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The molecular profile of *Paratrajectura longcementglandatus* Amin, Heckmann et Ali, 2018 (Acanthocephala: Transvenidae) from percid fishes in the marine waters of Iran and Iraq

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Summary

Paratrajectura longcementglandatus Amin, Heckmann et Ali, 2018 (Transvenidae) was recently described from two species of percid fishes collected from the marine territorial waters of Iraq and Iran in the Persian Gulf. The genus *Paratrajectura* Amin, Heckmann et Ali, 2018 is a close relative to transvenid genera *Trajectura* Pichelin et Crib, 2001 and *Transvena* Pichelin et Crib, 2001. Morphologically, *Paratrajectura* is characterised by having apical proboscis cone, long, tubular cement glands, short lemnisci, prominent roots on all proboscis hooks, subterminal female gonopore, and males with long pre-equatorial testes. Molecular studies of *P. longcementglandatus* using 18S rDNA and *cox1* genes compared with available data of members of other families of Echinorhynchida showed that *P. longcementglandatus* is grouped with species of the genus *Transvena* forming a clade within the family Transvenidae.

Keywords: Spiny headed transvenid worms; 18S rDNA; *cox1*; phylogeny; Perciformes; Middle East

Introduction

Pichelin & Cribb (2001) described the family Transvenidae with two genera: monotypic *Transvena* with *T. annulospinosa* Pichelin et Cribb, 2001, and *Trajectura* with two species, *T. ikedai* (Machida, 1992) and *T. perinsolens* Pichelin et Cribb, 2001. Specimens of the two genera were recovered from wrasses (Labridae, Perciformes) in the Pacific off southern Australia and southern Japan. Lisitsyna *et al*. (2019) described two other species of the family Transvenidae, namely *Transvena pichelinae* Lisitsyna, Kudlai, Cribb et Smit, 2019, and *Pararhadinorhynchus sodwanensis* Lisitsyna, Kudlai, Cribb et Smit, 2019 from the marine fishes from the Sodwana Bay, South Africa. The other genus of this family, *Paratrajectura*, was established by Amin *et al*. (2018). It comprises one species *Paratrajectura longcementglandatus* Amin, Heckmann et Ali, 2018, which was described on the basis of worms from the Japanese threadfin bream *Nemipterus japonicus* Bloch (Nemipteridae) and the tigertooth croacker, *Otolithes ruber* Bloch et Schneider (Sciaenidae, Perciformes) caught in the marine territorial waters of Iraq and Iran, the Persian Gulf (Amin *et al*., 2018). The genus *Paratrajectura* is characterised by having apical proboscis cone, long, tubular cement glands, short lemnisci, prominent roots on all proboscis hooks, subterminal female gonopore, and males with long pre-equatorial testes.

While, several studies have been published about sequence data for acanthocephalans including two *Transvena* spp. (Westram *et al*., 2011; Garcia-Varela, *et al*., 2013; Pinacho-Pinacho *et al*., 2014; Lisitsyna *et al*., 2019), no sequence data has been published for *P. longcementglandatus* whose phylogenetic relationship with other acanthocephalans and related families was unknown. In this paper, we report the molecular profile of *P. longcementglandatus*,

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validate its generic affiliations, and explore its evolutionary relationships with related and other species and taxa based on partial 18S rDNA and *cox1* genes.

Materials and Methods

DNA extraction and PCR amplification

For extraction of genomic DNA, five adult worms of *P. longcementglandatus* were washed with sterile distilled water several times to remove the ethanol residuals. Total DNA was extracted using Qiagen DNeasy Blood and Tissue kit (Qiagen Inc., Valencia, California, USA) according to manufacturer's instructions and kept at −20 °C until use.

