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REDESCRIPTION AND MOLECULAR ANALYSIS OF *NEOECHINORHYNCHUS* (*NEOECHINORHYNCHUS*) PLANILIZAI (ACANTHOCEPHALA, NEOECHINORHYNCHIDAE) *FROM PLANILIZA ABU* (MUGILIDAE) IN IRAQ

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Redescription and molecular analysis of *Neoechinorhynchus* (*Neoechinorhynchus*) *planilizai* (Acanthocephala, Neoechinorhynchidae) from *Planiliza abu* (Mugilidae) in Iraq. Amin, O. M., Chaudhary, A., Caracciolo, M. E., Ali, A. H., Al-Kuraizy, M. H., Rubtsova, N. Y. & Singh, H. S. — *Neoechinorhynchus* (*N.*) *planilizai* Al-Ayash, Gustinelli, Al-Nasiri, Caffara, 2021 was recently described from *Planiliza abu* (Heckel) in Iraq using line drawings. We update and complete that description using new ocular microscopy, SEM, and molecular analysis. New collections revealed structures that have not been previously reported including constricted proboscis, posterior hooks longer than middle hooks, bursa without sensory structures, spongy hypodermic tissue with lacunar channels under smooth cuticular surface, eggs with no fibrils, variable micropores, single ventral para-receptacle structure, large triangulate cephalic ganglion, a new papilla at the posterior tip of the proboscis receptacle, an anterior testis distinctly longer than posterior testis, short robust uterus a huge pot-shaped uterine bell, and two sets of ligament bundles emerging from the inner posterior tip of the trunk. No other taxonomic accounts of this species are known. The molecular analysis of *N. planilizai* is also described with sequences of the 18S nuclear ribosome generated and

aligned with closely related sequences from GenBank database. Maximum likelihood and Bayesian inference analyses demonstrated that *N. (N) planilizai* was nested within neoechinorhynchid clades. The presence of *N. (N) planilizai* with other Middle East species in the tree allowed its characterization for the first time using morphological and molecular features.

Key words: scanning electron microscopy, 18S rDNA, phylogenetic relationships, para-receptacle structure, Tigris River, fish parasites.

Introduction

Neoechinorhynchus (*N.*) *planilizai* Al-Ayash, Gustinelli, Al-Nasiri, Caffara, 2021 has been rarely reported since its morphological description from the Abu mullet *Planiliza abu* [Heckel] (Mugilidae) and infrequently from the Tigris catfish *Silurus triostegus* [Heckel] (Siluridae) by Al-Ayash (2021). It was first erroneously reported as *N. lizai* from *P. abu* by Al-Ayash (2020) in the Tigris River and from *Silurus triostegus* by Al-Ayash (2020) in his unpublished Ph. D. thesis and later formally by Al-Ayash et al. (2021). The description and 8 lines drawings in both accounts were very similar but the dissertation additionally included 15 uninformative microscopic images.

In his dissertation, Al-Ayash (2020) reported *N. planilizai* from 17.7% of *S. triostegus* and from 95.5% of *P. abu. Silurus triostegus* was infected with a large number of endohelminth parasites including 20 trematode species, 16 cestodes, 8 nematodes and seven acanthocephalans including *Echinorhynchus* sp., *Neoechinorhynchus iraqensis* Amin, Al-Sady, Mhaisen and Bassat, 2001, *N. planilizai* (reported as *N. lizai*), *N. rutili* (Müller, 1780) Hamann, 1892, *N. zabensis* Amin, Abdullah, Mhaisen, 2003, *Neoechinorhynchus* sp. and *Pomphorhynchus spindletruncatus* Amin, Abdullah, Mhaisen, 2003, (Mhaisen, Personal Comm.). In addition to the 3 new species of *Neoechinorhynchus* Stiles and Hassall, 1905 described by Al-Ayash et al. (2021) (*N. barbi*, *N. planilizai*, *N. tigrisensis*), they reported "additional" species of marine and freshwater acanthocephalans. Amin et al. (2023) reviewed the acanthocephalan species recorded from fishes off Iraq and pointed that some acanthocephalan species so far recorded come from misidentification.

In the present research, specimens of *Neoechinorhynchus planilizai* was collected from hosts in the Tigris River at Salah Al Din province, Iraq and characterize it by using the morphology and molecular data to study the phylogenetic position within the genus *Neoechinorhynchus*.

Material and Methods

Collections

Originally, A total of 451 fish belonging to 16 species and 5 families were collected from February to December, 2018 in the Tigris River at Salah Al Din province between Tikrit city (34°25'39" N and 44°20'45" E) and Al-Dhuluiya city (34°20'44" N and 44°15'40" E) north of Baghdad, mid Iraq. Sixty five of 68 (95%) and 6 of 34 (18%) individuals of *P. abu* and of *S. triostegus*, respectively, were infected with 361 and 15 specimens of *N. iraqensis* and *N. planilizai*, respectively (Al-Ayash, 2020; Al-Ayash et al., 2021). Thirteen of the 68 *P. abu* individuals (19%) were also infec-

ted with 15 specimens of *Neoechinorhynchus zabensis* and 2 of the 34 examined individuals of *S. triostegus* (6%) were infected with 3 specimens of *Neoechinorhynchus* (*N.*) *iraqensis*.

Most recently in 2024, one of us (OA) received 929 acanthocephalans collected from 274 of 884 examined individuals (31%) of *P. abu* (Table 1) in the Al-Gharraf River, Thi Qar Province, southern Iraq (46°17'45" to 46°11'64" E and 31°44'60" to 31°58'74" N) collected between December 2022 and December 2023 (Table 1). These specimens were mostly *N. iraqensis* but some belonged to *N. planilizai*.

Management of specimens

Whole mounted specimens of the first two collections were used for ocular microscopy images. Six specimens of the third collection were used for SEM, and three specimens for molecular analysis.

Processing for microscopy

Worms from representative samples of the original and subsequent collections were stained in Mayer's acid carmine, destained in 100% ethanol and 4% HCL, dehydrated in ascending concentrations of ethanol, cleared in 100% xylene and then in 50% xylene and 50% Canada balsam each for 24 hr. and whole mounted in Canada balsam.

