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Retraction

The editorial board of Brazilian Journal of Veterinary Parasitology communicates the formal publication of Retraction for extracting the article:

Abdel-Gaber R, Al-Quraishy S, Abdel-Gaber R, Dkhil MAM. *Neoechinorhynchus macrospinosus* (Acanthocephala: Neoechinorhynchidae) in Rabbit fish *Siganus rivulatus* (Siganidae): morphology and phylogeny. *Braz J Vet Parasitol* 2020; 29(3): e005120. https://doi.org/10.1590/s1984-29612020034.

The article is being retracted because the authors cited in the text (Amin and Nahhas, 1994) do not described "*Neoechinorhynchus*" *macrospinosus* but *Neorhadinorhynchus macrospinosus*. Specimens in figures 1 & 2, are certainly not *Neoechinorhynchus* and your phylogenetic tree (figure 3) is totally in error and corrupts the data base of GenBank.

Profa. Dra. Rosangela Zacarias Machado
Editor-chief

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Original Article

Neoechinorhynchus macrospinosus (Acanthocephala: Neoechinorhynchidae) in Rabbit fish *Siganus rivulatus* (Siganidae): morphology and phylogeny

Neoechinorhynchus macrospinosus (Acanthocephala: Neoechinorhynchidae) em peixes-coelho *Siganus rivulatus* (Siganidae): morfologia e filogenia

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Abstract

Siganids are the most important marine fish distributed along the African coast. Therefore, the current study aimed to investigate parasite fauna infects one of the most important mariculture fish species in the Red Sea, the Rabbit fish *Siganus rivulatus*. One acanthocephalan species has been isolated from the posterior region of fish intestine, belonging to the Neoechinorhynchidae family, and named as *Neoechinorhynchus macrospinosus* Amin & Nahhas, 1994 based on its morphological and morphometric features. In order to determine the accurate taxonomic position of this acanthocephalan species, molecular phylogenetic analysis was carried out based on the partial sequences of 18S rDNA gene region. The obtained data revealed that this species was associated with a close identity >71% for other species belonging to the Neoechinorhynchidae family. In addition, the recovered species deeply embedded in the *Neoechinorhynchus* genus, closely related to the previously described *Neoechinorhynchus* sp., *N. mexicoensis*, and *N. golvani* with identity percent of 95.14, 93.59, 93.59%, respectively. Therefore, the present study provide a better understanding about the taxonomic status of *N. macrospinosus* based on 18S rDNA that can be useful for achieving a proper assessment of biodiversity.

Keywords: Marine fish, molecular analysis, Neoechinorhynchidae, 18S rDNA.

Resumo

Os siganídeos são os peixes marinhos mais importantes distribuídos ao longo da costa africana. Portanto, o presente estudo teve como objetivo investigar a fauna de parasitas infectando uma das espécies mais importantes de peixes para maricultura no Mar Vermelho, o peixe-coelho *Siganus rivulatus*. Uma espécie de acantocéfalo foi isolada da região posterior do intestino de peixes pertencentes à família Neoechinorhynchidae, e denominadas *Neoechinorhynchus macrospinosus,* Amin & Nahhas, 1994, com base em suas características morfológicas e morfométricas. A fim de determinar a posição taxonômica precisa dessa espécie de acantocéfalo, a análise filogenética molecular foi realizada com base nas sequências parciais da região do gene 18S rDNA. Foi revelado que essa espécie estava associada a uma identidade próxima de até 71% para outras espécies pertencentes à família Neoechinorhynchidae, profundamente enraizada no gênero *Neoechinorhynchus* e intimamente relacionada a *Neoechinorhynchus* sp., *N. mexicoensis* e *N. golvani* descrito anteriormente com percentual de identidade de 95,14, 93,59, 93,59%, respectivamente. Portanto, o presente estudo fornece uma melhor compreensão sobre o status taxonômico de *N. macrospinosus* com base no 18S rDNA que pode ser útil para obter uma avaliação adequada da biodiversidade.

Palavras-chave: Peixe marinho, análise molecular, Neoechinorhynchidae, 18S rDNA.

