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ORIGINAL ARTICLE

MORPHOLOGICAL AND GENETIC VARIATION OF FRESHWATER LEECHES (ANNELIDA, CLITELLATA, HIRUDINEA) FROM EUPHRATES RIVER IN THE MIDDLE OF IRAQ

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ABSTRACT

Leeches are a group of segmented worms that play important roles in aquatic environment based on diversity, and abundance feeding habits. A total of 144 specimens of the leech were collected from two sites in Babylon Province on the Euphrates River including S1 in Al-Musayyib City site and S2 in Shatt Al-Hillah site, during the period from March 2023 to May 2024. Specimens were examined morphologically and by molecular analysis of genomic DNA, using to amplification of the conserved 18S rRNA encoding gene and universal primers ITS1 and ITS4, some environmental variables were measured, including water and air temperature (°C), pH, Electrical conductivity EC (µs cm), Total Dissolved Solid (TDS) and Turbidity, in addition to sediment texture analysis. The result showed that the collected specimens belonged to four species, including *Barbronia cf. gwalagwalensis* Westergen & Siddall, 2004, *Barbronia borealis* Bolotov, Eliseeva & Kondakov, 2023, *Barbronia weberi* Blanchard, 1897, and *Barbronia arcana* Richardson, 1970; all these species belong to the family Salilfidae and order Hirudinida. The significance of this discovery is the addition of four new record species to Iraqi fauna.

Keywords: 18SrRNA marker, Freshwater leeches, Genomic DNA, Hirudinea, Salifidae.

INTRODUCTION

Leech species are classified as a group of worms that belong to phylum Annelida, class Clitellata, and subclass Hirudinea, which have a worldwide distribution (Langer *et al*., 2017). Many species live in standing and flowing water, while some have adapted to a terrestrial lifestyle (Jordan and Verma, 2002; Borda and Siddall, 2004a). They are defined by the absence of chaetae and parapodia (except in Acanthobdella), having suckers on both ends of their elongated bodies with unpaired genital openings on the clitellum (Sket and Trontelj, 2008), and a number of eyes on the dorsal side of the head. Leeches are hermaphrodites, and they are extremely sensitive to light and shadows passing above them (Sladecek and Kosel, 1984). Food availability is of great importance for the distribution of leeches in the studied aquatic systems. Vegetation, both aquatic and growing in water bodies, should also be considered as an important factor in terms of leech distribution; submerged plants in particular can influence local habitat conditions, including food resources (Cichocka *et al*., 2015)

The subclass (one word) Hirudinea includes the order Hirudinida, which is divided into three suborders including Rhynchobdellida, Arhynchobdellida, and Acanthobdellida. The first one has three families called Glossiphoniidae, Ozobranchidae, and Piscicolidae. While the second one contains two groups one is called Hiruddiniformes, which has seven families named Cylicobdellidae, Haemopidae, Hirudinidae, Macrobdellidae, Haemadipsidae, Semeiscolecida, Xerobdellidae, and the other is called Erpobdelliformes, which has three families named Americobdellidae, Erpobdellidae, and Salifidae (Bielecki *et al*., 2011). The species that belong to Acanthobdellida are characterized by living as ectoparasites on salmon and lacking the anterior sucker (Borda and Siddall, 2004 b).

Leeches are considered an important component of the aquatic food web; they prey on chironomid flies, oligochaetes, amphipods, insect larvae, and snails (Bielecki *et al*., 2011), while other leeches are temporary sanguivorous ectoparasites (Dodds, 2002). They are also used as intermediate and definitive hosts for some fluke's worms and cystic tapeworms (Bush *et al*., 2001). In medicine, people widely use leeches for bloodletting or to draw blood from swollen areas due to venomous bites (Aluto and Etcha, 2018). On the other hand, leeches are used as a group as a basic element of monitoring in freshwater habitats and an indicator for biological assessment of environmental quality (Kazanci *et al*., 2015), as well as to target vertebrate DNA by using iDNA extracted from leech guts in order to monitoring animals that are unknown in aquatic system (Lynggaard *et al*., 2022).

In Iraq, there are many studies that dealt with recording leech species, including the redording of *Glossiphonia heteroclita* (Linnaeus, 1761) in Al Dalmaj Marsh (Jaweir and Al-Sarai, 2016). Then Al-Ameen and Jawair (2019) diagnosed three new record species of leeches in Al-Hindyia River, identified as *Erpobdella octoculata* (Linnaeus, 1758), *E. punctate* Leidy, 1872, and *Hemiclepsis marginata* (Müller, 1773). The genus *Theromyzon* Philippi, 1867, 1862 is considered a new record for the Iraq fauna (Al-Dharob, 2021). Also, the species *Dina prokletijaca* Grosser and Peši, 2016, was recorded by Zewayee and Ali (2024) in Bekhal stream, Erbil, for the first time in Iraq fauna. From Felaw pond near the Choman District about 160 Km north of Erbil, the species *Hirudo medicinalis* Linnaeus, 1758, was identified based on the 18SrDNA gene (Bilal, 2024).

