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Original Article

Tubificid worms (Oligochaetes: Naididae) in the sediments of the Euphrates River, Iraq

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Abstract: A total of 1557 tubificid worms were collected from the sediments of six sites on the middle sector of the Euphrates River and its branches. The collected species were identified according to their morphological characteristics as *Branchuria sowerbyi* Beddard, 1892; *Limnodrilus hoffmeisteri* Claparède, 1862; *L. hoffmeisteri* complex IX, *L. claparedianus* Ratzel, 1868; *L. cervix* Brinkhurst, 1963; *Tubifex tubifex* (Müller, 1774); *Ilyodrilus templetoni* (Southern, 1909) and *T. blanchardi* Vejdovský, 1891. *Ilyodrilus templetoni* and *T. blanchardi* were new records for the Iraqi fauna, and their identification was confirmed by molecular analysis using the 18S rRNA gene. Their sequences were deposited in GenBank under accession numbers PQ275504 and PQ275628, respectively.

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Introduction

The family Naididae comprises microdril oligochaete worms, divided into two subgroups: the naidid worms, which are further classified into the subfamilies of Tubificinae Eisen, 1879, Naidinae Ehrenberg, 1828, Telmatodrilinae Eisen, 1879, Limnodriloidinae Erséus, 1982, Phallodrilinae Brinkhurst, 1971, Pristininae Lastočkin, 1921, and Rhyacodrilinae Hrabě, 1963 (van Haaren and Soors, 2013). They are important components of the benthic communities of many freshwater and marine ecosystems. In freshwater aquaria, they may be referred to as detritus worms. Microdrile worms are generally small oligochaetes, typically less than 25 mm long, inhabiting marine, brackish, and freshwater habitats, as well as occasionally wet soils. A total of 13 aquatic microdrile families are known. They have a single-layered clitellum. In contrast, the megadriles, generically termed earthworms (Jamieson, 1988), are characterized by a multilayered clitellum, which is much more apparent than the single-layered clitellum of the microdriles. Members of the family Naididae inhabit the sediments of freshwater surfaces and constitute the largest proportion of freshwater macrofauna. In contrast to other oligochaete species, naidid worms inhabit the water column and graze on aquatic plants rather than burrowing in the sediment; a few species are also predatory (Rodriguez and Reynoldson, 2011).

Marszewska et al. (2017) note that even in a small area with very severe conditions for organisms, a relatively high diversity of tubificid worms can be observed. They are particularly useful as biological indicators of pollution and water quality assessment (Verdonscot, 2007), and they exhibit a wide range of environmental quality variation (Rodriguez and Reynoldson, 2011). They play an important role as food for birds, fish, shrimp, and other invertebrates (Kolesnyk et al., 2019; Lietz, 1987). Oligochaetes are hermaphroditic worms that reproduce by cross-fertilization, whereas some species can reproduce asexually via paratomy or archiotomy (Tim and Martin, 2015).

Morphological identification of naidid species based on many criteria includes body size, color, the number and shape of segments, the number and shape of chaetae in each segment, in addition to the anatomy of the reproductive and digestive system (Timm,

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Table 1. The coordinates of the sampling sites in the current study.

Site number	Latitude	Longitude
S1	33.33.84	43.76.09
S2	32.77.43	44.26.71
S3	32.62.19	44.03.56
S4	32.70.57	44.26.43
S5	32.51.06	44.44.00
S6	32.07.81	44.45.07

2009). Due to the importance of oligochaeta in the aquatic environment, it has been the subject of many research and studies in Iraq (Al-Abbad, 2012; Jaweir and Al-Janabi, 2014; Jaweir, 2014; Jawair and Al-Sarai, 2016; Al-Ameen and Jawair, 2020; Ali and Jaweir, 2020; Zaar and Jaweir, 2022; Obaed, 2022). The lack of molecular studies on them in Iraq, combined with the absence of specialized tubificid lists of Iraqi species, prompted the current study. Hence, this study aimed to identify tubificid worms from the Euphrates River in Iraq using the 18S rRNA gene.

