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First Record of Leech *Dina Punctata* (Annelida: Erpobdellidae) from Lesser Zab River in Northern Iraq: Morphological and Molecular Investigation

Samir J. Bilal[1,2], Luay A. Ali[3], Ladee Y. Abdullah[4], Rozhgar A. Khailany[5,6,*] , Sarah F. Dhahir[3] and Shamall M.A. Abdullah[1]

[1,2]*Department of Fish Resource and Aquatic Animal, College of Agriculture, Salahaddin University, Erbil, Iraq*

[2]*Department of Biology, Faculty of Eduaction, University of Ishik, Erbil, Iraq*

[3]*Department of Biology, College of Education, Salahaddin University, Erbil, Iraq*

[4]*Scienctific Research Center, Salahaddin University, Erbil, Iraq*

[5]*Department of Biology, College of Science, Salahaddin University, Erbil, Iraq.*

[6]*Department of Medical Biology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey*

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Abstract

A total of 17 specimens of the leech *Dina punctata* were collected from Lesser Zab River in Zirandul region near Qashqoli village during the period from July to October 2015. Specimens were examined either live using dissecting microscope, or after being fixed in 5% formaldehyde. Specimens used for molecular analysis of genomic DNA were fixed and preserved in absolute ethanol. Following DNA extraction, region of 28S rDNA was amplified by Polymerase Chain Reaction (PCR), and the nucleotides order was determined using genetic analyzer. Morphological and morphometric futures as well as molecular analysis showed that the collected specimens belonged to *D. punctata*. This is the first record for this species in Iraq.

Keywords: *Dina punctata*, 28S rDNA marker, DNA sequencing.

1. Introduction

Leeches belonging to the family Erpobdellidae constitute an important part of the freshwater benthic fauna of the Northern Hemisphere. Most erpobdellid species are predators on small invertebrates (Dall, 1983; Toman and Dall, 1997, Siddall, 2002). The seven genera of the family are chiefly characterized by two taxonomic characters: the presence or absence of a pre-atrial loop formed by paired male gono-ducts and the type of annulation (Sawyer, 1972). External characters like body shape, size, color and color patterns can be very variable depending on the method of fixation (Nesemann and Neubert, 1994). However, the phylogenetic relationship among genera of Family Erpobdellidae has been assessed based on morphological characters as well as molecular analysis of mitochondrial cytochrome c oxidase subunit I, mitochondrial 12S rDNA and nuclear 18S rDNA (Siddall, 2002). In the present report, the genera *Dina, Mooreobdella, Nephelopsis and Trocheta* are formally synonymised under the genus *Erpobdella*, the type genus of the family. In the present report, genus *Dina* is retained as it is still being used by many investigators (See Jeug (2008), Kutschera (2010) and Ahmad *et al*. (2015))**.**

The three most abundant and species-rich genera of erpobdellid leeches are *Erpobdella, Dina* and *Trocheta.* These have traditionally been distinguished by their annulation pattern. *Erpobdella* has five unsubdivided annuli per somite; in *Dina* the last annulus is widened and subdivided once, and in *Trocheta*, the last and often also the first annulus is further subdivided. Furthermore, the annulation pattern has proved to be inappropriate for the diagnosis of *Dina* (Neubert and Nesemann, 1995; Trontelj and Sket, 2000; Sket and Trontelj, 2008).

* Corresponding author. e-mail: rozhgar.mohammed@su.edu.krd or rozhgarbio@yahoo.com.

The present study reports for the first time on the occurrence of the leech *D. punctata* in Lesser Zab River, near Erbil City, northern Iraq. The leech was identified on the basis of morphological criteria and molecular analysis of 28S rDNA.

2. Materials and Methods

2.1. Study Site and Parasite Materials

In the present study, 17 samples of leeches were collected from Lesser Zab River from one site Zirandul region about 2 km before Qashqoli village, 109 km from Erbil City during the period July to October 2015. In the laboratory, 9 specimens were either examined alive using a dissecting microscope, or 5 specimens fixed in 5% formaldehyde for dissection further examination. Three specimens were fixed and preserved in absolute ethanol for molecular analysis.