The aim of this study is to record and identify species of leech in the Euphrates River and to add some of them as a new record to the Iraqi fauna and to compare them morphologically and genetically with the previously recorded species worldwide.

MATERIALS AND METHODS

Water and air temperature were measured by electronic thermometer. The thermometer is zeroed and then placed in water for a minute, and the same applies to air in the shade of the sample site, and then the results were reading. Potential of Hydrogen (pH), Total Dissolved Solid (TDS) (mg/l), Electrical Conductivity (µs/cm), and Turbidity were measured using multi- parameters type HANNA, Romanian model after calibrating each device (APHA, 2017). The sediment texture analysis was conducted in the laboratories of the Ministry of Science and Technology, Department of Environment. After the sediment material was left for several days exposed to air and after it had completely dried, the analysis was conducted.

Leech individual specimens were collected by using tweezers from the underside of stones, shells of mollusks, and leaves of submerged or floating plants from two sites in Babylon Province on the Euphrates River: Site (S1) on the main Euphrates River in Al-Musayyib (latitude 32.781889 and longitude 44.288679, and site (S2) on Shatt AL Hillah (a branch of Euphrates River) in Al- Hillah City, about 100 kilometers south of Baghdad (latitude 32.488651 and longitude 44.265848) (Map 1), during the period from March 2023 until May 2024. In the laboratory, leeches are first anesthetized by adding a few drops of carbonated water, 1 % (Yule and Sen, 2004). Relaxed leeches should be passed or wiped with a brush to remove excess of oozing out mucous, and then the sample is placed on a glass slide for examination by using a light microscope under magnification power 4X and possibly 10X to distinguish the number of pairs of eyespots and their location in the different body segments, as well as the location of the reproductive openings, and body color and then to be photographed using a microscope camera Sony a7ri Japanese origin. Then, the specimens were kept in 75-85% alcohol, and the back part of the worm's body was cut off and preserved in alcohol for later use in molecular analysis. The specimens were identified according to identification keys by Klemm (1982, 1995).

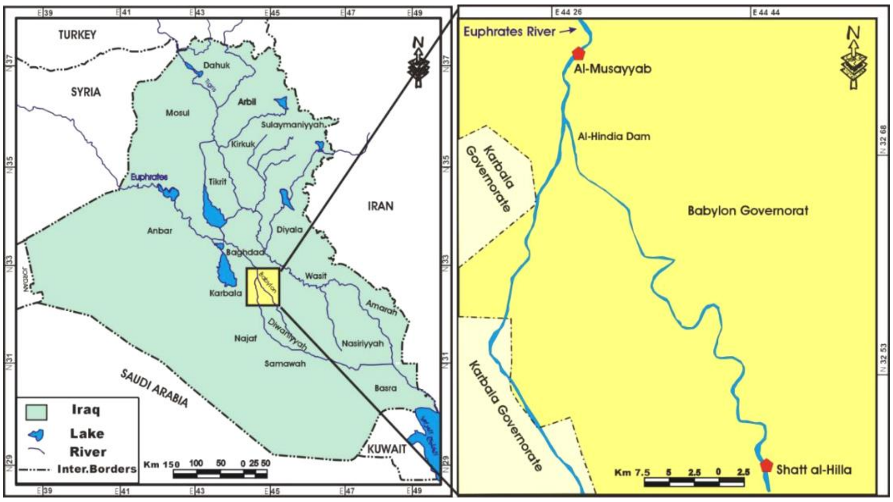
To confirm the morphological identification as well as to know the evolutionary relationships with related species based on the 18SrRNA gene, a portion of the worms, near the posterior end, which was diagnosed morphologically, was cut off and placed in special plastic tubes containing 85% ethyl alcohol, one for each individual. The molecular technique was used to identify the worm by amplifying the conserved 18S rRNA encoding gene using universal primers ITS1 and ITS4. Genomic DNA from the worm's specimens was prepared using a DNA extraction kit (Genomic DNA Mini Kit tissue GB100/300), and following the manufacturer's instruction. The isolated DNA was subjected to PCR (OWL Electrophoresis System Thermo, USA). The PCR products were separated by 1% agarose gel electrophoresis and visualized using the gel imaging system. A forward primer ITS1 and reverse primer ITS4 were used (Tab.1).

The primer set was set were provided by Macrogen, Korea. Each one of the investigated and construed sequences was submitted to the NCBI Bank it portal and all instructions described by the portal were followed as described by it. The sequencing was carried out at NCBI to obtain the unique GenBank accession number for the investigated sequences.

A specific inclusive tree was constructed in this study according to the neighbor-joining protocol. The observed variables were matched to their neighboring reference sequences using NCBI-BLAST (Zhang *et al*., 2000). Then, a complete tree, including the plotted variant, was constructed using the neighbor-joining method and visualized as a rectangle by BioEditor (version 7.2.5).

Table (1): primers designed for the current study.