PCR reactions were performed in 30 µL volumes containing 2 × red PCR premix (Ampliqon, Odense, Denmark), 20 pmol of each primer and 3 µL of extracted DNA. The partial 18S rRNA gene was amplified using the forward primer (5'-AGATTAAGCCATGCATG-CGTAAG-3') and reverse primer (5'- ACCCACCGAATCAAGAAA-GAG-3'). Also, primers used for the amplification of the partial mitochondrial cytochrome oxidase subunit1 (*cox1*) gene were COI-F (5'-AGTTCTAATCATAARGATATYGG-3') and COI-R (5'-TAAACT-TCAGGGTGACCAAAAAATCA-3') (Folmer *et al*., 1994). PCR conditions for 18S rRNA gene amplification included of an initial denaturing step of 95 °C for 5 min and 35 cycles followed by denaturing step at 95 °C for 30 s, annealing step of 61 °C for 30 s, and 60 s of extension at 72 °C, and 72 °C for 7 min as a final extension. The thermal PCR profiles for *cox1* gene consisted of initial denaturation at 95 °C for 6 minutes followed by 35 cycles of 95 °C for 30 s (denaturation), 55 °C for 30 s (annealing), and at 72 °C for 60 s (extension) with a final extension of 72 °C for 6 minutes. PCR products were analysed on 1.5 % agarose gel and visualized with UV transluminator. Next, the PCR products were sequenced in both directions using the same PCR primers with ABI 3130 sequencer.