Table 1. **Collections of *Neoechinorhynchus iraqensis* and a few *N. planilizai* from Abu mullet *Planiliza abu* (Heckel, 1843) in Al-Gharraf River, Thi Qar Province, Southern Iraq**

No	Months, years	Number examined fish	Number infected fish	Number of parasite	Prevalence of infection%	Intensity of infection
1	December–2022	23	–	–	–	–
2	January–2023	8	3	12	37.5	4.0
3	Feb–2023	16	2	4	12.5	2.0
4	March–2023	10	7	15	**70.0***	**21.4**
5	April–2023	22	12	44	**54.5**	3.7
6	May–2023	103	76	464	**73.8**	6.1
7	June–2023	174	94	262	**54.0**	2.8
8	July–2023	94	39	69	41.5	1.8
9	August–2023	96	4	4	4.16	1.0
10	September–2023	90	13	13	14.1	1.0
11	October–2023	106	–	–	–	–
12	November–2023	71	17	30	23.9	1.8
13	December–2023	71	7	12	9.9	1.7
Total or mean		884	274	929	31.0	3.4

*Extreme parameters are bolded.

Deposit and registration

Type specimens were deposited by Al-Ayash in the collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic (IPCAS A-124). ZooBank registration: urn:1sid:zoobank.org: act: FF60A87E-10F7-41D2-B1EB-26A53C66ED33.

Optical microscopy

Images created for this presentation using optical microscopy were acquired using a Zeiss Axioskop Transmitted Nomarski DIC Phase Contrast Microscope Trinocular (Munich, Germany) and a Canon T3i EOS 600D DSLR Camera (Melville, New York). Images from the microscope were transferred from the laptop to a USB and stored for subsequent processing on a computer.

Scanning Electron Microscopy (SEM)

Fixed parasite specimens in 70% ethanol were prepared following established protocols (Lopes Torres et al., 2013), which included sequential dehydration through an ethanol gradient (70% — absolute), critical point drying LEICA EM CPD 300 (Leica Microsystems, Wetzlar, Germany), and subsequent mounting on alumimium stubs using conductive double-sided carbon tape. Eggs samples were extracted from fixed adult females and adhered to glass coverslips using bovine skin gelatin (Sigma-Aldrich, St. Louis, Missouri, USA), then processed using the same protocol applied to the parasite specimens. All samples were sputter-coated with a gold layer approximately 20 nm in thickness using a DII-29010SCTR Smart Coater (JEOL, Akishima, Japan). Observations were performed using a JEOL JSM-IT500HR SEM (JEOL, Akishima, Japan). Images were taken at various magnifications. Samples were received under an acceleration voltage of 10 kV.

Experimental protocol

Fish were collected periodically in the field using seins then transferred on ice to the laboratory in the same day for processing. They were subsequently anesthetised before the intestinal tracks were removed for dissection, with a longitudinal slit, and parasite recovery. Acanthocephalans were placed in refrigerated water over night or until the proboscides were everted then placed in cold 70% ethanol for fixing and transport.

Molecular methods

Two specimens of *N. planilizai* that were placed in tubes and stored at –20 °C were used for the DNA extraction. The genomic DNA was extracted using DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. The 18S region of nuclear ribosomal DNA (rDNA) was amplified by polymerase chain reaction (PCR). The forward and reverse primers for the 18S gene were Worm A and 1270R and 930F and Worm B (Littlewood and Olson, 2001) respectively. PCR reactions (25 µl) consisted of 1.2 µl of each primer, 2.5 µl of 10 X buffer with $MgCl_2$, 2.0 µl of dNTPs (10 mM), 1 U of Taq DNA polymerase (Invitrogen) with 3.5 µl of the genomic DNA and 13.6 µl of distilled water. The PCR cycling parameters for 18S rDNA

amplifications were followed as: denaturation at 94 °C for 3 minutes; 30 cycles of 94 °C for 1 minute; annealing at 56 °C for 50 seconds and an extension at 72 °C for 1 minute with a final extension at 72°C for 7 minutes. The sequencing reactions were performed using an ABI BigDye Terminator Kit (Applied Biosystems, USA) and amplification reaction products were detected using an ABI 3730 capillary DNA Sequencer. The 18S sequences were assembled using Codoncode Aligner version 5.1.2.

The sequences generated for 18S were aligned with other sequences downloaded from GenBank database (available on https://www.ncbi.nlm.nih.gov/). *Polyacanthorhynchus* Travassos, 1920 was used as the outgroup. Sequences for 18S molecular marker were aligned by the software Clustal W with default parameters implemented in MEGA version 11 (Tamura et al., 2021). The Akaike Information Criterion (AIC) implemented in MEGA version 11 was used to detect the best-fitting nucleotide substitution model and GTR + G + I was estimated as the best model. The 18S phylogenetic analyses were run using the maximum likelihood (ML) and Bayesian Inference (BI) methods using MEGA version 11 and TOPALi version 2.5 (Milne et al., 2009) respectively. ML tree 10,000 bootstrap replicates were run to evaluate nodal support. For BI analysis, running two independent Markov Chain Monte Carlo (MCMC) chains runs of four chains each for 10^6 generations, with sampling every 1000 generations with a burn-in of 25%. The genetic divergence among taxa was assessed using uncorrected 'p' distances in MEGA version 11. The accession numbers for the sequences generated here for 18S rRNA gene have been deposited in NCBI GenBank with the following accession numbers: PX506235, and PX506229.

Results

The structures and measurements of our newly collected specimens of *N. planilizai* were comparable to those in the original description by Al-Ayash et al. (2021, p. 671) that were, however, incomplete and inadequately documented. Our account of the anatomical structures was generally similar but much more detailed and restructured compared to their line drawings figures 2A–G (p. 672), with marked variations. We describe new morphological and anatomical observations of structures that were not reported in the original description constituting a redescription of *N. planilizai.* In addition to the original measurements and inadequate description, we redescribe below our new observations of the external morphology of specimens using SEM (Figs 1, *a–f*; 2, *a–f*) and the internal anatomy using ocular microscopy (Figs 3, *a–e*; 4, *a–c*).