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



Map (1): The leeches sampling location within the Euphrates River in the middle of Iraq.

RESULTS AND DISCUSSION

A total of 144 individuals were sorted from 30 specimens collected from S1 and S2 during this study. They were identified as four species related to the genus *Barbronia* Johansson, 1918. Thirty specimens were collected during this study, chemical and physical factors are shown in Table (2). The sediment texture in S1 was sandy loam, and in S2 was sandy clay loam. *B. weberi* (Blanchard, 1897), *B. arcana* Richardson, 1970, *B. borealis* Bolotov, Eliseeva, and Kondakov, 2023, and *B. cf. gwalagwalensis* Westergren & Siddall, 2004. The percentage of each species to the total number of individuals is shown in Diagram (1).

The species *B. cf. gwalagwalensis* was recorded at the highest percentage of 33%, while only 6% was recorded for *B. borealis*, which was the lowest percentage. When preparing specimens for microscopic examination and after treating them with alcohol, the results are strong contraction of the body and even disappearance of some important taxonomic characters disappeared. So reliability of identification is dependent on suitable preservation.

The identification was based on molecular diagnosis because their morphological characters are very similar to each other except for the species *B. weberi* (Blanchard, 1897), which has minute papillae on the dorsal surface. All species belonging to this genus have three pairs of eyes: one labial and two buccal pairs. The comprehensive rectangular phylogenetic tree of intergenic spacer sequences that shows evolutionary relationships between the identified species and the species closely related to them in Diagram (2).

All individuals were found attached to the lower surface of small rocks, stones, and mollusk shells. Others were found associated with aquatic plants, especially *Ceratophyllum demersum*, *Hydrilla verticillata*, and filamentous algae, except for the species *B. arcana* which was collected while swimming on the surface of the water.

Liu *et al*. (2024) refer to the identification of species of *Barbronia*, as well as the phylogenetic placement of this genus within the Erpobdeliformes leech family Salifidae.

Table (2): Values of chemical and physical factors for all study sites and all seasons during the study period from March 2023 to June 2024 first row represents the range, the second mean and standard deviation.

Physical and chemical factors	S1	S2
Air temperature (C°)	18.0-47.3 2.78±30.60	11.7-46.2 2.46±27.92
PH	7.23-8.10 0.34±7.61	7.4-8.2 0.35±7.82
Electrical conductivity EC (µs cm)	875-1180 105±1080	580-1084 61.2±836.7
Total Dissolved Solid (TDS)	530-1050 58.1±826.3	399-942 42.5±755.7
Turbidity	4.5-12.7 0.73±8.59	1.5-10.2 0.45±6.98

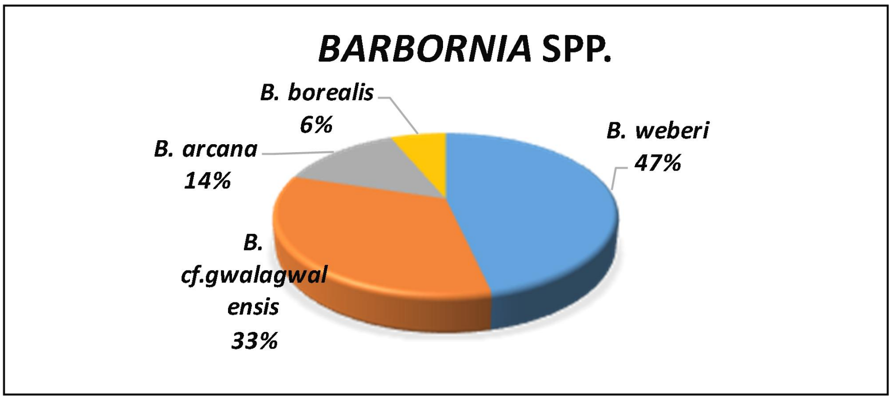


Diagram (1): The percentage of each identified species to the total individual number of *Barbronia* spp.

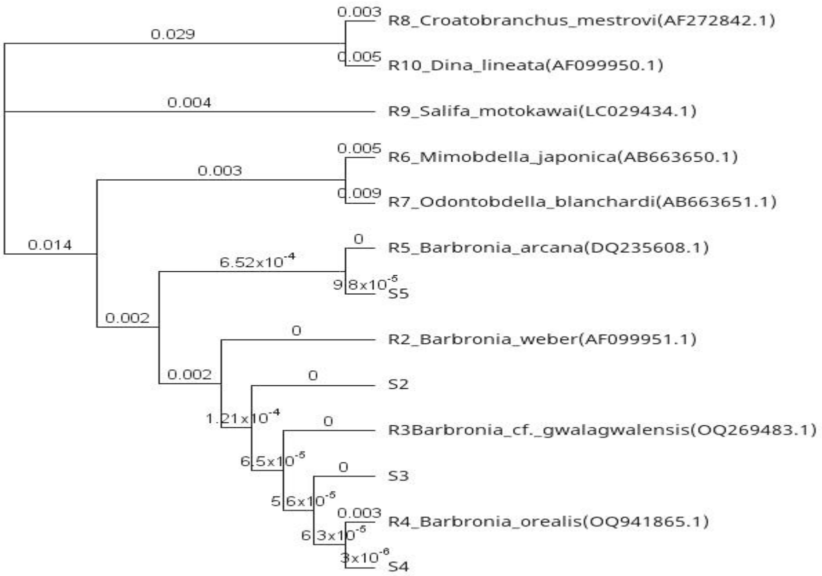


Diagram (2): The comprehensive rectangular phylogenetic tree of intergenic spacer sequences. All the mentioned numbers referred to the GenBank accession number of each referring species. The number at the bottom portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. The letter "S#" refers to the code of the investigated samples.