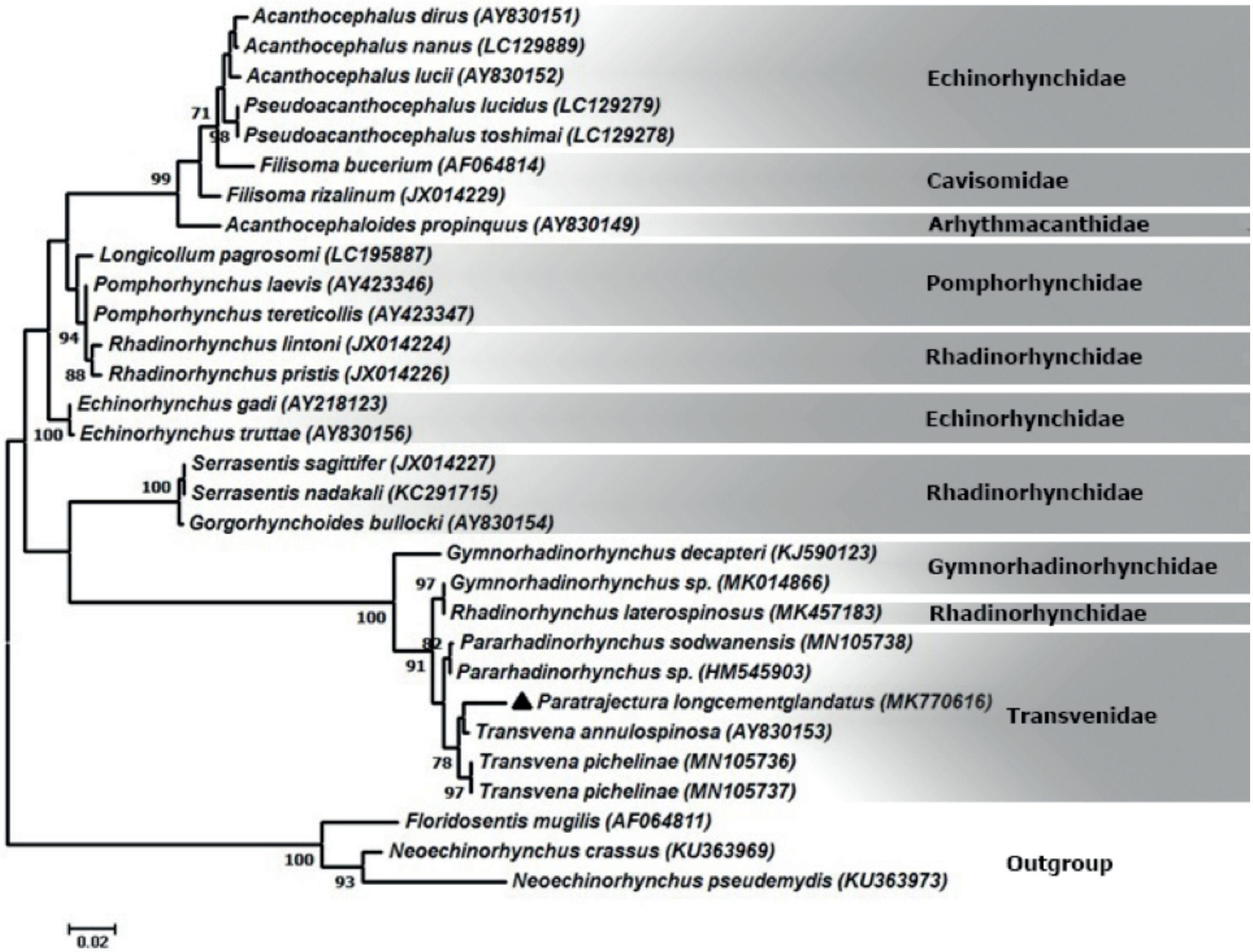

Fig. 1. Phylogenetic tree based on the Maximum likelihood analysis using 18S rDNA sequence of *Paratrajectura longcementglandatus* of current study and sequences of the closest-related members of the order Echinorhynchida deposited in the GenBank. Outgroup: *Floridosentis mugilis, Neoechinorhynchus pseudemydis* and *N. crassus*. Bootstrap values lower than 70 are omitted.