Morphology of *N. planilizai* using SEM

The use of SEM revealed additional features not previously reported in the original description and are herein described for the first time in figures 1, 2. Specimens were long, smooth, thin and cylindrical, showing considerable sexual dimorphism in trunk size, among other characters, with males reaching 28 mm long compared to females reaching 80 mm long. In males, the proboscis was constricted between the middle and posterior hooks; all hooks were deeply recessed (Fig. 1, *a*). The bulbous anterior proboscis had hooks in two circles with the apical hook reaching 40 μm in length. The

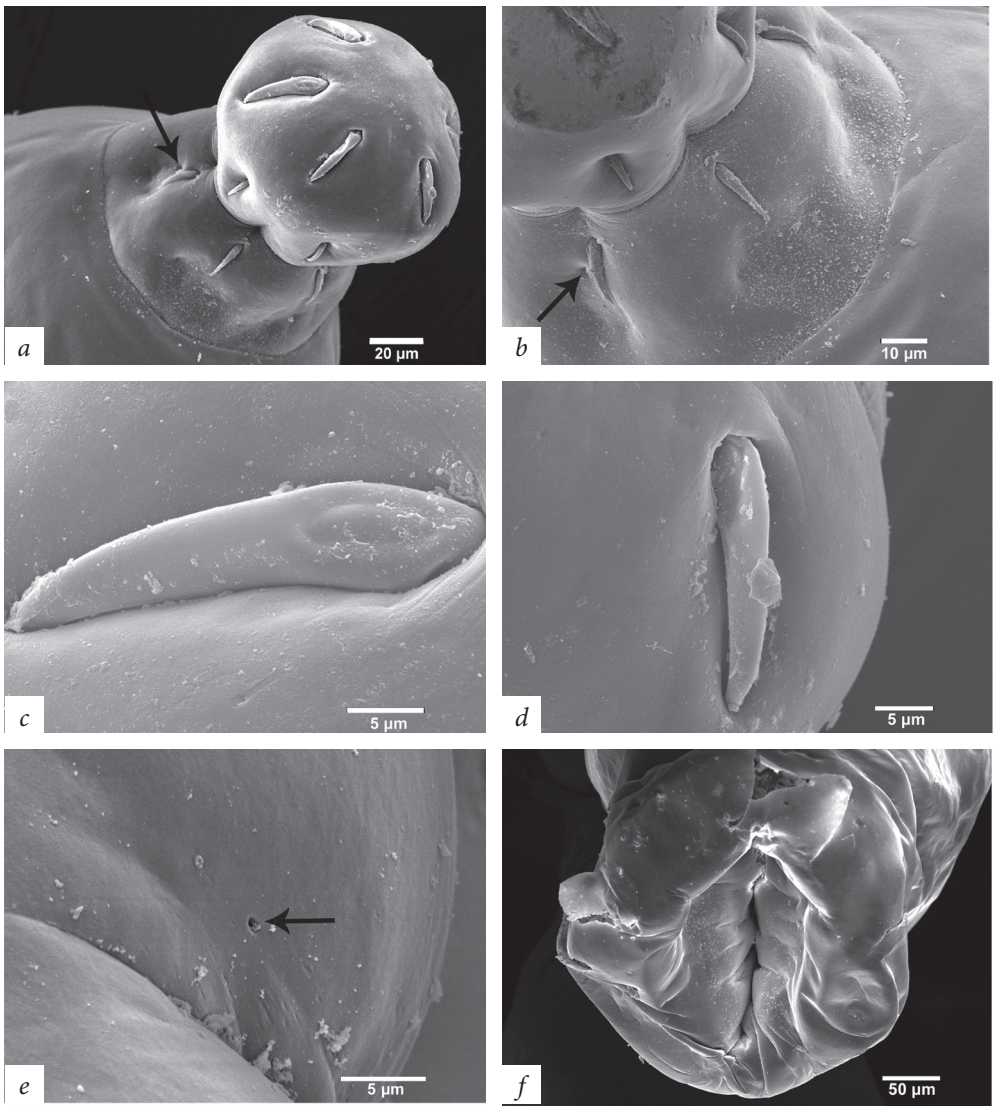

Fig. 1. SEM images of *Neoechinorhynchus planilizai* males collected from *Planiliza abu* in the Tigris River, Iraq; *a* — the proboscis showing recessed hooks, a constriction between middle and posterior hook circles, and the girdle. A sensory pore is marked with an arrow. Posterior hooks are larger than middle hooks. Note depressions in the widening neck; *b* — another perspective of the proboscis and neck marking different size hooks, proboscis constriction, and the girdle. A sensory pore is marked with an arrow; *c* — a high magnification of a recessed apical hook; *d* — a high magnification of a posterior hook; *e* — a sensory pore on the neck; & *f* — a ventral view of the bursa showing no evidence of sensory structures

posterior proboscis fans out into the neck which depicts multiple sensory pores (arrows) and is marked off the anterior trunk with a distinct cuticular thickening forming the girdle (Fig. 1, *a*). Another perspective of the proboscis constriction and the girdle (Fig. 1, *b*) also demonstrate the different size of the middle hooks compared to the longer posterior hooks also demonstrated in fig. 1, *a*. These observations are in stark contrast to the original description where the length of both middle hooks and poste-

