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**Research Article**

# The first record of *Dina prokletijaca* (Hirudinida: Erpobdellidae) from Bekhal stream, Erbil, Iraq

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## Abstract

The *Dina prokletijaca* was found in the samples that were obtained from the Bekhal stream for the current investigation. The samples were gathered throughout the course of nine months, from March to October 2021. The reduced number of papillae on the back of *D. prokletijaca* distinguishes it. It can be identified by its stock and short body, the dorsal side with two dark and wide longitudinal paramedian stripes, and the ovisacs are curled reaching the somite four beyond the females' genital pores and coiled throughout their entire length.

**Keywords**: Bekhal Stream, Dina Prokletijaca, Erbil, Hirudinea, Iraq, Leech

## Introduction

Leeches are widely distributed around the world, found in water and terrestrial, However, they are mostly found in freshwater. It contains about 300 species, the majority of which are freshwater ectoparasites that feed on fish and other organisms (Bilal et al., 2017; Hopkins et al., 2014; Jordan & Verma, 2010). Leeches are classified as members of the phylum: Annelida, class: Clitellata and subclass: Hirudinea. Most of them are benthic invertebrates distributed worldwide. Their diversity and distribution are affected directly by the ecological conditions, physical and chemical factors, and interference of food relationships among the benthic community (i.e., predation, competition and parasitism in ecosystem or the water where they found) (Langer, Vezsenyi, De Carle, Beresford, & Kvist, 2018). The link between taxa in the

Erpobdellidae Family is determined by morphological characteristics and molecular examination of mitochondrial cytochrome c oxidase subunit I, mitochondrial 12S rDNA, and nuclear 18S rDNA (Siddall, 2002). In Iraq and Kurdistan region, 16 leech species with unidentified one recorded from various source, as follow:

Herzog (Herzog, 1969) reported the first paper on leeches in Iraq which was *Piscicola sp.* recorded on *Barbus schejch* from various regions of Iraq. Kalifa (Khalifa, 1985) recorded *Hemiclepsis margunata* on *Barbus sharpeyi* caught from fish farms for first time in Iraq. Then, Rahemo (1989) found *Cystibranchus mastacembeli* on *Mastacembelus simach* (syn. *Mastacembelus mastacembelus*) obtained from the Tigris River in Mosul. *Piscicola geometra* recorded by Mhaisen et al. (Mhaisen, Al-Khateeb, Balasem, & Mutar, 1997) in the host *Aspius vorax* from Euphrates River near Anbar province. Bashê (Bashê, 2008) identified *Cystibranchus mammillatus* in the Aski Kalak area near Erbil, in the Greater-Zab River. Moreover, Ahmed (Ahmed, 2009) identified two more species, *Erobdella octoculata* and *Haemopis sanguisuga*, in aquatic regions of Erbil and its surrounds. Ali (2010) recorded *Fadejewobdella quinqueannulata* in Iraq from Greater-Zab River. *Dina lineata* was later discovered in Greater Zab River in Iraq (Ali, 2007). In Al-Qaim city, Al-Anbar region, Al-Salmany (Al-Salmany, 2015) conducted a study on the parasitic illnesses of several fish species from the Euphrates River and discovered *Acipenserobdella volgensis* on *Carasobarbus luteus* and *Cyprinion macrostomum*. Bilal et al. (Bilal et al., 2017) added another species of the Erpobdellid leech, Dina punctata for the first time to the Iraqi flora from Lesser Zab River near Qashqoli; leeches were investigated morphologically and molecular study. Morover, a study on morphology and molecular of leeches from various areas in Iraqi Kurdistan Region conducted by Hallaq (Hallaq, SH., 2020) and recorded *Erobdella bucera, Hellobdella stagnalis, Hirudo verbena* and *Piscicola geometra.*

Finally, Hallaq (2020) investigated the first sighting of Erpobdella mestrovi from Zalm stream in Sulaimani Province, Kurdistan Region of Iraqi (Hallaq & Ali, n.d.).

The current study sought to investigate the morphology and molecular biology of *D. prokletijaca* in Bekhal stream, Rawanduz District.

