis* larvae to induce a *T. spiralis* infection model (group III).

Induction of Parkinson's disease

Rotenone was purchased from Sigma (St. Louis, MO-USA), and suspended in 0.5 % carboxy methyl cellulose sodium salt. Each mouse in this group received 30 mg/kg/day by oral gavage for 28 days (Inden *et al*., 2009). The development of PD was confirmed by observing the development tremors, bradykinesia and rigidity in mice.

Induction of T. spiralis Infection

T. spiralis larvae were recovered from the muscles of the previously *T. spiralis* infected BALB/c mice that were bred in Tanta Medical Parasitology Department *T. spiralis* Life Cycle Maintenance Unit. The used strain had been previously genotyped and proved to be "*T. spiralis*" species by the European Union Reference Laboratory for Parasites in August 2015. The muscles of the euthanized infected mice were enzymatically digested and suspended in a measured inoculum of phosphate buffer solution (PBS) (Gamble, 1996). Each mouse in the infection group was left starving one night prior to the infection day, and then received on the next morning 0.25 ml of PBS loaded with 200 living muscle larvae by gastric gavage (Wang *et al*., 2011). After four weeks the experiment was terminated to combine between the presence of adult worms remnants in the intestine, and the larvae whether the migrating newborn ones in the circulation or the incompletely encapsulated nse in muscles to measure the changes after exposure to the maximal circulatory inflammatory products (Bruschi & Murell, 2002).

Behavioural Tests (Grid and bar tests)

By the end of the experiment, behavioural tests were done for each mouse in the three groups. Each test was repeated three times to record the average score. Afterward the animals were prepared for samples collection and scarification. Bar test was done where the mouse was placed with both fore paws on a bar 9 cm above and parallel from the base, then the time needed for removal of the paw was recorded. Grid test was done by hanging the mouse by its paws on a vertical grid 25.5 cm wide and 44 cm high with as pace of 1 cm between each wire), and then the time needed by the mouse to move its paws or any sort of first movement was recorded. Catalepsy score was calculated according to the time scale as follows: score 0: 0-10 sec, 0.5: 11 – 30 sec, 1: 31 – 60 sec, 1.5: 61 – 90 sec, 2: 91 – 120 sec, 3: 121 – 150 sec, 3.5: 151 – 180 sec, 4: 181 – 210 sec, 4.5: 211 – 240 sec, and 5: >240 sec. Each test was measured for 4 min and each animal underwent three consecutive trials with 5 – 10 min interval between the tests. Catalepsy score of the test (mobility time in seconds) was done by calculating the sum of the score of the ten animals for one trial in each group, then calculating the mean for the 3 trials (Alam & Schmidt, 2004; Ghosh *et al*., 2011; Makhija & Jagtap, 2014).

Samples collection

Plasma

After the induction of general anaesthesia by halothane, the ventral chest area was cleaned with 70 % ethanol. Afterward, blood was slowly aspirated by intracradiac puncture (Parasuraman *et al*., 2010) and sample was collected in sodium citrate treated tubes to be centrifuged at 2,000 x g. The resultant supernatant was equally divided and transferred into three clean eppendorf tubes to be stored at -20°C till use.

Brain extraction

While still under anaesthesia, each mouse was euthanized by cervical dislocation. First, the head was sprayed with 70 % ethanol, and then decapitation was done at the base of the brain stem. The head was held at the base of the skull, and then a cut was done between the two olfactory bulbs by placing one blade of a small pair of scissors into each eye cavity and cutting coronally. Another two lateral cuts were done at the base of the skull, followed by a longitudinal one along the sagittal suture. The brain was exposed by peeling back the skull with a pair of curved forceps, and then the brain was freed by using a small spatula. The brain was immediately placed in cold PBS to be rinsed and then transferred in 10 cm plastic Petri dish containing PBS. Each brain was positioned on its ventral surface and divided longitudinally into two halves (Walker & Kempermann, 2014). One half was wrapped in foil paper to be preserved under -20°C till homogenization, and the other half was preserved in 10 % formaldehyde for histopathological examination.

Biochemical study

Detection of advanced oxidation protein products (AOPPs) in plasma

AOPPs levels were determined spectrophotometrically according to Witko-Sarsat *et al*. (1996) by semi-automated method on micro-plate reader (Model MR 5000; Dynatech, France). A volume of 200 µl of plasma diluted 1:5 in 50 mM Phosphate buffer saline PBS, pH: 7.4 was placed in test wells, followed by the addition of 10 µL of 1.16 M potassium iodide (KI) to each well, and then 20 µl of absolute acetic acid 2 min later. In standard wells, 10 µl of 1.16 M KI were added to 200 µl of chloramine-T solution 0 – 100 µmol/liter (Sigma, St, Louis, MO) followed by 20 µl of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm against a blank containing 200 µl of PBS, 20 µl of acetic acid and 10 µl of KI.

IFN-γ and TGF-β detection in plasma

IFN-γ was detected by Mouse IFN Gamma PicoKine™ ELISA kits purchased from Boster biological technology,California, USA, Catalog Number: EK0375. TGF-β was detected by Mouse TGF-beta1 Platinum ELISA kits purchased from e-bioscience, Vienna, Austria, Catalog Number: BMS608/4. Procedures were done according to the manufacturer's protocol for each kit.

Detection of DNA fragmentation in brain samples

DNA fragmentation was measured by the diphenylamine (DPA) method described by Zhu *et al*. (1998). Briefly, 20 mg of brain tissue was cut and lysed in 250 µl of the lysis buffer Tris/HCl 5 mM pH 8.0, Triton X-100 0.5 % and EDTA 20 mM with vigorous stirring, and then the mixture was incubated for 10 min at 4°C. After centrifugation at 10,000 x *g* for 20 min at 4°C fragmented the intact chromatin pellet (by centrifugation at 10,000xg for 20 min at 4°C) was transferred into a new clean tube. The pellet was dissolved in 500 µl TEX buffer, treated with 500 µl 10 % trichloroacetic acid (TCA), then centrifuged at 5000 x *g* for 10 min at 4°C. The supernatant was discarded and the precipitated DNA pellet was resuspended in 250 µl of 5 % trichloroacetic acid and boiled for 15 min. Twenty µl of 6M perchloric acid was added to both the lysate supernatant and the treated pellet sample, in addition to 500 µl of a freshly prepared diphenylamine reagent 1.5g diphenylamine, 1.5 ml concentrated sulfuric acid and 19 µl acetaldehyde dissolved in 100 ml glacial acetic acid). The reaction mixture was incubated for 18 h at 37°C. Absorbance was measured by a semi-automated-spectrophotometer (Robonik-Prietest-Touch, India) at 600 nm, against freshly prepared diphenylamine reagent as a blank. The percentage of fragmented DNA was calculated as follows:

$$\text{Fragmented DNA} = \frac{\text{Amount of fragmented DNA in supernatant X 100}}{\text{Amount of fragmented DNA in supernatant + amount of DNA in pellet}}$$

Table 1. Catalepsy score of Grid & Bar tests in the study groups.

	Grid test						
	Mean	SD	Kruskal-Wallis Test		Dunn's Multiple Comparisons Test		
			f	*P*-value	G: I & II	G: I & III	G: II & III
G-I (N=10)	6.27	0.96					
G-II (N=10)	41.55	3.44	17.818	0.0001	<0.001	>0.05	>0.05
G-III (N=10)	25.49	2.24					
	Bar test						
	Mean	SD	Kruskal-Wallis Test		Dunn's Multiple Comparisons Test		
			f	*P*-value	G: I & II	G: I & III	G: II & III
G-I (N=10)	7.06	1.18					
G-II (N=10)	37.9	3.07	17.818	0.0001	<0.001	>0.05	>0.05
G-III (N=10)	22.99	1.6					

N= number, G-I: healthy control, G-II= Rotenone induced Parkinson's model, G-III= *T. spiralis* infected group

Dopamine level in brainby ELISA

The Dopamine Research ELISA kit (Rocky Mountains Diagnostics, Inc., Colorado Springs, CO) was used to detect the dopamine level in the brain homogenate according to the manufacturer protocol. Each sample was divided and processed in duplicates (Ustione & Piston, 2012). The dopamine concentration range in the standard curve was 36 – 3600 pg.

Histopathological study

Histopathological staining by H&E and evaluation by image J-analyzer

Formalin fixed brain samples were processed, and embedded in paraffin blocks. Tissue sections of 4 μm thick were prepared for the hematoxylin and eosin (H&E) and the immunohistochemical staining. First, the sections were blindly examined by two pathologists to observe any neuronal loss, apoptotic neurons, cytoplasmic vacuolation, and detect Lewy's body formation in the infected and healthy sections and compare them to the typically detected changes in the brain sections etrieved from the rotenone induced Parkinson's model (Westin *et al*., 2010). Apoptotic neurons were counted on images from 10 random fields per slide using image-J software (Java image processing program inspired by National institute of health NIH, USA) (Schneider *et al*., 2012).

Immunohistochemical staining for Tyrosine Hydroxylase

The previously prepared brain sections were deparaffinised in xylene, rehydrated in descending concentrations of alcohol then washed in PBS phosphate buffer saline. After antigen retrieval by boiling in 10 mmol/L citrate buffer pH 6.0) for 10 minutes in a microwave, slides were immersed in 3 % hydrogen peroxide in order to block endogenous peroxidase. Background staining was blocked by placing slides in Ultra V Block Labvision, TA-015-UB, USA) for 5 minutes. Afterward, the slides were incubated with anti-tyrosine hydroxylase rabbit monoclonal antibody (Abcam, ab75875, EP1533Y, UK) in 1/200 dilution for 10 minutes at room temperature. Ultravision detection kits (TA-015-HD) were used,

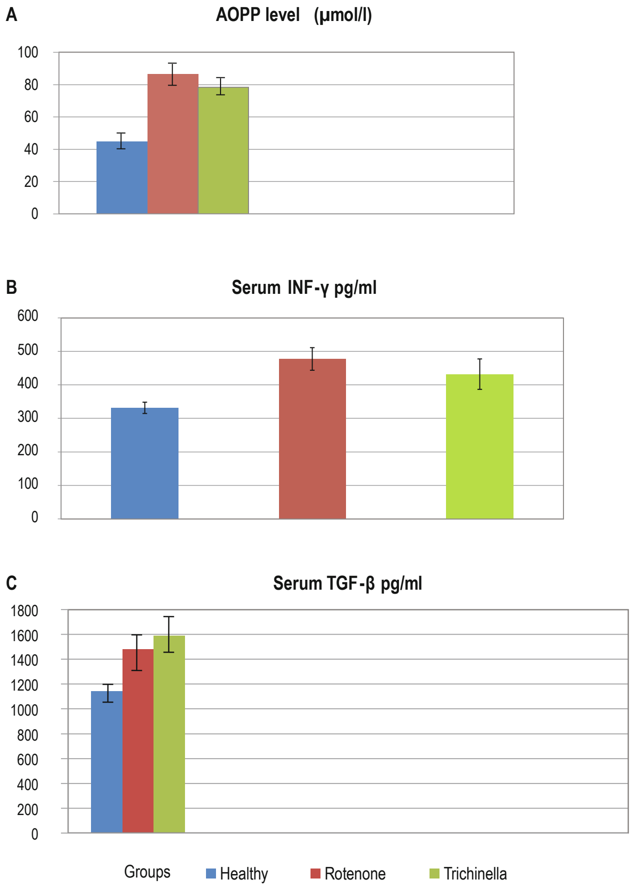

Fig. 1. A figure collects the bar charts that illustrate the statistical analysis for the AOPP (part A), IFN-γ (part B) and TGF-β (part C) levels in the different study groups. **A** – AOPP mean±SD reached 78.300±7.7331 μmol/L in the *Trichinella* infected group, 44.529±6.867 μmol/L in the healthy control group and, 86.543±9.416 μmol/L in the Parkinson's model. **B** – IFN-γ mean ±SD reached 477.69±33.73 pg/ml in the *Trichinella* infected group, 331.73±16.87 pg/ml in the healthy control group and, 432.30±45.43 pg/ml in the Parkinson's model. **C** – TGF-β mean±SD reached 1589.6±115.7 pg/ml in the *Trichinella* infected group, 1143.5±50.9 pg/ml in the healthy control group and, 1480.0±100.5 pg/ml in the Parkinson's model.

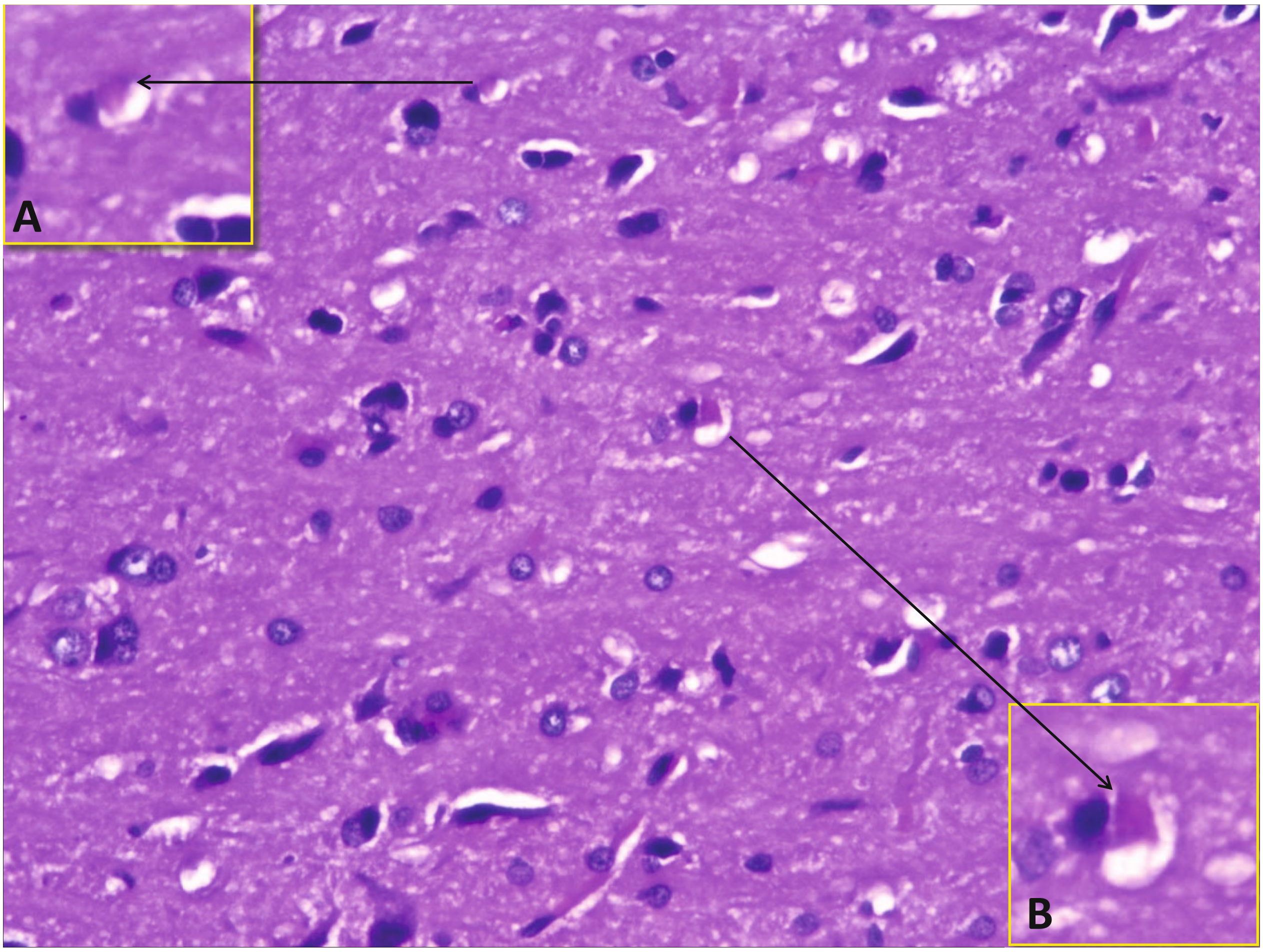

Fig. 2. H&E stained brain section (x400) from the rotenone induced Parkinson's model (group II) demonstrates the shape of Lewy's bodies that were used as a reference during examination of the other groups sections. By higher magnification (x1000 in A & B) Lewy's bodies appear as homogeneous eosinophilic cores surrounded by pale eosinophilic halos.

after incubating slides with biotinylated goat anti-polyvalent and then streptavidin peroxidase for 10 minutes with each of them. The DAB (diaminobenzidine) tetrachloride was used as a chromogen and the slides were counter stained with Meyer's haematoxylin.

Tyrosine hydroxylase immunoreactivity was determined as brownish staining on neurons and fibers. Image-J software was used to determine the number of TH immune reactive neurons and percentages of TH-stained tissue. For each slide, 10 random fields were captured then opened by image-J software. Mean area of all tissue included as well as TH-stained tissue were measured, and then the percentage of TH-stained tissue was calculated. The TH-stained neurons were counted as described by Fuduka *et al*. (1999).

Statistical analysis

Statistical analysis was performed using GraphPad prism 7. Data were expressed in terms of means±standard deviation) for continuous variables. Kruskal – Wallis test was performed to compare between groups followed by Dunn's multiple comparisons post-test. P value less than 0.05 was considered statistically significant.

Ethical Approval and/or Informed Consent

All procedures were performed according to the standards guidelines for researches on experimental animals, and after taking permission from Tanta University Faculty of Medicine Ethical Committee for Researches (permission code 31273/12/16).

Results

Induction of Parkinson's disease in group II was confirmed by the motor changes and the behavioural tests scores, while infection of group II mice was confirmed by visualization of *Trichinella* larvae in diaphragms specimens pressed between two glass slides and checked under microscope.

Table 2. Brain DNA fragmentation and dopamine level.

	Percentage of DNA fragmentation						
	Mean	SD	ANOVA		Dunn's Multiple Comparisons Test		
			f	*P*-value	G: I & II	G: I & III	G: II & III
G-I (N=10)	5.96	1.117	13.900	0.001	<0.01	<0.05	>0.05
G-II (N=10)	21.634	6.088					
G-III (N=10)	17.633	5.001					
	Tissue dopamine level ng/l						
	Mean	SD	ANOVA		Dunn's Multiple Comparisons Test		
			f	*P*-value	G: I & II	G: I & III	G: II & III
G-I (N=10)	1.066	0.026	14.542	0.0007	<0.001	<0.05	>0.05
G-II (N=10)	0.419	0.037					
G-III (N=10)	0.463	0.050					

N= number, G-I: healthy control, G-II= Rotenone induced Parkinson's model, G-III= *T. spiralis* infected group

Behavioural Test

As demonstrated in Table1; the mean score of rotenone induced Parkinson's group was significantly higher than in healthy and *Trichinella* infected groups. Still, the latter group showed a higher mean score in both tests (25.49 and 22.99) when compared with control (healthy) mice.

Biochemical study

AOPPs in plasma

AOPPs level was significantly elevated in *Trichinella* infected group III (78.300±7.7331) than in healthy control group I (44.529±6.867). No significant variation in AOPPs was recorded between rotenone induced Parkinson's model samples and *Trichinella* infected model mice (P>0.05).

IFN-γ and TGF-β in blood

IFN-γ showed a significant variation among the three groups (p=0.0006), with the highest level detected in *Trichinella* infected group (477.69 ± 33.73), followed by IPD (432.30 ± 45.43). IFN-γ was significantly higher in *Trichinella* infected group than healthy group (331.73 ± 16.87). Groups II and III also showed a significantly higher levels of TGF-β; 1480.0 ± 100.5, 1589.6 ± 115.7, respectively) than healthy control group (1143.5 ± 50.9), with no significant difference between them.

Figure 1 demonstrates bar charts for the statistical analysis of biochemical parameters measured in blood (AOPP, IFN-γ and TGF-β).

DNA fragmentation detection in brain tissue

There was a significant difference in brain DNA fragmentation among the study groups (p<0.001). It was significantly elevated inboth the Parkinson's and *Trichinella* infected groups, with no significant difference between them.

Dopamine level in brain homogenate

A marked reduction of dopamine level was detected in the brain samples from both the *Trichinella* infected mice and Rotenone induced Parkinson's groups. Still, the latter group showed a sig-

Table 3. Number of apoptotic neurons and Tyrosine hydroxylase (TH) immunoreactive neurons.

	Apoptotic neurons						
	Mean	SD	Kruskal-Wallis Test		Dunn's Multiple Comparisons Test		
			f	*P*-value	G: I & II	G: I & III	G: II & III
G-I (N=10)	26.86	3.04	14.74	<0.0001	0.029	0.001	0.734
G-II (N=10)	79.14	10.19					
G-III (N=10)	89.29	11.44					
	TH-immunoreactive neurons						
	Mean	SD	Kruskal-Wallis Test		Dunn's Multiple Comparisons Test		
			f	*P*-value	G: I & II	G: I & III	G: II & III
G-I (N=10)	18.71	3.15	14.38	<0.0001	0.001	0.019	0.999
G-II (N=10)	3.86	1.46					
G-III (N=10)	2.71	1.03					

N= number, G-I: healthy control, G-II= Rotenone induced Parkinson's model, G-III= *T. spiralis* infected group

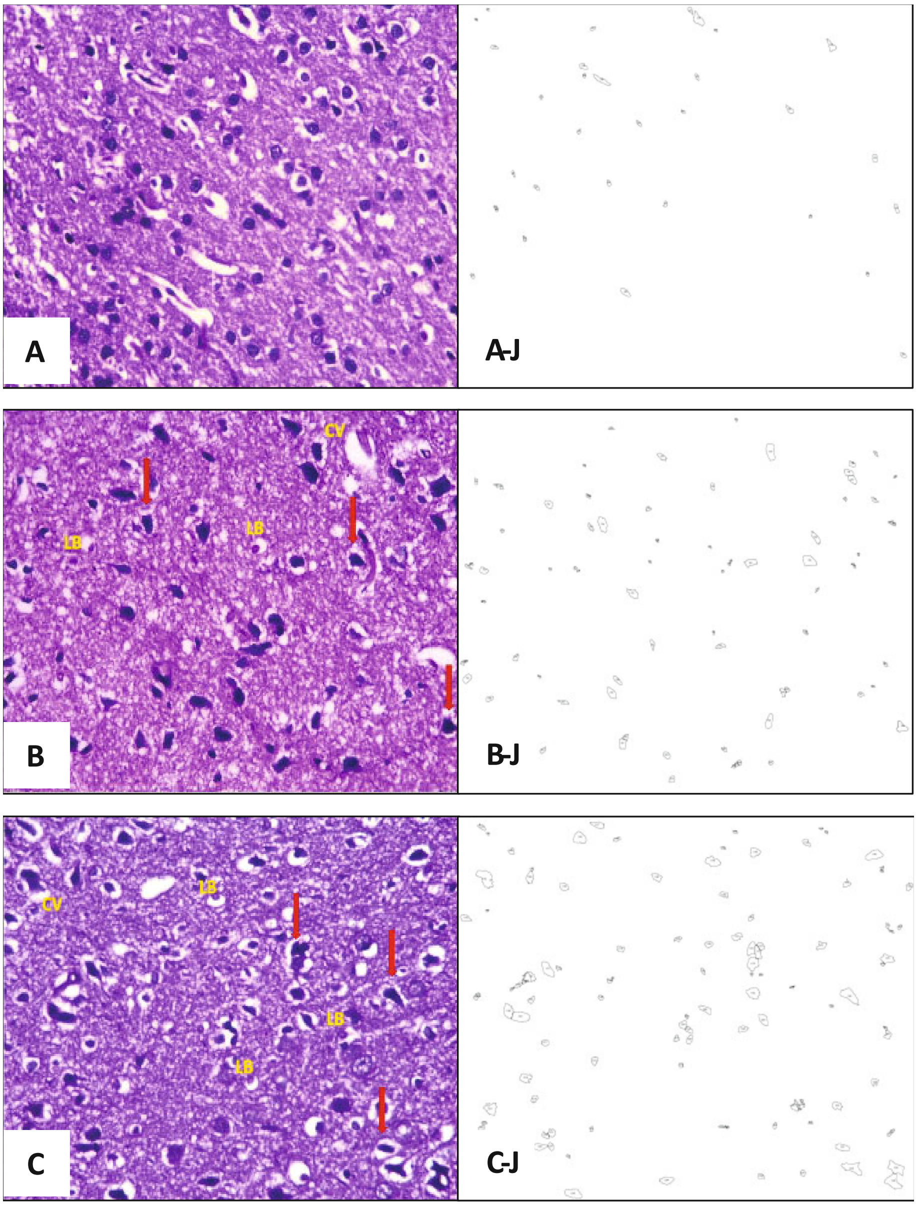

Fig. 3. A plate of H&E stained cortical sections (x400) from the different study groups (A, B &C) and the 8-bit images of the same sections (A-J, B-J & C-J) that show the outlined apoptotic neurons counted by image-J analyzer after normal neurons nuclei subtraction. **A** – section from cortical tissue of healthy control mouse (group I), showing normal neurons with round nuclei and prominent nucleoli. **B** – a section of cortical tissue from rotenone induced Parkinson's model mouse (group II), showing high density of the darkly stained pyknotic nuclei (arrow), cytoplasmic vacuolation, (CV), and Lewy's bodies (LB). **C** – a section from cortical tissue of *Trichinella spiralis* infected mouse (group III), showing higher number of the darkly stained pyknotic nuclei (arrow), cytoplasmic vacuolation, (CV), and Lewy's bodies (LB) in comparison to the section from the healthy group.

nificantly lower level. The results of brain DNA fragmentation and dopamine level are illustrated in Table 2.

Histopathological results

H&E

Sections from healthy control group showed apparently normal tissue that consists of large neurons with pale nuclei and prominent nucleoli. Sections from Rotenone group and *Trichinella* infected group revealed cortical atrophy with neuronal loss, increased apoptotic neurons and cytoplasmic vacuolation. Scattered Lewy's bodies, as exemplified in Figure 2, were detected in both groups. The number of apoptotic neurons in groups II and III (Mean+SD=79.14 ± 10.19, 89.29 ± 11.44, respectively)were significantly higher than in healthy mice (26.86 ± 3.04) as compared in Figure 3.

Tyrosine hydroxylase expression

Tyrosine hydroxylase (TH) immune reactivity was determined

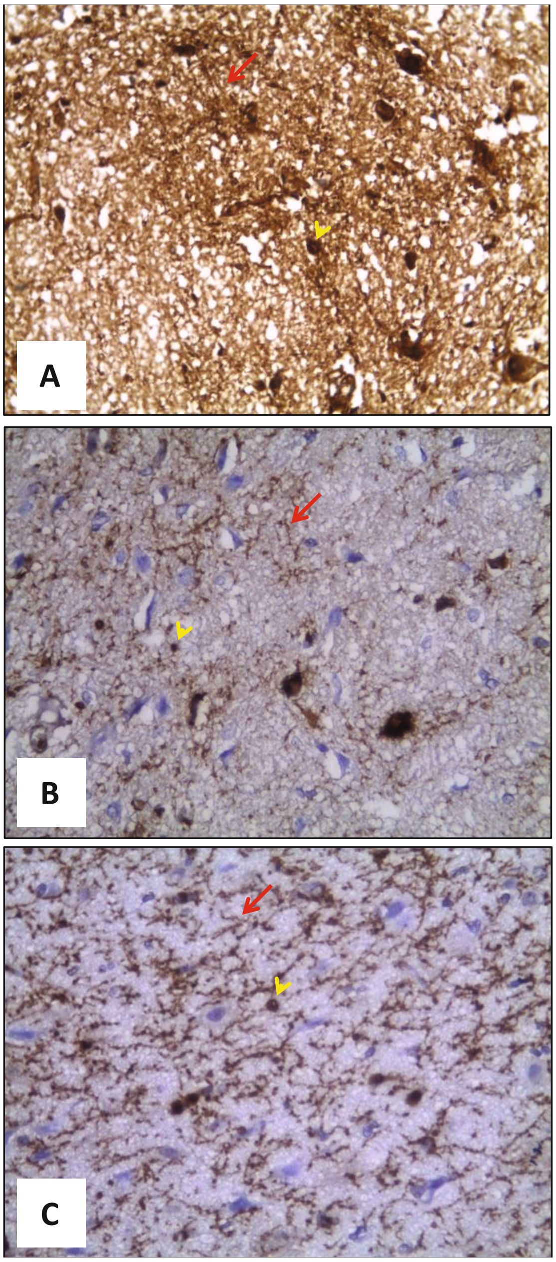

Fig. 4. Tyrosine hydroxylase immuno stained sections (x400) from cortical tissue of the different study groups. The yellow arrow heads are pointing at the neuron heads, while the red arrows at the fibers. **A** – a section from healthy control (group-I) with strong immune positivity of TH in both cortical neurons and fibers. **B** – a section from cortex of a Rotenone induced Parkinson's model mouse (group-II) with marked loss of TH immune stained neurons and fibers. **C** – a sections from cortex of a *T. spiralis* infected mouse (group-III) with marked loss of TH immune stained neurons and fibers in comparison to the healthy group.

as brownish staining in cortical neurons as well as in cortical fibers. A significant decrease in thepercentage of TH reactive neurons was detected in the cortical areas of *Trichinella* infected group (mean % III= 24.29 ± 2.19), in comparison to healthy one (mean % I= 73.71 + 6.45). No significant difference was observed between groups II and III in the percentage of reactive neurons (mean % II= 21.43 + 2.13). Tyrosine hydroxylase expression in the different study groups is exemplified in comparative sections in Figure 4. TH immunoreactivity was highly expressed in brain sections from the healthy group (Mean±SD=18.71+3.15). The least expression of TH reactive neurons was observed in *Trichinella* infected mice (2.71+ 1.03). The mean expression in the Parkinson's model mice was 3.86 ± 1.46, which is in between the expression in the control and the *T. spiralis* infected model.

The numerical data of the histopathological study are demonstrated in Table 3.

Discussion

Idiopathic Parkinson's (IP) is a neurodegenerative disease that develops after an unexplained death of the dopaminergic neurons. Many researchers have linked between IP and exposure to infectious agents during early life. Interestingly, some of the incriminated organisms are not brain pathogens e.g. *Helicobacter pylori* (Çamcı & Oğuz, 2016), *H5N1 influenza* virus and *Hepatitis C* virus (Jang *et al*. 2009; Wu *et al*., 2015). The mechanisms involved in the development of IP by these organisms vary between neurotoxins production (Schulz *et al*. 2006), microglial activation, phosphorylation of the presynaptic neuronal protein "α-Synuclein" (Senzolo *et al*., 2011), and the release of a massive amount of proinflammatory cytokines and oxidative stress products (Arai *et al*., 2004).

Regarding the relation between parasitic infections and IP; *Toxoplasma gondii* has been the only suspected parasite because of its ability to disrupt the dopaminergic neurotransmission, and the reported high anti-*Toxoplasma gondii* seropositivity in IP patients in previous studies (Celik *et al*., 2010; Cook *et al*., 2015; El Gendy *et al*., 2017). The current study is considered the first to explore if the inflammatory reactions induced by a systemic helminth like "*Trichinella spiralis*" can trigger Parkinson's like lesions in the brain. Actually the brain can be affected by the parasites in two ways; first by the establishment of parasitic niches, cysts or granulomatous lesions within its tissue as occurs in neurocysticercosis, amoebic encephalitis and toxoplasmosis (Masocha & Kristensson, 2012), and second by the generalized inflammatory reactions that some parasitic infections can induce like malaria, schitososmiasis and visceral larva migrans (Pittella, 2013).

Regarding our study findings of the behavioural motor affection; the catalepsy score of the grid and bar tests was higher in the *Trichinella* infected mice than in the healthy group, but lesser than in the Parkinson's group. In fact, various neurological manifestations like behavioural disorders, tetraparesis and oculomotor paralysis have been previously reported in *T. spiralis* infected patients. However, these are considered uncommon manifestations in trichinellosis, and the mechanisms involved in pathogenesis are not clearly elucidated yet (Compton *et al*., 1993; Neghina *et al*., 2012).

A significant increase in the apoptotic dopaminergic neurons and a significant decrease in both the brain dopamine level and the tyrosine hydroxylase (TH) expression were observed in the brain samples from the *T. spiralis* infected group of mice. The detected lesions in the infected mice were mainly cortical. In fact, cortical dopaminergic neurons apoptosis has been previously reported in Parkinson's and dementia patients (Fukuda *et al*., 1999; Marui *et al*., 2003). According to Asmus *et al*. (2008) there is a subpopulation of cortical interneurons that can produce TH. A little is still known about these cortical cells despite of their potential relevance to the dopaminergic cortical circuit across the mammalian species (Benavides-Piccione & DeFelipe, 2007).

In 1999 Olson documented that in cerebral trichinellosis the lesions can be limited to neurons apoptosis, non purulent meningitis, and cellular infiltration. The tight junctions of the blood brain barrier (BBB) prevent the passage of larvae from the circulation (Ginhoux-*et al*., 2013). This may explain why no larvae or granuloma were detected in the brain sections retrieved from the infected group of the current study. According to Kristensson *et al*. (2015) only severe endothelia irritation, arachnoidal vasculitis and microglial activation can be seen in CNS trichinellosis. In the other hand, Lewy's bodies (LB) were significantly detected in the cortical areas of the infected mice. They are considered a major parkinsonian histopathological finding that was observed in the brain sections from the infected mice (Filiano *et al*., 2015). The LB formation is caused by accumulation of insoluble fibrils of phosphorylated α-synuclein within the cytoplasm of the nerve cells (Prandovszky *et al*., 2016). α-synuclein is an important protein for the maintenance of the synaptic vesicles supply in the presynaptic terminals and regulation of dopamine release (Burn *et al*., 2004). Normally, a small fraction of α-synuclein (<4 %) becomes phosphorylated in healthy brains (Caggiu *et al*., 2016). In PD, neurons start to release small amounts of α-synuclein by exocytosis and the majority remains accumulated in the cytoplasm. Such abnormal accumulation of α-synuclein elicits a vigorous immune response (Lee *et al*., 2014). Interestingly, cross-reactivity between human α-synuclein peptides and some infectious agents like HSV1 is now suspected after the detection of high amounts of antibodies against HSV1 homologous polypeptides in PD patients (Caggui *et al*., 2016). Hence, the potentiality of the presence of a similar cross-reactivity between α-synuclein proteins and *T. spiralis* (or other parasitic) polypeptides (e.g. the somatic antigens and the secretory-excretory products of the adult worms and larvae) can't be excluded, especially after the detection of brain lesions in the *Trichinella* infected mice.

According to Allen Reish and Standiert (2015) both the innate and adaptive immunity are prominently activated in PD that even the interactions between them can modify the pathological process. In an experimental study on Parkinsonian monkeys; IFN-γ was documented as the most persistently high pro-inflammatory cytokine in the circulation (Mizuta *et al*., 2001). Also in clinical studies on the sera of PD patients; high IFN-γ level was reported (Barcia *et al*., 2011; Abdeldayem *et al*., 2014). Such elevation was explained as the result of glial cells activation by some infectious agents and the subsequent trials of inflammatory cells to pass the blood brain barrier and attack them (Banks, 2005; Mount *et al*., 2007). According to Perry (2004) IFN-γ is very injurious to the sensitively packed dopaminergic neurons. In the current study, IFN-γ blood level was significantly higher in the *T. spiralis* infected group in comparison to the healthy and the Parkinson's mice. In fact; IFN-γ is crucially related to the immune response against *Trichinella* larvae (Helmby & Grencis, 2003). According to Dvorožňáková *et al*. (2011) the increase in the circulatory IFN-γ becomes prominent two weeks after *T. spiralis* infection, which coincides with giving birth to newborn larvae and the beginning of the migratory phase (Dvorožňáková *et al*., 2005 and 2012). The increase in IFN-γ level during trichinellosis has been even proved to persist from day 10 to day 45 post-infection (i.e. during the phase of larval encapsulation in the muscles) even in the presence of a small number of larvae (Venturiello *et al*., 1995; Dvorožňáková *et al*., 2013). Moreover, Barbe *et al*. (2017) confirmed that the death of the encapsulated larvae can also enhance IFN-γ production.

TGF-β is another pro-inflammatory cytokine that strongly disturbs the function and decreases the number of the tyrosine hydroxylase (TH) immunoreactive neurons (Roussa *et al*., 2004). In 1996 Vawter *et al*. documented a significant elevation of TGF-β in the cerebrospinal fluid of the IP patients, which was explained by the reported elevation of TGF-β in the circulation of the studied cohort. In the current study; the *Trichinella* infected mice showed the highest TGF-β blood level, and the least TH expression. During trichinellosis, high level of TGF-β was proved that it helps in the down-regulation of the post enteric inflammation, and facilitates tissue invasion by the circulating larvae. TGF-β-1 receptors were observed to become up regulated in some of the organs that are not commonly invaded by *T. spiralis* (Toms & Powrie, 2001). Bliss *et al*. (2003) also confirmed this observation when they recorded an increase in the larval capability to invade the liver in the presence of a high TGF-β level. However, despite the high TGF-β-1 level that was detected in the infected mice in the current study; no brain invasion by the *T. spiralis* larvae was recorded.

During the circulatory phase of *T. spiralis* infection; the Th1 immune response predominates and promotes the classical pathway of macrophage activation and nitric oxide (NO) production (Cunningham, 2013). The high IFN-γ production in trichinellosis is responsible for the activation of NOS-2 enzyme that transforms L-arginine into nitric oxide to destroy the circulating larvae (Fabre *et al*., 2009). In the current study, the plasma advanced oxidation protein products (AOPPs) were significantly higher in the *Trichinella* infected mice. According to Capeillere-Blandin *et al*. (2004) on exposure of blood albumin to oxidative and carbonyl stresses products AOPPs become generated. AOPPs have a pro-inflammatory character, and act as inflammatory mediators that stimulate the recruitment of more neutrophils and monocytes and activate the T-lymphocytes oxidative ignition and dendritic cells (Cakir *et al*., 2016).

With the continuous trials of the migrating nematodal larvae to leave the circulation, and pass to the surrounding tissues by crossing the blood tissue barriers a massive amount of INF-γ is released. Subsequently, a cyclic inflammatory process of oxidative tissue damage, excitotoxicity, mitochondrial dysfunction, and proteolysis starts. In such case; areas nearby the blood tissue barriers show the highest tissue damage (Bruschi & Chiumiento, 2011). This can be an explanation for the significant high number of apoptotic dopaminergic neurons that were detected in the cortex. They are considered the nearest dopaminergic neurons to the highly vascular arachnoid matter and choroid plexus (Venditti *et al*., 2013). In addition, once the balance between the ROS production and cellular antioxidant activity is disturbed; massive tissue damage occurs in the brain areas that exhibit the highest level of oxidized lipids, proteins and DNA like the cerebral cortex (Alam *et al*., 1997; Badie *et al*., 2000; Niedzielska *et al*., 2016).

With the presence of a high and sustained IFN-γ production in the circulation the microglial cells and astrocytes become activated. The role of the astroytes in the BBB is mainly to encounter the passage of larvae and blood-derived leukocytes into the brain (Glass *et al*., 2010). In the presence of a neuroinflammatory condition as occurs in infections, the integrity and function of the BBB becomes modified to enable the passage of leukocyte to the CNS (Lee, & MacLean, 2015). Though this is important to help in elimination of the invading pathogens; it will trigger the release of two types of inflammatory molecules by the microglia; the anti- and the pro-inflammatory factors (Le *et al*., 2016). The first is beneficial and occurs soon after the CNS insult, while the second (which persists) occurs later, and is very notorious as it inhibits the neuronal regeneration, and establishes a sustained status of CNS inflammation and glial scar formation. Since in trichinellosis there is a persistent elevation of blood IFN-γ along the different phases of infection (McGeer *et al*., 2003), sustained activation of the microglia can be expected. The chronically activated microglia and astrocytes will release reactive oxygen intermediates, nitric oxide, inflammatory cytokines, and activate the Jak/STAT pathway with "Fas" death receptor and its ligand "FasL" up regulation (Badie *et al*., 2000; Hayley *et al*., 2004). These inflammatory products will be all toxic to the sensitively packed dopaminergic neurons. In addition, the dopaminergic neurons are very rich in ROS-generating enzymes like monoamine oxidase, which makes them more prone to damage and oxidative stress generation (Bruschi *et al*., 2013; Hwang, 2013).

A significantly higher level of DNA fragmentation was detected in the *Trichinella* infected mice. With massive ROS production the sensors of irreversible DNA damage response and protein kinases become activated, and DNA fragmentation occurs (Chen *et al*., 2012). Persistent DNA damage is considered one of the main pathogenic sign in the human neurodegenerative diseases since DNA integrity plays a cardinal role in the maintenance of the brain cells growth and regeneration (Andersen, 2004). According to Quian and Flood (2008) the accumulating brain insults due to the sustained microglial activation echoes with the slowly progressive destruction of the dopaminergic neurons that characterizes the development of idiopathic Parkinson's. Thus, there is a growing body of evidence that the presence of a pathogen in the circulation may initiate a ststus of microglial activation and neuroinflammation that will contribute IP development (Subramaniam & Federoff, 2017).

Conclusion

According to the previous findings; *Trichinella spiralis* infection was associated with significant brain DNA fragmentation, cortical neurodegenerative lesions in the form of Lewy's bodies, dopaminergic neurons apoptosis and a significant decrease in TH activity and brain dopamine level. These findings are all documented as pathognomonic signs for the neurodegenerative diseases generally and Parkinson's disease specifically. The reported brain changes may be explained by the significant increase in AOPP, and the proinflammatory cytokines "IFN-γ and TGF-β", which result from *Trichinella spiralis* infection. In the migratory phase of trichinellosis an intense immune stimulation is triggered by the presence of adult worms' traces in the intestine and the larvae (whether the free ones in the circulation or the incompletely encapsulated ones in the muscles). The localization of the brain lesions in the cortical areas of the dopaminergic circuit can be explained by being the closest to the meninges and the parasite induced inflammatory reactions in the circulation, and the richness of the cortical neurons with oxidized lipids, proteins and DNA. Generally, further studies are warranted to demonstrate the brain changes after different parasitic infections, infectious doses and longer duration; since Parkinson's disease is a long term disease with accumulative lesions and a progressive course. Moreover, cross-antigenecity between the different brain proteins and parasitic polypeptides must be investigated to discover any potentiality of cross-reactivity and autoimmune reaction, as was previously discovered with some viruses.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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The incidence of pinworm (*Enterobius vermicularis*) in pre-school and school aged children in the Eastern Slovakia

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Summary

Helminth infections caused by *Enterobius vermicularis* have a cosmopolitan character and most often affect the paediatric pre-school and school age population. The presented study was conducted to determine the prevalence of *E. vermicularis* in the analyzed population of children in the Eastern Slovakia. The Graham's scotch tape method was used to investigate the presence of *Enterobius vermicularis* eggs in 390 specimens. The analyzed set consisted of 218 girls and 172 boys, divided by age into three groups – aged from 5 months to 2 years, aged from 3 to 6 years, and aged from 7 to 15 years. Investigation of perianal scotch tapes of children for the presence of *E. vermicularis* eggs revealed the prevalence of *E. vermicularis* was P = 3.59 %. Depending on the incidence of *E. vermicularis* infection, we detected no statistically significant difference (p> 0.05). The prevalence of *E. vermicularis* in boys was P = 4.07 %, and in girls P = 3.21 %. The highest prevalence of *E. vermicularis* was recorded in the group of children aged from 3 to 6 years (P = 5.03 %). Most of the samples were positive at age 4 and 5. The lowest prevalence was in the group of children aged from 5 months to 2 years (P = 0.97 %), and the prevalence of *E. vermicularis* in the group of children aged from 7 to 15 was P = 3.91 %. The difference in the incidence of *E. vermicularis* infection among different age groups of children was not statistically significant (p> 0.05).
Enterobius vermicularis nematode infection and enterobiasis currently represents a major public health problem in Slovakia. At the present its occurrence is the most frequent in the paediatric population. Therefore it is important to introduce a targeted hygienic-epidemiological measure in children's collectives, what also should include proper and effective diagnostics and frequent recurrent therapy.
Keywords: *Enterobius vermicularis*; helminthiases; enterobiasis; children population; prevalence

Introduction

Infections with *Enterobius vermicularis* nematodes occur in the human population worldwide, although *E. vermicularis* infection and enterobiasis are more common in the temperate zone than in the tropic one. The species of *Enterobius gregorii* was recorded in Europe, Africa, and Asia, but it is probably not a different species (Nakano *et al*., 2006). Enterobiasis is one of the most frequent infections and affects nearly 1 billion people across all socioeconomic classes (Lohiya *et al*., 2000). The most commonly infected population is the infant population, and the individuals living in collectives. The prevalence decreases with the growing age of children and by gradual acquisition of hygienic habits. Infection transmission is carried out by the faecally-oral route of transmission by consuming of *E. vermicularis* infectious eggs. After ingestion of the infectious eggs, larvae are released in the small intestine

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and the adult individuals settle in the colon. Its common occurrence is in the caecum and terminal ileum of the small intestine. Transmission of infection can also occur after handling clothing or bed linen, contacting surfaces and objects in the environment that are contaminated with eggs. A small number of eggs can be transported by air, by inhalation and subsequent swallowing of infectious eggs. Their further development is the same as for ingested eggs. Coupling between adult individuals takes place in the colon. Pregnant females migrate to the perianal area, especially during the sleep of the host where they lay eggs. Each female produces approximately 10,000 eggs. Female migration causes marked itching (pruritus). Aautoinfection is often and occurs by transferring the infectious egg by hand into the mouth from the site of intense itching. The time interval from the infected eggs consumption to laying the eggs by adult females in the perianal area is approximately one month. The adult lifespan is about two months long. The larvae in eggs develop and the eggs become infectious within 4 to 6 hours under optimal conditions. Re-infection or migration of newly hatched larvae from anal skin back into the rectum may occur, but the frequency of occurrence is unknown. The resistance of eggs in the environment is relatively high. They can survive for 2 – 3 weeks on clothing, bed linen, or other objects (Burkhart & Burkhart, 2005).