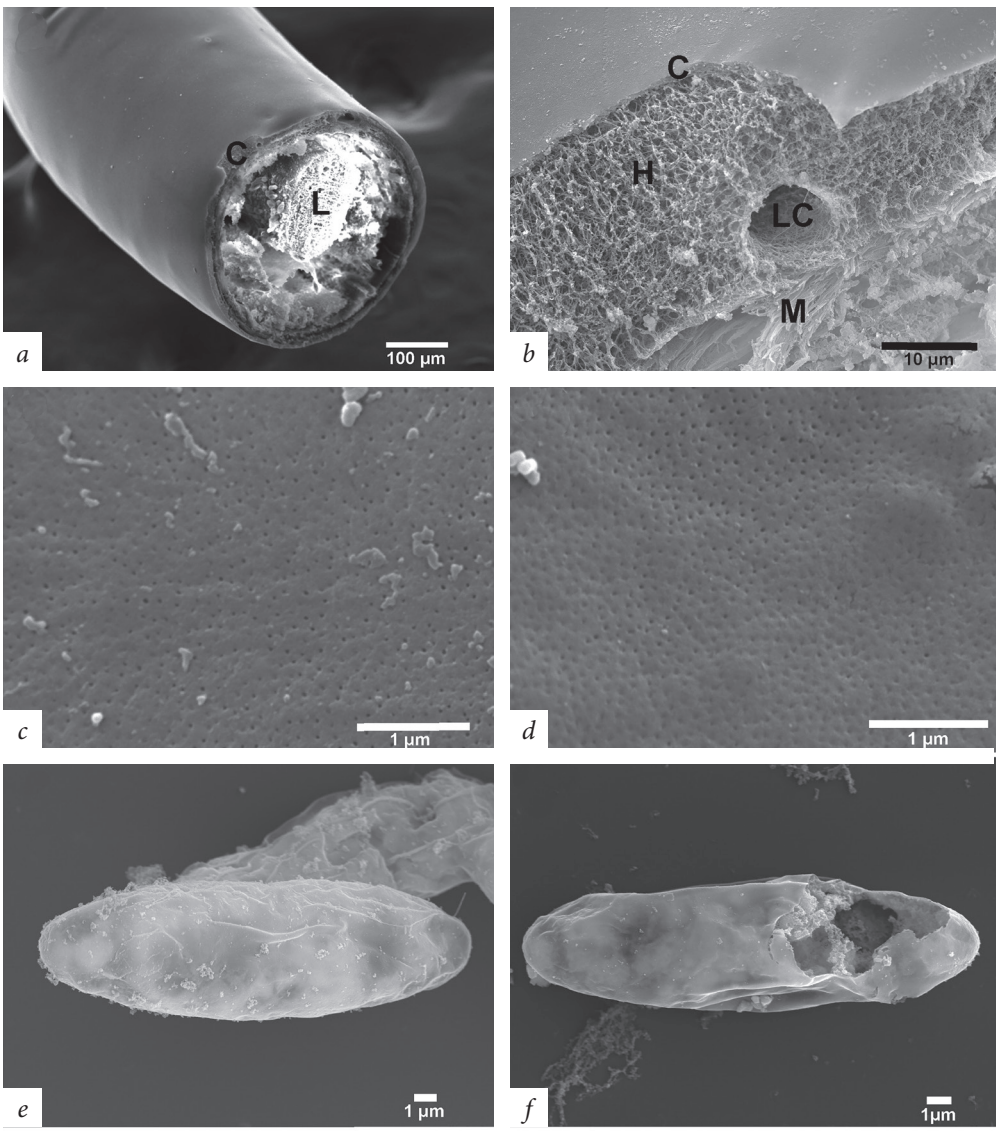

Fig. 2. SEM images of *Neoechinorhynchus planilizai* females collected from *Planiliza abu* in the Tigris River, Iraq; *a* — a cross section in the anterior trunk showing the smooth surface, thin cuticle (C) and disturbed hypodermis, a few eggs, and a section of the large lemniscus (L); *b* — a highly magnified section of the body wall showing the cuticle (C), relatively thick hypodermis (H), a lacunar channel (LC), and the muscular layer (M); *c–d* — micropores from the middle and posterior region of the trunk, respectively. Note differences in pore diameter and distribution corresponding with differential absorption of nutrients; *e* — a fully ripe egg with giant subcutaneous nuclei; *f* — a similar egg with intentionally broken shell to demonstrate the absence of fibrils

rior hooks were stated as equal at 13–19 (16) µm in length as also shown in their Fig. 2, B; they measured 20 males. The apical end of the proboscis did not show the depression that marks the position of the apical organ which is, however, present as seen in microscopic images. An anterior hook deeply recessed in the proboscis (Fig. 1, *c*) and a posterior hook (Fig. 1, *d*), and a neck sensory pore (Fig. 1, *e*, arrow) is