Materials and Methods

Sediment samples were collected from six sites along the main stream of the middle sector of the Euphrates River, and its branches, as well as from some small streams, from February 2023 until March 2024. Figure 1 shows the locations of the study sites, and their latitudes and longitudes are listed in Table 1. Site S1 is located in the main river in Fallujah; Site S2 is also in the main river stream in Al-Musayyib city near Ibn Saif Children's Hospital; Site S3 is the Al-Husseinyah River in Karbala city, which branches off the Euphrates River;, that its surrounding area was a mixture of residential and agricultural areas; Site S4 is located in the Al-Hindiyah Dam area, which is about 9 kilometers away from Al-Musayyib city, and it is characterized by irregular water flow and continuous dredging operation, and surrounded by agricultural land area; Site S5 was in Shatt AL-Hillah in Hillah city, about 100 kilometers south of Baghdad. Finally, Site S6, located in the Al-Abbasiya River in the city of Najaf, upstream of the Al-Abbasiya Dam, is an agricultural area.

A hand shovel was used to collect sediment

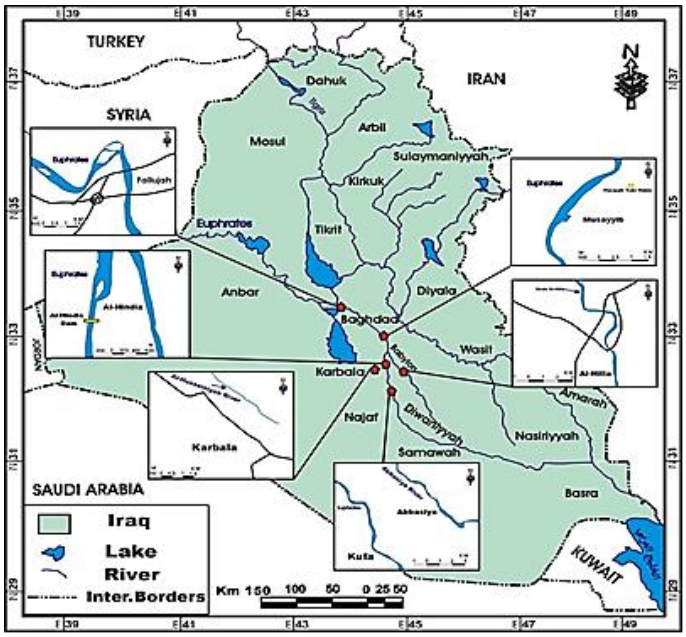

Figure 1. The sampling sites from the Euphrates River in the middle of Iraq.

samples approximately 50 cm from the riverbed at depths of 10 to 20 cm. In the laboratory, the sediment samples were sieved through a 0.25 mm mesh, and the residue was spread onto a white tray with a thin water layer (Witley, 1967). The tubificid worms were then hand-sorted with forceps under a stereomicroscope, identified by their red color, and collected into a small Petri dish containing a small amount of river water. A few drops of 70% alcohol were carefully added to anesthetize the worms, and the worms were then preserved in 70% alcohol (EPA, 1982).

For morphological identification, each preserved worm was examined on a clean glass slide under a microscope and washed with distilled water. A drop of lactophenol was used as mounting medium and covered with a coverslip, with slight pressure applied to the worm to demonstrate the diagnostic characteristics necessary for identification. The

Table 2. primers designed for the current study.

Primer	Sequence	Number	Annealing temp.(^{0}C)	Product size
Forward 18s	GGTCTGTGATGCCCTTAGATGT	22	60	375
Reward 18s	GATCCTTCCGCAGGTTCACCTAC	23	60	375

Table 3. The number of species in each site;, total number in each site, Percentage and Frequency at each species (* = new records).

