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Differential size-biased parasitism between *Polyacanthorhynchus nigerianus* (nomen nudum) and *Polyacanthorhynchus echiyensis* (nomen nudum) (Acanthocephala: Polyacanthocephala)

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Abstract. Seasonal occurrence of acanthocephalans is replete with controversies stemmed from hosts' activities and environmental conditions etc. Ergo, recent mitochondrial DNA resolved two new cystacanths sequences in a parentenic host *Polyacanthorhynchus nigerianus* NG1 KC904074 and *Polyacanthorhynchus echiyensis* NG5 KC9040745 has necessitated the present study between wet and dry seasons. Though the trend of infection followed a similar pattern, NG5 had higher prevalence than NG1 with highest prevalence 5.3% in January. However, the trend changed from March-July when the occurrence of NG1 had relatively much lower monthly prevalence $\leq$ 2.6%. Their differential body size NG5 994.61 $\pm$ 110.07 mm and NG1 237.58 $\pm$ 30.34 mm, respectively, resulted in a trade-off between their body sizes and numbers.

Keywords: Abiotic factors; Body size; Mitochondrial DNA; *Polyacanthohynchus*; Thorny-headed worms; Seasonality; *Synodontis batensoda*.

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Introduction

Polyacanthocephala as a separate class was first proposed by Schmidt and Canaris (1967) and Bullock (1969), and then more specific characters are being used to support the proposal. Out of the four earlier recognized species *Polyacanthorhynchus macrorhynchus* (Diesing, 1856), *Polyacanthorhynchus caballeroi* Diaz-ungria and Rodrigo, 1960, and *Polyacanthorhynchus rhopalorhynchus* (Diesing, 1851) inhabit the digestive tract of South American caimans whereas the fourth species, *Polyacanthorhynchus kenyensis* Schmidt and Canaris, 1967 is only known in the larval stage, infecting freshwater fish in Kenya (Amin, 1987).

Also, recently mitochondrial CO1 gene of two new cystacanths sequences *Polyacanthorhynchus nigerianus* (nomen nudum) and *Polyacanthorhynchus echiyensis* (nomen nudum) infecting some parentenic individuals of *Synodontis batensoda* Rüppell, 1832 in Nigeria were sequenced and compared with the three widely recognized classes of Acanthocephala, namely Archiacanthocephala, Palaeacanthocephala and Eoacanthocephala. It is notable that these two new sequences formed a common clade with *P. caballeroi*, which is the only representative of the new class Polyacanthocephala in the GenBank/NCBI (Echi et al., 2015).

However, there is little information about the seasonal occurrence of *P. macrorhynchus*, *P. caballeroi*, and *P. rhopalorhynchus.* In addition, available information on the seasonal occurrence of *P. kenyesis* among its potential hosts had no perceptible seasonal influence in its occurrence (Amin and Dezfuli, 1995).

The information on the seasonal influence with respect to the occurrence of the two new sequences is inexistent. Therefore, present study was to present the effects of environmental influence of various seasons in their occurrence and size differences in these thorny-headed worms on fish hosts.

Materials and method

152 individual samples of *S. batensoda* were randomly collected on monthly basis (September 2012-August 2013) from caught stocks by fishers at Otuocha, Anambra River (06° 21’ 29” N and 07° 52’ 22” E), Anambra State, Nigeria, following details in Olaosebikan and Raji (1998).