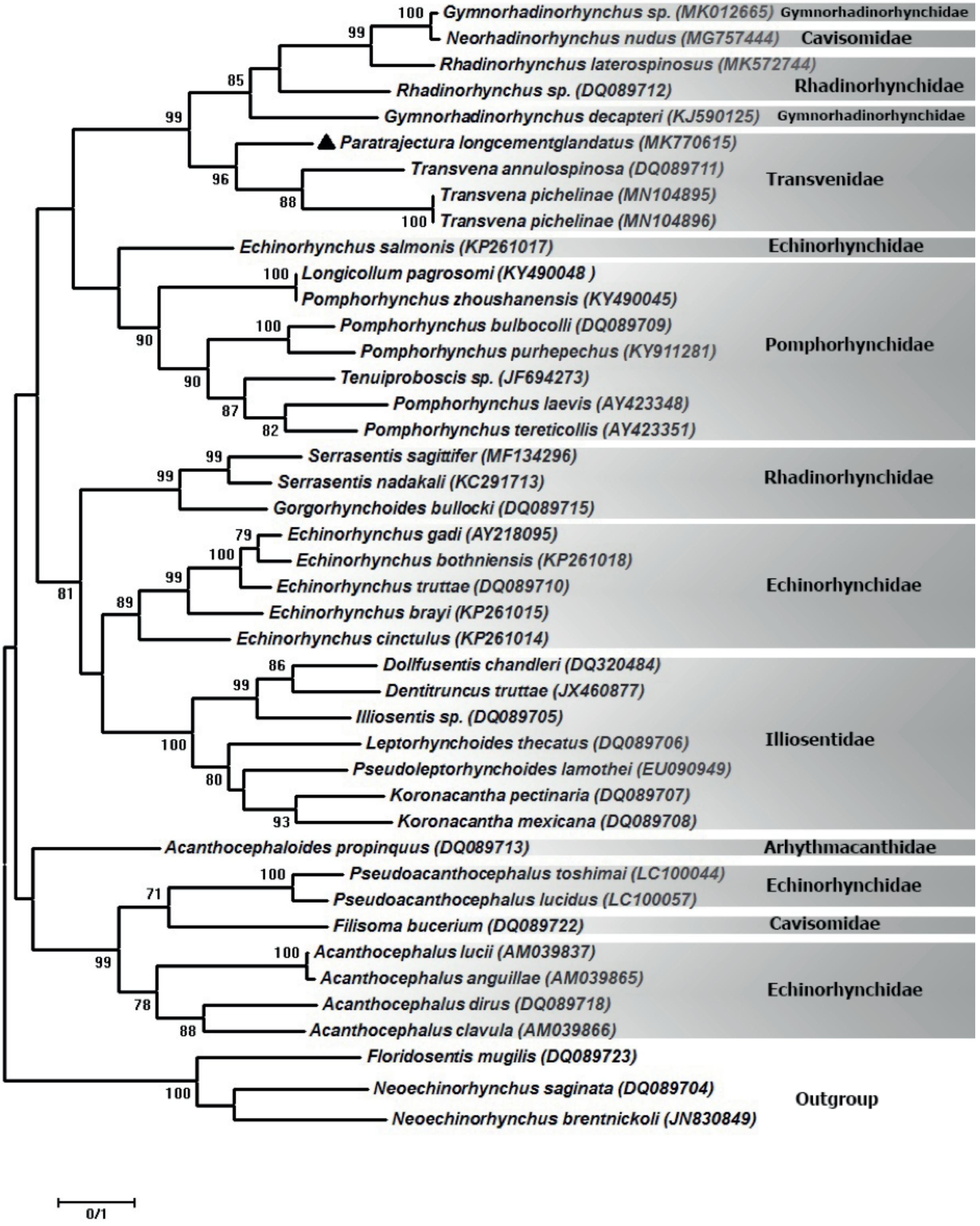

Fig. 2. Phylogenetic reconstruction based on the Maximum likelihood analysis using partial region of the *cox1* sequence of *Paratrajectura longcementglandatus* of current study and sequences of the closest-related members of order Echinorhynchida deposited in the GenBank. Outgroup: *Floridosentis mugilis, Neoechinorhynchus saginata* and *N. brentnickoli*. Bootstrap values lower than 70 are omitted.

The obtained sequence results were manually edited and trimmed using Chromas software v.2.01 (Technelysium Pty Ltd., Brisbane, Queensland, Australia). Next, generated sequences were compared with GenBank submitted sequences using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih. gov/). Also, Clustal W method of Bioedit software v.7.0.9 was used for multiple sequence alignment (Larkin *et al*., 2007). The sequences of 18S rRNA and *cox1* genes were submitted to GenBank database (Accession Numbers: MK770616 for 18S rRNA and MK770615 for *cox1*)

Phylogenetic analysis

The phylogenetic tree was constructed using Maximum-Likelihood model and Tamura-3-parameter model by Molecular and Evolution Genetic Analysis software v.6 (MEGA 6). The reliability of topology of the tree was supported with Bootstrap value based on 1000 replications. The whole scientific names of acanthocephalan species, names of host species, *localities,* and GenBank accession numbers used in the phylogenetic analysis are listed in Table 1.

Ethical Approval and/or Informed Consent

The authors declare compliance with all relevant ethical standards.

Results

The specimens of *P. longcementglandatus* successfully presented amplifications of about 1234 bp for the 18S rDNA gene and 664 bp for the *cox1* gene. Comparisons of the18S rDNA and *cox1* sequences from this parasite with other available acanthocephalan sequences in GenBank, using multiple sequence alignment, showed that it had the highest similarity with *T. annulospinosa* based on18S rDNA (98 %*)* and *cox1* (77 %) genes*.* The 18S rDNA dataset (1129 nt) included 26 sequences for species of seven families within the Echinorhynchida and the novel sequence of *P. longcementglandatus*. The *cox1* dataset (538 nt) included 39 sequences for species of nine families of Echinorhynchida and the sequence of *P. longcementglandatus*.