Description of species

Barbronia weberi (Blanchard, 1897)

About 67 individuals were collected from S1 and S2, 58 individuals from S1 and 9 from S2. The body length ranges between 20- 45mm, with a mean of 35±9.24, when the specimen is alive and when fixed from 16.55-19.24mm. The color was reddish-grey in a preserved condition, but when alive it is dark reddish green. There are papillae on the dorsal surface. It has four pores on the ventral surface. Male and the female gonopores and two accessory gonopores, one located in front of the male pore and the other in front of the female pore. The clitellum annuli are about 16. The distance from the tip of the head to the clitellum is about 1.87mm and to the male gonopore is 2.01mm, and of the caudal sucker is about 1.34mm (Pl. 1).

Molecular analysis of a partial and complete sequence of the 18s rRNA gene is indicated in the National Center for Biotechnology Information (NCBI). It was uploaded to one word (GenBank) with accession number PQ270273; also it has been registered in Iraq Natural History Research Center and Museum under the number H.S.27.24 (Tab. 3). Nucleic acid sequence alignment of the identified sample and *B. weberi* reference genome is shown in Digram (3).

In many European countries this species is considered as an invasive species and is known as the Asian species, but in the last two decades it has been recorded in several countries, including the Netherlands, Spain, Australia, Italy, Argentina, Brazil, Costa Rico, Mexico, North Carolina, China, and India (Pamplin and Rocha, 2000; Govedich *et al*., 2003; Van Haaren *et al*., 2004; Bandyopadhyay and Mandal, 2005; Pavluk *et al*., 2011; Garduño -Montes *et al*., 2016, Iwama and Arruda 2016; Sawyer and Sawyer, 2018; Zhao *et al*., 2020) . This species often lives in running water as well as lakes packed with nutrients. It is mostly attached to aquatic plants that use it as a substrate (Genoni and Fazzone, 2008). Moreover, this species is considered as a second intermediate host of the parasitic trematode *Australapatemon niewiadomskae* (Blasco-Costa *et al*., 2016).

According to Ludányi *et al*. (2019), this species was recorded for the first time in two sites, including the River Danobe and a stream called Perint in Hungary. The reproduction of *B. weberi* is lasted for a full year and for five generations; the result indicated that there was no evidence of cross-fertilization found. It is not obvious if this ability is facultative or obligatory (Sawyer, 2020). It is not possible to determine whether this species can reproduce sexually. Sawyer and Sawyer (2018) indicate that this species is useful us laboratory research tool, especially for developmental studies. Bolotov *et al*. (2023) indicated that the diagnosis of *B. weberi* that has been recorded in many European countries to species level needs to be confirmed by a molecular approach.

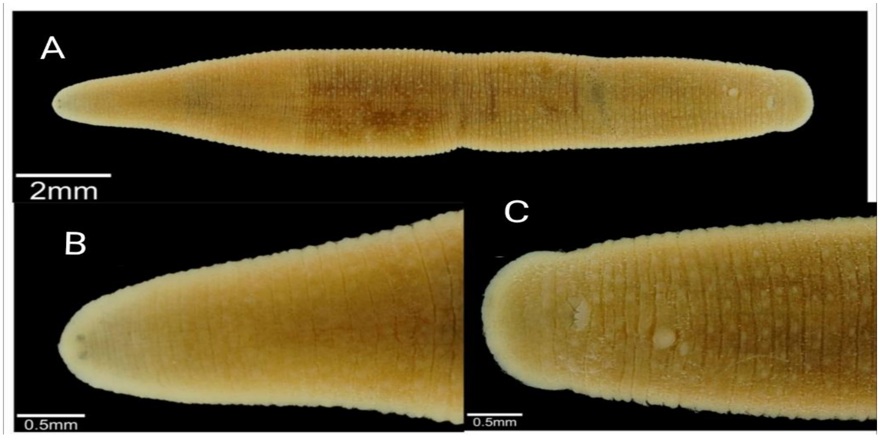
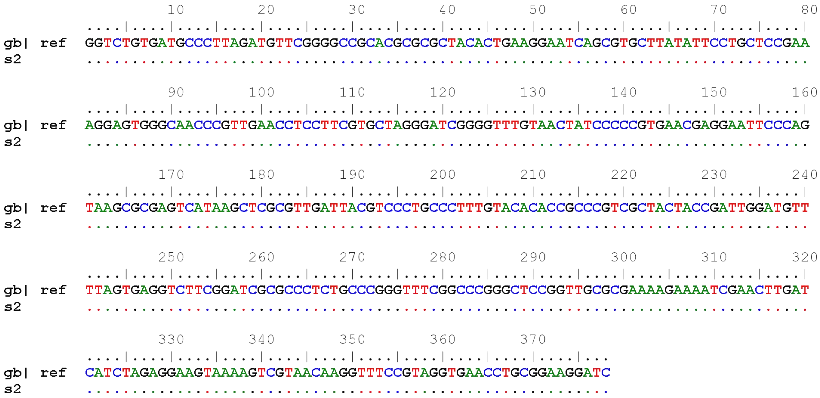