Diagnosis of enterobiasis is performed by applying a transparent adhesive tape in the perianal area immediately after waking up followed by subsequent microscopic diagnosis (Garcia, 2007).

The classic signs of the disease are perianal and vaginal pruritus caused by female migration and egg sticking. Most infections are asymptomatic. Cases of heavy nematode infestation cause insomnia, weight loss, hyperactivity, teeth grinding, abdominal pain, and vomiting. *E. vermicularis* are not vectors of any known pathogens. In children, especially in a case of heavy nematode load, the neurological symptoms such as nervousness, restlessness, irritability, and distraction that may affect the growth of children may occur (Burkhart & Burkhart, 2005; Smolyakov *et al*., 2003), emotional instability and enuresis (Brooker & Bundy, 2009). In addition to pronounced pruritus in the area of the rectum and the perineal region occur mainly at night, which is associated with continuous scratching of the area around the rectum that leads to small wounds what is associated with development of secondary bacterial infections including bacterial dermatitis and folliculitis (Gutiérrez, 2000). Occasionally, after laying the egg, the parasite in the skin in the perianal area can migrate into the vagina instead of back into the rectum. This causes vulvovaginitis. In severely infected women and girls, migration into the vagina may cause a mucosal vaginal discharge. Uterine infection caused by *E. vermicularis* can cause vaginal bleeding (Al-Rufaie *et al*., 1998; Burkhart & Burkhart, 2005; Smolyakov *et al*., 2003).

Hong *et al*. (2002) described the case of ovarian enterobiasis where the presence of a degenerate adult *E. vermicularis* with a number of viable eggs was detected in the parenchymal ovary.

A lot of studies indicate a possible association between *E. vermicularis* infection and the appendicitis (Mansouri, 2017; Aydin, 2007; Da Silva *et al*., 2007; Ramezani & Dehghani, 2007). Parasitic infections of the appendix are rarely the cause of acute appendicitis. Massive *E. vermicularis* infection can imitate the symptoms of acute appendicitis (Dunphy *et al*., 2017), without any histological evidence of acute inflammation (Risio *et al*., 2016). It should be considered in the children with abdominal pain whether this is a case of acute appendicitis and thus avoid unnecessary surgical removal of the appendix (Dunphy *et al*., 2017).

In addition to the occurrence in female genital organs, the other extraintestinal localizations were found. Such as kidney (Cateau *et al*., 2010), pelvis as a pelvic abscess caused by *E. vermicularis*, what causes peritonitis in the peritoneal cavity (Das *et al*., 2001), as well as in the genito-urinary tract of a man with the nematode found in the prostate and eggs in the prostate secretion (Zahariou *et al*., 2007).

Material and Methods

In this study, 390 samples of perianal scotch tapes were examined for the presence of *E. vermicularis* eggs in the examined children population originating in various regions of the Eastern Slovakia. The analyzed set consisted of 218 girls and 172 boys. By age, the file was divided into three age groups. The first group consisted of children aged between 5 months and 2 years, the second group of children aged 3 to 6 (pre-school age children), and the third group of children aged 7 to 15 years (school age children). To examine the presence of *Enterobius vermicularis* eggs, Graham's perianal scotch tapes method was used to observe the preparation microscopically for the presence of *E. vermicularis* eggs after pressing the transparent adhesive tape to the anal plicae (without washing the anal region for 1 – 2 days) and sticking the tape to the slide (Graham, 1941; Garcia, 2007). For the statistical evaluation of the results, a chi square test (χ^2) was used using SPSS. Statistical significance was confirmed when the p-value was less than 0.05.

Ethical Approval and/or Informed Consent

All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional ethical committee and with the 1964 Helsinki declaration and its later amendments.

Results

As determined by the ovoscopic examination of 390 samples of perianal scotch tapes in the studied children population, the prevalence of *E. vermicularis* was P = 3.59 % (Table 1).

Depending on the incidence of *E. vermicularis* infection, we detected no statistically significant difference ($p > 0.05$). The number of positive samples was the same in both sexes (n = 7). The prevalence of *E. vermicularis* in boys was P = 4.07 % and in girls

Table 1. Prevalence (P) of *Enterobius vermicularis* in the monitored children population.

Enterobius vermicularis	
Number of examined samples	N = 390
Number of positive samples	n = 14
P (%)	**3.59 %**

P = 3.21 %. The highest prevalence of *E. vermicularis* was recorded in the group of children aged from 3 to 6 years (P = 5.03 %). The most positive samples were in group of 4 and 5 years old kids. The lowest prevalence was in the group of children aged from 5 months to 2 years (P = 0.97 %) and the prevalence of *E. vermicularis* in the group of children aged from 7 to 15 was P = 3.91 %. The difference in the incidence of *E. vermicularis* infection among the different age groups of children was not statistically significant (p> 0.05). The results of the examinations are summarized in Table 2 and Fig. 1.

In 8 samples of perianal scotch tapes, the presence of *Ascaris lumbricoides*, and in 2 samples of *Trichuris* spp was detected. Positive samples came from the children aged 2, 3, and 6 years old.

Discussion

Enterobiasis is not considered to be a serious disease, but the morbidity level in the world is significant, especially in children. Eosinophilic enterocolitis, appendicitis and inflammatory bowel disease have been reported as a consequence of enterobiasis (Sah *et al*., 2006).

The prevalence of *E. vermicularis* P = 3.59 % was found in the investigated children population from the Eastern Slovakia. Similar results from an epidemiological study on the occurrence of enterobiasis in children and adolescents from the north-eastern region of Poland conducted in 2008 – 2009 showed 3 % prevalence (Zukiewicz *et al*., 2011), however, in 2013 – 2015 the overall prevalence in Poland was 10.1% (Kubiak *et al*., 2017). Crotti and D'Annibale (2006), in a 2002 – 2003 study conducted in Italy, found the presence of *E. vermicularis* eggs in 13.4 % children. In the western Europe, the prevalence of *E. vermicularis* may be 30 – 50 % (Scully, 2011; Burkhart & Burkhart, 2005). The prevalence among children in some communities is up to 61 % in India, 50 % in England, 39 % in Thailand, 37 % in Sweden, and 29 % in Denmark (Burkhart & Burkhart, 2005). The prevalence of enterobiasis in Turkey in children attending elementary schools varies between 5.4–67 % (Çeliksöz *et al*., 2005), and 42.6 % in children in Russia (Chernyshenko *et al*., 2003). Also in Tyrol, it has been shown to be the most frequently occurring intestinal helminthiasis, and representing about a half (49.7 %) of diagnosed cases (Tomaso *et al*., 2001). In the United States, 40 million infected people with the highest prevalence in children, individuals in close contact, in collectives, in families (Lohiya *et al*., 2000) are to be estimated. Thumb sucking (Burkhart & Burkhart, 2005) and fingernail bitting (Cook, 1994).has been shown to increase the incidence and rate of recurrence.

Depending on the incidence of *E. vermicularis* infection, there was no statistically significant difference (p> 0.05), what was consistent with the results of various other studies (Li *et al*., 2015; Wang *et al*., 2016).

Enterobiasis occurs predominantly in children at age 5 – 14 (Cook, 1994). The highest prevalence of *E. vermicularis* was recorded in the group of children aged from 3 to 6 years (P = 5.03 %). The most positive samples were in 4 and 5 years olds who are pre-school children. The sensitivity of the infant population to the prevalence of helminthic infections is declining. This change in the susceptibility may be partly due to a change in children's behaviour or activities. Five to six-year-olds are more frequently in contact in kindergartens than children at the age of one and two years (Sang *et al*., 2011). Our results also confirmed the lowest prevalence in the group from 5 months to 2 years (P = 0.97 %). The prevalence of *E. vermicularis* in the group of monitored children aged 7 – 15 years was P = 3.91 %. The difference in the incidence of *E. vermicularis* infection among different age groups

Table 2. The prevalence of *Enterobius vermicularis* (P) v in the monitored children population in dependence on sex and age.

Sex		**n**	**P (%)**	X^2
Boy	N= 172	7	4.07	
Girl	N= 218	7	3.21	0.649076
Age				
5 m – 2 years	N= 103	1	0.97	
3 – 6 years	N= 159	8	5.03	0.220356
7 – 15 years	N=128	5	3.91	

N – number of examined samples, n – number of positive samples, P – prevalence, X^2 – chí square test, p – value of p significance level (α=0.05)

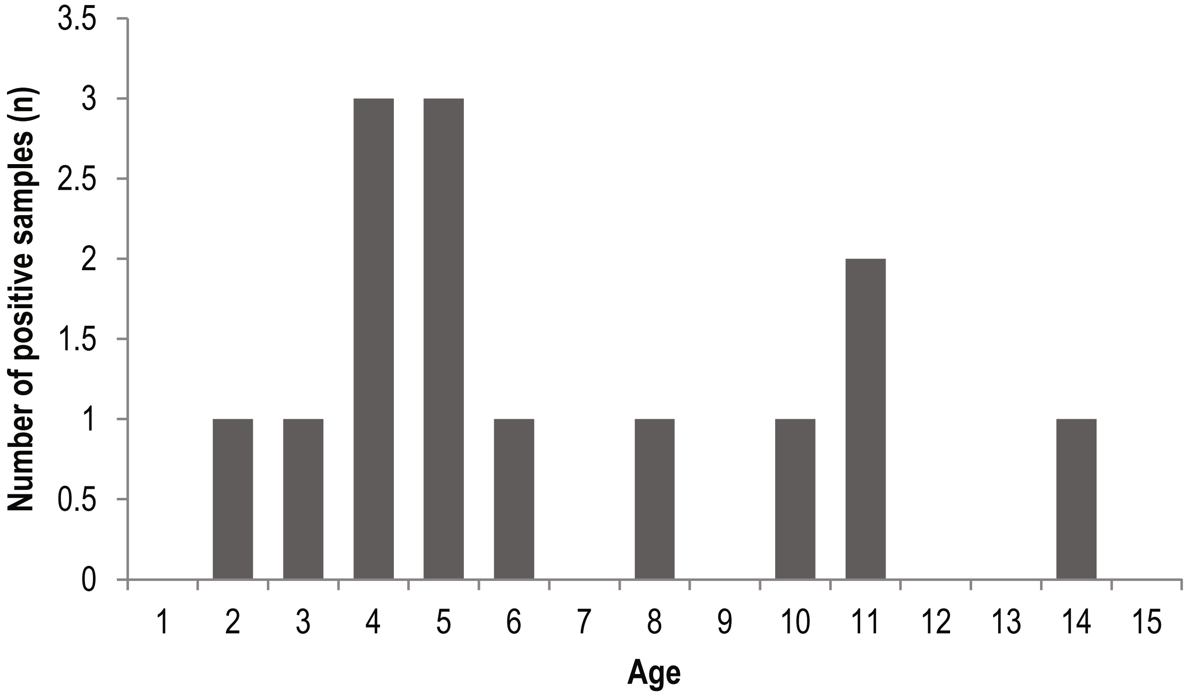

Fig. 1. Positive samples to *Enterobius vermicularis* in dependence on age.

of children was not statistically significant (p> 0.05). Older children are less likely to play on the floor, in clay, sand, put their fingers in their mouths, and wash their hands before the food and after toilet, what it is in not a typical case in younger children (Suraweer *et al*., 2015). Higher immune responses in older children as well as conscious hygienic habits, including personal hygiene, reduce the susceptibility to endoparasitic infection (Juriš *et al*., 2014).

Likewise, Hong-Mei *et al*. (2015) reported that younger children (aged two to six years) were more likely to have infections than older children (Hong-Mei *et al*., 2015). Anuar *et al*. (2016) found the prevalence of enterobiasis in children aged from 1 to 6 years. From their results, the overall rate of children's positivity for the presence of *E. vermicularis* eggs was 12.5 %. The infection prevalence is age dependent. A higher rate of infection was observed in children aged from 5 to 6 years. Significant risk factors influencing the transmission of infection were thumb sucking and a large family of positive children. The authors report that the cause could be inadequate personal hygiene of children and playing with toys contaminated by *Enterobius* eggs.

According to Juriš *et al*. (2014), in children patients with pulmonary diseases of infectious and non-infectious etiology, in whom endoparasitic infections were diagnosed, the increased levels of IgE (in 40 % of positive patients), elevated Eo levels (in 45 % positive patients), elevated Lym (in 40 % of positive patients) were statistically significantly higher in the patients positive to helminthiasis.

In 8 specimens of perianal scotch tape preparations, we detected the presence of *Ascaris lumbricoides*, and in 2 samples also *Trichuris* spp. eggs. Although the method of perianal scotch tape preparations is not a standard method for the diagnosis of geohelminths *A. lumbricoides* and *Trichuris* spp. that use flotation methods, it can indirectly point to the presence of a massive infection by these helminthiases and running ovulation of female *A. lumbricoides* when the eggs are secreted by the stool. *E. vermicularis*, unlike geohelmints, can be reproduced in humans without passing through the developmental cycle phase in the external environment in the soil. Therefore, interhuman transmission is easy (Lohiya *et al*., 2000).

E. vermicularis infection therapy should be biphasic with a single repetition after 2 weeks. Benzimidazole preparations of mebendazole (2 × 100 mg for 3 days), or albendazole (400 mg once) are used. Even here the dose was repeated after 3 weeks. Albendazole is not recomendet in children under two years of age. However, the therapy itself for cure is notsufficient. Aadequate personal hygiene, hygiene of the environment, thorough washing and ironing of underwear and bed linen are required. In case of positive control examination, it is necessary to repeat the therapy for the whole family. The time period for developing *E. vermicularis* eggs to adult stage is 14 days. To prevent autoinfection, it is necessary to administer two antihelminth doses (the second after 14 days). Mebendazole causes death only of adult helminth individuals, and not eggs or larvae. Surviving eggs and larvae in the host's gut can mature into new adults within 14 days. The second dose, which is 14 days after the first administration of the antihelminth, is crucial to the destruction of these new adults. The second dose given before 14 days would be ineffective for later mature adults and after 14 days female eggs would be produced (Russell, 1991; Kastner *et al*., 1992).

It is also important to treat contacts that may be infected but are undetectable, negative for the presence of oocytes in perianal scotch tape preparations (during the out-of-ovulation period of female helminths), because reinfection of the recently treated individuals may occur. For effective management of enterobiasis therapy, all contacts in the household should be treated with two-phase doses of antihelminthics, with repetition after 14 days (Lohiya *et al*., 2000).

Conclusion

Infection with *Enterobius vermicularis* and enterobiasis is currently a serious worldwide public health problem with the most common occurrence in the paediatric population. It is therefore important to introduce targeted intervention. The measures to prevent the spread of enterobiasis require two-phase therapy for the sick (with repetition after 14 days of the first dose of antihelminthics), therapy of infected individuals who are undetectable (negative for the presence of oocytes in perianal scotch tape preparations, including all-contact therapy. Health education should be done with a focus on the children population as the most risky group, parents, guardians, and teachers. Hygiene measures to prevent infection should be targeted at specific age groups of children, taking into account the highest rate of infection in pre-school children. Particular emphasis should be placed on keeping the personal hygiene, and environmental hygiene.

Because of the high rate of *E. vermicularis* recurrence in a collective environment such as nursery schools and primary schools, it is important to wash the hands with soap and warm water, disinfect hands after using the toilet, before eating and change diapers in the smallest children.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Helminths infecting the cat-eyed snake *Leptodeira annulata* Linnaeus 1758 (Squamata: Dipsadidae) in a semiarid region of Brazil

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Summary

Snakes have diverse feeding and living habits, being exposed to a variety of endoparasite communities. However, more studies are still necessary to document these relationships. We examined 18 specimens of the cat-eyed snake *Leptodeira annulata* from a semi-arid region in Northeast Brazil. Eight *taxa* of parasites were found, with higher prevalence of cystacanths (Acanthocephala). Five nematode species (*Hexametra boddaertii, Oswaldocruzia* sp*., Oxyascaris* sp., *Physaloptera* sp. and *Raillietnema spectans*) and the pentastome *Raillietiella furcocerca* represent a new parasitism record for the host studied. Our results also showed that *L. annulata* could act as paratenic host for acanthocephalans. These results contribute to the knowledge of the helminth fauna of *L. annulata*.
Keywords: parasites, nematoda, neotropical, Pentastomida, snakes, reptiles

Introduction

Parasitism is one of the most common life styles with parasites representing a considerable portion of the world's biomass, but these organisms were for a long time neglected in biodiversity surveys (Poulin & Morand, 2004; Dobson *et al*., 2008; Kuris, 2008). Given the importance of these organisms structuring communities in ecosystems, as well provide data on ecology of the host (Poulin, 1999; Brooks & Hoberg, 2000), there has been a recent increase of studies on the fauna of endoparasites especially of reptiles in Brazil (Anjos *et al*., 2011; Albuquerque *et al*., 2012; Ávila *et al*., 2012; Teles *et al*., 2015). Such studies provide information about the ecology, natural history, life cycle, and evolution of host-parasite systems. However, the lack of studies on helminths associated with vertebrate organisms is still evident, being necessary more studies in the area (Mati *et al*., 2015).
The endoparasite fauna can be related, among other factors, to the diet and microhabitat of hosts (Brito *et al*., 2014; Ribas *et al*., 1998). Snakes have very diverse feeding habits, being exposed to a wide variety of parasites (Aho, 1990; Jiménez-Ruiz, *et al*., 2002). *Leptodeira annulata* (Linnaeus 1758) is a semi-arboreal reptile, distributed from Mexico to eastern of South America (Duellman, 1958) and along all biomes of Brazil, such as the Amazon, Atlantic forest, Cerrado, and Caatinga (Bertoluci *et al*., 2009; Bernarde *et al*., 2012; Cole, *et al*., 2013; Mesquita *et al*., 2013). Studies on *L. annulata* address aspects like foraging, diet and reproduction (Martins & Oliveira, 1998; Mesquita *et al*., 2013; Silva-Neta *et al*., 2015), but data on the parasitic fauna are scarce with records only *Ophidascaris trichuriformis* Vaz, 1935 (Sprent, 1988) and *Renifer heterocoelium* Travassos, 1921 (Pinto *et al*., 2012).
In this context, species inventory are important tools serving as a base for ecological studies, enabling the knowledge of what and how many species are part of an ecosystem and providing essential information about the diversity of organisms (Poulin *et al*. 2015). Aiming at filling the gap in the knowledge of the parasite fauna of *L. annulata*, this study analyzed the helminth fauna as-

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sociated with individuals from the Southern region of Ceará State, Brazil.

Material and Methods

This study was carried out with samples from the Herpetological Collection of Universidade Regional do Cariri (URCA-H- 1981; 3279; 4532; 4907; 4910; 4911; 4913; 4914; 4915; 5541; 5631; 6742; 6847; 7521; 7889; 7900; 8014; 11228). The specimens were collected from 2012 to 2014 in the municipality of Aiuaba (n=4) (6° 34' 25" S, 40° 07' 25" W, WGS84), Barro (n=4) (7° 10' 36" S, 38° 46' 54" W, WGS84), Farias Brito (n=8) (6° 55' 50" S, 39° 33' 56" W, WGS84), Jati (n=1) (7° 41' 10" S, 39° 00' 57" W, WGS84) and Mauriti (n=1) (7° 23' 21" S, 38° 46' 28" W, WGS84) all located in the Southern region of Ceará State, Brazil (Fig. 1). Study area is characterized by hot semi-arid tropical climate and mild hot semi-arid tropical climate (IPECE, 2016).
A total of 18 specimens of *L. annulata* being eight females (mean snout-vent length 541.2 mm) and eleven males (447.5 mm SVL) were euthanized with a lethal injection of sodium thiopental (CFMV, 2013) necropsied and had the liver, lung, heart, mouth, larynx, stomach, large and small intestine, coelomic cavity, and kidneys checked for presence of parasites under the stereomicroscope. The parasites found were processed to separate them completely from the host tissue and stored in 70 % ethanol.
Aiming to perform the taxonomic identification of the helminths obtained, different preparation methods were carried out according to the taxonomic group. Cystacanths were, stained with carmine and preserved in 70 % ethanol. The cestode was also stained with carmine and fixed between slide and coverslip. The nematodes were mounted in temporary slides with Amman's lactophenol or latic acid. The pentastomes were cleared using Hoyer's solution and preserved in 70 % ethanol. The slides were examined with optical microscope and the specimens were identified using the keys for identification of Yamaguti (1959, 1961, 1963), Vicente *et al*. (1993), Gibbons (2010), Rego (1983). Samples of all parasites were deposited in the Helminthological Collection of the laboratory of Zoology of Universidade Regional do Cariri, URCA, Ceará State, Brazil.
The parasitological descriptors of prevalence (P), mean abundance (MA), mean intensity of infection (MII), richness, and range of intensity of infection (RII) were calculated according to Bush *et al*. (1997).

Ethical Approval and/or Informed Consent

The collection of specimens was authorized by Instituto Chico Mendes de Conservação da Biodiversidade-ICMBio (Authorization number 29613-1) and by the ethics committee of Universidade Regional do Cariri (CEUA/URCA, process No. 00260/2016.1), the

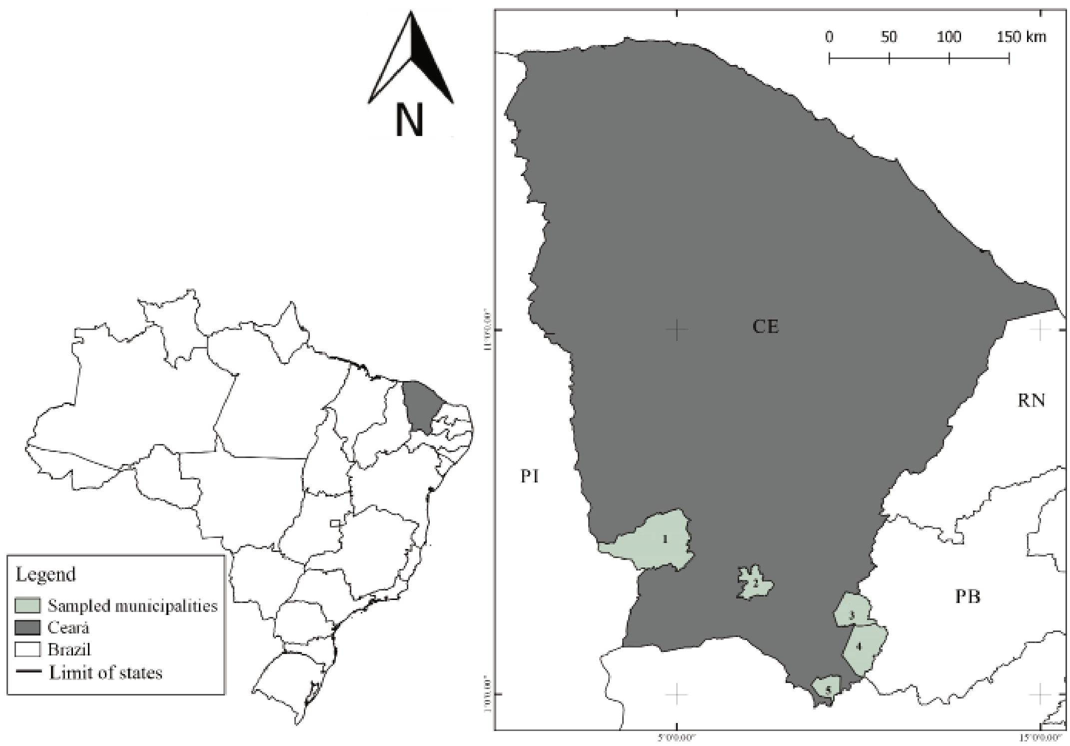

Fig. 1 Municipalities where the specimens of *Leptodeira annulata* were collected in the state of Ceará, Brazil. 1-Aiuaba, 2-Farias Brito, 3- Barro, 4- Mauriti, 5- Jati.

research related to animals has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Results

A total of 153 parasite specimens were collected with total prevalence of 78.9 % and mean intensity of infection of 10.20 ± 2.81. The component community associated with *L. annulata* was composed of eight *taxa*: 18 specimens of nematodes distributed in five *taxa* (*Hexametra boddaertii* Baird 1860, *Raillietnema spectans* Gomes 1964, *Oswaldocruzia* sp., *Oxyascaris* sp., and *Physaloptera* sp.), three pentastomes of the species *Raillitiella furcocerca* Diesing, 1863, one unidentified cestode, and 131 unidentified cystacanths (Acanthocephala) (Table 1). The cystacanths showed the highest prevalence (63.2 %), intensity (10.92 ± 3.28), and mean abundance (6.9). The cysts could not be identified at species level because the shape and number of the hooks in the proboscis could not be determined. The cestode was found in one female host specimen (SVL = 544.36), showing the lowest values of prevalence (5 %), intensity (1), and mean abundance (0.05), but the parasite specimen could not be identified at species level due to poor conditions of preservation.

Discussion

Studies that investigated the helminth fauna of some snake species from the Neotropical region such as McAllister *et al*. (2010a, 2010b), Bursey and Brooks (2011) did not record any infection in *L. annulata*. This fact may be due to the low number of individuals studied, because in the present study, *L. annulata* presented higher richness (8 parasite *taxa*) compared to studies of endoparasites for other snake species (Ávila *et al*., 2013; Nasiri *et al*., 2014).

Raillietnema spectans is a common parasite in amphibians (Vicente *et al*., 1990). The low prevalence of this parasite in the snake *L. annulata* could represent an accidental infection, since this nematode have a monoxenic life cycle and infection occurs through ingestion of eggs and/or larvae penetrating actively in host skin (Anderson, 2000). *Leptodeira annulata* is an active forager feeding mainly on amphibians (Martins & Oliveira, 1998; Bernarde & Abe, 2010; Mesquita *et al*., 2013) and could be exposed to this parasite while foraging.

The unidentified species of Acanthocephala found in this study showed the highest prevalence and were present in the coelomic cavity of 12 specimens of *L. annulata*. Similar results were found by Smales (2007) in nine colubrid species which also presented acanthocephalans encysted in coelomic cavity. The presence of cystacanths in the coelomic cavity of the snakes analyzed indicates that they are acting as paratenic hosts. The cysts were probably acquired from amphibians that are part of the diet of *L. annulata* (Mesquita *et al*. 2013). According to Baker (2007), acanthocephalans have indirect life cycle, with intermediate forms in arthropods and crustaceans, reaching their adult stage in fish or aquatic birds. In the case of infecting an unsuitable host, the parasites can encyst again until reaching a definite host.

The correct identification of the acanthocephalans found in the present study was not possible due to encysted form of the specimens which prevents the visualization of morphological characteristics necessary for identification, such as proboscis rows of hooks and reproductive organs (Smales, 2007).

The pentastome *R. furcocerca* belongs to a parasite genus commonly found in the respiratory system of squamates from South America (Almeida *et al*., 2008a). This parasite is known to infect snakes with Neotropical distribution, being already recorded in *Boa constrictor* (Linnaeus, 1758), *Clelia clelia* (Daudin, 1803), *Crotalus durissus* (Linnaeus, 1758), *Drymarchon corais* (Boie, 1827),

Table 1. Prevalence (P), mean intensity of infection (MII) with standard error (SE), (MA) mean abundance, (IS) infection site, and (RII) range of intensity of infection of the helminths associated with the snake *Leptodeira annulata* from the South region of Ceará State, Brazil.

	P (%)	MII ± SE	MA	IS	RII
Acanthocephala					
Cystacanth	66.7	10.92 ± 3.28	7.28	BC	2 – 37
Cestoda					
Unidentified cestode	5.6	1	0.05	SI	1 – 1
Nematoda					
Hexametra boddaertii	5.6	1	0.05	L	1 – 1
Oswaldocruzia sp.	5.6	2	0.11	LI	1 – 2
Oxyascaris sp.	5.6	2	0.11	SI	1 – 2
Physaloptera sp.	5.6	1	0.11	ST	1 – 2
Raillietnema spectans	5.6	11	0.61	LI	1 – 11
Pentastomida					
Raillitiella furcocerca	11.1	1.5 ± 0.5	0.17	L	1 – 3

Infection sites: body cavity (BC), large intestine (LI), small intestine (SI), stomach (ST), lung (L).

Lachesis sp. (Motta, 1963; Rego, 1983) *Mastigodryas bifossatus* (Raddi, 1820), *Philodryas nattereri* (Steindachner, 1870), *Pseudoboa nigra* (Duméril, Bibron and Duméril, 1854), *Thamnodynastes chaquensis* (Bergna and Alvarez, 1993), *Thamnodynastes chaquensis* (Bergna and Alvarez, 1993), *Thamnodynastes chaquensis* (Bergna and Alvarez, 1993), *Xenodon merremii* (Wagler, 1824), (Alcantara *et al*., 2014; Almeida *et al*., 2008b; Esslinger, 1986). The present study represents the first record of *R. furcocerca* infecting *L. annulata*.

This study presents new records for the nematodes *H. boddaertii*, *Oswaldocruzia* sp., *Oxyascaris* sp. and *R. spectans* in *L. annulata*, the first record of a cestode in *L. annulata*, and the first record of infection by the pentastome *R. furcocerca* in this snake species. These records have the importance of being part of the first studies for the Caatinga area in Northeast of Brazil with this species, and also contribute significantly to the knowledge of the parasitic fauna of *L. annulata* in the Neotropical region providing data on the helminths associated with this snake species.

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Conflict of interest

Authors state no conflict of interest.

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Helminths infecting *Dryadosaura nordestina* (Squamata: Gymnophthalmidae) from Atlantic Forest, northeastern Brazil

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Summary

We analyzed the patterns of infection by helminths in populations of the Gymnophthalmidae lizard *Dryadosaura nordestina* from three Atlantic Forest fragments in Northeast Brazil. Prevalence and mean intensity of infection by location showed the following results: ARIE Mata de Goiamunduba (60.8 % and 10.4 ± 8), RPPN Engenho Gargaú (83.3 % and 20.8 ± 19.7) and Benjamim Maranhão Botanical Garden (70.4 % and 7.78 ± 5.8). We provide the first records of helminth infection for the lizard *D. nordestina*, in which three species of nematodes, *Aplectana* sp., *Cosmocerca* sp. and *Physaloptera lutzi* and one trematode *Haplometroides odhneri* were recovered. Trematodes of the genus *Haplometroides* were previously known as parasites only in snake and amphisbaenian hosts in South America. Now, our study provides the first record of a species belonging to this genus parasitizing lizards. In conclusion, our study shows that *D. nordestina* have a depleted helminth fauna (three species at maximum), similar to other studies with lizards of this family in Brazil and that its parasite abundance is related to host snout-vent length, but not to the sex.
Keywords: nematode, trematode, lizard, parasitism

Introduction

Habitat fragmentation processes are identified as the main causes of extinction of animal and plant species (Pimm & Raven, 2000). This occurs because many species belonging to a particular habitat are restricted to small portions of the same. Thus, as areas are being lost, extinction rates tend to rise dramatically (Pimm & Raven, 2000; Rocha *et al.*, 2006). Considering parasite communities, environmental changes have a faster and devastating effect, compared to free-living organisms, and they may be extinct before their hosts (Lyles & Dobson, 1993).
According to Pinto *et al*. (2006) the main way to ensure the preservation of the biodiversity of the Brazilian Atlantic Forest will be with ecosystem management activities. However, the strategies, actions, and necessary interventions are hampered by the lack of knowledge about the ecology of the species and the functioning of the ecosystems where they are inserted (Pinto *et al*., 2006). In addition, knowing that parasites play an important role in the balance of ecosystems (Freeland & Boulton, 1992; Lafferty & Kuris, 2005), studies addressing the host-parasite relationships are also important to propose and develop strategies for management and conservation of biodiversity (Marcogliese, 2004).
Parasitological studies involving Gymnophthalmidae lizards in Brazil still remain scarce and mostly focused on records of new

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hosts (Ávila & Silva, 2010). Only 14 Gymnophthalmidae of the 93 species occurring in Brazil have some kind of investigation on the host-parasite relationship (Ávila & Silva, 2011; Ávila *et al.*, 2010b; Brito *et al.*, 2014; Bursey & Goldberg, 2004; Goldberg *et al.*, 2007a; Goldberg *et al.*, 2007b; Oliveira *et al.*, 2017; Neta & Ávila, 2018; Ribeiro *et al.*, 2018).

Dryadosaura Rodrigues, Xavier Freire, Machado Pellegrino & Sites, 2005 is a genus composed of only one species that occurs in the Northeast part of the Atlantic Forest of Brazil, distributed in the states of Rio Grande do Norte to Bahia (Oliveira & Pessanha, 2013). *Dryadosaura nordestina* Rodrigues, Xavier Freire, Machado Pellegrino & Sites, 2005 is a small lizard, with body size (Snout-vent length) ranging from 45.4 ± 5.7 mm for males and 42.7 ± 7.1 mm for females, it is an active forager with generalist diet, composed mainly of arthropods, being ants and insect larvae the main food items (Garda *et al.*, 2014). However, there are no records on parasites associated with *D. nordestina*, possibly because it is an uncommon lizard, the difficult to its collection, and because there are still few specimens housed in Herpetological Collections. Thus, the main objective of our study is to know the composition of helminth species associated with *D. nordestina*, evaluating effects of sex and host body size, using samples from the Atlantic Forest, northeastern Brazil.

Materials and Methods

We collected 56 specimens of *D. nordestina* during expeditions in three localities. We collected 23 lizards in the ARIE Mata de Goiamunduba (ARIE-MG), area of 67.5 ha, located in the Bananeiras municipality (-06°45'03.78"S – -35°38'00.06"W) in October 2016; six in the RPPN Engenho Gargaú (RPPN-EG) area of 1058.62 ha, in the Santa Rita municipality (-06°59'52"S – -34°57'30"W) in September 2016 and 27 lizards at the Benjamim Maranhão Botanical Garden (BMBG) area of 471ha, in the João Pessoa municipality (-07°08'08"S – -34°51'37"W) in November 2016 (Dias *et al.*, 2006). All areas are remnants of Atlantic Forest located in the Paraíba state, Brazil. The average annual rainfall of the areas is 1490 mm and the average annual temperature of 24.6° C. All specimens were captured manually or using pitfall-traps (25 sets in each area), constructed with four buckets (20L) arranged in "Y" shape, totalizing 100 buckets per area (Oliveira *et al.*, 2017).

Lizards were killed with a lethal injection of 2 % lidocaine hydrochloride and measured snout-vent length (*SVL*) with digital calipers. Subsequently, they were sexed, preserved in 10 % formalin, and stored in 70 % alcohol. In the laboratory, we removed the respiratory and gastrointestinal tracts that were surveyed for endoparasites under a stereomicroscope. The endoparasites were cleared in Hoyer's solution (Everhart, 1957), counted, registered the site of infection, and subsequently identified in accordance to Anderson (2009) for nematodes and (Silva *et al.*, 2007) for trematode species. Subsequently, they were preserved in 70 % alcohol and deposited in "Coleção de Invertebrados Paulo Young", in the Universidade Federal da Paraíba, Brazil (UFPB-NEM: 03, 04; UFPB-DIG: 03, 04, 05).

The infection rates were calculated according to Bush *et al.* (1997), where prevalence of infection corresponds to the number of infected hosts divided by the total number of hosts in the sample x 100 (it appears in percentages throughout the text) and mean intensity of infection (*MII*) is the total number of parasites found in a sample, divided by the number of hosts infected with that parasite; finally, parasite abundance is defined as the total number of parasites found in a sample (individual host, host population and/or host community) Throughout the text, means appear ± 1 SD.

To verify the influence of sex and host size (SVL) on the abundance of endoparasites Generalized linear mixed models – GLMM were used. This model was chosen due to the possibility of introducing into the equation random factors, thus removing possible effects that they may exert on the fixed variables (Bates, 2014).

Knowing that our sampling corresponds to three distinct component communities, to test the hypotheses suggested above, we adopted the study areas as random factors, in order to remove any influence of the locality on our dataset.

In order to verify if the abundance of endoparasites varies between the sexes of the hosts, we performed a GLMM adopting the locality and the body size (SVL) of the hosts as random factors. On the other hand, to verify the relationship between the abundance of endoparasites and the body size of the hosts (SVL), we adopted the locality and sex of the hosts as random factors.

Also, GLMM's were performed in R software with the help of packages "lme4" (Bates, 2014) and "MuMIn" (Barton, 2009) adopting the Poisson (link:log) distribution family (Wilson & Grenfell, 1997).

Ethical Approval and/or Informed Consent

The present research has complied with all the relevant national regulations and institutional policies for the care and use of animals. Permits for capturing of the lizards and analyzing of the endoparasites used in this study were released by SISBIO-IBAMA (no: 54378/3, authentication code: 78752298; no: 56863-1, authentication code: 47783645), SUDEMA (no: 004/2016, process no. 5376/16), and Benjamim Maranhão Botanical Garden-BMBG (no: 003/2016/JBBM/SUDEMA).

Results

We examined 56 *D. nordestina* specimens, of which 33 were adult males (SVL: 40.4 ± 10.6), 14 adult females (SVL: 39.4 ± 10.5), and nine juveniles (SVL: 20.8 ± 10.2). Prevalence and mean intensity of infection in lizards by location, respectively, were: ARIE-MG (60.8 % and 10.4 ± 8), RPPN-EG (83.3 % and 20.8 ± 19.7), and BMBG (70.4 % and 7.78 ± 5.8).

We identified three nematode species, *Aplectana* sp. Railliet and Henry, 1916; *Cosmocerca* sp. Diesing, 1861, and *Physaloptera lutzi* Cristofaro, Guimarães and Rodrigues, 1976; and also, one

Table 1. Parasitological data from populations of *Dryadosaura nordestina* from Atlantic Forest, northeastern Brazil, infected by helminths.

	Aplectana sp.			*Cosmocerca* sp.			*Physaloptera lutzi*			*Haplometroides odhneri*		
Locality	%	II (Range)	SI	%	II (Range)	SI	%	II (Range)	SI	%	II (Range)	SI
RPPN-EG	83.3	17.8 ± 5.7	S; S.I; L.I	16.7	1	S.I	-	-	-	33.3	(3 – 11)	S; S.I
ARIE-MG	56.5	5.2 ± 3.7	S; S.I; L.I	-	-	-	26.1	12 ± 4.8	S	13	6	S
BMBG	70.4	7 ± 5	S; S.I; L.I	-	-	-	-	-	-	14.8	4 ± 2.3	S.I

Prevalence (%), Mean intensity of infection – II, Site of infection – SI: Stomach – S, Small intestine – S.I and Large intestine – L.I.

trematode species, *Haplometroides odhneri* Ruiz and Perez, 1959 infecting *D. nordestina* (Table 1).

The GLMM did not find significant differences in relation to the sex of the hosts and the endoparasites abundance (R^2= 0. 01235937; $Z_{1,34}$= 0. 668; P= 0.504), however, revealed a significant relationship between endoparasites abundance and SVL (R^2= 0.4674977; $Z_{1,34}$= 8.377 P < 2e-16).

Discussion

Our results provide the first records of helminth infection for the lizard *D. nordestina*, in which three species of nematodes (*Aplectana* sp., *Cosmocerca* sp. and *P. lutzi*) and one trematode (*H. odhneri*) were recovered.

Nematodes belonging to the family Cosmocercidade infect amphisbaenians (Amorim *et al.*, 2017), lizards (Ávila & Silva, 2010), and frogs (Gomez *et al.*, 2017) and they have a monoxenic life cycle, in which their infective stages are transmitted directly via tegument or accidental ingestion (Anderson, 2000). This characteristic of transmission may explain the high prevalence of infection by *Aplectana* sp. in all sampled areas (Table 1), considering that the adaptations presented in Gymnophthalmidae lizards for fossorial and semi-fossorial life (Garda *et al.*, 2014; Grizante *et al.*, 2012; Oliveira *et al.*, 2018) may facilitate the process of infection with parasites that have a direct life cycle (Oliveira *et al.*, 2017). On the other hand, *Cosmocerca* sp. presented low infection rates, corroborating the results obtained by Bursey & Goldberg (2004) for Gymnophthalmidae lizards from Amazonia, which showed prevalence varying between 16-36 % and mean infection of intensity inferior to two. Thus, knowing that monoxenic nematodes can have their infection process favored by the ecology of Gymnophthalmidae lizards (Garda *et al.*, 2014; Grizante *et al.*, 2012; Oliveira *et al.*, 2018), in case of *Cosmocerca* sp., historical factors (phylogeny) can be pointed out as one of the main determinants that explain the low infection rates presented here (Poulin, 2007; Brito *et al*., 2014).

Poulin (1998) states that there is a trade-off between the performance and range of host species that a parasite can exploit. This can easily be perceived for nematodes of the genus *Physaloptera*, since they infect a wide range of lizards in South America (Ávila & Silva, 2010; Teixeira *et al.*, 2017), however, almost always followed by low prevalence of infection: *P. lutzi* (0.9 %) parasitizing *Ameivula ocellifera* (Spix, 1825) (Ribas *et al.*, 1995); *P. lutzi* (2 %) and *P. retusa* Rudolphi, 1819 (3.9 %) in *Ameiva ameiva* (Linnaeus, 1758) (Ribas *et al.*, 1998); *P. retusa* (5.3 %) and *P. lutzi* (19.3 %) in the lizard *Tropidurus hispidus* (Spix, 1825) (Anjos *et al.*, 2012); *P. retusa* (9 %) registered in *Polychrus acutirostris* Spix, 1825 (Araujo Filho *et al.*, 2014); *P. retusa* in the sympatric lizards *Hemidactylus mabouia* (Moreau de Jonnès, 1818) (3.94 %) and *Phyllopezus pollicaris* (Spix, 1825) (2.54 %) (Sousa *et al.*, 2014); *Physalopreta* sp. (5 %) in the stomach of *Iguana iguana* (Linnaeus, 1758) (Teles *et al.*, 2017) and more recently, *P. lutzi* registered by Lima (2017) parasitizing the gecko lizards *P. pollicaris* (3 %), *Hemidactylus brasilianus* (Amara, 1935) (4.1 %), *Hemidactylus agrius* Vanzolini, 1978 (1.6 %), *Gymnodactylus geckoides* Spix, 1825 (12.6 %), and *Lygodactylus klugei* (Smith, Martin e Swain, 1977) (1.5 %), from northeastern Brazil. Thus, the low infection rates presented here by the generalist nematode *P. lutzi* apparently obey the standard cited above. However, further testing is needed to verify if this hypothesis truly fits the patterns of infection exhibited by the parasitic nematode species of lizards in the Neotropical region.

Trematodes of the genus *Haplometroides* Odhner, 1910 were previously known as parasites only in snake and amphisbaenian hosts in South America (Silva & Barrella, 2002;Silva *et al.*, 2005a,b; 2007;2008; Santos *et al.*, 2008). Now, our study provides the first record of *H. odhneri* parasitizing lizards.

