Uttar Pradesh Journal of Zoology

Volume 45, Issue 7, Page 135-143, 2024; Article no.UPJOZ.3359
ISSN: 0256-971X (P)

Molecular Identification of *Neoechinorhynchus iraqensis* (*Acanthocephala*: *Neoechinorhynchidae*) from *Planiliza abu* in Darbandikhan Lake

Muqdad Kamal Ali [a*] and Shamall Mohammad Amin Abdullah [b]

[a] Department of Animal Science, College of Agricultural Engineering Science, University of Sulaimani, Iraq.
[b] Department of Fish and Aquatic Resource, College of Agricultural Engineering Science, University of Salahaddin, Iraq.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2024/v45i73985

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3359

Original Research Article

Received: 08/03/2024
Accepted: 26/03/2024
Published: 29/03/2024

ABSTRACT

A total of 100 fishes belonged to *Planiliza abu* (Heckel, 1843) were taken from Darbandikhan Lake in Sulaimani Province, Kurdistan Region, Iraq from December 2023 to February 2024. The fish were checked for parasitic acanthocephalan. The research showed that *Neoechinorhynchus iraqensis* existed. In this study, 18S rDNA and DNA sequencing were used to identify *N. iraqensis*. The findings of the molecular analysis show that 533 bp was the PCR product of *N. iraqensis* and

**Corresponding author: Email: muqdad.ali@univsul.edu.iq;*

observed the smallest genetic distance with *Neoechinorhynchus* sp. GL-2015 (KU363972.1) and *Neoechinorhynchus* sp. XL-2014 (KM507363.1) was (0.025), and the highest genetic distance was recorded with *Tenuisentis niloticus* (MZ727194.1) was (0.194). Neighbor - Joining trees inferred for 18S rDNA showed that *N. iraqensis* as a separate clade in the trees, demonstrating that this species originated in Iraq. This finding indicates that the species is the same species that was morphologically identified and described for the first time in Iraq.

Keywords: Neoechinorhynchus iraqensis; Planiliza abu; 18S rDNA; Darbandikhan Lake; DNA sequencing.

1. INTRODUCTION

There are several different types of parasites that belong to the main groups, such as trematoda, cestoda, acanthocephala, and monogena, infest fish [1]. According to earlier research, the fish culture sector in Iraq was having a significant effect and beneficial impact on the parasite populations of freshwater fish [2]. The significance of fish parasites is closely related to the significance of the fish they may infect. Consequently, the previous knowledge of fish's breeders, parasites, and their effects is crucial from an economic perspective [3].

Acanthocephala, often known as thorny-headed worms, have an ever-present proboscis that is equipped with hooks and spines. It frequently attaches to the intestine of the final host and is an obligatory endoparasite [4]. The basic life cycle and developmental stages are the same for all acanthocephalans. For the development of the larva, all require an arthropod as an intermediate host, and all use vertebrates as their only host [5].

Regarding the genus *Neoechinorhynchus* 11 species have been recorded in freshwater fish in Iraq [6]. Amin et al [7] Described for the first time *N. iraqensis* in intestine of the mugilid fish *Planiliza abu* (as *Liza abu*) from Euphrates River in Al-Anbar Province, Iraq.

The family Mugilidae is distributed widely. Its major species are found in temperate and tropical coastal waters. Some species survive all or part of their lifespan in lakes, rivers, and coastal lagoons [8]. Only 80 of the 304 species that were available in this family were valid [9].

There isn't any extensive research on the molecular of *N. iraqensis* in the Iraq and Kurdistan region. The objective of this research is to use DNA sequencing by Polymerase chain reaction (PCR) to identify *N. iraqensis* in Darbandikhan Lake.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 100 fish belonging to mugilid species (*Planilizaabu*) (Fig.1) were collected from the Darbandikhan Lake in Sulaimani, Kurdistan region, Iraq. It is confined to latitudes 35º 06' 58''-35º 21' 07'' N and longitudes 45º 40' 59''-45º 44' 42'' E, 65 kilometers southeast of the city of Sulaimani (Fig. 2).

