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First Report of *Hemiclepsis marginata* Parasitism on *Alburnoides holciki* in Uzbekistan

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Abstract Parasitic freshwater leeches may affect fish health by producing feeding lesions, weakening the host and creating favourable conditions for secondary infections; however, host records from Central Asian inland waters remain limited. We report a new host-parasite association of the freshwater leech *Hemiclepsis marginata* with the riffle minnow *Alburnoides holciki* in the Surkhandarya basin, Uzbekistan. In late May 2025, leeches and fish were collected from shallow freshwater biotopes at 38°19'03.3"N, 67°54'27.2"E. Twenty-four leech specimens were examined using external morphology, body measurements and standard taxonomic characters. Leeches were attached to the soft external tissues of *A. holciki*, mainly the gills, fins and skin. The locality was characterized by 30-70 cm water depth, 15-20 °C water temperature, pH 6.9 and dissolved oxygen of 8.11 mg/L. The bottom substrate was sandy and slightly marshy and contained insect larvae, mollusks and other benthic invertebrates. A review of available host records for the genus *Hemiclepsis* revealed no previous record of *H. marginata* parasitizing *A. holciki*. This finding expands the known host range of *H. marginata* and represents the first documented association between this leech and *A. holciki*. The occurrence of the species at a locality showing visible signs of anthropogenic disturbance provides additional information on its habitat use in Uzbekistan. Further studies should assess prevalence, seasonal dynamics, pathological effects and the potential role of *H. marginata* in pathogen transmission within native fish communities.

Keywords Glossiphoniidae, Host-parasite interaction, Cyprinid fish, Central Asia, Inland waters, Host record

1. Introduction

Leeches (Annelida: Clitellata: Hirudinida) are an ecologically diverse group of annelids inhabiting freshwater, marine and terrestrial ecosystems. More than 900 species have been described worldwide, although recent taxonomic, molecular and genomic studies indicate that hirudinean diversity remains incompletely documented, particularly in poorly investigated regions [11,13,19,20]. Freshwater leeches occupy a range of trophic niches, including predation, temporary ectoparasitism and associations with other aquatic organisms. The family Glossiphoniidae Vaillant, 1890 is an exclusively freshwater lineage that includes numerous species with specialized host associations [21].

Parasitic and blood-feeding leeches are associated with various vertebrate hosts, particularly fishes, amphibians and reptiles. Fish-associated leeches usually act as temporary ectoparasites and may attach to the skin, fins, oral cavity and gills [4,23]. Their feeding activity can cause localized tissue damage, haemorrhage and blood loss, while severe infestations may lead to epithelial damage, inflammatory responses and reduced host condition [1,24]. Gill attachment may be particularly important because injury to highly vascularized branchial tissues can potentially affect respiratory function. Feeding lesions may also facilitate secondary infections, and some fish-associated leeches have been implicated as mechanical or biological vectors of aquatic pathogens [2,4,6]. Thus, fish-leech interactions are relevant to parasite biodiversity, fish health and freshwater disease ecology.

The genus Hemiclepsis Vejdovsky, 1884 comprises dorsoventrally flattened glossiphoniid leeches occurring mainly in freshwater habitats. Members of the genus are associated with freshwater fishes and other vertebrates, while several species also use freshwater mussels as shelters or secondary hosts [3,18]. Recent studies show that host associations within Hemiclepsis are more diverse than previously recognized. *Hemiclepsis yangtzenensis* Xu et al., 2021, for example, was described from Asian swamp eel (Monopterus albus) stocks in China [18], while hirudiniasis caused by *Hemiclepsis marginata asiatica* has been documented in aquarium-reared red-bellied pacu, Piaractus brachypomus [22]. These findings emphasize the importance of new host records for understanding host specificity and ecological plasticity in freshwater leeches.

Hemiclepsis marginata (O. F. Müller, 1774) is one of the most widely distributed members of the genus and has been reported from Palearctic and Oriental freshwater systems. Its

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morphology, taxonomy, geographic distribution and host associations have been discussed in both classical and recent studies [10,17]. In Uzbekistan, the species was historically reported from the Zarafshan River basin and other inland waters [14]. More recent morphological and molecular studies confirmed H. marginata in the Fergana Valley and provided additional information on its taxonomy and biology in Central Asia [17]. Associations with the Aral basin snowtrout Schizothorax eurystomus and the marsh frog Pelophylax ridibundus have also been documented in Uzbekistan [17]. Nevertheless, information on the host spectrum, attachment sites and ecological characteristics of H. marginata in Central Asian inland waters remains limited.

