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First report and record of nematode *Eustrongylides africanus* larvae in a vertebrate host mudfish *Clarias* species from Bida floodplain of Nigeria

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ABSTRACT: The nematode specimens from *Clarias* species were confirmed to be the larval forms of nematode *Eustrongytides africanus* that occurs in a wide diversity of fish-eating aquatic animals. And its effective third- and fourth-stage larval development was also described. The physical presence of this parasite in *Clarias* species is manifested by swellings or undulations on skin surfaces as grub-like. On close inspection, they were grossly observed as tiny white or pink (third-stage) or bright red (fourth-stage) blood-sucking larvae, at different depths of the body cavity, with relatively long bodies. The third-stage larvae are shorter than the fourth-stage larvae both larval stages retained the second-stage cuticles. There was no significant difference ($P>0.05$) in body length between male and female third-stage larvae. Female fourth-stage larvae were significantly ($P<0.05$) longer than males fourth-stage larvae. The most useful characteristics for describing the third-and fourth-stage larvae are body length and head shape, which were easily observed. But the characteristics of the cephalic and labial papillae, although more difficult to observe, were the most definitive. This is the first report and record of the confirmation of the larval forms of *Eustrongylides africanus* and the description of its third- and fourth-stage larval development from a vertebrate host *Clarias* species from Bida floodplain of Nigeria (or West Africa) with natural infection.

Key words: *Eustrongylides africanus* larvae, *Clarias* species, Bida floodplain, Nigeria.

Introduction

Parasitic nematode of the genus *Eustronglyides* reach maturity in piscivorous (fish-eating) birds. Infection results in mortality of the ovian hosts. Third- and especially foruth-stage larvae of *Eustrongylides* species are known to parasitise a wide variety of vertebrate intermediate hosts, but fourth-stage larvae from fish invadethe tissues of various reptilian and mammalian hosts (including man) if infected fish are ingested uncooked. An invertebrate as the first intermediate host has been confirmed for *Eustrongylides excisus*, Jaegerskiold, 1909 in freshwater oligochaetes (measures, 1988). In the United States of America, a

frequent vertebrate intermediate host for *Eustrongylides ignotus* is the benthic mummichog *Fundulus heteroclitus.*

The third-stage larvae of *Eustrongylides* species have been reported in aquatic annelids (oligochaetes) as first intermediate hosts (Lichenfels and Stroup, 1985) and fish as second intermediate hosts where the fourth-stage larvae develop. The identification of the nematode larvae of the genus *Eustrongylides* is based on the characteristics of adults (Lichtenfels and Pilitt, 1986) and has been reported from 17 orders of fish worldwide (Spalding *et al*., 1993). It is apparent from such a broad host range that *Eustrongylides* is not restricted to a particular taxonomy group of fish with particular feeding habits. The present report is part of a study of undertaken in Bida floodplain to determine whether *Clarias* were infected with larvae *Eustrongylides*. This is the first report and record of the confirmation of the larval forms of *Eustrongylides africanus* (Khalil and Thurston, 1973) and the description of the third- and fourth-stage larval development (Khalil, 1998) from a vertebrate host *Clarias* species (Holden and reed, 1972) from Bida floodplain of Nigeria (or West Africa) (Khalil and Polling, 1997) with natural infection.

