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Research Article

Molecular Identification of *Baylisascaris transfuga* from Gobiid Fishes: Parasite Prevalence and Fish Condition

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ABSTRACT
Molecular biology methods are advocated for specific and accurate diagnosis of parasites. We examined gobiid fishes, *Bostrychus africanus* and *Periophthalmus papilio*, from four locations in Rivers State, Nigeria, for parasite identification using molecular biology techniques. Basket traps were used to capture the fish; gobiids were identified using specific guides, and meristic measurements were taken. Parasite isolation was done using standard procedures. The parasites were sorted based on morphological differences by wet microscopy, stored in absolute ethanol and transported to the Regional Centre for Biotechnology and Bioresources Research (RCBBR), University of Port Harcourt, for molecular identification. Two parasites were isolated: an acanthocephalan which could not be sequenced due to inadequate amount of DNA (<0.2mol), and ascaridoid nematodes identified using the NCBI database as *Baylisascaris transfuga* (accession number PV400747; with 97.0% similarity). Prevalence of *B. transfuga* and the acanthocephalan in *B. africanus* was 2.0% and 3.3%, respectively. In *P. papilio*, the prevalence of *B. transfuga* was 2.4% to 17.9%, while the Acanthocephalan had a prevalence of 17.1%. The gobiids are accidental hosts for *B. transfuga*, whose definitive hosts are bears. The parasite might have been a sub-species infective to non-bear carnivores in Port Harcourt Zoo. Research gaps include the examination of the zoo animals for the presence of *B. transfuga*.

Keywords: Acanthocephalan; *Bostrychus africanus;* New Calabar River; *Periophthalmus papilio*; Upper Bonny Estuary

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INTRODUCTION

The use of molecular biology techniques in parasite identification has been promoted due to the challenge of identifying larval stages of several parasites (Mueller *et al*., 2020) which is largely impossible due to absence of diagnostic features (Ahmed *et al*., 2011). Even where diagnostic features are fully developed, molecular identification enables researchers to ascertain the identity of parasitic isolates for specific diagnosis (Knoll *et al*., 2021). Behera *et al*. (2022) mentioned the following as methods that have been used in the study of parasitic pathogens: nucleic acid-based approach, real-time polymerase chain reaction (RT-PCR), Luminex-based assays, microsatellites etc.

Prevalence is an important measure in disease ecology, transmission cycles, risk factors and control strategies. It represents the proportion or percentage of individuals infected with a particular pathogen over a period of time (Miller *et al*., 2018). Since prevalence is an expression of the number of infected individuals in a given population, it includes both existing and new infections. Arnold *et al*. (2020) showed that prevalence is influenced by the number of new hosts, the number of hosts recovering from the infection and the number of hosts dying from the infection.

Condition factor is globally recognized as an indicator of the health status of fishes. It provides information for the assessment of the feeding intensity of fish samples in varying environmental conditions (Igwela *et al*., 2011), thereby indicating the suitability of the habitat for the fish species (Ragheb, 2023). Getso *et al*. (2017) showed that the condition factor reflects how biotic and abiotic factors interact to impact on the wellbeing of aquatic organisms. Ragheb (2023) proposed that each species of fish has a particular range of Fulton's condition factor. Helminth parasitism is a key biotic factor that could affect the condition factor of fish (Lagrue and Poulin, 2015).

This research investigated the molecular identification and prevalence of endoparasitic helminths as well as the condition factor of sleeper gobies (*Bostrychus africanus*) and mudskippers (*Periophtahlmus papilio*) caught from four locations situated along the New Calabar River and Upper Bonny Estuary, Rivers State, Nigeria.

MATERIALS AND METHODS

Study Locations

Samples of *B. africanus* were fished from Mgbuodohia River (N4°47'24.4083" E6°58'18.2909") axis of New Calabar River (Ezienyi *et al.,* 2022). The river has mangrove vegetation and is an important source of water and a means of transportation for the local inhabitants. *Periophthalmus papilio* samples were harvested at Rumuolumeni (N4°49'4", E6°57'24"), Borokiri (N4° 51'53.88' and E6° 51'54.87') and Ogbakiri (N4° 47' to N4° 38' and E6°54' to E6°39') mudflats. Rumuolumeni is a suburb of Port Harcourt, Rivers State, Nigeria. Several companies as well as Ignatius Ajuru University of Education are situated in Rumuolumeni. Together with Mgbuodohia River, the waterfront at Rumuolumeni from where the fishes were trapped are tributaries of the New Calabar River and are characterized by extensive mangrove swamps, tidal mudflats, influenced by semi-diurnal tidal regime (Ezienyi *et al*., 2022). Borokiri is a neighbourhood of Port Harcourt, Rivers State, Nigeria. It is characterized by a very dense population along the river system which extends into the Upper Bonny Estuary (Nwoha *et al*., 2019). Ogbakiri is situated in Emohua Local Government Area, Rivers State, Nigeria. The Ogbakiri peninsula, as it is often regarded, is located by the New Calabar River which separates it from parts of Port Harcourt and Degema (Michael and Lale, 2021).