## Martial and methods

Bekhal is one of the major springs in the Iraqi Kurdistan area, draining the Korak mountain catchments of around 65 km2; it is loca ted approximately 500m from Bekhal village at an altitude of 630 m.a.s.l. (Bekhal village consists of about 110 houses located 6 Km. south west of

Rawanduz district). Downstream of Bekhal spring 400m far from spring water flow at an altitude 545 m.a.s.l. (Fig. 1), the total yearly discharge is 30×106 m3 of water about 1000 l/s (Stevanovic & Iurkiewicz, 2009).

In the present study (10) leech samples were collected with tweezer or by hand underside stones, roots and in submerged plants in the water, as well on the shore. Leech samples (some of them) were preserved in ethanol (96%) for molecular study, some in 5% formaldehyde for (morphology) and remains alive for dissection and further examination in the laboratory. *D. Prokletijaca* was recognized using the identification key developed by (Grosser, Pešić, Berlajolli, & Gligorović, 2016). Ruler was used for taking measurements (however, the accuracy of this measurement is acceptable, because leeches rely heavily on bodily contraction). Material was examined using Motic S-10 dissecting microscope, electrical microscope (SWIFT Microscope Trinocular Compound SW350T With Camera). Leech samples were collected from March to October.

Additionally, some physical and chemical parameters Air and water temperature (AT, WT), pH, Alkalinity (Alk.), Total Hardness (TH) Dissolved oxygen (DO), biological oxygen demand for five days ($BOD_5$) and other nutreint component (i.e Sodium $Na^{+}$, Potacium $K^{+}$, reactive phosphate $PO4^{-3}$, nitrite $NO^{2-}$, nitrate $NO^{3-}$ and magnisium $Mg^{+2}$) were measured in Bekhal stream.

**Figure 1.** Bekhal stream (Soran District) Erbil

## Results

### Morphological study:

The annual average mean of physical and chemical parameters were as follows: air and water temperature values (22.9 and 11.8 $C^{o}$), pH (8.0), total hardness and alkalinity were (219.3 and 99.3 mg $CaCO3.l^{-1}$), DO and $BOD_5$ (12.2 and 1.5 $mg.l^{-1}$) respectivly, while nutreint element values of $Na^{+}$, $K^{+}$, $NO3^{-}$,$NO2^{-}$ and $Mg^{+2}$ were (19.7, 1.5, 5.4, 0.4 and 11.7 $mg.l^{-1}$) subsequently, reactive phosphate $P\text{-}PO_4^{-3}$ (21.1 $\mu g.l^{-1}$) during the studied period (Fig. 2). The *D. prokletijaca* is a short and stocky leech that has a cylindrical body along its whole length. In the final third of its length, it has minor lateral keels, but they are not elevated. specimens of adults can reach lengths of up to 26–27 mm and widths of up to 4.7–4.9 mm. mouth that opens widely, large cranial sucker, and upper lip that is not extended at all (Fig. 3). The breadth of the body is obviously greater than the width of the caudal sucker on the medium-sized specimen. There are no papillae

that are easily noticeable; nevertheless, annulus 2 contains a large number of papillae that are so minute that they are scarcely apparent. These papillae are evident both ventrally and dorsally. An annulation that is analogous to the one seen on a typical *Dina* leech. Pentannulated somites in the middle of the body, with an annulus b6 that is somewhat broadened, while the back half of the body is noticeably more enlarged than the front. It appears that the annulus b6 was not subdivided into the annuli c11 and c12, or if it was, the division was only very small. The males' genital pore is found in the furrow b2/a2, whereas the females' genital pore is found in the furrow b5/b6. Both pores are separated by two annuli, which are the genital pores. When opposed to the dorsal surface, the ventral surface is simpler and has a brighter appearance. The number of eyes in the specimens that were investigated either significantly decreased or were not visible. The male reproductive system is characterized by a short cornua that is thick and a short atrial body that is relatively small. The cornua makes a sudden ventrally turn, elongating proximally to the b1/b6 border or to b6 of the preceding somite, with the apical part of the structure remaining uncoiled (Fig. 4 B and C). After the fourth ganglion, the vasa deferentia begin to become thicker and more firmly coiled, and the paired vasa deferens continue to stretch until the seventh ganglion, which is positioned beyond females' genital pore. The ovaisacks, which are a pair structure that lies dorsally to the vas deferens, are what distinguish the female reproductive system from the male system. These sacks continue to the fourth somite beyond the female genital pore. The ovarian sacks have a prominent coiled shape throughout their whole length (Fig. 4A).