Our study did not reveal significant differences between the sexes of the lizards in relation to the abundance of endoparasites, similar to the results obtained for other Gymnophthalmidae lizards by Neta & Ávila (2018) for *Colobosauroides cearensis* Cunha, Lima-Verde e Lima, 1991 and Ribeiro *et al*. (2018) for *Nothobachia ablephara* Rodrigues, 1984. However, to populations of *Anotosaura vanzolinia* Dixon, 1974 from Caatinga, females were more infected them males, since they tend to use more humid habitats during their reproductive period, potentializing the process of infection by the monoxenic nematode *Oswaldocruzia brasiliensis* Lent et Freitas, 1935 (Oliveira *et al.*, 2017).

Usually, juvenile hosts tend not to be parasitized or harbor a reduced parasite load when compared to adult individuals, due to the shorter exposure time the sources of infection (Kuris *et al.*, 1980). In conformation with this theory, no juvenile lizard in our sample was parasitized.

According to Nascimento (2004), larger hosts harbor a larger parasitic load, because they present a larger area and possibly more resources for the establishment of parasite populations. Our study corroborates this hypothesis since we observed a significant relationship between *SVL* and the abundance of parasites in the lizard *D. nordestina*, similar to Neta & Ávila (2018) for *C. cearensis* in a Caatinga area, northeast of Brazil.

Small lizards may restrict the diversity of associated endoparasites, since niche differentiation and microhabitat segregation by competing parasite species may be impaired by the reduced size of the host (Ávila *et al.*, 2010a; Kuris *et al.*, 1980; Oliveira *et al.*, 2017). For lizards, this hypothesis is supported mainly by Gymnophthalmidae: *Cercosaura eigenmanni* (Griffin, 1917) and *C. oshaughnessyi* (Boulenger, 1885) both parasitized by three taxa of parasites (Bursey & Goldberg, 2004); *Micrablepharus maximiliani* (Reinhardt e Luetken, 1862) also parasitized by three taxa of parasites (Brito *et al*., 2014); *A. vanzolinia* parasitized by only one species of nematode (Oliveira *et al.*, 2017); *N. ablephara* with three gastrointestinal helminth taxa identified (Ribeiro *et al*., 2018) and *C. cearensis* infected with five gastrointestinal helminth taxa (Neta & Ávila, 2018).

In conclusion, our study shows that *D. nordestina* have a depleted helminth fauna (three species at maximum) composed of nematodes and trematodes and that its parasite abundance is related to host *SVL*, but not to the sex.

Conflict of Interest

Authors state no conflict of interest.

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Helminth community dynamics in a population of *Pseudopaludicola pocoto* (Leptodactylidae: Leiuperinae) from Northeast-Brazilian

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Summary

Climatic variation in low latitudes influences the dynamics and structure of parasite communities. Environmental changes caused by dry and rainy seasons alter prevalence and abundance of endoparasite communities. In addition to providing a list of the helminth species associated with the swamp frog *Pseudopaludicola pocoto*, this study aimed to investigate the effects of rainfall and temperature on parasitological descriptors of helminths associated with *P. pocoto* in an area of the semiarid zone. A total of 817 swamp frog specimens were collected between 2013 and 2017, with four sampling expeditions during the dry season and four during the rainy season. Environmental parameters of temperature and rainfall were compared to the parasitological descriptors of prevalence, abundance and mean infection intensity of the parasite community using a multivariate linear regression. A richness of eight parasite species was identified, including Nematoda (*Rhabdias* sp., *Cosmocerca parva*, *Oxyascaris oxyascaris*, *Physaloptera* sp., *Brevimulticaecum* sp., *Spiroxys* sp. and unidentified nematode) and Acanthocephala (cystacanths). Rainfall levels had a significant effect on the infection intensity of *Rhabdias* sp. being the presence of this species higher during the rainy season, whereas no influence of temperature was observed on the helminth community.
Keywords: Anura; Caatinga; helminthfauna; Neotropical; seasonality; semiarid

Introduction

Leptodactylidae is one of the ubiquitous frog families in the Neotropics, with great richness and abundance in the Caatinga biome (Roberto *et al*., 2013; Ávila, 2015). Leptodactylids occur in a wide variety of habitats, becoming exposed to several degrees of helminth infections (Goldberg *et al*., 2007; Bursey & Brooks, 2010; Hamann & González, 2010). The genus *Pseudopaludicola* currently comprises 22 species of small swamp frogs distributed in South America (Cardozo *et al*., 2018). To date, only two species had their helminth fauna investigated – *Pseudopaludicola boliviana* Parker, 1927, in which a richness of ten taxa was found, including trematodes, cestodes, nematodes and acanthocephalans (Duré *et al*., 2004; González & Hamann, 2012), and *Pseudopaludicola falcipes* Hensel, 1867, in which only the nematode *Cosmocerca podicipinus* Baker and Vaucher, 1984 was recorded (González & Hamann, 2004; 2009). *Pseudopaludicola pocoto* Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014 was recently described from Northeast-Brazilian and is widely distributed in the Caatinga biome, however, there are still no studies on its ecology, only on

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its geographical distribution (Magalhães *et al*., 2014; Pereira *et al*., 2015; Silva *et al*., 2015; Lantyer-Silva *et al*., 2016 and Silva *et al*., 2017).

Several factors contribute to the dynamics and structure of parasite communities, like seasonality, environmental heterogeneity or factors associated with the host, such as spatial distribution, population density, and body size (Aho, 1990). Climate changes can cause some effects upon biological communities, like alterations on the abundance and transmission rates of helminths and also have an influence on host-parasite relations (Altizer *et al*., 2006; Koprivnikar *et al*., 2006; King *et al*., 2007; Koprivnikar & Poulin, 2009; Schotthoefer *et al*., 2011; Pizzato *et al*., 2013 and Brito *et al*., 2014). The prevalence and abundance of helminths are more influenced by seasonal variations in regions of median latitudes because cold seasons alter the acquisition of the parasite by the host (Pizzato *et al*., 2013). Meanwhile, in tropical areas, the prevalence and abundance of parasites can either increase or decrease between dry and rainy season (Choudhury & Dick, 2000). Thus, climatic factors, such as temperature, humidity, and rainfall levels can exert different effects upon parasitological descriptors of helminth infections.

Identifying what are the environmental factors that influence the helminth community can contribute towards the comprehension of how infection dynamics changes through time and space in order to unravel the mechanisms involved in host-parasite interactions. Besides providing a list of the helminths associated with *P*. *pocoto*, this study aims (I) to compare the similarity between the helminth communities associated with species of *Pseudopaludicola* and (II) to investigate the effects of rainfall and temperature upon the parasitological descriptors of prevalence (P), mean intensity of infection (MI), abundance, diversity, and migration of parasites between sites of infection in the helminths community associated with *P. pocoto* in Brazilian Northeast.

Material and Methods

This study was carried out in the Benguê Reservoir, Aiuaba, Ceará, Brazil (06°35'35"S, 40°08'31"W). Host samplings were performed from September 2013 to March 2017, with four expeditions during the dry season and four during the rainy season. This region is located in one of the driest areas of Ceará State, with mean annual rainfall levels of 560 mm (Funceme, 2016).

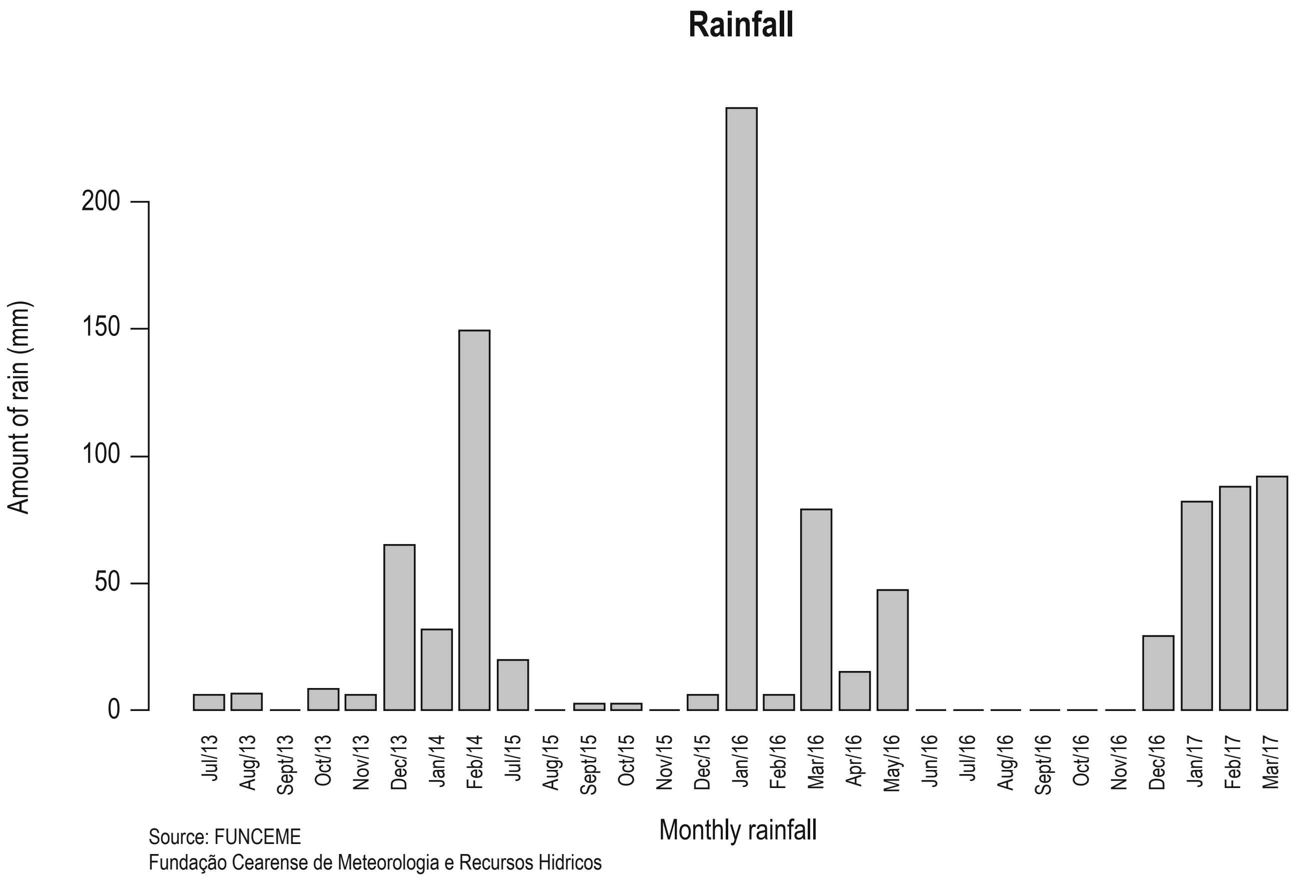

Fig. 1. Monthly rainfall levels related to the sample period of *P. pocoto* in the municipality of Aiuaba, Ceará State, Brazil.

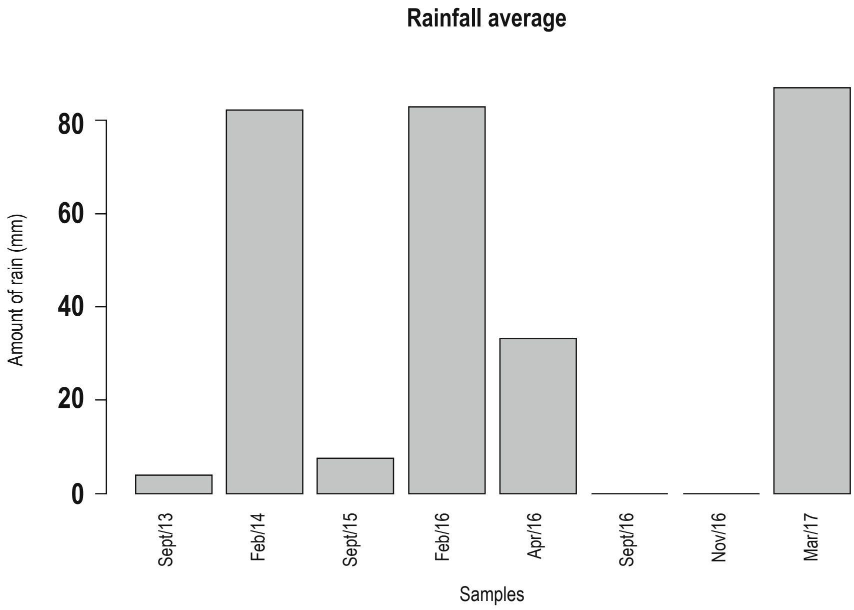

Fig. 2. Quarterly means of rains related to the sampling months of *P. pocoto* in the municipality of Aiuaba, Ceará State, Brazil.

A total of 817 specimens of *P. pocoto* (573 males, mean snout-vent length [SVL] ± SD 13.97 ± 1.56 mm, range: 10.15 – 16.5 mm, 244 females, SVL: 15 , 1 ± 1,57 mm, range: 11,41 – 18,46 mm) were used for this study, being collected by hand for this parasitological and also specimens collected for other purposes and deposited in the Herpetology Collection of the Universidade Regional do Cariri - URCA-H, Crato, Ceará State were used. Specimens were euthanized with sodium thiopental, necropsied for helminths, fixed in 10 % formaldehyde and stored in 70 % ethanol.

Data on rainfall levels were gathered using monthly means from the Fundação Cearense de Meteorologia e Recursos Hídricos – FUNCEME (Foundation of Meteorology and Hydric Resources of Ceará State). For statistic analyses between rainfall and parasitological descriptors, a mean rainfall of every three months was extracted related to the period between the samplings (Figs. 1 and 2).

The following parasitological descriptors: prevalence, mean intensity of infection, and abundance were calculated following Bush *et al*. 1997 using standard error and range. Aggregation of parasites was calculated using the Discrepancy Index (D) by Poulin 1998 which ranges from 0 to 1, where D = 0, all hosts harboring the same number of parasites; D = 1, all parasites found in a single host. This index was calculated in the software Quantitative Parasitology 3.0 (Rózsa *et al*., 2000).

Nematodes were found alive, washed in saline solution (0.9 % NaCl), fixed and preserved in 70 % ethanol. The nematodes were cleared in lactophenol or lactic acid while acanthocephalans were removed from their cysts, stained in carmine, and cleared in creosote. All endoparasites were observed and identified to the lowest possible level under a light microscope DMLB (Leica) and DM 5000B with interferential phase contrast, according to the litera-

Table 1. Helminth component community associated with *Pseudopaludicola pocoto* from the municipality of Aiuaba, Ceará State, Brazil.

Helminth	P (%)	MI	MA	IS	Stage	Range
Nematoda						
Rhabdias sp.[a]	22.6	1.49 ± 1.7	0.34	Lungs	Adult	1 – 8
Cosmocerca parva [a,b]	25.5	1.35 ± 1.4	0.34	St, SI and LI	Adult	1 – 5
Oxyascaris oxyascaris [a,b]	12.3	1.54 ± 1.8	0.2	SI and LI	Adult	1 – 7
Physaloptera sp.[a]	0.1	1 ± 1	0	St	Larva	1
Spiroxys sp.[a]	0.1	1 ± 1	0	Cav	Larva	1
Brevimulticaecum sp.[a,b]	0.1	1 ± 1	0	Cav	Larva	1
Unidentified nematode	2.9	1.5 ± 2.3	0.4	St, SI and Cav	Larva	1 – 4
Acanthocephala						
Cystacanth	1.1	1.43 ± 1.79	0	Cav	Cyst	1 – 3

P (%) - prevalence; MI – mean intensity of infection; MA – mean abundance; IS – infection site; St – stomach; SI – small intestine; LI – large intestine; Cav – body cavity; a – new record; b – new locality ture (Yamaguti, 1961; Sprent, 1979; Vicente *et al*., 1991; Anderson, 2000 and Gibbons, 2010). All parasites were deposited at the Coleção Parasitológica do Laboratório de Zoologia from Universidade Regional do Cariri – LZ-URCA (Parasitological Collection of the Laboratory of Zoology).

Richness (total number of helminths) and Brillouin's index of diversity were used to describe the parasite community. Richness was estimated using species accumulation curve, in which the number of observed species is a function of the sampling effort, measured in number of individuals using the R software packages Biodiversity R and Vegan (R core team, 2014). The Shapiro-Wilks test was applied to evaluate the normality of prevalence data, mean intensity of infection, mean abundance (MA) and diversity. Thereby, diversity between seasons was compared using *Wilcoxon's* test for paired samples and differences of prevalence, mean intensity of infection, and mean abundance between seasons was tested by Student *t*-test.

Using a data matrix with the presence/absence variables for the parasite species related to the genus *Pseudopaludicola*, the degree of similarity among these helminth communities was calculated using the Sorensen's index (So), with a posterior analysis of clustering using the Cluster method using the unweighted pair-group average (UPGMA).

A multivariate regression was performed to assess the influence of rainfall levels and temperature and their interaction on prevalence, mean intensity of infection and abundance of the helminth community of *P. pocoto*. To evaluate whether there were alterations of infection sites between dry and rainy seasons, a contingency table was made with data on the abundance of species infection/sites. The helminths that did not vary sites between the seasons and the ones that were not frequent were excluded from the analyses not to have influence of these values upon the species that showed greater abundance and occupied different sites. To evaluate the significance of the results, a chi-square test was performed with the data organized as a contingency table of two factors (Gotelli & Ellison, 2011). All statistical analyses were performed using the software PAST 3.0.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed according collection authorization issued by Chico Mendes' Institute (ICMBio/SISBio) Nº 29613 – 1; 55467 – 1 for scientific activities aims and authorized by council from Universidade Regional do Cariri-Urca nº 00260/2016.1.

Results

From the 817 hosts necropsied, 406 were parasitized with at least on helminth taxon (P = 49.7 %, MI = 1 ± 0.51, MA = 0.49 ± 0.4, range = 1 – 8). From the 405 hosts sampled in the dry season, 193 were parasitized with at least one helminth taxon (P = 47.7 %,

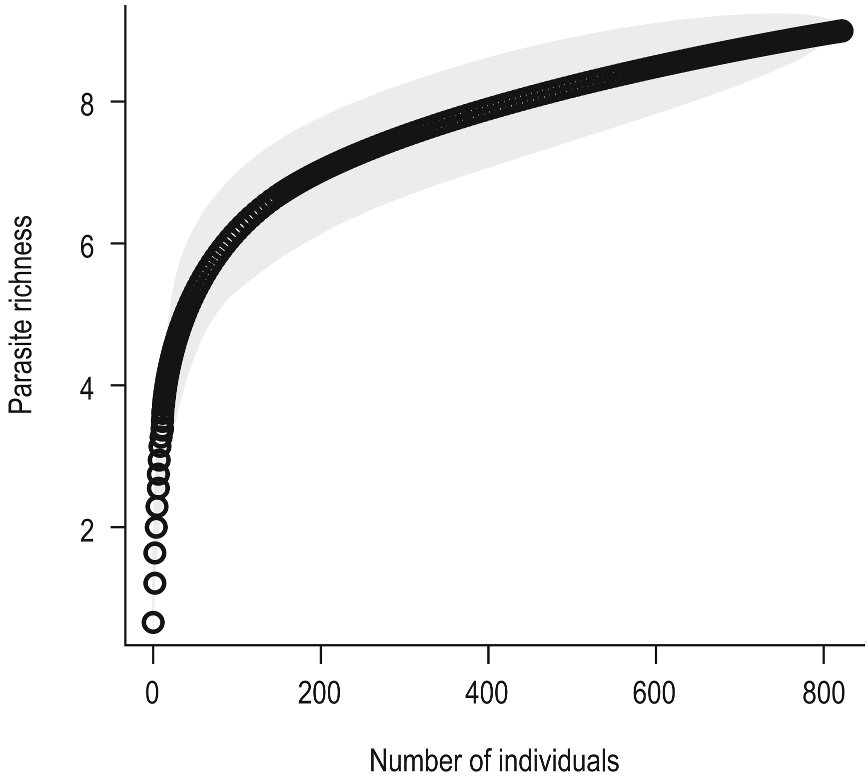

Fig. 3. Species accumulation curve (black line) and confidence interval (gray) for the richness of the helminths associated with *P. pocoto*, from the municipality of Auiaba, Ceará State, Brazil.

Table 2. Effects of rainfall levels and temperature on the infection descriptors of the helminth community of *Pseudopaludicola pocoto*, from the municipality of Aiuaba, Ceará State, Brazil.

		Prevalence	Infection Intensity	Abundance
Rainfall				
Helminth community	*r*	0.195	0.632	0.427
	p	0.64	0.09	0.29
Rhabdias sp.	*r*	0.464	0.91	0.556
	p	0.24	0.0014*	0.15
C. parva	*r*	0.118	0.574	0.284
	p	0.78	0.14	0.49
O. oxyascaris	*r*	0.298	0.02	0.372
	p	0.47	0.95	0.36
Temperature				
Helminth community	*r*	-0.428	0.672	-0.462
	p	0.29	0.07	0.25
Rhabdias sp.	*r*	-0.582	-0.41	-0.5
	p	0.13	0.31	0.2
C. parva	*r*	-0.485	-0.611	-0.54
	p	0.22	0.11	0.17
O. oxyascaris	*r*	-0.265	0.371	-0.31
	p	0.52	0.36	0.45
Rainfall: Temperature				
Helminth community	*r*	0.03	0.4	0.18
	p	0.59	0.14	0.48

*Effects of rainfall levels on infection intensity of *Rhabdias* sp. (p-value < 0.05)

MI = 1 ± 0.52, MA = 0.48 ± 0.6, range = 1 – 5), and from the 412 hosts sampled in the rainy season, 213 were parasitized with at least one helminth taxon (P = 51.7 %, MI = 1 ± 0.48, MA = 0.52 ± 0.6, range 1 – 8).

A total of 803 helminths specimens were collected, including nematodes and acanthocephalans, showing a richness of eight taxa (*Rhabdias* sp., *Cosmocerca parva* Travassos, 1925, *Oxyascaris oxyascaris* Travassos, 1920, *Physaloptera* sp., *Brevimulticaecum* sp., *Spiroxys* sp., unidentified nematode, and cystacanths) (Table 1). The infracommunity richness varied from one to three helminth species by host. The most abundant species recorded in this study were found in adult stage: *Rhabdias* sp. (N = 279), *C. parva* (N = 285), and *O. oxyascaris* (N = 157).

The aggregation index of the helminths showed moderate values in the dry season (*D* = 0.52±0.5) and rainy season (*D* = 0.48±0.48). The Brillouin's diversity index for the dry and rainy season were *i* = 1.42 and *i* = 1.29, respectively. There was no difference between seasons for prevalence (t= -0.0439; p = 0.96), mean intensity of infection (t = 1.359; p = 0.27) and mean abundance (t = -0.824; p = 0.47). The accumulation curve and the confidence interval showed a tendency to stabilization of richness of the helminths associated with this host in that location (Fig. 3).

The similarity between the communities of helminths of *P. pocoto* and *P. boliviana* and between *P. pocoto* and *P. falcipes* was of (So = 0), totally differing in their compositions of parasitic species. The proximity between *P. boliviana* and *P. falcipes* was of (So = 18.1 %). The distance between the communities analyzed and compared with the present study are represented in Figure 4.

There was no influence of rainfall or temperature or the interaction between both environmental variables on the community of

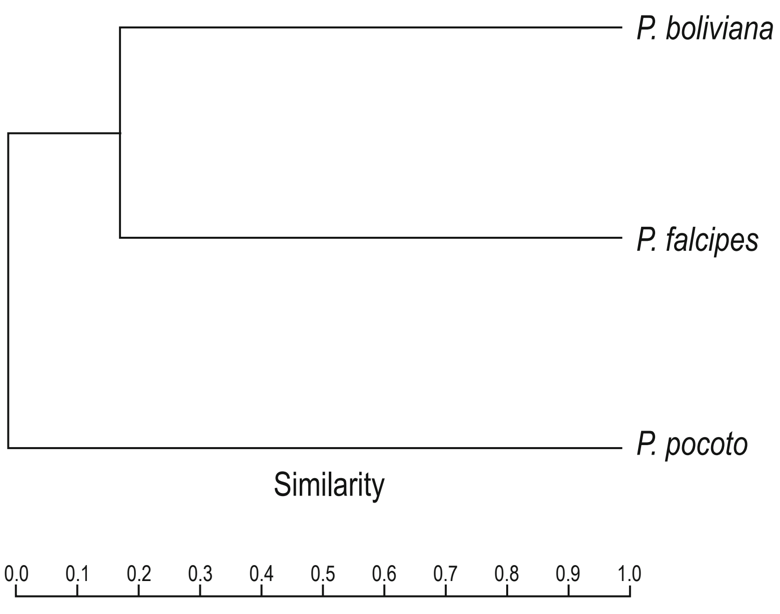

Fig. 4. Dendogram based on Sorensen similarity index comparing the helminth communities associated by genus *Pseudopaludicola*.

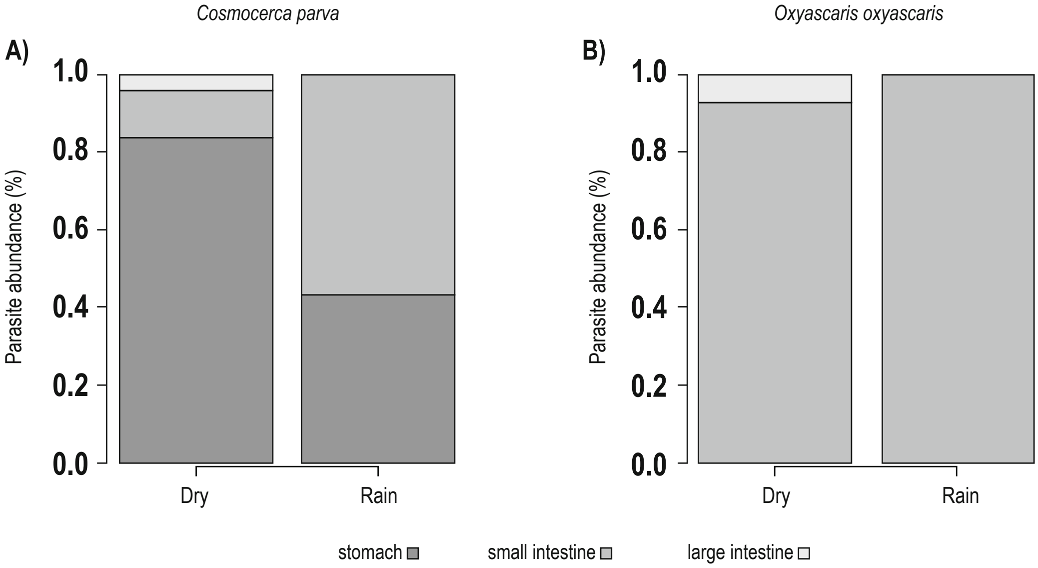

Fig. 5. Species/site relation between dry and rain season.

helminths *P. pocoto* (Table 2). However, the rainy season had a significant influence on intensity of infection of *Rhabdias* sp. ($p = 0.0014$), which was not found for the other abundant species (Table 2).

Regarding range of infection sites, Figure 5 shows the results of the most abundant species and the ones with greatest variation of sites. The chi-square test showed significant differences of the parasites migration patterns among the infection sites of the hosts between dry and rainy season for *C. parva* ($p = 9.84e^{-17}$). *Cosmocerca parva* was more related to the stomach (n=129) in the dry season, while in the rainy season this species used both stomach (n=67) and the intestines (n=89). However, for *O. oxyascaris* ($p = 0.0024$), although significant, the test found variation in the abundance of infection of this species between dry and rainy season, but no changes in the infecting site. The values of abundance in each host's site infection by season and total values are presented in Figure 6.

Discussion

Similar to other members of the Leptodactylidae, *P. pocoto* prefers semi-aquatic habitats (Frost, 2013), which is mirrored in the

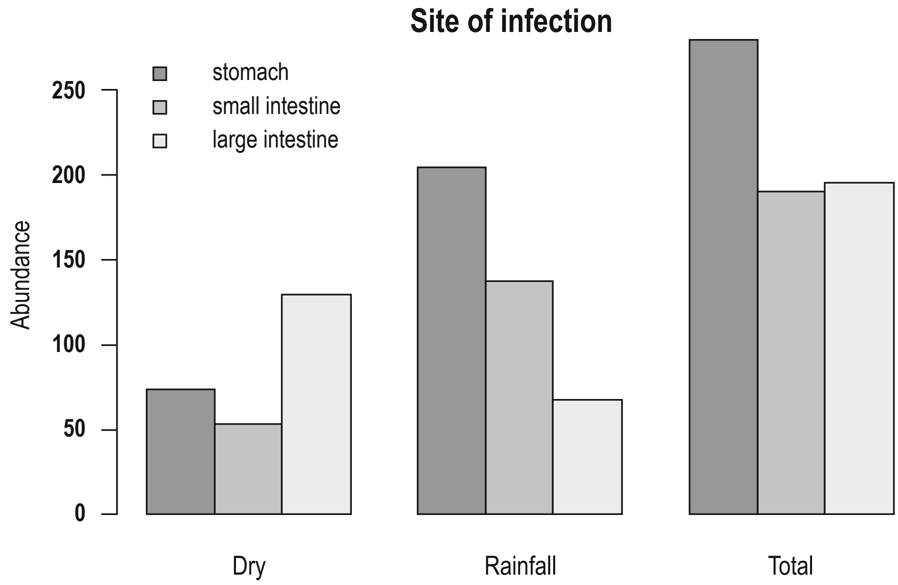

Fig. 6. Abundance values of infection by host site.

Table 3. A selection of parameters in male individuals of *Cosmocerca parva*.

	Cosmocerca parva	
	Travassos, 1925	Present study
Body length	1.42 – 2.01	1.38
Spicule length	90 – 110	89
Gubernaculum length	85 – 108	69
Number of plectanes	5 – 7	5
Lateral alae	Present	Present

Column 1 represents the metric range in µm of the morphometry of *C. parva*. (Revised from Bursey et al., 2015)

infection routes of its helminths, since the most abundant parasite species found in this study have direct life cycle. Nematodes were the most frequent taxa in the helminth community of *P. pocoto* in this study, with representatives of six families (Rhabdiasidae, Cosmocercidae, Oxyascarididae, Physalopteridae, Gnathostomatidae, Heterocheilidae). As in the present study, cosmocercids are the most frequently nematodes found among Leptodactylidae (Duré *et al*., 2004; González & Hamann, 2004; 2012; Santos & Amato, 2013; Campião *et al*., 2014).

The genus *Rhabdias* currently includes approximately eighty species, of which fifteen are valid for the Neotropical region (Kuzmin *et al*., 2016). From the species that occur in Brazil, seven are found in the amphibians and reptiles: *Rhabdias androgyna* Kloss, 1971, *Rhabdias fuelleborni* Travassos, 1926, *Rhabdias hermafrodita* Kloss, 1971, *Rhabdias galactonoti* Kuzmin, Melo, Silva-Filho and Santos, 2016, *Rhabdias paraenses* Santos, Melo, Nascimento, Nascimento, Giese and Furtado, 2011, *Rhabdias breviensis* Nascimento, Gonçalves, Melo, Giese, Furtado and Santos, 2013, and *Rhabdias stenocephala* Kuzmin, Melo, Silva-Filho and Santos, 2016 (Kuzmin *et al*., 2016).

The morphological and morphometric characters often used for the characterization of *Rhabdias* species largely overlap (Tkach *et al*., 2014). Currently, the most promising morphological characters with a tendency to accompany molecular data results are the shape and structure of the apical region, which are classified into five categories: (a) absence of lips, (b) six lips uniform in size and shape (c) four submedian and two pseudolabial lips, (d) two lateral pseudolabia and four in protuberance forms, and (e) species with only two pseudolabia (Tkach *et al*., 2014; Kuzmin, 2013). From the specimens collected, it was possible to identify that the apical morphological characteristics are consistent with the characteristics of the neotropical species, like the presence of six lips uniform in length and shape (Tkach *et al*., 2014). Morphometric analyzes performed for the *Rhabdias* specimens collected in this study demonstrate morphological characters of the apical region and morphometrics different from the species recorded for Brazil, which led us to the implementation of molecular analyzes for the certification of a possible new species for the genus and a later description (Figs. 7a and 7b).

Species of the genus *Cosmocerca* are widely distributed throughout all continents. The species *Cosmocerca parva* has a large distribution throughout Central and South America, thus having morphological and morphometric variations regarding body length, width and number of plectanas (5 – 7) (Rizvi *et al*., 2011). According to González and Hamann 2011 these variations occur according to the host and sometimes within the same individual. Even though the specimens found in this study present the same morphological characteristics as the ones described by Travassos 1925, the phenotypic plasticity observed is wide and the morphometric characters observed are smaller than those reported in the literature (Bursey *et al*., 2015) (Table 3), which may be related to the size of the host (Fig. 7c).

There are currently 30 species described for *Cosmocerca*, of which 10 are described for the Neotropical region (Bursey *et al*., 2015). Peru, Argentina and Brazil are the countries in South America with the highest numbers of infection records of *C. parva* in amphibians, respectively (Santos & Amato, 2013). In Brazil, the records of this helminth are mainly concentrated in the South and Southeast regions, infecting species of Brachycephalidae, Leptodactylidae, Hylodidae, Hylidae, and Bufonidae (Campião *et al*., 2014).

Oxyascaris oxyascaris was initially described parasitizing the snake *Mastigodryas bifossatus* Raddi, 1820 (= *Drymobius bifossatus*) in Rio de Janeiro (Vicente *et al*., 1991). Currently, the genus is composed of four other *Oxyascaris* species: *Oxyascaris similis* Travassos, 1920 (= *Pteroxyascaris similis*), *Oxyascaris caudacutus* Freitas, 1958, *Oxyascaris mcdiarmidi* Bursey and Goldberg, 2007 (Bursey & Goldberg, 2007). In Brazil, there are records of *O. oxyascaris* infecting amphibians of the Leptodactylidae family in the South and Southeast regions (Vicente *et al*., 1991). In the Northeast of Brazil, the records are restricted to the states of Bahia and Pernambuco (Teles *et al*., 2015). This species is identified by having a mouth with three lips, muscular esophagus followed by a glandular ventricle, equal spines, and gull-wing and caudal wings absent (Vicente *et al*., 1991) (Figs. 7d and 7e).

Physaloptera are parasites of all classes of terrestrial vertebrates (Anderson, 2000; Gorgani *et al*., 2013). Currently, the following species have been registered for South America and Brazil, infecting reptiles and mammals: *Physaloptera liophis* Vicente and Santos, 1974, *Physaloptera obtusissima* (= *P. monodens*) Molin, 1860, *Physaloptera tubinambae* Pereira, Alves, Rocha, Lima and Luque,

Table 4. List of helminths related to the species of the genus *Pseudopaludicola*.

Host	Helminths	Locality	References
Pseudopaludicola boliviana	**Trematoda**		
	Catadiscus sp.		
	Haematoloechus sp.		
	Gorgoderina sp.		
	Bursotrema sp.		
	Plagiorchiata sp.		
	Travtrema sp.		
	Echinostomatidae sp.	Corrientes, Argentina	Duré, 2004; González and Hamann, 2012
	Cestoda		
	Eucestoda sp.		
	Nematoda		
	Cosmocerca sp.		
	Cosmocerca podicipinus		
	Acanthocephala		
	centrorhynchus sp.		
Pseudopaludicola falcipes	**Nematoda**	Corrientes, Argentina	González and Hamann, 2004; 2009
	Cosmocerca podicipinus		
Pseudopaludicola pocoto	**Nematoda**		
	Rhabdias sp.		
	Cosmocerca parva		
	Oxyascaris oxyascaris		
	Physaloptera sp.	Ceará, Brazil	Present study
	Spiroxys sp.		
	Brevimulticaecum sp.		
	Unidentified nematode		
	Acanthocephala		
	Cystacanth		

2012, *Physaloptera praeputialis* Linstow, 1889, *Physaloptera lutzi* Cristofaro, Guimarães and Rodrigues, 1976, *Physaloptera retusa* Rudolphi, 1819 and *P. bainae* Pereira, Alves, Rocha, Lima and Luque, 2014 (Ávila & Silva, 2010; Ávila *et al*., 2012; Pereira *et al*., 2014 and Ramos *et al*., 2016). As for amphibians there is a record of infection by Physalopteridae larvae in the municipality of Angicos (RN) in the host *Rhinella granulosa* Spix, 1824 (Madelaire *et al*., in press). The specimens of *Physaloptera* sp. found in this study present a cephalic colarete formed by the cuticle reflected on the lips and having a mouth with two large, lateral, simple, triangular lips, each provided with a variable number of apical teeth and externally with papillae (Vicente *et al*., 1991) (Fig. 7f).

The genus *Spiroxys* is widely distributed throughout the Eurasian Palearctic, North Africa, North America and Neotropical countries (Hasegawa *et al*., 1998; Mascarenhas & Muller, 2015). Two species of the genus are found in Brazil, *Spiroxys contortus* Rudolphi 1819, described for the South and Southeast and *Spiroxys figueiredoi* Freitas and Dobbin 1962, with records for the North-Northeast, Southeast and Central-West regions infecting species of chelonians and snakes (Vicente *et al*., 1993; Bernadon *et al*., 2013; Mascarenhas and Muller, 2015 and Viana *et al*., 2016). Species of the genus *Spiroxys* are currently divided into three groups: (a) characterized by the presence of teeth in each lobe of the pseudolabium, (b) with teeth only in the median lobe and finally Roca and García, 2008 proposed a third group (c) that are without teeth, found in the Eastern, Australian and Ethiopian zoogeographic regions (Purwaningsih, 2015). The species that occur in Brazil, *S. contortus* and *S. figuereidoi*, are included in the second group (Mascarenhas & Muller, 2015; Fig. 7g).

Brevimulticaecum species are described occurring in the continents of Africa, America and Oceania (Vieira *et al*., 2010). The genus is characterized by having smooth lips with winged margins and absence of dentigerous furrows, excretory pore located anterior to the nerve ring and ventricle with short appendages (González

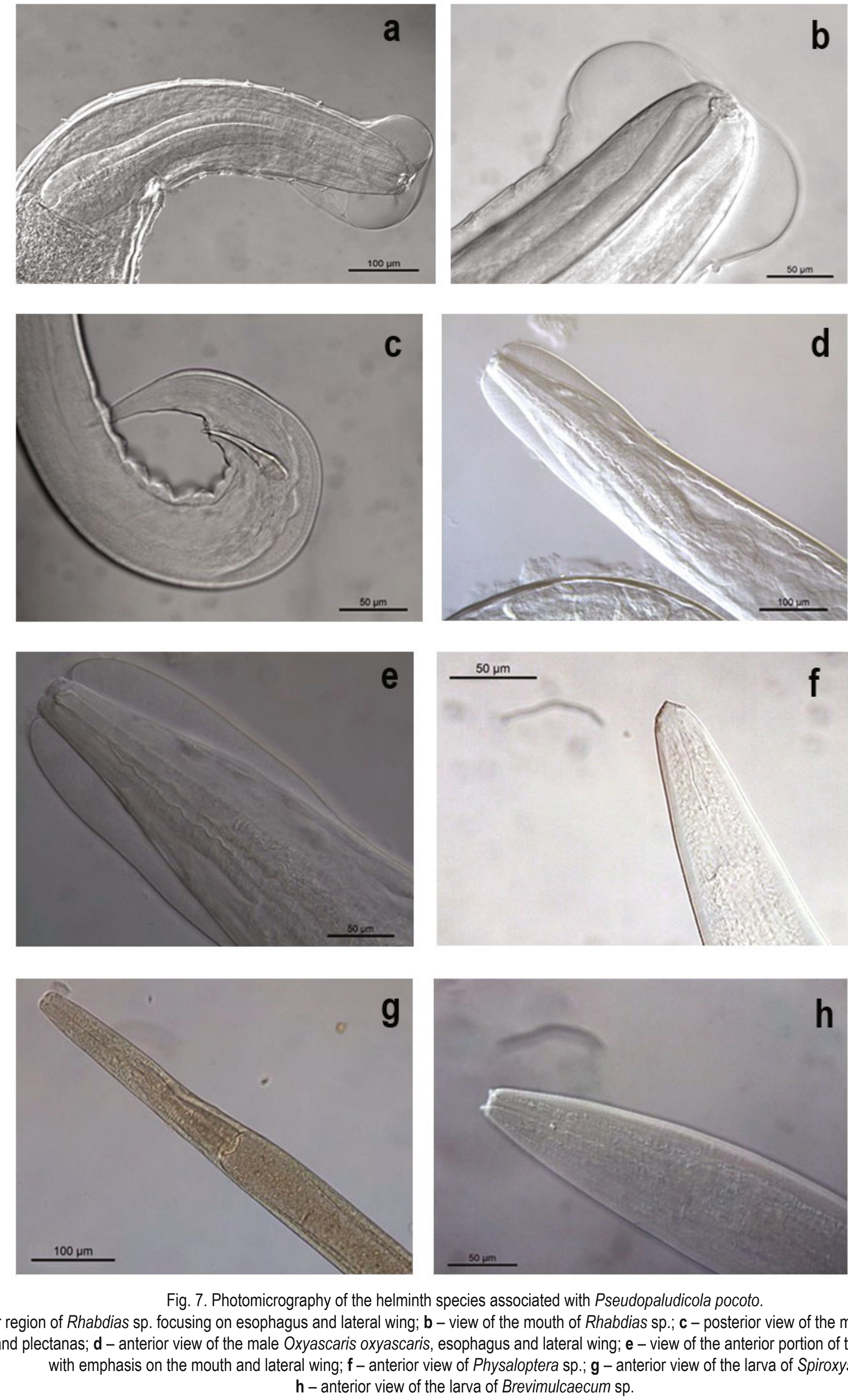

Fig. 7. Photomicrography of the helminth species associated with *Pseudopaludicola pocoto*.
a – anterior region of *Rhabdias* sp. focusing on esophagus and lateral wing; **b** – view of the mouth of *Rhabdias* sp.; **c** – posterior view of the male *Cosmocerca parva*, spicules and plectanas; **d** – anterior view of the male *Oxyascaris oxyascaris*, esophagus and lateral wing; **e** – view of the anterior portion of the male *O. oxyascaris* with emphasis on the mouth and lateral wing; **f** – anterior view of *Physaloptera* sp.; **g** – anterior view of the larva of *Spiroxys* sp.; **h** – anterior view of the larva of *Brevimulcaecum* sp.

& Hamann, 2013). Immature individuals of *Brevimulticaecum* were recorded infecting species such as the Brazilian snake *Bothrops neuwiedi* Wagler in Spix, 1824, the treefrog *Dendropsophus minutus* Peters, 1872 and in the freshwater fishes *Gymnotus carapo* Linnaeus 1758 and *Loricariichthys brunneus* Hancok 1828 (Sprent, 1979; Moravec and Kaiser, 1994; Moravec *et al*., 1997 and Vieira *et al*., 2010) (Fig. 7h).

Accumulation curve based on sampling effort proved to be satisfactory, since the sample reached the asymptote and was representative for sampling the helminth species associated with *P. pocoto*. The helminths richness in *P. pocoto* (S=8) is higher among species of *Pseudopaludicola* (Duré *et al*., 2004; González & Hamann, 2004; 2012). The richness of the helminth infrapopulation of *P. pocoto* varied from one to three species per host, which may be explained by the body size that is a factor influencing the richness and composition of helminth communities (Campião *et al*., 2015).

Duré *et al.* 2004 studied the helminth fauna of *P. boliviana* and found a greater species diversity, with representatives of Trematoda (70 %) showing the greatest richness and intensity of infection (Table 4). The cluster analysis indicates that there is more proximity between the helminth communities of *P. boliviana* and *P. falcipes* than between *P. pocoto* and this is due to the fact that *P. boliviana* and *P. falcipes* are sympatric species, and the low similarity between them can be explained by the low sampling of helminths in *P. falcipes* (Fig. 4). In addition, we must also consider that this difference between the community of helminths of *P. pocoto* and the other species of the genus may be due to geographical and environmental differences. The Argentine province of Corrientes, where the species *P. boliviana* was studied, is characterized by wide habitat heterogeneity, many temporary and permanent water bodies, and gleyic arenosols (Duré *et al*., 2004; IUSS, 2015). Thus, aquatic environments can facilitate the occurrence of trematodes, considering that the life cycle of these helminths is heteroxenic, and that in at least one of the phases of parasite transmission is found free in a liquid environment (Travassos, 1950).