Collection of Samples

Basket traps were used to capture the sleeper gobies and mudskippers. Trapped fishes were stored in ice and transported to the Entomology and Parasitology Laboratory, Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Nigeria, for identification and laboratory investigation. Sampling was done in September, 2022.

Identification of Fish Samples

Fish identification was achieved using morphological descriptive keys (Idodo-Umeh, 2003). The total and standard lengths of each fish specimen were taken using a meter rule calibrated in mm and cm, while the wet body weight was determined using a sensitive weighing balance (Camry, model EK5350).

Parasitic Examination of Fish Samples

Dissection and examination of fish samples for parasites was done as described by Ifegwu and Amuzie (2025). Parasites isolated were counted and records were taken. Nematodal and acanthocephalan parasites were fixed in 100% ethanol.

Molecular Identification of Parasites

Nematodal and acanthocephalan parasites were transported in vials to the Regional Centre for Biotechnology and Bioresources Research (RCBBR), University of Port Harcourt, Nigeria, for molecular identification. The process involved DNA extraction using Quick-DNA™ Miniprep Plus Kit (Zymo Research, Irvine, CA, USA) which has 28S rDNA gene as the target gene, following the manufacturer's instructions with modifications as reported by Ifegwu and Amuzie (2025).

Polymerase chain reaction (PCR) amplification targeting the 28S rDNA locus was achieved using One Taq Quick Load 2X Master Mix with Standard Buffer (New England Biolabs, USA). The sequences of primers used were: Forward-LCO1490 (GGTCAACAAATCATAAAGATATTGG) and Reverse-HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA). The 16S PCR cycling protocol was employed for the amplification. Agarose gel electrophoresis was done on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) using SafeView FireRed DNA Dye as stain and visualized using E-Box (Vilber Lourmat, Italy). Instrumentation used was Mastercycler nexus gradient (Eppendorf, Germany).

The sequences generated were edited using Bio-Edit. An alignment search for similar sequences was done using Basic Local Alignment Search Tool (BLAST) on the National Centre for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The generation of evolutionary history and a phylogenetic tree was achieved through the Neighbor-Joining method (Saitou and Nei, 1987) and Jukes-Cantor method (Jukes and Cantor, 1969) as detailed in Ifegwu and Amuzie (2025). These analyses were done with MEGA X (Kumar *et al*., 2018).

Computation of the Fish Condition Factor and Parasite Prevalence and Mean Intensity

The condition factor (CF) of fish specimens was computed according to Ridanovic *et al*. (2015) using the formula: CF = (Wet Weight (g) / L^3(cm)) x 100; where L is the total length.

Prevalence and mean intensity of parasitic infection were calculated after Bush *et al*. (1997). The prevalence was computed as percentage of infected hosts, while mean intensity was computed as number of parasites per infected host.

RESULTS

Molecular Identification of Helminthic Parasites of *Bostrychus africanus* and *Periophthalmus papilio*

Two helminthic parasites (nematodes and acanthocephalans) were isolated from the infected fish hosts. The nematodal parasites were successfully identified using the molecular technique described herein. The acanthocephalans isolated were taken through the process of DNA extraction and polymerase chain reaction, but could not be sequenced due to the very small amount of DNA (<0.2mol) extracted. The closest gene bank match to the nematodal isolate, tagged RCBBR_WA1 (accession number PV400747), was *Baylisascaris transfuga voucher VPG101* with a percentage similarity of 97% (Figure 1). The gene sequence of the isolate is as shown below:

> *Baylisascaris transfuga* isolate RCBBR_WA1

TTGGGTTTTGGTAGGATAGTTGGTACGAGTCTGTCTTTA
ATTATTCGTCTTGAGTTGGCTAAGAGGGTCTTTTATTAG
GTAACGGGCAACTTTATAATGGGTGATTACTTCACATG
CTATTTTAATAATTTTTTTTATGGTTATGCCTACTATGAT
TGGGGTTTTGGAAATTGGATGTTGCCTTTGATGTTGGG
GGCACCAGATATAAGTTTTCCTCGTTTAAATAATTTAAG
TTTCTGATTGTTACCTACGGCTATATTTCTTATTCTTGAC
GCTTGTTTTGTTGATGCGGGTGGGACTAGTTGGACTGT
TATCCCCTTTAAGTACTATGGGTCATCCTGGCAGGAGTG
TAGACTTAGCTTTTTTAGTCTTCATTGTGCTGGTGTAAG
GTCGATTCTTGGTTCTATTAATTTTATGACAACTACCAA
AAATTTGGGAGAAGTTCTATTTCTTTGGAGCATATAAAC
TTTTTGTGTGAACTGTTTTTGTTACTGTTTTTCTTTTACG
GCTTTCTTTGCCTGTTTTGGGGGAGCTATTACTATGCTT
TTAACTGACGTAATTTAATACTCTTTTTTTGATCCTAGAC
AGGGGGGTAACCCTTTATTTACCACATTTATTTTTGATTT
TTTGGCACCCTGAATTTAAA.

Prevalence of Helminthic Parasites in *Bostrychus africanus* and *Periophthalmus papilio*

One hundred and fifty samples of *Bostrychus africanus* were examined at Mgbuodohia. *Baylisascaris transfuga* and the acanthocephalan were isolated from eight infected fish hosts (Table 1). Three hosts were infected with nematodes, while five others were infected with acanthocephalan parasites. Sixty-seven samples of *P. papilio* were obtained at Borikiri. Twelve were infected with *B. transfuga* (with a prevalence of 18%) with twenty-one parasites being isolated thus mean intensity of about two parasites per infected host (Table 1). Forty-one *P. papilio* samples were examined at Rumuolumeni. One host was infected with *Baylisascaris transfuga*, while seven were infected with acanthocephalan parasites. At Ogbakiri, eighteen *P. papilio* samples were examined and two were infected with *B. transfuga* (Table 1).

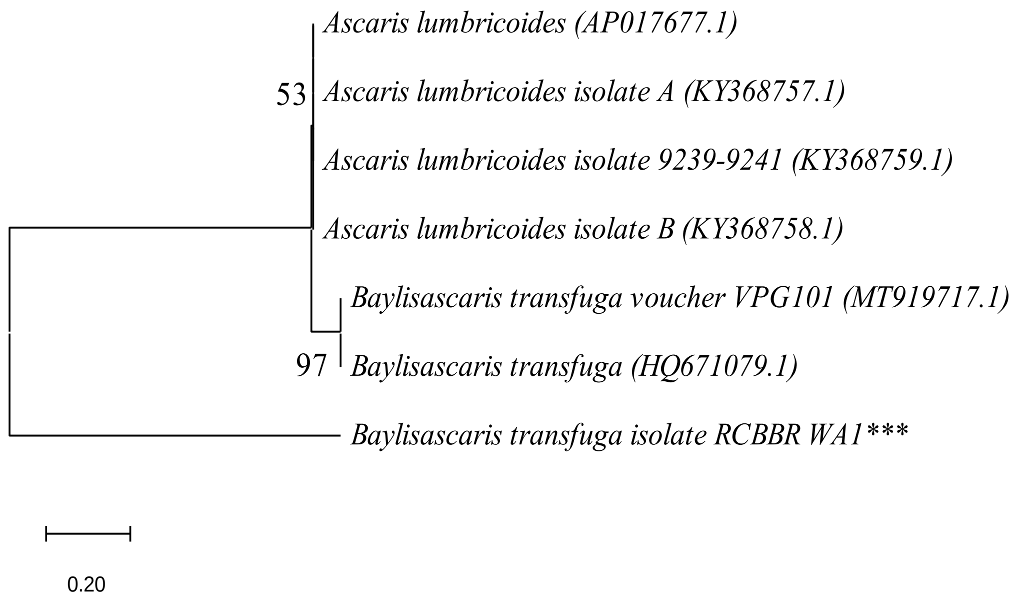

Figure 1. Phylogenetic tree of isolate RCBBR_WA1 based on neighbour -joining method. The values in parenthesis represent the accession numbers; the asterisked organism is the isolate used in the study.