The phylogenetic reconstruction based on the partial sequence spanning the 18S rDNA showed that our sequence of *P. longcementglandatus* is clustered with *Transvena annulospinosa* (AY830153), *T*. *pichelinae* (MN105736 and MN105737), *P. sodwanensis* (MN105738) and an unidentified species of *Pararhadinorhynchus* (HM545903) with strong support forming a clade of the family Transvenidae. Also, the species of *Gymnorhadinorhynchus* sp. (MK014866) (Gymnorhadinorhynchidae) and *Rhadinorhynchus laterospinosus* (MK457183) (Rhadinorhynchidae) are very closely related with the family Transvenidae in the tree with 100 % of bootstrap support. The sequence of *Gymnorhadinorhynchus decapteri* (KJ590123) (Gymnorhadinorhynchidae) is located at the basal position to the members of the clade. Other families of the order Echinorhynchida including Rhadinorhynchidae, Pomphorhynchidae, Cavisomidae, Arhythmacanthidae and Echinorhynchidae located in a major sister clade (Fig. 1). Inter-generic differences are noted between *P. longcementglandatus* and *T. annulospinosa, T. pichelinae, P. sodwanensis* and *Pararhadinorhynchus* sp. from Transvenidae based on partial 18S rDNA sequence were 2.4 % (18 nt), 2.8 % (21 nt), 2.9 % (22 nt) and 2.9 % (22 nt), respectively. According to phylogenetic analyses based on the *cox1* gene, our sequence of *P. longcementglandatus* (MK770615) is grouped with *T. annulospinosa* (DQ089711) and *T*. *pichelinae* (MN104895 and MN104896) with strong support in a clade of the family Transvenidae. The species of *G. decapteri* (KJ590125) and *Gymnorhadinorhynchus* sp. (MK012665) (Gymnorhadinorhynchidae), *Neorhadinorhynchus nudus* (MG757444) (Cavisomidae), *R. laterospinosus* (MK572744) and *Rhadinorhynchus* sp. (DQ089712) (Rhadinorhynchidae), appear as a sister group of the family Transvenidae (Fig. 2). The interspecific divergence between *P. longcementglandatus* and *T. annulospinosa, T. pichelinae* based on partial *cox1* gene was 23.4 % (141 nt), 27.3 % (144 nt), respectively.

Discussion

Recently, molecular methods are applied for species identification, classification and phylogenetic analysis of acanthocephalan species (García-Varela *et al*., 2002). To date, molecular profile has been provided for few species of the family Transvenidae including *T. annulospinosa, T. pichelinae*, *P. sodwanensis* and *Pararhadinorhynchus* sp. (Pichelin & Cribb, 2001; Lisitsyna *et al*., 2019). In the current study, phylogenetic relationships of *P. longcementglandatus* as another genus of this family is described based on partial 18S rDNA and *cox1* genes determining relationships with other acanthocephalan families.

This study showed that the interspecific variation between *P. longcementglandatus* and species of *Transvena* based on partial 18S rDNA was 2.4 % – 2.8 % (18 – 21 nt) and between it and species of *Pararhadinorhynchus* was 2.9 % (22 nt). Also based on *cox1* gene, inter-generic variations between *P. longcementglandatus* and *T. annulospinosa* was 23.4 % (141 nt). These results illustrate that sequence differences between the genera of the family based on *cox1* gene is higher than18S rDNA and it is appropriate to consider for taxonomic studies at the generic level.

The phylogenetic analysis of the 18S rDNA sequence (Fig. 1) showed that *P. longcementglandatus* is grouped in a highly supported clade with *T. annulospinosa* (AY830153), *T. pichelinae* (MN105736 and MN105737), *P. sodwanensis* (MN105738) and *Pararhadinorhynchus* sp. (HM545903) forming a clade of the family Transvenidae. In the clade, the family Transvenidae grouped close to *R. laterospinosus* (MK457183) and *G. decapteri* (KJ590123) (Gymnorhadinorhynchidae). Our phylogenetic tree for 18S rDNA is similar to those of García-Varela *et al*. (2002) and Lisitsyna *et al.* (2019) where the family Transvenidae grouped close to different species of *Rhadinorhynchus* (Rhadinorhynchidae) and *Gymnorhadinorhynchus* (Gymnorhadinorhynchidae).

Table 1. Acanthocephalan species represented in the phylogenetic analysis with their family, host species, GenBank accession numbers, locations, and references.