Pseudopaludicola pocoto showed a component community mainly composed of nematodes, which may be related to the characteristics of its habitat. The municipality of Aiuaba shows low rainfall levels, annual temperature typical of semiarid climate, and soil composed of arenosols-argillaceous matter (Ipece, 2016). Nematodes are abundant in terrestrial habitats (Ruppert & Barnes, 1996), and the occurrence of these worms in the same habitat as *P. pocoto* enable the encounter of parasites and hosts. The greater abundance of *Rhabdias* sp., *C. parva* and *O. oxyascaris* found in all samplings of this present study suggest that the larvae of these nematode species are present in the habitat throughout the year. This fact can be favored by some habitat characteristics such as high soil humidity, allowing the eggs of these parasites to remain viable in the soil throughout the year giving rise to new larval forms and thus allowing continuity to infection by penetration through the skin in the host (Anderson, 2000; Brito *et al*., 2014).

Although the study period is insufficient to access long-term bioclimatic predictions on parasite infections of amphibians, the results found herein showed a significant effect of environmental changes on the parasite community, which corroborate other results for the Brazilian semi-arid. Brito *et al.* 2014 found a significant effect of climate variations on the abundance of the helminth community infecting lizards of the family Tropiduridae. In this present study, the environmental changes had an effect on the intensity of infection of *Rhabdias* sp. (Table 2). This lung parasite showed high prevalence in all sampling periods, but was even higher during the rainy season, which may be due to the high humidity in this period. However, climate interaction and rainfall levels did not have effects on the helminth community and species *C. parva* and *O. oxyascaris* in the present study. The investigation of which other variables, like sun radiation, salinity, humidity, and soil pH, are influencing the maintenance of parasite communities is an interesting premise for future studies.

Koprivnikar & Poulin 2009 observed the effects of temperature on cercariae species, in which the temperature was a significant variable increasing the growth rate of these parasites. However, the behavior of these helminths differs in subtropical areas as compared to tropical areas (Choudhury & Dick, 2000; Pizzato *et al*., 2013). For the present study, the temperature did not have a significant influence on the helminth infection rates. Although, a suppressing effect could be observed on the helminth as the temperature increased, even without statistic significance (Table 2).

Environmental changes influence the feeding behavior of hosts (Brito *et al*., 2014), which can affect the infracommunity diversity and infrapopulation abundance of helminths. The migration pattern between the seasons of *C. parva* and *O. oxyascaris* within the infection sites of *P. pocoto* can indicate greater resource availability within the host during the rainy season. This justifies an increase in the occurrence of *C. parva* in the intestinal tract of the host, being found simultaneously with *O. oxyascaris* in the same infection site during that season. However, there might be a greater resource competition in the intestinal tract during the rainy season which may lead *C. parva* to unexplored sites within the host (Esch *et al*., 1990). Still, the occurrence of *C. parva* in the stomach during the dry season can indicate that this species is not a good competitor and by occupying the first organ of the digestive tract this parasite has more chances to obtain food resources (Fig. 5).

The low occurrence of the species *Brevimulticaecum* sp., *Spiroxys* sp. and *Physaloptera* sp. can be explained by the climate conditions of the habitat and also by their life cycle since the three species were recorded during high levels of rainfall. However, the low prevalence and abundance of these nematodes can be related to the fact that species with complex life cycles suffer decrease in population density during trophic transmission (Poulin & Largrue, 2015). Another explanation that justifies the low values of the parasitological descriptors for these genera recorded in this work is the sample effort, which was important to reveal rare species associated with this host. These parasite larvae can be found infecting amphibians that act as second intermediate or paratenic hosts (Sprent, 1979; Vicente *et al*., 1993; Moravec *et al*., 1997; Goldberg & Bursey, 2007; Goldberg *et al*., 2009 and González & Hamann, 2013). The precise identification of these helminths was not possible because only one specimen of each was found.

Investigating and describing the effects of environmental changes on endoparasites associated with members of the family Leptodactylidae can help to elucidate such effects on the dynamics of parasite communities. Besides, inventories of parasite fauna can contribute to studies on host-parasite relation of leptodactylids because they generate new information on the helminths associated with these hosts and provide new guides to identification (Poulin *et al*., 2015). The present study provides new helminth records for *Pseudopaludicola*, and also new records on the range and distribution of some helminth species in the Northeast-Brazilian. The Caatinga biome, with its habitat heterogeneity concentrated in the semiarid, encompasses a diverse fauna of amphibians (Camurugi *et al*., 2010; Andrade *et al*., 2014; Borges-Leite *et al*., 2014 and Cavalcanti *et al*., 2014). Nevertheless, there is still a scarce knowledge of diversity of helminths endoparasites associated with these amphibians.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Dactylogyrids (Platyhelminthes: Monogenea) from Sudanese *Labeo* spp., with a description of *Dogielius sennarensis* n. sp. and a redescription of *Dogielius flosculus* Guégan, Lambert & Euzet, 1989

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Article info

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Summary

Dactylogyrid monogeneans of *Labeo horie* Heckel, 1847 and *L. niloticus* (Linnaeus, 1758) (Cyprinidae) were surveyed at two sites on the River Nile in Sudan. The present study reports the presence of ten species of *Dactylogyrus* Diesing, 1850 and three species of *Dogielius* Bychowsky, 1936 based on a morphometric evaluation of the sclerotised structures. The species found include: *Dactylogyrus nathaliae* Guégan, Lambert & Euzet, 1988; *D. rastellus* Guégan, Lambert & Euzet, 1988; *D. retroversus* Guégan, Lambert & Euzet, 1988; *D. senegalensis* Paperna, 1969, *D. yassensis* Musilová, Řehulková & Gelnar, 2009 and five other undescribed species of *Dactylogyrus*. The genus *Dogielius* was represented by *Dogielius flosculus* Guégan, Lambert & Euzet, 1989; the newly identified *D. sennarensis* n. sp., and one undescribed *Dogielius* species. While *D. sennarensis* n. sp. resembles *D. intorquens*, it differs from this species and other congeners by having a longer ventral bar and anchor points and nosclerotised vagina. In addition, this study redescribes *D. flosculus* based on the morphology of specimens collected from *L. horie*. All specimens studied had a single large vagina, in contrast to the original description which reported a vagina composed of two unconnected parts. All dactylogyrid species in this study represent new host and geographical records.
Keywords: Dactylogyridae; *Dactylogyrus*; *Dogielius*; Sudan; River Nile

Introduction

Monogenean parasites represent an important part of the parasite community in most fish species. Whittington (1998) estimated that the world fish fauna may ultimately prove to harbour 25 000 monogenean species, of which just 3000–4000 have been described so far. Monogenean parasite diversity generally increases with decreasing latitude or increasing water temperature (Poulin, 2007). In essence, generation time is shorter, and evolution occurs more rapidly in warmer conditions (Cribb *et al*., 2002). Therefore, recently there is a trend of increasing number of new monogenean species being described predominantly from the tropical regions (e.g. Musilová *et al*., 2009; Přikrylová *et al*., 2009; Knoff *et al*., 2015; Pulido-Flores *et al*., 2015; Soo *et al*., 2015; Nitta & Nagasawa, 2016). Dactylogyrid monogeneans mainly infect freshwater cyprinids (Teleostei: Cypriniformes), with the suborder Dactylogyrinea Bychowsky, 1937 being the most numerous and diverse group (Cribb *et al*., 2002). Whilst Gibson *et al*. (1996) published a list of over 900 nominal species of *Dactylogyrus* Diesing, 1850, it is clear from the historical summary that the number of valid dactylogyrid species will continue to increase significantly (Cribb *et al*., 2002). Representatives of the genus *Dactylogyrus* were reported mainly

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from the Paleartic region, South Asia, Africa and North America and form about one quarter of the 3000-4000 monogenean species known to date (Cribb *et al*., 2002).

In comparison, the genus *Dogielius* Bychowsky, 1936 includes only 27 species, of which 17 have been described in Africa, four in China, one in India and five from the Palaearctic to date (Pugachev *et al*., 2009). Species of the genus *Dactylogyrus* do not naturally occur in the Neotropics, where, among others, other dactylogyrid genera such as *Anacanthorus* or *Urocleioides* are present (Kohn & Cohen, 1998).

Fish of the large genus *Labeo* Cuvier, 1817 are widely distributed in Africa and Southeast Asia. More than 100 species are known from both continents (Skelton, 2001; Froese & Pauly 2018), of which at least 80 occur in Africa (Skelton, 2001). Along the Sudanese stretch of the White and Blue Nile, *Labeo* are represented by five species: *L. niloticus* (Linnaeus, 1758); *L. horie* Heckel, 1847; *L. coubie* Rüppel, 1832; *L. forskalii* Rüppell, 1835 (El Moghraby & Abd el Rahman, 1984) and *L. meroensis* Moritz, 2007. The area of occurrence of *L. horie* and *L. niloticus* overlaps within the Blue and White Nile basin, in lakes Albert, Kyoga, Turkana and Rudolf, and in the River Omo (Froese & Pauly, 2018). Both species are predominantly herbivorous and constitute a considerable part of the catch of inland fisheries in the Sudan, making them a commercially important species (el Moghraby & Abd el Rahman, 1984; Azeroual *et al*., 2010).

Up until now, there have been no intensive fish parasite studies undertaken along the Sudanese stretch of the Nile. As such, there are no records of dactylogyrid monogeneans parasitizing *L. horie* and *L. niloticus*, and just four monogenean species have been reported from the five host species, *i. e. Macrogyrodactylus polypteri* Malmberg, 1957 from *Polypterus bichir* Lacepède, 1803 and *Polypterus senegalus* Cuvier, 1829; *Gyrodactylus malalai* Přikrylová, Blažek & Gelnar, 2012 from *Coptodon zillii* (Gervais, 1848); *Gyrodactylus* sp. from *Clarias gariepinus* Burchell, 1822) and *Afrogyrodactylus girgifae* Přikrylová & Luus-Powell, 2014 from *Brycinus nurse* (Rüppell, 1832) (Malmberg, 1957; Khalil, 1964, 1969, 1970; Přikrylová *et al*., 2012; Přikrylová & Luus-Powell, 2014). On the other hand, 39 dactylogyrid species have been recorded on fish from the Lower Nile in Egypt. To date, 48 monogenean species from five genera (including three subspecies) have been described for African *Labeo* spp.: *Dactylogyrus* (30 species), *Dogielius* (14 species, including two subspecies), *Paradiplozoon* (three species), *Diplozoon* (one species) and *Afrodiplozoon* (one species) (Khalil & Polling, 1997; Musilová *et al*., 2009; Crafford *et al*., 2012).

The aim of the present study was to examine and classify monogenean parasites from *L. horie* and *L. niloticus* from the Sudanese stretch of the Blue and White Nile. To the best of the authors' knowledge, this is the first parasitological survey of these two fish species; hence, the data will contribute significantly to our understanding of parasite diversity in tropical regions.

Material and Methods

Live individuals of *L. horie* and *L. niloticus* were obtained from fishermen at two sites in Sudan. Two specimens of *L. horie* and one specimen of *L. niloticus* were obtained in Kosti, a major city located on the western bank of the White Nile (13°10' 18,58" N, 32°40'19,24" E), while one specimen of *L. horie* and one specimen of *L. niloticus* were obtained at the Sennar Dam on the Blue Nile near the town of Sennar (13°32' 50,61" N, 33°38'4,23" E). The fish were identified according to Bailey (1994); the species' names following FishBase (Froese & Pauly, 2018).

The gills were examined under a binocular microscope for presence

Table 1. Records of dactylogyrids collected in this study, reported from different *Labeo* spp. hosts.

	Labeo coubie	*Labeo horie*[5]	*Labeo niloticus*[5]	*Labeo roseopunctatus*	*Labeo senegalensis*
Dactylogyrus nathaliae	-	-	+[K,S]	+[2]	-
Dactylogyrus rastellus	-	+[S]	-	-	+[2]
Dactylogyrus retroversus	+[2,4]	+[K]	-	-	-
Dactylogyrus senegalensis	+[4]	+[K,S]	+[S]	-	+[1,2]
Dactylogyrus yassensis	+[4]	+[K,S]	+[K]	-	+[2]
Dactylogyrus sp. 1	-	+[K]	-	-	-
Dactylogyrus sp. 2	-	-	+[K]	-	-
Dactylogyrus sp. 3	-	-	+[K,S]	-	-
Dactylogyrus sp. 4	-	-	+[K]	-	-
Dactylogyrus sp. 5	-	-	+[K]	-	-
Dogielius flosculus	-	+[S]	-	-	+[3]
Dogielius sennarensis	-	-	+[S]	-	-
Dogielius sp. 1	-	+[S]	+[K]	-	-

[1]Paperna, 1969; [2]Guégan *et al*., 1988; [3]Guégan *et al*., 1989; [4]Musilová *et al*., 2009; [5]Present study

of metazoan parasites according to standard methods (Ergens & Lom, 1970). All monogeneans collected were preserved in glycerine ammonium-picrate mixture (GAP; Malmberg, 1957) and later mounted in Canada balsam for further analysis (Lim & Gibson, 2008). Individual specimens were deposited in the helminthological collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic (IPCAS).
A motorised Olympus BX61 light microscope equipped with phase contrast and digital image analysis (Stream Motion 1.9.2) was used for species identification and measurements (Olympus Corporation, Japan). Drawings were made with the aid of a drawing tube attached to the microscope. All measurements are given in micrometres (μm) with the means followed by the range and number (n) of specimens measured in parentheses. The measurement scheme for the hard structures (i.e. haptoral attachment components, vaginal armament and male copulatory organ) follows that of Musilová *et al*. (2009). The numbering of hook pairs (in Roman numerals) follows the recommendations of Mizelle (1936). When all hooks were of the same size and morphology, only one was depicted. Where the hooks differed in size and morphology, all were depicted, with the smallest and largest hooks depicted in specific cases.
For morphological comparisons, specimens from the National Museum of Natural History, Paris (MNHN), and the Royal Museum for Central Africa, Belgium (RMCA), were examined, specifically *D. nathaliae* Guégan, Lambert & Euzet, 1988, holotype (MNHN 267 HC Ti 208); *D. longiphallus* Paperna, 1973, syntype (RMCA M.T.35.702); and *D. flosculus*, voucher (MNHN 268 HC Tj 209).

Ethical Approval and/or Informed Consent

The conducted research is not related to either human or animal use.

Results

Ten *Dactylogyrus* species and three *Dogelius* species were collected from the gills *L. horie* and *L. niloticus* (Table 1). Morphometric descriptions of all species found are provided below.

Dactylogyridae Bychowsky, 1933
***Dactylogyrus* Diesing, 1850**

***Dactylogyrus nathaliae* Guégan, Lambert & Euzet, 1988** (Fig. 1)

Type host and locality: *Labeo roseopunctatus* Paugy, Guégan & Agnèse, 1990; River Niger in Bamako, Mali; River Baoule at Missira, Mali (Guégan *et al*., 1988)
Present record: *Labeo niloticus* White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Blue Nile Sudan, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan
Site: Gill lamellae
Type specimen: Holotype MNHN 267 HC
Voucher specimen: IPCAS M-647
Material examined:
15 flattened specimens (four specimens from *L. niloticus* (Kosti) and 11 specimens from *L. niloticus* (Sennar))
Redescription: Body length 326 (315–334; n = 3); greatest width

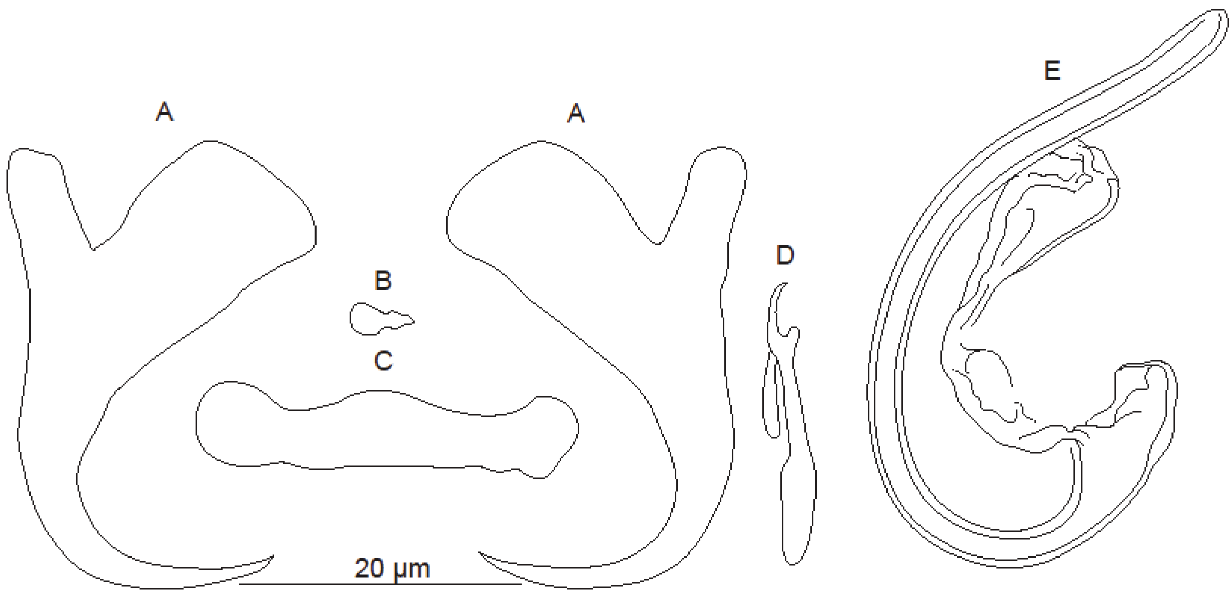

Fig. 1. *Dactylogyrus nathaliae* Guégan, Lambert & Euzet, 1988. A = anchor; B = ventral bar; C = dorsal bar; D = hook; E =copulatory organ

60 (48–70; n = 3). Haptor 43 (33–59; n = 3) long; 58 (36–72; n = 3) wide. Single pair of anchors (dorsal): inner length 31 (29–34; n=15); outer length 31 (29–34; n=15); inner root 15 (13–17; n=15) long; outer root 10 (9–13; n=15) long; point 12 (10–15; n=13) long. Two bars, dorsal: 29 (27–31; n=14) long, 5 (4–7; n=14) wide; ventral: 4 (3–6; n=6). Hooks seven pairs, dissimilar in size; hook lengths (n=6): pair I =19; pair II=14; pairs III, IV, V, VI and VII=14. One pair of needles located near hooks of pair V. No sclerotised vagina observed. Male copulatory organ (MCO) total length 42 (39–47; n=15); tube trace-length 67 (63–71; n=15).

Remarks: Comparison of our specimens from *L. niloticus* with morphometric characters observed from the holotype *D. nathaliae* (MNHN 267 HCTi 208) and measurements given in the original description (Guégan *et al*., 1988) supports the identity of the newly collected specimens. The haptor is composed of anchors, dorsal and vestigial ventral bars and, typically for this species, marginal hooks of embryonal type with button-extended base. *L. niloticus* represents a new host species for *D. nathaliae*.

Dactylogyrus rastellus **Guégan, Lambert & Euzet, 1988** (Fig. 2)

Type host and locality: *Labeo senegalensis* Valenciennes, 1842; River Niger in Bamako, Mali; River Baoule at Missira, Mali (Guégan *et al*., 1988)

Present record: *Labeo horie* Blue Nile, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan

Site: Gill lamellae

Voucher specimen: IPCAS M-648

Material examined: Two flattened specimens

Redescription: Body length 329; greatest width 55. Haptor 65 long; 72 wide. Single pair of anchors (dorsal): inner length 50 (n=2); outer length 37.5 (37–38; n=2); inner root 17.5 (17–18; n=2); outer length 3.5 (3–4; n=2); point 18.5 (18–19; n=2) long, sharply angled relative to the shaft. Shaft double, swollen and long. One bar, dorsal: 20.5 (20–21; n=2) long, 3.5 (3–4; n=2) wide. Hooks seven pairs, dissimilar in size; hook lengths (n=2): pair I = 23; pairs II, III and IV18 (16–21); pairs V, VI and VII 29 (26–31). No sclerotised vagina observed. MCO total length 28.5 (27–30); tube trace-length 32 (n=2), formed by short copulatory tube, curved into an arch and accessory piece from its middle extending laterally. Ventral bar absent.

Remarks:

This specimen was determined as *D. rastellus* based on morphological characters of sclerotised structures. The size of sclerotised structures corresponds well to the original description of *D. rastellus*; however, inner length and outer length of anchors are shorter with respect to the original species description. *L. horie* represents a new host species for *D. rastellus*.

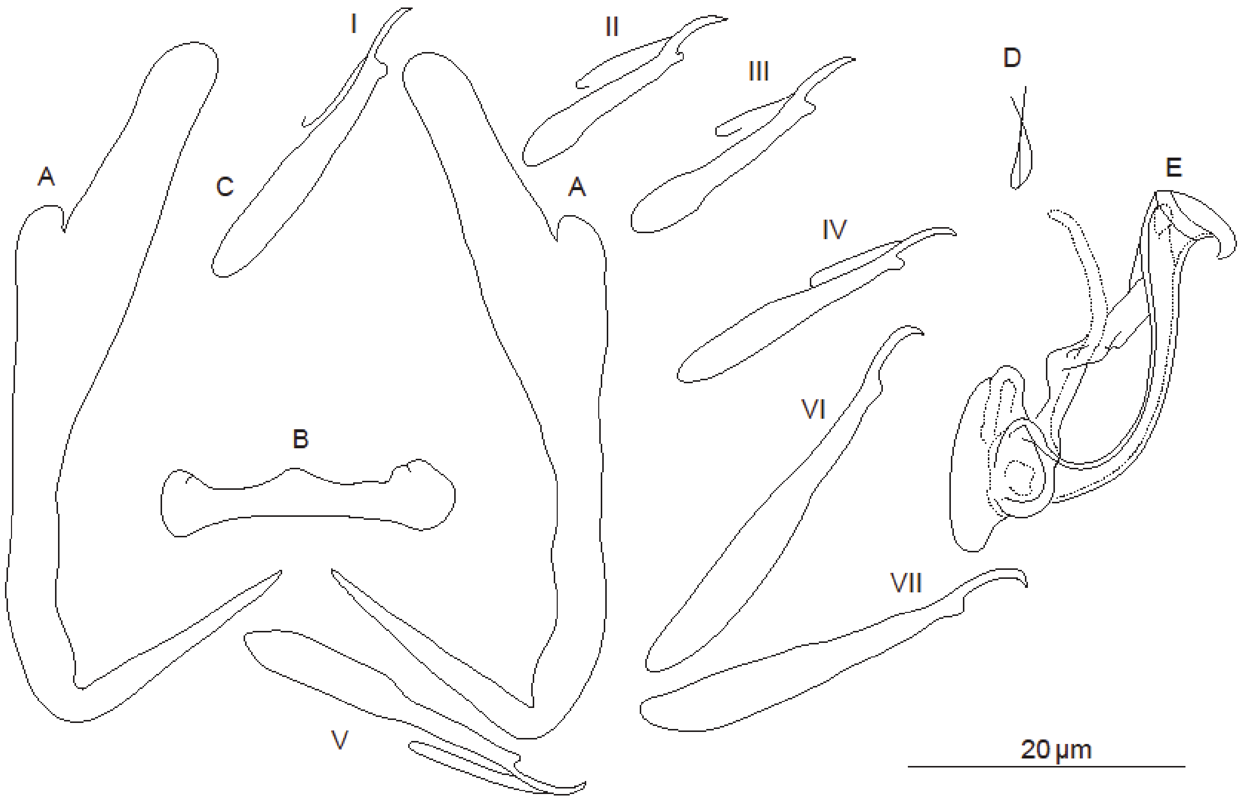

Fig. 2. *Dactylogyrus rastellus* Guégan, Lambert &Euzet, 1988. A = anchor; B = dorsal bar; C = hooks; D = needle; E =copulatory organ

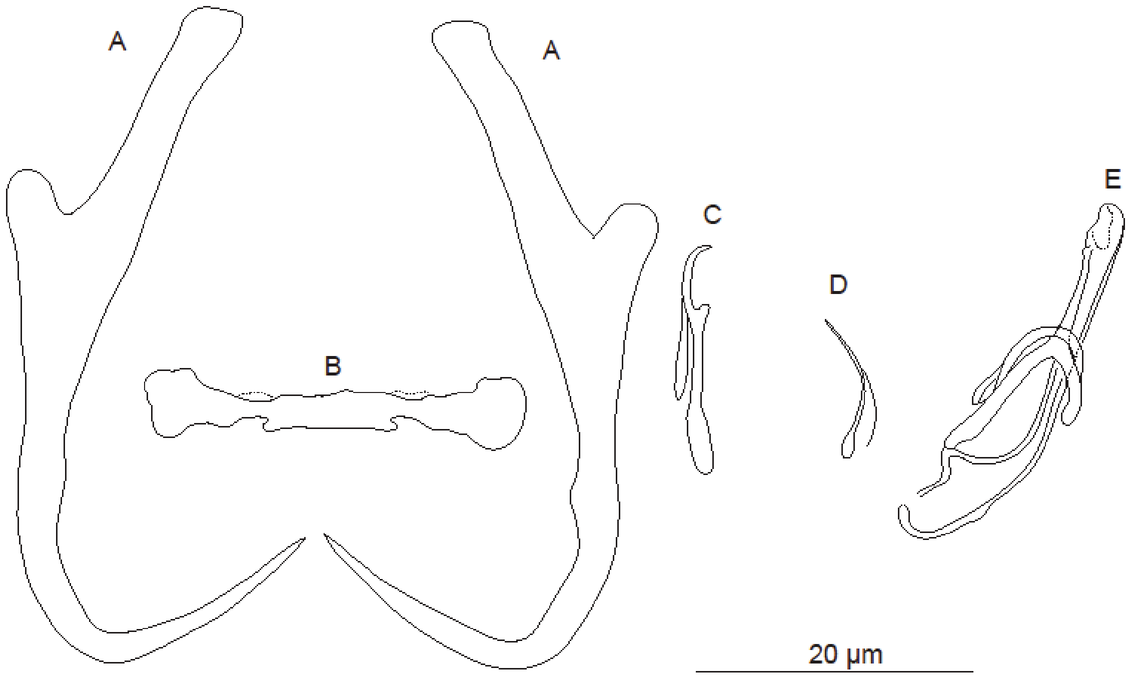

Fig. 3. *Dactylogyrus retroversus* Guégan, Lambert & Euzet, 1988. A = anchor; B = dorsal bar; C = hooks; D = needle; E = copulatory organ

Dactylogyrus retroversus **Guégan, Lambert & Euzet, 1988 (Guégan *et al.*, 1988)** (Fig. 3)

Type host and locality: *Labeo coubie* Rüppel, 1832; River Niger in Bamako, Mali (Guégan *et al.*, 1988)

Other records: *Labeo coubie* River Baoule at Missira, Mali (Guégan *et al.*, 1988); *Labeo coubie*, River Gambia, Senegal (Musilová *et al.*, 2009)
Present record: *Labeo horie*, White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Sudan

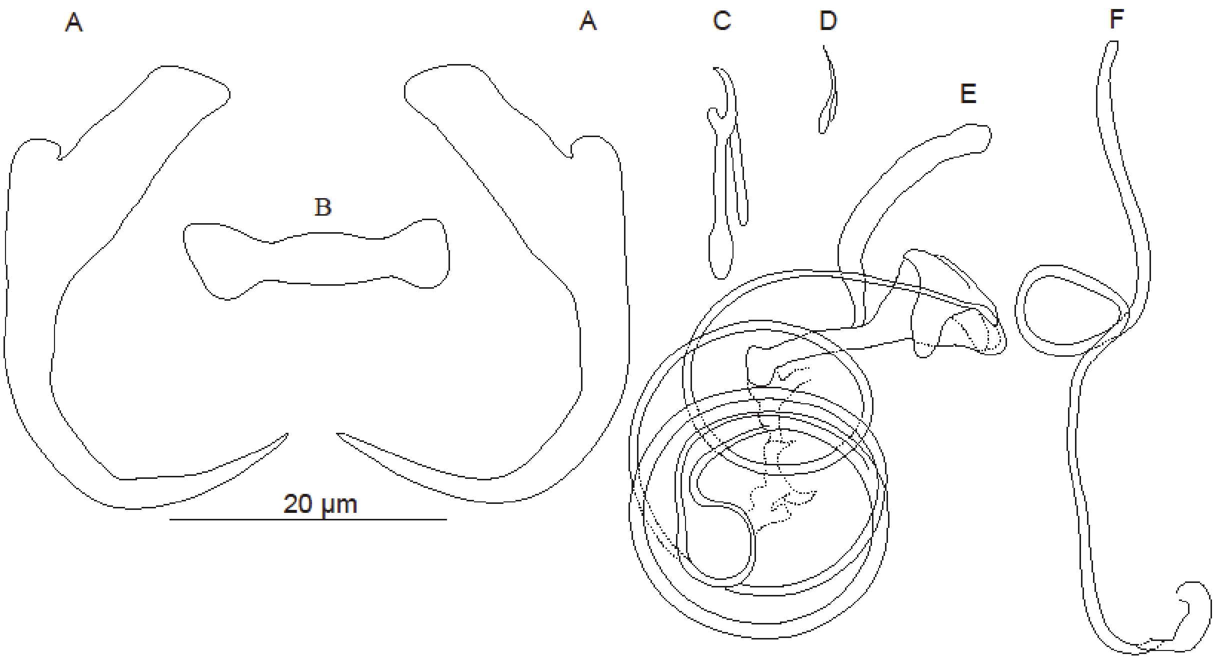

Fig. 4. *Dactylogyrus senegalensis* Paperna, 1969. A = anchor; B = dorsal bar; C = hooks; D = needle; E = copulatory organ; F = vagina

Site: Gill lamellae
Voucher specimen: IPCAS M-650
Material examined: 13 flattened specimens
Redescription: Body length 344 (329–358; n=2); greatest width 82 (60–103; n=2). Haptor 65 (55–74; n=2) long; 183 (174–192) wide. Single pair of anchors (dorsal): inner length 45 (41–47; n=13); outer length 32 (29–36; n=13); inner root 17 (15–19; n=13); outer root 4 (2–5; n=13); point 17 (16–18; n=13) long. One bar (dorsal): 27 (24–29; n=13) long, 3 (2–4, n=13) wide. Hooks seven pairs, similar in shape and size; hook lengths 16 (15–16; n=6). Needles (1 pair) located near hooks of pair V. Vagina non-sclerotised. MCO total length 26 (24–28; n=10), tube trace-length 27 (27–28; n=5).
Remarks: The specimens collected during the present study were identified based on morphology of anchors and MCO, which were identical to those presented by Guégan *et al*. (1988) in the original species description. *Dactylogyrus retroversus* can be confused with *D. titus* Guégan, Lambert & Euzet, 1988 as the morphology of both species is almost identical. Both species have a double swollen shaft and the MCO of both species has a short copulatory tube (shorter in *D. titus*) associated with an accessory piece shaped like the letter Y. However, the species can be distinguished by the size of the sclerotised structures, with all features being larger in *D. retroversus* (Dorsal anchors: *D. retroversus* - inner length 42; outer length 29; inner root 18–19 long; outer root 2–3 long; point 17–18 long; *D. titus* - inner length 39; outer length 26; inner root 17 long; outer root 2 long; point 14 long). Note, however, that the total length and trace-tube length of the copulatory organ is closer to that of *D. titus*. (*D. retroversus* - MCO total length 30–34; tube trace-length 34–35; *D. titus* - MCO total length 20; tube trace-length 27). The finding of *D. retroversus* on *L. horie* represents a new host record.

Dactylogyrus senegalensis **Paperna, 1969** (Fig. 4)

Type host and locality: *Labeo coubie*, Lake Volta at the Black and White Volta confluence, Ghana (Paperna, 1969)
Other records: *Labeo senegalensis*, Lake Volta at Yeji, Ghana (Paperna, 1969); *Labeo senegalensis*, River Niger in Bamako, Mali; River Baoule at Missira, Mali; Lake Guiers, Senegal (Guégan *et al*., 1988)
Present records: *Labeo horie*, White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Blue Nile, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan; *Labeo niloticus*, Blue Nile, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan
Site: Gill lamellae
Voucher specimen: IPCAS M-649
Material examined:
Nine flattened specimens (two specimens from *L. horie* (Sennar), four specimens from *L. horie* (Kosti), three specimens from *L. niloticus* (Sennar))
Redescription: Body length 491; greatest width 81. Haptor 74 long; 93 wide. Single pair of anchors (dorsal): inner length 32 (31–33; n=9), outer length 27 (25–28; n=9), inner root 12 (11–14;

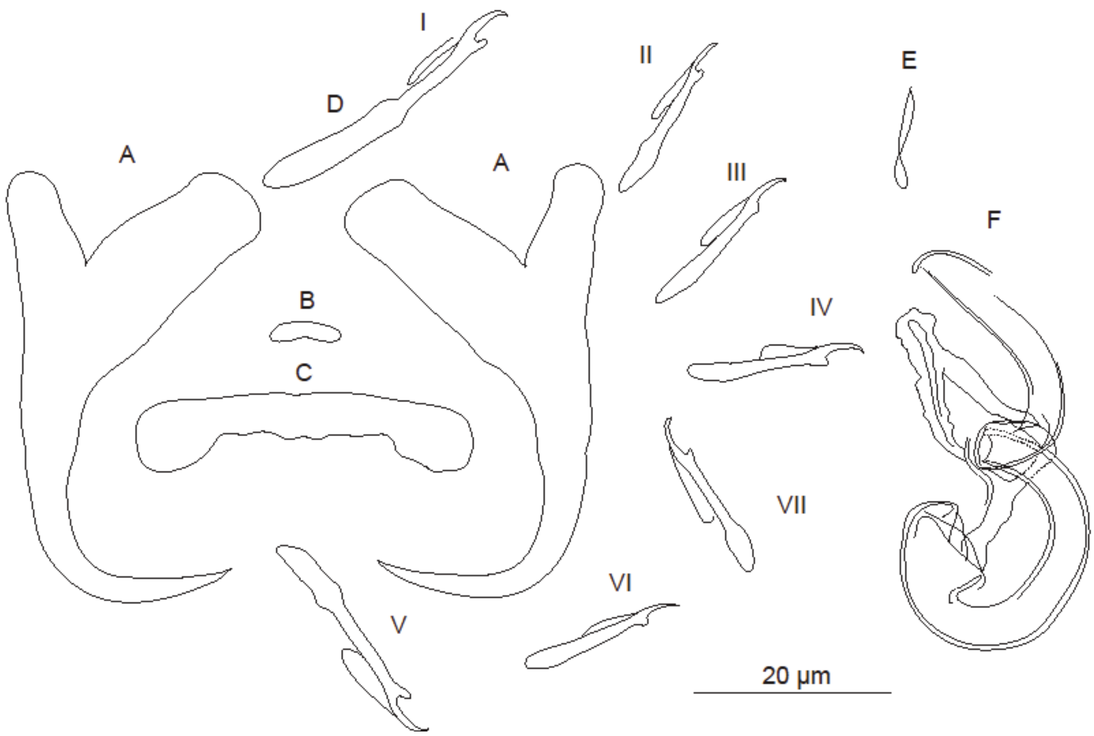

Fig. 5. *Dactylogyrus yassensis* Musilová, Řehulková & Gelnar, 2009. A = anchor; B = ventral bar; C = dorsal bar; D = hook; E = needle; F = copulatory organ

n=9), outer root 3 (2–4; n=9), point 13 (12–15; n=9) long. One bar, dorsal: 19 (17–21; n=9) long, 4 (3–6; n=9) wide. Hooks seven pairs, similar in shape and size; hook length 15 (13–16; n=6). One pair of needles located near hooks of pair V. MCO total length 40 (34–45; n=8); tube trace-length 177 (149–195; n=7); with a coiled tube of three rings passing through the middle. Distal part of the accessory piece characterised by a lateral extension. Vagina sclerotised, long tube forming coiled spiral; total length: 44 (36–48; n=7). Ventral bar absent.

Remarks: The morphology and measurements of the specimens found on *L. horie* and *L. niloticus* in the present study correspond to the descriptions of *D. senegalensis* found on the gills of *L. coubie* and *L. senegalensis* from Ghana (Paperna, 1969) and *L. senegalensis* from Mali and Senegal (Guégan *et al*., 1988). Both *L. horie* and *L. niloticus* represent new host species for *D. senegalensis*.

***Dactylogyrus yassensis* Musilová, Řehulková & Gelnar, 2009** (Fig. 5)

Synonym: *Dactylogyrus cyclocirrus* Paperna, 1973 of Guégan *et al*. (1988)

Type host and locality: *Labeo coubie*, River Gambie, Campement du Lion, Niokolo Koba National Park, Senegal (Musilová *et al*., 2009).

Other records: *Labeo. senegalensis*, River Niger in Bamako, River Baoule at Missira, Mali; Lake Guiers, Senegal, (Guégan *et al*., 1988)

Present records: *Labeo horie* While Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Sudan, Blue Nile, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan; *Labeo niloticus* White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Sudan

Site: Gill lamellae

Voucher specimen: IPCAS M-651

Material examined:

14 flattened specimens (eight specimens from *L. horie* (Sennar), four specimens from *L. horie* (Kosti), two specimens from *L. niloticus* (Kosti))

Redescription: Body length 690 (586–854; n=4); greatest width 166 (91–231; n=4). Haptor robust 105 (79–132; n=4) long; 145 (112–197; n=4) wide. Single pair of anchors, dorsal: inner length 43 (40–46; n=14); outer length 43 (42–46; n=14); inner root, wide 15 (13–17; n=14) long; outer root 9 (7–11; n=14) long; point 13 (10–15; n=14) long. Two bars: dorsal bar 35 (31–38; n=14) long, 6 (5–7; n=14) wide; ventral bar reduced in size, vestigial 8 (7–9; n=10) long; 2 (n=10) wide. Hooks dissimilar in size; hook lengths (n=11): pair I = 28; pair V = 23; pairs II, III, IV, VI and VII = (16–20). One pair of needles located near hooks of pair V. Sclerotised vagina not observed. MCO total length 41 (35–47, n=12), forms a coiled wide tube of about 1.5 ring, which is supported by accessory piece extending from large basal ampulla.

Tube trace-length 74 (62–80; n=12). Accessory piece slightly sclerotised, articulated to base.

Remarks: Specimens were determined as *D. yassensis* based on morphology and size of sclerotised structures. *Dactylogyrus*

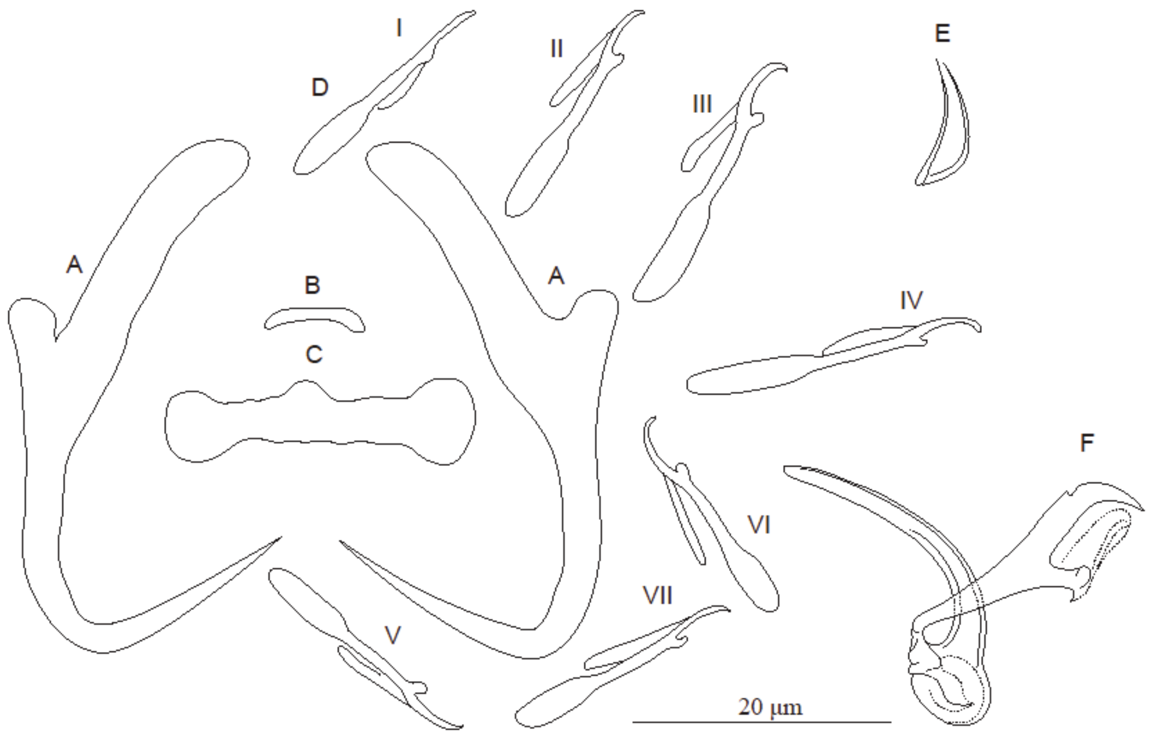

Fig. 6. *Dactylogyrus* sp.1. A = anchor; B = ventral bar; C = dorsal bar; D = hooks; E = needle; F =copulatory organ

yassensis was described based on the revision of type material of *D*. *cyclocirrus* Guégan *et al*. (1988), now considered the senior subjective synonym (Musilová *et al*., 2009). The species mentioned above can be easily confused with *D*. *omega* Guégan & Lambert, 1991, which differs in just some morphological features and measurements. In contrast to *D*. *omega*, *D*. *yassensis* possess larger anchors, the shorter point not reaching the level of the inner anchor root tip (extending past the level of inner anchor root tip in *D*. *omega*), a dorsal bar smaller in relation to anchor size, and a copulatory tube with a noticeably larger diameter (Musilová *et al*., 2009). The sclerotised structures of *D*. *yassensis* described here are larger than those of *D*. *cyclocirrus*, *D*. *omega* and *D*. *yassensis* from *L*. *coubie*; however, they are closest to those of *D*. *yassensis*. Both *L*. *horie* and *L*. *niloticus* represent new hosts species for *D*. *yassensis*.

***Dactylogyrus* sp. 1** (Fig. 6)

Present record: *Labeo horie* White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Sudan
Site: Gill lamellae
Material examined: One flattened specimen
Description: Body length 307; greatest width 80. Haptor 88 long; 125 wide. Single pair of anchors (dorsal): inner length 41 (n=1); outer length 28 (n=1); inner root 21 (n=1) long, terminally rounded internal process; outer root 4 (n=1) long, rounded; point 18 (n=1) long, sharply hooked. Two bars: dorsal bar 20 (n=1) long, wide with the protrusion in the middle, 5 (n=1) wide; ventral bar reduced in size, vestigial 8 (n=1) long; 1 (n=1) wide. Hooks dissimilar in size; hook lengths (n=1): pairs I, V, VI and VII = 18; pairs II, III and IV = 23. Needles (1 pair) located near hooks of pair V. No sclerotised vagina observed. MCO total length 23 (n=1); tube trace-length 32 (n=1), shaped like the letter V.

Remarks: The morphology and measurements of our specimen, especially the anchors and bars, are very similar to those in the original description of *D. longiphallus* Paperna, 1973, syntype (RMCA M.T. 35.702). However, some differences were observed in copulatory organ morphology. The MCO of *D*. *longiphallus* consists of basal ampulla followed by a narrow, tubular copulatory tube tapering distally. The accessory piece is fixed to the basal ampulla, forming a forked branch that continues on to affinely sclerotised sheath, which serves as a tube guide. *Dactylogyrus* sp. 1 unlike *D. longiphallus*, a vagina was not observed. Moreover, the copulatory tube in the specimen studied (23 µm long) was almost half the size of those in *D. longiphallus* specimens measured by Paperna (1973) from Nzoia (39µm), L. Albert (38–40), Mobuku (36–40), Kzinga (40), Ruaha (n=35–38) and Ghana (n=27–36).