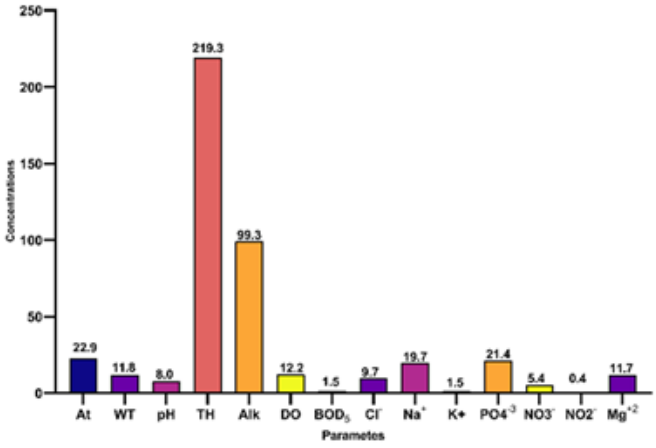


**Figure 2.** physicochemical parameters and nutrein elements. Air and water temperature (AT, WT), pH, Alkalinity (Alk.), Total Hardness (TH) Dissolved oxygen (DO), biological oxygen demand ($BOD_5$), Sodium Na+, Potacium K+, reactive phosphate $PO4^{-3}$, nitrite NO2-, nitrate NO3- and magnisium Mg+2