Tubificids worms	S1	S2	S3	S4	S5	S6	Total number	Percentage %	Frequency %
Branchuria sowerbyi	95	101	18	204	381	45	844	54.21	76.66
Limnodrilus hoffmeisteri	7	166	36	66	84	18	377	24.21	93.33
Limnodrilu claparedianus	-	62	-	17	-	-	79	5.07	26.67
Limnodrilu cervix	-	4	-	-	-	-	4	0.26	6.67
*Ilyodrilus templetoni**	-	3	3	-	6	-	12	0.77	16.66
*Tubifex blanchardi**	-	66	20	21	127	5	239	15.35	60
Tubifex tubifex	-	-	-	-	2	-	2	0.13	3.33
The number of species in each site	2	6	4	4	5	3	Total number = 1557		

prepared slide was left for at least overnight before examination. It was examined under a light microscope to clarify the chaetae, particularly the penial chaetae and the penis sheathes, if present. The prepared slides were analyzed using a compound microscope (Novel model X57-N107T) at 4x, 10x, 40x, and, if necessary, 100x magnification. The morphological criteria were photographed using a Sony a7R IV camera mounted on the compound microscope. The specimens were identified using appropriate keys (Brinkhurst and Jamieson, 1971; EPA, 1982; Timm, 2009; Van Haaren and Soors, 2013).

To confirm the identification of some confused species, molecular identification was performed using genomic DNA extracted with a Genomic DNA Mini Kit (GB100/300) and subjected to PCR amplification and sequencing. The 18S rRNA gene was amplified by PCR using universal primers (Table 2). The sequencing results for the PCR products were obtained using the protocol described by Al-Shuhaib and Hashim (2023). The sequences were edited, aligned, and analyzed in conjunction with the corresponding sequences in the reference database using the BioEdit Sequence Alignment Editor (Version 7.1; DNASTAR, Madison, WI, USA). The sequences were submitted to NCBI to obtain unique GenBank accession numbers. A comprehensive phylogenetic tree was constructed using the neighbor-joining method. The observed variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server (Zhang et al., 2000). Then, a complete, inclusive tree, including the observed variant, was reconstructed using the neighbor-joining method and visualized in BioEdit (v7.2.5).

Results and Discussions

A total of 1557 tubificid worms were collected from six sites, representing seven species; *clusccc*ccc (subfamily Rhyacodrilinae) was the most abundant species, occurring at all study sites. The lowest percentages and frequencies were recorded for *Tubifex tubifex.* This table also shows that S2 was the most diverse site, whereas S1 was the least diverse. Three species of the genus *Limnodrilus* were observed: *L. hoffmeisteri*, the most abundant species with a percentage of 24.21% and frequency of 93.33%, as well as *L. claparedianus* and *L. cervix*.

Two varieties of *L. ho*ffmeisteri were observed, one with the long penis sheath and the other with the short penis sheath. Later, the identification of the latter was confirmed by molecular study as *L. hoffmeisteri* complex IX. This result was also previously recorded by Zaar and Jaweir (2021). Regarding the percentage of two types of *L. hoffmeisteri*, the first one with a long penis sheath was 84.35%, and *L. hoffmeisteri* complex IX, which has a short penis sheath, was 15.64%. In addition, two new record species to the Iraqi fauna, viz, *Ilyodrilus templetoni* Southern, 1909, and Tubifex