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Introduction

A variety of wild freshwater and marine fish are subjected to infection by different species of parasites (Abdel-Ghaffar et al., 2014). Acanthocephala Koelreuter, 1771 is a group of permanent parasites of most vertebrates, including humans; having over 1.150 species, belonging to 125 genera and 19 families are known (Kennedy, 2006). Acanthocephalans have received little attention in the fields of human and veterinary medicine (Shih et al., 2010). Cases of serious illness or high mortality induced by acanthocephalan infections in fish were seldom reported due to lower infection intensity compared with other helminth parasites (Shih et al., 2010). The name of the phylum as thorny- or spiny-headed worms referred to the presence of an evertable proboscis, armed with hooks, which they use to pierce and hold the gut wall of their gnathostome definitive host (Mehlhorn et al., 1988a, b; Taraschewski, 1989, 1990). They typically have complex life cycles, including arthropods as the intermediate hosts and fish, amphibians, birds, and mammals as the definitive hosts (Taraschewski, 2000). Adult acanthocephalans infect fish as definitive hosts belong to two classes: Eoacanthocephala Van Cleave, 1936 and Palaeacanthocephala Meyer, 1931. The former includes two orders: Cyracanthocephala Van Cleave, 1936 and Neoacanthocephala Van Cleave, 1936; and the latter consists of two orders: Echinorhynchidea Cobbold, 1876 and Polymorphida Petrochenko, 1956. Key exclusively based on the morphological features for classes, orders, families, and subfamilies of Acanthocephala was described previously by Amin (1987). Different species of acanthocephalans are known to occur in Persian Gulf and Red Sea as *Sclerocollum rubrimaris* Schmidt & Paperna, 1978, *Neoechinorhynchus qatarensis* Amin, Saoud & Alkuwari, 2002, *Neoechinorhynchus dimorphospinus* Amin & Sey, 1996, and *Serrasentis sagittifer* Linton, 1932 (Amin et al., 1984, 2002). Garey et al. (1996) mentioned that the identification of acanthocephalan parasites based on morphological characters is not fully reliable which may lead to their misclassification. For accurate and effective identification for different parasites, various studies have recently demonstrated that the internal transcribed spacers (ITS) and nuclear ribosomal DNA (rDNA) provide distinguishing genetic markers (Zhu et al., 1998; García-Varela et al., 2000, 2005; Shih, 2004).

The rabbit fish *Siganus rivulatus* Forsskål & Niebuhr, 1775 (Teleostei: Siganidae) is one of the most common marine fish in the Red Sea with well-known intestinal helminths (Nagaty, 1956; Schmidt & Paperna, 1978; Fusco & Overstreet, 1979; Diamant, 1989; Dzikowski et al., 2003; Hassanine, 2006; Hassanine & Al-Jahdali, 2007). The purpose of this study was to provide more information of an acanthocephalan parasite of the rabbit fish using light and scanning electron microscopy to establish its morphology and combine this data with molecular analysis to clarify its taxonomic position.

Material and Methods

Fish collection and parasitological examination

A total of forty specimens of the Rabbit fish *Siganus rivulatus* (Family Siganidae) were collected (n=20, in winter; n=20 in summer) from the Gulf of Suez coasts, Red Sea, Egypt. Fish were transported to the Laboratory and dissected within 48 h of collection for registering of parasitic infections using a stereo-dissecting microscope (Nikon SMZ18, NIS ELEMENTS software). All procedures that contribute to this work comply with the ethical standards authorized by ethical committee (CUFS-S-Para-38-14) at Cairo University, Cairo, Egypt. Parasitological terms (prevalence and intensity) were calculated according to Bush et al. (1997). Acanthocephala parasites were left over-night in refrigerator to allow protrusion of proboscis to come out then fixed using hot glycerol-alcohol (5% glycerin in 70% ethyl alcohol), cleared in clove oil, stained using Semichon's acetocarmine (Pritchard & Kruse, 1982), then examined and photographed for internal details using Leica DM 2500 microscope (NIS ELEMENTS software, ver. 3.8, Leica Microsystems, Morrisville, USA). In preparation for scanning electron microscopy (SEM), some of the collected parasites were kept in 4% buffered glutaraldehyde, then washed thoroughly with the same buffer and post-fixed for 4 h with aqueous osmium tetroxide, dehydrated through acetone, and dried in a critical point drying apparatus (BOMER-900, Leica Microsystems, Morrisville, USA) using liquid CO_2, mounted on an aluminum stub, coated with gold palladium in a JEOL, JEC-3000FC, and then subjected with JSM-6060LV microscope (JEOL, Tokyo, Japan) using 10kV. Measurements were performed with an Olympus ocular micrometer (Olympus Corporation, Tokyo, Japan) and given as the range in millimeters followed by means ± SD in parentheses.

