PANAMJAS
Pan-American Journal of Aquatic Sciences

First report of nematodes parasitizing *Clarias gariepinus* (Pisces, Siluriformes) in the Americas

JULIANA SOUZA RIBEIRO[1], FRANCISCO CARLOS RODRIGUES DE OLIVEIRA[1] & NICOLE BRAND EDERLI[2*]

[1]Laboratório de Sanidade Animal, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, RJ, Brazil, 28013-602
[2]Instituto do Noroeste Fluminense de Educação Superior (INFES), Universidade Federal Fluminense (UFF), Av. João Jasbick, s/n, Aeroporto, Santo Antônio de Pádua, RJ, Brazil, 28.470-000

**Corresponding author*: nicole.ederli@gmail.com

Abtract. *Clarias gariepinus* is native to North and South Africa and is widely bred in Europe, Asia and the Middle East. The species was introduced in Brazil by commercial breeders in the 1980s, and its cultivation in unorthodox tanks without protective barriers near river and lake beds resulted in escape and invasion of the surrounding environment. The present work aims to evaluate the nematodes that attack *C. gariepinus* from Brazil. For this, 30 fish were captured, necropsied and the internal organs were dissected under a stereomicroscope. The collected helminths were fixed in AFA and stored in 70% ethanol. The nematodes were clarified in Amann's lactophenol, mounted between the lamina and coverslip, observed, and schematized using a light microscope; the ecological indexes were analyzed. During the necropsy, we observed third larval stage of *Contracaecum* sp. in the viscera (mucosa of the stomach, mesentery, pancreas and peritoneal fat). These larvae have a cuticular tooth and excretory pore located at the anterior extremity with no distinction of sex. The present work is the first report of parasitism in *C. gariepinus* in the Americas as well as the first report of the presence of *Contracaecum* larvae in this fish outside of Africa.

***Keywords**:* African catfish, Nematoda, larvae

Resumo: Primeiro relato de nematoide parasitando *Clarias gariepinus* (Pisces, Siluriformes) nas Américas. *Clarias gariepinus* é nativo do Norte e Sul da África e é amplamente criado na Europa, Ásia e Oriente Médio. A espécie foi introduzida no Brasil por criadores comerciais na década de 80 e o seu cultivo em tanques irregulares sem barreiras protetivas peto de rios e lagos o que resultou no escape e invasão do ambiente ao redor. O presente trabalho teve como objetivo avaliar os nematoides que afetam *C. gariepinus* no Brasil. Para tanto, 30 peixes foram pescados, necropsiados e os órgãos internos foram dissecados sob estereomicroscópio. Os helmintos coletados foram fixados em AFA e conservados em etanol 70%. Os nematoides foram clarificados em lactofenol de Amann, montados entre lamina e lamínula, observados e esquematizados sob microscópio ótico; os índices ecológicos foram analisados. Durante a necropsia, observamos larvas de terceiro estádio de *Contracaecum* sp. nas vísceras (mucosa do estômago, mesentério, pâncreas e gordura peritoneal). As larvas apresentaram dente cuticular e poro excretor localizado na extremidade anterior do corpo sem distinção de sexo. O presente trabalho é o primeiro relato de parasitismo em *C. gariepinus* bem como o primeiro relato da presença de larvas de *Contracaecum* neste peixe fora da África.

***Palavras-chave**:* Bagre-Africano, Nematoda, larva

Introduction

The fish of the order Siluriformes have an ornamented head with long barbels arranged in pairs, as well as leather-like skin (Anzuategui & Valverde 1998). *Clarias gariepinus* (Burchell, 1822) is popularly known as the African catfish and can exceed 140 cm in length and 60 kg in weight (Burgess 1989). These fish have an elongated, scaleless, and sturdy body with a slightly flattened, bony head. They have the ability to breathe out of the water due to the presence of a suprabranchial organ (Ersoy & Ozeren 2009).