Material and Methods

The study area is the freshwater wetlands or floodplains, created by rivers Niger and kaduna around Bida, located between Longitude 5°45’ to 6°15’E and 8°30’ to 9°10’N within the southern Guinea Savannah zone of Nigeria (Areola *et al*., 1992). *Clarias gariepinus* and *C. anguillaris* of different sizes and sexes caught from four, including a variety of pristine and human-altered, fish landing sites in Bida floodplain were randomly selected and placed in floodplain water in buckets and transported live to the Fish Health Diagnostic Laboratory at NIFFR. Specimens usually diagnosed as worm infected by the undulations on the skin surfaces as grub-like presentations were killed in an human manner by cervical dislocation or decapitation and either examined immediately or frozen and examined as soon as possible. Worms recovered from different sites of infections were fixed in steaming glacial acetic acid alcohol (1 part steaming glacial acetic acid and 3 parts of 95% ethyl alcohol) to narcotize straighten and relax then instantly (Meyer and Oslen, 1975), stored in alcohol-glycerine and studied fresh. Some larvae were cleared in lactophenol studied, measured the morphological characteristics and photographed under light binocular dissection microscope between 10 to 30X magnification in phenol-alcohol. Two hundred and twenty-two (222) of the specimens recovered from *Clarias* species were passed to Dr. L.F. Khalil in 1998 through the International Institute of Parasitology, England to confirm the species and to describe the stages of the larval development using standard procedures and identification keys and tools. The analysis between the body length of the third- and fourth-stage larvae and sex of larvae was done using a statistical computer software programme at level of $P = 0.05$.

Results and Discussion

The physical presence of *Eustrongylides* larvae in free catches *Clarias* is manifested by swellings or undulations on the body skin as grub-like (Figure 1). Necropsy of affected fish as a sure means of isolating these tiny blood-sucking larvae grossly observed on close inspection at different depths of the body cavity (muscles or elsewhere) by their white or pink (third-stage larvae)
Or bright red (fourth-stage larvae) colouration due to the presence of haemoglobin within them (Yanong, 2002) and relatively coiled, long bodies(Figure 2). Larvae nematodes belonging to the genus *Eustrongylides* (Jaegerskiold, 1909) have been recorded from freshwater fishes of the genus B*agrus, Clarias, Dinotoperus, Engraulicypris, Haplochromis Lamparologus, Mormyrus, Protopterus* and Tilapias from east (tabzania and Uganda) and central (Zaire) African Lakes: Lake Albert, Lake Edward, Lake George, Lake Kivu, Lake Tanganyika and Lake Victoria (Khalil and Polling, 1997). Also, found in several other freshwater fishes of the genus *Micropterus, Leponus, Lutra, Perch* and Ompok from Bangladesh, Mexico, United States of America and Venezuela (Khanum *et al*., 1996; Hoberg *et al*., 1997; Moravec *et al*., 1997; Rojas *et al*., 1997; Rosinski *et al*., 1997; Coyner *et al*., 2002).

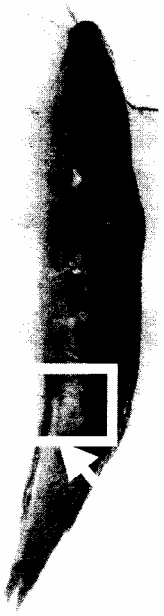
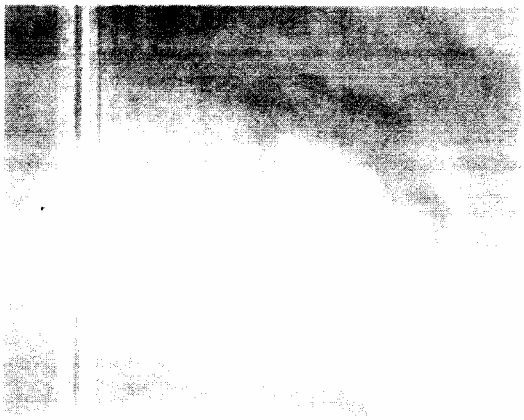
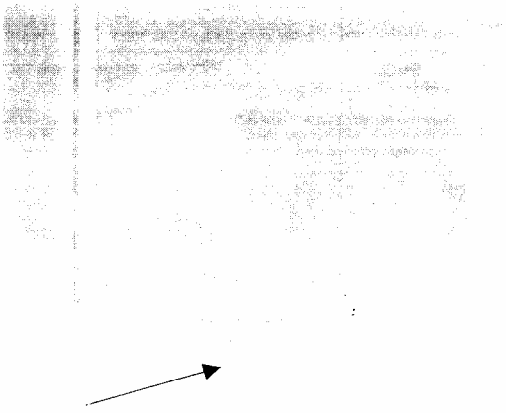


Figure 1: *Eustrongylides africanus* larvae infected *Clarias* species (whole)



Undulation due to *Eustrongylides africanus* larval infection.

