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Mitochondrial DNA resolution of two new sequences *Polyacanthorhynchus echiyensis* n. sp. and *Polyacanthorhynchus nigerianus* n. sp. (Polyacanthocephala: Acanthocephala) in a parentenic host from a tropical River

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Abstract

Acanthocephalan fauna are distributed globally as visceral parasites of vertebrates and arthropods. Morphological description of four known species of the newly described class Polyacanthocephala has been replete with controversies. However, Mitochondrial COI gene of two new cystacanths sequences; *Polyacanthorhynchus nigerianus* n.sp. (NG_1 KC904074) and *Polyacanthorhynchus echiyensis* n.sp. (NG_5 KC904075) infecting some parentenic individuals of *Synodontis batensoda* in Nigeria were sequenced. The resulting sequences were aligned with 36 other sequences of acanthocephalans representing three widely recognized classes; Archiacanthocephala, Palaeacanthocephala and Eoacanthocephala. The only representative of the new class Polyacanthocephala in the GenBank/NCBI *Polyacanthorhynchus caballeroi* (DQ089724) formed a common clade with these two new sequences. This study thus supports existence of the new class Polyacanthocephala as an independent class within Acanthocephala.

Keywords: COI gene, gene data base, parasites, Synodontis batensoda

Introduction

Acanthocephalans (thorny-headed worms) are tripoblastic pseudocoelomates; a phylum of visceral parasites of invertebrates and vertebrates. Possession of integument, lining the entire body surface by this group of invertebrates is a common attribute they share with other helminthes parasitic groups. Many studies on the systematics of Acanthocephala over the years formed the basis for current systematics across the recognized classes: Archiacanthocephala, Eoacanthocephala, Palaeacanthocephala and Polyacanthocephala. Reasonably, up-to-date taxonomy of the phylum has 4 classes, 10 orders, 22 families, 147 genera, and 1194 species; fossil taxa include 1 family, 3 genera, and 5 species (Monks and Richardson 2011, Van Cleave 1936). From the foregoing, evolutionary and phylogenetic origins of acanthocephalans were closely related to rotifers (Phylum Rotifera), free living organisms occurring in aquatic environments (Garey *et al*. 1996). Although, the morphological features of members were not too discrete to clarify these relationships, the application of molecular techniques in understanding phylogeny to unknot the concern, which has added new means to reveal relationships of the taxa using their own genetic data. Molecular phylogenies of Acanthocephala using different genetic sequences have all, so far provided support for Rotifera / Acanthocephala relationships (Gracia-Varela *et al*. 2002, Gracia-Varela and Nadler 2005, Verweyen *et al*. 2011). Nevertheless, these molecular analyses have been applied and consistently revealed Polyacanthocephala a new class. For instance, molecular probes have proved the only representative of genus *Polyacanthorhynchus* in the GenBank/NCBI, *P. caballeroi* and representatives of other classes to support the existence of the new class (Near *et al*. 1998, Gracia-Varela *et al*. 2002).

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Aside morphometric identification, delineation and characterization of species, a wide variety of protein and DNA based methods have been evaluated for identification of fish species and parasites using molecular markers. (Waters and Cambray 1997). Its successful application for both species identification and discovery has been demonstrated in many studies, involving many taxonomic groups, for example fish (Ward *et al*. 2009), fish parasites (Locke *et al*. 2010), nematodes (Elsasser *et al*. 2009). Molecular markers using 5' end of the mitochondrial cytochrome c oxidase subunit I (COI) gene has been used as a global bio-identification sequence for animal groups. A DNA barcode is a standardized portion of the gene used to identify species. The utilization of such short DNA sequences for species identification for quick and reliable species-level identifications pertains to all forms of life. This technology known as DNA barcoding relies on the observation that a 'barcode' sequence divergence within species is typically much lower than divergence exhibited between species. DNA barcoding has been effectively applied for many organisms' unambiguous recognition as a standardized genetic marker in many studies and has gained global support as a rapid, accurate, cost effective and broadly applicable tool for species identification (Hebert *et al*. 2003a). The aim of this study was to determine mitochondrial DNA COI gene of two new cystacanth sequences of acanthocephalan as an effective tool to support existence of the new class Polyacanthocephala.

Materials and methods

A total of 45 individuals of *Synodontis batensoda* were collected in River Niger at Otuocha (06^0 21' N and 7^0 52' E) sampling port during two different sampling periods in dry season, Jan – March 2012 and Oct – Dec 2012. The periods coincided with contraction of habitat in a seasonally flooding aquatic ecosystem (Echi and Ezenwaji 2010). The helminthes consisting of 9 *Polyacanthorhynchus echiyensis* n.sp. (NG_5) and 13 *Polyacanthorhynchus nigerianus* n.sp. (NG_1) were preserved in analytic grade ethanol prior to molecular analysis.