Clarias gariepinus originated in North and South Africa (Burgess 1989) and is widely bred in other parts of Africa, Europe, Asia and the Middle East (Froese & Pauly 2003). The species was introduced in Brazil by commercial breeders in the mid-1980s, and its breeding in atypical tanks without protection barriers near river and lake beds resulted in escape and invasion of the surrounding environment. When compared to other farmed freshwater fish, the yield of the African catfish fillet is high (40 to 45%), and its flesh has a soft, firm texture that is ideal for grilling. When sold as a whole fish, its appearance is considered unappealing, which generates marketing difficulties (Ozório *et al*., 2004).

According to Teugels (1986), *C. gariepinus* is an omnivorous species. Due to this diversified diet, this fish can serve as a host to several species of parasites, but there are no reports on its parasitic fauna in the Americas. In Africa, these fish have been reported to serve as hosts to several parasitic species, including trematodes, cestodes, acanthocepla and nematodes. In Brazil, although *C. gariepinus* is widely distributed, the parasitic fauna is unknown. Thus, the present study aims to characterize the nematodes that infect *C. gariepinus*, captured in the municipality of Campos dos Goytacazes, Rio de Janeiro, Brazil

Material and Methods

Thirty *C. gariepinus* were captured from a lake in the disctrict of Tocos, in the city of Campos dos Goytacazes, State of Rio de Janeiro, Brazil. Fish were captured with nylon line, number 10 (10-pound resistance) with 0.25 mm diameter, 5/0 stainless hook (5 cm in size) with various baits such as live annelids, fish fillet and flour mass with crushed fish. Shortly following capture, the fish were packed into isothermal boxes containing ice, in an effort to reduce stress by desensitization, and transported to the laboratory. The fish were euthanized by freezing and stored at -20 °C. Fish identification was performed based on the morphological characteristics. Biometric data (weight, total length, and standard length) and the sex of each specimen were recorded.

The necropsy procedures, as well as the collection, fixation, conservation and processing of the parasites were performed according to the protocols described by Amato *et al*. (1991) and Eiras *et al*. (2000). The collected nematode larvae were fixed in AFA (70o GL ethanol, 93%; formaldehyde, 5%; glacial acetic acid, 2%) for 48 hours and stored in 70% ethanol and later clarified with Amann lactophenol (one part distillated water, two parts glycerin, one part lactic acid, one part phenic acid) between the lamina and cover slip.

Measurements were performed to the nearest micron (range (mean ± S.D.)) and were based on eight larvae specimens collected from several fish. Measurements were performed with the Axioplan Zeiss light microscope (Carl Zeiss, Germany), equipped with a Canon Power-Shot A640 digital camera (Canon, China), and utilizing the Zeiss Axion Vision Sample Images Software (Carl Zeiss, Germany) for image analysis. Drawings were performed using an Axioplan Zeiss light microscope (Carl Zeiss, Germany) equipped with a camera lucida and were digitized using Adobe Photoshop Elements 8.0 software and an Intuos4 Wacom pen tablet (Wacom Co. Ltd, Japan).

Results

Of the 30 *C. gariepinus*, nine were male and 21 were female. Among them, we observed the third larval stage (L3) of *Contracaecum* sp. (Fig. 1) encapsulated in the viscera (stomach mucosa, mesentery, pancreas and peritoneal fat) of three males and seven females. Twenty larvae specimens were collected. Nine were found in the female catfish, and eleven in the males. Among the analyzed fish, a prevalence of 30%, a mean abundance of 2.22 and a mean intensity of 0.67 were observed. The collected larvae presented a filiform body, a whitish appearance in vivo, and a tiny cuticular tooth centralized in the anterior extremity of the delicate contour body and two lips (Fig. 1b). The total length ranged from 12,409 to 24,459 (18,289 ± 3,764.4), with a greater width observed in the final third of the organism, measuring 536 to 853 (685 ± 99.1). An excretory pore was located near the oral opening (Fig. 1b). The distance from the nerve ring to the anterior extremity measured at 217 to 338 (293 ± 40.1). The muscular esophagus measured from