Table 1. Prevalence and mean intensity of parasitic infection in gobies (*Bostrychus africanus*) and mudskippers (*Periophthalmus papilio*) in Rivers State, Nigeria

Location	Fish Host	Parasites	Number of Hosts Examined	No. of Infected Hosts	Number of Parasites Isolated	P(%)	MI
Mgbuodohia River	*Bostrychus africanus*	*Baylisascaris transfuga*	150	3	3	2.0	1.0
		Acanthocephala	150	5	5	3.3	1.0
Borikiri	*Periophthalmus papilio*	*Baylisascaris transfuga*	67	12	21	17.9	1.7
Rumuolumeni	*P. papilio*	*Baylisascaris transfuga*	41	1	1	2.4	1.0
		Acanthocephala	41	7	8	17.1	1.1
Ogbakiri	*P. papilio*	*B. transfuga*	18	2	2	11.1	1.0

Keys: P(%) = Prevalence, MI = Mean Intensity

Condition Factor (CF) of *Bostrychus africanus* and *Periophthalmus papilio*

One hundred and fifty samples of *B. africanus* harvested from Mgbuodohia River had total length ranging between 5.0cm and 13.3cm; the wet body weight ranged between 1.0g and 31.0g; while the condition factor had a mean value of 1.21, with values ranging between 0.60 and 2.31 (Table 2). The total length and wet body weight of the sixty-seven mudskippers obtained from Borikiri ranged from 8.10cm to 14.7cm and 4.0g to 26.0g, respectively. The fishes were generally in good condition having a mean condition factor of 0.94, which ranged from 0.70 to 1.74. The condition factor of the mudskippers obtained from Ogbakiri ranged from 0.93 to 1.31 with a mean value of 1.09. The minimum weight was 5.0g while the maximum value was 22.0g. The total length, on the other hand, had a mean value of 10.83cm, with values ranging from 8.0cm to 13.0cm. The fish samples (n=41) from Rumuolumeni had mean condition factor of 0.9. The total length ranged between 9.9cm and 12.5cm, while the wet body weight ranged from 8.0g to 16.0g (Table 2).

Table 2. Condition factor and morphometric values of *Bostrychus africanus* and *Periophthalmus papilio* from the study locations, Rivers State, Nigeria

Location (Fish Species)	Condition Factor	Minimum	Maximum	Mean ± standard deviation
Mgbuodohia (*B. africanus*)		0.60	2.31	1.21 ± 0.20
	Wet body weight (g)	1.0	31.0	8.23 ± 4.4.6
	Total length (cm)	5.0	13.3	8.55 ± 1.34
Borikiri (*P. papilio*)		0.70	1.74	0.94 ± 0.14
	Wet body weight (g)	4.0	26.0	12.07 ± 5.59
	Total length (cm)	8.10	14.7	10.65 ± 1.61
Ogbakiri (*P. papilio*)		0.93	1.31	1.09 ± 0.09
	Wet body weight (g)	5.0	22.0	14.72 ± 5.72
	Total length (cm)	8.0	13.0	10.83 ± 1.62
Rumuolumeni (*P. papilio*)		0.68	1.32	0.90 ± 0.12
	Wet body weight (g)	8.0	16.0	12.14 ± 2.0
	Total length (cm)	9.9	12.5	11.04 ± 0.65

DISCUSSION

The genus *Baylisascaris* refers to intestinal nematodal parasites that commonly infect wild mammals, including marsupials and rodents (Sheikh *et al*., 2018; Strkolcova *et al*., 2018). Adeniyi *et al*., 2015, reported eggs of *Baylisascaris* sp. from jackals and foxes kept in some University zoological gardens in Nigeria. Their larval stages have been largely reported to cause varying degrees of larval migrans (including visceral, ocular, and neural larval migrans) in a wide variety of hosts, including mice, rabbits, domestic birds, humans, etc (Sheikh *et al*., 2018; Strkolcova *et al.*, 2018). Though *B. transfuga* has not been reported in humans, *B. procyonis* which uses raccoons as definitive hosts has been reported in humans (Bauer, 2013). *Baylisascaris transfuga* specifically infect bears (Strkolcova *et al*., 2018; Jurankova *et al*., 2022). According to a review by Sheikh *et al*. (2018), several vertebrates function as paratenic hosts for *Baylisascaris* spp. However, there is no report of *Baylisascaris transfuga* in fish. Since bears are not common in the vicinity of the river systems from where the gobies were harvested, it is difficult to hypothesize the source of fish infection, except that they were carried in water currents over long distances, especially as *B. transfuga* eggs have been shown to survive up to five years in conducive conditions (Sheikh *et al.,* 2018). In research on soil geohelminths, Imafidor and Owhoeli (2018) isolated *Baylisascaris* sp. from farmlands at Isiodu, in Emohua, Rivers State, Nigeria. It is possible, then that the gobies examined in this research swallowed embryonated eggs from the environment. The eggs could have been washed away from the Port Harcourt Zoo, located beside Azuabie Creek off the Bonny Estuary, where wild, carnivorous animals including lions and tigers, are kept. However, Sapp *et al*. (2017) reported that 'no non-bear definitive hosts are known' for this parasite. It is possible, though, that there could be sub-species of *B. transfuga* which could infect other carnivorous hosts besides bears. While this is yet to be ascertained, we hypothesize that these eleoterids are accidental hosts of the parasite.