Reliable documentation of new host–parasite associations is particularly important in regions where freshwater parasite communities are insufficiently studied. Recent studies of fish-associated leeches have shown that well-documented host records can provide useful information on parasite distribution, host use and regional parasite diversity [23]. Studies of other fish-parasitic leeches also indicate that geographic and host ranges may be broader than suggested by historical records [25]. Therefore, additional verified host records are needed to better understand the host range of H. marginata.

The riffle minnow *Alburnoides holciki* Coad & Bogutskaya, 2012 (Leuciscidae) is a Central Asian freshwater fish originally described from the Hari River basin [5]. Subsequent morphological and molecular studies expanded its known distribution in Central Asia [9], and DNA barcoding confirmed its occurrence in the inland waters of Uzbekistan [15]. Some populations previously identified as *A. eichwaldii* were shown to represent *A. holciki*, demonstrating that its distribution extends farther north than previously recognized [15]. Despite increasing knowledge of its distribution and molecular identity, information on the parasites and ectoparasitic associations of *A. holciki* remains very limited.

The Surkhandarya basin in southern Uzbekistan forms part of the upper Amu Darya drainage and supports diverse native freshwater communities. However, host-parasite relationships involving freshwater leeches and native fishes of this region remain poorly documented. Establishing such records is important for improving regional biodiversity inventories and providing baseline information for future studies of parasite distribution and fish health.

Therefore, the aim of the present study was to document a previously unreported host–parasite association between *Hemiclepsis marginata* and *Alburnoides holciki* in the Surkhandarya basin, Uzbekistan. We provide morphological evidence for leech identification, document its attachment to the external tissues of *A. holciki*, characterize the environmental conditions of the collection locality, and evaluate the record in the context of previously published host associations within the genus Hemiclepsis. This study expands current knowledge of the host range of *H. marginata* and contributes new information to the parasitological and biodiversity inventory of Central Asian freshwater ecosystems.

2. Materials and Methods

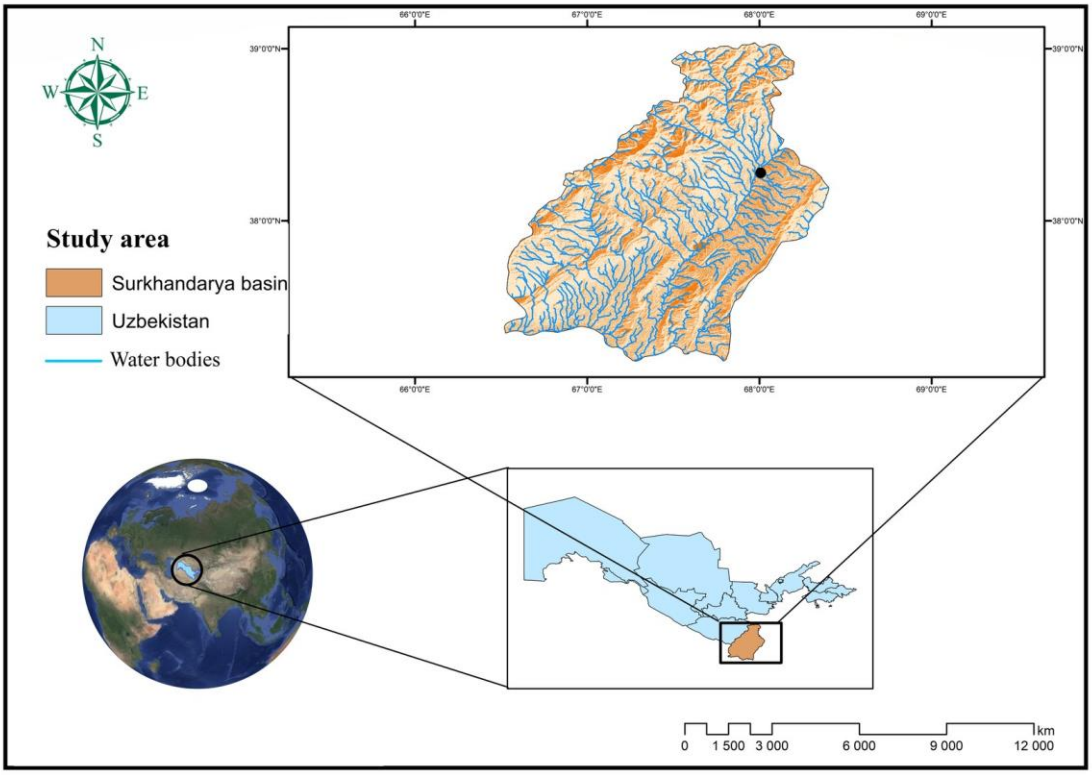

Figure 1. Sampling Locality of *Hemiclepsis marginata* in the Surkhandarya Basin, Uzbekistan