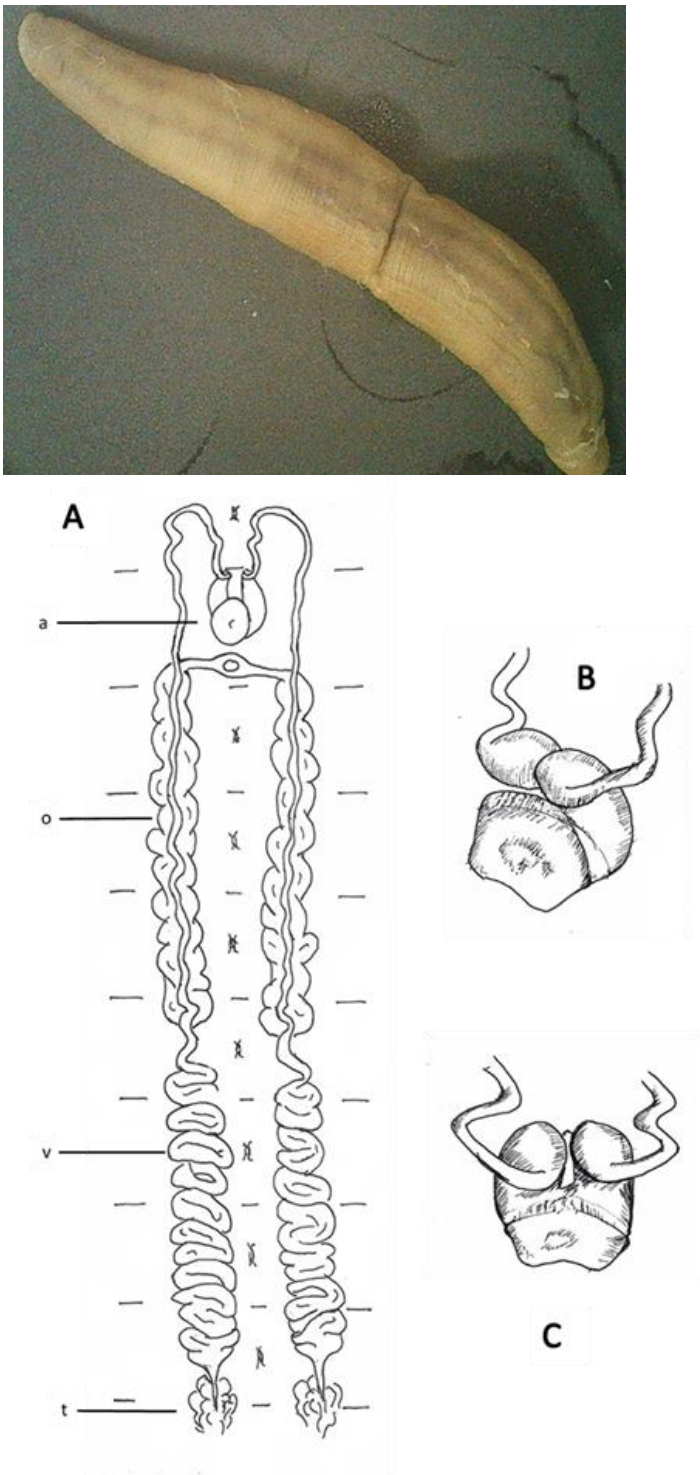


**Figure 4.** Sexual organs of *Dina prokletijaca*, A: internal anatomy (Abbreviations: a: atrium; o: ovarian sacks; v: vasa deferens; t: testisacs). B-C: atrium: B: ventrolateral view, C: ventral view

## Genetic investigation

*Extraction of genomic DNA*

The adult specimen leech's genomic DNA was extracted in its entirety through the GeneAll® ExgeneTM for Clinic Cell SV small kit., which was acquired from GeneAll® (Songpa-gu, Seoul, KOREA) and extracted using a 50-L elution buffer according to the manufacturer's instructions. Before conducting PCR, the extracted genomes were kept at -20°C.

*PCR amplification*

PCR approach was used for detection, and sequencing of *D. prokletijaca* based on mitochondrial regions. The variation of mtDNA was examined using amplified products of PCR. Mitochondrial areas were amplified with slight differently than Bouga et al. (Bouga, Harizanis, Kilias, & Alahiotis, 2005).

5' - GATTACTTCCTCCCTCATTA -3' and 5' - AATCTGGATAGTCTGAATAA -3' primers were used to amplify COI gene areas (Rahimi, Mirmoayedi, Kahrizi, Zarei, & Jamali, 2016).

PCR cocktail was performed in 50 µL reaction mixture composed of 25 µL of 2× PCR master mix, each primer (1.0 µL), 1.5 µL template of genomic DNA and adding water of free nuclease to completed 50 µL. The PCR was then carried out as follows: initial denaturation at 94°C for 4 minutes, 35 cycles of 94°C for 1 minute, annealing at 50°C for 1 minute, extension at 72°C for 2 minutes, and a final extension step at 72°C for 10 minutes. The amplified products were then electrophoresed on 1.5% agarose gel to confirm fragment size (Hamasalih & Abdulrahman, 2020).

*Analysis of DNA Sequencing*

The products of DNA from the PCR were primed for sequencing utilising forward COI gene primers and sent to Macrogen Company (238, Teheran-ro, Seoul, Korea). Sequence productes were aligned and compared to previously deposited sequences to at the National-Centre for Biotechnology Information (NCBI) GenBank. D. prokletijaca sequences were submitted in NCBI-GenBank (Fig. 5).