Figure 2: *Eustrongylides africanus* larvae infected *Clarias* species (incised)



Eustrongylides africanus larvae (magnified).

Mean values of the morphological measurements of nematode *Eustrongylides africanus* larvae in *Clarias* species from Bida floodplain of Nigeria is shown on Table 1. The body length for the male, female and total sampled third-stage larvae were 92±19, 90±22 and 91±21mm, respectively. The males have longer body length than the females. But there was no significant difference (P>0.05) in the body length between the male and female third-stage larvae. The third-stage larvae from *Clarias* were longer than those described from oligochaetes (Measures, 1988). The body width, oesophagus length and tail length were 705.2 ± 68.9; 125.1 ± 25.2 and 491.2 ± 55.9µ for respectively for males and 710.8 ± 30.8; 122.3 ± 28.7 and 495.5 ± 54.7µ for the females respectively. The total sampled had 706.3 ± 51.7; 123.5 ± 27.1 and 492.8 ± 54.7µ for the body width, oesophagus length and tail length respectively. The body length for the male, female and total sampled fourth-stage larvae were 95±21; 101 ± 25 and 97 ±mm respectively. Female fourth-stage larvae were significantly (P<0.05) longer than males' fourth-stage larvae. The body width, oesophagus' length and tail length were 708 ± 72.5¸128.2 ± 26.3 and 493.2 ± 56.2µ respectively for males and 715.3 ± 35.2, 126.5 ± 29.4 and 498.1 ± 57.5µ for the body width, oesophagus length and tail length, respectively. The third-stage larvae are shorter than the fourth-stage larvae similar to the report of Lichtenfels and Pilitt (1986). Size seemed to be the most useful characteristics in separating the two larval stages. The total sampled had 711.2 ± 58.5, 127.2 ± 28.1 and 495.3 ± 56.9µ for the body length, oesophagus' length and tail length respectively.

Table 2 showed the description of the third- and fourth-stage larvae of nematode *Eustrongylides africanus* from mudfish *Clarias* retained the second-stage cuticle. The first-stage cuticle was not seen and presumably had been shed. The cephalic extremity was conical and labial papillae were similar in shape and position to third-stage larvae from oligochaetes and *E. tubifex* (Lichtenfels and Stroup, 1985). However, lateral labial papillae were more anterior than dorsal and ventral labial papillae. Ventral papillae were seen slightly anterior to the outer circle of labial papillae. The indentation of the posterior extremity at the anus in male third-stage from mudfish *Clarias* was more marked than seen in those from oligochaetes. The spicule primordial entered the rectum dorsally. The genital primordial entered the rectum ventrally and at a position anterior to that of the spicule primordial. The genital primordial in male and female larvae were supported by minute process to the body wall and extended anterior and terminated after a short distant in a blunt extremity. In female third-stage larvae the genital primordial extended a greater distant than seen in larvae oligochaetes. The female genital primordial then ended posteriorly and terminated in a blunt extremity at a position slightly posterior to the rectal-intestinal junction. The intestinal lumenof male and female larvae was wide and contained numerous cells and cellular debris.