Phylogenetic analysis

Ethanol-preserved samples were used to extract the gDNA by using Qiagen DNeasy™ tissue kit (Qiagen, Inc., Valencia, California) according to instructor protocol. The 18S rDNA was targeted and amplified by polymerase chain reaction (PCR) using 18FP-1 (5'-AGA TTA AGC CAT GCA TGC GTA AG-3') and 18RP-1 (5'-TGA TCC TTC TGC AGG TTC ACC TAC-3') as described by Verweyen et al. (2011). PCR reactions (25 µL) were performed in 2 mM $MgCl_2$, 0.2 mM each of dNTPs, 2.5 µL 10× rTaq DNA buffer, 2.5 µM of each primer, 1.25 U rTaq polymerase buffer, 1 µL of DNA sample and completed to 25 µl with dist. H_2O in a thermocycler (BioRad) under the following conditions: 94 °C for 5 min (initial denaturation), then 35 cycles of 1 min at 94 °C (denaturation), 1 min at 50 °C (annealing), and 1 min at 72 °C (extension) and finally post-PCR extension was carried out for 7 min at 72 °C. Each amplicons was visualized by 1% TBE agarose gel stained with 1% ethidium bromide through UV trans-illuminator. PCR products with predicted size were purified using Pure link™ Quick Gel Extraction Kit (Invitrogen) following manufacturer's instructions. Amplicons were bidirectional sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) using 18FP-2 (5'-GTA AAA CGA CGG CCA G-3') and 18RP-2 (5'-CAG GAA ACA GCT ATG AC-3') as described by Verweyen et al. (2011). Each sequence was manually edited for accuracy by using ABI Editview (Perkin-Elmer). A BLAST search was conducted on the NCBI database (https://www.ncbi.nlm.nih.gov/genbank) to classify associated acanthocephalan sequences and especially the *Neoechinorhynchus* species. The obtained sequences were aligned with others obtained from GenBank™ using CLUSTAL-X (Thompson et al., 1997). The phylogenetic tree was constructed using MEGA 7.0 (Kumar et al., 2016) by using Maximum Likelihood (ML) method based on the Kimura 2-parameter model (Kimura, 1980) and Close-Neighbor-Interchange algorithm (Nei & Kumar, 2000) with branch support was given using 1000 bootstrap replicates. *Macrochaetus collinsi* (gb| DQ297705.1) was employed as the out-group.

Results

Twelve (15%) out of forty specimens of the Rabbit fish *Siganus rivulatus* were found to be naturally infected with an acanthocephalan parasite *Neoechinorhynchus macrospinosus*. The infection was recorded in the posterior region of the fish intestine. The intensity of infection was recorded to be 3-7 parasite specimens per infected fish. The infection was increased during winter to be 50% (10/20; with an intensity of 4-6) and fall to 10% (2/20; with an intensity of 5-7) during summer season with no observations on other seasons.

Microscopic examination (Figures 1, 2)

Worm body elongated and cylindrical with truncate ends. It covered with a thin tegument followed by a much thicker hypodermis. Proboscis wider than long and globular with 9-11 rows of 8-16 hooks on each row that similar in size and shape and gradually decreased in length posteriorly. Proboscis receptacle double walled. Trunk very long, cylindrical, and widest in the anterior region and tapering ends without trunk spines.

Body of male worm (based on 10 mature specimens)

Body 3.7-4.4 (4.1±0.1) long and 0.28-0.34 (0.30±0.2) wide. Proboscis 0.21-0.25 (0.22±0.1) long and 0.15-0.19 (0.17±0.1) wide. Trunk (include lemniscus) 2.2-2.6 (2.4±0.2) long and 3.1-3.6 (3.4±0.2) wide. Male reproductive system occupied up to two thirds of trunk. Testes spherical to ovoid in shape and tandem in position situated one behind the other in posterior half of trunk with a copulatory bursa located at posterior end of the body.