2.2. DNA Extraction, PCR Amplification and Nucleotide Sequencing

Genomic DNA from leech specimens was prepared using a DNA extraction kit (GeNet Bio, KOREA) and following the manufacturer's instructions with minor modification. Briefly, leech specimens were macerated in mortar and pestle, and the contents were transferred into sterile tubes containing 200-250 µL tissue lysis buffer and kept in incubator for 4 hours. Qualification and quantification of DNA concentration was performed by using Nano Drop (ND- 1000, USA). Samples of genomic DNA with (A260–A320) / (A280–A320) ratio more than 1.7 and outputs more than 30 ng/µL were obtained.

A region of 28S rDNA was amplified by polymerase chain reaction (PCR). The primers were universal, forward primer C1 (ACCCGCTGAATTTAAGCAT at position 25), and reverse primer C3 (CTCTTCAGAGTACTTTTCAAC at position 390), as designed and selected by Mollaret *et al.* (2000). PCR reaction and conditions were performed using MJ Research, Applied Biosystem (AB) thermal cycler. Fifty µL reaction mixture was prepared in PCR tubes containing 2.5 µL DNA templates, 25 µL OnePCR^{TM} master mix (GENEDIREX, KOREA), 1 µL for each primer and 20.5 µL double deionized water (ddH_2O). The cycling conditions comprised of initial denaturation at 94˚C for 5 min, 35 cycles of denaturation at 94˚C for 45 sec, annealing temperatures at 51˚C for 45 sec and extension at 72˚C for 45 sec, and final extension at 72˚C for 5 min. Agarose gel electrophoresis was employed to check the efficiency of PCR reactions. The samples were prepared and run in 2% gel of agarose then stained with SYBR green that makes the DNA visible under UV light.

The ABI 3130X nucleotide sequence analyzer (SINGAPORE) was used to find nucleotides order of 28S rDNA from the specimens. The PCR fragments of the specimens were excised from the agarose gel and used as a source of DNA template for sequence specific PCR amplification.

3. Results

3.1. Morphological Characterization

Thick and large leeches up to 75 mm length, with colors that vary from dark brown or reddish brown to greenish with one pair of dirty paramedian stripes and yellowish spots were arranged transversally on each annulus. As for annulations, the somites consist of four short, and one long, annuli, with annulation formula (b1, b2, a2, b5 and b6), b6 annuli is larger than the other and divided by a shallow furrow (c11, c12). Genital pores separated by 1.5 to 2 annuli; male gonopore in the furrow of XII b2/a2, female pore in XII b5.b6. Atrium thick with curved cornua and simple coiled ends (Nesemann, 1993).

Nine specimens were used to determine the morphometric features of the collected leeches. The leeches were greenish in color with bright transverse yellow spots in life specimens (Fig. 1: A; B; C). Their dimension ranges and (means) were as follows: length, 20.3-70.7 mm (50.6 mm), width, 6.2-8.9 mm (7.5 mm), atrium, 2.1-2.3 mm (2.25 mm) on smoite XII (reaching from XI/XII to XII b2/a2) (Fig. 1 E), pseudognaths highly developed, 1.8-2.2 mm (1.92 mm) (Fig. 1 A; C). Morphometric features conform well to *Dina punctata* descriptions as per Nesenann (1993).

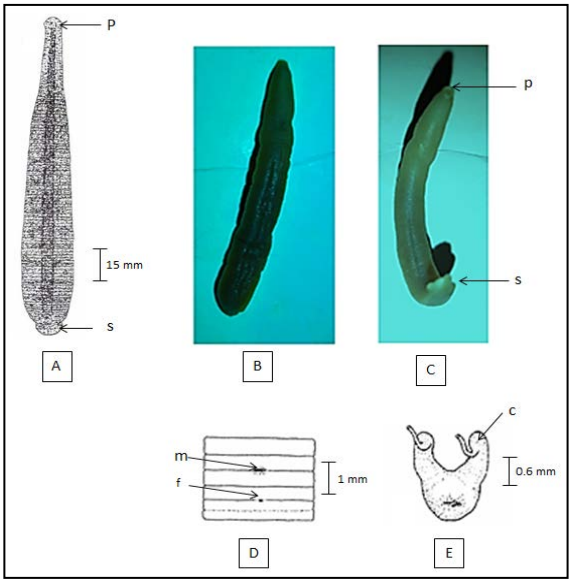

Figure 1. *Camera Lucida* drawings and photomicrographs of a leech specimen that were identified as *Dina punctata*.