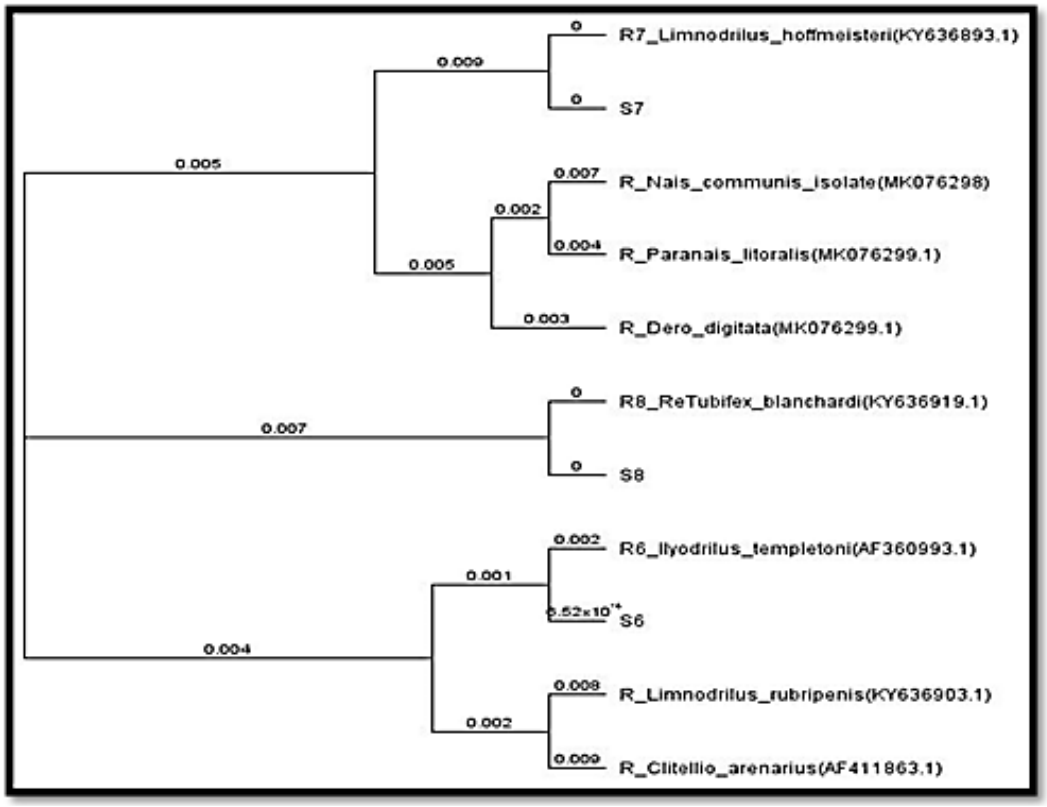

Figure 2. The phylogenetic tree of the collected tubificid species. The numbers at the branches indicate the degree of scale range among the comprehensive tree-categorized organisms. The letter "S#" refers to the code of the investigated samples.

blanchardi Vejdovsky, 1891, were recorded (Table 3). Neighbor-joining tree of the collected tubificid species are presented in Figure 2.

Description of new records

Ilyodrilus templetoni Southern, 1909

A total of 12 individuals belonging to this species were collected from S2, S3, and S5 (Table 3). The length ranges from 10 to 30 mm, the number of segments ranges from 86 to 122, dorsal bundles with 3-4 pectinate chaeta, which have equal teeth with a length ranging from 88.91 to 115.67 µm, and 1-4 hair chaeta with a length of 220-330 µm; sometimes they are all bifid setae. Ventral bundles with 3-4 setae per bundle, with a length from 67.65 to 122.89 µm, anterior-most setae with the upper tooth longer and thinner than the lower tooth. In segment number XI, only one ventral chaetae is present, spermathecal absent, penis sheaths conical in shape and tapering end with asymmetrical opening (Fig. 3). It was deposited in GenBank under accession number PQ275504.

According to Brinkhurst (1971), this species is generally widespread and may be a variant of *I. perrieri*, *I. fragilis*, or both. *Ilyodrilus templetoni* is similar to *Tubifex tubifex*, *Varichaetadrilus harmani*, and *Tasserkidrilus spec*., but all these species have a different type of penis sheath. The anterior ventral chaetae of *I. templetoni* have a longer terminal tooth without an intermediate tooth, and the penial chaetae are absent in mature specimens. A single bifid ventral seta is seldom observed in XI (Van Haaren and Soors, 2012); this is consistent with the current study. This species is often found together with *L. claparedianus* and *L. hoffmeisteri* (Bremnes and Sloreid, 1994), as in the current work. This species was recorded by Atanackovic et al. (2023) from various water bodies across 181 locations, including mountain streams, upper reaches of rivers, and downstream reaches of large lowland rivers in Serbia. This species was first recorded in Korean freshwater by Lee and Jung (2014).