DNA Extraction and PCR

DNA was extracted from alcohol preserved parasite tissue (~25 mg) by using Qiagen DNeasy Blood and Tissue kit. Universal primers (forward and reverse); LCO 5' GGTCAACAAATCATAAAG 3', HCO 5' TAAACTTCAGGGTGACCA 3', VRd1 5' TAGACTTCTGGGTGGCCR 3' and VFd1 5' TCTCAACCAACCACAAR 3'were used for amplifying CO1 gene.

PCRs were performed In 25 µl reactions consisting of 2.5 µl each of 10x PCR buffer, MgCl2 (25 mM) and 0.5 µl dNTPs (2 mM), 0.25 µl of each primer (10 µM), 1 µl of Taq DNA polymerase, 14 µl of dH2O and 4 µl of template DNA (10-20 ng) in a Thermocycler (ABI 9700). The following thermo cycling conditions were used for amplifications: initial denaturation at 95^{o}C for 5 minutes, followed by 40 cycles of 95 ^{o}C for 30 seconds, 52 ^{o}C for 40 seconds, 72 ^{o}C for 1 minute, and a final extension at 72 ^{o}C for 7 minutes. 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 Big Dye Terminator V.3.1 Cycle sequencing Kit and sequenced in an ABI 3730 capillary sequencer following manufacturer's instructions. Pair wise evolutionary distance was determined by the Kimura-2-parameter model using the software programme Mega 5. Neighbor Joining (NJ) tree was constructed to provide graphic representation of the species divergence (Tamura *et al.* 2011). The new sequences have been deposited in GenBank/NCBI.

Results and Discussion

Only 13 hosts examined were infected with the two parasites. This represents a prevalence of (7) 0.32 %, mean intensity of 1.4 and (6) 0.27 %, mean intensity of 2.8 for *Polyacanthorhynchus echiyensis* n.sp. and *Polyacanthorhynchus nigerianus* n.sp. respectively DNA from the 2 samples visualized with 2.0% agarose gel electrophoresis and ethidium bromide staining were successfully amplified using a standard protocol (Figure 1). In addition, the samples were successfully sequenced using the forward and reverse primers to obtain robust forward and reverse sequences of approximately 700 bp. VRd1/VFd1 amplified both parasites during PCR while LCO/HCO only worked on *Polyacanthorhynchus nigerianus* n.sp. These two sequences are so related that they differed by one

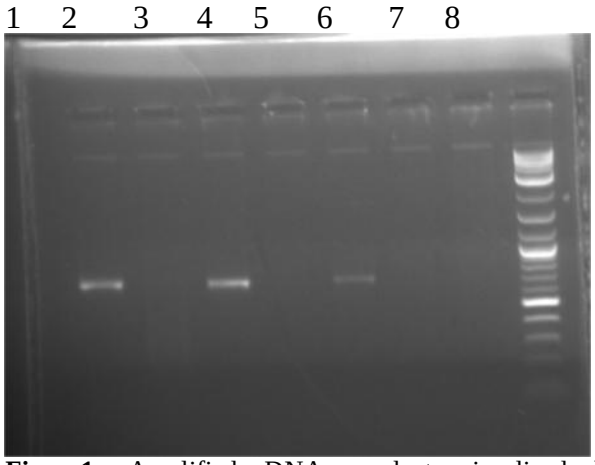

Figure1. Amplified DNA product visualized by ethidium bromide staining. Lanes 1 & 2 represent *Polyacanthorhynchus echiyensis* n.sp using primers VRd1/VFd1 and LCO/HCO respectively. Lanes 3 & 5 represents *Polyacanthorhynchus nigerianus* n.sp primers LCO/HCO and VRd1/VFd1 respectively. Lane 8 = 700-bp ladder

gene only. Although, 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 – *Polyacanthorhynchus caballeroi* (DQ089724) formed a common clade with these parasites. 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 2). Molecular basis of characterizing organisms remains valid. All the same, currently, there is emerging consensus that a combination of both long-established morphology and molecular techniques be paired with newer molecular methods to generate an even more powerful data set to better understand the relationships of taxa (Perkins *et. al*. 2011). However, the increasing use of molecular methods in systematics, through easy integration of DNA sequence data into phylogeny analysis programs, means that molecular method has quickly overtaken traditional morphological taxonomy as the standard method for generating phylogenies and understanding the systematics of different taxa. Accordingly, a DNA sequence tag must be an essential part of any new species descriptions in the future. (Marek *et. al*. 2002). Meanwhile, although, the genera *Tilapia* and *Oreochromis*, well known parentenic hosts to *Polyacanthorhynchus kenyesis* in Kenya (Amin 1987, Amin and Dezfuli 1995), are readily available in River Niger, infection by the two species appeared specific to *Synodontis batensoda* to the best of our knowledge. Also, whereas the South American species utilize caimans as their definitive hosts, no caimans exist in River Niger and other water bodies in Nigeria to best of our knowledge.