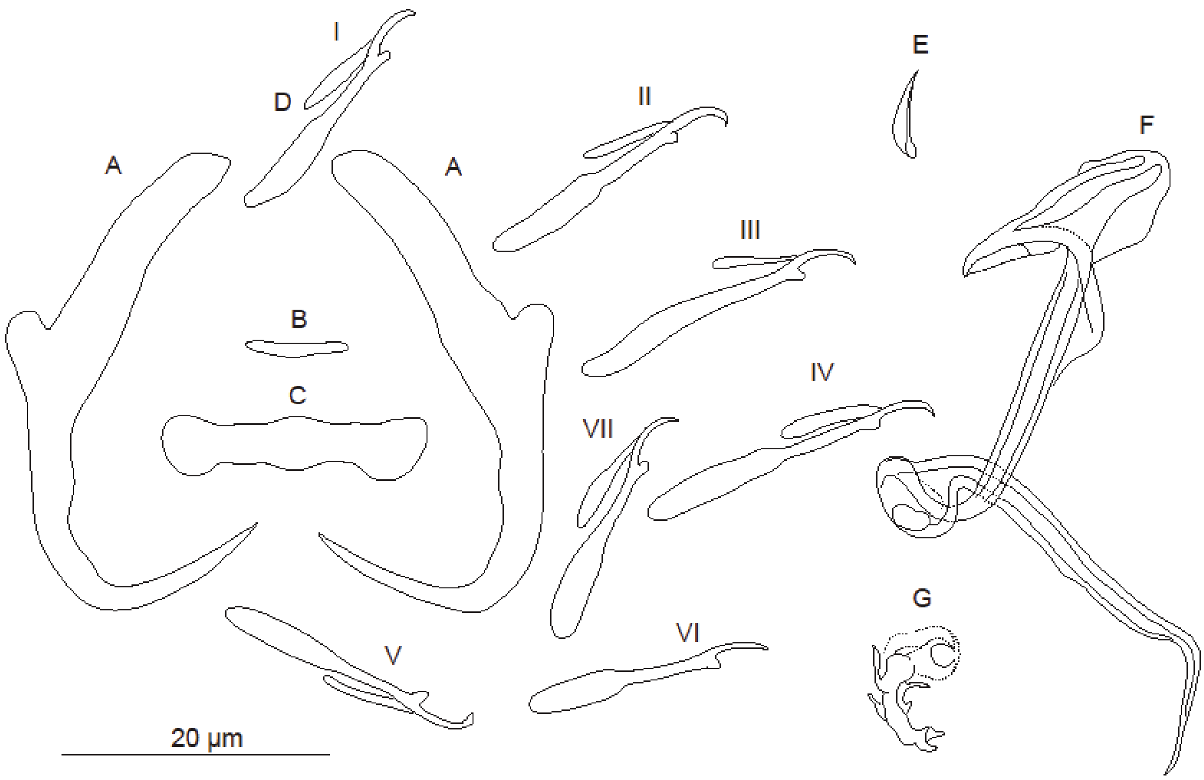

Fig. 7. *Dactylogyrus* sp.2. A = anchor; B = ventral bar; C = dorsal bar; D = hooks; E = needle; F = copulatory organ; G = vagina

***Dactylogyrus* sp. 2** (Fig. 7)

Present record: *Labeo niloticus* White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Sudan
Site: Gill lamellae
Voucher specimen: IPCAS M-642
Material examined: One flattened specimen
Description: Single pair of anchors (dorsal): inner length 34 (n=1); outer length 22 (n=1); inner root 18 (n=1) long; outer root 3 (n=1) long, rounded; point 13 (n=1) long. Two bars (dorsal) 21 long, 4 (n=1) wide; ventral bar reduced in size, vestigial 8 (n=1) long; 1 (n=1) wide. Hooks seven pairs, similar in size 20 (18–22). Needles (one pair) located near hooks of pair V. No sclerotised vagina observed. Copulatory organ total length 34 (n=1); tube trace-length 38 (n=1); MCO forms long tube without accessory piece shaped like the letter L.
Remarks: *Dactylogyrus* sp. 2 was dissimilar to any previously described African *Dactylogyrus* species; however, description of a new species was not possible due to the availability of only one specimen.

***Dactylogyrus* sp. 3** (Fig. 8)

Present record: *Labeo niloticus* White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Blue Nile, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan
Site: Gill lamellae
Voucher specimen: IPCAS M-643
Material examined: Two flattened specimens (one specimen from *L. niloticus* (Kosti)
one specimen from *L. niloticus* (Sennar))
Redescription: Body length 412; greatest width 126. Haptor 74 long; 78 wide. Single pair of wide anchors (dorsal): inner length 39.5 (n=2); outer length 23.5 (n=2); inner root 21.5 (n=2) long; outer root 4 (n=2) long; roundish curved point 15 (n=2) long. Two bars (dorsal) 20 (n=2) long with medially located protrusion; 6.5 (n=2) wide; ventral bar reduced in size, vestigial 6 (n=2) long; 1.5 (n=2) wide. Hooks dissimilar in size (n=2); hook lengths pair I = 20.5; II = 23.5; III = 24.5; IV = 25.5; V = 18.5; VI = 17; VII = 17. Needles (1 pair) located near hooks of pair V. Vagina total length 10. Copulatory organ total length 26, tube trace length 31.5; MCO accessory piece terminates with three finger-like projections. Copulatory tube waved and begins by funnel, passing through the projection.
Remarks: Morphologic features are similar to *Dactylogyrus* sp. 4, mentioned below. Based on morphology and measurements of haptor and male copulatory organ, *Dactylogyrus* sp.3 is similar to *D. dembae* Musilová, Řehulková & Gelnar, 2009, which differs from our study material in sharp angulation of points, leaner anchors, proximal part of shaft being slightly swollen and median width of dorsal bar narrower.

***Dactylogyrus* sp. 4** (Fig. 9)

Present record: *Labeo niloticus* White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Sudan

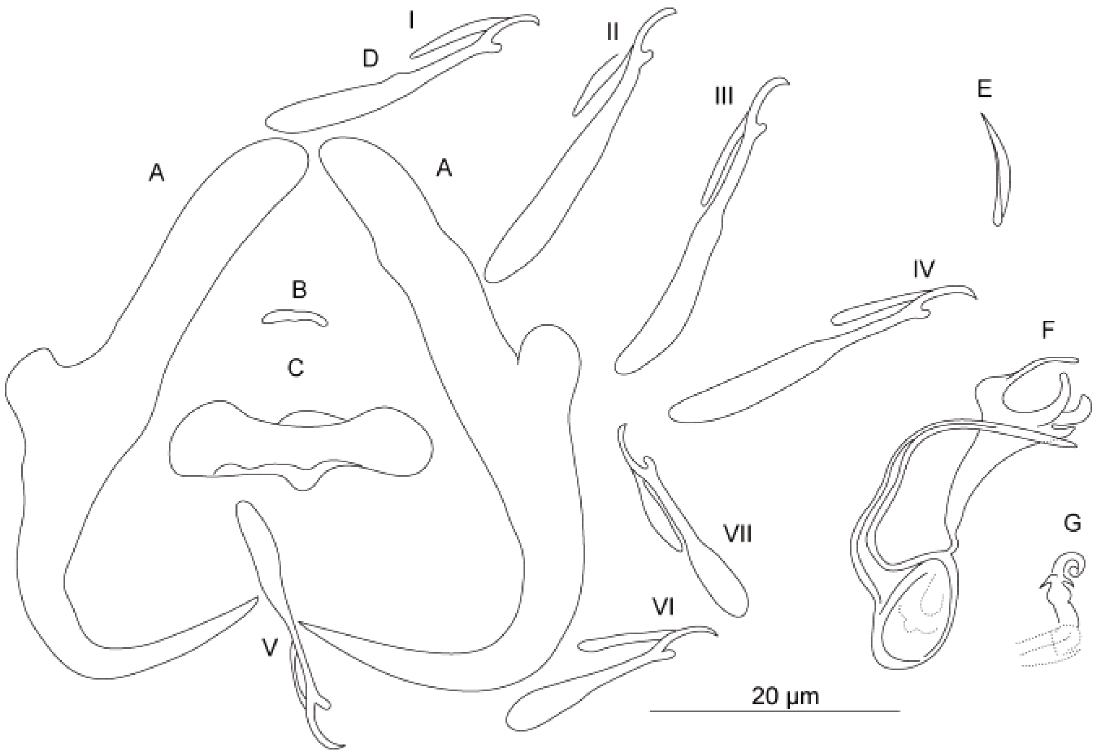

Fig. 8. *Dactylogyrus* sp.3. A = anchor; B = ventral bar; C = dorsal bar; D = hooks; E = needle; F = copulatory organ; G = vagina

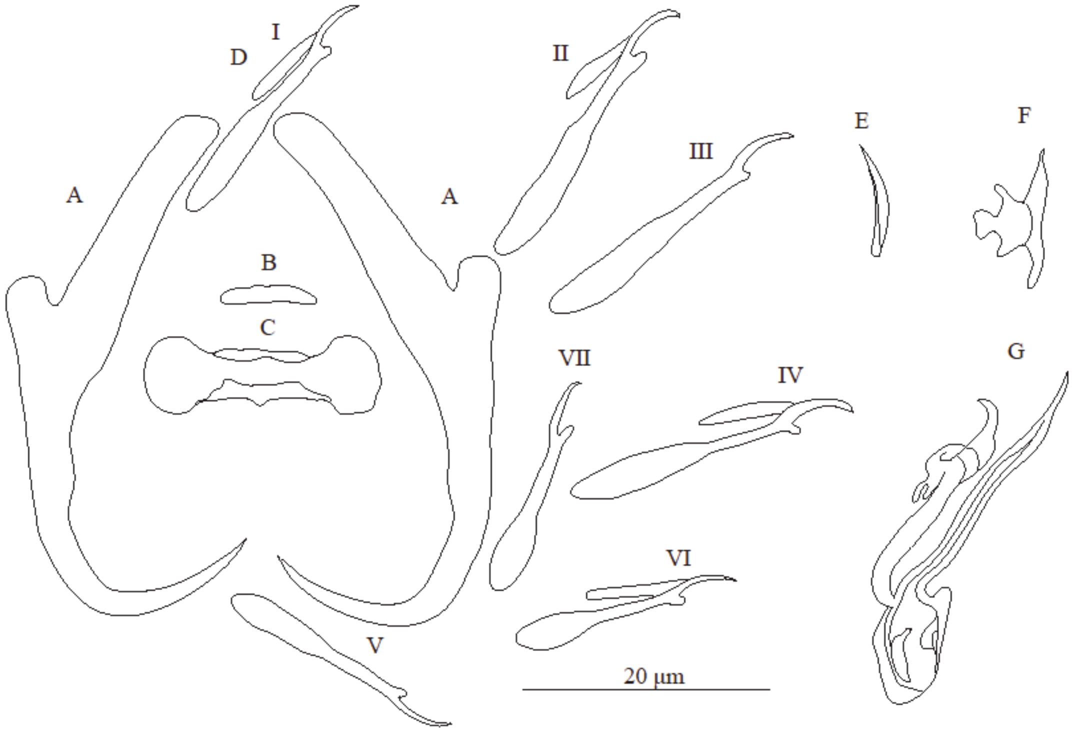

Fig. 9. *Dactylogyrus* sp.4. A = anchor; B = ventral bar; C = dorsal bar; D = hooks; E = needle; F = copulatory organ; G = vagina

Site: Gill lamellae
Voucher specimen: IPCAS M-644
Material examined: One flattened specimen
Description: Single pair of anchors (dorsal): inner length 47 (n=1); outer length 25 (n=1); inner root 30 (n=1) long; outer root 4 (n=1) long; point 15 (n=1) long. Two bars, dorsal 20 (n=1) long, 6 (n=1) wide; ventral bar reduced in size, vestigial 5 (n=1) long; 1 (n=1) wide. Hooks dissimilar in size; hook lengths pair I = 23; II = 27; III = 28; IV = 28; V = 20; VI = 18; VII = 18. Needles (one pair) located near hooks of pair V. Vagina total length 10. Copulatory organ total length 32, tube trace length 35.
Remarks: Morphology of *Dactylogyrus* sp. 4 is similar to *Dactylogyrus* sp. 3 mentioned above. Unlike the latter species, *Dactylogyrus* sp. 4 has sharply hooked points, and a longer inner length of anchor and inner root length. These features also distinguish the material from another similar species *D. dembae*. Further, the anchor inner root length of *Dactylogyrus* sp. 4 is almost twice as long as that of *D. dembae* (*D. dembae* - inner root of dorsal anchor 16 µm).

***Dactylogyrus* sp. 5** (Fig. 10)

Present record: *Labeo niloticus* White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E), Sudan
Site: Gill lamellae
Present specimen: IPCAS M-645
Material examined: One flattened specimen
Description: Body length 364; greatest width 83. Haptor 83 long; 119 wide. Single pair of anchors (dorsal): inner length 45 (n=1); outer length 32 (n=1); inner root 20 (n=1) long, swollen; outer root 4 (n=1) long; point 13 (n=1) long, roundish, curved to the shaft. Two bars (dorsal) 20 (n=1) long, at the edges balloon-shaped 4 (n=1) wide; (ventral) 8 (n=1) long, reduced in size. Hooks seven pairs, dissimilar in size; hook lengths (n=1): pair I =18 (n=1), pairs II, III, IV = 23 (n=1); pairs V, VI, VII = 18 (n=1). Needles located near hooks of pair V. Vagina total length 11 (n=1). Copulatory organ total length 32 (n=1), tube of MCO is slightly wave, connected to the accessory piece in its proximal part. Accessory piece forms large ampulla in its base, from which it extends in tube. Distal part forms a hook with finger-like projections.
Remarks: Morphometrics of sclerites are dissimilar to any other African dactylogyrid. *Dactylogyrus* sp. 5 may represent a new species; however, description of a new species was not possible due to the availability of only one specimen.

***Dogielius* Bychowsky, 1936**

***Dogielius flosculus* Guégan, Lambert & Euzet, 1989** (Fig. 11)

Type host and locality: *Labeo senegalensis*, River Niger in Bamako, Mali; River Baoule at Missira, Mali (Guégan *et al*., 1989)
Present record: *Labeo horie* Blue Nile, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan

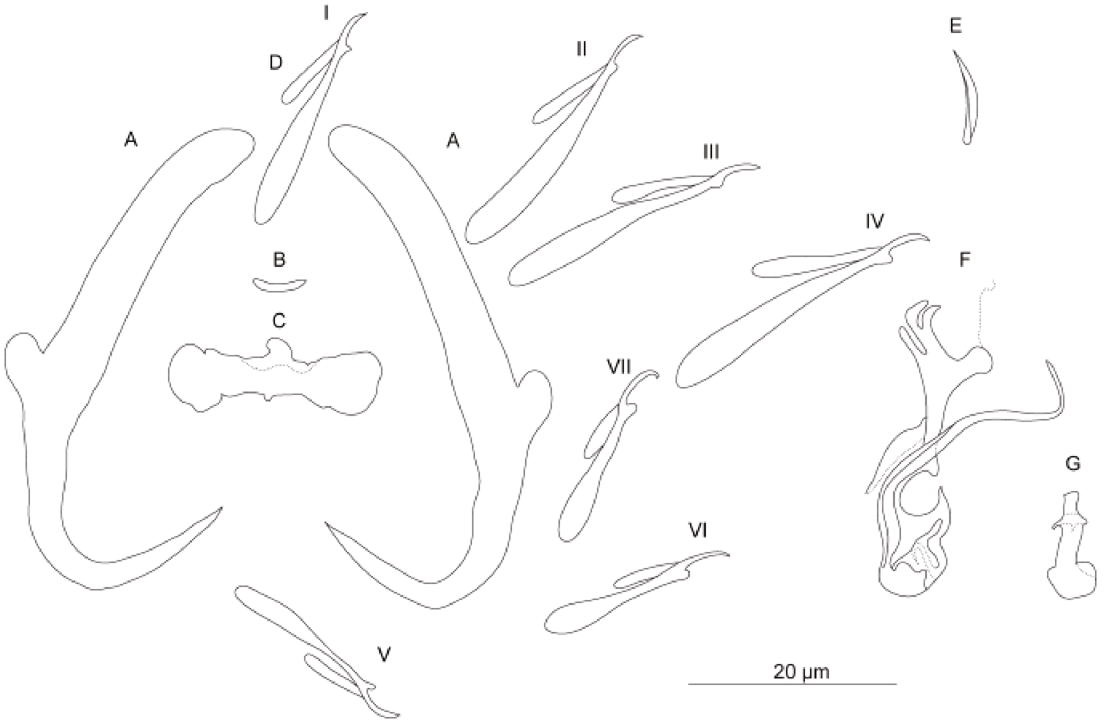

Fig. 10. *Dactylogyrus*sp.5. A = anchor; B = ventral bar; C = dorsal bar; D = hooks; E = needle; F = vagina; G = copulatory organ

Site: Gill lamellae
Type specimen: holotype MNHN 268 HC (Tj 209)
Voucher specimen: IPCAS M-640
Material examined: 10 flattened specimens from *L. horie* (Sennar)
Redescription: Body length 356 (295–390; n=3); greatest width 107 (95–116; n=3). Haptor 80 (52–109; n=3) long; 129 (98–170; n=3) wide. Shaft is long and thin. Single pair of anchors (ventral): inner length 45 (43–47; n=10); outer length 50 (48–53; n=10); point 22 (17–25; n=10) long, thin at the end, bent. Robust bar (ventral) 47 (45–50; n=9) long, 9 (8–10; n=9) wide. Hooks seven pairs, similar in size 19 (14–21; n=5) long. Needles located near hooks of pair V. Vagina total length 30 (25–35; n=9), bulb-shaped

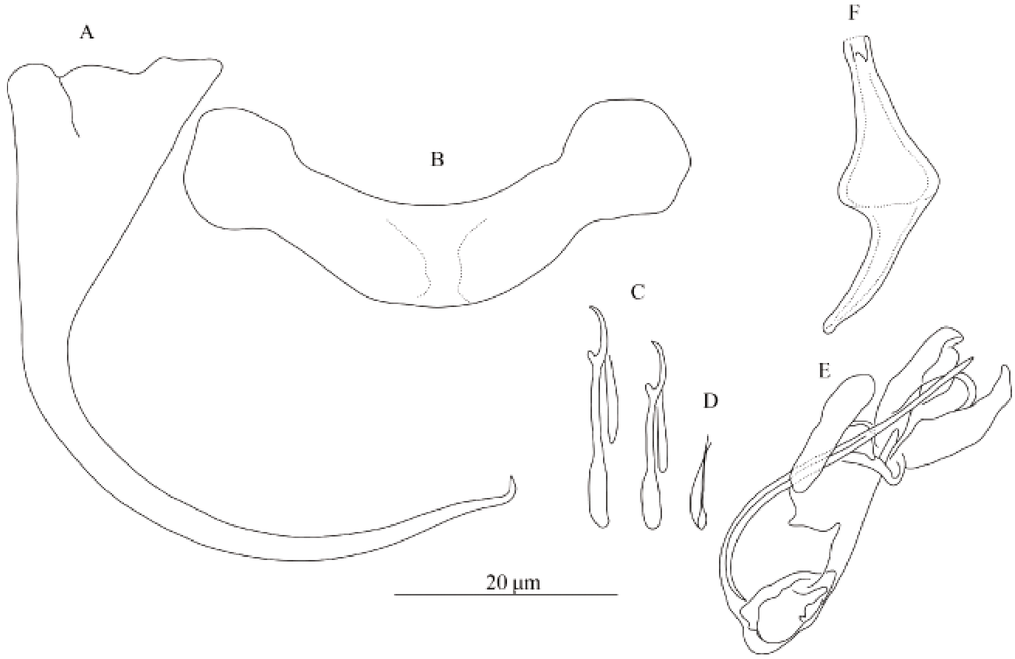

Fig. 11. *Dogielius flosculus* Guégan, Lambert & Euzet, 1989. A = anchor; B = ventral bar; C = hooks; D = needle; F = vagina; G = copulatory organ

in the middle, from which tapering projections extend on both sides. Copulatory organ total length 33 (22–45; n=10), a thin tube with a clearly defined funnel; tube with accessory piece forms a number 8, which opens on one side.

Remarks: This specimen was determined as *D. flosculus* based on morphology and size of sclerotised structures. The anchors are narrow and long, and the measurements correspond well to the original description. The MCO comprises a thin tube connected with the funnel. The holotype MNHN 268 HC (Tj 209) from *L. senegalensis* was partially damaged and thus the copulatory organ and vagina could not be observed. Here, we provide a redescription of *D. flosculus* with illustrations. Morphology and measurements of the haptor and copulatory organ shows these specimens to be conspecific. The vagina of our *D. flosculus* specimen had a unique shape among the species congeners, being very large vagina and composed of two parts connected in the middle. The original description of *D. flosculus* shows the vagina composed of two unconnected parts, however, when the two parts are connected, it closely resembles the vagina of our specimens, both morphologically and by size. In the case of the genus *Dogielius*, a disconnected vagina has not yet been observed and, in terms of functionality, it is unlikely that it ever will be. A comparison of the sclerotised structure dimensions for *Dogielius* from this study and those of *D. flosculus* described by Guégan *et al.* (1989) are provided in Table 2. The finding of *D. flosculus* on *L. horie* in Sudan represents a new host and geographic record for this species.

***Dogielius sennarensis* n. sp.** (Fig. 12)

Type host and locality: *Labeo niloticus* Blue Nile, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan
Site: Gill lamellae
Type specimens: Holotype and four paratypes IPCAS M-639
Material examined: Seven flattened specimens
Etymology: The species name refers to the type locality
Description: Anchors, hooks and bars stretched across the haptor, dominant ventral bar. Four eyes located on the front of the body. Whole body relatively short and wide. Body length316 (216–387; n=6); greatest width 87 (62–120; n=6). Haptor 80 (45–134; n=6) long; 109 (58–158; n=6) wide. Single pair of anchors (ventral): inner length 32 (31–33; n=7); outer length 46 (43–50; n=7); point 32 (28–35; n=7) long. Shaft relatively distinct from the point. Point quite short and rounded, ending with a small bend. One bar (ventral) 72 (70–76; n=7) long, 7 (7–8; n=7) wide. Hooks seven pairs, similar in size 18 (16–20; n=1) long. Needles located near hooks of pair. Copulatory organ total length 32 (30–34; n=6). MCO comprises a very thin tube passing through the fork-shaped accessory piece.
Remarks:
The haptor in the material studied was comprised of anchors with long shafts, long thin points and a huge bar. *D. sennarensis* n. sp. mainly differs from other members of the genus *Dogielius* in the length of the points, its 32 µm (mean) point being longer than most

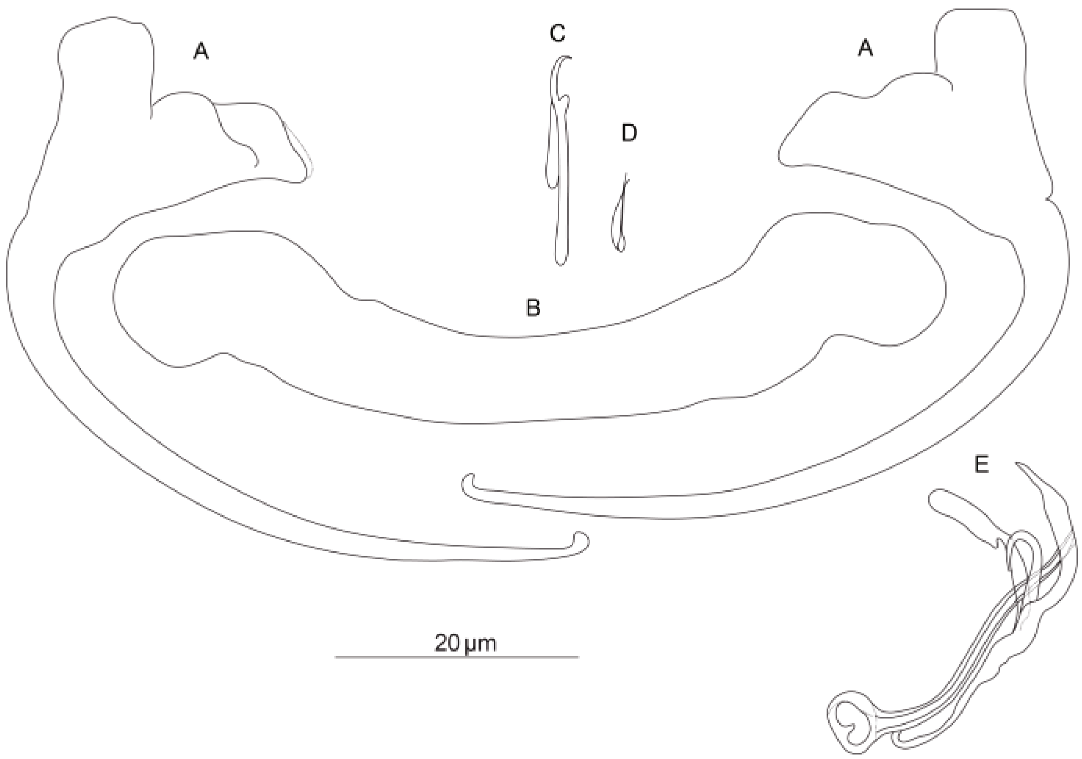

Fig. 12. *Dogielius sennarensis* n.sp. A = anchor; B = ventral bar; C = hooks; D = needle; E = copulatory organ

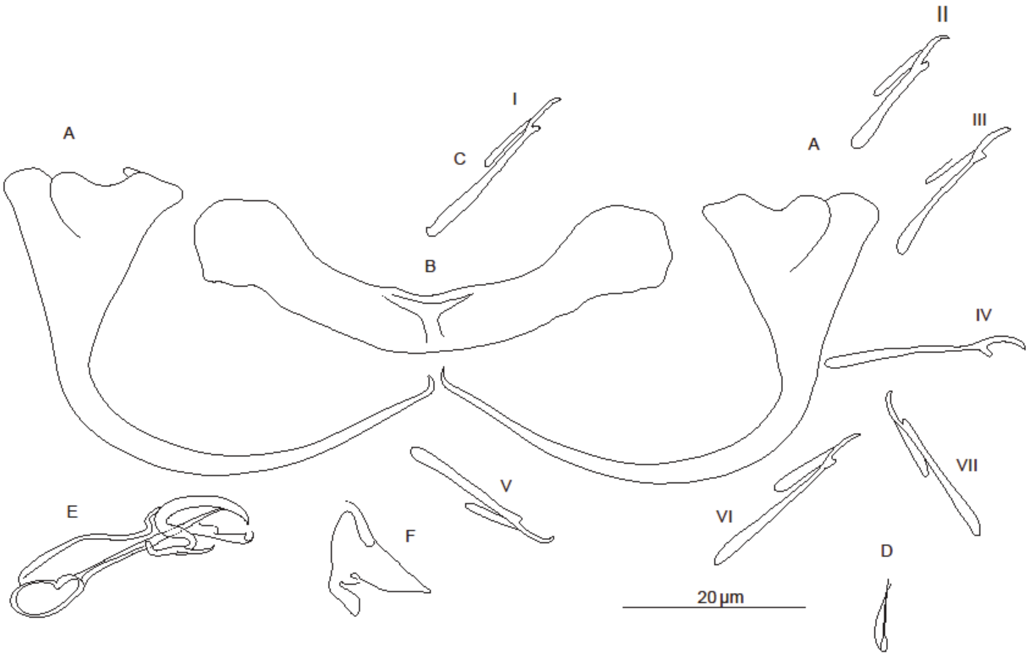

Fig. 13. *Dogielius* sp.1. A = anchor; B = ventral bar; C = hooks; D = needle; F = vagina; G = copulatory organ

other congeners. The ventral bar (70–76 µm long) is longer than the vast majority of African *Dogielius* species (except *D. grandijugus* Guégan, Lambert & Euzet (1989) (80–100 µm) and *D. phrygieus* Guégan & Lambert, 1990 (74 µm), from which it differs in the size of the other structures (Guégan *et al*., 1989; Guégan & Lambert, 1990; Musilová *et al*., 2009). The most obvious feature distinguishing *D. sennarensis* n. sp. from other congeners is the absence of a non-sclerotised vagina. Nevertheless, the majority of *Dogielius* spp. exhibit similar copulatory organ morphology, except for those that are coiled. The copulatory organ of *D. sennarensis* n. sp. is relatively small in comparison with other *Dogielius* spp., though the shape of the *D. sennarensis* n. sp. MCO and morphology of its sclerotised parts resembles those of *D. intorquens* Crafford, Luus-Powell & Avenant-Oldewage, 2012, found on *L. umbratus* (Smith, 1841) and *L. capensis* (Smith, 1841) at the Vaal Dam in South Africa (Crafford *et al*., 2012). In this case, however, it differs in the size of its copulatory organ (larger in *D. sennarensis* n. sp.; *D. intorquens*, accessory piece 25 µm long; *D. sennarensis* 32 µm). Morphometric differences were also observed in the haptor sclerites, having a longer outer root and anchor point length and a significantly larger ventral bar than *D. intorquens*. A comparison of all measurements for the species mentioned above is provided in Table 2.

***Dogielius* sp. 1** (Fig. 13)

Present records: *Labeo horie* Blue Nile, Sennar (13°32' 50,61" N, 33°38'4,23" E), Sudan; *Labeo niloticus* White Nile, Kosti (13°10' 18,58" N, 32°40' 19,24" E)
Site: Gill lamellae
Voucher specimen: IPCAS M-646
Material examined: Two flattened specimens
Description: Body length 324 (323–325; n=2); greatest width 88 (83–93; n=2). Haptor 61 (56–66; n=2) long; 142 (138–145; n=2) wide. Single pair of anchors (ventral): inner length 32 (n=2); outer length 43 (n=2); point length 22 (20–24; n=2) long. Shaft of anchor clearly distinguished from point, which is thin and long. Anchors elongated in width and flattened. One bar (ventral) 54.5 (53–56; n=2) long, huge; 6.5 (6–7; n=2) wide. Hooks seven pairs, similar in size 19 (16–22; n=1) long. Needles located near hooks of pair V. observed. Vagina total length 12 (n=1). Copulatory organ total length 30 (28–33; n=3), comprising a tube extending from the funnel supported by the accessory piece and ending in a fork-like hook.
Remarks: The haptor and copulatory organ are most similar to those of *D. flosculus*, though the vagina observed in our specimen differs significantly. Moreover, the inner length of the anchors is shorter, the ventral bar longer and the copulatory organ shorter than in *D. flosculus*. *Dogielius* sp.1 probably represents a new species; however, description of a new species was not possible due to the availability of only two specimens.

Discussion

While the River Nile is the longest river in Africa and supports more than 800 fish species (including Lake Victoria; Witte *et al*., 2009), current knowledge on monogeneans parasitising those fish is relatively poor, despite many of them being of commercial importance (El Moghraby & Abd el Rahman, 1984). This study presents first records on the occurrence of 13 dactylogyrid species from two sites

Table 2. Comparison of measurements (µm; mean with range in parentheses) of the haptoral hard parts of *Dogielius flosculus* collected from *Labeo horie* (this study) and *L. senegalensis* (Guégan *et al*., 1989), *Dogielius intorquensis* collected from *L. umbratus* and *L. capensis* (Crafford *et al*., 2012) and *Dogielius sennarensis* n. sp. collected from *L. niloticus* (this study). n = number of individuals measured.

	Dogielius intorquens Crafford, Powell & Oldewage, 2012 (n=11)	***Dogielius sennarensis* n.sp.** (present study)	***Dogielius flosculus*** (present study)	***Dogielius flosculus*** Guégan, Lambert & Euzet, 1989 (n=10)
Host and locality	*Labeo umbratus* - SouthAfrica; *Labeo capensis* - SouthAfrica	*Labeo niloticus* - Sudan - Sennar	*L. horie* -Sennar, Sudan	*L. senegalensis* - Mali
Anchor	Mean (Range)	Mean (Range; number of individuals measuered)		
Inner length	29 (23–34)	32 (31–33; n=7)	45 (43–47; n=10)	43 (40–46)
Outer length	37 (30–44)	46 (43–50; n=7)	50 (48–53; n=10)	45 (42–49)
Point length	23 (17–28)	32 (28–35; n=7)	22 (17–25; n=10)	25 (23–29)
Ventral bar				
Total length	45 (33–53)	72 (70–76; n=7)	47 (45–50; n=9)	47 (45–50)
Median width	6 (3–7)	7 (7–8; n=7)	9 (8–10; n=9)	8
Hooks	18 (13–20)	18 (16–20; n=1)	19 (14–21; n=5)	19.5 (18–21)
I	16 (11–20)			
II	15 (12–20)			
III	19 (12–21)			
IV	17 (13–21)			
V	18 (13–23)			
VI	19 (15–23)			
VII	19 (16–25)			
Copulatory organ				
Total length	26 (16–31)	32 (30–34) (n=6)	33 (22–45) (n=10)	42 (37–47)
Tube trace length	23 (14–31)			
Vagina			30 (25–35) (n=9)	18

along the Sudanese Nile parasitizing two new hosts, the African cyprinids *L. horie* and *L. niloticus*. The data presented is the first for dactylogyrid parasites from Sudan and for both *Labeo* species. The previous lack of parasite species reported from this region arises from an absence of parasite studies in Sudan, rather than any absence of species. Indeed, to the best of our knowledge, this is the first such study in the region. The lack of previous studies has been due, in large part, to difficulties in gaining access to certain African regions and problems associated with collecting material.

Our data supplements the monogenean diversity parasitizing *Labeo* spp. with five undescribed *Dactylogyrus* spp. and one undescribed *Dogielius* spp. and also provides a new description of the species of *Dogielius*. Both morphology and measurements of the sclerotised parts in the undescribed species did not correspond with any of the known *Dactylogyrus* and *Dogielius* species; hence, we suggest that these species are new to science. Unfortunately, owing to the limited number of specimens available, we were unable to provide full species descriptions.

Despite the low number of host individuals examined (i.e. 3 individuals of *L. horie* and 2 of *L. niloticus*), monogenean diversity appears relatively high in the two fish species examined, with seven species collected from *L. horie* and 10 species from *L. niloticus*. On the other hand, diversity was comparable with that for *L. victorianus* (nine spp.), *L. senegalensis* and *L. parvus* (both eight spp.) (Khalil & Polling, 1997; Musilová *et al*., 2009); and lower than that for the widespread *L. coubie* (Khalil & Polling, 1997), which has the highest monogenean diversity (18 spp.) of any African *Labeo* spp. According to Guégan *et al*. (1989), presence of similar parasites on *L. coubie* and *L. senegalensis* suggests a close phylogenetic affinity between the two cyprinids. In fact, both species, along with *L. roseopunctatus*, belong to a monophyletic group, and thus are highly likely to share the same parasites. Similarly, *L. horie* and *L. niloticus* belong to the same group as *L. senegalensis* (close relationship between *L. horie* and *L. senegalensis*; Lévéque *et al*., 1991), which would also explain the presence of the same monogenean species, despite the geographic distance between populations. Monogeneans are known as host-specific parasites and may even be the most host-specific of all fish parasites (Whittington *et al*., 2000). This high degree of host specificity is mainly explained through the coevolution of specialist parasites and their host. In general, specificity is inversely related to host range, with specificity decreasing as host range increases (Desdevises *et al*., 2002). Further, species that use more resources (generalists) tend to be more widespread and abundant than specialised species limited to a narrow range of resources (Mendlová & Šimková, 2014). Species mentioned in present study partly meet the definition, while undescribed *Dactylogyrus* spp. and *Dogielius* sp. require further studies.

Taking into account that the estimated number of monogenean species parasitising one host ranges from three (Lim, 1998) to five (Whittington, 1998), and that there are approximately 80 *Labeo* species in Africa (Skelton, 2001), it can be assumed that the possible number of parasitic monogeneans on these hosts could approach 400 species. This indicates that current knowledge of monogenean fauna (i.e. 59 spp., including the species reported in this paper) parasitising African *Labeo* fishes presently represents just 12–19% of the actual amount, and descriptions of new species from this area are highly anticipated.

Conflict of Interest

Authors state no conflict of interest

Acknowledgements

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Soil nematode abundances were increased by an incremental nutrient input in a paddy-upland rotation system

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Article info

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Summary

To study the effect of fertilization on soil nematode communities in a paddy-upland rotation system, an ongoing thirty-three years long-term fertilizer experiment is conducted which includes seven treatments; an unfertilized treatment (control), nitrogen (N), phosphorus (P), potassium (K) fertilizer treatments (N, NP, NPK) and organic manure (M) combined with chemical fertilizer treatments (MN, MNP, and MNPK). The soil nematode community structure and crop yields were determined in 2012 and 2013. Overall total nematode abundance was increased by an incremental nutrient input both in the rice and wheat fields. Total number of nematode was 1.25 – 2.37 times greater in the rice field and was 1.08 – 2.97 times greater in wheat field in the fertilization treatments than in the unfertilized treatment. Soil free-living nematode abundances was significantly ($P < 0.001$) increased in organic manure combined with chemical fertilizer treatments in rice field. Fungi-feeders and plant-feeding nematodes abundances were not significantly different among treatments in rice and wheat fields. Omnivorous and predatory nematodes were the most dominant groups in the present study. Omnivores, predators and *Prodorylaimus abundances* were significantly ($P < 0.05$) higher in organic manure combined with NPK fertilizer treatments than in chemical fertilizer alone and unfertilized treatments both in rice and wheat fields. Stepwise regressions revealed that soil free-living nematodes were significant predictors of rice grain yields ($R^2 = 0.56$, $P < 0.001$) and omnivorous and predatory nematodes were significant predictors of the wheat grain yield ($R^2 = 0.89$, $P < 0.001$). Therefore, long-term application of organic manure combined with chemical fertilizer could increase nematode abundances and crop yields. Organic manure combined with chemical fertilizer application was recommended in agricultural ecosystem.
Keywords: soil nematode community; trophic group; *prodorylaimus*; crop yields; long-term fertilizer experiment

Introduction

Maintaining sustainable soil utilization in developing countries is essential to supply the demand for food grain production. However, steady crop yields cannot be maintained without the application of some soil amendments (Gruzdeva *et al.*, 2007). Long-term and large numbers of chemical fertilizer applications have led to the loss of soil fertility, a reduction in soil biodiversity, and polluted farm products (Singh *et al.*, 2016; Ding *et al.*, 2017). Therefore, the application of organic fertilizer has come under consideration. The benefits of long-term manure application to soil include better tilth, improved water-holding capacity and cation exchange capa-

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city, moderated soil temperature, enhanced crop performance, and increased soil organic matter and biological fertility (McSorley & Gallaher, 1997). Accordingly, soil fertility and crop yields are increased and good physical or chemical soil properties and biological communities are maintained. However, the long-term application of chemical fertilizers leads to soil hardening, weak aeration, and weak biological activity (Hopkins & Shiel, 1996).

Paddy rice-upland wheat crop rotation systems are one of the largest agricultural production systems in the world, mainly distributed in southern and eastern Asia (Brar *et al.*, 2013). The 13.5 million hectares were in South Asia, and the other 10.5 million hectares are in South China, widely disseminated throughout the provinces of Jiangsu, Zhejiang, Hubei, Guizhou, Yunnan, Sichuan and Anhui, etc. (Hu *et al.*, 2015). This area accounts for roughly half of the total rice cultivated area in China. Therefore, the protection of the soil environment in the rice-wheat crop system is crucial for the maintenance of sustainable crop yields and to ensure China's food security (Zhang *et al.*, 2016).

Long-term field experiments are indispensable sources of knowledge and provide a tool for examining soil environment changes (Debreczeni & Körschens, 2003). Furthermore, long-term fertilizer experiments could reflect the influence of fertilizer benefit, crop growth and soil environment due to annual changes in climate (Miao *et al.*, 2011; Wei *et al.*, 2016). Some information could be gained regarding sustainable agriculture and humanity's influence on the soil fertility, landscape and agricultural environment (Diacono & Montemurro, 2010; Miles & Brown, 2011; Peterson *et al.*, 2012; Geisseler & Scow, 2014).

Soil free-living nematode communities are known to be sensitive to agricultural management practices such as fertilization, irrigation, and tillage (Porazinska *et al.*, 1999; Zhang *et al.*, 2017a). Furthermore, soil nematodes can be placed into at least five functional or trophic groups. They occupy a central position in the soil detrital food web and play a crucial role in soil organic-matter decomposition, plant-nutrient mineralization, and nutrient cycling (Neher, 2001). Therefore, soil nematode diversity, species composition, trophic group, and biomass may be useful bioindicators for monitoring soil environmental changes (Gutiérrez *et al.*, 2016; Scharroba *et al.*, 2016; Zhao *et al.*, 2016; Čerevková *et al.*, 2017). Liang *et al.* (2009) reported that total nematode abundance responded positively to manure treatments in monoculture maize fields. Hu & Qi (2013) found that long-term application of micro-organic compost could increase nematode abundance in wheat fields. So far, all studies about the effects of fertilization on the nematode community have mainly focused on the upland cropping systems. However, few investigations have reported the effects of long-term application of inorganic and organic fertilizer on soil nematode communities in the paddy-upland rotation system. Therefore, the aims of this study are to (1) determine the impacts of long-term fertilization on the soil nematode community, (2) compare the differences of the nematode communities under organic manure and inorganic fertilizer treatments in the paddy rice-upland wheat rotation systems.

Materials and Methods

Study site

An ongoing long-term (thirty-three years) fertilizer experiment in a rice-wheat rotation system was initiated from rice cultivation in 1981, belonging to the National Fertilizer Experiment Monitoring Network at Nanhu Experimental Station, Hubei Academy of Agricultural Sciences in Wuchang China, located at latitude 30°28'N, longitude 114°25'E and at an altitude of 20 meters in central China. The experimental site lies in a subtropical monsoon zone, which

Table 1. Soil properties (0–20 cm) in different fertilization treatments in October 2012.

Treatment	Organic Matter (g kg^{-1})	Total N (g kg^{-1})	Alkaline N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	pH
Control	21.69c	1.64c	112.22b	8.21c	105.63bc	7.17ab
N	22.02c	1.79bc	113.88b	8.71c	94.77bc	7.37ab
NP	26.02b	1.86bc	129.51b	18.71c	93.12c	7.57a
NPK	26.54b	1.88b	134.35b	18.33c	117.66bc	7.22ab
MN	36.74a	2.71a	188.98a	130.69b	139.14bc	7.22ab
MNP	34.72a	2.61a	173.51a	136.73b	141.27b	7.30ab
MNPK	34.04a	2.70a	189.01a	151.32a	205.94a	7.03b

Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$) among treatments according to LSD multiple comparison.

is characterized by hot summers and severe winters, and occasional snowfall during winter. The mean annual temperature is 13 °C, ranging from a minimum of 3.7 °C in January to a maximum of 28.8 °C in July. The mean annual precipitation is 1300 mm, and the annual non-frost period is 240 days from 1981 to 2013. The soil at the experimental site is yellow-brown soil, belonging to the Albic Luvisol in the FAO classification, which has a clay-loam texture with 15 % sand, 36 % silt, and 49 % clay. Soil physical-chemical properties in October 2012 at an 0 – 20 cm depth were shown in Table 1.

Experimental design

The field experiment was constituted of seven treatments and three replicates in a randomized complete block design. Each plot was 40 m^2 (5 m width × 8 m length). The seven treatments were as follows: (1) the unfertilized treatment, control; (2) the inorganic N fertilizer treatment, N; (3) the inorganic N and P fertilizer treatment, NP; (4) the inorganic N, P and K fertilizer treatment, NPK; (5) the manure plus inorganic N fertilizer treatment, MN; (6) the manure plus inorganic N and P fertilizer treatment, MNP; and (7) the manure plus inorganic N, P and K fertilizer treatment, MNPK. The chemical fertilizers were applied at an annual rate of 150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, and 150 kg K_2O ha^{-1} (Zhang et al., 2017b). The N, P, and K fertilizers were applied as urea, ammonium phosphate, and potassium chloride, respectively. 22,500 kg ha^{-1} of organic fertilizers from pig dung compost was applied to the MN, MNP and MNPK treatments. The pig dung compost contained an average of 282.05 g kg^{-1} organic C, 15.08 g kg^{-1} total N, 20.84 g kg^{-1} P_2O_5, 13.56 g kg^{-1} K_2O, and 69 % water. 60 % of inorganic fertilizers were applied during the rice growth season and the other 40 % during the wheat growth season, while manure was applied equally (50:50) to the two crops. The N fertilizer was applied three times in the rice growth season. Namely, 40 % of the N fertilizer as basal fertilizer was applied before the cultivating rice, 40 % was applied during the rice tillering stage and 20 % was applied during the rice booting stage. Similarly, the N fertilizer was also applied three times in the wheat growth season. Namely, 50 % of N fertilizer as basal fertilizer was applied before the planting wheat, 25 % during the wheat seedling stage and 25 % was applied during the jointing stage in the wheat growth season. Every year, the P, K fertilizers and manure were applied as basal fertilizer, prior to plough. All basal fertilizers and manure were evenly sprinkled on the soil surface by hand and were incorporated into the plough layer by tillage as soon as possible. Tillage was performed to a depth of 20 cm by a plough, followed by a harrow. The fertilized and unfertilized plots were tilled similarly.