A. Camera lucida drawings of the leech (Dorsal side), showing pseudognaths (P) and posterior sucker (S).
B. Photomicrography of the leech (Dorsal side).
C. Photomicrography of the leech (Ventral side).
D. Male (m) and female (f) gonopores on somite XII (Ventral view).
E. Atrium with cornua (c) (Ventral view).

3.2. Molecular Characterization

The sequence from 28S rDNA of *leech specimens* was made of 300 bp (amplified fragment was 365bp, while after sequencing 65 miss-nucleotides were excluded, related to quality of sequencing analysis) and put to BLAST then compared with other stored species of *Dina* sequences from GenBank database. The BLAST results indicated that the query sequence was more than 99% identical to *D. punctata* (Fig. 2 and 3).

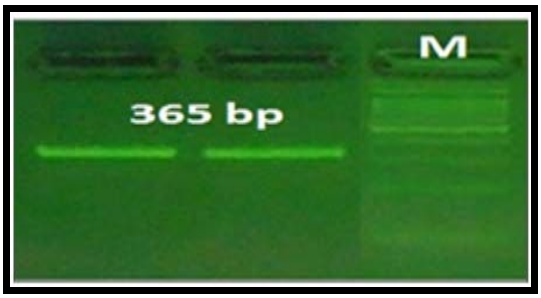

Figure 2. The result of 2% agarose gel stained with SYBR green of 28S rDNA of leech specimens following molecular analysis.

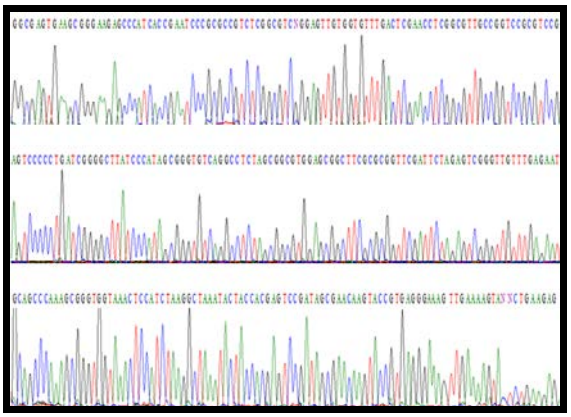

Figure 3. The chromatography sequence result of 28S rDNA sequence of leech specimens which proved to be more than 99% identical *to Dina punctata.*

4. Discussion

The morphological characters, body part measurements and colorations of the examined specimens conform to the descriptions of *D. punctata* recorded by Moore (1939), Nesemann (1993) and Ahmed *et al*. (2015). Previously, only one species of the present genus, *D. lineata* was recorded in Kurdistan Region, Iraq from Greater Zab River by Ali and Jaweir (2013). Since, there are no previous reports for this species in Iraq, the present records regarded as the first for *D. punctata* in this country.

Dina lineata was recorded from Spain and two assortments notata and punctata was recognized (Nesemann, 1993). Johansson (1927) described the new assortment *punctata*, but subsequent authors did not segregate the two assortments. Ahmed *et al*. (2015) reported that the scientific classification of the genus *Dina* in the western Mediterranean requires revision. Additionally, Minelli (1979) reported that *D. lineata* recorded from Italy is most likely *D. punctata*. Indeed, Jueg (2008) indicated that *D. punctata* in the Iberian Peninsula is exceptionally regular and *D. lineata* is absolutely truant. This investigator recommended that *D. lineata* reported from the Iberian Peninsula by García-Más and Jiménez (1984) and García-Más *et al*. (1998) was actually *Dina punctata*. Furthermore, Nesemann and Neubert (1994) described *D. punctata maroccana* as another subspecies from Morocco and they suggested that some of the specimens described as *Dina lineata* by Moore (1939) from Morocco can be considered to be co-unspecific to their new subspecies. Ahmed *et al*. (2015) thought that *D. punctata maroccana* may be synonymous with *D. punctata*. The taxonomic status of D. lineata and the geographic conveyance of *D. punctata* stay unverifiable till (Nesemann, 1993; Ahmed *et al*., 2015).

Most external characters and dorsal coloration of *Dina punctata* and *Dina stschegolewi* are similar (Nesemann, 1993). In addition to this, for proper identification of some taxa the cocoons need to be examined. Indeed, Kutschera (2010) mentioned that the new described species *Trocheta intermedia* a leech from Germany resembles the taxon *D. punctata* from Switzerland, but they differ in colorations of cocoons, and in most cases collection of these cocoons is difficult.