NG-01 *Polyacanthorhynchus nigerianus* n.sp.

TGTCATATATTTCATGTTAAGGGTCTGGTGC
GGTTTGGTCGGGTTTAGGTTGAGTGGGCTT
ATCCGGTTGGAGTTGGGTGCAAGTGGTTGT
TGACTAAACAGAGAAAGGTTGTACAACAGT
ATTGTTACTATACACGCTGTTATTATAGTCT
TCTTTTTGGTTATACCAAGTTTTATAGGGGG
TTTTGCTAATTGATTAATTCCGGTGATGCTA
TCAAAGGGTGATATGATTTACCCACGATTG
AACAATGTAAGATTTTTGTTAGTGCCTACTT
CTTTAATTTTATTTATTTTTTCGGCTTTTCTT
GGGGGGCCTCAAGCAGGTTGGACTTTTTAT
CCGCCTTTGAGGGCAAAAGAATTCATAGGG
GGCTTAGAGATAGACAGGAGAATTTTGAGT
CTTCACTTATTGGGTGTTTCGTCAATTTTAG
GTGCTATTAATATTTTAGCTACTGTATTTTC
AGCAGTTGGAGAAAAAGTGTTAGGGTTTGA
ACAGGTGCCTTTGTTTATCTGAGCTTTGGTG
ATTACTTCGTTAATGGTGCTTTTAGTCATCC
CGGTGCTTGCTGCGGCTTTGTTAATGTTACT
ATTAGATCGTAATTTTAATTCCAGATTTTTT
GACCCAGCGGGTGGTGGGAGTTTAATCTTG
TATCAGCATTTATTT

NG-05 *Polyacanthorhynchus echiyensis* n.sp.

GGTGTCATATATTTCATGTTAAGGGTCTGGT
GCGGTTTGGTCGGGTTTAGGTTGAGTGGGC
TTATCCGGTTGGAGTTGGGTGCAAGTGGTT
GTTGACTAAACAGAGAAAGGTTGTACAACA
GTATTGTTACTATACACGCTGTTATTATAGT
CTTCTTTTTGGTTATACCAAGTTTTATAGGG
GGTTTTGCTAATTGATTAATTCCGGTGATGC
TATCAAAGGGTGATATGATTTACCCACGAT

TGAACAATGTAAGATTTTTGTTAGTGCCTAC
TTCTTTAATTTTATTTATTTTTTCGGCTTTTC
TTGGGGGGCCTCAAGCAGGTTGGACTTTTT
ATCCGCCTTTGAGGGCAAAAGAATTCATAG
GGGGCTTAGAGATAGACAGGAGAATTTTGA
GTCTTCACTTATTGGGTGTTTCGTCAATTTT
AGGTGCTATTAATATTTTAGCTACTGTATTT
TCAGCAGTTGGAGAAAAAGTGTTAGGGTTT
GAACAGGTGCCTTTGTTTATCTGAGCTTTGG
TGATTACTTCGTTAATGGTGCTTTTAGTCAT
TCCGGTGCTTGCTGCGGCTTTGTTAATGTTA
CTATTAGATCGTAATTTTAATTCCAGATTTT
TTGACCCAGCGGGTGGTGG