Table 2. The number of soil nematodes (individuals 100 g^{-1} dry soil) after different fertilization treatments in rice and wheat fields.

	Treatment	**FN**	**BF**	**FF**	**PF**	**OP**	***Prodorylaimus***
Rice	Control	262.8d	71.9a	6.9a	110.7a	184.1d	82.3d
field	N	270.2d	75.2a	8.4a	195.2a	186.6d	93.6d
	NP	339.0d	104.9a	11.6a	181.4a	222.5d	128.7cd
	NPK	477.2c	141.2a	6.0a	173.7a	330.0c	197.6c
	MN	619.1b	113.2a	7.2a	151.7a	498.7b	446.8b
	MNP	704.1ab	66.0a	5.2a	117.0a	633.0a	561.0a
	MNPK	772.2a	129.5a	5.9a	111.7a	636.7a	588.7a
Wheat	Control	470.2c	65.9bc	10.0a	210.8a	394.3c	178.6d
field	N	562.2c	89.0bc	2.5a	173.9a	470.7c	170.0d
	NP	578.6c	134.0ab	0.0a	213.0a	444.7c	176.5d
	NPK	942.8bc	195.2a	11.2a	202.2a	736.3bc	499.7cd
	MN	1238.2b	162.1a	0.0a	232.4a	1076.1b	971.3bc
	MNP	1293.0b	58.8c	3.9a	195.2a	1230.2ab	1103.3ab
	MNPK	1907.2a	195. 6a	4.0a	115.9a	1707.6a	1591.2a

FN: the number of free-living nematodes in soil; BF: bacterial feeders; FF: fungal feeders; PF: plant feeders; OP: omnivores and predators. Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$) among treatments at the same sampling times according to LSD multiple comparison.

All plots were transplanted with rice seedlings (*Oryza sativa* L.) in summer and were sown with wheat seeds (*Triticum aestivum* L.) in winter, which has been done annually since 1981. The rice was transplanted in June and harvested in October, and the wheat was directly sown in November and harvested in May of the next year. The above-ground crops were harvested with a sickle and removed; thus, no straw returned into the soil in any plot. Nevertheless, rice or wheat stubble and roots were incorporated into the soil with a plow before the subsequent rice or wheat planting. In addition to the fertilizer treatments, all other agronomic managements were identical in the fertilized and unfertilized plots.
Rice and wheat grains were separated from straws using a plot thresher. Grains and straw were weighed after sun-drying and recorded from the whole plot.

Soil sampling
Soil samples from the upper (0 – 20 cm) soil layer were collected in the rice and wheat maturity stages in October 2012 and in May 2013, respectively. Composite soil samples consisting of ten cores (2.5 cm diameter × 20 cm depth) were collected from each plot. Soil samples were stored in tied and insulated plastic bags to prevent moisture loss. They were transferred to 4 °C storage as soon as possible until they were used to analyze the soil nematode community structure.

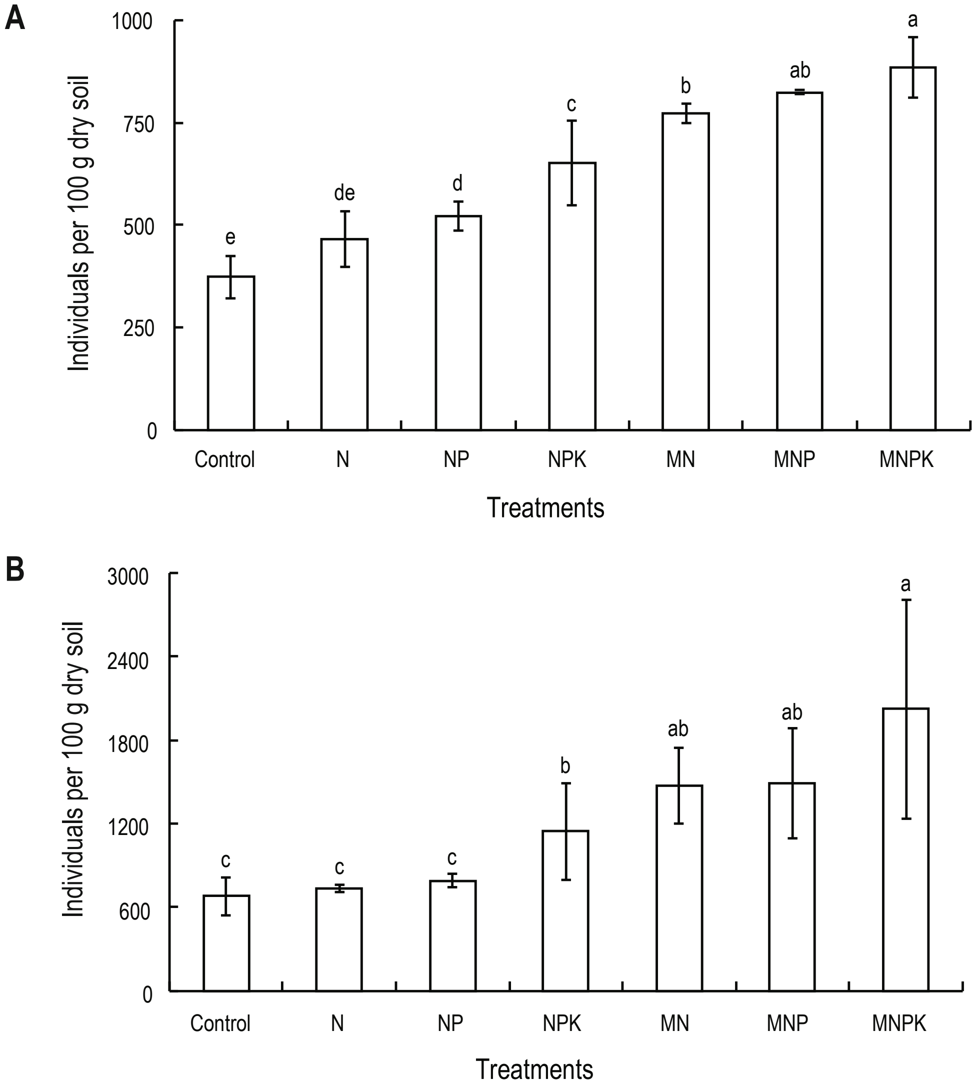

Fig. 1. Total nematode abundance after different fertilization treatments in the rice field (A) and wheat field (B). Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment. Data are means ± SD (n = 3). Different letters (a, b, c) above the column indicate significant differences ($P < 0.05$) among treatments according to LSD multiple comparison.

Soil physical-chemical properties analysis

Soil samples collected from the 0 – 20 cm were air-dried at room temperature, mixed, sieved through a 1-mm screen, and sub-samples were used to analyze soil physical-chemical properties. The air-dried sub-samples were ground to pass through a 0.25-mm sieve to determine soil organic matter and total N contents. The potassium dichromate external heating method was used to determine soil organic matter content (Blakemore *et al*., 1972). The Kjeldahl method and the alkaline-hydrolysable diffusion method were used to determine total N and alkaline-hydrolysable N content (Bremner, 1996). Soil available P was extracted with 0.5 mol L^{-1} $NaHCO_3$ (soil: solution=1:20) and measured with the Olsen method (Olsen *et al.*, 1954). Soil available K was extracted with 1 mol L^{-1} NH_4Ac (soil: solution=1:10) and measured with the flame photometry method (Carson, 1980). Soil pH was measured with 0.01 mol L^{-1} $CaCl_2$ slurry (soil: solution=1:2.5) using a glass electrode (Lu, 1999).

Nematode analysis

Field moist subsamples (100 g) of each sample were used to extract nematodes with the sugar flotation and centrifugation method (Barker *et al*., 1985). The nematode populations were expressed as 100 g^{-1} dry soil. After counting the total number of nematodes using an anatomical lens, two hundred nematodes per sample were randomly selected and identified to genus according to esophageal and morphology characteristics using an inverted compound microscope (Mal & Lyon, 1975; Ying, 1998).

The characteristics of the nematode communities were described using the following approaches: (1) total number of nematodes per 100 g dry soil; (2) the number of soil free-living nematodes per 100 g dry soil; (3) trophic groups: (a) bacterial feeders; (b) fungal feeders; (c) plant feeders; and (d) omnivore-predators (Yeates *et al.* 1993).

Statistical analysis

One-way variance analysis (one-way ANOVA) was used to detect differences between different treatments. Differences at the $P < 0.05$ level were considered statistically significant under the least significant difference (LSD) test. All statistical analyses and the stepwise regression were performed using SPSS 18.0 version software package.

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals by any of the authors.

Results

Soil physical-chemical properties

Soil organic matter, total N, alkaline-hydrolysable N and available P content were significantly (F = 82.892, P < 0.001 in soil organic matter; F = 39.068, P < 0.001 in soil total N; F = 18.383, P < 0.001 in alkaline-hydrolysable N; F = 224.353, P < 0.001 in available P) higher in the manure combined with chemical fertilizer treatment than in the chemical fertilizer alone and unfertilized treatment. Soil available K content were significantly (F = 6.443, P = 0.002) higher in the manure combined with NPK fertilizer treatment than in the other fertilization treatments and unfertilized control (Table 1).

Total nematode abundance

Total number of nematodes had an increasing tendency with soil nutrient input increments in rice and wheat fields (Fig. 1). Total number of nematode was 1.25 – 2.37 times greater in rice field and was 1.08 – 2.97 times greater in wheat field in the fertilization treatments than in the unfertilized treatment. In particular, the increase was significant in the NPK and manure combined with the chemical fertilizer treatments (F = 31.452, P < 0.001 in rice field; F = 5.253, P = 0.005 in wheat field).

Nematode trophic group abundance

Like the total number of nematodes, the number of soil free-living nematodes (bacterial-feeders + fungal-feeders + omnivores + predators) had an increasing tendency with soil nutrient input increments in rice and wheat fields. In particular, the increase was significant (F = 41.455, P < 0.001) in the manure combined with chemical fertilizer treatments in rice field. Moreover, the number of soil free-living nematodes were significantly (F = 9.890, P = 0.005) higher in the NPK treatment than in the NP, N and unfertilized treatments under the rice field (Table 2).

The bacteria-feeding nematode abundance was not significantly different among treatments in rice field; however, the bacteria-feeding nematode abundances were significantly (F = 6.000, P = 0.003) higher in the MNPK, MN, NPK treatments than in the MNP, N and unfertilized treatments in wheat field (Table 2). The fungi-feeding nematode were the least abundant trophic group in this study. Plant-feeding nematodes were the second-most dominant trophic groups. The number of fungi-feeding and plant-feeding nematodes were not significantly different among treatments in rice and wheat fields. The omnivorous and predatory nematodes were the most dominant groups in the present study (Table 2). The number of omnivore-predators was 1.01 – 3.46 times greater in rice field and was 1.19 – 4.33 times greater in wheat field in the fertilization treatments than in the unfertilized treatment. In particular, omnivore and predator abundances were significantly (F = 58.636, P < 0.001) higher in the manure combined with the chemical fertilizer treatments than in the chemical fertilizer alone and the unfertilized treatments in rice field. Omnivore and predator abundances were significantly (F = 5.553, P = 0.013) higher in the MNPK treatment than in the chemical fertilizer alone and unfertilized treatments in wheat field. Omnivore and predator abundances were significantly (F = 9.640, P = 0.005) higher in the NPK treatment than in the NP, N and unfertilized treatments in rice field. A total of 39 nematode taxa were identified in the present study, of which 11 were bacterial feeders, 5 were fungal feeders, 6 were plant feeders, 12 were omnivores and 5 were predators

Table 3. The number of soil nematode taxa (individuals 100 g^{-1} dry soil) for each treatment in rice field.

Genus	Guild	Control	N	NP	NPK	MN	MNP	MNPK
Protorhabditis	Ba1	0.0	0.0	1.9	0.0	0.0	0.0	0.0
Rhabditis	Ba1	0.0	0.0	0.0	0.0	0.0	0.0	3.1
Panagrolaimus	Ba1	1.3	3.3	3.7	4.0	7.3	2.6	42.4
Cephalobus	Ba2	4.2	6.6	1.9	0.0	0.0	2.6	0.0
Eucephalobus	Ba2	3.7	1.7	3.4	4.8	4.8	2.6	6.3
Acrobeloides	Ba2	3.3	3.8	0.0	0.0	9.6	0.0	3.1
Monhystera	Ba2	0.0	0.0	0.0	1.9	2.4	0.0	0.0
Plectus	Ba2	3.8	6.7	8.7	17.5	24.8	26.6	13.7
Prismatolaimus	Ba3	4.6	4.6	10.0	30.6	14.7	10.6	30.8
Pseudoaulolaimus	Ba3	48.9	44.2	73.5	80.3	39.8	18.2	24.7
Amphidelus	Ba4	2.0	4.2	1.9	2.0	9.8	2.7	5.2
Aphelenchoides	Fu2	0.0	0.0	1.9	0.0	7.2	5.2	5.9
Tylencholaimus	Fu4	2.5	0.0	1.9	0.0	0.0	0.0	0.0
Doryllium	Fu4	3.1	8.4	7.8	6.0	0.0	0.0	0.0
Leptonchus	Fu4	1.3	0.0	0.0	0.0	0.0	0.0	0.0
Malenchus	H2	20.8	14.9	20.1	4.3	12.5	5.4	0.0
Basiria	H2	43.5	126.4	96.5	98.9	35.3	48.0	39.2
Psilenchus	H2	22.9	22.5	22.3	4.8	4.8	0.0	0.0
Tylenchorhynchus	H3	0.0	0.0	0.0	0.0	32.5	13.4	11.1
Hirschmanniella	H3	22.1	24.8	33.3	56.0	66.6	50.2	61.4
Belondira	H5	1.4	6.6	9.3	9.7	0.0	0.0	0.0
Pristionchus	Om1	0.0	0.0	0.0	0.0	4.8	2.6	5.6
Lordellonema	Om4	0.0	0.0	0.0	0.0	0.0	5.4	0.0
Prodorylaimus	Om4	82.3	93.6	128.7	197.6	446.8	561.0	588.7
Mesodorylaimus	Om4	26.5	18.4	21.1	45.6	2.4	5.3	6.3
Eudorylaimus	Om4	13.9	12.1	0.0	14.2	15.0	15.9	3.1
Thorneella	Om4	1.4	0.0	0.0	0.0	0.0	0.0	0.0
Pungentus	Om4	2.5	1.3	0.0	0.0	0.0	2.7	0.0
Aporcelaimus	Om5	7.1	4.7	3.4	2.0	22.4	13.5	5.4
Sectonema	Om5	1.3	0.0	0.0	2.4	0.0	0.0	0.0
Tripyla	Ca3	0.0	0.0	0.0	2.4	0.0	0.0	0.0
Iotonchus	Ca4	0.0	0.0	0.0	0.0	0.0	2.7	5.4
Mylonchulus	Ca4	0.0	1.3	3.1	2.0	2.6	8.0	10.9
Laevides	Ca5	49.1	55.2	66.1	63.7	4.8	15.9	11.3

Functional guild classifications are made based on the feeding habits and life-cycle characteristics (Ba: bacterivores, Fu: fungivores, Ca: Carnivores, Om: omnivores, H: herbivores) (Yeates, 1993). Suffix numbers are c-p values for the taxon (Bongers & Bongers, 1998). Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Table 4. The number of soil nematode taxa (individuals 100 g^{-1} dry soil) for each treatment in wheat field.

Genus	Guild	Control	N	NP	NPK	MN	MNP	MNPK
Protorhabditis	Ba1	0.0	4.8	0.0	0.0	0.0	0.0	0.0
Rhabditis	Ba1	0.0	0.0	2.6	0.0	0.0	0.0	0.0
Panagrolaimus	Ba1	0.0	11.8	10.5	7.8	23.3	4.0	4.0
Cephalobus	Ba2	2.7	2.3	2.7	6.2	4.0	0.0	0.0
Eucephalobus	Ba2	0.0	0.0	0.0	0.0	4.0	4.0	0.0
Acrobeloides	Ba2	0.0	4.8	2.6	0.0	4.0	0.0	0.0
Monhystera	Ba2	4.7	0.0	2.5	3.2	0.0	0.0	13.9
Plectus	Ba2	0.0	14.7	28.3	69.8	30.7	22.2	93.9
Prismatolaimus	Ba3	9.7	21.1	56.5	32.6	47.3	15.7	54.9
Pseudoaulolaimus	Ba3	48.9	29.4	28.3	72.5	49.1	13.0	28.8
Amphidelus	Ba4	0.0	0.0	0.0	3.2	0.0	0.0	0.0
Aphelenchoides	Fu2	2.3	0.0	0.0	0.0	0.0	0.0	0.0
Ditylenchus	Fu2	0.0	0.0	0.0	0.0	0.0	3.9	0.0
Tylencholaimus	Fu4	0.0	0.0	0.0	4.8	0.0	0.0	0.0
Doryllium	Fu4	7.7	2.5	0.0	6.4	0.0	0.0	4.0
Malenchus	H2	0.0	2.3	5.1	14.7	0.0	0.0	0.0
Basiria	H2	136.4	115.7	105.2	75.4	106.1	76.6	36.9
Psilenchus	H2	5.2	7.3	23.0	12.6	5.7	0.0	0.0
Tylenchorhynchus	H3	2.7	0.0	0.0	0.0	34.2	58.3	18.1
Hirschmanniella	H3	66.5	43.8	77.0	88.8	86.4	60.3	60.9
Belondira	H5	0.0	4.9	2.7	10.7	0.0	0.0	0.0
Pristionchus	Om1	6.4	16.9	12.8	3.2	7.9	11.8	13.9
Coomansinema	Om4	0.0	0.0	2.7	0.0	4.0	4.0	0.0
Ecumenicus	Om4	2.3	0.0	0.0	0.0	0.0	0.0	0.0
Prodorylaimus	Om4	178.6	170.0	176.5	499.7	971.3	1103.3	1591.2
Mesodorylaimus	Om4	57.8	79.7	65.0	63.4	13.6	23.4	18.1
Eudorylaimus	Om4	6.4	0.0	2.5	0.0	0.0	7.9	26.7
Thorneella	Om4	7.3	0.0	0.0	0.0	0.0	0.0	0.0
Pungentus	Om4	13.5	12.3	15.3	6.4	0.0	7.9	0.0
Aporcelaimus	Om5	10.5	7.4	2.7	34.5	24.1	18.3	33.9
Labronema	Om4	0.0	7.3	0.0	0.0	0.0	7.9	0.0
Sectonema	Om5	5.0	4.8	5.1	0.0	0.0	0.0	0.0
Tripyla	Ca3	2.3	0.0	10.5	9.6	8.8	3.9	5.9
Miconchus	Ca4	8.0	9.7	12.6	24.1	9.7	16.9	4.0
Iotonchus	Ca4	2.3	9.7	0.0	0.0	9.6	10.4	0.0
Laevides	Ca5	93.7	152.9	139.1	95.5	27.2	14.3	13.9

Functional guild classifications are made based on the feeding habits and life-cycle characteristics (Ba: bacterivores, Fu: fungivores, Ca: carnivores (predators), Om: omnivores, H, plant feeders (herbivores) (Yeates, 1993). Suffix numbers are c-p values for the taxon (Bongers & Bongers, 1998). Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.

Table 5. Crop grain yields after different fertilization treatments.

Treatment	Rice grain yield (t/ha)	Wheat grain yield (t/ha)
Control	3.79d	2.24c
N	4.66cd	2.32c
NP	5.13bc	3.13b
NPK	5.17bc	3.23b
MN	6.04ab	4.56a
MNP	6.10ab	4.65a
MNPK	6.55a	4.74a

Control, the unfertilized treatment; N, the inorganic nitrogen fertilizer treatment; NP, the inorganic nitrogen and phosphorus fertilizer treatment; NPK, the inorganic nitrogen, phosphorus and potassium fertilizer treatment; MN, the manure plus inorganic nitrogen fertilizer treatment; MNP, the manure plus inorganic nitrogen and phosphorus fertilizer treatment; MNPK, the manure plus inorganic nitrogen, phosphorus and potassium fertilizer treatment.
Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$) among treatments according to LSD multiple comparison.

(Table 3, 4). The number of *Prodorylamus* were significantly (F = 46.0201, $P < 0.05$) higher in the manure combined with the chemical fertilizer treatment than in the chemical fertilizer alone and the unfertilized treatments in rice field (Table 2).

Crop yield

Rice and wheat yields were significantly (F = 3.764, P = 0.019 in rice; F = 91.648, $P < 0.001$ in wheat) influenced by the different fertilization treatments (Table 5). Rice grain yields were 1.23 – 1.73 times greater in the fertilization treatments than in the unfertilized treatment. In particular, rice grain yields were significantly (F = 5.265, P = 0.015) higher in the MNPK treatment than in the chemical fertilizer alone and the unfertilized treatments. Rice grain yields were significantly (F = 5.415, P = 0.014) higher in the MNPK, MNP, MN treatments than in the N and unfertilized treatments; nevertheless, there were no significant differences between the N and unfertilized treatments.

Wheat grain yields were 1.04 – 2.12 times greater in the fertilization treatments than in the unfertilized treatment. Wheat grain yields were significantly (F = 91.648, $P < 0.001$) higher in the manure combined with the chemical fertilizer treatments than in the chemical fertilizer alone and in the unfertilized treatments. Moreover, wheat grain were significantly (F = 19.641, $P < 0.001$) higher in the NPK, NP treatments than in the N alone and in the unfertilized treatments; nevertheless, there was no significant difference between the N alone and the unfertilized treatments.

Regression analysis

Stepwise regression equations were conducted to evaluate soil nematode community parameters in rice and wheat fields. These parameters were significantly associated with rice and wheat grain yields. The number of soil free-living nematode was found to be a significant (F = 23.968, $P < 0.001$) predictor of rice grain yields. The number of omnivorous and predatory nematodes was found to be a significant (F = 150.214, $P < 0.001$) predictor of wheat grain yields (Table 6).

Discussion

Compared to the unfertilized treatment, the total number of nematode was increased in rice and wheat fields due to fertilizer application. In particular, there was a significant increase in the NPK and manure combined with the chemical fertilizer treatments. Similarly, increasing total nematode abundances after application of manure were also reported by Villenave *et al.* (2003), Forge *et al.* (2005) and Hu & Qi (2013). The increase of nematode populations could possibly be linked directly to higher food resources associated with the input of organic matter (Villenave *et al.*, 2003). Increasing total number of nematodes due to application of chemical fertilizer was also reported by other studies (Li *et al.*, 2016; Hu *et al.*, 2017). Villenave *et al.* (2010) also observed that soil free-living nematodes were increased due to organic and mineral fertilizer use.

Table 6. Stepwise regression of grain yields of rice and wheat with the soil nematode community.

	Predictor variable	Constant	Coefficient	R^2	F	P
Rice	FN	3.368	0.004	0.558	23.968	< 0.001
Wheat	OP	1.613	0.005	0.888	150.214	< 0.001

FN: the number of soil free-living nematodes; OP: the number of omnivorous and predatory nematodes.
The values are fitted in the equation: $y = a + bx_1 + cx_2$; y = grain yield; x_1 and x_2 are the independent variables; and a, b and c are coefficients.

Fungi-feeding and plant-feeding nematode abundances were not significantly different among treatments in the present study. This result is not consistent with previous studies showing that organic materials application resulted in an increase of fungal feeders abundances (Ferris & Matute, 2003; Wang *et al.*, 2006; Villenave *et al.*, 2010) and that plant-feeding nematodes were increased due to compost and chemical fertilizer application in a maize field (Hu & Qi, 2010).

The omnivorous and predatory nematodes were the most dominant groups in the present study. In contrast, bacterial feeders were the most abundant group in paddy field (Ou *et al.*, 2005), in yellow squash field (Wang *et al.*, 2006), and in Horqin sandy land (Jiang *et al.*, 2007; Guan *et al.*, 2015). Plant-feeding nematodes were prevalent in maize and wheat fields (Hu & Qi, 2010, 2013; Zhang *et al.*, 2012), in sorghum field (Villenave *et al.*, 2010), and in millet field (Diakhaté *et al.*, 2013). Compared to the unfertilized treatment, the abundance omnivore-predators was increased in rice and wheat fields due to fertilizer use. A similar study also showed that the application of manure to an agricultural field could increase the abundance of omnivorous nematodes (Villenave *et al.*, 2004). Li *et al.* (2010) reported that organic fertilizer application increased the relative abundance of omnivore-carnivores, but different levels of applied nitrogen fertilizer decreased their relative abundance under greenhouse conditions. The composition and abundance of omnivores and carnivores were not affected by the soil amendments (Villenave *et al.*, 2010) and the effect of organic amendment on omnivorous and predatory nematodes was minimal (Wang *et al.*, 2006). However, Forge *et al.* (2005) reported that high amount manure and chemical fertilizer application reduced omnivorous nematode abundance, but predacious nematodes were more abundant in manure-treated soil than in fertilized soil. Abundant omnivorous and predatory nematodes in the manure-treated soils suggested that more complex soil food webs are expected to result in greater biodiversity of the belowground organisms mediating multiple soil ecosystem functions (Ferris *et al.*, 2012). For example, carnivores, through preying on plant-parasitic nematodes, were regarded as having potential for the biological regulation of pest species (Steel & Ferris, 2016). The increase of omnivorous and predacious nematodes were beneficial for soil health because omnivores and predators play an important role in further mineralizing soil nutrients tied up in bacteria-feeders or fungi-feeders, as well as preying on other nematodes including plant-parasitic nematodes (Wang *et al.*, 2006; Khan & Kim, 2007). *Prodorylamus* were the dominant genera in the present study. However, *Helicotylenchus* and *Scutellonema* were predominant in millet field (Diakhaté *et al.*, 2013), *Pratylenchus* and *Tylenchorhynchus* were dominant in sorghum field (Villenave *et al.*, 2010) and *Tylenchorhynchus, Pratylenchus,* and *Rotylenchus* were the dominant genera in wheat field (Hu & Qi, 2013). The number of nematode genera (39) in the present study was lower than in paddy field (45) (Lü *et al.*, 2017), in maize field (48) (Liang *et al.*, 2009), and in banana field (56) (Zhong *et al.*, 2017), but similar to the result in a continuous sorghum field (38) (Villenave *et al.*, 2010), in soybean field (37) (Pan *et al.*, 2016), and in Tibetan plateau grassland (41) (Hu *et al.*, 2017).

The number of soil free-living nematode was a significant predictor of rice grain yields and the number of omnivorous and predatory nematode was a significant predictor of wheat grain yields. Similarly, Mandal *et al.* (2007) reported that alkaline phosphatase activity was significant ($P < 0.001$) for predicting the grain yields of wheat at the dough stages of wheat growth. Hu & Qi (2013) observed that soil free-living nematode was a significant ($P < 0.05$) predictor of wheat grain yields during the jointing stage of wheat growth.

Conclusions

Based on these results, we deduced that long-term application of organic manure and chemical fertilizer modified the soil nematode community structure, increased crop yields. Total nematode abundance had an increasing tendency with soil nutrient input increments in rice and wheat fields. The number of total soil nematode was significantly increased due to organic manure combined with the chemical fertilizer application in rice field. Omnivores and predators and *Prodorylaimus* were significantly higher in the organic manure combined with NPK fertilizer treatments than in the chemical fertilizer alone and in the unfertilized treatments in rice and wheat fields. Stepwise regressions revealed that the number of soil free-living nematode was a significant predictor of rice grain yields ($R^2 = 0.56$) and the number of omnivorous and predatory nematode was a significant predictor of wheat grain yield ($R^2 = 0.89$). In conclusion, long-term application of organic manure combined with chemical fertilizer could increase nematode abundances and raise crop yields. Organic manure combined with chemical fertilizer application was recommended in agricultural ecosystem.

Conflict of interest

The authors declare there is no conflict of interest.

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Research Note

Morphological and molecular characterization of *Haplorchoides mehrai* Pande and Shukla 1976 (Digenea: Heterophyidae) from Chiang Mai province

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Article info

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Summary

Cyprinoid fish in Chiang Mai province has been reported the presence of a large number of metacercariae, particularly the metacercariae of *Haplorchoides* and those not identified to species. This study aims to investigate morphological and molecular characteristic of the minute intestinal fluke *H. mehrai* metacercariae in two cyprinoid fish species from Chom Thong district, Chiang Mai province, Thailand: the Tinfoil barb (*Barbonymus schwanenfeldii*) and the White eye barb (*Cyclocheilichthys repasson*). A total of 180 fish (90 from *B. schwanenfeldii* and 90 from *C. repasson*) were collected over three seasons: cool, hot and the rainy season (December 2015 to August 2016). Fish were examined for *H. mehrai* metacercariae infection, including areas such as muscle and the inner side of body scales, by using a light microscope. The prevalence of *H. mehrai* metacercariae in *B. schwanenfeldii* and *C. repasson* was 73.33 % and 100 % respectively. *Haplorchoides* metacercariae were identified as *H. mehrai* based on the morphological characteristics; the position of the acetabulum and the number and arrangement of the acetabular spines. Phylogenetic analysis based on Cytochrome c Oxidase subunit I (COI) gene showed that *H. mehrai* metacercariae from *B. schwanenfeldii* and *C. repasson* were the same species as the adult stage of *H. mehrai* from *Hemibagrus nemurus* and *Mystus multiradiatus*. Both morphological and molecular characteristic could indicate that *Haplorchoides* metacercariae originated from this study were *H. mehrai*. Furthermore, it is a new record of the minute intestinal fluke *Haplorchoides mehrai* in Chiang Mai Province.
Keywords: *Haplorchoides mehrai*, *Barbonymus schwanenfeldii*, *Cyclocheilichthys repasson*, Metacercariae, COI, Chiang Mai province

Introduction

Haplorchoides mehrai is a minute intestinal fluke, first described by Pande and Shukla (1976). The *Haplorchoides* genus belongs to the subfamily Haplorchiinae, family Heterophyidae (Chen, 1949; Pearson & Ow Yang, 1982; Yamaguti, 1958). Freshwater fish, particularly cyprinoid fish served as the second intermediate host of *H. mehrai* metacercaria (Scholz *et al*., 1991; Manpratum *et al*., 2017). The adult stage of *H. mehrai* have been first recorded in the small intestines of *Mystus vittatus* from India (Pande & Shukla, 1976). Some previous studies, *H. mehrai*, adult stages have been reported from Yellow catfish, *Hemibagrus nemurus* in Khon Kaen Province, Northeast Thailand (Manpratum *et al*., 2017). In the Northern Thailand, the high prevalence of *Haplorcoides* spp. metacercariae in cyprinoid fish have been recorded in Phitsanulok (Noikong *et al*., 2011) and Chiang Mai province (Saenphet *et al*., 2001; Nithikathkul & Wongsawad, 2008). Moreover, the adult stage of *Haplorchoides* spp. have been reported to infect the Yel-

* – corresponding author

low catfish, *H. nemurus* in Chiang Mai (Wongsawad *et al*., 2004) and Chiang Rai province (Purivirojkul & Areechon, 2008). The Ping River is an important river in Chiang Mai province, containing many aquatic animals, particularly cyprinoid fish which act as the second intermediate hosts for *Haplorchoides* spp. The Ping river flows through the Chom Thong district, Chai Mai province an area that supports a large amount of agriculture and many fisheries. Kumchoo *et al.* (2005) recorded that cyprinoid fish, *Barbonymus schwanenfeldii* and *Cyclocheilichthys repasson* were infected with *Haplochoides* sp. metacercaria in Chom Thong district.

Mitochondrial DNA based Polymerase Chain Reaction (PCR) methods have been effective for identification and studying the phylogenetics of trematodes in the family Heterophyidae (Chontananarth *et al*., 2014). A COI primer was used in the differentiation of COI fragments from Heterophyidae (*Haplorchis taichu*i) with fragments from Opisthorchiidae (*Opisthorchis viverrini*) by Thaenkham *et al*. (2007). The COI gene is useful for assessing the genetic variation in *H. taichui* (Dung *et al*., 2013).

This study aimed to examine the prevalence of *Haplorchoides mehrai* metacercariae in *B. schwanenfeldii* and *C. repasson* from Chom Thong district, Chiang Mai province, Thailand. A phylogeny based on the COI gene of *H. mehrai* and other heterophyid trematodes was also reconstructed. This data provides useful information for the control and prevention of *H. mehrai* infection in Chiang Mai province and for Thailand in general.

Materials and Methods

Parasite specimens

A total of 180 fish (90 from *B. schwanenfeldii* and 90 from *C. repasson*) were collected in the same river area (N18.403918, E98.702038) from Chom Thong district, Chiang Mai province, Thailand. Fish were collected over 3 seasons: cool (n = 30), hot (n = 30) and the rainy season (n = 30), from December 2015 to August 2016. Fish were transferred to the laboratory at the Department of Biology, Faculty of Science, Chiang Mai University. Standard length (cm) and weight (g) of the fish were recorded. The fish were individually examined, which included an examination of their body scales (30 scales per fish) and meat (2g). The scales were directly examined for metacercariae under light microscope. The fish meat was ground by blender, then mixed with pineapple juice and incubated at 37 °C for 1 – 2 hours. The processed meat was filtrated with graded sieves to remove large particles, rinsed twice with water and examined under light microscope.

Adults of *Haplorchoides mehrai* were collected from Yellow catfish (*Hemibagrus nemurus*), Asian redtail catfish (*Hemibagrus wyckioides*) and Iridescent mystus (*Mystus multiradiatus*).

For the species identification, encysted and excysted metacercariae and adults of *Haplorchoides mehrai* were fixed and flattened in 4 % formalin for preparation of permanent slides. The trematodes were stained with Delafield' hematoxylin, then dehydrated in alcohol series, cleared in xylene and permanently mounted in permount. Specimens on permanent slides were illustrated using a compound microscope with a drawing tube. Measurements were obtained using an ocular micrometer and expressed in micrometers (μm). The identification was based on morphology according to Pande and Shukla (1976) and Shameem and Madhavi (1988). The prevalence of infection was calculated based on the equation of Margolis *et al*. (1982).

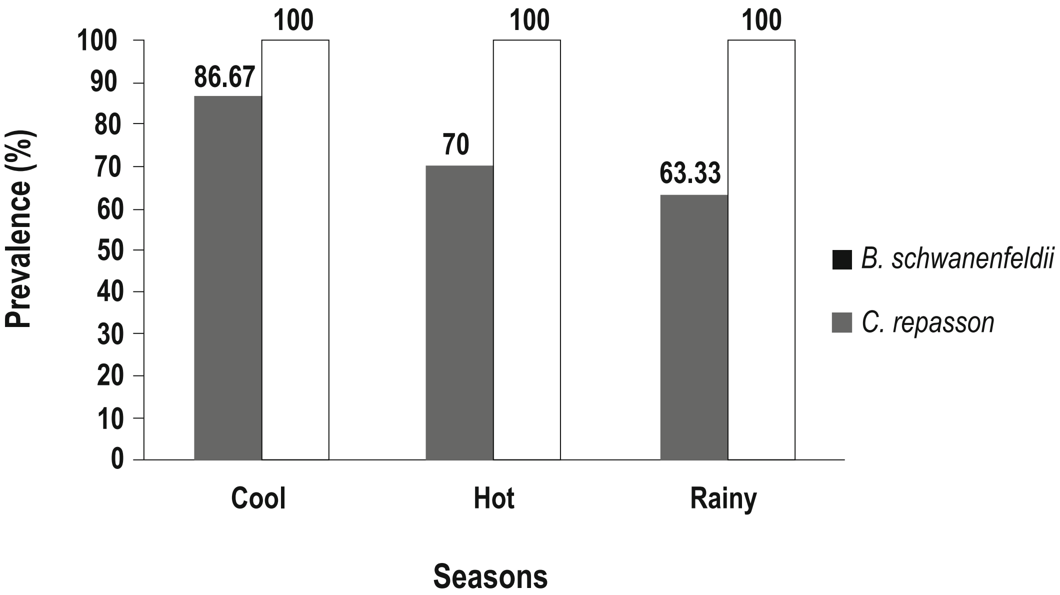

Fig. 1. Prevalence of *Haplorchoides mehrai* metacercaria during 3 seasons.

Table 1. Prevalence of *Haplorchoides mehrai* metacercariae in Cyprinoid fish from Chiang Mai province.

Cyprinoid fish	No. of examined fish	No. of infected fish	Prevalence	Intensity
Barbonymus schwanenfeldii	90	66	73.33	14.86
Cyclocheilichthys repasson	90	90	100	129.85

Genomic DNA extraction

Genomic DNA of all parasites was extracted from both adults and metacercariae based on the Chelex method used by Caron *et al.* (2010). The genomic DNA of the flukes was stored at -20 °C until used.

COI PCR

The PCR amplification of Cytochrome c Oxidase subunit I (COI) was followed the methods described in Chontananarth *et al*. (2014). It consists of a pair of primers: forward primer (JB3) 5' TTTTTTGGGCATCCTGACGTTTAT 3' and reverse primer (JB 4,5) 5' TAAAGAAAGAACATAATGAAAATG 3'. The final volume of 20 µl PCR product mixture consisted of 1.0 µl genomic DNA, 2.0 µl PCR buffer, 2.0 µl (10 mM) of dNTPs, 0.7 µl (50mM) of $MgCl_2$, 1 µl of primer and 0.3 µl of *Taq* polymerase. PCR amplification followed an initial denaturation of 3 min at 95 °C, followed by 40 cycles, which consisted of denaturation for 1 min at 95 °C, 1 min of annealing at 50 °C, 1 min of elongation at 72 °C and a final elongation step for 7 minutes at 72 °C. The COI PCR product was checked using DNA Dye Non Tox (AappliChem) staining and separated on 1.4 % TBE agarose gel electrophoresis. All COI PCR products were subjected for purify and sequencing.

Phylogenetic tree construction

Phylogenetic trees were constructed using the program Mega version 6.06, and molecular data were analyzed using Maximum likelihood (ML) and Neighbor joining (NJ) methods. The reliability of internal branches in both methods was estimated using 1000 bootstrap replications. Sequences of the fluke *Fasciola gigantica* (Fasicolidae) were used as an outgroup for phylogenetic analysis.

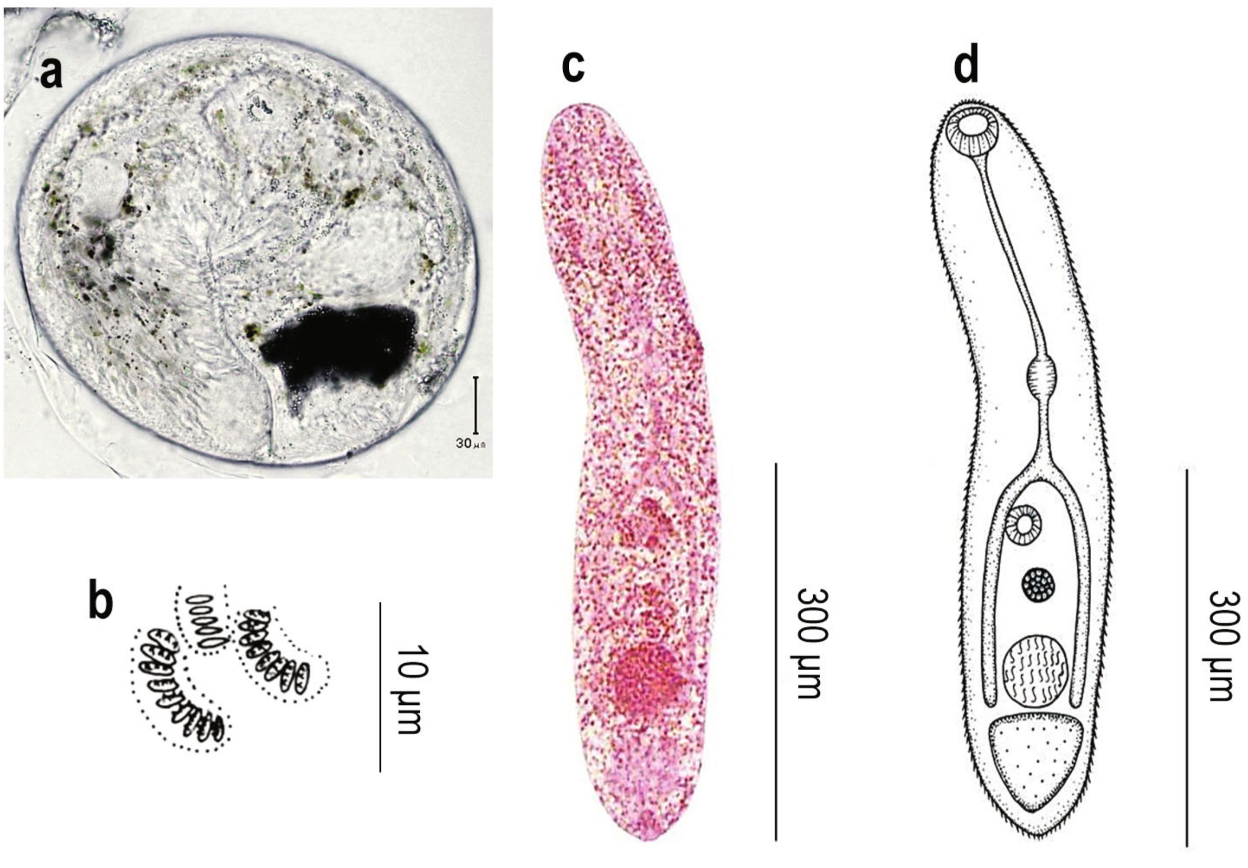

Fig. 2. Encysted metacercariae of *H. mehrai* from *B. schwanenfeldii* (a) Acetabular spine (b). Excysted metacercariae stained Delafield' hematoxylin (c,d).

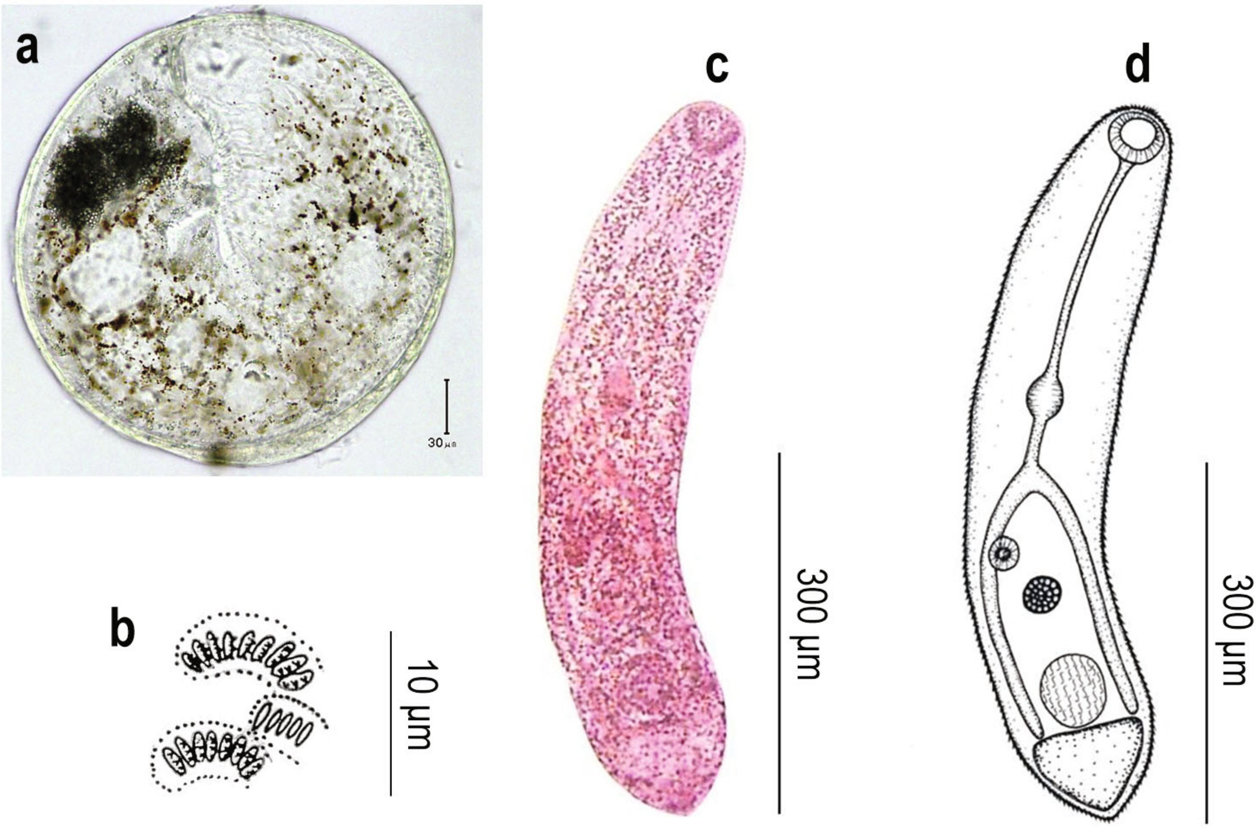

Fig. 3. Encysted metacercariae of *H. mehrai* from *C. repasson* (a) Acetabular spine (b). Excysted metacercariae stained Delafield' hematoxylin (c,d)

Ethical Approval and/or Informed Consent

There are no the use of animal for experimentation but use only for surveyed research. We have animal use license number of U1-07209-2560 that issued by the Institute of Animal for Scientific Purpose Development (IAD), Thailand. However, this research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Results

Prevalence of infection

The results revealed that the prevalence of *H. mehrai* metacercaria infection (Table 1) in *Barbonymus schwanenfeldii* was 86.7 % in the cool, followed by 70 % in the hot and 63.3 % in the rainy season (Fig. 1). The average prevalence and intensity across all three seasons was 73.3 % (66/90) and 14.86 respectively. Prevalence in *Cyclocheilichthys repasson* was 100 % for all three seasons (90/90) (Fig. 1) with an intensity of 129.85. The metacercariae of *H. mehrai* were recovered from the inner side of body scales and the general muscular tissue of *B. schwanenfeldii* and *C. repasson*. Body scales and fish meat of *B. schwanenfeldii* respectively contained 17.21 % and 10 % of all metacercariae found, whereas the body scales and fish meat of *C. repasson* contained 43.39 % and 16.66 % of all metacercariae respectively.