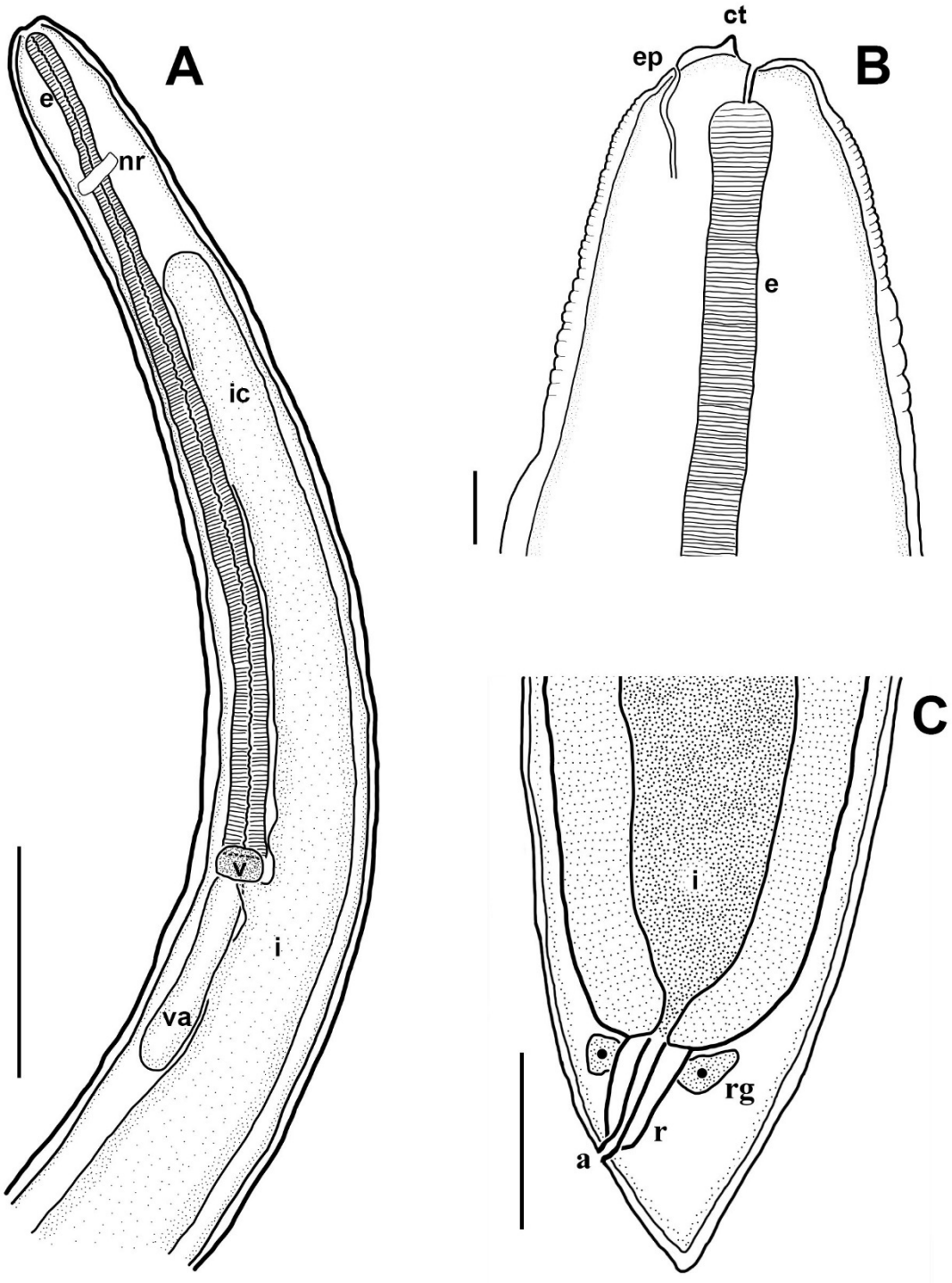

Figure 1. Third larval stage of *Contracaecum* sp. from *Clarias gariepinus* from Brazil. **A.** Anterior region evidencing (e) esophagus; (nr) nerve ring; (ic) intestinal cecum; (v) ventricle; (i) intestine; (va) ventricular appendix. Bar: 500 µm. **B.** Anterior end evidencing (ct) cuticular tooth; (ep) excretory pore; (e) esophagus. Bar: 50 µm. **C.** Posterior end evidencing (i) intestine; (rg) rectal glands; (r) rectum; (a) anus. Bar: 200 µm.