The abiotic factors - surface water temperature, dissolved oxygen and pH were determined using details in Echi and Ezenwaji (2010). The standard length (SL) of each sample was measured to the nearest 0.1 cm with the use of a meter rule and sex determined by dissection and gonad examination. The parasites lengths were obtained using stage and ocular micrometers (Erma Inc., Tokyo, Japan). DNA was extracted from alcohol preserved parasite tissue (~25 mg) by using Qiagen DNeasy Blood and Tissue kit. Universal (VF1d & VR1d) primers were used in the present study for amplifying CO1 gene (Ivanova et al., 2006). PCRs were performed in 25 µL reactions consisting of 2.5 µL each of 10x PCR buffer, $MgCl_2$ (25 mM) and 0.5 µL dNTPs (2 mM), 0.25 µL of each primer (10 µM), 1 unit of AmpliTaq Gold DNA polymerase, 14 µL of dH_2O and 4 µL of template DNA (10-20 ng) in a Thermocycler (ABI 9700). The following thermo cycling conditions were used for amplifications: 95 °C, 5 min, 95 °C, 30 s, 54 °C, 45 s, 72 °C, 45 s, for 40 cycles, 72° C, 7 min, 4 °C ∞.

The PCR products were visualized on 1% agarose gels and the most intense products were purified using Exo Sap IT (USB). Bidirectional sequencing was performed using the PCR primers and products were labeled with BigDye Terminator V. 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) and sequenced in an ABI 3730 capillary sequencer following manufacturer’s instructions. The sequences were aligned using ClustalW and potentially misaligned sequences were excluded. The extent of sequence differences between species was calculated averaging pairwise comparisons of sequence differences across all individuals. Pairwise evolutionary distance was determined by the Kimura-2-Parameter Method, using the software programme Mega 5 (Tamura et al., 2011).

The number of polymorphic sites and nucleotide diversity (pi), nucleotide composition and number of transition and transversion between species were determined. Gaps were considered as missing data on the phylogenetic reconstructions. Neighbour Joining (NJ) tree was constructed to show intraspecific and interspecific relationships among the new sequences and related sequences in GenBank/NCBI.

Results

Comparatively, there is a significant difference between the two parasites. Also, *P. echiyensis* had more population 222 (162 from males and 60 from females hosts) than *P. nigerianus* 114 (85 from males and 29 from females hosts) (Table 1) and relatively occupied the small intestine more whereas *P. nigerianus* often extended up to the large intestine owing to its larger body size. Larger size of *P. nigerianus* was too obstructive to the gastro-intestinal tract of the infected individuals. The intestine appeared entirely blocked and continually contracted in affected individuals owing to massive size of the parasite. Digestive accessories like the liver had no infection of these acanthocephalans.

While identifications are usually made by comparing unknown sequences against the DNA barcodes of known species via distance-based tree construction sequence identification engine, the present sequence showed no homology identifications through BOLD and GenBank/NCBI. The closest Polyacanthocephala *P. caballeroi* (DQ089724) formed a common clade with these parasite sequences. Other classes; Archiacanthocephala, Palaeacanthocephala and Eoacanthocephala indicated a clear close species clade relationships with various members gene relationships in the GenBank/NCBI database. A data set of 36 taxa and bootstrap values (higher than 50%) are presented on equivalent branches of the NJ tree where the relationships among classes of Acanthocephala were supported by high bootstrap values (Figure 1). The new sequences are deposited in GenBank/NCBI with accession numbers KC904074 and KC9040745.