Fig. 1. *Palaniliza abu*

Fig. 2. Map of Darbandikhan Lake

Gill nets or cast nets were used for collecting fish, from December 2023 to February 2024. Shortly after being caught, newly obtained fish were checked in the lab for parasites. Fish names are based on Froese and Pauly [10]. The recovered acanthocephalans had been first cleaned in saline solution, then chilled in chilly water for 12 hours, and finally preserved in seventy percent ethanol [11].

2.2 DNA Extraction

The AddPrep Genomic DNA Extraction Kit (ADD BIO INC. Korea) was used to extract DNA from *N. iraqensis* samples according to the instructions provided by the manufacturer.One percent agarose gel was used to determine the DNA purity, and the DNA concentration was determined using a Nanodrop spectrophotometer. In preparation for use, DNA Samples had been stored at -20 °C.

2.2.1 Primer sequence and PCR conditions

The primers were used to amplify the nuclear ribosomal DNA's 18S rDNA region, (forward: 5'-: CGGGGGGAGTATGGTTGC-3') and (reverse: 5'TGATCCTTCTGCAGGTTCACCTAC-3'). The thermo cycling conditions were as follows: an initial denaturation at 94 °C for 8 min, followed by 35 cycles for 30 s at 94 °C, for 30 s at 56 °C for annealing, an extension for 30 s at 72 °C, a final extension for 10 min, and then storage at 4 °C.

The genomic DNA had been diluted into a volume of 25 µl l for the PCR reaction, and it also contained 2 µl of each primer, 12.5 µl of Master Mix 10 X buffer containing MgCl2, 3 l of dNTPs (10 mM), 0.9 l of 1 U of Taq DNA polymerase (Biotools, Spain), 7 µl of the genomic DNA, and 3.5µl of distilled water.

Using the same primer as above, the sequencing processes were carried out using a Genetic Analyzer 3500, Applied Bio Systems (USA), in accordance with the instructions provided by the manufacturer.The 18S rDNA sequences were obtained, and using Mega X's default ClustalW [12], they were matched with sequences from other similarly associated species.

3. RESULTS

During the current study period, *Planiliza abu* was surveyed for parasitic acanthocephalans. One acanthocephalan, a species of the genus *Neoechinorhynchus*, was found during the survey. A brief description of this parasite is given below.

***Neoechinorhynchus iraqensis* Amin, Al-Sady, Mhaisen and Bassat, 2001**

Host: *Planiliza abu* (Heckel, 1843).
Prevalence of infection: 7 %.
Mean intensity: 2.4 worm/ fish.
Site infection: Intestine.
Locality: Darbandikhan Lake

In this present research, the DNA sequences of *Neoechinorhynchus iraqensis* have been compared to the sequences of other acanthocephalans and closely associated species that belong to the same genus obtained from GenBank (Table 1).

The 18S rDNA fragment was 533 bpafter editing by using the BioEdit program 7.2 (Fig 3). The genetic distance estimated between *acanthocephalan* species used for phylogenetic analysis varied from 0.025 to 0.194 (Table 2). The nucleotide composition was as follows: 24.95% T, 28.57% C, 25.67% A, and 20.79% G are shown in (Table 3).

As a result, Fig 4 shows the Neighbor Joining tree. The 18S rDNA dataset's phylogenetic tree reveals that *N. iraqensis* in the separation position in this tree.

4. DISCUSSION

There have not been any studies on molecular identification for *Neoechinorhynchus iraqensis* to classify it; it was only characterized morphologically by Amin et al [7,13] and Ali [14]. This study investigated *N. iraqensis*, which was identified for the first timeby molecular technique and compared tothe same genusand otheracanthocephalan species recorded in the Genbank.

The eleven species of *Neoechinorhynchus* reported according to GBIF [15] so far in freshwater fish in Iraq are: *N. australis* (Van Cleave, 1931), *N. chilkaensis*, (Podder, 1937), *N. cristatus* (Lynch, 1936), *N. dimorphospinus* (Amin and Sey, 1996), *N. iraqensis* (Amin, Al-Sady, Mhaisen& Bassat, 2001), *N. macronucleatus* (Machado Filho, 1954), *N. rutili* (Mu¨ller, 1780 re described by Hamann in Stiles and Hassall, 1905), *N. zabensis* (Amin, Abdullah &Mhaisen, 2003), *N. tigrisensis* (Al-Ayash, Gustinelli, Al-Nasiri & Caffara, 2021), *N. planilizai*(Al-Ayash, Gustinelli, Al-Nasiri & Caffara, 2021) and *N. Barbi* (Al-Ayash, Gustinelli, Al-Nasiri & Caffara, 2021).

