Piscicola geometra (Linnaeus, 1761) leech parasitizing two fish species hosts from Greater Zab near Aski-Kalak, Kurdistan-Iraq, morphological and molecular investigations

Sh. J. Hamad-Ali1 and L. A. Ali2

1Fish Res. And Aquatic Animals Dept., College of Agricultural Engineering Sciences, Salahaddin University – Erbil.
2Biology Dept., College of Education, Salahaddin University – Erbil.

*Corresponding author's e-mail: shayan.ali@su.edu.krd

Abstract. Samples of two fish species, Cyprinus carpio and Silurus triostegus were collected from Aski-Kalak and inspected for the presence of parasitic leeches from March 2018 to February 2019 with a prevalence of 6.66 and 8.33 and mean intensity 0.10 and 0.11 respectively. Both hosts’ species were regarded as new hosts for this parasite in Iraq since no previous reports in this country. Collected leeches were fixed, preserved, and identified based on morphological and molecular techniques. For molecular identification, amplification and sequencing of 18S rDNA, with forward primer C1, (ACCCGCTGAACTTTAAGCAT, position 25), and reverse primer C3, (CTCTTCAGAGTACTTTTCAAC, position 390) were studied, while morphological identification based on well described, and distinguished by focusing on color patterns, number vis arrangement of eyes, body annulations, gonopores shape and location, shape, location and size of body parts, in addition to some species-specific characters, criteria’s were documented by photographing and drawing illustrations.

Key words: Piscicola geometra, parasitic leeches.

1. Introduction
Leeches can be defined as members of phylum Annelida, class Clitellata, subclass Hirudinea, they closely related to Oligochaeta [1]. They are characterized by presence of 33 - 34 segments, designated with Roman numbers (I-XXIV), each somite contains a ganglion in the superficial furrows into 2 - 16 annuli [2], [3].

Leeches primarily are restricted to freshwater, also, several species are estuarine, marine, and terrestrial. It includes more than 300 species, most are ectoparasites on freshwater forms but rarely marine, feeding upon fishes and other animals [4], [5], [6].

Leeches are one of the threats in wild and aquacultures waters. Heavy infestations of fishes may cause chronic anemia. Skin infestations can change the fish behavior, like rubbing the body against objects around causing injurious ulcers on the skin or/and the mouth that regards as a source for secondary infections [7], [8]. Moreover, leeches are the main vector for other parasites (transporting of hemoparasites during their feeding)[8] [9]. The human can be infected with different disease causatives during leech using for treatment, especially if these leeches were not from cured cultures or when re-use of them with no proper disinfection process [4].

The present work aimed to survey morphologically as well as molecularly for the presence of the parasitic leech species on fishes from Greater Zab at Aski-Kalak District.
2. Materials and Methods

Greater Zab River is one of the Tigris River sub-basins. Leech samples were collected by surveying fish skin and fins, once captured, leeches were transported with the river water for processing in the laboratory of Fish Resources and Aquatic Animals Department/College of Agricultural Engineering Sciences in Salaladdin University-Erbil [10].

For the morphological study, specimens were relaxed by putting leeches into a small to medium size jar with tap water, then replaced with distilled water, and relaxed by using dropwise additions of 10% ethanol, hence when no response of leech seen to the touch (mostly 15-30 minutes). The extended length and diameter were measured, color patterns recorded, clitella and gonopores position detected, eyes counted, suckers shape and lines of each specimen enumerated [11],[12]. Leeches fixed by gently pressing (between two glass slides), put in 10% buffered formalin (for at least 3 hrs), and preserved in 70% ethanol [13], [14] [15] protocol was followed for the molecular characterization and identification. DNA Extracted from life deep freeze in ddH2O at -22°C fixed samples. Samples melted for extraction of the genomic DNA with a DNA extraction kit (GeNet Bio, KOREA). For amplification of 18S rDNA region with a polymerase chain reaction (PCR). Universal primers were used, forward primer C1 (ACCCGCTGAATTTAAGCAT at position 25), and reverse primer C3 (CTCTTCAGAGTACTTTTCAAC, position 390) were used. All products that of the PCR machine was verified on a 2% agarose gel. Gel electrophoresis was employed to check the validity of PCR products.

3. Results and Discussion

*Piscicola geometra* (Linnaeus, 1761)

Seven leech specimens of the fish parasite *Piscicola geometra* were noticed on the skin and fin base of the cyprinid fish *Cyprinus carpio* and the silurid fish *Silurus triostegus* from Ask-Kalak (Table 1).

The body length was 19.41-21.68 mm, width 4.33-6.05 mm, oral sucker dimensions 3.42 X 3.86 mm, Posterior disk 6.82-7.27 mm (Fig 1). Body coloration, striated dark altered by light rings. *P. geometra* specimens caudal sucker have had 12-14 punctiform ocelli and dark rays, this identification characteristic for this species (Fig 1).

Many previous works reported the same measurements and morphologic characters of the present studied specimens [16], [17], [18].

The molecular characterization of this species showed clear results, 99% similarity of 18S rDNA (comparing of 507 bp) with that of the Gene Bank (Fig. 2; 3; 4). *P. geometra* has a Palaearctic distribution, according to Bere in 1929, it is reported on the trout from lakes in Wisconsin, and Moore in 1898 isolated it in Germany on the common carp from Columbia District, later [16] reported this species parasitizing *Lepomis gibbosus* in Michigan. Oliver in (1958) reported the first occurrence of the present species in Canada were. Also, *P. geometra* is a very common leech in Europe and North America [19], [20], [21],[22].
Table 1. Showing the Prevalence, mean intensity and site of infection.

| Fish Host         | No. of examined fishes | No. of infected fishes | Prevalence % | Mean intensity | Site of infection |
|-------------------|------------------------|------------------------|--------------|----------------|------------------|
| *Cyprinus carpio* | 26                     | 2                      | 6.66         | 0.10           | Skin             |
| *Silurus triostegus* | 36                     | 3                      | 8.33         | 0.11           | Fin base         |

**New host.

In Iraq, [23] reported Piscicola sp. for the first time on Barbus schejch from Tigris River near Baghdad. Exactly, for *P. geometra*, [17] reported it from Leuciscus vorax (as Aspius vorax); [24] reported it on Luciobarbus esocinus (as Barbus esocinus) and [25] recorded it on Arabibarbus grypus (as Barbus grypus). Worth to mentioning, Ali in 1989 mentioned unidentified species on the skin of Barbus esocinus (synonym Lucibarbus esocinus) from the Greater Zab River, while Shamsuddin et al. in 1971 reported another species on Arabibarbus grypus (as Barbus grypus), and according to the present results that compared to the previous reports, both fish hosts *C. carpio* and *S. triostegus* are new hosts for this leech species in Iraq [26].

Fig. 1: Piscicola geometra, Lucida drawing.
Fig. 2: Pair-wise alignment of 18S rDNA sequence of Piscicola geometra. Query is the study or sample sequence and Sbjct is in the GenBank sequence.

Fig. 3: The sequencing result of 18S rDNA of Piscicola geometra.
4. Conclusion
The present work concluded that both fish hosts, *C. carpio* and *S. triostegus* are new hosts for *P. geometra* in Iraq that added to the three previous hosts were reported for it, *Arabibarbus grypus*, *Leuciscus vorax*, and *Luciobarbus esocinus*.

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