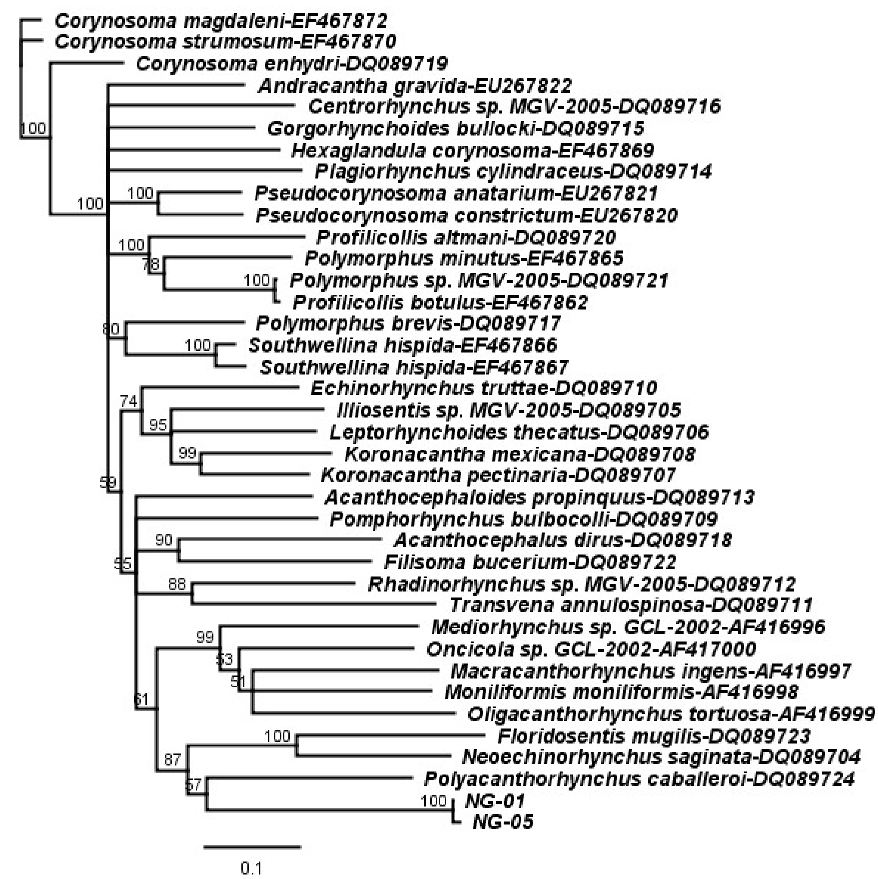

Figure 1. NJ tree showing high bootstrap values on equivalent branches of the tree, with *P. caballeroi* forming a common clade with NG-01 *P. nigerianus* and NG-05 *P. echiyensis*.

Out of 152 individual samples of *S. batensoda* comprising 91 males (59.8%) and female 61 (40.1%), 57 (37.5%) and 54 (35.5%) had co-infection of *P. echiyensis* and *P. nigerianus* respectively. The parasites mean size NG1 237.58 mm $\pm$ 30.34 mm of *P. echiyensis* was lower than NG5 994.61 mm $\pm$ 110.07 mm *P. nigerianus* (Figure 2). Similarly, out of 57 (37.5%) hosts infected, 43 males (75.4%) and 14 females (24.6%) had *P. echiyensis* with total recovered parasites 222. Also, 54 (35.5%), males 40 (74%) and females 14 (25.9%) had *P. nigerianus* and a total 114 parasites recovered. The overall prevalence was high; 37.5% for *P. echiyensis* whereas *P. nigerianus* had 34.8%. The length groups in each case showed very young < 10 cm (2.0%), and much older individual hosts above 21 cm (3.9%) had least level of the infections, respectively. Conversely, 10 cm-13 cm and 14 cm-17 cm length groups had high levels of infections with highest within 10 cm-13 cm range (23.0%).