Plate (1): *Barbronia weberi*; (A) Complete worm body showing dorsal papillae, (B) Anterior end showing first pair of eyespots, (C) Posterior dorsal view showing anal pore.



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s2       ................................................................................

                 90       100       110       120       130       140       150       160
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
gb| ref  AGGAGTGGGCAACCCGTTGAACCTCCTTCGTGCTAGGGATCGGGGTTTGTAACTATCCCCCGTGAACGAGGAATTCCCAG
s2       ................................................................................

                170       180       190       200       210       220       230       240
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
gb| ref  TAAGCGCGAGTCATAAGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGGATGTT
s2       ................................................................................

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         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
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s2       ................................................................................

                330       340       350       360       370
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gb| ref  CATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATC
s2       ..........................................................
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Diagram (3): Nucleic acid sequence alignment of the identified sample and *Barbronia weberi* reference genome.

Barbronia arcana Richardson, 1970

Twenty individuals were collected from S1 (13 individuals) and S2 (7 individuals) during the study period. The species represent 13.8% of the total number of leech individuals (Dig.1). The individuals had a greyish-red body color in a preserved condition; the body length ranges between 18-42 mm when the specimen is alive with a mean of 29.5±9.5 and when fixed from 14.36-18.24mm (Pl. 2). This species was identified by a molecular approach by using the 18S rRNA gene, since it was closely similar in morphology to *B. webri*, its DNA sequence was residing in NCBI, and uploaded to one word (GenBank) with accession number PQ274900. Also, it has been registered in the Natural History Research Center and Museum Iraq-University of Baghdad under the number H.S.29.24 (Tab.3). Nucleic acid sequence alignment of the identified specimens and *B. weberi* reference genome is shown in (Dig 4). According to Liu *et al*. (2024), this species and *B. wutttkei* are synonyms of *B. weberi* depending on COI.

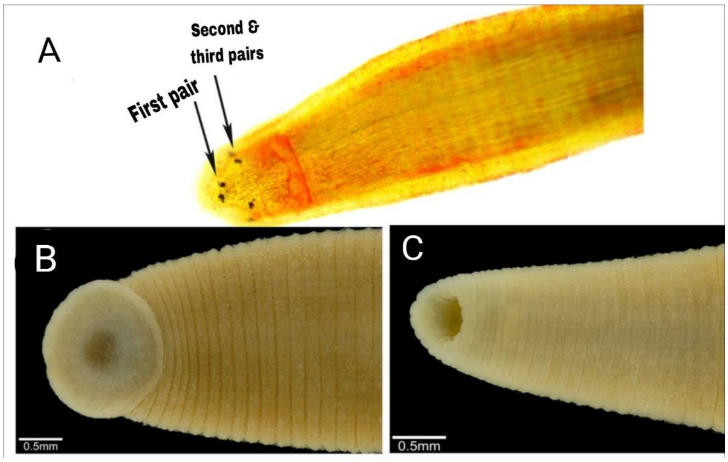
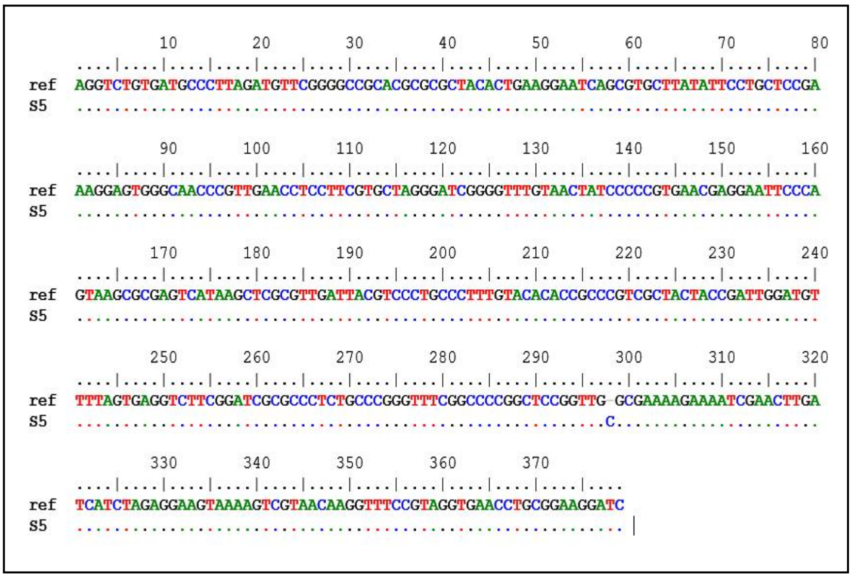


Plate (2): *Barbronia arcana*; (A) Anterior end dorsal view, (B) Posterior sucker, (C) Anterior sucker.