1,521 to 2,459 (2,002 ± 313.5). The distinct ventricle, measured from 60 to 146 (108 ± 30.6), an intestinal cecum, directed toward the anterior region, measured from 930 to 1,643 (1,312 ± 221.6), and a relatively small ventricular appendix measured from 389 to 577 (475 ± 66.5). The tail with conical tip measured 104 to 193 (142 ± 33.3) and was devoid of a caudal spine, with a body width of 87 to 215 (140 ± 41.9) at the level of the anus (Fig. 1c).

Representative specimens were deposited to the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, RJ, under the registration number CHIOC 38549.

Discussion

The nematode larvae of the genus *Contracaecum* are similar to the larvae of *Hysterothylacium* spp. in having an intestinal cecum and ventricular appendix. However, the location of the excretory pore allows for the differentiation of the two genera. In *Contracaecum* spp., this structure is located at the anterior end, just after the cuticular tooth, whereas in *Hysterothylacium* spp., the excretory pore is located in the esophageal region, at the level of the nerve ring (Køie & Fagerholm 1995). Thus, the nematode larvae collected in the present study belong to the genus *Contracaecum*. In addition to the morphological characteristics common to the two genera, the analyzed larvae also contained an excretory pore in the anterior region, close to the oral opening, as displayed in Figure 1b.

The morphology of the specimens collected in the present study is characteristic of L3 larvae because they do not have a developed reproductive system. Huizinga (1967) described the second larval stage (L2) as thin and elongated, with a cuticular tooth on the ventral face of the mouth, an underdeveloped intestinal cecum, esophagus, ventricule and nerve ring that is difficult to visualize at 400x magnification. Moreover, the intestine is characteristically thin and granular.

Identification of the collected larvae at the species level was not possible: the specific identification of nematodes is carried out according to a set of morphological characteristics of the reproductive system, and the morphometry of male and female specimens (Barson 2004). Furthermore, there is a shortage of papers describing the morphology and ultrastructure of the nematode larvae. Further investigations focused on the molecular biology or experimental infections will be required to provide a clearer identification of the nematodes affecting the fish under study (Barson & Avenant-Oldewage 2006), which was not possible in this work. The occurrence of the genus *Contracaecum* outside of Africa is important because it shows that these nematodes can affect these fish outside of their natural habitat. The present study discovered that African catfish from Brazil were infected with third stage larvae of *Contracaecum* sp., suggesting that along with the introduction of these fish to Brazil in the 1980s, a concurrent introduction of their parasites occurred, which may disseminate in natural environments. Another hypothesis is that the specimens of *C. gariepinus* analyzed in this study were infected with a species of *Contracaecum* that is common to other fish native to the study area and autochthonous to Brazil.

This work is the first report on parasitism in *C. gariepinus* in the Americas and the presence of *Contracaecum* larvae in this fish outside of Africa. *Contracaecum* larvae have already been reported in *C. gariepinus* in many water bodies from South Africa (Prudhoe & Hussey 1977, Whitfield & Heeg 1977, Mashego & Saayman 1981, Boomker 1982, Boomker 1994, Barson & Avenant-Oldewage 2006), Zimbabwe (Barson 2004, Barson *et al*., 2008) and Uganda (Mwita & Nkwengulila 2004, 2010, Akoll *et al*., 2011).

Prudhoe & Hussey (1977) identified *Contracaecum* spp. larvae in *C. gariepinus* from the Elands and Olifants Rivers in Transvaal and concluded that these larvae are extremely common in African freshwater fish. Several studies carried out in Africa have reported that infections by *Contracaecum* larvae are highly prevalent in *C. gariepinus*, with infection occurring at high intensities of up to 700 to 2000 larvae per fish in almost 100% of the fish analyzed (Mashego & Saayman 1981, Boomker 1982, 1994). Thus, *Contracaecum* infections are considered to be the most prevalent in South African fish (Barson & Avenant-Oldewage 2006). The prevalence observed in our study (30%) was low when compared to the studies carried out in Africa. Akoll *et al*. (2011) and Mwita & Nkwengulila (2004) described a lower prevalence, of 2 and 4%, respectively. Boomker (1994) and Barson (2004) reported the prevalence of *Contracaecum* to be similar to those observed by our group (Table I). Other studies reported a prevalence of greater than 50% (Table I). The low prevalence of *Contacaecum* larvae found in *C. gariepinus* in Brazil may suggest that the fish are adapting to the *Contacaecum* species already present in the country. According to Boomker (1994), *Contacaecum* larvae have a low specificity to an intermediate or paratenic host and can infect several species of marine and freshwater fish. In the adult phase, these nematodes display some degree of host specificity (Thomas 1937; Whitfield & Heeg 1977). Another hypothesis is that the contracaecum larvae, if introduced to Brazil together with the African catfish, did not find a suitable and preferred host in the region, which can result in a low rate of environmental infection and,