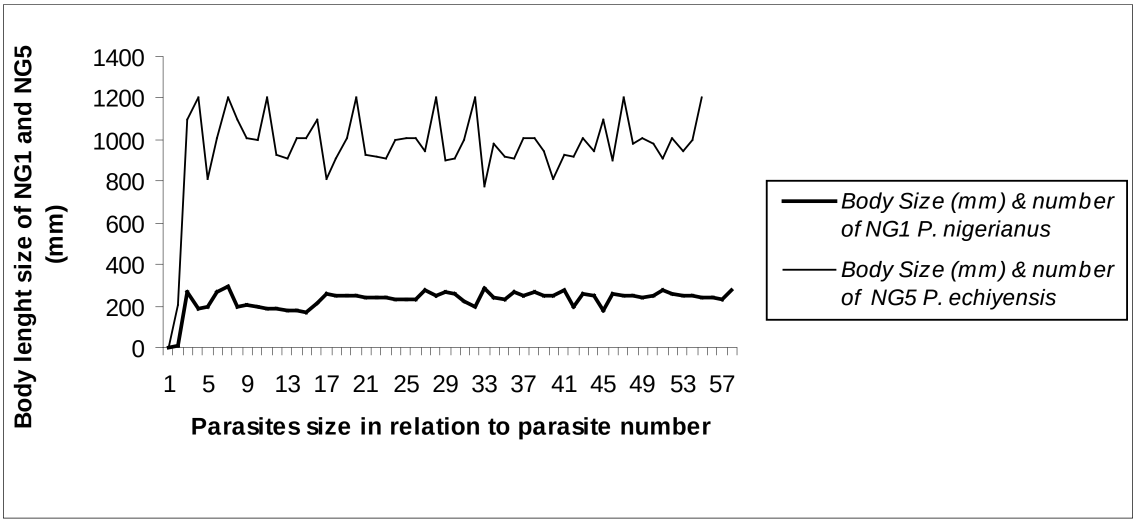

Figure 2. Parasite size and number in the hosts *S. batensoda.*

Although, fewer individual samples were caught by fishers during the dry season months, the occurrence of the parasites was distinctly more than during the rains. Though the trend of infection followed a similar pattern, NG5 had higher prevalence than NG1 with the highest prevalence 5.3% in January. However, this similarity in the pattern of infection failed from March-July when the occurrence of NG1 had relatively much lower monthly prevalence $\leq$ 2.6% than NG5, i.e. 2.6%, 2.6%, 2.0%, 2.0%, 3.3%, respectively (Figure 3).

The abiotic factors, dissolved oxygen (8.0-14.0 mg.L^{-1}) and pH (5.5-7.0) influenced the occurrence of the parasites. For instance, during floodplain fullness with higher oxygen concentration fewer parasites occurred whereas during habitat contraction and lower pH more parasites occurred (Table or Figure showing this data). However, the surface water temperature (20.1–27.5 °C) appeared not to show any perceptible effect. The occurrence of the parasites peaked when the water was slightly acidic pH (6.6-6.7) and dissolved oxygen approximately equal to 10.0 mg.L^{-1} which coincided with dry season months and floodplain contraction whereas lower infection rates were recorded during floodplain fullness when the water pH was 7.4-7.0 was definitely neutral and the oxygen content was approximately greater than 12 mg.L^{-1} (Table.1).