Morphological analysis

<u>Metacercariae of *Haplorchoides mehrai* from *Barbonymus schwanenfeldii* and *Cyclocheilichthys repasson*</u>

Encysted metacercariae of *H. mehrai* from *B. schwanenfeldii* (Fig. 2) and *C. repasson* (Fig. 3) are nearly spherical, with a double layered cystic wall. Both excysted metacercariae of *H. mehrai* from *B. schwanenfeldii* (Fig. 2c, 2d) and *C. repasson* (Fig. 3c, 3d) have lance-shaped bodies, with a scale like spine on the body surface. Oral sucker subterminal. Prepharynx longer than esophagus. Caeca extends slightly beyond posterior border of testes. Acetabulum submedian, located near intestinal bifurcation. Acetabulum with spines present in three groups. Testes rounded, median, located between the caecal ends and posterior body. Ovary spherical, pre-testicular, median. Excretory bladder saccular, post-testicular. The measurements of the excysted metacercariae were shown in Table 2.

Table 2. Comparing the Organs size (in μm) of *H. mehrai* excysted metacercariae from *B. schwanenfeldii* and *C. repasson.*

Organs	Previous study	This study	
	H. mehrai (Pande and Shukla,1976)	*H. mehrai* from *B. schwanenfeldii*	*H. mehrai* from *C. repasson*
Body length	225 – 565	499.6 (355 – 630)	494.4 (390 – 620)
Body width	90 – 180	120.3 (90 – 160)	130.3 (110 – 160)
Number of acetabular spines	15 – 32	19 – 27	19 – 27
Anterior group	5 – 14	5 – 7	5 – 7
Median group	5 – 9	7 – 10	7 – 10
Posterior group	5 – 9	7 – 10	7 – 10
Oral sucker length	25 – 50	41.6 (32.0 – 49.4)	41.3 (32.5 – 52.0)
Oral sucker width	32 – 54	46.6 (39.0 – 54.6)	48.3 (41.0 – 59.8)
Acetabulum length	18 – 40	27.9 (20.8 – 36.4)	27.8 (20.8 – 38.8)
Acetabulum width	14 – 40	27.4 (20.8 – 34.0)	27.1 (20.8 – 36.4)
Prepharynx length	14 – 94	139.8 (70.2 – 187.2)	136.6 (78.0 – 195.0)
Pharynx length	25 – 47	34.5 (23.4 – 44.2)	31.2 (25.0 – 44.2)
Pharynx width	14 – 36	31.5 (20.9 – 44.2)	30.6 (25.0 – 43.2)
Esophagus length	11 – 58	44.2 (23.4 – 78.0)	40.5 (23.4 – 65.0)
Ovary length	11 – 47	27.7 (20.8 – 36.4)	26.8 (15.6 – 36.4)
Ovary width	18 – 47	28.2 (20.8 – 39.0)	28.2 (18.2 – 39.0)
Testis length	29 – 58	46.1 (26.0 – 67.6)	44.0 (28.6 – 62.4)
Testis width	29 – 90	51.1 (33.8 – 70.2)	47.9 (28.6 – 62.4)

( ) = average value

<u>Adult of *Haplorchoides mehrai* from *Hemibagrus nemurus* and *Mystus multiradiatus*</u>

H. mehrai from *H. nemurus* (Fig. 4a, 4b) and *M. multiradiatus* (Fig. 4c, 4d) have a small body size. Body is lance-shaped. The body tegument has a scale like spine. Oral sucker subterminal. Prepharynx longer than esophagus. Caeca extend slightly beyond posterior border of testes. Acetabulum small, submedian, located near intestinal bifurcation. Acetabulum with spines in three groups. Seminal vesicle with two-chambers, behind intestinal bifurcation. Testes rounded, median, between caecal ends posterior to the body. Ovary spherical, pretesticular. Seminal receptacle pretesticular, lateral of ovary. Vitelline follicles around testes. Eggs small, numerous, operculate, with fully embryonated. The measurements of adult stages were shown in Table 3.

Molecular analysis

Our COI sequence data revealed the partial size of 396 bp. in all specimens. Phylogenetic trees were constructed using the Neighbor joining method and the Maximum likelihood method (Fig 5). Bootstrap values were computed independently for 1000 replications. Both methods revealed the monophyletic group of *Haplorchoides* which separated from related group (*Haplorchis* and *Metagonimus*). In *Haplorchoides* group, *Haplorchoides* metacercariae originated from *B. schwanenfeldii* and *C. repasson* were clustered with the *H. mehrai* from *Hemibagrus nemurus*, *H. wyckioides* and *Mystus multiradiatus*, with high bootstrap support. The *H. mehrai* group in this study was separated from *Haplorchoides* sp. from previous studies with high bootstrap support.

Discussion

In this study, a high prevalence of *H. mehrai* metacercaria infection was found in *C. repasson*. This result was similar to Kumchoo *et al*. (2005), in which 100 % of *C. repasson* was infected by *Haplorchoides* sp. metacercariae, whereas the prevalence in *B. schwanenfeldii* was much lower. However, this result is quite different from Noikong *et al*. (2011), which reported 76.23 % and 56.26 % prevalences of *Haplorchoides* sp. metacercaria infection in *C. re-*

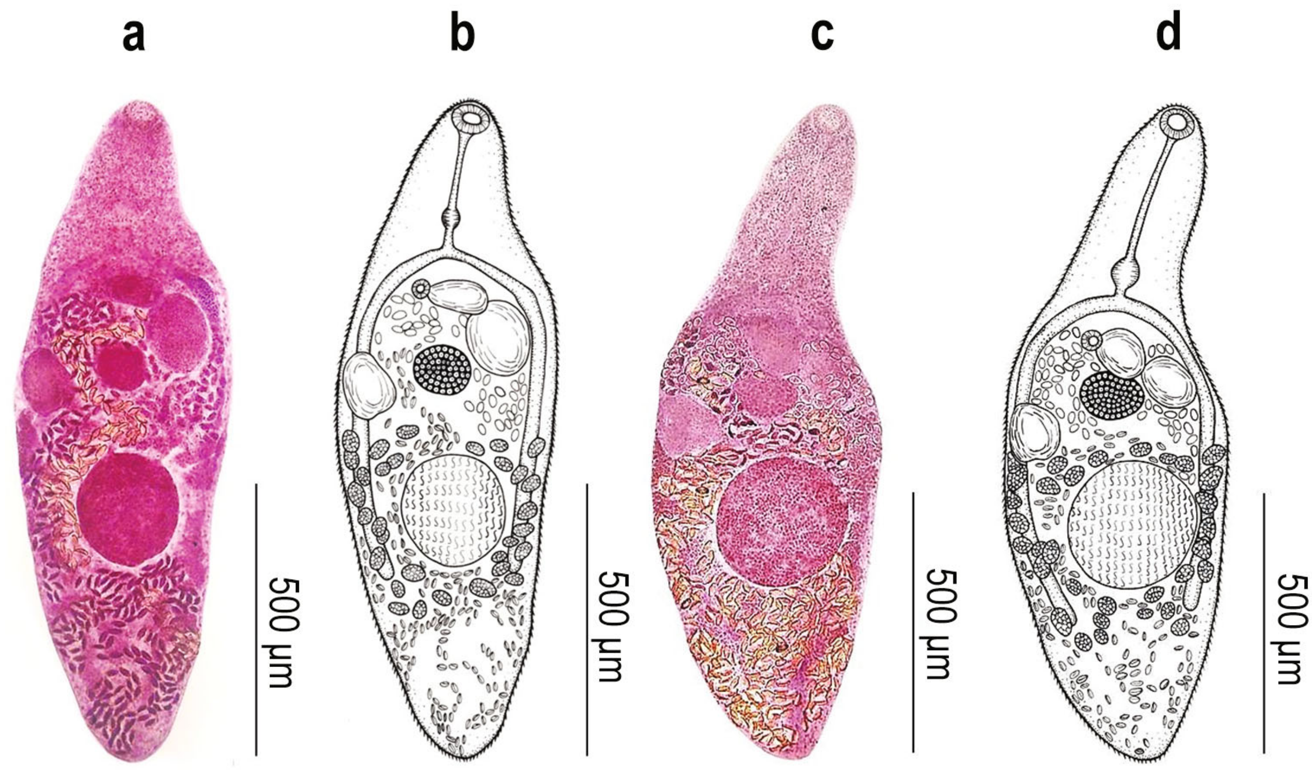

Fig. 4. Adult of *H. mehrai* from *H. nemurus* (a,b) and adult of *H. mehrai* from *M. multiradiatus* (c,d).

passon and *B. Schwanenfeldii* from the Kwae Noi Bamroongdan dam, Wat Bot district, Phitsanulok province, northern Thailand, respectively. The prevalence of infection of *H. mehrai* metacercariae in this study was higher than Noikong *et al*. (2011). However, prevalences over the three seasons were similar to Noikong *et al.* (2011); *H. mehrai* metacercariae were most prevalent in cool, followed by the hot and the rainy season respectively. Metacercariae of *H. mehrai* were found on the inner side of body scales and in muscle, which is in concordance with previous studies, such as Namue *et al*. (1998) Saenphet *et al*. (2001) and Kumchoo *et al*. (2005). *Haplorchoides* spp. metacercariae are found in common species of freshwater fish particularly cyprinoid fish. In Chiang Mai, they are often found together with metacercariae of *Haplorchis taichui* (Namue *et al*., 1998; Boonchot & Wongsawad, 2005; Nithikathkul & Wongsawad, 2008; Wongsawad *et al*., 2013).

In the morphological analysis, excysted *H. mehrai* from *B. schwanenfeldii* and *C. repasson* were similar to the adult stage of *H. mehrai* from *Hemibagrus nemurus* and *M. Multiradiatus* collected from Chiang Mai province, Thailand and other countries (Pande & Shukla, 1976; Shameem & Madhavi, 1988; Manpratum *et al*., 2017). They show the same position of ceaca, acetabulum and the same number of acetabular spines in three groups. However, the numbers of acetabular spines in *H. mehrai* from the four different fish species in this study were also some out of range at posterior and median group. The prepharynx length of both excysted and adult stage *H. mehrai* were longer than described in previous studies by Pande and Shukla (1976) and Shameem and Madhavi (1988). The body size of adult *H. mehrai* in this study was bigger than reported for *H. mehrai* in Northeast Thailand by Manpratum *et al*. (2017).

In previous studies, the High Annealing Temperature Random Amplified Polymorphic DNA (HAT-RAPD) technique was used to identify *Haplorchoides* spp. and other heterophyid species (Chuboon & Wongsawad, 2009; Wongsawad *et al*., 2013). HAT-RAPD was also used to compare metacercariae of *Haplorchoides* sp. from cyprinoid fish with the adult stage of *Haplorchoides* sp., which infect the same fish as those used in this study, such as the Yellow catfish, *Hemibagrus nemurus.* (Wongsawad & Wongsawad, 2011). Likewise, the COI gene can be used to identify *H. mehrai* metacercariae originated from this study. Phylogenetic trees using Neighbor joining and Maximum likelihood methods showed the monophyletic group of *Haplorchoides*. *H. mehrai* metacercariae clustered with *H. mehrai* adults and separated from *Haplorchoides* sp. originated from previous study (Chontananarth *et al*., 2014), with high bootstrap support. The COI gene can also be used to distinguish *H. mehrai* from other trematodes in family Herterophyidae. Our study could indicate that *H. mehrai* metacercariae originated from *B. schwanenfeldii* and *C. repasson* tended to be

Table 3. Comparing the organs size (in μm) of adult *H. mehrai* from *H. nemurus* and *M. multiradiatus.*

Organs	Previous study		This study	
	H. mehrai (Pande and Shukla, 1976)	*H. mehrai* (Shameen and Madhavi, 1988)	*H. mehrai* from *H. nemurus*	*H. mehrai* from *M. multiradiatus*
Body length	255 – 720	928 – 1360	1,243.8 (830 – 1,975)	1,285 (910 – 1,625)
Body width	75 – 390	384 – 512	319 (250 – 470)	327.0 (230 – 450)
Number of acetabular spines	15 – 32	18 – 26	19 – 27	19 – 27
Anterior group	5 – 14	6 – 10	5 – 7	5 – 7
Median group	5 – 9	6 – 8	7 – 10	7 – 10
Posterior group	5 – 9	6 – 8	7 – 10	7 – 10
Oral sucker length	25 – 54	72 – 80	49.7 (33.8 – 67.6)	49.6 (31.2 – 65.0)
Oral sucker width	36 – 65	80 – 96	59.6 (44.2 – 72.0)	56.8 (41.6 – 70.2)
Acetabulum length	19 – 65	48 – 56	36.3 (27.0 – 46.8)	38.3 (31.2 – 47.0)
Acetabulum width	17 – 40	56 – 80	37.1 (27.0 – 42.6)	40.3 (26.0 – 52.0)
Prepharynx length	11 – 65	80 – 96	236.4 (91 – 350)	252.1 (122.2 – 400)
Pharynx length	14 – 54	52 – 56	42.6 (31.2 – 52.0)	46.8 (36.4 – 57.2)
Pharynx width	14 – 40	46 – 48	42.8 (28.6 – 59.8)	43.0 (31.2 – 56.9)
Esophagus length	108	?	69.2 (27.0 – 117.0)	54.9 (13.0 – 132.6)
Seminal vesicle 1 length	36 – 79	96 – 128	74.7 (31.2 – 119.6)	83.7 (41.6 – 113.0)
Seminal vesicle 1width	29 – 90	80 – 112	50.8 (28.6 – 80.6)	60.0 (31.2 – 91.0)
Seminal vesicle 2 length	29 – 72	64 – 88	88.4 (52.0 – 153.4)	90.1 (57.2 – 140.4)
Seminal vesicle 2 width	25 – 79	48 – 56	61.5 (20.8 – 140.2)	73.7 (31.2 – 127.4)
Seminal receptacle length	32 – 72	92 – 112	87.0 (46.8 – 128.2)	98.1 (59.8 – 132.6)
Seminal receptacle width	25 – 72	90 – 110	71.7 (44.2 – 120.5)	84.9 (46.8 – 148.2)
Ovary length	25 – 76	96 – 112	93.9 (52 – 135.2)	101.4 (75.4 – 130.0)
Ovary width	36 – 90	96 – 128	103.8 (49.4 – 140.4)	105.5 (62.4 – 135.2)
Testis length	72 – 126	250 – 264	202.5 (124.8 – 280)	207.8(111.8 – 265)
Testis width	65 – 252	248 – 304	201.1 (130.0 – 270)	206.7 (101.4 – 266)
Egg length	30.6 – 37.7	36 – 38	28.0 (25.0 – 31.2)	28.1 (25.0 – 31.2)
Egg width	17 – 21.4	20	17.7 (15.0 – 19.5)	17.7 (15.0 – 19.5)

( ) = average value

H. mehrai associated with the similar morphology (three groups of acetabular spines).

In conclusion *Haplorchoides mehrai* metacercariae were found on the inner side of body scales and in the muscle of the cyprinoid fish, *Barbonymus schwanenfeldii* and *Cyclocheilichthys repasson* from Chom Thong district Chiang Mai province, Thailand. Both the prevalence and intensity of infection was high. Therefore, this is a high-risk area for *H. mehrai* infection in freshwater animals. This study revealed new records of both *H. mehrai* metacercaria (from *B. schwanenfeldii* and *C. repasson)* and adult stage (from *Hemibagrus nemurus* and *Mystus multiradiatus)* in Chiang Mai province, Northern Thailand.

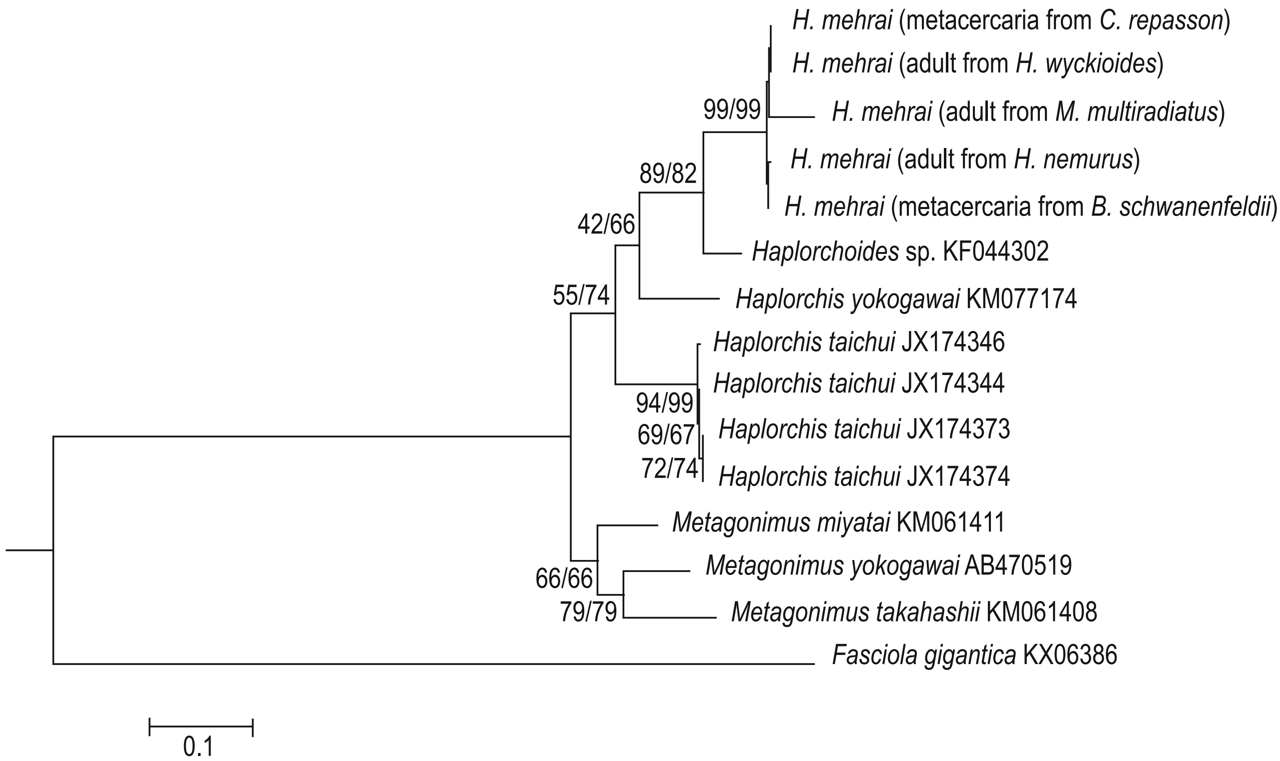

Fig. 5. Phylogenetic tree of *Haplorchoides* spp. and related groups constructed using Neighbor joining (NJ) and Maximum likelihood (ML) (Tamura-Nei model for ML method) analysis of COI gene, with 1,000 bootstrap replicates. Statistic support values for individual nodes are shown on the tree (based on NJ/ML method).

Conflict of Interest

The authors state no conflict of interest.

Acknowledgements

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Research Note

Ithyoclinostomum dimorphum Diesing, 1850 (Digenea, Clinostomidae) in *Hoplias malabaricus* (Erythrinidae) with the first report of infection of the eyes

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Summary

The present study investigated the occurrence of metacercariae of *Ithyoclinostomum dimorphum* in *Hoplias malabaricus* in the basin of the São Francisco River in the state of Minas Gerais (Brazil). Twenty-nine fish were examined in June 2012 during a survey of fish and parasitic fauna. Of the fish examined, 34.5 % had infected eyes, intestine and musculature, with a mean intensity of 1.1 and an abundance of 0.4 per fish. The prevalence in the intestine was 31.0 %, with mean intensity of 1.1 and mean abundance of 0.3. In the eyes, the prevalence was 3.4 %, with a mean intensity of 1.0 and mean abundance of 1.0. The metacercaria found in the right eyeball was lodged between the cornea and iris. The low parasitism did not affect the condition factor (Kn) of the parasitized fish. This was the first report of *I. dimorphum* in the eyes of *Hoplias malabaricus*, a secondary intermediate host for this endoparasite
Keywords: Brazil; condition factor; eye parasite; Trematoda

Introduction

Hoplias malabaricus Bloch, 1794 (Trahira) is widely distributed in South America (Costa *et al.,* 2015), and is abundant in several environments of the Amazon, São Francisco and Paraná hydrographic basins. The dispersion of this piscivorous fish is associated with its capacity to survive for long periods without feeding and to remain in environments with a low concentration of dissolved oxygen (Malabarba, 2006). This Erythrinidae is of considerable economic importance for commercial artisanal fishing in several regions of Brazil (Rodrigues *et al.,* 2017).

A total of 662 species of Trematoda have been found among South American fish, of which 266 species are registered in Brazil (Luque *et al.,* 2017). Among these are the Digenea *Ithyoclinostomum dimorphum* Diesing, 1850 (Clinostomidae), an endoparasite found during adulthood in the esophagus of birds as such as *Ardea cocoi*, *Nyctiocorax* sp. and *Tigrisoma brasiliense*. Various species of fish, including *H. malabaricus*, and mollusks act as intermediate hosts (Dias *et al.,* 2003; Gallio *et al.,* 2007; Benigno *et al.,* 2014). This species of Clinostomidae is the largest worm in this family (Dias *et al.,* 2003), and metacercariae are commonly found in the musculature, gills, pericardium, external wall and esophagus of fish (Gallio *et al.,* 2007; Belei *et al.,* 2013; Benigno *et al.,* 2014). However, there has been no record of ocular infection of fish by this digenea until now. Thus, the objective of this study was to describe the occurrence of *I. dimorphum* in *H. malabaricus* and the first record of infection in the eyes of this host from a tributary of the São Francisco River basin (Brazil).

Materials and Methods

In June 2012, twenty-nine specimens of *H. malabaricus* (19 fema-

* – corresponding author

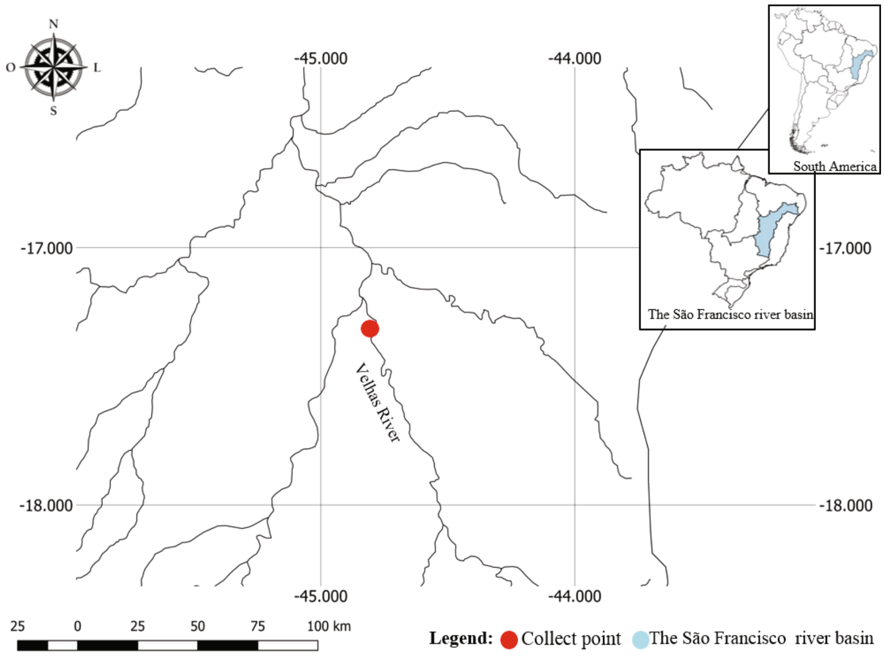

Fig. 1. Geographical location of the *Hoplias malabaricus* collection site in a tributary of the São Francisco River, Minas Gerais (Brazil).

les and 10 males) were captured in the upper part of the das Velhas River, a tributary of the São Francisco River, in the state of Minas Gerais, Brazil (44°49'11.20"W, 17°15'8.00"S) (Fig. 1) using gill nets of various mesh sizes. The fish were collected during a survey on the fish and parasitic fauna of the São Francisco River hydrographic basin by the Centro Nacional de Pesquisa e Conservação de Peixes Continentais (CEPTA). Following collection, all fish were weighed (g) and their standard length (SL, cm) was measured. They were then necropsied *in loco* to collect specimens of the Digenea parasites. All the parasites collected were quantified and stained with carmine acid of Langeron (Amato *et al.,* 1991) and identified in accordance with Travassos *et al.* (1969). The permanent slides of the parasites were assembled in Canadian balsam and used to determine the measurements of the reproductive structures in a stereomicroscope (Zeiss model STMI DV 4), with camera and ZEN software. The specimens of *H. malabaricus* were deposited in the fish collection of the Genetics Museum of the Universidade Estadual Paulista (Paulista State University) (UNESP), Botucatu, São Paulo. The ecological terms were those proposed by Bush *et al.* (1997). Specimens of *I. dimorphum* deposited on fixed glass slides were deposited at the "Adão José Cardoso" Zoology Museum, University of Campinas (UNICAMP), São Paulo, Brazil (N°. ZUEC PLA 155 - *I. dimorphum* in eyes; N°. ZUEC PLA 156 - *I. dimorphum* in intestine and N°. ZUEC PLA 157 - *I. dimorphum* in muscles).

The body weight (g) and total length (cm) data were used to calculate the relative condition factor (Kn) of the parasitized and non-parasitized fish, which was compared using the Mann-Whitney U-test (Zar, 2010). In addition, weight and length data were used to calculate the length-to-weight ratio ($W = aL^b$) of parasitized and non-parasitic fish, after logarithmic transformation of length and weight, and later adjustment along two straight lines, thus obtaining $\ln y = \ln A + B \ln x$ (Le Cren, 1951).

Ethical Approval and/or Informed Consent

The study was carried out in accordance with the principles adopted by the Colégio Brasileiro de Experimento Animal (the Brazilian College of Animal Experimentation) (COBEA). All the fish were collected pursuant to a collection authorization granted by IBAMA/ICMBio - Nº 27447-1/2011.

Results

Of the twenty-nine specimens of *H. malabaricus* examined, the eyes (Fig. 2A and B), gut and muscles of 34.5 % (n=10) of the fish were infected by *I. dimorphum* (Fig. 2C), with a mean intensity of 1.1 and mean abundance of 0.4 per host. The prevalence in the intestine was 31.0 % (n=9), with mean intensity of 1.1 and mean abundance of 0.3 per host. In the eyes of the hosts, the prevalence was 3.4 % (n=1), with a mean intensity of 1.0 and a mean abundance of 1.0 per host.

In the right eyeball the metacercaria of *I. dimorphum* was lodged between the cornea and the iris, occupying an area of 0.4 cm^2, corresponding to 78.0 % of the total organ (Fig. 2C and D).

In *H. malabaricus* analyzed, we found only one *I. dimorphum* infected one eye. Only this *I. dimorphum* we will describe:

Ithyoclinostominae Yamaguti, 1958

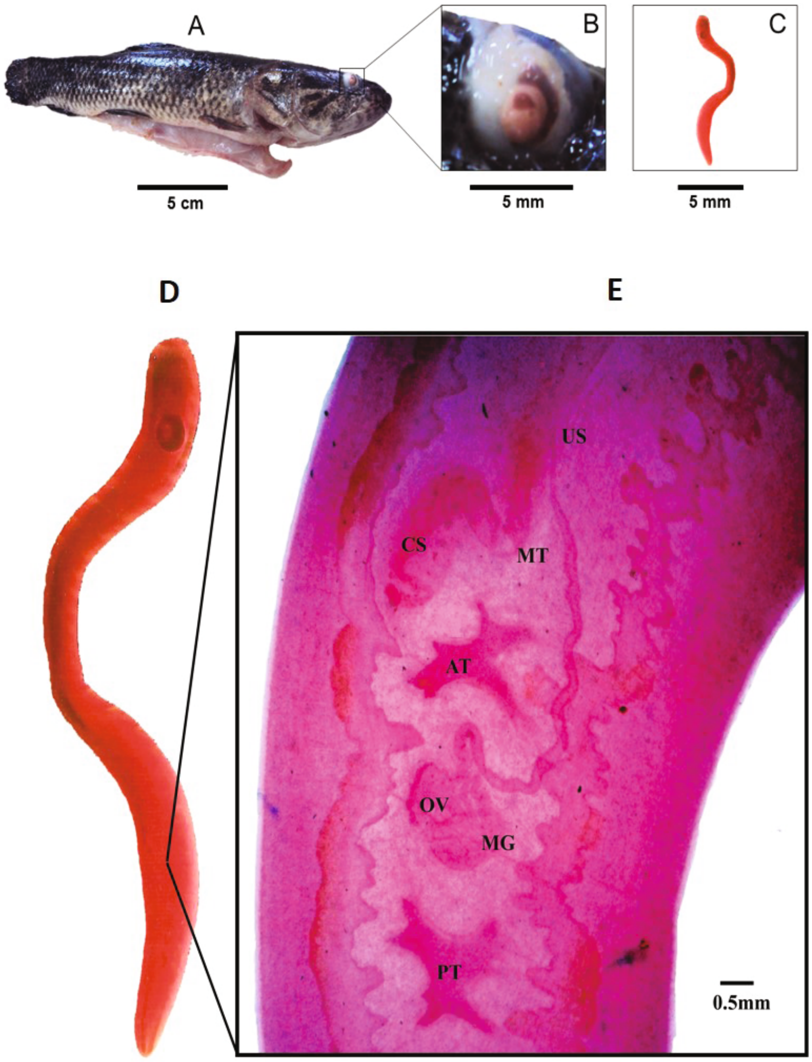

Fig. 2. *Hoplias malabaricus* from a tributary of the São Franscisco River, Minas Gerais, Brazil (A). Eye infection of the right eye caused by *Ithyoclinostomum dimorphum* (B). *Ithyoclinostomum dimorphum* stained with Langeron acid carmine (C). *Ithyoclinostomum dimorphum* from *Hoplias malabaricus* (D). Internal organs of the reproductive system (E). US: Uterine sac, MT: Metraterm, CS: Cirrus sac, AT: Anterior Testis, PT: Posterior Testis, OV: Ovary, MG : Mehlis Gland.

Ithyoclinostomum Witenberg, 1925
Ithyoclinostomum dimorphum (Diesing, 1850) Witenberg, 1926 (Fig. 3a, b)

General description

Body elongated, flattened (Fig. 3a). Body surface with rounded sensory papillae, furrows and rings forming superficial annulations, dorsal and ventral. Oral sucker, terminal, triangular aperture, surrounded by an expansion of the body wall such as collar-like and radial furrows in the surface (Fig. 3a); pharynx present. Caeca simple, long, without lateral branches or diverticula. Ventral sucker, near anterior extremity of body, close to oral sucker, subtriangular aperture (Figs. 3a). Testes lobed, medians, intercaecals, in the posterior half of body; cirrus-sac, destro anterolateral to anterior testis, intercaecal, internal seminal vesicle coiled (Fig. 3a, b). Genital pore, ventral to cirrus-sac, slightly prominent, surrounded by tegumental rugosities and papillae (Fig. 3b). Ovary, intertesticular (Fig. 3a, b). Uterus, intercecal, originating from the Mehlis' gland, ascending sinistral to anterior testis reaching uterine sac (Fig. 3a-b). Uterine sac elongated, median, intercaecal. Metraterm, ventro-lateral to cirrus-sac, converging in a genital atrium (Fig. 3b). Vitelline follicles, caecals, extending from hindbody to the end of the first third of body, below cecal bifurcation, confluent on posterior end; vitelloduct anterior to ovary; considerable space free of internal organs between ventral sucker and anterior limit of vitellarium (Fig. 3a). Mehlis' gland larger than ovary, median, between testis, latero-dorsal to ovary (Fig. 3a, b).

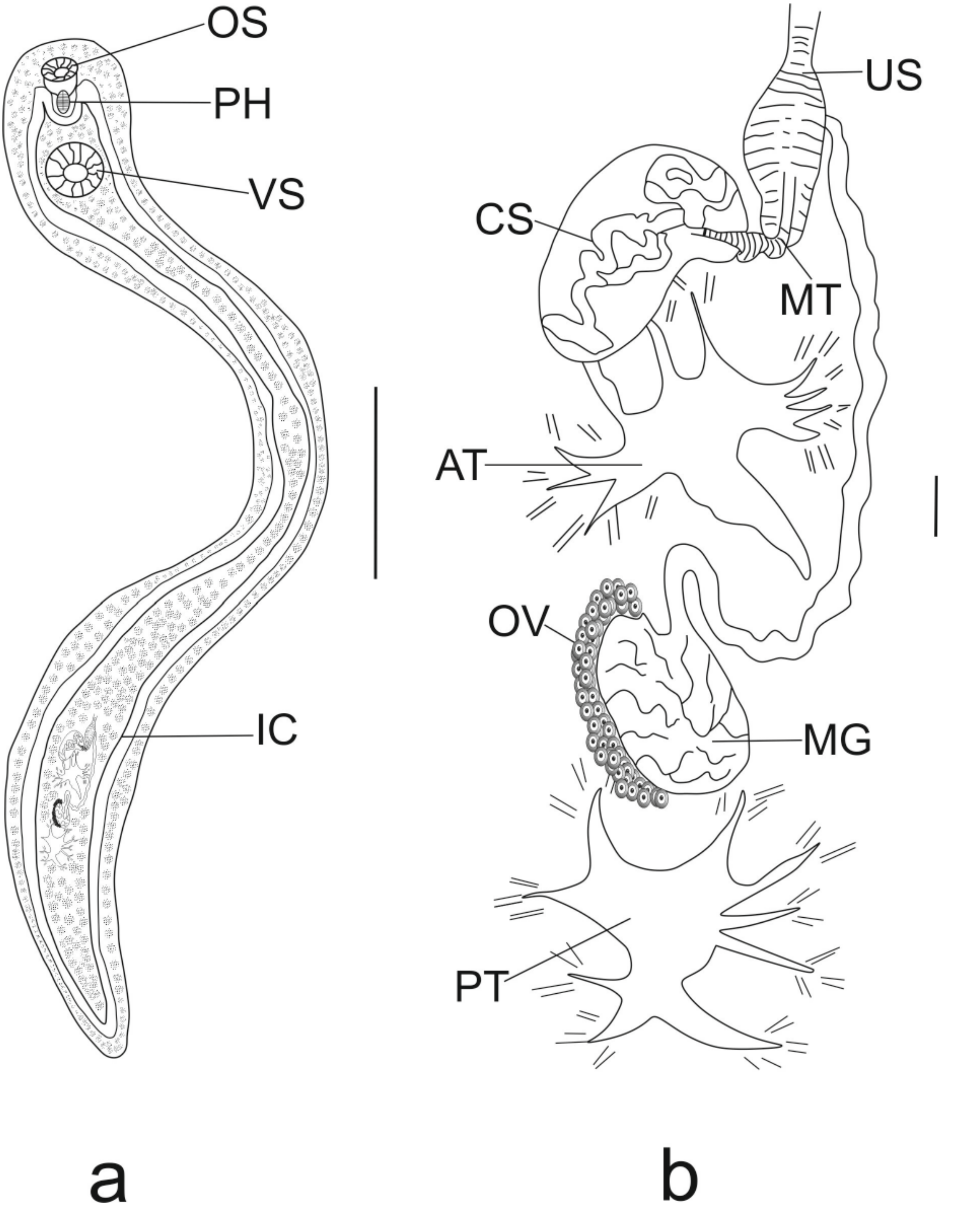

Fig. 3. Metacercaria of *Ithyoclinostomum dimorphum* from *Hoplias malabaricus* from a tributary of the São Franscisco River, Minas Gerais, Brazil. a. Total, ventral view, oral sucker (OS), pharynx (PH), ventral sucker (VS) and intestinal cecum (IC). b. Detail of genital organs, anterior testis (AT), ovary (OV), Mehlis' gland (MG), uterine sac (US), metraterm (MT), cirrus-sac (CS) and posterior testis (PT). Scale bars in a = 5.0 mm and b = 0.5 mm.

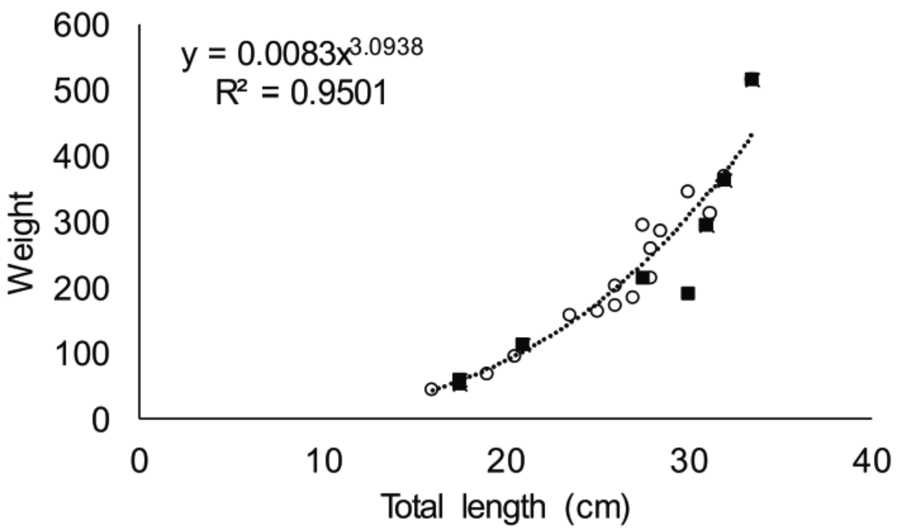

Fig. 4. Relationship between total length-weight of *Hoplias malabaricus* parasitized by *Ithyoclinostomum dimorphum* metacercariae (■) and non-parasitized (○) collected in a tributary of the São Francisco River, Minas Gerais (Brazil).

Measurements
Of one specimen from *H. malabaricus* in eye: Body 33.42 long, 3.01 maximum width. Oral sucker 0.62 long, 0.48 wide; Pharynx 0.48 long, 0.42 wide. Ventral sucker 1.34 long, 1.26 wide. Uterine sac 0.23 long, 0.18 wide. Anterior testicle 0.32 long, 0.48 wide. Posterior testicle 0.48 long, 0.63 wide. Ovary 0.30 long, 0.12 wide.

Taxonomic Summary
Hosts: *Hoplias malabaricus*.
Locality: São Francisco River in the state of Minas Gerais, Brazil.
Site of infection: Eye of *H. malabaricus*.
Numbers of collected specimens: 1 *I. dimorphum* from *H. malabaricus* in eye.

Table 1. Morphometry (mm) of reproductive structures of metacercariae of *Ithyoclinostomum dimorphum* of the intestine, muscles and right eye of *Hoplias malabaricus* from the tributary of the São Francisco River, Minas Gerais (Brazil).

Measured structures	Das Velhas River		Arari Lack, Pará*
	Cavity parasites (n = 4)	Eye parasite (n = 1)	Cavity parasites (n = 1)
	Mean (range) (mm)	(mm)	(mm)
Width of uterine sac	0.30 (0.24 – 0.37)	0.18	0.25
Length of uterine sac	–	-	2.5
Width of metraterm	0.24 (0.20 – 0.31)	0.11	-
Cirrus sac width	0.48 (0.43 – 0.52)	0.38	0.27
Length of cirrus sac	0.83 (0.71 – 1.10)	0.66	0.45
Width of anterior testicle	0.76 (0.50 – 0.95)	0.48	0.34
Length of anterior testicle	0.69 (0.45 – 1.00)	0.32	0.47
Width of posterior testicle	0.74 (0.55 – 0.91)	0.63	0.23
Length of posterior testicle	0.47 (0.34 – 0.56)	0.48	0.45
Width of ovary	0.18 (0.15 – 0.25)	0.12	0.12
Ovary length	0.36 (0.27 – 0.45)	0.30	0.18
Width of Mehlis Gland	0.59 (0.48 – 0.67)	0.22	-
Length of Mehlis Gland	0.53 (0.44 – 0.69)	0.41	-

* Benigno *et al*. (2014)

Prevalence: 3.4 %
Abundance: 0.3 per host
Material deposited: From *H. malabaricus* N°. ZUEC PLA 155 - *I. dimorphum* in eye.
The Kn of fish with eyes, intestine and muscles parasitized with *I. dimorphum* (Kn = 0.99) did not differ (*U* = 86.0, p = 0.68) from that of the non-parasitized fish (Kn = 1.00). The growth of parasitized and non-parasite hosts was allometrically positive (Fig. 4).
For the morphometric study of metacercariae of *I. dimorphum* the internal reproductive structures were measured (Fig. 2E). Measurements were taken in mm based on parasites of the eyeball (length 28.43 mm, width 2.54 mm), intestine and muscles (mean length 48.91 mm, mean width 3.35 mm) of the host (Table 1).

Discussion

In *H. malabaricus* from the upper das Velhas River, the total prevalence of *I. dimorphum* was similar to that reported for the same host (30.8 %) from the Lages Reservoir in the state of Rio de Janeiro (Paraguassu & Luque, 2007). However, it was higher than that reported for *H. malabaricus* from the São Francisco River (10.6 %) infected by *Ithyoclinostomum* sp. (Costa *et al.,* 2015) and from the Arari Lake, in the Ilha do Marajó in the state of Pará (0.9 %), infected by *I. dimorphum* (Benigno *et al.,* 2014). However, these last two studies examined a greater number of *H. malabaricus* specimens than the present study. Poulin (1993) reported that higher prevalence values are related to a more uniform distribution of parasites among hosts, as there is increasing exploitation of the available hosts.
While infection of *H. malabaricus* by metacercariae of *I. dimorphum* has been described in several regions of Brazil, this is the first report of this endoparasite in the ocular region of this host. As the presence of metacercariae of *I. dimorphum* was reported only in the mesentery, musculature, heart, esophagus, cloaca, gills and operculums of host fish (Belei *et al.,* 2013), eyeball parasitism is not fully known. This new site of infection may be due to the sporadic occurrence or behavior of this parasite. Due to the low parasitism of *I. dimorphum*, which occupied almost all of the eyeball, no damage to the prey capturing performance by the host was observed, and the body characteristics of the examined host population were not affected. However, when migrating to the host fish eyes other metacercariae may cause exophthalmos, retinal detachment, cataracts and blindness or even death (Zago *et al.,* 2013; Belei *et al.,* 2013; Ramos *et al.,* 2016) when at high levels of abundance.
The condition factor is based on the relationship between the individual's weight and length and is an important indicator of fish health, reflecting recent nutritional conditions and interactions between the fish and the biotic and abiotic factors (Le Cren, 1951; Zago *et al.,* 2013). No difference was observed in the Kn of *H. malabaricus* parasitized by *I. dimorphum* and non-parasitized fish due to the low parasite infection in the intestine, eyes and muscles. Similarly, other studies have also shown that low parasitism by the metacercariae of digeneas did not affect the host condition factor (Paes *et al.,* 2010; Ramos *et al.,* 2016). On the other hand, an increased abundance of metacercariae of *Sphincterodiplostomum musculosum* in *Steindachnerina insculpta* caused a reduction in the condition factor of the hosts (Zago *et al.,* 2013), because digenea larvae can be highly pathogenic.
Metacercariae of *I. dimorphum* of the *H. malabaricus* musculature and intestine, the width of the uterine sac, the width and length of the cirrus sac, the anterior and posterior testis length were similar to those described by Benigno *et al.* (2014); while the values of the width of the anterior and posterior testes were lower. However, the metacercariae of *I. dimorphum* of the ocular region of *H. malabaricus* had smaller measurements to the specimens collected here in the intestine and muscles, as well as in relation to the measurements of the uterine sac and the testis length of the specimens reported by Benigno *et al.,* (2014); while the cirrus sac and width of the anterior and posterior testes had higher values, although the width of the ovary was similar. Such differences may be due to the different methods and measurement tools used in these studies, as well as the methodologies of the treatment of metacercariae for identification. Therefore, standardization of preparation techniques and morphometric measurements in morphometric studies of *metacercariae* of *I. dimorphum* is required, as this parasite has high body plasticity.