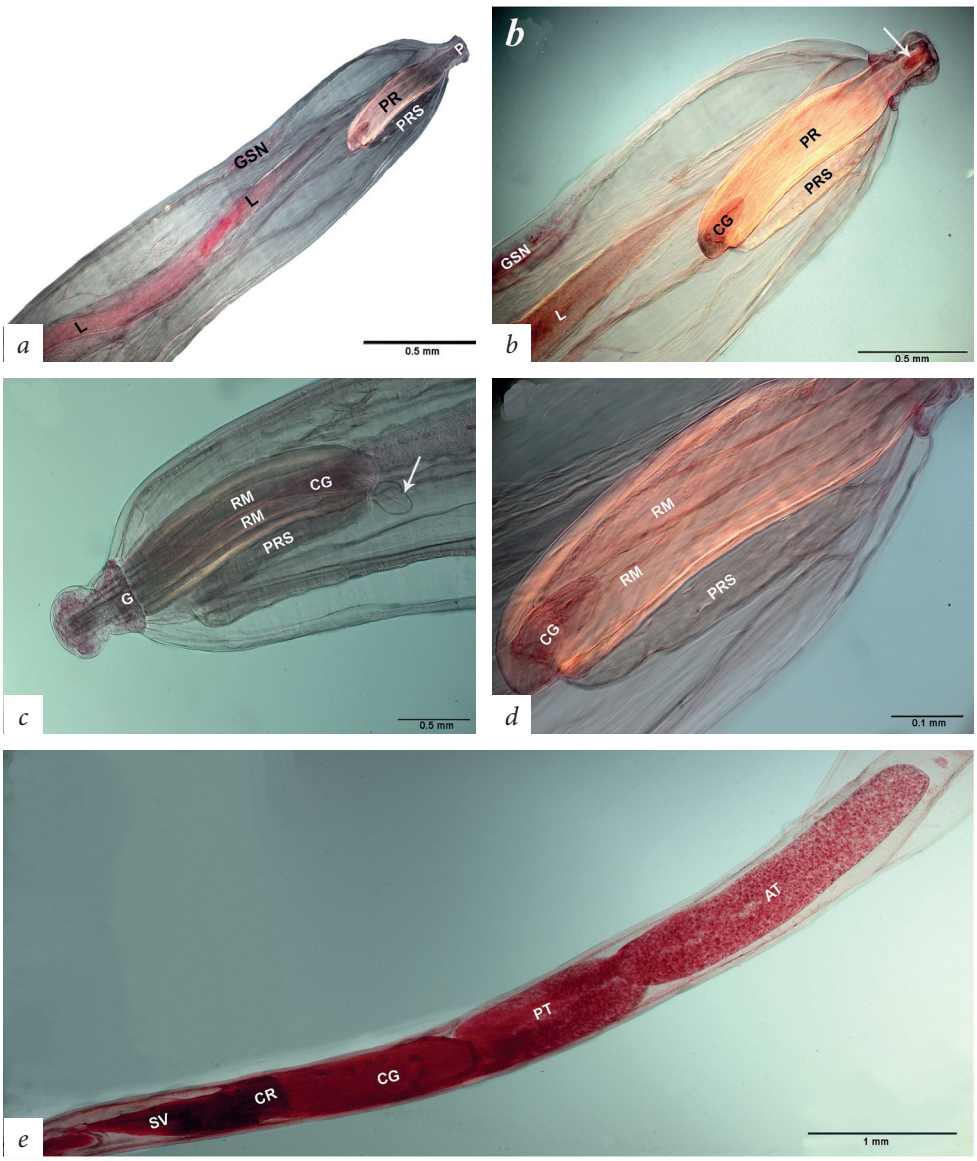

Fig. 3. Ocular microscopy images of *Neoechinorhynchus planilizai* females collected from *Planiliza abu* in the Tigris River, Iraq; *a* — The anterior portion of a female specimen showing the relative size proportions of the proboscis (P), the much larger proboscis receptacle (PR) and the ventrally adjacent para-receptacle structure (PRS), the huge large lemniscus (LL) with one giant nucleus at that location, and one dorsal giant subcutaneous nucleus (GSN); *b* — a higher magnification of the specimen in Fig. 3, *a* showing the proboscis receptacle (PR), para-receptacle structure (PRS) cephalic ganglion (CG), a giant subcutaneous nucleus (GSN), and the lemniscus (L). The arrow points to the apical organ; *c* — a higher magnification of another female specimen emphasizing the retractor muscles (RM), the para-receptacle structure (PRS), the girdle (G), the cephalic ganglion (CG), and the receptacle papillae (arrow); *d* — detail of the receptacle with two sets of retractor muscles (RM), Para-receptacle structure (PRS), and cephalic ganglion (CG); *e* — the male reproductive system including the anterior testis (AT), posterior testis (PT), cement gland (CG), cement gland reservoir (CR), and sperm vesicle (SV)

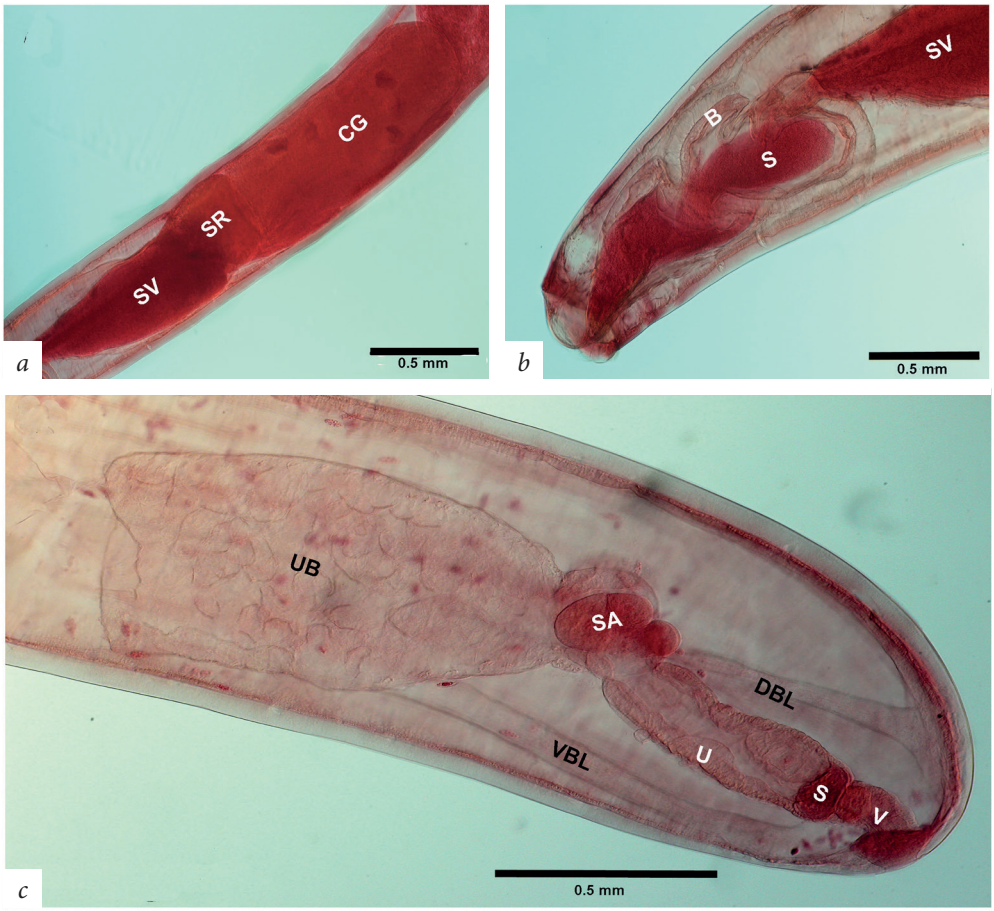

Fig. 4. Ocular microscopy images of *Neoechinorhynchus planilizai* females collected from *Planiliza abu* in the Tigris River, Iraq; *a* — a part of the male reproductive system emphasizing the cement gland (CG) with at least 5 giant nuclei, the cement gland reservoir (CR), and the seminal vesicle (SV); *b* — the posterior part of the male genital terminalia showing the posterior limb of the seminal vesicle (SV), sperm (S), and the invaginated bursa (B; *c* — the complete female reproductive system showing the vagina (V), swelling resembling a sphincter (S), short, thick-walled and robust uterus (U), selective apparatus with a few cells (SA), and large pot-shaped uterine bell (UB). The primary dorsal and ventral bundles of ligaments (DBL and VBL, respectively)

revealed. A basal view of a partially contracted bursa (Fig. 1, *f*) demonstrates its smooth surface and the absence of any characteristic sensory structures.

