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 *Parahadinorhynchus 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′-AGATTAAGCCATGCATGCGTAAG-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′-AGTTCTAATCATAARGATATYGGG-3′) and COI-R (5′-TAAACTTCAGGTTGACCCAAAAATCA-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 longcemengl glandatus of current study and sequences of the closest-related members of order Echinorhynchida deposited in the GenBank. Outgroup: Floridosentis mugilis, Neoechinorhynchus saginata and N. brentnickoi. 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 the 18S 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 on 18S 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. picheliniae* (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 deceptri* (KJ590123) (Gymnorhadinorhynchidae) is located at the basal position to the members of the clade. Other families of the order Echinorhynchida including Rhadinorhynchidae, Pomphorhynchidae, Cavisomididae, Arhythmacanthidae and Echinorhynchidae located in a major sister clade (Fig. 1). Inter-generic differences are noted between *P. longcementglandatus* and *T. annulospinosa*, *T. picheliniae*, *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. picheliniae* (MN104895 and MN104896) with strong support in a clade of the family Transvenidae. The species of *G. deceptri* (KJ590125) and Gymnorhadinorhynchus sp. (MK012665) (Gymnorhadinorhynchidae), Neorhadinorhynchus nudus (MG574444) (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. picheliniae* 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 (Garcia-Varela et al., 2002). To date, molecular profile has been provided for few species of the family Transvenidae including *T. annulospinosa*, *T. picheliniae*, *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 than 18S 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. picheliniae* (MN105736 and MN105737), *P. sodwanensis* (MN105738) and an unidentified species of *Pararhadinorhynchus* (HM545903) forming a clade of the family Transvenidae. In the clade, the family Transvenidae grouped close to *R. laterospinosus* (MK457183) and *G. deceptri* (KJ590123) (Gymnorhadinorhynchidae). Our phylogenetic tree for 18S rDNA is similar to those of Garcia-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              | Decapterus punctatus          | KJ590123                  | KJ590125               | Brazil     | Braicovich *et al.* (2014)             |
| (Braiovich, Lanfranchi, Farber, Marvaldi, Luque et Timi, 2014) |                               |                           |                        |            |                                        |
| **Cavisomidae**                              |                               |                           |                        |            |                                        |
| Neorhadinorhynchus nudus (Harada, 1938)      | Auxis thazard                 | MG757444                  |                        | China      | Li *et al.* (2018)                     |
| Filisoma bucerium (Van Cleave, 1940)         | Kyphosus elegans              | AF064814                  | DQ089722               | Na*        | Garcia-Varela *et al.* (2000), Garcia-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         | Garcia-Varela *et al.* (2002), Garcia-Varela and Nadler (2006) |
| Serrasentis sagittfer (Linton, 1889)         | Lutjanus sebae                | MF134296                  |                        | Australia  | Barton *et al.* (2018)                 |
| Serrasentis sagittfer                         | 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 Crumm-opht halmus      | JX014224                  | -                      | USA        | Verweyen *et al.* (2011)               |
| Rhadinorhynchus pristis (Rudolphi, 1802)     | Selar Crumm-opht halmus       | JX014226                  | -                      | USA        | Verweyen *et al.* (2011)               |
| **Transvenidae**                          |                     |                     |                     |                     |
|------------------------------------------|---------------------|---------------------|---------------------|---------------------|
| *Paratrajectura longecementglandatus*    | Percid fishes       | MK770616            | MK770615            | Marine waters of Iraq and Iran | Present study |
| (Amin, Heckmann et Ali, 2018)            |                     |                     |                     |                     |
| *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 fuscens*   | 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 dinus* (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 pyrhhogaster* | 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) |
| Species                                      | Host                          | GenBank Accession | Location                      | Authors                           |
|----------------------------------------------|-------------------------------|-------------------|-------------------------------|-----------------------------------|
| Echinorhynchus truttae (Schrank, 1788)       | Thallassus thymallus          | AY830156          | Na                            | Garcia-Varela and Nadler (2005, 2006) |
| Echinorhynchus brayi (Wayland, Sommerville et Gibson, 1999) | Pachycara crassisceps        | 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         | -                 | China                         | Li et al. (2017)                  |
| Pomphorhynchus zhoushanensis (Li, Chen, Amin et Yang, 2017) | Oplegnathus fasciatus        | -                 | China                         | Li et al. (2017)                  |
| Pomphorhynchus bulbocollii (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          | France                        | Perrot-Minnot (2004)              |
| Pomphorhynchus tereticollis (Rudolphi, 1809) | Gammarus pulex                | AY423347          | France                        | Perrot-Minnot (2004)              |
| Acanthocephaloides propinquus (Dujardin, 1845) | Gobius bucchichii             | AY830149          | Na                            | Garcia-Varela and Nadler (2005, 2008) |
| Diplosentidae                                |                               |                   |                               |                                   |
| Sharpilosentis peruviensis (Lisitsyna, Scholz et Kuchta, 2015) | Duopalinus cf. peruanus       | KP967562          | Peru                          | Lisitsyna et al. (2015)           |
| Illiosentidae                                |                               |                   |                               |                                   |
| Dolphusentis chandleri (Golvan, 1969)        | Na                            | DQ320484          | Na                            | Baker and Sotka (unpublished)     |
| Dentiruncus truttae (Sinzar, 1955)           | Salmo trutta                  | JX460865          | Croatia                       | Vardić Smržić et al. (2013)       |
| Illiosentis sp.                              | Na                            | AY830158          | Na                            | Garcia-Varela and Nadler (2005, 2006) |
| Species / Genus | Species / Genus | Accession | Accession | Author(s) | Reference |
|----------------|----------------|-----------|-----------|-----------|-----------|
| *Leptorhynchoides thecatus* (Linton, 1891) | *Lepomis cyanellus* | AF001840 | DQ089706 | Na | Near *et al.* (1998), Garcia-Varela and Nadler (2006) |
| *Pseudolepotohyphonoides lamothei* (Salgado-Maldonado, 1976) | *Ariopsis guatemalenis* | EU090950 | EU090949 | Na | Near *et al.* (1998), Garcia-Varela and Nadler (2006) |
| *Koronacantha pectinaria* (Van Cleve, 1940) | *Microlepidotus brevipinnis* | AF092433 | DQ089707 | Na | Garcia-Varela and Nadler (2005, 2006) |
| *Koronacantha Mexicana* (Monks et Pérez-Ponce de León, 1996) | *Haemulopsis leuciscus* | AY830157 | DQ089708 | Na | Garcia-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 | Garcia-Varela and Nadler (2006) |
| *Floridosentis mugilis* (Machado Filho, 1951) | Na | AF064811 | DQ089723 | Na | Garcia-Varela and Nadler (2006), Garcia-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 Cavismoridae appear as a sister group with the clade of family Transvenidae. Other families of Echinorhynchida such as Pomphorhynchidae, Echinorhynchidae, Cavismoridae, 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 (Garcia-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 (Garcia-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; Garcia-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 than 18S 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.

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