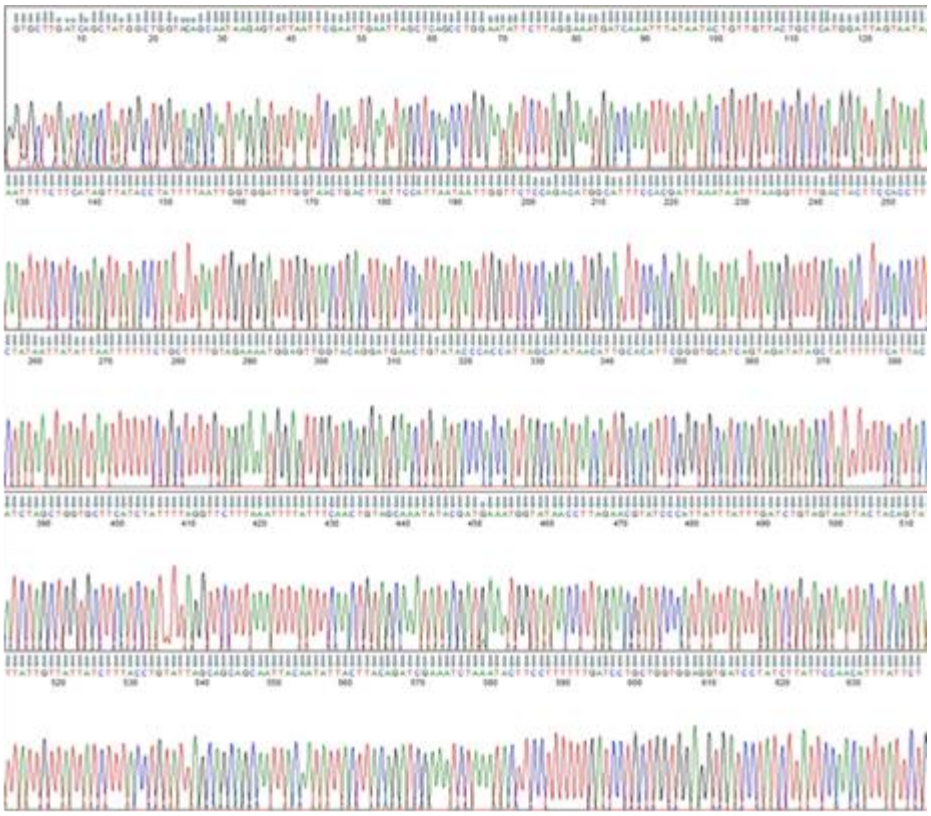

**Figure 5**. Chromatographic sequence of CO1 gene sequence of leech sample which proved to be more than 99% identical to *D. prokletijaca*

## Discussion

Leeches of the species *D. prokletijaca* can be recognised from leeches of the species *Dina lineata* by their larger size and more slender bodies, as well as their smaller mouth openings. *Dina lineata*-complex is the most diverse category, and it contains taxa that are found across a wider range (Nesemann & Neubert, 1999). The female genital pore of the small-sized *Dina lineata lacustris* differs in that it is always situated on b6 (either in the centre or at the end of the annulus), and it is never positioned in the furrow b5/b6 (Sket, 1968). Additionally, a reduction in the amount of pigmentation found in the eyes is easily observed in *D. prokletijaca*. Some immature specimens have a small eye, while the eyes of adults are either diminished or not visible. Variations in the extent of the ovarian sacks were also detected across the specimens. One specimen's ovarian sacks extend to the furrow b6/a1 between somites 3 and 4 behind the

female genital pore, while another specimen's ovarian sacks reach the fourth somite ganglion. Both of these locations are behind the female genital pore.

There was also evidence of an intermediate position, and in one specimen, the right ovarian sack extends to the ganglion of the fourth somite, whereas the left ovarian sack extends to the furrow b5/b6 of the third somite after the female genital opening, both of these positions were discovered by (Grosser et al., 2016).

Because this study is regarded to be the first one of its kind in Iraq and only the second one of its kind internationally on *D. prokletijaca*, there is not a lot of information available about this species. According to the ecological analysis of the water properties, we oberved that *D. prokletijaca* prefer living in cold water stream that characterized by cold water temperature (11.8 $C^{o}$), moderately hard water with medium nutrient (mesotrophic) stream, pH on alkaline side (8.0) and high disolved oxygen content.

## Conclusion

According to our finding, *D. prokletijaca* favours cold water mesotrophic streams with cold water temperature, moderately hard water, alkaline pH, and high disolved oxygen concentrations.

## References

Ahmed, S. T. (2009). Biological studies on leeches in Erbil and its suburbs. Ph. D. Thesis, Coll. Sci.., Univ. Salahaddin: 113pp.

Al-Salmany, S. O. K. (2015). Parasitic infections of some fish species from Euphrates River at Al-Qaim city, Al-Anbar province. M. Sc. Thesis, Coll. Sci., Univ. Al-Anbar, 193 pp.(In Arabic).

Ali, L. A. (2007). First record of Fadejewobdella quinqueannulata (Hirudinea: Erpobdellidae) in Greater Zab River-Iraq. Zanco the Scie. J. of the Univ. of Salahaddin, 2(22), 47–50.

Bashê, S. K. R. (2008). The parasitic fauna of spiny eel Mastacembelus mastacembelus (Banks and Solander, 1794) from Greater Zab River-Kurdistan region-Iraq. M. Sc. Thesis, Coll. Sci. Educ., Univ. Salahaddin: 62pp.