Females had similar but slightly larger proboscis and hooks than males as shown in our figures 1, *a-d* even though in the original description, the apical hooks were smaller in females (21–35 μm long) compared to 22–40 μm long in males. The morphology of the presoma was similar in both sexes. As in males, females have long cylindrical smooth cuticular surface (Fig. 2, *a*). A cross section of the trunk in the anterior middle section passes by the large lemniscus (L) (Fig. 2, *a*) and a thin section of the sub-cuticular body wall layers that are greatly magnified in Fig. 2, *b*. In that Figure (2, *b*) we observe the thin cuticle (C), the much thicker spongy hypodermis (H) innervated by lacunar channels (LC) over laying the muscle layers (M). Note

that lacunar channels are smoothly drilled emulating blood vessels. Micropores were observed on all anatomical structures. Note differences in size and distribution of micropores in relation to the metabolic absorption of nutrients through the body wall. Absorption rates clearly vary between the middle region of the trunk (Fig. 2, *c*) compared to the posterior region (Fig. 2, *d*). Wright and Lumsden (1969, 1970) and Byram and Fisher (1973) further reported that these are pores of peripheral canals that are continuous, with canalicular crypts. These crypts appear to "constitute a huge increase in external surface area implicated in nutrient up take".Whitfield (1979) estimated a 44-fold increase at a surface density of 15 invaginations per 1 μm^2 of *Moniliformis moniliformis* (Bremser, 1811) Travassos 1915 tegumental surface (Byram and Fisher, 1973). The egg of *N. planilizai* is fusiform-ellipsoid with a smooth surface and apparent subcutaneous giant nuclei (Fig. 2, *e*) which is usually characteristic of the early acanthella indicating that this acanthocephalan species is of precocious nature. A forced break into eggs (Fig. 2, *f*) does not demonstrate any fibrils which is not the case with the very similar *N. iraqensis* eggs, thus adding one more distinguishing factor between the two species.

Anatomy of *N. planilizai* using optical microscopy

Thorough optical microscopic observations of the new specimens revealed many features that have not been previously reported and are herein described for the first time. Figures 3, *a*–4, *c* supplement the original description and overcome the limitations of the 8-line drawings used there. The anterior trunk of a female specimen (Fig. 3, *a*) shows the small size of the proboscis compared to the trunk, the extensive size of the large lemniscus (L), the relatively large receptacle (PR) with the triangulate cephalic ganglion, and a giant subcutaneous nucleus (GSN). The darkly stained para-receptacle structure (PRS) can also be seen on the ventral side of the receptacle. Only one ventral PRS is seen in each specimen. A higher magnification of the anterior trunk (Fig. 3, *b*) shows more detail of the anterior bulbous proboscis enclosing the darkly stained apical organ (arrow), the proboscis receptacle (PR), triangulate cephalic ganglion (CG), and the PRS. Parts of the subcutaneous giant nucleus (GSN) and a lemniscus (L) can also be seen. Figure 3, *c* is comparable to Figure 3, *d* that emphasizes additional structures including the retractor muscles (RM), the girdle (G), and a new structure that is herein named the receptacle papillae (RP) (arrow). The RP are paired ovoid shaped structures that are somewhat constricted at junction with the receptacle and appear to have a branching internal framework. They are noted in the original description (Fig. 2, C), but this structure is not mentioned or discussed. We do not know if the RP have any functional utility, but it certainly does have a taxonomic value to distinguish *N. planilizai* from related species such as *N. iraqensis*. A higher magnification of the proboscis receptacle (Fig. 3, *d*) emphasizes the cephalic ganglion (CG), retractor muscles (RM) and the PRS. Major components of the male reproductive system include the testes a syncytial cement gland (CG), cement reservoir (CR) and ducts, seminal vesicle (CV) and the bursa (Fig. 3, *e*). The anterior testis (AT) is invariably larger and longer than the posterior testis (PT) and the cement gland includes up to 8 giant nuclei. Fig-

ure 4, *a* gives a higher magnification of the cement gland (CG) showing at least 5 giant nuclei, the cement reservoir (CR) and the seminal vesicle (SV). A higher magnification of the posterior end of the male terminalia (Fig. 4, *b*) showing the posterior end of the seminal vesicle (SV) and Saefftigen's pouch (S) also shows the invaginated bursa (B) with sperm. A detail of the female reproductive system (Fig. 4, *c*) demonstrates the angular vagina (V), swelling resembling sphincter (S), short and robust uterus (U), prominent selective apparatus (SA) with a few cells, and the large pot-shaped thick-walled uterine bell (UB) which is longer than the rest of reproductive system. The primary dorsal and ventral bundles of ligaments (DBL & VBL) are also prominent. All the above-mentioned structures are specific and are herein described for the first time in *N. planilizai*, and hence, have important taxonomic value.

Molecular results

The 18S dataset alignment includes sequences representing species of the genus *Neoechinorhynchus*, and *Floridosentis*. The 18S phylogenetic tree inferred from ML and BI analyses shows that the sequences obtained in the present study of *N. planilizai* are nested with other congeners with well supported bootstrap and posterior probability support values (Fig. 5). In the clade I, *N. planilizai* was placed as sister species of *N. iraqensis* from Iraq as the closest relative, while the isolates of *N. planilizai* also revealed the clear inclusion of *N. saginata* (KU363970) and *N. crassus* (KU363971, KU363974) both from Iran and *Neoechinorhynchus* sp. (MK507363) from China, along with *Neoechinorhynchus* sp. (KU363972) from Iran respectively that appears to be most closely related (Fig. 5). The 18S tree reconstruction portrayed clades I, II and III to accommodate *Neoechinorhynchus* species with good bootstrap and posterior probabilities values (BS = 94; PP = 1.00) (Fig. 5). In both analyses, *Neoechinorhynchus* seemed as paraphyletic, clustered in different main clades (Fig. 5). Clade I obtained in this study is nested with species of *Neoechinorhynchus* (from Iran, Iraq, China, Brazil and Vietnam) along with clade II that included the *Neoechinorhynchus* species (from USA, Iran, and China that also comprises *Floridosentis* species from Mexico (Fig. 5). The clade III formed by *N. tumidus* from Russia is in a separate phylogenetic clade with well supported values (Fig. 5). Due to the molecular data constrained by the partial number of sequences and genetic markers employed for *Neoechinorhynchus* species from Iraq, the phylogenetic analysis needs further revisions in the future. The status of the species of the genus *Neoechinorhynchus* required additional information from more representative members of the class Eoacanthocephala (Ru et al., 2022). The present species belongs to clade I and the data related to the species (Clade I, II and III) shows in the table (Table 2). The clade I consists of species *N. planilizai* from present study from Iraq shows the genetic divergence with *N. iraqensis* (from Iraq), *N. crassus, N. saginata* and *Neoechinorhynchus* sp. (KU363972), all from Iran and *Neoechinorhynchus* sp. (MK507363) from China showing the genetic divergence ranging from 4.2 to 8.2%, while only the Iraqi species *N. iraqensis* shows a genetic divergence of 4.2%.