The fourth-stage larvae from naturally infected *Clarias* retained the second and third-cuticles, which were larger than third-stage from oligochaetes. Female fourth-stage larvae were significantly longer than the males' fourth-stage larvae. Labial papillae were similar in shape and position to those of third-stage larvae except labial and somatic papillae were longer and more prominent than those of third-stage larvae. The caudal sucker of male larvae was more developed than that of third-stage and had a small cuticular hem around the perimeter. The genital primordial of male larvae consisted of four regions. From the rectum a thick-walled genital tube (ejaculatory duct) with circular muscles extended anteriorly often looping several times. The genital tube (seminal vesicles) then widened and consisted of longitudinal muscles and circular muscles. These longitudinal muscles ended shortly before the genital tube narrowed and its walls thickened. The genital tube then extended further anteriorly and the walls beam thin with small or imperceptible linen; the tube (vas efferens) lacked muscles. The genital tube then curved posteriorly near the oesophageal-intestinal function and after a short distant expended into a large, thin-wall, hologonic testis with a blunt testis. The spicule was not sclerotized completely. The dorsal wall of the posterior part of the rectum had small spiny projections, which were directed posteriorly to the genital primordial of the female larvae with of regions. The vaginal wall if thick and consisted of cuboidal ephithelium and circular muscles with an inner layer of cuticle. The genital tube (uterus) then widened with narrow lumen. The genital tube (oviduct) consisting circular muscle and a small or imperceptible lumen that further extended anteriorly. The genital tube then curved posteriorly and extended into a large thin-walled hologonic ovary with a tapered terminus. The genital tube of male and female larvae was supported by well-developed process of the body wall. Numerous small papillae some bifid or trifid distally were present in small groups on the posterior extremity of male and female fourth-stage larvae. The larvae head cuticle is coarse, marked annulated with transverse striation (Figure 3a), mouth small but surrounded by two papillae coronas (Figure 3b). Fourth-stage larvae of *Eustrongylides africanus* could be distinguished from the third-stage larvae by their larger size (32 – 83mm long), the number of cuticles present (three) and the marked development of the reproductive system.

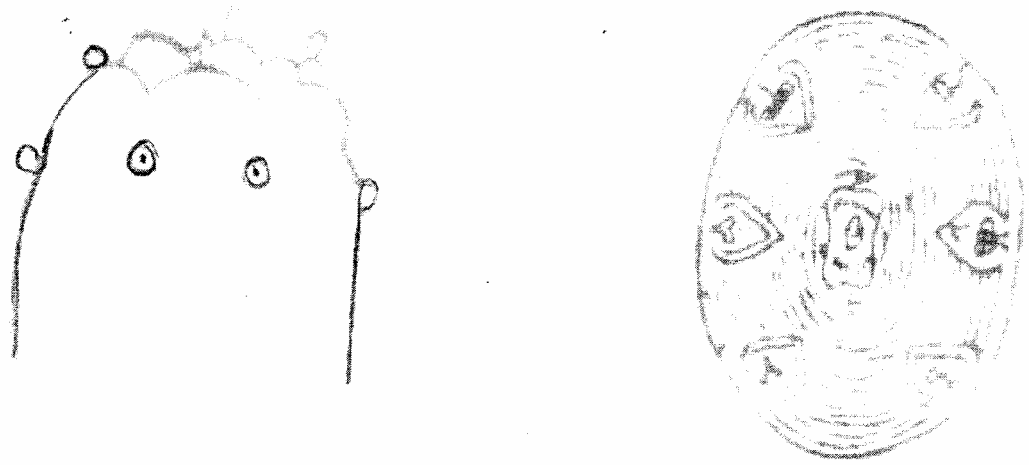
Table 1: Mean values of the morphological measurements of nematode *Eustrongylides africanus* larvae in *Clarias* species from Bida floodplain of Nigeria.

	Third-stage larvae						**Fourth-stage larvae**					
	Male		**Female**		**Total**		**Male**		**Female**		**Total**	
Parameters	**(n=52)**		**(n=58)**		**(n=110)**		**(n=47)**		**(n=53)**		**(n=110)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Body length (mm)	92	19	90	22	91	21	95	21	101	25	97	23
Body width (µ)	705.2	68.9	710.8	30.8	706.3	51.7	708.5	72.5	715.3	35.2	711.2	58.5
Oesophagus length (µ)	125.1	25.2	122.3	28.7	123.5	27.1	128.2	26.3	126.5	29.4	127.2	28.1
Tail length µ)	491.2	55.9	495.5	53.3	492.8	54.7	493.2	56.2	498.1	57.5	495.3	56.9

Table 2: Description of two larval stages of nematode *Eustrongylides africanus* from mudfish *Clarias* in Bida floodplain of Nigeria.