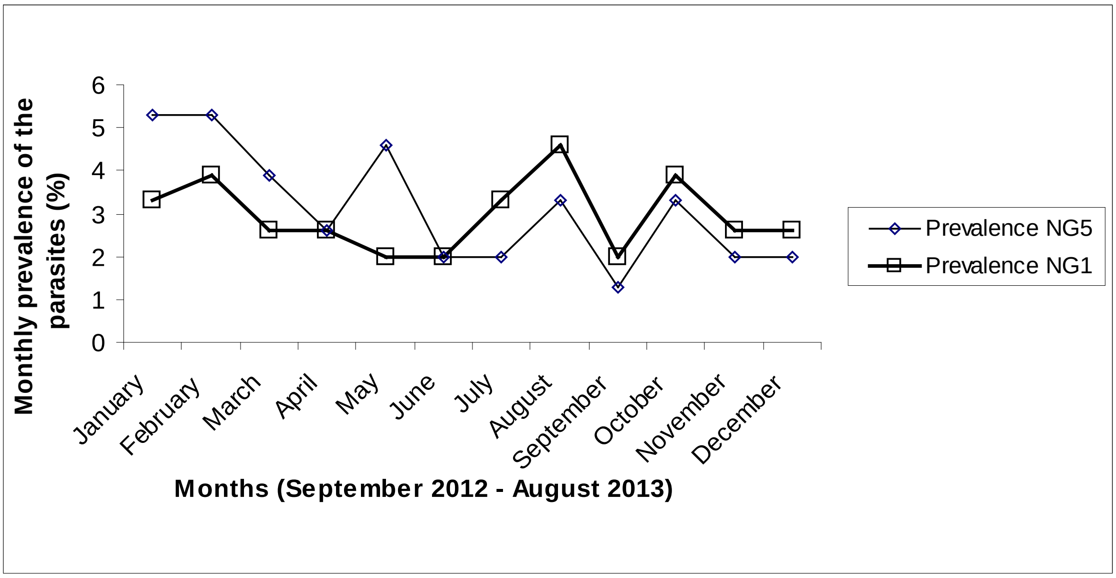

Figure 3. Seasonal occurrence of *P. nigerianus* and *P. echiyensis*.

Table 1. Monthly prevalence and physical environmental factors.

Month	Number of parasites	Prevalence of NG1	DO mgL^{-1}	pH	Month	Number of Parasites	Prevalence of NG5
January	16	3.3	10.0	6.6	January	37	5.3
February	14	3.9	10.0	6.6	February	24	5.3
March	10	2.6	10.0	6.6	March	16	3.9
April	9	2.6	12.0	7.0	April	11	2.6
May	5	2.0	14.0	7.2	May	22	4.6
June	3	2.0	13.7	7.4	June	17	2.0
July	11	3.3	13.2	7.4	July	13	2.0
August	14	4.6	14.0	7.0	August	25	3.3
September	6	2.0	13.0	7.0	September	5	1.3
October	9	3.9	10.0	6.7	October	15	3.3
November	7	2.6	9.8	6.7	November	21	2.0
December	10	2.6	9.0	6.7	December	19	2.0

Discussion

For the reasons that all organisms have access to limited energy cum other life dependent resources, there is a swap between the number and size of offspring; individuals so as to produce larger offspring are constrained to produce fewer, conversely those to produce smaller sized offspring produce larger numbers. Consequently, densities of microscopic organisms are much higher than macroscopic organisms. In effect, microscopic organisms tend to allocate more energy for reproduction to increase survival rate than larger organisms. In nature, the densities of a wide variety of organisms are highly correlated with body size. Therefore, in general, densities of animals and plants populations decrease with increasing size (Molles, 2002).

Temperature appeared not to exert much effect on the occurrence of the parasite species recovered. Seasonal occurrence of Acanthocephala in fish hosts differ among individual species hosts and their ecosystem (Chubb, 1982), for example, differences in hosts' diet habits, abiotic factors and adaptation to these changes by the intermediate and definitive hosts. However, there was no seasonality in the occurrence of *P. kenyensis* (Amin and Dezfuli, 1995), hitherto, the only known *Polyacanthorhynchus* sp in Africa. Nevertheless, seasonality influences fullness and contraction of aquatic ecosystems in Otuocha sampling port, Anambra River Basin.

Also, increased human activities along water banks have caused enormous and indiscriminate influx of refuse which could support infection in a contracted condition as infective larvae of parasites clump in patches of debris that form food materials of their hosts (Echi, 2005). Occurrence of the parasites was dependent on the habitat conditions which encouraged more detritus and it's clumping during dry season months than during rainy season months. The infective larvae in the food materials of the hosts were made available in the decaying organic matter contents of both fauna and flora origins. Infections of these acanthocephalan cystacanths maintained its definite infection cycle at early and late dry season months. Enormous detritus that influxes the river from heavy human activities such as rice mills, organic food materials etc along the banks collect and connect these infective larvae along with their hosts. Infection perhaps occurs at clumped portions where hosts' feeding activities were high.

Conclusion

Occurrence of the new sequences was season dependent based on the habitat conditions which encouraged more detritus and it's clumping during dry season months than during rainy season months. The differences in size of the new sequences produced differential parasitic effects on the hosts.

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Conflict of interest statement

Authors declare that they have no conflict of interests.

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