Table 2. **Acanthocephala species, GenBank accession numbers, origin and source used for phylogenetic analysis based on the 18S region. Asterisk* shows data available as unpublished and species sequenced during this study are shown in bold**

Species	GenBank accession no.	Location	Reference
Neoechinorhynchus personatus	MN149067	Spain	Sarabeev et al., 2020
Neoechinorhynchus personatus	MN149068, MN149069, MN149070	Ukraine	Sarabeev et al., 2020
Neoechinorhynchus personatus	MT020794, MT020795	Tunisia	Amin et al., 2020
Neoechinorhynchus agilis	MN148895, MN148896, MN148897, MN148898	Spain	Sarabeev et al., 2020
Neoechinorhynchus yamagutii	MN149220	Russia	Sarabeev et al., 2020
Neoechinorhynchus sp. JYW-2010	HM545898	China	Wang et al., 2010 *
Neoechinorhynchus aldrichettae	OM103593, OM103594, OM103595	Austra-lia	Huston et al., 2022
Neoechinorhynchus dimorphospinus	MK510080	Thailand	Amin et al., 2019 b
Neoechinorhynchus cephali	MN992024	India	Kaur et al., 2020 *
Neoechinorhynchus johnii	MK260005, MK260007	Vietnam	Amin et al., 2019 a
Neoechinorhynchus pseudemydis	KU363973	Iran	Dadar and Adel, 2016 *
Neoechinorhynchus pseudemydis	U41400	USA	Near and Nadler, 1996 *
Neoechinorhynchus qinghaiensis	MW144440	China	Pan, 2020*
Neoechinorhynchus crassus	AF001842	USA	Near et al., 1998
Neoechinorhynchus crassus	KU363969	Iran	Dadar and Adel, 2016 *
Neoechinorhynchus tumidus	KF156876	Russia	Malyarchuk et al., 2014
Neoechinorhynchus cylindratus	MF974925	USA	Blubaugh and Gauthier, 2018 *
Neoechinorhynchus saginata	AY830150	USA	Garcia-Varela and Nadler, 2005
Neoechinorhynchus buttnerae	MK249749	Brazil	Souza and Benavides, 2018 *
Neoechinorhynchus buttnerae	MW590330	Brazil	Soares et al., 2021 *
Neoechinorhynchus sp.	KM507363	China	Liu et al., 2014 *
Neoechinorhynchus sp.	KU363972	Iran	Adel and Dadar, 2016 *
Neoechinorhynchus saginata	KU363970	Iran	Dadar and Adel, 2016 *
Neoechinorhynchus crassus	KU363971	Iran	Dadar and Adel, 2016 *
Neoechinorhynchus crassus	KU363974	Iran	Dadar and Adel, 2016 *
Neoechinorhynchus planilizai	PX506235, PX506229	Iraq	Present study
Neoechinorhynchus iraqensis	PV776638, PV776640	Iraq	Amin et al., 2026
Floridosentis pacifica	MT527841	Mexico	Rosas-Valdez et al., 2020
Floridosentis mugilis	AF064811	Mexico	García-Varela et al., 2000

Discussion

In the present study, *N. planilizai* was identified based on integrated approach, use of morphology and molecular data. Initial differential diagnosis of freshly collected materials was challenging as specimens of this species are often readily confused with the very similar species *N. iraqensis* which is also collected from the same host species *P. abu* in the same waters (Table 1). Worms of both species are similarly long, cylindrical, cord-like, smooth and with similar proboscides. To pick specimens for research purposes, we can only distinguish the two species apart under a dissecting scope against a back-light source when we can find the para-receptacle structure which is found only in *N. planilizai* and not in *N. iraqensis*. Subsequent microscopic examination brings about more anatomical differences such as the pot-shaped uterine bell and the dorsal and ventral ligament bundles in *N. planilizai* compared to the egg fibrils and the beady lining on the inner body wall in *N. iraqensis*, among other differences.

The present study thus advances our knowledge of the diversity of *Neoechinorhynchus* species in West Asia, high-lightening the requirement for keen studies on this group of parasites. Despite the many reports on fish parasites from various Arabian Gulf waterways, ex., Mhaisen (2002) and Mhaisen and Abdul-Ameer (2024), including a few descriptions, none has been so thoroughly studied like *N. iraqensis*. Nevertheless, our study of a good collection of new specimens, demonstrated that the original description of *N. planilizai* did not cover most facets of its morphology and anatomy. This presentation completes its morphological description using ocular microscopy and SEM images for the first time with special references to proboscis hooks, anterior trunk landscape, micropores, para-receptacle structure, lemnisci, reproductive structures topography, and egg anatomy. In the original description, Al-Ayash et al. (2021) mention that "Trunk end (genital terminalia) like round bulb, with big rounded or ovoid sensory papillae." We believe that their "sensory papillae" were misidentified and their Fig. 2, E clearly mislabeled the invaginated bursa and their external "bsp" (bursa sensory papillae).

Our study also adds a molecular confirmation of its identity using the 18S gene. (Fig. 5). Molecular analyses of new acanthocephalan taxa, especially of the genus *Neoechinorhynchus* Stiles and Hassall, 1905 have not been used in the Arabian Gulf region in the past except very recently study on *N. iraqensis* (Amin et al., 2026). We hope that we are introducing a trend of new taxonomic research of the acanthocephalans of fishes in this important region of the Middle East. Within acanthocephalans, *Neoechinorhynchus* is the most diverse genus with about 117 species described already and they are distributed globally (Amin, 2013; Smales, 2013; Pinacho-Pinacho et al., 2014). Despite that, with regard to the molecular data, and compared to the species already described, there is still a gap in molecular studies that needs to be filled. Molecular data from other congeneric species of *Neoechinorhynchus* need to be generated in order to understand their phylogenetic relationships, which will provide a more comprehensive view of the genus. The previous studies of the genus *Neoechinorhynchus* using molecular data, predicted that the genus is paraphyletic (Pinacho-Pinacho et al., 2018), with which we are also agree and it is coherent with present study.