Characteristics	**Third-stage larvae**	**Fourth-stage larvae**
Males	Longer than females Caudal sucker less developed with indentation of the posterior extremity at the anus. Spicule primordial entered the rectum dorsally. Genital primordial entered the rectum ventrally at a position anterior to the spicule primordial and extended anterior and terminated after a short distance in a blunt extremity.	Shorter than females Caudal sucker more developed with a smaller cuticular hem around the perimeter. Spicule is selerotized completely. Genital primordial consisted to four regions; ejaculatory duct, seminal vesicle, vas efferens and testis.
Females	Shorter than males Genital primordial extended with a greater distance anteriorly, curved posteriorly and terminated in a blunt extremity slightly posterior to the rectal – intestinal junction.	Longer than males Genital primordial consisted of four regions; vagina, utesur, oviduct and ovary.
Both sexes	Live larva is whiter or pink in colour Conical cephalic extremity Retained second-stage cuticle Lateral labial papillae were more anterior than dorsal and ventral labial papillae. Ventral papillae slightly anterior than the outer circle of the labial papillae Genital primordial are supported by minute process to the body wall. Intestinal lumen is wide and contained numerous cells and debris.	Live larva is bright red in colour. Bluntly rounded cephalic extremity. retained second-stage cuticle. Labial papillae similar to the third-stage in shape and position except the labial and somatic papillae were longer and more prominent. Numerous small papillae present in small groups on the posterior extremity. Genital tube supported by well developed process to the body wall.

Khalil, (1998).



a b

a. Lateral view
b. Frontal view

Figure 3: Head of *Eustrongylides africanus* larva

The cuticle of the third molt was retained. Thus, three cuticles were visible on fourth-stage larvae, the loose cuticles of the second and third-stages and the new cuticle of the fourth-stage. Karmanova (1968) reported third-stage larvae (8-30mm long), molting third-stage larvae (30-32mm long) and fourth-stage larvae of *E. excisus* in naturally infected fish but the later larvae were not described. Sprinkle (1973) described the third-stage (9-32mm long) with one cuticle) and fourth-stage (33-93mm long, with two cuticles) larvae of *E. tubifex* in yellow perch. Crites (1982) disntinguished third- and fourth-stage *E. tubifex* based on morphology of the inner and outer labial papillae. The number of cuticles present was not indicated. Lichtenfels and Pilitt (1986) distinguished third- and fourth-stage larvae *Eustrongylides* from *Fundulus* species. The practical value of the morphology of the labial and cephalic papillae for separating the third- and fourth-stage larvae is limited because it is difficult to be observed with light microscopy. This is compounded by the fact that the papillae of the fourth-stage can be seen through the third-stage cuticle prior to the third molt. However, this character can be used to identify the larvae for most purposes. In the present study, molting specimens were not available, but the morphological differences in papillae indicated that a molt occurred in female specimen. Three characters have therefore been found to be useful for separating *Eustrongylides* third- and fourth-stage larvae in this study. Body length and head shape were the most easily observed, but the characteristics of the labial and cephalic papillae, although more difficult to observe were the most definitive. This is the first report and record of the confirmation of the larval forms of *Eustrongylides africanus* (Khalil and Thurston, 1973) and the description of the third- and fourth-stage larval development (Khalil, 1998) from a vertebrate host *Clarias* species (Holden and reed, 1972) from Bida floodplain of Nigeria (or West Africa) (Khalil and Polling, 1997) with natural infection.

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References

Areola, O.; Ituehe, O.; Ahmed, K.; Adeleke, B. and Leong, G.C. (1992). Certificate physical and human geography for secondary schools. University Press Plc., Ibadan, 406pp.

Coyner, D.F.; Spalding, M.G. and Forester, D.J. (2002). Epizootiology of *Eustrongylides ignotus* in Florida: distribution density and natural infections in intermediate host. J. Wildlife Dis. 38(3): 483-499.

Crites, J.L. (1982). Impact of the nematode parasite *Eustrongylides tubifex* on yellow perch in Lake Erie. Commercial Fisheries Research and development Project, Ohio, U.S. Department of Commerce, national Oceanic and Atmospheric Administration, Washington, D.C., 84pp.