Thus, there is a need for more reliable methods including molecular analysis to identify species belonging to genera of Family Erpobdellidae (Siddall, 2008). *D. punctata* species is molecularly well distinguished from the other available species of *Dina.* The primary sequence analysis using universal primers of studied specimens revealed that the leech from northern Iraq belongs to species *D. punctata* (Fig. 3). Its rDNA conforms to the same rDNA sequence fragment marker, available at the GeneBank in National Center for Biotechnology Information (NCBI).

In conclusion, that is the first record for the existence of *Dina punctata* in Iraq. The morphological characters, body parts and colorations of the specimens and DNA sequence based analysis revealed the identity of *D. punctata.*

References

Ahmed RB, Romdhane Y and Tekaya S. 2015. Checklist and distribution of marine and freshwater leeches (Annelida, Clitellata, Hirudinea) in Tunisia with identification key. Eco. Mont, 2(1): 3-19.

Ali AA and Jaweir. 2013. First record of *Dina lineata* (Hirudinea: Erpobdellidae) in Greater Zab river Kurdistan region-Iraq. J. U. Zakho, 1: 207-209.

Dall PC. 1983. The natural feeding and resource partitioning of *Erpobdella octoculata* L. and *Erpobdella testacea* Sav. in Lake Esrom, Danmark. Int. Rev. Ges. Hydrobiol, 68: 473–500.

García MI and Jiménez JM. 1984. Introducción al estudio de las comunidades macrobentónicas de losrios asturianos : Hirudineos. Limnetica, 1:179–186.

García MI, López FM, Mora AM and Ortega GT. 1998. Hirudineos (Annelioda, Hirudinea) de las aguas continentales de la Comunidad Valenciana (Espana). Boletín de la Real Sociedad Española de Historia Natural Madrid, 94 (1-2): 149–161.

Johansson L. 1927. Hirudineen aus dem nördlichen und östlichen Spanien. Gesammelt von Dr. F. Haas in den Jahren 1914-1919, Abh. Senckenb. Naturforsch.Ges, 39, 217–231. (In German).

Jueg, U. 2008. Beitrag zur Egelfauna (Hirudinea) der Iberischen Halbinsel. Lauterbornia, 65, 83–104, Dinkelscherben.

Kutschera U. 2010. A new leech species from Southern Germany, *Trocheta intermedia* nov. sp. (Hirudinea: Erpobdellidae). Lauterbornia, 70: 1-9.

Mollaret, I, Jamieson, BG and Justine J. 2000a. Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. Int. J. Parasitol, 30: 171–85.

Minelli A. 1979. Hirudinea (Fauna d'Italia, 16), Calderini, Bologna.

Moore JP. 1939. Leeches (Hirudinea) from the Atlas Mountains of Morocco. Annals and Magazine of Natural History, 3: 80–87.

Nesemann H. 1993. Identification key to the Hungarian leeches of the subfamily Trochetinae Pawlowski, 1954, with notes on systematics of the subfamily Erpobdellinae Blanchard, 1894 (Hirudinea). Annls hist-nat Mus. nam. Hung, 85:19-35.

Nesemann H and Neubert E. 1994. New data to the leeches of the subfamily Trochetinae (Hirudinea, Erpobdellidae). Miscellanea Zoologica Hungarica, 9, 19–28.

Neubert E and Nesemann H. 1995. Contribution to the knowledge of the genus Dina Blanchard (1892) (Hirudinea: Erpobdellidae). Hydrobiologi, 315 (2): 89-94.

Siddall, M. 2002. Phylogeny of the leech family Erpobdellidae (Hirudinida : Oligochaeta). Invertebrate systematics, 6: 1-6.

Sawyer RT. 1972. North American Freshwater Leeches, exclusive of the Piscicolidae, with a key to all species. Illinois Biol. Monogr, 56: 155 pp.

Sket B and Trontelj P. 2008. Global diversity of leeches (Hirudinea) in freshwater. Hydrobiologia, 595:129-137.

Toman MJ and Dall PC. 1997. The diet of *Erpobdella octoculata* (Hirudinea: Erpobdellidae) in two Danish lowland streams. Arch. Hydrobiol, 140: 549-563.

Trontelj P and Sket B. 2000. Molecular re-assessment of some phylogenetic, taxonomic and biogeographic relationships between the leech genera *Dina* and *Trocheta* (Hirudinea: Erpobdellidae). Hydrobiologia, 438: 227-235.