In the present study, the low value of genetic distance recorded between*N. iraqensis*, *Neoechinorhynchus* sp. GL-2015(KU363972.1) and *Neoechinorhynchus* sp. XL-2014 (KM507363.1) was0.025 andthe high value of genetic distance was recorded between *N. iraqensis* and *Tenuisentisniloticus* (MZ727194.1) was 0.194. Phylogenetic study revealed that *N. iraqensis* takes a separate location within the

Table 1. Acanthocephala, host and accession numbers used for phylogenetic analysis based on the 18S rDNA region

Acanthocephala	Host	GenBank accession no.
Neoechinorhynchus sp. GL-2015	*Capoeta aculeata*	KU363972.1
Neoechinorhynchus saginata	Na	AY830150.1
Neoechinorhynchus pseudemydis	Na	U41400.1
Neoechinorhynchus crassus	*Capoeta aculeata*	KU363974.1
Neoechinorhynchus qinghaiensis	Na	MW144440.1
Neoechinorhynchus cylindratus	*Micropterus salmoides*	MF974925.1
Neoechinorhynchus buttnerae	Na	MW590330.1
Neoechinorhynchus buttnerae	Na	MK249749.1
Neoechinorhynchus sp. XL-2014	BH-liaoning	KM507363.1
Neoechinorhynchus crassus	Na	AF001842.1
Neoechinorhynchus sp. JDC-2005	Na	DQ181946.1
Neoechinorhynchus personatus	*Mugil cephalus*	MT020795.1
Neoechinorhynchus personatus	*Mugil cephalus*	MT020793.1
Neoechinorhynchus pseudemydis	Capoeta aculeata	KU363973.1
Neoechinorhynchus agilis	*Mugil cephalus*	MN705824.1
Neoechinorhynchus simansularis	Na	KF156877.1
Neoechinorhynchus sp. JYW-2010	*Siganusfuscescens*	HM545898.1
Neoechinorhynchus aldrichettae	*Aldrichettaforsteri*	OM103595.1
Neoechinorhynchus salmonis	Na	KF156878.1
Neoechinorhynchus tumidus	Na	KF156876.1
Neoechinorhynchus beringianus	Na	KF156875.1
Neoechinorhynchus sp. AC3	*Heteropneustesfossilis*	MF784256.1
Tenuisentis niloticus	*Lates niloticus*	MZ727194.1

❖ *Na= Not Available*

Table 2. Genetic distance between *Neoechinorhynchus iraqensis* and other genus of *Neoechinorhynchus* and Acanthocephalan species

Acanthocephalan species	Genetic distance
Neoechinorhynchus iraqensis	
Neoechinorhynchus sp. GL-2015	0.025
Neoechinorhynchus saginata	0.059
Neoechinorhynchus pseudemydis	0.054
Neoechinorhynchus crassus	0.057
Neoechinorhynchus qinghaiensis	0.054
Neoechinorhynchus cylindratus	0.050
Neoechinorhynchus buttnerae	0.045
Neoechinorhynchus buttnerae	0.047
Neoechinorhynchus sp. XL-2014	0.025
Neoechinorhynchus crassus	0.057
Neoechinorhynchus sp. JDC-2005	0.071
Neoechinorhynchus personatus	0.086
Neoechinorhynchus personatus	0.086
Neoechinorhynchus pseudemydis	0.053
Neoechinorhynchus agilis	0.089
Neoechinorhynchus simansularis	0.057
Neoechinorhynchus sp. JYW-2010	0.091
Neoechinorhynchus aldrichettae	0.093
Neoechinorhynchus salmonis	0.065
Neoechinorhynchus tumidus	0.061
Neoechinorhynchus beringianus	0.064
Neoechinorhynchus sp. AC3	0.095
Tenuisentis niloticus	0.194

Table 3. Nucleotide composition for *Neoechinorhynchus iraqensis* and other acanthocephalan species recorded in Genbank