Tubifex blanchardi Vejdovsky', 1891

A total of 239 individuals were collected from all sites except S1 (Table 3). The body length ranges from 4 to 22 mm, and the number of segments ranges from 59

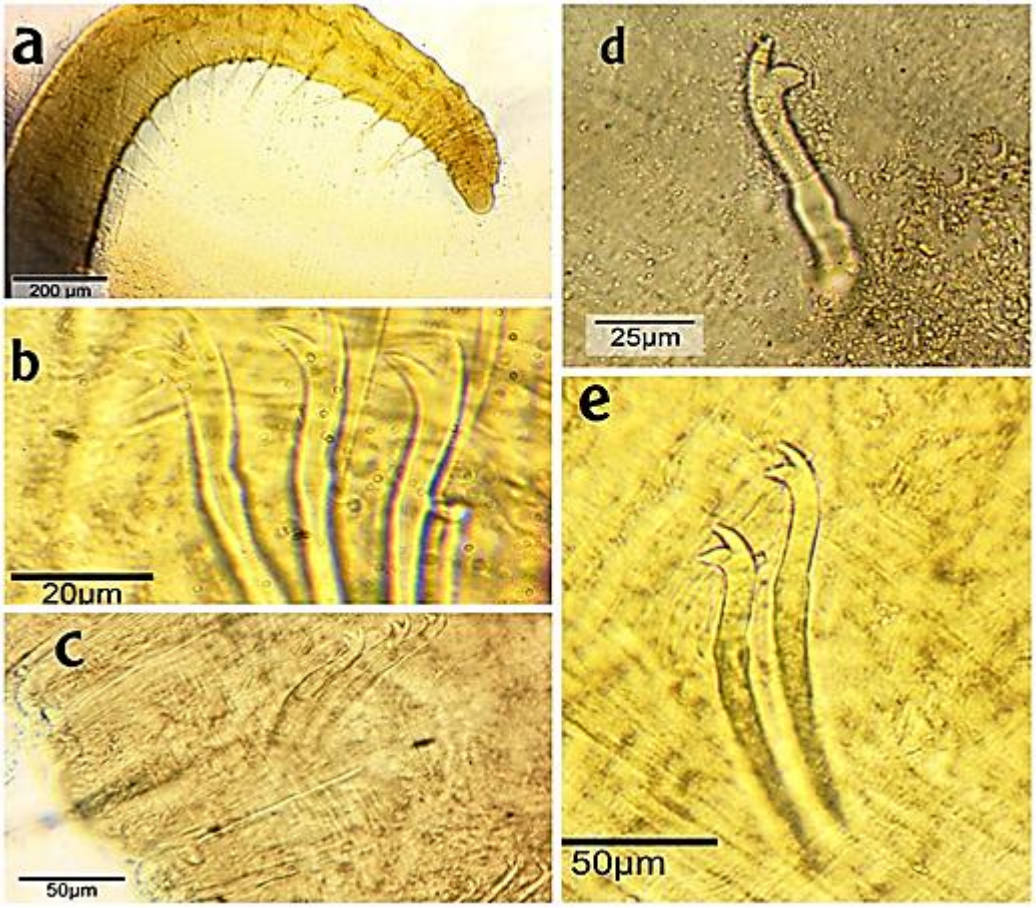

Figure 4. *Ilyodrilus templetoni*: (a) whole worm body, (b) dorsal hair chaetae and bifid chaetae showing intermediate teeth, (c) anterior ventral chaetae, (d) one ventral chaetae in segment XI, and (e) posterior ventral chaetae.

to 118. All bundles have only bifid chaetae with the upper tooth slightly longer than the lower one in the anterior bundle. In the posterior segment, there are 2-3 chaetae in each bundle with teeth of more or less equal length. The length of the crotchets ranged from 41.13 to 63.15 µm; male pores in XI; the penis sheath cannot be seen easily, only seen in mature individuals, as barrel-shaped with a width of 31.06 µm at the base, and 59.24 µm at the top, with a length of about 73.05 µm (Fig. 4). Its 18S rRNA gene sequence was deposited in GenBank under accession number PQ275628.