Hoberg, M. and Henny, C.J.; Hedstrom, O.R. and grove, R.A. (1997). Intestinal helminthes of river Otters (*Lutra Canadensis*) from the Pacific Northwest. J. Parasitol. 83(1): 105-110.

Holden, M. and Reed, W. (1972). West African Freshwater Fish. West African Nature handbook. London, Longman.

Jaegerskiod, L.A. (1909). Nematoden aus Aegypten und dem Sudan. results of the Swedish Zoological Expeditions to Egypt and the White Nile, 1901, 3: 1-66.

Karmanova, E.M. (1968). Dioctophymidea of animals and man disease caused by them. In: Fundamentals of Nematodology, vol. 20, Academy of Sciences of the USSR. Translated and Published for the U.S. Dept. of Agriculture, Amerind Publishing Co., Pvt. Ltd., New Delhi, India, 383pp.

Khalil, L.F. (1998). Description and confirmation of the nematode parasites specimens from *Clarias* species in Bida floodplain of Nigeria. Personal Communication.

Khalil, L.F. and Polling, L. (1997). Checklist of the helminth parasites of African freshwater fishes. University of the North, Republic of South Africa. River Pprinters, Pieterburg, South Africa, 185pp.

Khalil, L.F. and Thurston, J.P. (1973). Studies on the helminth parasites of freshwater fishes of Uganda including the description of two new species of digeneans. Revue de Zoologie et de Botanique Africaines. 7(2): 209-248.

Khanum, H.; Ahmed, A.T.A. and Zaman, Z. (1996). Endoparasite community of two species of genus Ompok (Laceped). J. Bengal Nat. Hist. Soc. 15(2): 32-36.

Lichtenfels, J.R. Pilitt, P. (1986). *Eustrongylides sp.* (Nematoda: Dioctophymatoidea): Differentiation of third- and fourth-stage larvae from Killifish. *Fundulus sp.* collected in Chesapaeke Bay area, U.S.A. Proceedings of Helminthological Society of Washinngton. 53: 144-148.

Lichtenfels, J.R. and Stroup, C.F. (1985). *Eustrongylides sp.* (Nematode Dioctophymatoidea) in North America. Proceedings of Helminthological Society of Washington, 52: 320-323.

Measures, L.N. (1988). The development and pathogenesis of *Eustrongylides tubifex* (Nematoda: Dictophymatoidea) in oiligochaetes. J. Parasitol. 74: 294 – 304.

Meyer, M.C. and Oslen, W.O. (1975). Essentials of Parasitology, 2nd edition. Wim. C. Brown Co. Publisher, Iowa, 303pp.

Moravee, F.; Prouza, A. and Reyero, R. (1997). Some nematodes of freshwater fishes in venezuela. Folia Parasitologica. 44(1): 33-47.

Rojas, E.P.; Perez-Ponce-de-Leon, G. and Prieto, L.G. (1997). Helminth community structure of some freshwater fishes from Patzcuaro, Michoacan, Mexico. Trop. Ecol. 38(1): 129 – 131.

Rosinki, J.L.; Muzzall, P.M. and Haas, R.C. (1997). nematodes of yellow perch from Saginaw bay, Lake Huron, with emphasis on *Eustrongylides tubifex* (Dioctophymatidae) and *Philometra cylindracea* (Philometridae). J. Helminthol. Soc. Wash. 64(1): 96-101.

Spalding, M.G.; Bancroft, G.T. and Forrester, D.J. (1993). The epizootiology of eustrongylidosis in wading birds(Ciconiiformes) in Florida. Journal of Wildlife Diseases. 29: 237-249.

Sprinkle, D.J. (1973). Life history studies of *Eustrongylides tubifex* (Nitzsh, 1819) Jaegerskiold, 1909 (Nematoda: Dictophymatida). M.Sc. Thesis, Ohio State University, Colombus.

Yanong, R.P.E. (2002). nematodes (Roundworm) infections in fish. 1st edition. Cooperative Extension Service, Institute of Food and Agriculture Sciences, University of Florida, U.S.A., 16pp.