Acanthocephalan species	T	C	A	G
Neoechinorhynchus iraqensis	24.954	28.571	25.678	20.795
Neoechinorhynchus sp. GL-2015	25.836	20.057	27.252	26.856
Neoechinorhynchus saginata	25.845	19.542	27.908	26.705
Neoechinorhynchus pseudemydis	26.441	19.379	27.627	26.554
Neoechinorhynchus crassus	26.509	20.069	26.740	26.682
Neoechinorhynchus qinghaiensis	25.899	19.927	27.300	26.874
Neoechinorhynchus cylindratus	25.916	19.987	26.849	27.249
Neoechinorhynchus buttnerae	26.442	20.052	27.065	26.442
Neoechinorhynchus buttnerae	25.888	19.853	27.580	26.678
Neoechinorhynchus sp. XL-2014	25.730	20.000	27.303	26.966
Neoechinorhynchus crassus	25.622	20.474	26.778	27.126
Neoechinorhynchus sp. JDC-2005	25.634	20.828	26.569	26.969
Neoechinorhynchus personatus	26.615	19.844	26.848	26.693
Neoechinorhynchus personatus	26.714	20.016	26.556	26.714
Neoechinorhynchus pseudemydis	26.507	20.364	26.962	26.166
Neoechinorhynchus agilis	26.416	19.976	26.237	27.370
Neoechinorhynchus simansularis	26.452	19.140	26.667	27.742
Neoechinorhynchus sp. JYW-2010	26.545	19.613	26.814	27.028
Neoechinorhynchus aldrichettae	27.314	19.364	26.405	26.917
Neoechinorhynchus salmonis	26.372	19.268	26.480	27.879
Neoechinorhynchus tumidus	25.781	19.531	26.674	28.013
Neoechinorhynchus beringianus	26.316	19.261	26.316	28.108
Neoechinorhynchus sp. AC3	25.952	19.940	27.555	26.553
Tenuisentis niloticus	26.811	20.289	26.268	26.630
Average	26.238	20.019	26.924	26.820