Body of female worm (based on 10 mature specimens)

Body 4.5-5.1 (4.8±0.1) long and 0.29-0.35 (0.31±0.2) wide. Proboscis 0.23-0.28 (0.25±0.1) long and 0.17-0.21 (0.19±0.1) wide. Trunk 0.34-0.39 (0.35±0.2) long and 0.38-0.41 (0.39±0.2) wide. Posterior end of female characteristic in shape, and tapered from about level of uterus.

Molecular analysis

The amplified and sequenced 18S rDNA gene regions and GC content for the recovered *Neoechinorhynchus* species were 350 (39%), 490 (42.7%), 490 (42.7%) bp and deposited in GenBank™ with accession numbers KP300036.1, MT321075.1, MT321076.1, respectively. Pairwise distance for these isolates was 0.09 with a

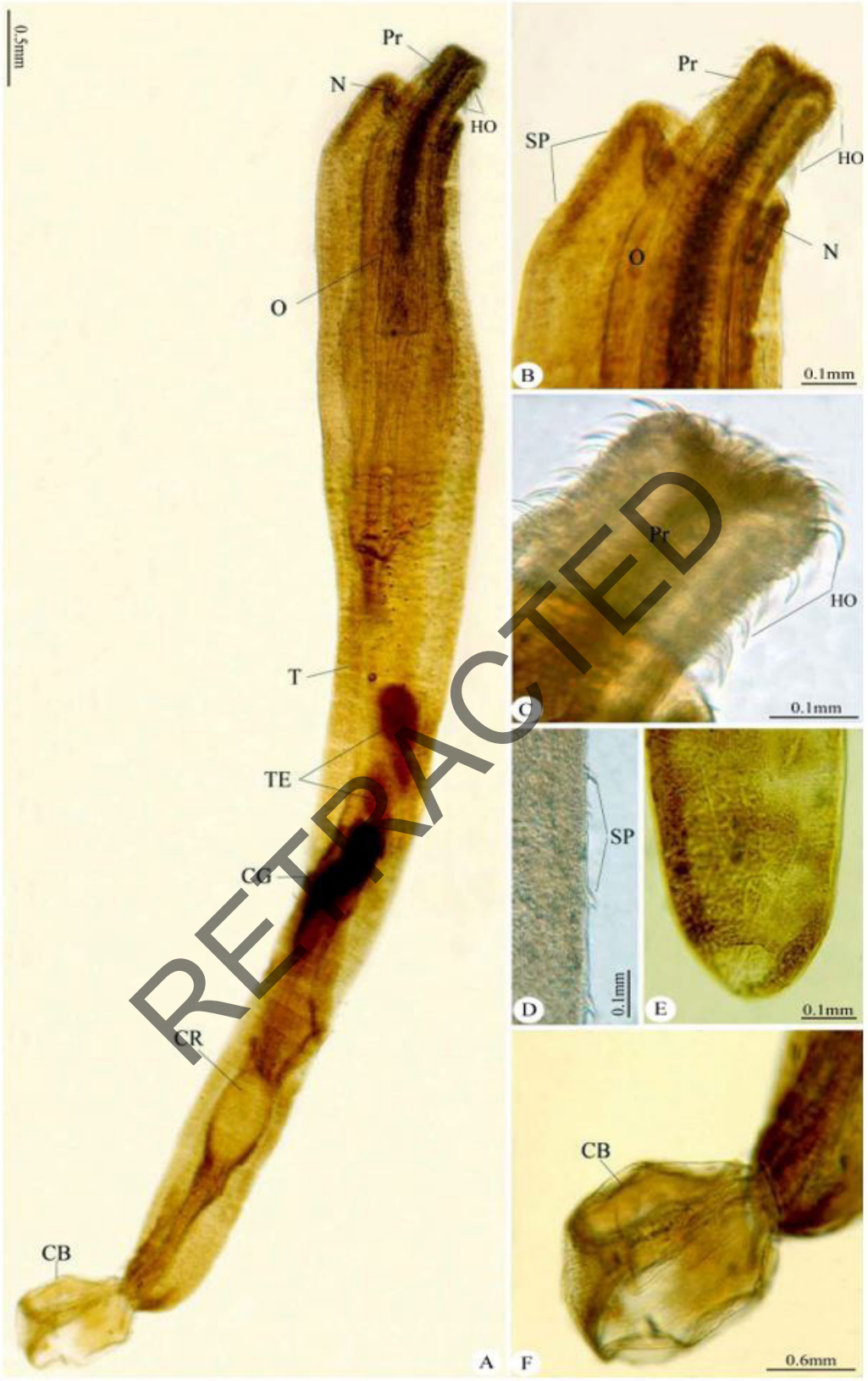

Figure 1. Photomicrographs of the acanthocephalan parasite *Neoechinorhynchus macrospinosus* infecting *Siganus rivulatus*. (A) Adult male worm with anterior end supplied by a retractable proboscis (Pr) and hooks (HO) followed by neck region (N) and note the presence of oesophagus (O) and the trunk region (T) having two testes (TE), cement gland (CG), cement reservoir (CR), and ended by copulatory bursa (CB); (B-D) High magnifications of different body parts of: (B, C) Proboscis (Pr) provided with hooks (SP), followed by neck (N) surrounded by spines (SP), and note the presence of oesophagus (O); (D) Neck region provided with spines (SP); (E) Posterior end of female worm; (F) Posterior end of Male worm with copulatory bursa (CB).

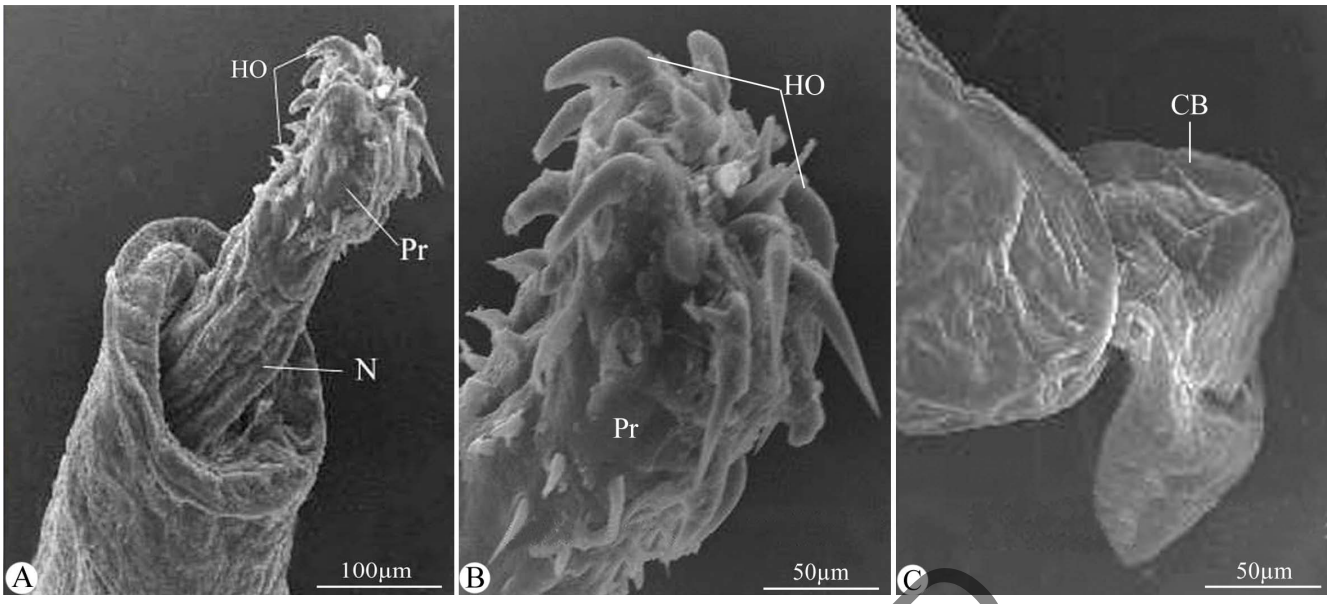

Figure 2. Scanning electron micrographs of the acanthocephalan parasite *Neoechinorhynchus macrospinosus* infecting *Siganus rivulatus*, showing: (A) Anterior end supplied by a retractable proboscis (Pr) provided with hooks (HO) and followed by neck region (N); (B, C) High magnifications of: (B) Proboscis (Pr) provided with hooks (HO). (C) Posterior end of male worm with copulatory bursa (CB).