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S5   ................................................................................

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S5   ................................................................................

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     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
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S5   ................................................................................

            250       260       270       280       290       300       310       320
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref  TTTAGTGAGGTCTTCGGATCGCGCCCTCTGCCCGGGTTTCGGCCCCGGCTCCGGTTG-GCGAAAAGAAAATCGAACTTGA
S5   .........................................................C......................

            330       340       350       360       370
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ref  TCATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATC
S5   ........................................................... |
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Diagram (4): Nucleic acid sequences alignment of identified sample and *Barbronia arcana* reference genome.

Barbronia borealis Bolotov, Eliseeva & Kondakov, 2023

About nine individuals were collected from S2. This species is considered one of small leeches, distinguished by a body length ranging between 20.23 mm, with a mean of 21.6±1.24, and a width of the posterior sucker of about 1.76 mm. Body elongated, reddish brown color on the dorsal surface and light brown on the ventral surface. Body surface is smooth without papillae. There are three pairs of eyes at the front of the worm's body. The first pair is located in annulus number 3, and the last two pairs are located in annuli six and seven (Pl.3). The clitellum is located in the segments from X to XIV. Gonopores are large; they can be easily noticed. The male accessory pore is in front of the male gonopore, and the female accessory pore is behind the female gonopore. Male gonopore in XII, female gonopore in XIII, anus dorsally at XXVII**.**

Molecular analysis of a partial and complete sequence of the 18SrRNA gene is indicated in the NCBI. It was uploaded to one word (GenBank) with accession number PQ275723. Also, it was deposited in the Iraq Natural History Research Center and Museum under the number H.S.28.24 (Tab.3). Nucleic acid sequence alignment of the identified sample and *B. weberi* reference genome is shown in (Dig.5).

This species was described by Bolotov *et al.* (2023) as a new species depending on molecular identification by using mitochondrial cytochrome c oxidase subunit I (COI) and 18SrRNA genes. According to Bolotov *et al.* (2023), this species is similar to *B. weberi and B. gwalagwalensis* in external characteristics.

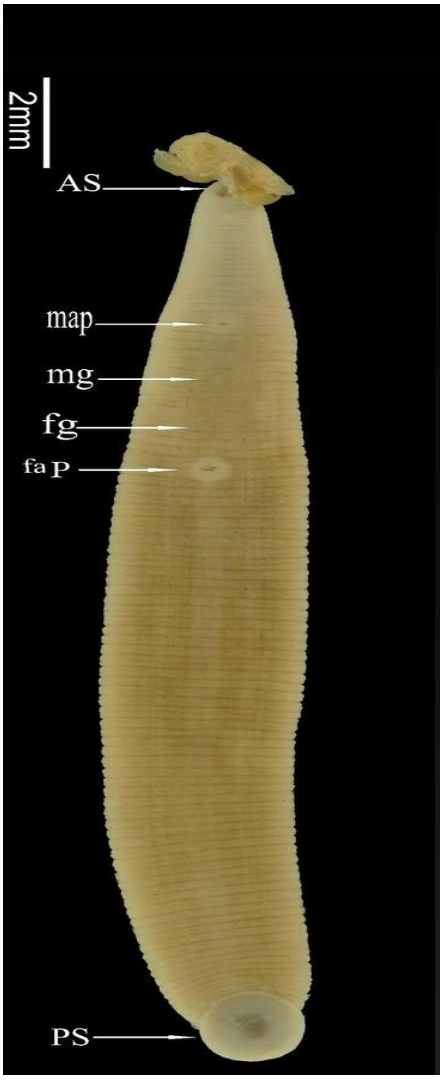
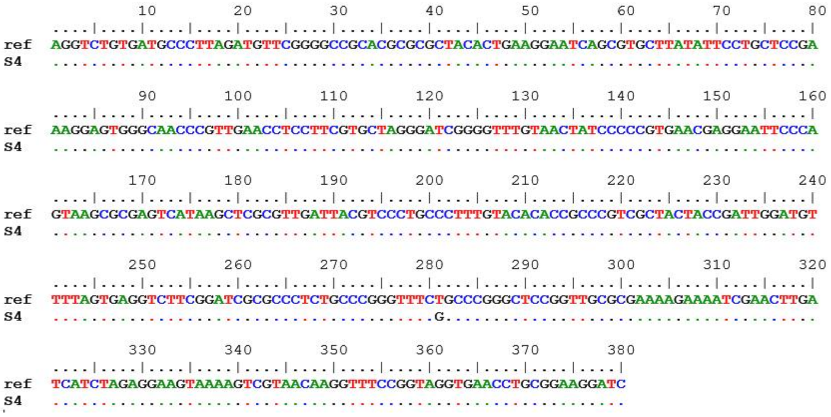


Plate (3): *Barbronia borealis* ventral view [AS, anterior sucker with some food from digestive tract; PS, posterior sucker; map, male accessory pore; fap, female accessory pore; mg, male gonopore; fg, female gonopore].