Prevalence of *B. transfuga* was 2.0% in *B. africanus* from Mgbuodohia, and ranged from 2.4% to 17.9% in *P. papilio*. The Acanthocephalan occurred at a prevalence of 3.3% in *B. africanus* of Mgbuodohia and 17.1% in *P. papilio* of Rumuolumeni. Ugbomeh *et al.* (2018) reported prevalence of ascaridoid nematodes in *B. africanus* ranging from 21.8% to 91.6% from the New Calabar River. Robert *et al.* (2022) reported *Raphidascaroides africanus* from market-derived samples of *B. africanus*, Rivers State, Nigeria, at a prevalence of 6.7% and 100%, respectively. Thus, lower prevalence of infection was reported in the present research. This could be due to the fact that *B. transfuga* is not usually infective in the host organisms examined. For instance, Adeniyi *et al.* (2015) recorded a prevalence of 100% by *Baylisascaris* sp. in jackals and 50% in foxes, respectively. In Slovakia, Strkolcova *et al*. (2018)

reported *Baylisascaris transfuga* at a prevalence of 52.9% in brown bears. The highest prevalence of *B. transfuga* (17.1%) in *P. papilio* of Borikiri could further suggest that the infective eggs of the parasite might have been sourced from the Port Harcourt Zoo which is located along the same Bonny Estuary as the Borikiri area.

The condition factor of aquatic organisms varies depending on such factors as the availability of food organisms and the general suitability of the water body. In this research, the condition factor of *B. africanus* ranged from 0.6 to 2.31 at one of the study locations. This shows that the ability of individual fish specimens to obtain and harness food organisms can lead to individual variations of the condition factor, within the same environment. *Periophthalmus papilio*, on the other hand, had condition factor values ranging from 0.68 at Rumuolumeni to 1.74 at Borikiri. These fish species have been reported to exhibit a range of condition factors, though being very tolerant of varying environmental conditions. The mean values from all locations generally indicated good health status: 1.21 at Mgbuodohia, 0.94 at Borikiri, 1.10 at Ogbakiri and 0.90 at Rumuolumeni. Moslen and Daka (2017) reported a condition factor of between 1.12 to 1.45 for *P. papilio* from Azuabie Creek located along the Bonny Estuary, Nigeria. Chukwu and Ansa (2019) reported that *B. africanus* from other locations of the New Calabar river, had condition factor ranging from 1.342 to 1.414, with a mean value of 1.353 ± 0.0047. Similarly, in lakes of western Benin, Stanislas *et al*. (2023) reported that the condition factor of *B. africanus* ranged from 0.77 to as high as 5.60. They noted that the ranges of the condition factor indicated that these eleotrids adapt to diverse habitat conditions.

CONCLUSIONS

Molecular diagnostic techniques revealed the infection of *Bostrychus africanus* and *Periophthalmus papilio* by an accidental nematode parasite, *Baylisascaris transfuga* (accession number: PV400747), with a similarity index of 97%. Acanthocephalan parasites were isolated but could not be sequenced due to insufficient amount of DNA (<0.2mol). The prevalence of parasite infection ranged from 2.0 to 17.9% for *B. transfuga*, and between 3.3 and 17.1% for the acanthocephalan. Fish condition factor ranged from 0.6 to 22.31 for *B. africanus* and 0.68 to 1.74 for *P. papilio*.

It is important to examine the ascaridoid parasites of gobies harvested from the river systems in Nigeria to ascertain the distribution of *B. transfuga* in these hosts. The histopathological impact of this parasite in infected fish hosts should also be investigated.

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