nucleotide divergence of 26 nt. Nucleotide sequence data were analyzed by maximum likelihood method to obtain a phylogenetic tree that representing three classes of Eoacanthocephala, Archiacanthocephala, and Palaeacanthocephala (Table 1, Figure 3). Pairwise comparison with the GenBank™ 18S rDNA gene data set confirmed the genus *Neoechinorhynchus* identification, but not the species identification with any *Neoechinorhynchus* species available (Table 1). The phylogenetic tree is constructed by two main clades (Figure 3). The major clade divided into two subclades, the first one clustered species within Eoacanthocephala

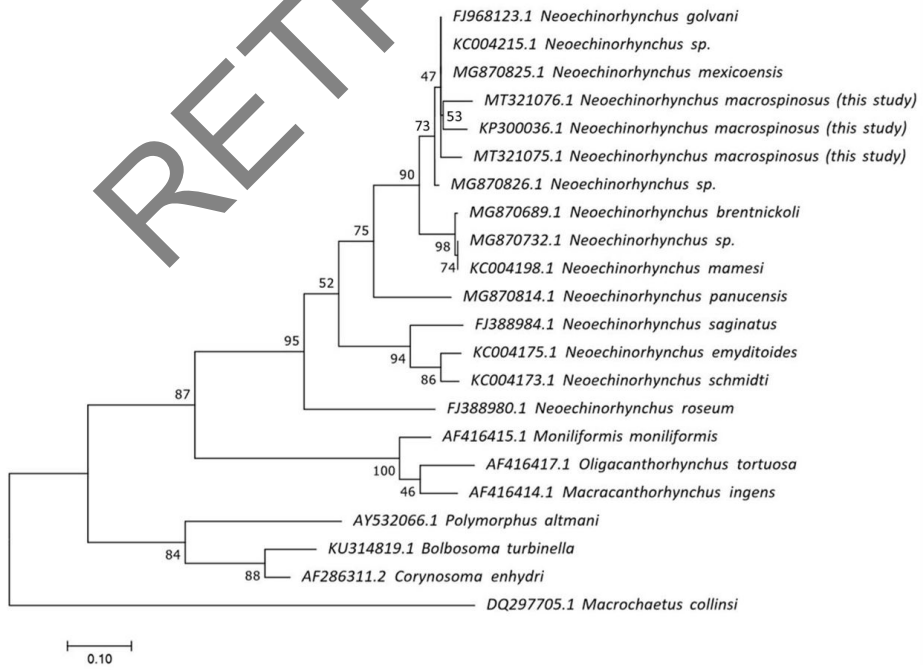

Figure 3. A phylogenetic analysis based on the 18S rDNA sequence demonstrating the position of *Neoechinorhynchus macrospinosus* with other Acanthocephala species. The tree was generated by Maximum Likelihood method based on Kimura 2-parameter model. Numbers at nodes indicate bootstrap values (1000 replications). The tree with the highest log likelihood (-2852.92) is shown. All positions containing gaps and missing data were eliminated. There were a total of 553 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Table 1. Acanthocephalans species used in the 18S rDNA analysis for *Neoechinorhynchus macrospinosus*.