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S4       ................................................................................

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ref      AAGGAGTGGGCAACCCGTTGAACCTCCTTCGTGCTAGGGATCGGGGTTTGTAACTATCCCCCGTGAACGAGGAATTCCCA
S4       ................................................................................

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S4       ................................................................................

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S4       ........................................G.......................................

               330       340       350       360       370       380
         ....|....|....|....|....|....|....|....|....|....|....|....|
ref      TCATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGGTAGGTGAACCTGCGGAAGGATC
S4       ............................................................
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Diagram (5): Nucleic acid sequence alignment of the identified sample and *Barbronia borealis* reference genome.

Barbronia cf. gwalagwalensis Westergren & Siddall, 2004

A total of 48 specimens were collected from S1 and S2, 35 individuals from S1and 13 from S2 during the study period. The length ranged from 12-16mm in preserved condition, while in alive specimen a ranged from 20-35 mm with a mean of 26.8±5.76 when alive. The width of the sucker is about 1.58mm. Body with one word (subcylidrical), vermiform, ribbon -shaped. Dorsal surface light brown, with three pairs of eyespots. The first pair found in the front end begins on the midline of the body, the last two pairs are located on segment IV, and it is segregated from the first pair by four segments.

Caudal sucker directed ventrally. Clitellum unclear in juveniles with male and female gonopores separated by seven annuli with two accessory gonopores, one at X/XI, and the second posterior to the female gonopore at XIII/XIV (Pl.4). Molecular analysis of a partial and complete sequence of 18SrRNA gene is indicated in the NCBI. It was uploaded to one word (GenBank) with accession number PQ276816, also it was deposited in Iraq Natural History Research Center and Museum under the number H.S.26.24 (Tab. 3). Nucleic acid sequence alignment of the identified sample and *B. weberi* reference genome is shown in (Dig. 6).

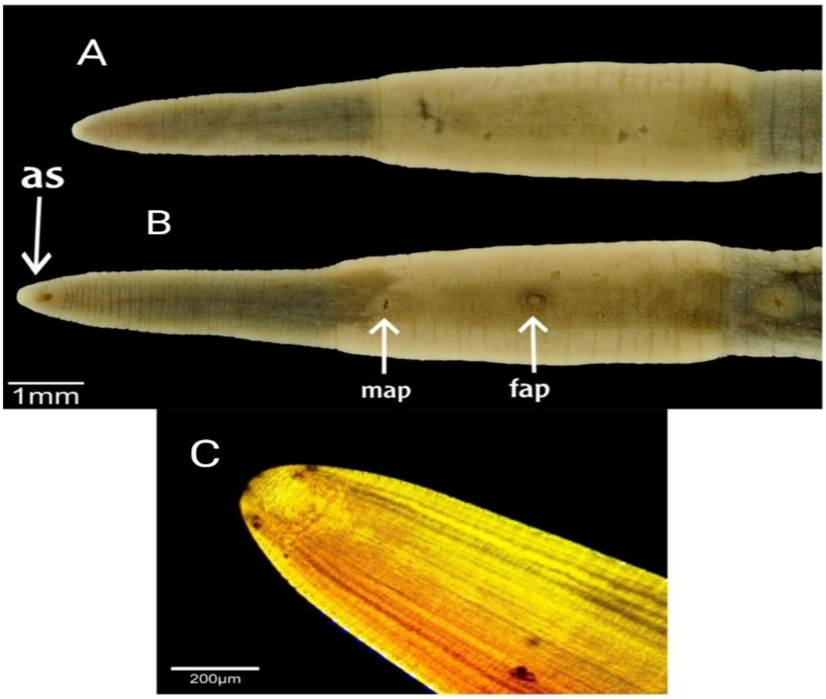
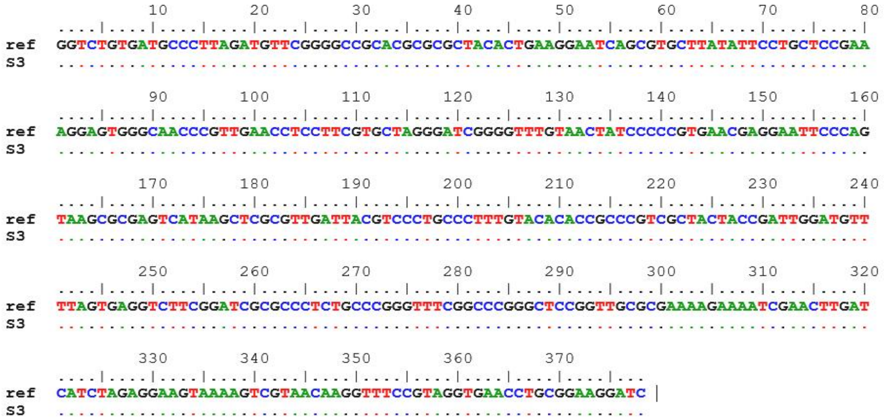