Bilal, S. J., Ali, L. A., Abdullah, L. Y., Khailany, R. A., Dhahir, S. F., & Abdullah, S. (2017). First Record of Leech Dina Punctata (Annelida: Erpobdellidae) from Lesser Zab River in

Northern Iraq: Morphological and Molecular Investigation. Jordan Journal of Biological Sciences, 10(2).

Bouga, M., Harizanis, P. C., Kilias, G., & Alahiotis, S. (2005). Genetic divergence and phylogenetic relationships of honey bee Apis mellifera (Hymenoptera: Apidae) populations from Greece and Cyprus using PCR–RFLP analysis of three mtDNA segments. Apidologie, 36(3), 335–344.

Grosser, C., Pešić, V., Berlajolli, V., & Gligorović, B. (2016). Glossiphonia balcanica n. sp. and Dina prokletijaca n. sp.(Hirudinida: Glossiphoniidae, Erpobdellidae)-two new leeches from Montenegro and Kosovo. Ecologica Montenegrina, 8, 17–26.

Hallaq, SH., J. (2020). Morphological and Molecular Investigations of Leeches (Annelida: Hirudinea) from Different Localities in Kurdistan Region/ Iraq.

Hallaq, S. J. H.-A., & Ali, L. A. (n.d.). First appearance of the cave leech Erpobdella mestrovi (Annelida: Erpobdillidae) in Iraq (Morphological and Molecular investigation) from Zalm stream, Sulaimani Province, Kurdistan Region, Iraq.

Hamasalih, R. M., & Abdulrahman, Z. F. A. (2020). Antibiofilm potency of ginger (Zingiber officinale) and quercetin against staphylococcus aureus isolated from urinary tract catheterized patients. Applied Ecology and Environmental Research, 18(1), 219–236. https://doi.org/10.15666/aeer/1801_219236

Herzog, P. H. (1969). Untersuchungen über die parasiten der süكwasserfische des Irak. Arch. Fischereiwiss, 20(2/3), 132–147.

Hopkins, W. A., Moser, W. E., Garst, D. W., Richardson, D. J., Hammond, C. I., & Lazo-Wasem, E. A. (2014). Morphological and molecular characterization of a new species of leech (Glossiphoniidae, Hirudinida): Implications for the health of its imperiled amphibian host (Cryptobranchus alleganiensis). ZooKeys, (378), 83.

Jordan, E. L., & Verma, P. S. (2010). Invertebrate zoology. New Delhi, India: S. Chanda and Company LTD.

Khalifa, K. A. (1985). Leeches on freshwater farmed fishes in Iraq. Journal of Wildlife Diseases, 21(3), 312–313.

Langer, S. V, Vezsenyi, K. A., De Carle, D., Beresford, D. V, & Kvist, S. (2018). Leeches (Annelida: Hirudinea) from the far north of Ontario: distribution, diversity, and diagnostics. Canadian Journal of Zoology, 96(2), 141–152.

Mhaisen, F. T., Al-Khateeb, G. H., Balasem, A. N., & Mutar, A. J. (1997). On a collection of some fish parasites from Euphrates River, Anbar province, Iraq. Babylon Univ. J., Pure Appl. Sci, 2(3), 267–272.

Nesemann, H., & Neubert, E. (1999). Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea. Süكwasserfauna von Mitteleuropa 6/2. Spektrum Akademischer Verlag Berlin.

Rahimi, A., Mirmoayedi, A., Kahrizi, D., Zarei, L., & Jamali, S. (2016). Genetic diversity of Iranian honey bee (Apis mellifera meda Skorikow, 1829) populations based on ISSR markers. Cellular and Molecular Biology, 62(4), 53–58.

Siddall, M. E. (2002). Phylogeny of the leech family Erpobdellidae (Hirudinida: Oligochaeta). Invertebrate Systematics, 16(1), 1–6.

Sket, B. (1968). K Poznavanju Favne Pijavk (Hirudinea) v Jugoslaviji, Zur Kenntnis der Egel-Fauna (Hirudinea) Jugoslawiens. Academia Scientiarum et Artium Slovenica Classis IV: Historia Naturalis et Medicina, Diss, 9(4), 127–197.

Stevanovic, Z., & Iurkiewicz, A. (2009). Groundwater management in northern Iraq. Hydrogeology Journal, 17(2), 367.