Conflict of Interest

Authors state no conflict of interest.

Acknowledgements

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Update on selected topics in acanthocephalan parasites research

Article info

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Summary

The respectable community of parasitologists aimed at the broad-spectral research of acanthocephalan parasites met at the 9th Acanthocephalan Workshop. The workshop took place in the beautiful surroundings of the High Tatras, Slovakia in the Congress Centre Academia, Stará Lesná near Tatranská Lomnica on September 9 – 13th. This special event was hosted by the Slovak Society for Parasitology, the Institute of Parasitology of the Slovak Academy of Sciences, Košice, Slovakia, and the Czech University of Life Sciences Prague, Czech Republic. It consisted of nearly three dozen lectures presented by distinguished acanthocephalan specialists who came from 13 countries and five continents. Vibrant discussions and creating new plans for future collaborations were accompanied by local mountain touring that offered the venue richly endowed with nature, deep forests and beautiful mountains. The contributions were addressed to resolve current systematic, taxonomic, biological, behavioural, ecological, and related topics. Presented results showed the most recent progressive developments comparable with all the other parasitic worm groups. The 10th Acanthocephalan Workshop will be hosted by Dr. Marie-Jeanne Perrot-Minnot, Université de Bourgogne Franche-Comté, Dijon, Bourgogne, France, in 2022.

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Authors of the cited part (2018): Title of the cited part. In: Update on selected topics in acanthocephalan parasites research. *Helminthologia*, 55(4): pages. DOI: 10.2478/helm-2018-0023

see the example below:
Amin, O.M. (2018): Variability in the Acanthocephala. In: Update on selected topics in acanthocephalan parasites research. *Helminthologia*, 55(4): 350 – 361. DOI: 10.2478/helm-2018-0023

SYSTEMATICS AND TAXONOMY

Variability in the Acanthocephala

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Unique and unusual features in the many species of acanthocephalans described by Amin from fish, amphibians, reptiles, birds, and mammals, in various parts of the world including South America, Vietnam, Japan, the United States, the Middle East, and North and East Africa, are described. The presentation is in five parts. (1) An introductory section dealing with the classification, general morphology, ecology, and life cycles of the Acanthocephala. (2) Unusual anatomical features of taxonomic or of questionable taxonomic importance addressing variations in the proboscis, proboscis hooks, male and female reproductive organs, and lemnisci. Newly described structures including (a) Para-receptacle structure (PRS) and hoods in certain species as well as a new order of Acanthocephala from Vietnamese birds, are also featured. (3) Structural and functional relationships explaining the relationship between the metamorphosis of the giant nuclei in Eoacanthocephala and worm reproductive cycle. (4) Host-parasite relationships elucidating the relationships between worm anatomy and biology during worm growth. (5) Curiosities in reviews and revisions highlighting taxonomically based zoo-geographical patterns and trends in the genera *Neoechinorhynchus*, *Polymorphus*, and *Pallisentis*. A comprehensive treatment of the acanthocephalans of South America and those marine forms off the Eastern United States is also included here. A look at the September, 2013 classification scheme of the Acanthocephala is included covering 4 classes, 26 families, 157 genera, and 1298 species are included

Acanthocephalans in the Helminthological collection of the Institute of Parasitology (IPCAS), České Budějovice, Czech Republic

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The Institute of Parasitology of the Biology Centre of the Czech Academy of Sciences (IPCAS) is a governmental research institution established in Prague in 1962 and relocated to České Budějovice (Budweis) in South Bohemia in 1985. It is the principal institution in the Czech Republic devoted exclusively to basic parasitological research including helminthology. Thanks to the effort of František Moravec, world expert in the systematics of fish nematodes and former curator, the Institute hosts one of the largest helminthological collections in Central Europe. The collection is extraordinarily rich mainly in fish helminths such as nematodes and monogeneans. However, collection of acanthocephalans is also worth mentioning as it includes a total of 107 species (92 identified to the species level) of 7 orders and 18 families; as many as 80 species are from fishes. Type specimens of 16 species (holotypes of 8 taxa) are also deposited in the collection; 14 of them are parasites of teleosts, one of amphibians and one of mammals. In two cases, the new species were type species of newly erected genera, namely *Pseudogorgorhynchus* Moravec, Wolter et Körting, 2000 and *Sharpilosentis* Lisitsyna, Scholz et Kuchta, 2015, both from fishes. Specialists in acanthocephalan taxonomy are much welcome to request specimens deposited in the IPCAS. Deposition of type and voucher material of any acanthocephalans in the future would be much appreciated.

An integrated taxonomic approach to understanding species diversity of fish-parasitizing *Neoechinorhynchus* species

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Neoechinorhynchus Stiles and Hassall, 1905 is a highly diverse and widespread genus of acanthocephalan that parasitizes fishes and turtles on six continents. The literature base for the genus grew rapidly throughout the 20th century, mostly as a result of original descriptive work recognizing the diversity across the world. There have been, however, relatively few contributions regarding North American species of *Neoechinorhynchus* since the turn of the 21st century, and a particular lack of use of modern molecular tools. Several recent studies used DNA sequence data to understand species relationships and uncover cryptic species of *Neoechinorhynchus* in Mexico, but there is still no molecular phylogenetic analysis focused on species of *Neoechinorhynchus* from the USA or Canada. Here we present the phylogenetic results of the first such study, based on nuclear DNA from the internal transcribed spacer (ITS) and large ribosomal unit (LSU) regions, including molecular data from multiple specimens of each of 17 total species (two from turtles and 15 from fishes) from the United States and Canada. We also present morphological evidence supporting the boundaries of several of these species. In addition to giving the first look at relationships between these species, this work has uncovered three previously uncharacterized species from white suckers of Central New York State. Plus, we have found that a species of another neoechinorhynchid genus, *Octospinifer* van Cleave, 1919 nests among species of *Neoechinorhynchus*, lending further support to the recently recognized paraphyly of this large and somewhat enigmatic genus.

***Pomphorhynchus* spp. in the Rhine system – recent situation and future perspectives**

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Over the last few years contrasting results regarding the distribution of two previously synonymised acanthocephalan species, *Pomphorhynchus laevis* and *Pomphorhynchus tereticollis*, have been published for the Rhine river system. Shortly after its redescription, Emde et al. (2012) stated a high invasive potential for *P. tereticollis*, due to its occurrence in the invasive round goby. Recently, a contradictory study by Hohenadler et al. (2018) showed a gradual replacement of *P. tereticollis* by *P. laevis* in eel. However, a distinction between the two congeners remains difficult, since both studies used either a morphological or molecular approach for the differentiation and furthermore, they only took account of unsuitable hosts. Hence, fish of different taxa from the Upper Rhine near Karlsruhe were examined for acanthocephalan infestation to clarify the recent distribution of the congeners.
To facilitate distinguishing between the two species, randomly selected *Pomphorhynchus* worms were verified molecularly by

sequencing the COI-locus. Proboscis hooks of the same specimens were counted and measured, allowing the analysis of hook patterns with the Proboscis Profiler (Wayland, 2010). Significant differences between the proboscis armature of *P. laevis* and *P. tereticollis* allowed a sufficient species separation. Principal Component Analysis of the hook profiles revealed a correlation between molecular and morphometric traits, thus yielding a potential new option for morphological determination.

Examination showed high infection rates with mature *P. laevis* in common barbel and chub, while *P. tereticollis* was only found at low abundance in chub and brown trout. For the first time co-infections of both species are recorded in an individual host. Unsuitable hosts, such as catfish, eel and round goby, were exclusively infected with immature *P. laevis*. In light of the recent findings the distribution of *Pomphorhynchus* spp. in the Rhine River and tributaries is discussed, as well as the influence of invasive Ponto-Caspian fish and amphipod species as acanthocephalan hosts have been re-evaluated.

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Never-ending story of *Pomphorhynchus* spp. – back to the type material?

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Pomphorhynchus laevis (Zoega in Müller, 1779) was considered to be the predominant species in European fishes for a long time, characterized by high morphological and physiological intraspecific variability (e.g. Kennedy,1984). During the first decade of the 21st century, previously synonymised *Pomphorhynchus tereticollis* (Rudolphi, 1809) was ressurrected (Bombarová et al., 2007; Špakulová et al., 2011): taking into account the ICZN rules, the Rudolphi´s type material was compared with newly collected worms from the type locality (Baltic coast) and the type fish host (*Platichthys flessus*), and the worm morphology was combined with new comparative genetic characteristics (gene sequencing, karyotype). The existence of these species is apparent but their distinction is difficult. The same applies to other congeners described so far in Europe using only morphological tools. The latest extensive study of *P. laevis* and *P. tereticollis* phylogeography throughout Western Palaearctic (Perrot-Minnot et al., 2018) showed that *P. tereticollis* occurs within Western and Central parts of Europe showing weak geographical and genetic structuring, while *P. laevis* sensu lato is representing by five lineages partially matching several major biogeographical regions. That means we have a big task ahead of us – i.e. to harmonize so far existing European *Pomphorhynchus* species with the recently described lineages, re-describe their morphology, and to re-establish species spectrum within the whole Europe including its Peri-Mediterranean and Ponto-Caspian parts. The best way may probably be to use newly acquired acanthocephalans from type localities and type fish hosts (in the absence of ethanol-fixed material in museum collections). Further, re-descriptions on basis of integrative approaches should include morphology, gene sequencing and maybe also chromosome analysis. A promising beginning may be *Pomphorhynchus* spp. from Sava River, the Işıklı Lake, the Volga River etc.

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Integrative taxonomy of acanthocephalans from Brazilian mammals

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Since the early twentieth century, species of acanthocephalans have been reported in mammals from different geographic regions in Brazil (Vieira et al. 2008). However, there is still a lack of adequate, complete taxonomic, phylogenetic and ecological information regarding these species. As part of a PhD project carried out with the support of the Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil, we aimed to identify acanthocephalan species collected from wild mammals in Brazil and investigate their phylogenetic relationships. Specimens of acanthocephalans were identified based on morphological and morphometric features using light and scanning electron microscopy and DNA analyses. We identified *Gigantorhynchus echinodiscus* (Gigantorhynchidae) in a giant anteater, *Myrmecophaga tridactyla*, from the Cerrado in the state of São Paulo. Although the species has been previously reported in this host, genetic data were absent. Our study provides details on the structures as a bipartite cylindrical proboscis with a crown of 18 hooks and numerous small hooks, pseudo-segmented body, long leminisci, large testes and ovoid eggs. Such features agree with the genus *Gigantorhynchus* characteristics (Yamaguti, 1963). Our genetic study included new sequences of partial 28S rRNA gene. Phylogenetic analyses were inferred using maximum-likelihood in PhyML 3.0 and Bayesian analysis in MrBayes version 3.2.6, this last using the CIPRES platform. Results showed that *G. echinodiscus* is closely related to *Mediorhynchus* sp., both forming a well-supported monophyletic group (scores=0.92/0.95). The family Gigantorhynchidae includes two genera, *Gigantorhynchus* and *Mediorhynchus*, both possessing bipartite proboscis with large hooks and small hooks, pseudo-segmented body, long or short and slender leminisci, large testes, eight compact cement glands and ovoid eggs. Genetic data were congruent with morphological studies, classifying both species within the family Gigantorhynchidae (Amin, 2013). This study extends our knowledge about acanthocephalans from Brazilian mammals, emphasizes the importance of integrative taxonomic studies to better understand their taxonomy and evolution.

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Redescription of *Acanthogyrus* (*Acanthosentis*) *maroccanus* (Dollfus, 1951) (Acanthocephala: Quadrigyridae) from the Algerian barb *Luciobarbus callensis* (Cypriniformes: Cyprinidae) from Algeria

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The fresh water acanthocephalan *Acanthogyrus* (*Acanthosentis*) *maroccanus* Dollfus, 1951 was insufficiently described in *Luciobarbus setivimensis* (Cypriniformes: Cyprinidae) in Morocco. Although this acanthocephalan has been reported from cyprinid fishes in northern Africa, its detailed morphological description has never been provided. In this study, we present novel morphological data including scanning electron microscopy micrographs (SEM), based on specimens found in the Algerian barb *Luciobarbus callensis* from the Oued Charef Dam Lake, Algeria. The most typical characteristics of *A. maroccanus* are: (i) short proboscis armed with 3 incomplete circles of 6 rooted hooks each; (ii) proboscis hooks of different lengths; (iii) trunk shorter than 8.5 mm; (iv) trunk with 17–19 circles of spines, covering almost completely the anterior part of the trunk; (v) 3–4 ventral and 6–8 dorsal giant hypodermal nuclei; and (vi) male reproductive structures (sperm duct and Saefftigen's pouch) extending into bursa. Our observation also revealed the presence of the parareceptacle structure and vaginal sleeve in females of *A. maroccanus* from Algeria. Additionally, we obtained partial sequences of the large ribosomal subunit (28S) and mitochondrial cytochrome c oxidase 1 (*cox*1) genes for this species; representing the first molecular data for the species-rich genus *Acanthogyrus* Thapar, 1927.

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Acanthocephalans from California sea lions (*Zalophus californianus*): New records and evidence for synonymy of *Corynosoma obtuscens* with *Corynosoma australe*

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California sea lions (CSLs) (*Zalophus californianus*) are one of the most abundant and recognized pinniped species in the North Pacific. To increase the limited knowledge addressing acanthocephalans parasitizing CSLs, 33 animals including pups, juvenile and adult sea lions were examined at The Marine Mammal Center (TMMC), California, USA, in 2012, 2015 and 2016. Totally, 2,268 specimens of acanthocephalans representing 5 species from three genera *Andracantha* (*A. phalacrocoracis* and *Andracantha* sp.), *Corynosoma* (*C. strumosum*, *C. obtuscens*) and *Profilicollis* (*P. altmani*) were found. The total prevalence was 73 % and intensity of infection varied from 1 to 1,225 (average = 94.5; median = 19). *P. altmani* and *A. phalacrocoracis*, predominantly parasites of fish-eating birds, were registered in CSLs for the first time and only in juvenile CSLs. Significant differences were observed in the species diversity in different age groups of CLSs. Detailed morphological and molecular examinations of *C. obtuscens* collected from CSLs gave us solid evidences for a synonymy of this species with *Corynosoma australe*. Comparison of the metrical and quantitative characters of 51 specimens of *C. obtuscens* (24 males, 27 females) from CSLs from California, USA and published data on *C. australe* from different hosts and regions, including type material of the both species (Johnston, 1937; Smales, 1986; Hernández-Orts et al., 2017) did not reveal any significant differences between these species. The level of the genetic divergence in the *cox*1 sequences from *C. obtuscens* from CSLs and *C. australe* from others marine mammals and penguins reaching 1.4 – 1.6 % can be considered as an intraspecific variability. Thus, according to the rule of priority the name *C. obtuscens* should be recognized as the junior synonym of *C. australe*.

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Acanthocephalans from common carp (*Cyprinus carpio* L.) from the Yangtze River basin, China

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Within the years 2009–2018, a total of 1,687 fish specimens belonging to 36 species of 11 families were collected and examined for intestinal parasites throughout the Yangtze River basin. The present work resumes acanthocephalan parasites from the family Illiosentidae Golvan, 1960 collected from the intestine of common carp (*Cyprinus carpio* L.) from the Tai Hu Lake in Wuxi, Jiangsu Province, China. A total of 17 market-size common carps (total length of 30 – 50 cm) were purchased at the fish market or from local fishermen and examined. In total, 21 specimens of acanthocephalans were found in 6 carps (prevalence of 35 %). Worms were washed in tap water, relaxed in refrigerator and then fixed in 4 % formalin solution under slight pressure. Several specimens were fixed in 96 % molecular-grade ethanol for molecular analyses. For final morphological examination, temporary microscopic preparations in Berlese's medium were made; line drawings were made using a drawing attachment on a Zeiss Axio Imager M1 compound microscope equipped with DIC optics and a digital imaging system. All the studied specimens were assigned to the Illiosentidae based on the family-specific morphology of the worms and the presence of 8 cement glands in males. However, specimens recently found in the common carp differed from all the existing genera of the family Illiosentidae by the i) structure of the reproductive system of females, i.e. vagina lacking a muscular sphincter; ii) presence of the terminally pointed protruding tail end in the form of a dome with a muscular base; iii) proboscis receptacle having a goblet-shaped widening in the anterior part of worms of both sexes, absent in representatives of other genera. In addition, recently found specimens are the only representatives of the family Illiosentidae hitherto found in freshwater ecosystems; all other species of the family were referred to parasitize marine fish. Therefore, a new acanthocephalan genus and species are to be proposed from the carp *C. carpio* from the Yangtze River basin, China.

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DIVERSITY AND GEOGRAPHICAL DISTRIBUTION

Acanthocephala from sharks and rays (Elasmobranchii)

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Acanthocephalan parasites, although relatively common in bony fish, are not usually found in cartilaginous fish (sharks and rays) and parasite-host switching between bony and cartilaginous fishes has not been reported. On occasion infections of elasmobranchs have been recorded, but usually the infection has been light, the worms not mature, or a single worm only recovered. In such instances the infections have either been considered accidental, or, in some circumstances, the result of an infected definitive host being consumed by the shark or ray. At least 14 species of acanthocephalan have been so recorded. A further 4 species have been described only from elasmobranchs (Weaver & Smales, 2014). Dissection of elasmobranchs from Australian waters is continuing and here we present preliminary results. Firstly, the probable accidental infection of *Sphyrna mokarran,* the great hammerhead, with *Corynosoma cetaceum* whose usual hosts are dolphins. Secondly, an as yet undescribed species from the wobbegong, *Sutorectus tentaculatus*. In this case the worms, males and females up to 50 mm long, were mature and the females were gravid. The species cannot be easily placed in any family and the morphologically similar species have not been recorded from any other fish found in Australian waters. This represents the fifth example of a species known only from elasmobranchs and indicates that in some circumstances elasmobranchs may act as suitable definitive hosts for acanthocephalan parasites.

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Acanthocephalans in northern fur seals (*Callorhinus ursinus*) from St. Paul Island, Alaska: results of four-year observations

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Several species of acanthocephalans (Acanthocephala: Polymorphidae) are known to parasitize in northern fur seals (NFSs) (*Callorhinus ursinus*). Acanthocephalans from the genera *Corynosoma* and *Bolbosoma* were found in NFSs in different parts of their range, including the NFSs from the Pribilov Islands (St. Paul and St. George Islands), where about 50 % of the NFS world population have breeding rookeries. The aim of the present study was to examine a species diversity of acanthocephalans parasitizing the NFSs at different rookeries of St. Paul Island, Alaska. During 2011–2014, gastrointestinal tracts of 756 humanely harvested NFS subadult males were examined during the annual Aleut subsistence harvest at five rookeries: Lukanin (165), Polovina (164), Gorbatch (125), Zapadnyj (182) and Morzhovyi (120). All acanthocephalans (1,170 specimens) were collected manually, fixed in 70 % ethanol and identified by the morphology after their clarification in the Berlese medium. Totally, 8 species of acanthocephalans from two genera *Corynosoma* and *Bolbosoma* were found in NFSs with a total prevalence of 43.4 %. *Corynosoma strumosum* was the most prevalent species (20.9 %); the prevalence of the other species was lower: *C. similis* – 13.5 %, *C. semerme* – 14.6 %, *C. villosum* – 12.8 %, *C. validum* – 1.9 %, *C. alaskensis* – 0.1 %, *C. cameroni* – 0.7 %, *B. nipponicum* – 1.6 %. The intensity of infection of acanthocephalans in NFSs was low – from 1 to 31 (average = 3.6±4.3; median = 2) parasites per seal. Differences in the species composition and prevalence were observed in NFS subpopulations from the five rookeries. The highest biodiversity of acanthocephalans was observed in subpopulations from Morzhovyj rookery (8 species); the lowest – in Lukanin (6 species). Comparison of our results with data previously collected in Alaska and other parts of the North Pacific revealed a decrease of the NFS infection with acanthocephalans during last four decades.

Diversity of Acanthocephala in some teleost fishes from Kalaat El Andalous, Tunisia

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In Tunisia, the first record of Acanthocephala was reported in 2015 from the Bizerte lagoon. In order to improve knowledge about this group, we extended our investigations to other localities such as lagoon and sea of Kalaat El Andalous (NorthernTunisia). During two years (April 2015 to May 2017), a total of 671 fishes belonging to four families, Gobiidae (*Gobius niger*, *G. paganellus and G. geniporus*), Scorpaenidae (*Scorpaena porcus*), Sparidae (*Lithognathus mormyrus*) and Belonidae (*Belone belone* and *B. gracilis*) were examined. Acanthocephalans were collected from the digestive tract of the fish and identified on the basis of the morphological features. The present study allowed us to identify three families of Acanthocephala including Illiosentidae Golvan, 1960: *Telosentis exiguus* von Linstow, 1901, Arhythmacanthidae Yamaguti, 1935: *Acanthocephaloides propinquus* Dujardin, 1845 and *A. irregularis* Amin, Oğuz, Heckmann, Tepe & Kvach, 2011, and Echinorhynchidae Cobbold, 1879: *Acanthocephalus lucii* Müller, 1776. The solution of parasitic infra-communities showed that they are species-poor; 75.90 % of fishes harboured only a single parasite species, whilst 23.09 % of them were infected with two species of Acanthocephala. *Telosentis exiguus* and *A. propinquus* were found along the entire digestive tract, whereas *A. irregularis* and *A. lucii* were restricted to its posterior part. The overall dynamics of the parasitism showed that *T. exiguus* is the most frequent species with the highest percentage of the prevalence (23.58 %) in *G. niger*. *Acanthocephaloides irregularis* and *A. lucii* were reported only in *S. porcus* and *L. mormyrus*, respectively. *Acanthocephalus lucii* was recorded in *L. mormyrus* from the Mediterranean Sea for the first time. The data of the present study were compared with results available from other localities and countries.

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Acanthocephalans of Carangidae and Mullidae from Northern Tunisia: Ecology and morphology.

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The acanthocephalan fauna of fish is poorly known in Tunisian waters and published data are restricted to those of Gargouri et al. (2015). In order to fill this gap, our survey is focused on acanthocephalans of commercially important fish in Bizerte Lagoon (Northern Tunisia). A total of 470 fish specimens belonging to Carangidae (*Trachurus trachurus*, *Caranx rhonchus*, *Trachinotus ovatus*) and Mullidae (*Mullus surmuletus*) were collected and examined for thorny headed worms. Five acanthocephalan species belonging to four different families were recovered, namely two species of Arhythmacanthidae Yamaguti, 1935: *Acanthocephaloides* sp. Meyer, 1932 and *Breizacanthus irenae* Golvan, 1969, and one of Illiosentidae Golvan, 1960: *Telosentis exiguus* (von Linstow, 1901), Polymorphidae Meyer, 1931: *Southwellina hispida* Van Cleave, 1925 and Pomphorhynchidae Yamaguti, 1939: *Longicollum pagrosomi* Yamaguti, 1935, respectively. The species showed flexibility in the spatial distribution within the host intestine being present in different parts of the gut, except for the cystacanth of *S. hispida*, which often occupied the mesentery of the anterior intestine. The latter species was recorded in *T. trachurus* from the Mediterranean Sea for the first time with a prevalence of 2.5 %. A new host record is similarly noted for *Acanthocephaloides* sp. (P = 1.7 %) recovered from *C. rhonchus*. This carangid also harboured the pomphorhynchid *L. pagrosomi* with a very low prevalence (1.11 %). *Breizacanthus irenae,* with the relatively important prevalence (P = 25 %), was collected from *M. surmuletus.* The most widespread acanthocephalan species (generalist) was *T. exiguus* (P = 35 % in *T. trachurus* and P = 27.8 % in *C. rhonchus*). The light and scanning electron microscopy showed that *T. exiguus* exhibited variations in the trunk size and number of proboscis hooks, compared with other previously reported morphological observations (Dezfuli & Sbrenna, 1990, Kvach & Sasal, 2010,). These preliminary data enhance the understanding the plasticity of acanthocephalans and promote further taxonomical research within this parasite group in the Mediterranean area.

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Acanthocephalans of the genus *Neoechinorhynchus* of the Baikal rift zone

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Neoechinorhynchus Stiles et Hassal, 1905 represents one of the largest genera within Acanthocephala. The latest revision of the genus (Amin, 2002) provided information on 88 species split into two subgenera. Only two species of this genus, namely *N. rutili* and *N. crassus*, were listed in the Key to the parasites of the freshwater fish fauna of the USSR (1987). However, more recent study on *Neoechinorhynchus* in northeastern Russia (Atrashkevich et al., 2016) showed that the genus is more species-rich there. We examined acanthocephalans of this genus in various cyprinid fishes from the water-bodies of the Baikal basin and found the fragmented presence of a single species *N. rutili*. Its occurrence was restricted to the mouths of the rivers Selenga and Upper Angara and adjacent shallow water zones, and it was absent in the open Baikal as well as the upper and middle streams of the abovementioned rivers. The prevalence of *N. rutili* differed in various fish hosts, being high in *Leuciscus idus* (P = 18.4 %), lower in *L. leuciscus baicalensis* (P = 1.4 %) and low in the *Rutilus rutilus* (P = 0.6 %). In general, the acanthocephalans of the Baikal Lake, including *N. rutili*, are limnophilic species and we suppose that the favorable conditions could be associated with a high water velocity in the rivers of the Baikal area and the drift of infected intermediate hosts into the lake. Another congeneric species, *N. tumidus*, was found far from the Baikal in the isolated Lake Baunt (northern Transbaikalia, the River Lena Basin) in *Lota lota* (70.3 %), *Coregonus lavaretus* (75.3 %) *and L. leuciscus baicalensis* (26.3 %).

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LIFE CYCLE AND ECOLOGY

Divergent selective pressures on male and female acanthocephalans: morphological and ecological consequences

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Acanthocephalans are dioecious parasites with a polygynous mating system. Both experimental and observational data drawn from several species suggest that males have a shorter life-span and intensely compete for the access to non-mated females, with larger body sizes likely rendering greater reproductive pay-offs. Regardless of whether females are choosy (this is not known), they live longer than males to produce the offspring in suitable microhabitats where nutritional resources are abundant. Accordingly, adult males and females should develop different strategies to face divergent selective pressures. In this presentation, we explore this question in two key aspects, i.e., the investment in holdfast structures, and microhabitat selection, using species of *Corynosoma* as a model. Firstly, we examined the size of body spines in cystacanths and adults of *C. australe* and *C. cetaceum*. We found that spines grow in both species, but only in females, which also have significantly larger spines than males. This sexual dimorphism does not result from a pure allometry and conform to the hypothesis that females need to withstand the extreme flow conditions prevailing in marine mammals for longer. Secondly, we compared the intestinal distribution of non-mated and mated females, and males, of *C. australe* and *C. strumosum* in two pinniped species. We found that both species share a strikingly similar pattern: the distribution of non-mated females is significantly more anterior than that of mated females, which concentrate in intestinal regions where a nutrient absorption occurs. The distribution of males, however, is significantly more anterior than that of females. Apparently, males, but no females, experience a trade-off between growing in suitable microhabitats and contacting non-mated females that migrate towards these microhabitats. A comparison of body sizes between sexes in *C. strumosum* along the intestine conforms to this interpretation.

Cryptic species and unexpected intermediate host specificity in the acanthocephalan *Polymorphus minutus*

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Polymorphus minutus is a bird-infecting acanthocephalan and is the most commonly reported species of this genus in central Europe. Previous data suggests that *P. minutus* comprises of different lineages or even cryptic species exploiting different intermediate hosts. Our aim was to test the genetic diversity of *Polymorphus* c.f. *minutus* depending on locality and intermediate host species, to examine if *P. minutus* can be considered a cryptic species. We sampled amphipod intermediate hosts infected with *Polymorphus* cf. *minutus* cystacanths originating from 27 sites in Germany and France. Parasites and hosts were identified using integrated datasets (COI and/or morphology for hosts and COI + ITS1-5.8S-ITS2 and morphology for parasites). Mitochondrial and nuclear genetic data strongly supported the existence of three cryptic *Polymorphus* cf. *minutus* (type 1-3) species. The morphological analyses indicated minor differences in hook dimensions between the types, while no difference was observed in the analysis with "Proboscis Profiler". The three types revealed a high degree of intermediate host specificity: *Polymorphus* type 1 was only encountered in *Gammarus fossarum* type B, *Polymorphus* type 2 in *Echinogammarus* sp. and *Echinogammarus berilloni*, and *Polymorphus* type 3 in *Gammarus pulex* and *Gammarus roeselii*. These results point to a so far neglected cryptic diversity of the genus *Polymorphus* in Central Europe. Furthermore, *Polymorphus* type 2 is most likely a non-native parasite in Germany that co-invaded with *E. berilloni* from the Mediterranean area. Potentially, type 3 originated from South-East Europe and migrated to Germany by *G. roeselii*, where it was captured in *G. pulex* as intermediate host. Therefore, our findings can be seen in the context of ecological globalization in terms of the anthropogenic displacement of intermediate hosts and its impact on the dispersal of parasite species. Moreover, our data clearly point to a taxonomic revision of *Polymorphus* c.f. *minutus*.

Massive infections of *Neoechinorhynchus buttnerae* in an Amazonian fish, *Colossoma macropomum*, with notes on the parasites biology

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For over a decade, an acanthocephalan *Neoechinorhynchus buttnerae* has caused production losses for *Colossoma macropomum* producers in Brazil. In some cases, the prevalence of the parasite reaches up to one hundred percent being accompanied with high infections causing a morphological damage of the intestine and compromise the growth of the fishes. The life cycle of this parasite was recently described under experimental conditions using intermediates hosts fed with the eggs of adult specimens of *N. buttnerae* collected from *C. macropomum*. Every stage of the development was photographed, measured, drawn and described. In total, ten larval developmental cycles were studied. This information might help future studies in hopes to control the massive infections in the most produced native fish of Brazil.

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Host-switch of *Corynosoma australe* (Polymorphidae) from mammal seals to bird penguins: exploiting an ancestral resource?

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Corynosoma australe is common acanthocephalan specific to otariid pinnipeds in temperate and sub-Antarctic waters of the Southern Hemisphere. This acanthocephalan is trophically-transmitted and is regularly exposed to hosts and non-hosts through complex food web interactions. In fact, immature specimens of *C. australe* have been reported in higher trophic levels hosts (e.g. sharks, birds and cetaceans) in which apparently, they cannot grow or reproduce. In this study we investigate an apparent host-switching event of *C. australe*, since gravid worms were collected from the intestine of Magellanic penguins *Spheniscus magellanicus* from Brazil. A morphological study showed that the acanthocephalans from penguins belong to *C. australe*. Partial fragments of the 28S rRNA and mitochondrial *cox*1 genes were amplified from isolates from penguins and two pinniped species (i.e. South American sea lion *Otaria flavescens* and South American fur seal *Arctocephalus australis*) to confirm this identification. Infection parameters clearly differed between penguins and the two pinniped species, which were significantly lower in the former. The sex ratio of *C. australe* also differed between penguins and pinnipeds; in penguins it was strongly biased against males, while in pinnipeds it was close to 1:1. Interestingly, females of *C. australe* from the South American sea lion were significantly smaller than those from penguins and the South American fur seal, but fecundity was lower and more variable in females collected from penguins. At first glance, the occurrence of reproductive individuals of *C. australe* in Magellanic penguins could be interpreted as an adaptive colonization of a novel avian host through favourable mutations. However, it could also be considered, perhaps more likely, as an example of ecological fitting through the use of a plesimorphic (host) resource, since the ancestors of *Corynosoma* infected aquatic birds.

BIOLOGY, ACANTHOCEPHALANS IN POLLUTED ENVIRONMENTS

Characterization of nutritional relationships between acanthocephalans and their hosts as compared to other host-parasite associations using stable isotope analyses

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Stable isotope analysis of carbon and nitrogen can deliver insights into trophic interactions between organisms. While many studies on free-living organisms are available, the number of those focusing on trophic interactions between hosts and their associated parasites still remains scarce. Information about some taxa such as acanthocephalans or monogeneans is either very limited or even completely missing. Additionally, available data revealed different and occasionally contrasting patterns, which is most likely depending on the parasite's taxonomic position and its degree of development. Among others, we have determined $\delta^{13}C$ and $\delta^{15}N$, in different acanthocephalan-host systems considering larval as well as adult stages of aquatic and terrestrial hosts. Herewith, we are able to evaluate the trophic position of acanthocephalans with respect to their hosts and to analyse a potential trophic level shift associated to the transmission from intermediate to definitive hosts.
In the context of a meta-analysis we have added this information to already existing data for approximately 100 host-parasite associations considering many taxonomic groups of parasites. This data compilation allowed us to describe patterns of nutritional relationships between hosts and their parasites. Taxa such as cestodes, trematodes and acanthocephalans as well as larval nematodes were mostly depleted in ^{15}N. Parasitic crustaceans (Copepoda, Isopoda, Cirripedia) and gastropods showed usually similar or lower isotope values, whereas parasitic insects, arachnids, fish as well as adult nematodes were enriched in ^{15}N (on average 4.6 ‰). In the taxa Cestoda, Trematoda and Acanthocephala processed nutrients provided by the host are assimilated via the body surface. Taxa such as Nematoda, Crustacea, Insecta, Arachnida and others, actively feed on tissues or fluids of their host organisms. Whereas the latter behave similar to micropredators, the nutrient absorbing parasite taxa appear to be commensal-like organisms

(acanthocephalans, cestodes) with respect to their nutritional strategy.

Combined effects of parasitism and anthropogenic stressors in the freshwater amphipod *Gammarus fossarum*: Impacts on multiple traits

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The interest in acanthocephalan fish parasites as model organisms in ecotoxicology increased ever since pioneer studies more than 20 years ago. More recently, the need for assessing multiple stressors has been emphasized, as well as the interest in combining behavioural and physiological responses with life-history traits. We address here whether the infection of the freshwater amphipod *Gammarus fossarum* with *Pomphorhynchus tereticollis* interacts with chemical pollutant and temperature. Using a fully crossed factorial design, we investigated whether infection at the population level (naïve populations without parasite historical record *vs* non-naïve populations with historical records) and at the individual level (infected and uninfected organisms) changes the effect of the carbamate insecticide (methomyl) and the temperature. For this, we assessed the expression of various behavioural and physiological traits (general defence systems), and food intake and survival as components of functional response and fitness respectively.
Infection with *P. tereticollis* has pervasive effects on most of the traits measured. At the population level, uninfected gammarids from non-naïve populations exhibited decreased AChE and anti-oxidant potential, and increased Glutahion-S-transferase activity, lipid perodixation and Pro-phenoloxidase activity. At the individual level, infection induced decreased feeding rate, increased AChE and GST activity, and decreased anti-oxidant potential, lipid peroxidation, and phenoloxidase activity. By contrast, methomyl alone had limited effect, restricted to decreased AchE activity (its target) and feeding rate. We evidenced very few interactions between stressors; however an additive effect of population infection history and methomyl exposure was evidenced on AChE activity and feeding rate. Temperature mainly modulates the effects of other factors – methomyl, population, and infection- therefore making the predictions about the outcome of global changes in temperature complex. Given these pervasive effects of population parasite history and individual infection, parasitism should be taken into account when running ecotoxicological monitoring, to gain realism in their conclusions when adressing ecological risk.

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Mechanistically modelling metal accumulation in fish-parasite systems: Ongoing attempts

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Fish are affected by both, exposure to metals and infection with parasites. Each of these stressors might have effects on the response of fish to the other. Some efforts have recently been done in developing kinetic models for a prediction of the metal accumulation in fish-parasite systems. Our previous model allows the investigation of relationships between its accumulation in the fish and acanthocephalans, but does not include the mechanisms how metals are accumulated in parasites. Physiologically-based pharmacokinetic (PBPK) model has been used for simulating the organ-specific accumulation of chemicals. However, the capacity of this model for mimicking fish-parasite systems has not been investigated. We aimed to integrate mechanisms for the simulation of metal accumulation in the system of the chub *Squalius cephalus* and acanthocephalan *Pomphorhynchus tereticollis* by developing of PBPK model, in which acanthocephalans were considered a compartment, similar to the blood, storages (muscle, skin, bone, and carcass), gill, kidney, liver, and intestine. The metal accumulation in the system was modelled as a function of internal (i.e. exchange between different compartments) and external (i.e. exchange with water) factors. The flow from the blood to the other compartments depends on diffusive exchange and fraction of metals dissolved in the blood plasma. The flow to the blood is a function of diffusive exchange and blood-tissue partitioning. The model was calibrated by MATLAB-based AMIGO Toolbox. As a detoxifying organ, the liver was found to accumulate Ag at the highest level. Initial results from the model calibration for Ag show a good potential of the model for simulation the accumulation in the muscle, gill, and liver. However, further attempts are being examined to improve the efficiency of the model in simulation of Agdelayed accumulation in the intestine as well as in acanthocephalans.

Comparison of the metal accumulation capacity of acanthocephalans from fish and mammalian definitive hosts

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Recent research on the metal accumulation potential of parasites provided strong evidence that various taxa can be used as sentinels for metal pollution. Among the taxa of parasites tested so far with respect to their metal accumulation capacity acanthocephalans have been found to accumulate metals to a much higher degree than the host and the host's surrounding environment. However, until now, most studies focused on limnetic host-parasite associations, whereas only few papers considered marine acanthocephalan-host systems. Moreover, most studies were performed on fish as definitive hosts, information about the accumulation potential of acanthocephalans from (marine) mammals is scarce. Here, we summarize data of several metal accumulation studies that were performed on marine acanthocephalans (belonging to the genera *Neoechinorhynchus* and *Corynosoma*) collected from their definitive fish (different species of mullets, Mugelidae) and mammalian hosts (common seal, *Phoca vitulina*). Moreover, based on a well-studied limnetic acanthocephalan species (*Pomphorhynchus laevis* from its cyprinid host) we also address the usefulness of acanthocephalans as sentinel organisms for rare earth elements (REE), which are – although being present only in low environmental concentrations – mainly introduced into ecosystems due to anthropogenic activities. The results confirmed that acanthocephalans from fish accumulated various elements to a higher extend than the host itself. This trend was found for both essential (e.g. Cu, Mn, Zn) and non-essential metals (e.g. Cd, Pb) as well as for some REE, especially when compared to host's muscle tissue. Acanthocephalans from the mammalian host did not show such pronounced accumulation patterns and the concentrations of most elements were lower or comparable to those found for kidney or liver tissue. Interestingly, several elements such as Ag, Cd, Mn, Ni and V were accumulated to a higher degree in the parasite compared to muscle, intestine and stomach tissue of the host. The differences in accumulation patterns will be discussed with respect to differences in pollution levels of the host's habitats and also to differences in uptake mechanisms and metabolism of metals in mammalian and fish hosts.

Insights from chub – acanthocephalan system in polluted environments: Towards host decontamination?

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Few studies have investigated the accumulation and metabolisation of organic micropollutants in host-parasite systems despite their ubiquity, chronic release and potentially higher toxicity of their metabolites. The fate of organic pollutants in host-parasite systems was examined in a freshwater fish, the chub *Squalius cephalus*, naturally infected by acanthocephalan *Pomphorhynchus laevis*. Infected (N = 74) and uninfected fish (N = 55) were caught by electrofishing operations from the Marne River and its tributaries in September 2016. The occurrence and levels of several persistent micropollutant families (polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl-ethers (PBDEs)) and metabolizable ones (polycyclic aromatic hydrocarbons (PAHs), phthalates, pyrethroid pesticides), as well as their metabolites, were characterized in parasites and various tissues of the fish (muscle, liver and stomach content). Infected chub were significantly less contaminated by OCPs ($P \leq 0.01$) but had higher levels of pyrethroid pesticides ($P \leq 0.05$) than uninfected fish. PAHs and phthalates reached higher levels in the acanthocephalans than in chub tissues ($[C_{[P.laevis]} / C_{[chub\ tissue]}] > 1$). The highest concentrations of PAHs were detected in parasites (Median [Min ; Max], in $ng.g^{-1}$ of dry weight (dw), 1922 [875.64 ; 9779.30]), followed by the stomach content (591.80 [321.28 ; 1714.17]), liver (92.29 [0.34 ; 637.39]) and muscle (6.74 [0.89 ; 147.76]), with levels up to 5,000 times higher in parasites than in the host muscle. Our results suggest (1) a potential transfer of organic pollutants from the host to parasites, (2) a lower ability

for parasites to metabolize these compounds and/or (3) a higher sensitivity of parasites to the pollution compared to their fish host. Experimental validations will be conducted to confirm this host decontamination by acanthocephalans and the potential benefits for chubs, through the analysis of suitable biomarkers for a host health assessment (oxidative stress, immunity, body condition).

Application of acanthocephalans as bioindicators of metal exposure in the karst Krka River influenced by industrial and municipal wastewaters

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Naturally present trace metal concentrations in rivers are low, even extremely low in karst rivers, which make them very sensitive to the anthropogenic influence. The Krka River is a typical karst river in the Republic of Croatia whose lower part was proclaimed a National Park due to its exceptional natural beauty. The aim of the present study was to evaluate metal exposure in the Krka River few kilometres upstream of the park border, in the area threatened by industrial and municipal wastewaters, using bioindicator organisms: fish brown trout (*Salmo trutta* Linnaeus, 1758), gammarids (*Gammarus fossarum* Koch, 1836) and fish intestinal parasites, acanthocephalans (*Dentitruncus truttae* Sinzar*, 1955*). During the last few decades, acanthocephalans were recognized as potential indicators of metal exposure in the aquatic environment due to their effective metal accumulation, which is order of magnitude higher, compared to commonly used bioindicator organisms (Sures et al., 2017). Concentrations of 16 microelements and 4 macroelements were measured in digested fish intestine, gammarids and acanthocephalans from the Krka River source as a reference site and downstream with the wastewater impact. Prior to metal measurement by high resolution inductively coupled plasma-mass spectrometer (HR ICP-MS), samples were acid digested in a drying oven at 85°C for 3.5 hours using concentrated HNO_3 and H_2O_2. The prevalence of acanthocephalans in fish from the reference site was 100 % and from the polluted area 80 %, while the mean intensity of infection per fish was around 30 in both locations. More effective accumulation of most metals was found in acanthocephalans compared to fish and gammarids in both locations, especially of toxic Cd, Pb, Sr and Tl, in which average levels were 4-39 times higher in acanthocephalans. Metal concentrations were mostly higher in organisms from the wastewater impacted area, confirming anthropogenic influence and useful contribution of acanthocephalans as sensitive bioindicators of bioavailable metal levels.

References:

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Accumulation of heavy metals in fishes and its parasites from the Ružín water reservoir, biomonitoring study

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In this study, the actual content of 10 heavy metals was analysed in different organs of European perch (*Perca fluviatilis* L.) as well as its parasites (*Acanthocephalus lucii, Proteocephalus percae*) from the Ružín water reservoir. Heavy metals from reservoir sediments accumulated mostly in the kidneys (almost 30-times more than in fish muscles). Toxic lead was mostly concentrated in fish bones. In the muscles of perch, the only mercury exceeded the maximum permissible level (1.05 $mg.kg^{-1}$; limit 0.5 $mg.kg^{-1}$). Concerning toxic element accumulation, acanthocephalans, in comparison to the tapeworms and fish organs, absorbed them most intensively.
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