The species examined in the present study as *N. planilizai* from Tigris River, Iraq were nested within clade I that encompasses most of the fish acanthoceph-

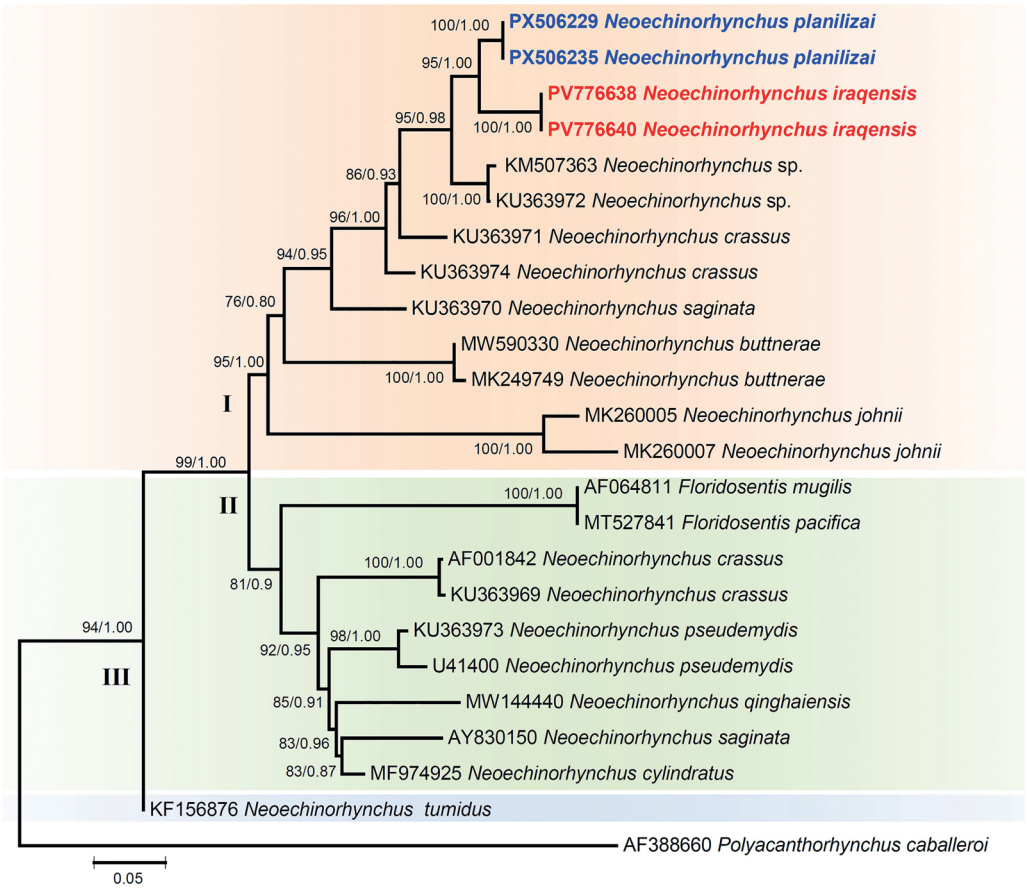

Fig. 5. Phylogenetic relationship among acanthocephalans based on the Maximum Likelihood (ML) and Bayesian Inference (BI) analysis of 18S rDNA gene sequences. Bootstrap values and Posterior probabilities on nodes are shown as maximum likelihood/Bayesian inference. The new sequences generated in this study are in blue. The species mentioned in red belongs to *Planiliza abu* (as same host as *Neoechinorhynchus planilizai*) from the Euphrates River, Iraq. The GenBank accession numbers are provided before the species names and the scale bars indicates substitutions per site. Unsupported nodes by BI are marked with a hyphen

alans. Clade I comprised species analyzed from the Middle East (*Neoechinorhynchus iraqensis, N. pseudemydis*, *N. crassus*, *Neoechinorhynchus* sp. GL-2015 and *N. saginata*) including the present species. The present study's phylogenetic analyses concluded with the 18S gene sequences established the presently studied species *N. planilizai* within congeners. The interspecific genetic divergence estimated on the present study between the isolates of *N. planilizai* with Middle East species in clade I ranged from 4.2 to 8.2%. Furthermore, the phylogenetic trees inferred with the 18S dataset reliably designate that the genus *Neoechinorhynchus* is not monophyletic. Although, in regard to *Neoechinorhynchus* status, polyphyletic or paraphyletic, it still remains an open question. Previous studies support its status as polyphyletic (Ru et al., 2022; Sereno-Uribe et al., 2022), while in another study, *Neoechinorhynchus* was suggested to be paraphyletic (Pinacho-Pinacho et al., 2017). However, Barger (2004) and Barger & Nickol (2004) demonstrated that *Neoechinorhynchus* associated with emydid turtles formed a monophyletic assemblage as did Near

& Nadler (1996 in an un-sourced paper). Our phylogenetic results are consistent with the studies of Malyarchuk et al. (2014), Pinacho-Pinacho et al. (2017), Amin et al. (2019 b), and Kaur & Sanil (2021).

In summary, further study of the distribution of *Neoechinorhynchus* spp. in different Middle East countries necessitates the collection of additional molecular data for a more comprehensive study. The availability of sequence data would lead to a better understanding of their phylogenetic relationships, and to clarify the species systematic positions.

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Authors contribution. Omar Amin did the research, confirmed species validity, wrote the manuscript, and created the optical microscopy images. Anshu Chaudhary did and wrote the molecular part and critically revised the manuscript. Makoto Enoki Caracciolo carried out the processing and execution of the SEM analysis. Atheer Ali and Mohammed Al-Kuraizy proposed the revision and provided research specimens. Nataliya Rubtsova refined and organized all images. Wanderley de Souza provided the SEM microscopy laboratory facilities and scientific support. Singh provided molecular lab facilities in India. All authors reviewed the manuscript and recommended submission.

Conflict of Interest. The authors declare no conflicts of interest.

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