Species	Host	GenBank Acc. no. 185 rDNA	GenBank Acc. no. *cox1*	Location	Reference
Gymnorhadinorhynchidae					
Gymnorhadinorhynchus sp.	*Regalecus russelii*	MK014866	MK012665	Japan	Steinauer *et al*. (2019)
Gymnorhadinorhynchus decapteri (Braicovich, Lanfranchi, Farber, Marvaldi, Luque et Timi, 2014)	*Decapterus punctatus*	KJ590123	KJ590125	Brazil	Braicovich *et al*. (2014)
Cavisomidae					
Neorhadinorhynchus nudus (Harada, 1938)	*Auxis thazard*	-	MG757444	China	Li *et al*. (2018)
Filisoma bucerium (Van Cleave, 1940)	*Kyphosus elegans*	AF064814	DQ089722	Na*	García-Varela *et al*. (2000), García-Varela and Nadler (2006)
Filisoma rizalinum (Tubangui et Masilungan, 1946)	*Scatophagus argus*	JX014229	-	Indonesia	Verweyen *et al*. (2011)
Rhadinorhynchidae					
Rhadinorhynchus laterospinosus (Amin, Heckmann et Van Ha, 2011)	*Auxis rochei*	MK457183	MK572744	Vietnam	Amin *et al*. (2019a)
Rhadinorhynchus sp.	*Sciaenidae*	AY062433	DQ089712	Na	García-Varela *et al*. (2002), García-Varela and Nadler (2006)
Serrasentis sagittifer (Linton, 1889)	*Lutjanus sebae*	-	MF134296	Australia	Barton *et al*. (2018)
Serrasentis sagittifer	*Johnius coitor*	JX014227	-	Indonesia	Verweyen *et al*. (2011)
Serrasentis nadakali (George et Nadakal, 1978)	Na	KC291715	KC291713	Na	Paul *et al*. (unpublished)
Gorgorhynchoides bullock (Cable et Mafarachisi, 1970)	*Eugerres plumieri*	AY830154	DQ089715	Na	Garcia-Varela and Nadler (2005, 2006)
Rhadinorhynchus lintoni (Cable et Linderoth, 1963)	*Selar Crumrn- opht halmus*	JX014224	-	USA	Verweyen *et al*. (2011)
Rhadinorhynchus pristis (Rudolphi, 1802)	*Selar Crumrn- opht halmus*	JX014226	-	USA	Verweyen *et al*. (2011)

Transvenidae					
Paratrajectura longcementglandatus (Amin, Heckmann et Ali, 2018)	Percid fishes	MK770616	MK770615	Marine waters of Iraq and Iran	Present study
Transvena annulospinosa (Pichelin et Cribb, 2001)	*Anampses neoguinaicus*	AY830153	DQ089711	Na	Garcia-Varela and Nadler (2005, 2006)
Transvena pichelinae sp. n. (Lisitsyna, 2019)	*Thalassoma purpureum*	MN105736, MN105737	MN104895, MN104896	South Africa	Lisitsyna *et al*. (2019)
Pararhadinorhynchus sodwanensis sp. n. (Lisitsyna, 2019)	*Pomadasys furcatus*	MN105738	-	South Africa	Lisitsyna *et al*. (2019)
Pararhadinorhynchus sp.	*Siganus fuscescens*	HM545903	-	China	Wang *et al*. (unpublished)
Echinorhynchidae					
Pseudoacanthocephalus toshimai (Nakao, 2016)	*Rana pirica*	LC129278	LC100044	Japan	Nakao (2016)
Pseudoacanthocephalus lucidus (Van Cleave, 1925)	*Rana ornativentris*	LC129279	LC100057	Japan	Nakao (2016)
Acanthocephalus lucii (Müller, 1776)	*Perca fluviatilis*	AY830152	-	Na	Garcia-Varela and Nadler(2005), Benesh *et al*. (2006)
Acanthocephalus lucii	*Perca fluviatilis*	-	AM039837	England	Garcia-Varela and Nadler (2005), Benesh *et al*. (2006)
Acanthocephalus anguillae (Müller, 1780)	*Perca fluviatilis*	-	AM039865	Austria	Benesh *et al*. (2006)
Acanthocephalus dirus (Van Cleave, 1931)	*Asellus aquaticus*	AY830151	DQ089718	Na	Garcia-Varela and Nadler (2005, 2006)
Acanthocephalus clavula (Dujardin, 1845)	*Perca fluviatilis*	-	AM039866	Ireland	Benesh *et al*. (2006)
Acanthocephalus nanus (Van Cleave, 1925)	*Cynops pyrrhogaster*	LC129889	-	Japan	Nakao (2016)
Echinorhynchus salmonis (Müller, 1784)	*Coregonus lavaretus*	-	KP261017	Finland	Wayland *et al*. (2015)
Echinorhynchus gadi (Müller, 1776)	Na	AY218123	AY218095	Na	Giribet *et al*. (2004)
Echinorhynchus bothniensis (Zdzitowiecki et Valtonen, 1987)	*Osmerus eperlanus*	-	KP261018	Finland	Wayland *et al*. (2015)