Table I. Species of *Contracaecum* in *Clarias gariepinus* from Africa and from the present study.

AUTHORS	N*	Local	Stage	ECOLOGICAL INDEX		
				Prevalence	Mean Intensity	Mean Abundance
Mashego and Saayman (1981)	337	Various localities	-	57%	-	-
Boomker (1982)	43	-	L_2	33%	0.7	2.3
Boomker (1982)	43	-	L_2	100%	355.6	355.6
Boomker (1994)	67	Sabie river	L_3	43%	4.0	-
Boomker (1994)	2	Olifants river	L_3	100%	2.0	-
Boomker (1994)	45	Crocodilo river	L_3	49%	32.0	-
Barson (2004)	202	-	L_2	43%	2.2	-
Mwita and Nkwengulila (2004)	1071	-	L_3	4%	-	-
Barson & Avenant-Oldewage (2006a)	-	-	L_2	86%	16.3	-
Akoll et al. (2011)	128	-	L_3	2%	6.3	-
Present study	**30**	**Brazil**	**L_3**	**30%**	**0.67**	**2.22**

consequently, a low rate of infection in the intermediate hosts. It is common to find a higher parasitic load in older (larger) fish than in young fish, which demonstrates an accumulative infection. According to Madi & Silva (2005), piscivorous birds cannot predate larger fish due their size. As a result of the longer survival associated with the lower impact of bird predation, larger catfish may accumulate more *Contracaecum* larvae due to successive predation of the infected smaller fish. Our study confirms this statement. Only one fish had a total length (113 cm) and weight (5 kg) that was significantly higher than that of the other fish (average of 52 cm and 1 kg). As mentioned by Madi & Silva (2005), the longest fish had a significantly higher *Contracaecum* parasitic load (n = 6) in comparison to the other specimens (average n = 2). According to Boomker (1994), a high prevalence of *Contracaecum* spp. larvae is found in bodies of water that have a large piscivorous bird population that can serve as the final host.

In the African catfish collected in Brazil, *Contracaecum* larvae were found encysted in the mesentery, pancreas, adipose tissue and the mucosa of the stomach. A large number of reports on parasitism in *C. gariepinus* by *Contracaecum* spp. larvae describe the presence of the parasites in the mesentery and the stomach mucosa (Boomker 1982, Boomker 1994, Barson *et al*., 2008, Akoll *et al*., 2011). Mashego & Saayman (1981) found the larvae in the liver, adipose tissue, musculature of the stomach and intestinal lumen. Prudhoe & Hussey (1977) collected larvae from the bile ducts and body cavity of the African catfish. In our study, we found one larva of *Contracaecum* encysted in the pancreas in a single fish. The phenotype of this larva was similar to the other larvae. This study is the first report of this parasite in the pancreas of *C. gariepinus*. Studies on parasites in Africa, where these fish are native, report other nematode species that parasitize *C. gariepinus*, including *Paracamallanus cyathopharynx* in the intestine (Mashego & Saayman 1981, Boomker 1982, 1994, Mwita & Nkwengulila 2004, Akinsanya & Otubanjo 2005, Barson *et al*., 2008, Moyo *et al*., 2009), *Procamallanus laeviconchus* in the stomach (Mashego & Saayman 1981, Boomker 1982, Mwita & Nkwengulila 2004, Oniye *et al*., 2004, Barson & Avenant-Oldewage 2006, Ayanda 2009), *Skrjabinocara* sp. in the stomach (Boomker 1982), *Rhabdochona* spp., *Capillaria* sp. and unidentified nematode larvae in the gut (Boomker 1994), *Spinitectus petterae* in the stomach (Boomker 1994, Mwita & Nkwengulila 2004), *Eustrongyloides* sp. (Mwita & Nkwengulila 2004) and *Rhabdochona congolensis* (Mwita & Nkwengulila 2010) in the celomatic cavity. However, in this study, no other species of nematodes were identified.