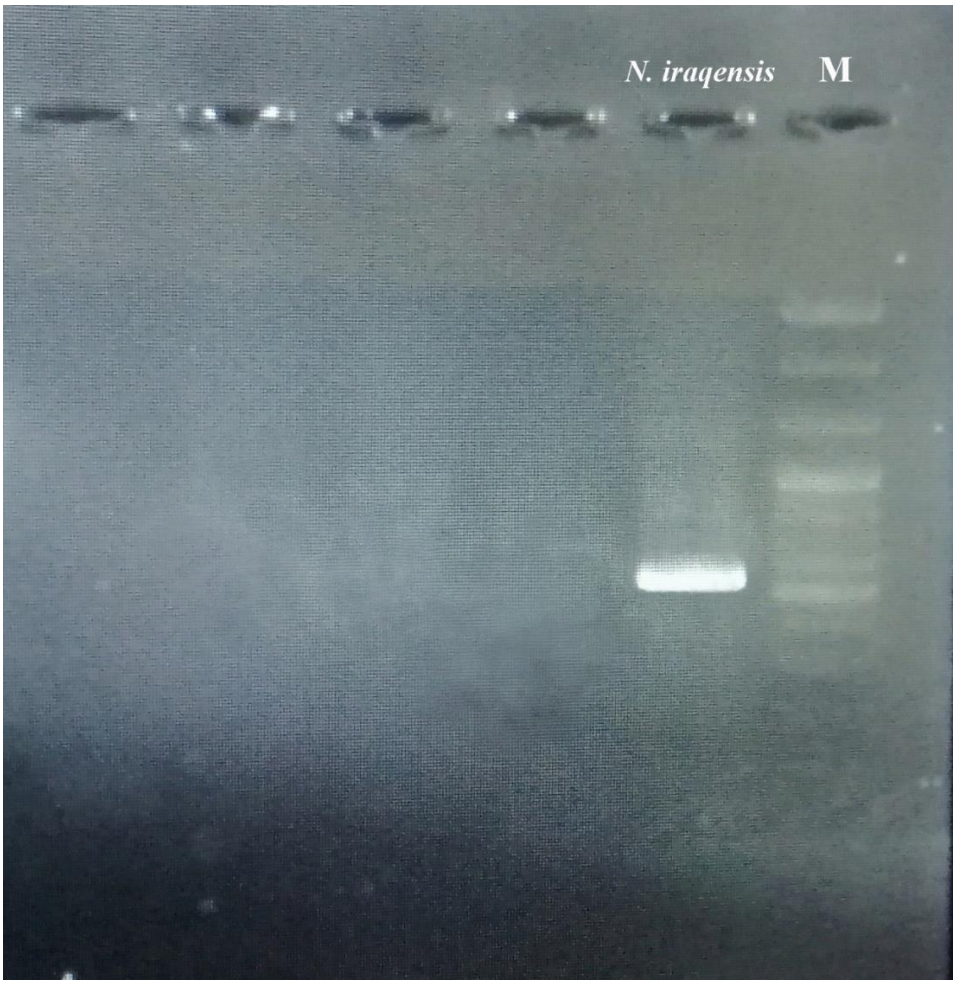

Fig. 3. PCR product for *Neoechinorhynchus iraqensis* from *Planiliza abu* fish. M: DNA ladder. The size of band = 533 bp

trees, denoting that this species originated in Iraq because it wasoriginally discovered and describedby [7]. *Neoechinorhynchus* is a genus that contains a wide variety of species, as already noted in several studies [16,17] and [18]. According to a phylogenetic tree study, *N. zabensis* is in a separate position from other species [14]. Phylogenetic study showed that *N. johnii* has a distinct location in the trees, most likely indicating that this species originated in Asia [19]. Nuclear DNA sequences of the 18S rDNA gene and mitochondrial DNA sequences of COX1 of *N. poonchensis* sp. n. have been amplified and matched with other sequences on GenBank.

N. poonchensis sp. n. was shown to be nested in a separateposition based on ML and BI method calculated for 18S rDNA and cox1 [20].For the 18S rRNA gene, the estimated genetic difference between the *Neoechinorhynchus* species varied from 0 to 2.5%. [21]. The genetic identification of the species *N. personatus* and *N. yamagutii* in gray mullets fish that are caught in the Atlantic and Pacific Oceans was reported by Sarabeev et al [22].The intra specific differences ranged from 0.01 to 0.02%, while the genetic divergence between *N. schmidti* and *N. emyditoides* was 4%. The pairwise variations ranging from 9.5 to 33% were found amongthese two species and four other congeners that are parasitic in fresh and brackish water fishes: *Neoechinorhynchus golvani*, *Neoechinorhynchus roseum*, *Neoechinorhynchus saginatus* and *Neoechinorhynchus* sp. [23]. Using a partial 18S rDNA dataset, the relatively large genetic differences among *N. ponticus* n. sp. and other *Neoechinorhynchus* species confirm its status of independence. *Neoechinorhynchus personatus* and *Neoechinorhynchus ponticus* n. sp. share an ancestry with *Neoechinorhynchus* species that are gathered from saltwater fish [24].