Echinorhynchus truttae (Schrank, 1788)	*Thymallus thymallus*	AY830156	DQ089710	Na	Garcia-Varela and Nadler (2005, 2006)
Echinorhynchus brayi (Wayland, Sommerville et Gibson, 1999)	*Pachycara crassiceps*	-	KP261015	Atlantic Ocean: Porcupine Seabight	Wayland *et al*. (2015)
Echinorhynchus cinctulus (Porta, 1905)	*Lota lota*	-	KP261014	Finland	Wayland *et al*. (2015)
Pomphorhynchidae					
Longicollum pagrosomi (Yamaguti, 1935)	*Pagrus major*	LC195887	-	Japan	Mekata *et al*. (unpublished)
Longicollum pagrosomi	*Oplegnathus fasciatus*	-	KY490048	China	Li *et al*. (2017)
Pomphorhynchus zhoushanensis (Li, Chen, Amin et Yang, 2017)	*Oplegnathus fasciatus*	-	KY490045	China	Li *et al*. (2017)
Pomphorhynchus bulbocolli (Linkins, 1919)	*Lepomis macrochirus*	-	DQ089709	Na	Garcia-Varela and Nadler (2006)
Pomphorhynchus purhepechus (García-Varela, Mendoza-Garfias, Choudhury et Pérez-Ponce de León, 2017)	*Moxostoma austrinum*	-	KY911281	Na	Garcia-Varela *et al*. (2017)
Tenuiproboscis sp.	*Epinephelus malabaricus*	-	JF694273	Na	Vijayan *et al*. (unpublished)
Pomphorhynchus laevis (Zoega in Müller, 1776)	*Gammarus pulex*	AY423346	AY423348	France	Perrot-Minnot (2004)
Pomphorhynchus tereticollis (Rudolphi, 1809)	*Gammarus pulex*	AY423347	AY423351	France	Perrot-Minnot (2004)
Arhythmacanthidae					
Acanthocephaloides propinquus (Dujardin, 1845)	*Gobius bucchichii*	AY830149	DQ089713	Na	Garcia-Varela and Nadler (2005, 2006)
Diplosentidae					
Sharpilosentis peruviensis (Lisitsyna, Scholz et Kuchta, 2015)	*Duopalatinus cf. peruanus*	-	KP967562	Peru	Lisitsyna *et al*. (2015)
Illiosentidae					
Dollfusentis chandleri (Golvan, 1969)	Na	-	DQ320484	Na	Baker and Sotka(unpublished)
Dentitruncus truttae (Sinzar, 1955)	*Salmo trutta*	JX460865	JX460877	Croatia	Vardić Smrzlić *et al*. (2013)
Illiosentis sp.	Na	AY830158	DQ089705	Na	Garcia-Varela and Nadler (2005, 2006)