Parasite species	Class: Family	Host species	Accession no.	% Identity	% Guanine-Cytosine (GC) content
Neoechinorhynchus sp.	Eoacanthocephala: Neoechinorhynchidae	*Dormitator maculatus*	KC004215.1	95.14	42.1
Neoechinorhynchus mexicoensis	Eoacanthocephala: Neoechinorhynchidae	*Dormitator maculatus*	MG870825.1	93.59	43.4
Neoechinorhynchus golvani	Eoacanthocephala: Neoechinorhynchidae	*Cichlasoma urophthalmus*	FJ968123.1	93.59	43.3
Neoechinorhynchus sp.	Eoacanthocephala: Neoechinorhynchidae	*Dormitator maculatus*	MG870826.1	93.18	43.3
Neoechinorhynchus sp.	Eoacanthocephala: Neoechinorhynchidae	*Dormitator latifrons*	MG870732.1	89.43	42.4
Neoechinorhynchus mamesi	Eoacanthocephala: Neoechinorhynchidae	*Dormitator latifrons*	KC004198.1	89.43	42.6
Neoechinorhynchus schmidti	Eoacanthocephala: Neoechinorhynchidae	*Trachemys scripta*	KC004173.1	87.80	45.8
Neoechinorhynchus brentnickoli	Eoacanthocephala: Neoechinorhynchidae	*Dormitator latifrons*	MG870689.1	87.64	42.2
Neoechinorhynchus emyditoides	Eoacanthocephala: Neoechinorhynchidae	*Trachemys scripta*	KC004175.1	86.34	44.6
Neoechinorhynchus saginatus	Eoacanthocephala: Neoechinorhynchidae	--	FJ388984.1	84.74	44.9
Neoechinorhynchus panucensis	Eoacanthocephala: Neoechinorhynchidae	*Herichthys* sp.	MG870814.1	83.13	44.4
Neoechinorhynchus roseum	Eoacanthocephala: Neoechinorhynchidae	*Achirus mazatlanus*	FJ388980.1	83.12	47.5
Macracanthorhynchus ingens	Archiacanthocephala: Oligacanthorhynchidae	--	AF416414.1	79.22	36.2
Moniliformis moniliformis	Archiacanthocephala: Moniliformidae	--	AF416415.1	78.16	35.8
Oligacanthorhynchus tortuosa	Archiacanthocephala: Oligacanthorhynchidae	--	AF416417.1	77.92	37
Bolbosoma turbinella	Palaeacanthocephala: Polymorphidae	*Paralichthys isosceles*	KU314819.1	70.91	49.7
Corynosoma enhydri	Palaeacanthocephala: Polymorphidae	--	AF286311.2	70.91	47.3
Polymorphus altmani	Palaeacanthocephala: Polymorphidae	--	AY532066.1	65.77	47

and Archiacanthocephala, and the second one including species of Polymorphidae within Palaeacanthocephala. Whereas, *Macrochaetus collinsi* (gb| DQ297705.1) in the minor clade representing phylum Rotifera. Comparison of nucleotide sequences and divergence showed that the partial 18S rDNA gene sequence of the recovered parasite species revealed sequence identities with taxa belonged to Eoacanthocephala as 95.14-83.12%, Archiacanthocephala as 79.22-77.92%, and 70.91-65.77% with Palaeacanthocephala. Monophyly of *Neoechinorhynchus* species was strongly supported. This phylogeny demonstrated that Archiacanthocephala is forming sister group to Palaeacanthocephala and Eoacanthocephala clade with high bootstrap nodal support. This analysis investigated the placement of the examined neoechinorhynchid species within Eoacanthocephala with close similarity to *Neoechinorhynchus* sp. (gb| KC004215.1), *N. mexicoensis* (gb| MG870825.1), and *N. golvani* (gb| FJ968123.1) as a more related sister taxons.

Discussion

Acanthocephala may have more of an impact upon intestinal parasite communities than other kinds of helminths and they are more likely to exhibit negative interactions with their own and other species, under both field and experimental conditions (Byrne et al., 2003). In the present study, *S. rivulatus* fish was found to be naturally infected with an acanthocephalan parasite *N. macrospinosus* which isolated from the fish intestine with a percentage of 15%. These results agreed with Abdou & Mahfouz (2006) who stated that the percentage of infection for acanthocephalan parasites ranged from 5.6% to 59%.

Concerning seasonal variation of the parasitic infection, it was clear that the highest prevalence found in winter to be 50% and fall to lowest value in summer season to be 10%, these variations between seasons may be attributed to the change in the climatic conditions. These results disagreed with Ali et al. (2012) who recorded a percentage of infection (20.68%) in three fish species belonging to genus *Schizothorax* (*Schizothorax niger*, *Schizothorax esocinus*, and *Schizothorax curvifrons*) infected with *Pomphorhynchus kashmirensis*, and the seasonal cycle was observed with summer showing the highest prevalence (39.62%) and the lowest prevalence was recorded during winter (10.25%). In addition, Radwan (2012) who stated that the infection with the acanthocephalan parasite *Sphaerirostris picae* (Centrorhynchidae) from the hooded crow *Corvus corone cornix* increased during summer season and reached to lowest levels during winter.