Leech specimens were collected in late May 2025 from freshwater biotopes of the Surkhandarya basin, Uzbekistan (38°19'03.3"N, 67°54'27.2"E). A total of 24 leech individuals of different body sizes were obtained using hydrobiological collecting instruments. Fish specimens were collected from the same biotopes where the leeches were recorded. The sampling locality and specimen photographs were documented by Salokhiddin Usmonov and Khayrulla Solijonov (Figure 1).

For microscopic examination, part of the material was fixed in 10% ethanol and preserved in 96% ethanol. Body dimensions were measured with a caliper. External morphology and diagnostic characters were examined in glycerin medium using a biological research microscope (XPS-500Ye). Microphotographs were taken with a Bresser Science ETD-201 stereomicroscope equipped with a BRESSER MicroCam SP 5.0. Species identification was based on the taxonomic keys and descriptions of Harding [7] and Lukin [10]. The fish species was identified following Coad and Bogutskaya [5].

Water depth, temperature, pH and dissolved oxygen were recorded at the sampling site. Because the study reports a first host record based on field observations and morphological identification, the results are presented descriptively. The host record was evaluated against published information on host associations in the genus *Hemiclepsis*.

3. Results

3.1. Taxonomic Position

Phylum: Annelida Lamarck, 1809
Class: Clitellata Michaelsen, 1919
Subclass: Hirudinea Lamarck, 1818
Order: Hirudinida Siddall et al., 2001
Suborder: Glossiphoniiformes Tessler & de Carle, 2018
Family: Glossiphoniidae Vaillant, 1890
Genus: *Hemiclepsis* Vejdovsky, 1884
Species: *Hemiclepsis marginata* (O. F. Müller, 1774)

3.2. Material Examined

Uzbekistan, Surkhandarya basin, 38°19'03.3"N, 67°54'27.2"E, late May 2025, 24 specimens, collected from shallow freshwater biotopes and from the external surface of *Alburnoides holciki*. The material was collected and photographed by Salokhiddin Usmonov and Khayrulla Solijonov.

3.3. Morphological Identification

The examined specimens corresponded to *Hemiclepsis marginata*. Adult individuals were medium-sized, dorsoventrally flattened and somewhat transparent. Body length in adults was 16-18 mm, body width was approximately 2.5-8.0 mm, and fully extended individuals reached 27-30 mm. The body was claviform. The margins and anterior extremity were almost colorless or hyaline, whereas the ground coloration was pale yellow.

The dorsal surface was variegated with orange, reddish-brown, lemon-yellow and green pigments. Lemon-yellow spots formed longitudinal rows on the dorsal surface. Low papillae were visible in positions corresponding to the outer paramedian and intermediate sense organs. The ventral surface lacked distinct spots and papillae. The posterior sucker bore a paramarginal row of lemon-yellow spots with reddish-brown radial streaks. The body consisted of 72 annuli; the first pair of eyes was situated on annulus 3 and the second, larger pair on annulus 4. The male gonopore was located between annuli 29 and 30, and the female gonopore was two annuli posterior to it, between annuli 31 and 32.

3.4. Host Association and Habitat

Leeches were observed attached to the soft external tissues of *A. holciki*, particularly the gills, fins and skin (Figure 2). This record represents the first documented host-parasite association between *H. marginata* and *A. holciki* from the Surkhandarya basin.

The sampling site was a shallow freshwater habitat with a water depth of 30-70 cm, water temperature of 15-20 °C, pH 6.9 and dissolved oxygen concentration of 8.11 mg/L. The bottom was sandy and slightly marshy. Insect larvae, mollusks and other benthic invertebrates were abundant. The site was located near a residential area, where plastic waste, food debris and construction materials were observed in the water.