Leptorhynchoides thecatus (Linton, 1891)	*Lepomis cyanellus*	AF001840	DQ089706	Na	Near *et al*. (1998), Garcia-Varela and Nadler (2006)
Pseudoleptorhynchoides lamothei (Salgado-Maldonado, 1976)	*Ariopsis guatemalenis*	EU090950	EU090949	Na	Near *et al*. (1998), Garcia-Varela and Nadler (2006)
Koronacantha pectinaria (Van Cleave, 1940)	*Microlepidotus brevipinnis*	AF092433	DQ089707	Na	García-Varela and Nadler (2005, 2006)
Koronacantha Mexicana (Monks et Pérez-Ponce de León, 1996)	*Haemulopsis leuciscus*	AY830157	DQ089708	Na	García-Varela and Nadler (2005, 2006)
Neoechinorhynchidae (Outgroup)					
Neoechinorhynchus brentnickoli (Monks, Pulido-Flores and Violante-González, 2011)	*Dormitator latifrons*	-	JN830849	Na	Pinacho-Pinacho *et al*. (2012)
Neoechinorhynchus saginata (Van Cleave & Bangham, 1949)	Na	-	DQ089704	Na	García-Varela and Nadler (2006)
Floridosentis mugilis (Machado Filho, 1951)	Na	AF064811	DQ089723	Na	García-Varela and Nadler(2006), García-Varela *et al*. (2000)
Neoechinorhynchus crassus (Van Cleave, 1919)	Na	KU363969	-	Iran	Dadar and Adel (unpublished)
Neoechinorhynchus pseudemydis (Cable and Hopp, 1954)	*Capoeta aculeata*	KU363973	-	Iran	Dadar and Adel (unpublished)

*Na = not available

Our phylogenetic analysis of the *cox1* gene (Fig. 2) confirmed that *P. longcementglandatus* is grouped with *T. annulospinosa* (DQ089711) and *T. pichelinae* (MN104895 and MN104896) which made the clade of the family Transvenidae with good statistical support. Also, the families Rhadinorhynchidae, Gymnorhadinorhynchidae and Cavisomidae appear as a sister group with the clade of family Transvenidae. Other families of Echinorhynchida such as Pomphorhynchidae, Echinorhynchidae, Cavisomidae, Illiosentidae, Rhadinorhynchidae, Gymnorhadinorhynchidae, Diplosentidae and Arhythmacanthidae are well separated in the later clade.

In the present study, the higher level of variation in *cox1* gene compared to the 18S rDNA gene provides better resolution of the relationships within closely related taxa. While Amin *et al*. (2019a) presented relationships in their analysis of *Rhadinorhynchus* based on *cox1* sequences, it was not clearer than 18S rDNA due to the lack of sufficient sequences of this gene in GenBank.

One of the most commonly used molecular markers for classification of acanthocephalans is the small subunit from RNA ribosomal gene or 18S rRNA. This gene displays a slow evolution rate and is highly conserved. It was used to infer phylogenetic relationships among the major classes of Acanthocephala (García-Varela & Pérez-Ponce de León, 2015). Most of phylogenetic studies of acanthocephalans similar to this research showed that 18S rDNA sequences appear to be suitable marker for phylogenies among acanthocephalans (García-Varela *et al.,* 2000; Near, 2002; Herlyn *et al*., 2003; Verweyen *et al*., 2011; Amin *et al*., 2019b). Also, *cox1* gene is commonly used for phylogenetic studies and to recognize and establish species limits in acanthocephalans (Guillen-Hernández *et al*., 2008; Alcántar-Escalera *et al*., 2013; García-Varela, *et al*., 2013). The present study confirmed that this gene has high genetic diversity among genera of the family and other families of Echinorhynchida which would be more particularly useful for phylogenetic analysis.

Finally, the genetic data collected in the current study provide a better understanding of the taxonomic status of *P. longcementglandatus*. Sequence variations within the family Transvenidae and among other families of Echinorhynchida based on *cox1* gene is higher than18S rDNA that can be useful for achieving a proper assessment of biodiversity. More sequence data from other geographical isolates using more gene targets will be useful for exploring the phylogenetic relationships among species. On the other hand, using of molecular tools for identification of acanthocephalan species is still scarce due to the lack of sequences of different genera of acanthocephalans in GenBank (Amin *et al*., 2013; Salgado-Maldonado, 2013; Weaver & Smales, 2013; Amin *et al*., 2014; Smales, 2014; Gomes *et al*., 2015; Steinauer & Nickol, 2015). More molecular studies are recommended in order to elucidate acanthocephalans classification.

Conflict of Interest

The authors declare no conflict of interest.

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