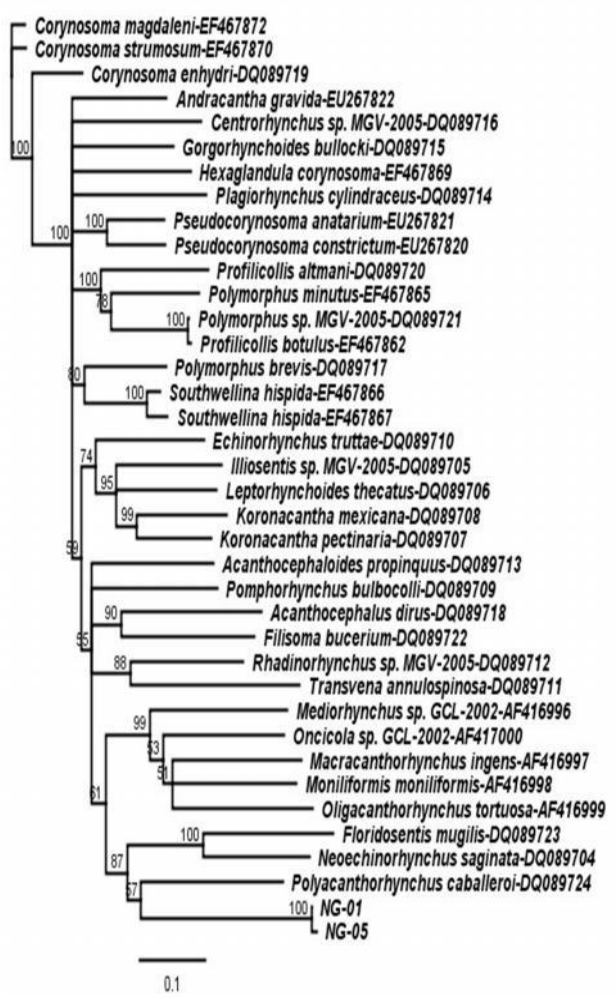

Figure 2: NJ tree showing high bootstrap values on equivalent branches of the tree, with *P. caballeroi* forming a common clade with NG-01 *P. nigerianus* n.sp and **NG-05** *P. echiyensis* n.sp

In conclusion, our results though support the existence of the new class Polyacanthocephala, suggest a follow up study on the structural similarities between the new findings and established species of the new class Polyacanthocephala.

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References

Amin, O.M. 1987. Key to the families and subfamilies of Acanthocephala with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). ***J of Parasitol,*** 73: 1216 - 1219.

Amin, O. M and Dezfuli,S.B. 1995. Taxonomic notes on *Polyacanthocephala kenyensis* (Acanthocephala: Polyacanthorhynchidae) from Lake Naivasha, Kenya. ***J of Parasitol,*** 81: 76 - 79.

Echi, P. C and Ezenwaji, H. M. G. 2010. The parasites fauna of characids' (Osteichthyes: Characidae) Anambra River, Nigeria. ***Afr J of Ecol,*** 48: 1 - 4.

Elsasser S.C., Floyd, R., Hebert, P.D and Schulte-Hostedde, A. I. 2009. Species identification of North American guinea worms (*Nematoda*: *Dracunculus*) with DNA barcoding. ***Mol Ecol Res,*** 9:707-712.

Gracia-Varela, M., Cummings, M. P., Pe´rez-Ponce, D. L. G., Gardner, S. L and Laclettea, P. J. 2002. Phylogenetic analysis based on 18S ribosomal RNA gene sequences supports the existence of class polyacanthocephala (acanthocephala). ***Mol Phylo and Evol,*** 23: 288 - 292.

Gracia-Varela, M and Nadler, S. 2005. Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU LSU rDNA gene sequences. ***J of Parasitol,*** 91:1401-1409.

Garey, J., Near, T., Nonnemacher, M and Nadler, S. 1996. Molecular evidence of Acanthocephala as a subtaxon of Rotifera. ***J of Mol Evol,*** 43: 287-292.

Hebert, P.D.N., Cywinska, A., Ball, S.L and Dewaard, J.R. 2003a. Biological identifications through DNA barcodes. ***Proceedings of the Royal Society B: Biological Sciences,*** 270: 313-321.

Locke, S. A, Mclaughlin, J. D and Macroglose, D. J. 2010. DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among *Diplostomoidea* (*Platyhelminthes*: *Digenea*) parasitizing freshwater fish in the St Lawrence River canal. ***Mol Ecol Res,*** 19: 2813-2827.

Marek, S., Zie, T and Jaakko, L. 2002. Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (monogenea, gyrodactylidae). ***Evol,*** 56: 2445–2458.

Monks, S and Richardson, D. J. 2011. Phylum Acanthocephala Kohlreuther, 1771 *In:* Zhang, Z.-Q. (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. ***Zootax,*** 3148: 234–237.

Near, J. T., Garey, J. R and Nadler, S. A. 1998. Phylogenetic relationships of the acanthocephala inferred from 18S ribosomal DNA sequences. ***Mol Phyl and Evol,*** 10: 287–298.

Perkins, S., Martinsen, E and Falk, B. 2011. Do molecules matter more than morphology? Promises and Pitfalls in parasites. ***Parasitol,*** 138: 1664-1674.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M and Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. ***Mol Biol and Evol,*** 28: 2731-2739.

Van Cleave, H. 1936. Recognition of a new order in the Acanthocephala. ***J of Parasitol,*** 22: 202-206.

Verweyen, L., Klimpel, S and Palm, H. 2011. Molecular phylogeny of the Acanthocphala (Classs Palaeacanthocephala) with paraphyletic assemblage of the orders Polymorphida and Echinorhynchida. ***PLo,*** 6: e28285.

Ward, R. D., Hanner, R and Hebert, P. D. N. 2009. The campaign to DNA barcode all fishes, FISH-BOL. ***J of Fish Biol,*** 74: 329-356.

Waters, J. M and Cambray, J. A. 1997. Intraspecific phylogeography of the *Cape galaxias* from South Africa: evidence from mitochondrial DNA sequences. ***J of Fish Biol,*** 50: 1329-1338.