4. Discussion

The present study documents *Alburnoides holciki* as a previously unreported host of *Hemiclepsis marginata* and expands the known host range of this freshwater leech. Species of Hemiclepsis are associated with a broad spectrum of aquatic vertebrates, particularly freshwater fishes, while some species also use freshwater mussels as shelters or secondary hosts (Table 1) [3,18]. However, no previous record of *H. marginata* parasitizing *A. holciki* was found in the available literature.

Previous studies have reported *H. marginata* from several cypriniform fishes, including *Capoeta capoeta gracilis*, *Squalius cephalus*, *Cyprinus carpio*, *Barbus spp.* and *Schizothorax eurystomus* [1,8,17]. The present record from *A. holciki* further indicates that H. marginata is not restricted to a single fish genus and may have a relatively broad host spectrum among freshwater fishes. Similar observations in other fish-associated leeches have shown that verified host records can considerably expand knowledge of parasite host ranges [23,25]. Nevertheless, additional sampling is required to determine whether *A. holciki* is a regular or occasional host.

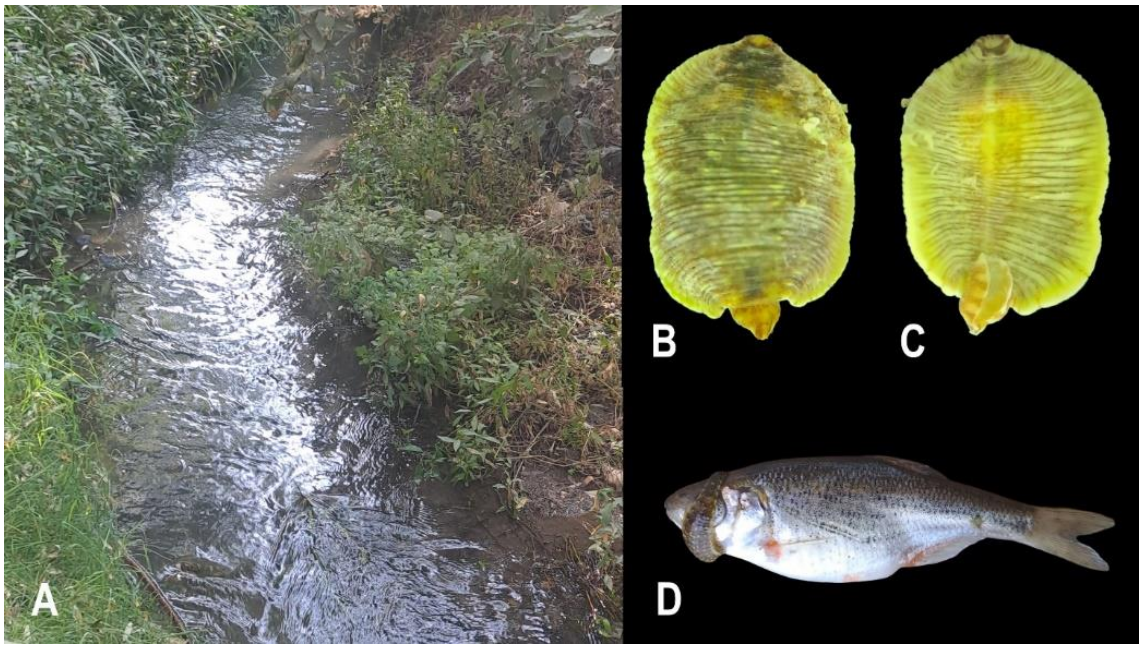

Figure 2. *Hemiclepsis marginata* and Its Habitat: (A) Natural Habitat; (B) Dorsal View; (C) Ventral View; (D) Host-Parasite Association Between *A. holciki* and *H. marginata*. Photographs by Salokhiddin Usmonov