Neoechinorhynchus Stiles & Hassall, 1905 is known to be the most diverse acanthocephalan taxon with worldwide distribution inhabiting fresh- and brackish water fish; its species are characterized by uniformity of anatomical organization (Shih, 2004; García-Varela et al., 2005; Smith et al., 2005). The morphological characteristics of the present specimen, shape and size of proboscis, the low number and position of the hooks on the proboscis clearly place this species within the *Neoechinorhynchus* genus and confirm to the descriptions for *N. macrospinosus* by Amin & Nahhas (1994). Also, it is similar in measurements and morphological characters to that obtained by Pichelin & Cribb (2001) and Shih et al. (2010) for the same parasite species. Amin & Nahhas (1994) reported that *N. macrospinosus* and *Diplosentis amphacanthi* Tubangui & Masiluñgan, 1937 isolated from *Siganus* species are synonyms as they were similar in having four cement glands and coiled lemnisci rather than enveloped in membranous sac. On the contrary, the present study reported that *N. macrospinosus* and *D. amphacanthi* as two separate species, as the former has neck with two small pits, which could be openings of solitary gland cell ducts and two sensory papillae, helping the proboscis in participating in the attachment and nourishment as well as a defensive role.

Identification and differentiation of acanthocephalans based on their morphological characters is not always feasible or reliable (Weber et al., 2013). García-Varela & Pérez-Ponce de León (2015) stated that 18S rDNA was used to infer phylogenetic relationships among the major classes of Acanthocephala. Therefore, the PCR analysis in the current study amplifying 18S rDNA gene to be used as a genetic marker to establish a more robust species delimitation criterion among populations of the genus *Neoechinorhynchus*. In addition, most of phylogenetic studies of acanthocephalan parasites similar to this study revealed that 18S rDNA sequences appear to be useful marker for phylogenies among acanthocephalans (García-Varela et al., 2000; Near, 2002; Herlyn et al., 2003; Verweyen et al., 2011; Amin et al., 2019). In the current study, molecular analysis obtained from the partial 18S rDNA gene sequences explain the morphological differences between *N. macrospinosus* and other *Neoechinorhynchus* species. Previous studies by Amin (1987), Garey et al. (1996), and Verweyen et al. (2011) proposing a monophyletic origin of acanthocephalans and their separation into four distinct classes. While, present phylogenetic tree constructed from 22 taxa of Acanthocephala species by Maximum Likelihood (ML) method. The current phylogeny supports the notion that acanthocephalans were monophyly in origin with three distinct classes Archiacanthocephala, Eoacanthocephala, and Palaeacanthocephala. These results agreed with data obtained by García-Varela et al. (2005), Weber et al. (2013) and Abdel-Ghaffar et al. (2014) whom reported that this classification based on the size and shape of proboscis spines, the position of lacunar canals, the number and type of cement glands in males, the persistence of ligament sacs in females, and host taxonomy and ecology. The ML tree showed that the monophyly Archiacanthocephala represented by *Macracanthorhynchus ingens*, *Moniliformis moniliformis* and *Oligacanthorhynchus tortuosa* appear as the sister taxon to a clade comprising monophyletic Eoacanthocephala represented by species of Neoechinorhynchidae and the other clade comprising monophyletic species of Palaeacanthocephala, which agreed with García-Varela et al. (2000), Near (2002) and Malyarchuk et al. (2014). In the present study, phylum Rotifera represented by *Macrochaetus collinsi* was used as the out-group in the ML tree, this agreed with other previous studies by Welch (2000) reported that the substantial phylogenetic evidence from combined data of morphology and molecular revealed that acanthocephalans have a close evolutionary relationship with Rotifera. Molecular analysis of *N. macrospinosus* using 18S rDNA gene demonstrated a high degree of similarity with other Acanthocephala species and grouped especially with species of the genus *Neoechinorhynchus* forming a supported clade within the family Neoechinorhynchidae.

Conclusion

Recent field study report the molecular profile of *N. macrospinosus* that validate its generic affiliations and explore its evolutionary relationships with other related species based on partial 18S rDNA gene. More molecular studies are recommended using other gene targets for exploring the phylogenetic relationships among species.

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