Plate (4): *Barbronia cf. gwalagwalensis*; (A) Dorsal view, (B) ventral view showing (as: anterior sucker, map: male accessory pore, fap: female accessory pore), (C) Dorsal view showing arrangement of three eyespot pairs.



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ref   GGTCTGTGATGCCCTTAGATGTTCGGGGCCGCACGCGCGCTACACTGAAGGAATCAGCGTGCTTATATTCCTGCTCCGAA
S3    ................................................................................

              90       100       110       120       130       140       150       160
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref   AGGAGTGGGCAACCCGTTGAACCTCCTTCGTGCTAGGGATCGGGGTTTGTAACTATCCCCCGTGAACGAGGAATTCCCAG
S3    ................................................................................

             170       180       190       200       210       220       230       240
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref   TAAGCGCGAGTCATAAGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGGATGTT
S3    ................................................................................

             250       260       270       280       290       300       310       320
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref   TTAGTGAGGTCTTCGGATCGCGCCCTCTGCCCGGGTTTCGGCCCGGGCTCCGGTTGCGCGAAAAGAAAATCGAACTTGAT
S3    ................................................................................

             330       340       350       360       370
      ....|....|....|....|....|....|....|....|....|....|....|....|...
ref   CATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATC |
S3    ..........................................................
```

Diagram (6): Nucleic acid sequence alignment of the identified sample and *Barbronia cf. gwalagwalensis* reference genome.

Table (3): Accession number of the sample that were compared with it in National Center for Biotechnology Information and the percentage of compatibility between them and Museum number in the Iraq Natural History Research Center & Museum.

Identified species	Accession number	Reference sequence NCBI	Identities %	Museum number
Barbronia weberi	PQ270273	AF099951.1	100%	H.S.27.24
Barbronia cf. gwalagwalensis	PQ276816	OQ269483.1	100%	H.S.26.24
Barbronia borealis	PQ275723	OQ941865.1	99.5%	H.S.28.24
Barbronia arcana	PQ274900	DQ235608.1	99.6%	H.S.29.24

CONCLUSIONS

The authors conclude that the above results indicates that the four species *Barbronia cf. gwalagwalensis*, *B. borealis*, *B. weberi*, *and B. arcana*, are newly recorded to the Iraqi fauna and they are morphologically close matches with those previously recorded by the authors from different localities worldwide. Genetically they are also improved by the NCBI. The match with NCBI reference sequence ranged from 99.5 to 100%.

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CONFLICT OF INTEREST STATEMENT

This paper is a component of a Ph. D. thesis in the Biology Department, College of Science for Women, University of Baghdad. There are no conflicts of interest with any other parties aside from that.

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التباين المظهري والوراثي لعلقيات المياه العذبة (الديدان الحلقية: السرجيات: العلقيات) من نهر الفرات في وسط العراق

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الخلاصة

تُعد العلقات مجموعة من الديدان الحلقية التي تلعب دوراً مهماً في البيئة المائية اعتماداً على وفرتها وعاداتها الغذائية المتنوعة، تم جمع 144 نموذج للعلقيات leeches من موقعين على نهر الفرات هما موقع مدينة المسيب م1 وموقع شط الحلة م2 خلال الفترة من اذار 2023 ولغاية حزيران 2024، فُحصت العينات مظهرياً وكذلك بوساطة التحليل الجزيئي للحمض النووي الجينومي باستخدام تقنية تضخيم جين 18SrRNAالمحفوظ وباستخدام بوادئ عالمية هي ITS1 و ITS4، وكذلك قُيست بعض المتغيرات البيئية والتي شملت درجة حرارة الماء والهواء(C°)، الرقم الهيدروجيني PH،التوصيلية الكهربائية EC(مايكروسمنز/سم)، اجمالي المواد الصلبة (TDS)، العكارة بالإضافة الى تحليل نسجه الراسب، أظهرت النتائج أن العينات التي تم جمعها تنتمي إلى أربعة أنواع وهي :

Barbronia cf. gwalagwalensis Westergen & Siddall, 2004
Barbronia borealis Bolotov, Eliseeva & Kondakov, 2023
Barbronia weberi Blanchard, 1897
Barbronia arcana Richardson, 1970

و تنتمي جميع هذه الأنواع الى العائلة و Salifidae رتبة Hirudinida. أن أهمية هذا الاكتشاف هو إضافة أربعة أنواع تُسجل لأول مرة للفونا العراقية.