There is only one study in the literature, performed by Barson & Avenant-Oldewage (2006), that reports on the morphometric data of third instar larvae of *Contracaecum* sp. in *C. gariepinus*. The

authors observed some of the same characteristics that were found in the present study, including a total length from 22,000 to 35,000 µm (mean of 27,600), a greater body width from 680 to 780 µm (710), an intestinal cecum from 1,240 to 2,200 µm (1,720) and a ventricular appendage from 510 to 1040 µm (790). When compared to the present study, these measurements were not significantly different. Barson & Avenant-Oldewage (2006) observed three lips and a caudal spine. In the Contracaecum larvae collected from the African catfish in our study, we observed two lips and a cuticular tooth, which may have been misidentified as a third lip and absence of a caudal spine by Barson & Avenant-Oldewage (2006). Thus, *C. gariepinus* participates in the epidemiological chain of at least one species of *Contracaecum* in Brazil, as the parasite in question has a heteroxenous life cycle. Further studies are required for two main purposes: to identify the species of *Contracaecum* larvae isolated during this study; and to discover if these parasites were introduced together with the African catfish or if they are autochthonous species of *Contracaecum* spp. and *C. gariepinus* participates in their life cycle.

Acknowledgements

Financial support: This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Conselho Nacional de Pesquisa (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

References

Akinsanya B. & Otubanjo O. A. 2005. Helminth parasites of *Clarias gariepinus* (Clariidae) in Lekki Lagoon, Lagos, Nigeria. Department of Zoology, Parasitology Unit, University of Lagos, Nigeria. **Revista Biología Tropical**, 54(1): 93-99.

Akoll P., Konecny R., Mwanja W. W., Nattabi J. K., Agoe C. & Schiemer. 2011. Parasite fauna of farmed Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) in Uganda. **Parasitololy Research**, 110(1): 315-323.

Amato J. F. R., Boeger W. A. & Amato S. B. 1991. **Protocolos para laboratório – coleta e processamento de parasitos de pescado. Imprensa Universitária, Universidade Federal Rural do Rio de Janeiro**. Seropédica, Brazil, 81 p.

Anzuategui I. A. & Valverde C. C. 1998. **Rações pré-calculadas para organismos aquáticos: peixes tropicais, trutas, rãs e camarões de água doce**. Agropecuária, Guaíba, Brazil, 135 p.

Ayanda O. I. 2009. Comparison of parasitic helminthes infection between the sexes of *Clarias gariepinus* from Asa dam Ilorin, north-central Nigeria. **Scientific Research and Essay**, 4(4): 357-360.

Barson M. 2004. The occurrence of *Contracaecum* sp. larvae (Nematoda: Anisakidae) in the catfish *Clarias gariepinus* (Burchell) from Lake Chivero, Zimbabwe. **Onderstepoort Journal of Veterinary Research**, 71(1): 35-39.

Barson M. & Avenant-Oldewage A. 2006. Nematode parasites of *Clarias gariepinus* (Burchell, 1822) from the Rietvlei Dam, South Africa. **Onderstepoort Journal of Veterinary Research**, 71(1): 35-39.

Barson M., Bray R., Ollevier F. & Huyse T. 2008. Taxonomy and faunistics of the helminth parasites of *Clarias gariepinus* (Burchell, 1822), and *Oreochromis mossambicus* (Peters, 1852) from temporary pans and pools in the Save-Runde River Floodplain, Zimbabwe. **Comparative Parasitology**, 75(2): 228-240.

Boomker J. 1982. Parasites of South African freshwater fish. I. Some nematodes of the catfish [*Clarias gariepinus* (Burchell, 1822)] from the Hartbeespoort Dam. **Onderstepoort Journal of Veterinary Research**, 49(1): 41-51.