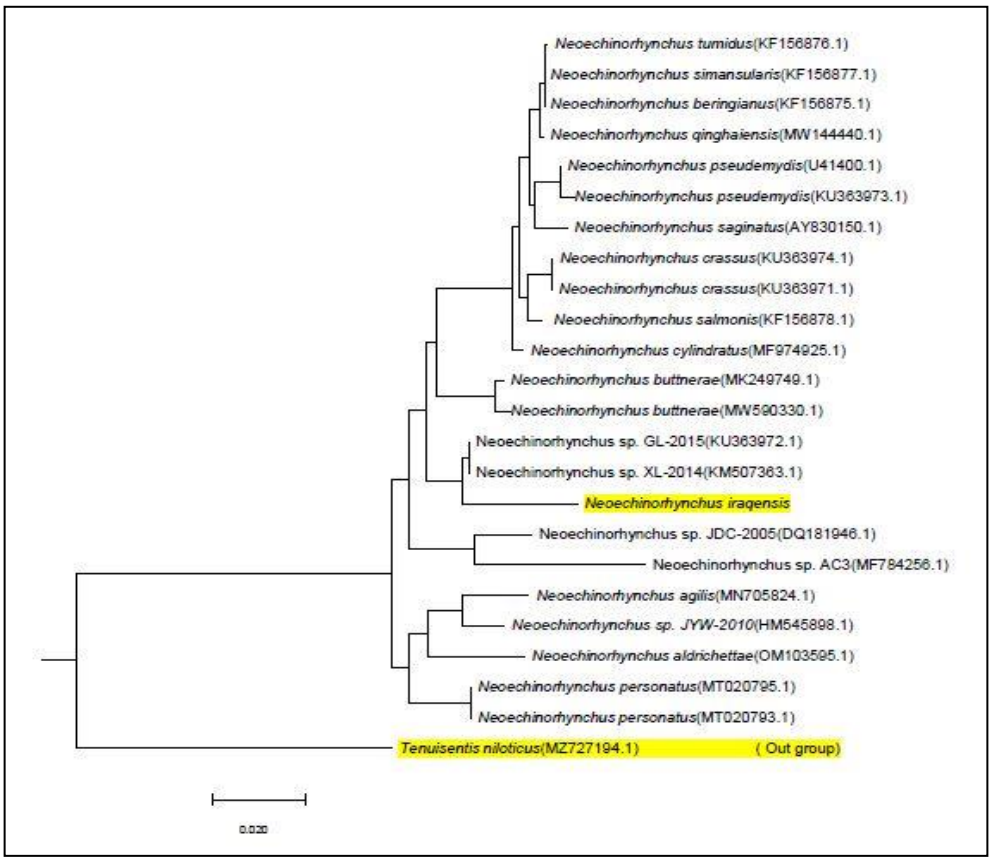

Fig. 4. The phylogenetic tree of *Neoechinorhynchus iraqensis* obtained for the present research and the other genus of *Neoechinorhynchus* were received from GenBank based on the partial 18S rDNA gene. This phylogenetic tree has been created dependent on the neighbor joining tree. *Tenuisentis niloticus*is out group

5. CONCLUSION

The findings of this study demonstrate the importance of identifying *N. iraqensis* using molecular techniques. For a study of the upper-level phylogeny, 18S rDNA was well preserved and appropriate. The results of this research show that *N. iraqensis* occupied an independent position within the trees; this result confirms that the species is the same type that was initially found and identified morphologically, showing that Iraq was the location where this species first appeared.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hoffman GL. Parasites of North American freshwater fishes. 2nd Edition. Cornell Univ. Press, London; 1998.
2. Abdullah SM, Rasheed ARAM. Parasitic fauna of some freshwater fishes from Dokan Lake, North of Iraq. I: Ecoparasites; 2004.
3. Okaeme AN, Olufemi BE, Obiekezie A. Parasites and non-fish predators of tilapia with particular reference to the sustainable management of fisheries of Lake Kainji Nigeria; 2000.
4. Taraschewski H. Host-parasite interactions in Acanthocephala: A morphological approach. Advances in Parasitology. 2000; 46:1-179.
Available:http://doi.org/10.1016/s0065-308x (00)46008-2.
5. Kennedy CR. Ecology of the Acanthocephala. Cambridge: Cambridge University Press. 2006;249.
6. Mhaisen FT. Index-catalogue of parasites and disease agents of fishes of Iraq; 2023.

Available:(Unpublished: xxx@yahoo. co. uk)