Tubifex blanchardi often occurs together with *T. tubifex*. Under certain conditions, changes in pH, salinity, mercury, and hardness concentrations cause *T. tubifex* to experience a gradual loss of hairs and pectinate chaetae, resulting in individuals resembling the "blanchardi" form (Chapman and Brinkhurst, 1987). In subsequent studies, it was recognized as a separate species based on morphological characteristics (Crottini et al., 2008; Marotta et al., 2009). This species is globally widespread, and it was recorded in the Sakarya River, Türkiye (Zeybek et al., 2018), Polish brackish waters (Marszewska et al., 2017), the Aegean River, Türkiye (Özbek et al., 2023), in Korean freshwater (Lee and Jung, 2014), in Germany (Eggers and Kirchberger, 2011), and in some Southern European countries (Giani, 2013).

Conclusions

The current study revealed two new records of Tubificid worms (Naididae: Tubificinae) for the Iraqi fauna, viz. *Ilyodrilus templetoni* Southern, 1909, and *Tubifex Blanchard* Vejdovsky, 1891. In addition, four other species of the subfamily Tubificinae and one species of the subfamily Rhyacodrillinae were recorded in the middle sector of the Euphrates River, Iraq.

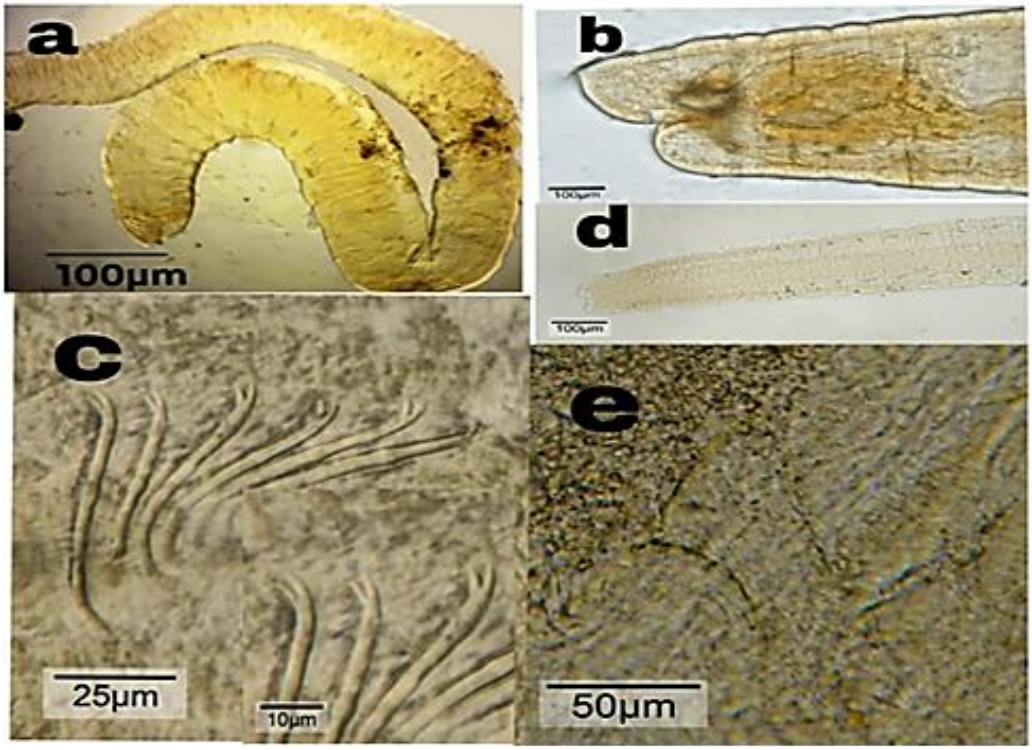

Figure 4. *Tubifex blanchardi*: (a) whole worm body, (b) anterior end shows the prostomium, (c) anterior bifid chaetae, (d) posterior end, and (e) penis sheath.

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