Table 1. Host Records for Species of the Genus *Hemiclepsis* Vejdovsky, 1884

No.	Species	Recorded hosts
1	*H. marginata* (O. F. Müller, 1774)	*Capoeta capoeta gracilis*, *Squalius cephalus* (Adel & Salimi, 2017); *Clarias* spp., *Tilapia*, *Cyprinus carpio*, *Barbus sharpeyi*, B. xanthopterus (Khalifa, 1985); *Schizothorax eurystomus*, marsh frog *Pelophylax ridibundus*, amphibian larvae and occasionally mollusks (Solijonov et al., 2024); *Alburnoides holciki* (this study).
2	*H. kasmiana* (Oka, 1910)	Secondary host and shelter: freshwater mussels *Aculamprotula*, *Buldowskia*, *Cristaria*, *Inversunio*, *Middendorfinaia*, *Nodularia*, Sinanodonta, *Lamprotula*, *Pronodularia*, *Obovalis* and *Margaritifera dahurica*; primary host: freshwater fishes *Perccottus* and *Silurus* (Bolotov et al., 2019).
3	*H. asiatica* (Moore, 1924)	Freshwater fishes.
4	*H. japonica* (Oka, 1932)	Unknown.
5	*H. viridis* Chelladurai, 1934	Frogs.
6	*H. bhatiai* Baugh, 1960	Unknown.
7	*H. hubeiensis* Yang, 1981	Freshwater fishes.
8	*H. erhaiensis* Yang, 1981	Freshwater fishes.
9	*H. guangdongensis* Tan & Liu, 2001	Amboina box turtle *Cuora amboinensis* (Geoemydidae).
10	H. chharwardamensis Mandal, 2013	Unknown; association with freshwater mollusks and fishes was suggested by Mandal (2013).
11	*H. khankiana* Bolotov et al., 2019	Secondary host and shelter: freshwater mussels *Nodularia*; primary host: freshwater fishes *Rhodeus* (Bolotov et al., 2019).
12	*H. myanmariana* Bolotov et al., 2019	Secondary host and shelter: freshwater mussels Lamellidens and Indonaia; primary host: freshwater fishes *Labeo* (Bolotov et al., 2019).
13	*H. schrencki* Bolotov et al., 2019	*Barbatula* and *Phoxinus* (Bolotov et al., 2019).
14	*H. tumniniana* Bolotov et al., 2019	*Pungitius* (Bolotov et al., 2019).
15	*Hemiclepsis* sp. 1 Bolotov et al., 2019	Unknown.
16	*H. yangtzenensis* Xu et al., 2021	*Monopterus albus* (Xu et al., 2021).

Note: The record of *A. holciki* as a host of *H. marginata* is reported for the first time in the present study.

Leeches were observed attached to the gills, fins and skin of A. holciki. These sites are biologically important because feeding by leeches may cause localized haemorrhage and tissue damage, while lesions may facilitate secondary infections [1,4,24]. Gill attachment may be particularly relevant because branchial tissues are delicate and highly vascularized. Previous studies have associated leech parasitism with tissue damage and secondary pathogens such as Saprolegnia and Trypanosoma [1,8]. However, pathological and histological effects were not examined in the present study, and therefore no direct adverse effect on *A. holciki* can be concluded.

The record is also relevant to the ecology of *A. holciki*. Morphological and molecular studies have demonstrated that this Central Asian riffle minnow has a broader distribution in regional inland waters than previously recognized [9,15]. Despite increasing knowledge of its taxonomy and distribution, information on its parasite fauna remains limited. The present finding therefore provides baseline information on the biotic interactions of *A. holciki* in southern Uzbekistan.

The sampling locality was characterized by shallow water, a sandy and slightly marshy bottom, abundant benthic invertebrates and visible signs of local anthropogenic disturbance. The occurrence of *H. marginata* under these conditions indicates that the species can persist in habitats affected by some degree of human activity. However, because environmental degradation was not quantitatively assessed, this observation should not be interpreted as evidence of high ecological tolerance. The presence of mollusks and other benthic organisms may also be relevant, as some Hemiclepsis species use freshwater mussels as shelters or secondary hosts [3,18].

The present study represents an initial documentation of a new host association based on a single sampling period and locality. Prevalence, infestation intensity, seasonal dynamics and pathological effects were not quantified. Future studies should therefore include repeated seasonal sampling, larger numbers of fish, quantitative assessment of infestation and histopathological examination of affected tissues. Such data would help clarify the ecological significance of the *H. marginata* - *A. holciki* association and improve understanding of fish-leech interactions in Central Asian freshwater ecosystems.

5. Conclusions

This study documents the first host-parasite association between *Hemiclepsis marginata* and *Alburnoides holciki* from the Surkhandarya basin, Uzbekistan. The record expands the known host range of *H. marginata* and contributes to parasitological and biogeographic knowledge of Central Asian freshwater ecosystems. The occurrence of the leech at a locality showing visible signs of anthropogenic disturbance provides additional ecological information on the species in Uzbekistan.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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