7. Amin OM, Al-sady RS, Mhaisen TF, Bassat SF. *Neoechinorhynchus iraqensis* sp. n. (Acanthocephala: Neoechinorhynchidae) from the Freshwater Mullet, *Liza abu*. *Comp. Parasitol*. 2001;68(1):108-111.
8. González-Castro M, Ghasemzadeh J. Morphology and morphometry based taxonomy of Mugilidae. In: Crosetti D,Blaber S, editors. Biology, ecology and culture of grey mullet (Mugilidae). CRC Press, Boca Raton, USA. 2015;1-21.
9. Fricke R, Eschmeyer WN, Fong JD. Eschmeyer's Catalog of Fishes Species by family/subfamily. Online Version; 2021.
   Available:http://researcharchive.calacademy.org/ research/ ichthyology/ catalog/ Species ByFamily.asp).
10. Froese R, Pauly D. (eds.) FishBase. World Wide Web Electronic Publication; 2023. Available:www.fishbase.org, version (02/2023).
11. Amin OM, Abdullah SMA, Mhaisen FT. *Neoechinorhynchus* (Neoechinorhynchus) *zabensis* sp. n. (Acanthocephala: Neoechinorhynchidae) from freshwater fish in northern Iraq. *Folia parasitologica*. 2003;50(4):293-297.
    Available:https://doi.org/10.14411/fp.2003.048
12. Kumar S, Stecher G, Tamura K. Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 2016;33:1870–1874.
13. Abdullah YS. Study on the parasites of some fishes from Darbandikhan Lake in Kurdistan Region, Iraq. Master of Science, Collage of Science Biology, University of Sulaimani; 2013.
14. Ali MK. Morphological and molecularly studies on the Genus *Neoechinorhynchus* Stiles and HASSALL, 1905 from two freshwater fishes in sulaimani province. doctor of philosophy in fish parasitology, collage of agricultural engineering sciences, University of Sulaimani; 2020.
15. GBIF.org. GBIF Home page; 2023.
    Available:http://www.gbif.org.
16. Amin OM. Classification of the Acanthocephala. Folia Parasitologica. 2013;60(4):273-305.
    Available:https://doi:https://doi.org/10.14411/fp.2013.031
17. Pinacho-Pinacho CD, García-Varela M, Sereno-Uribe AL, Pérez-Ponce de León G. A hyper-diverse genus of acanthocephalans revealed by tree-base and non-tree based species delimitation methods: Ten cryptic species of *Neoechinorhynchus*in Middle America freshwater fishes', Molecular Phylogenetics and Evolution. 2018;127:30-45.
18. Pinacho-Pinacho CD, Hernández-Orts JS, Sereno-Uribe AL, Pérez-Ponce de León G, García-Varela M. *Mayarhynchus karlae* n. g., n. sp. (*Acanthocephala: Neoechinorhynchidae*), a parasite of cichlids (Perciformes: Cichlidae) in southeastern Mexico, with comments on the paraphyly of *Neoechynorhynchus* Stiles & Hassall, 1905'Systematic Parasitology. 2017;94(3): 351-365.
19. Amin OM, Chaudhary A, Heckmann R, Ha NV, Singh HS. Redescription and molecular analysis of (*Neoechinorhynchus*) *johniiYamaguti*, 1939 (*Acanthocephala, Neoechinorhynchidae*) from the Pacific Ocean off Vietnam. Parasite. 2019;26:43.
20. Amin OM, Ahmed M, Chaudhary A, Heckmann RA, Singh HS. The morphological and molecular description of *Neoechinorhynchus* (*Neoechinorhynchus*) *poonchensis*sp. n. from *Schizothoraxrichardsonii* (Gray) in Poonch, Jammu and Kashmir, India. Folia Parasitol. 2022;69: 001.
21. Malyarchuk B, Derenko M, Mikhailova E, Denisova G. Phylogenetic relationships among *Neoechinorhynchus* species (*Acanthocephala: Neoechinorhynchidae*) from North-East Asia based on molecular data', Parasitology International. 2014;63:100–107.
22. Sarabeev V, Tkach Ie, Sueiro RA, Leiro J. Molecular data confirm thespecies status of *Neoechinorhynchus personatus* and *N. yamagutii* (*Acanthocephala, Neoechinorhynchidae*) from the Atlantic and pacific grey mullets (Teleosteı, Mugilidae). Zoo Diversity. 2020;54(1):1–10.
23. García-Varela M, García-Prieto L, Pérez Rodríguez R. Molecular identification and first description of the male of *Neoechinorhynchusschmidti* (Acanthocephala:Neoechinorhynchidae), a parasite of *Trachemys scripta* (Testudines) in

México. Parasitology International. 2011; 60(4):433-439.

24. Amin OM, Sharifdini M, Heckmann RA, Rubtsova N, Chine HJ. On the *Neoechinorhynchus agilis* (*Acanthocephala : Neoechinorhynchidae*) complex, with a description of *Neoechinorhynchus ponticus*n. sp. from *Chelon auratus*in the Black Sea. Parasite. 2020;27(48): 119.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3359