Boomker J. (1994). Parasites of South African freshwater fish. VI Nematode parasites of some fish species in the Kruger National Park. **Onderstepoort Journal of Veterinary Research**, 51(2): 129-139.

Burgess W. E. 1989. **An Atlas of Freshwater and Marine Catfishes: a preliminary survey of the Siluriformes**. TFH Publications, Neptune City, 784 p.

Eiras J. C., Takemoto R. M. & Pavanelli G. C. 2000. **Métodos de estudo e técnicas laboratoriais em parasitologia de peixes**. EDUEM, Maringá, Brazil, 333 p.

Ersoy B. & Ozeren A. 2009. The effect of cooking methods on mineral and vitamin contents of African catfish. **Food Chemistry**, 115: 419-422.

Froese R. & Pauly D. 2003. **Fish Base** accessible at http://www.fishbase.us/country/CountryList.p

hp?ID=1934&GenusName=Clarias&SpeciesName=gariepinus. (Accessed 17/01/2016).

Huizinga H. W. 1967. The Life Cycle of *Contracaecum multipatillatum* (von Drasche, 1882) Lucker, 1941 (Nematoda: Heterocheilidae). **The Journal of Parasitology**, 53(2): 368-375.

Køie M. & Fagerholm H. 1995. The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto (Nematoda, Ascaridoida, Anisakidae) in view of experimental infections. **Parasitology Research**, 81(6): 481–489.

Madi R. R. & Silva M. S. R. 2005. *Contracaecum* Railliet & Henry, 1912 (Nematoda, Anisakidae): o parasitismo relacionado à biologia de três espécies de peixes piscívoros no reservatório do Jaguari, SP. **Revista Brasileira de Zoociências**, 7(1): 15-24.

Mashego S. N. & Saayman J. E. 1981. Observations on the prevalence of nematode parasites of the catfish, *Clarias gariepinus* (Burchell, 1822), in Lebowa, South Africa. **South African Journal of Wildlife Research**, 11: 46-48.

Moyo D. Z., Chimbira C. & Yalala P. 2009. Observations on the helminth parasites of fish in Insukamini Dam, Zimbabwe. **Research Journal of Agriculture and Biological Sciences**, 5(5): 782-785.

Mwita C. & Nkwengulila G. 2004. Parasites of *Clarias gariepinus* (Burchell, 1822) (Pisces: Clariidae) from the Mwanza Gulf, Lake Victoria. **Tanzania Journal of Science**, 30(1): 53-62.

Mwita C. & Nkwengulila G. 2010. Phylogenetic relationships of the metazoan parasites of the Clariid fishes of lake Victoria inferred from partial 18S rDNA sequences. **Tanzania Journal of Science**, 36: 47-57.

Oniey S. J., Adebote D. A. & Ayanda O. I. 2004. Helminth parasites of *Clarias gariepinus* (Teugels) in Zaria, Nigeria. **Journal of Aquatic Sciences**, 19(2): 71-75.

Ozório R. O. A., Avnimelech Y. & Castagnolli N. 2004. Sistemas intensivos fechados de produção de peixes. Pp. 11-15. *In*: Cyrino JEP *et al*. **Tópicos especiais em piscicultura de água doce tropical intensiva**. Tecart, São Paulo, 533 p.

Prudhoe S. & Hussey C. G. 1977. Some parasitic worms in freshwater fishes and fish-predators from the Transvaal, South Africa. **Zoologica Africana**, 12(1): 113-147.

Teugels G. G. 1986. A systematic revision of the African species of the genus *Clarias gariepinus* (Pisces; Clariidae). **Zoologische Wetenschappen: Sciences Zoologiques**, 247: 2-192.

Thomas L. J. 1937. On the life cycle of *Contracaecum spiculigerum* (Rud.). **Journal of Parasitology**, 23: 429-431.

Whitfield A. H. & Heeg J. 1977. On the life cycles of the cestode *Ptychobothrium belones* and nematodes of the genus *Contracaecum* from Like St Lucia, Zululand. **South African Journal of Science**, 73: 121-122.

Received: July 2017
Accepted: September 2017
Published: December 2017