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Naim Sağlam

Fırat University, nsaglam@firat.edu.tr, Elazığ-Türkiye Mustafa Dörücü Fırat University, mdorucu@firat.edu.tr, Elazığ-Türkiye Mustafa Sarıeyyüpoğlu Fırat University, msari@firat.edu.tr, Elazığ-Türkiye Gonca Keser Adıyaman University, gkeser@adiyaman.edu.tr, Adıyaman-Türkiye Sibel Doğan Barata Fırat University, sbarata@firat.edu.tr, Elazığ-Türkiye Shabnam Farzali Fırat University, shebnem.farzali@gmail.com, Elazığ-Türkiye

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ORCID ID	0000-0002-3163-8432		0000-0002-1330-4965	0000-0001-8841-1098		
	0000-0002-0004-4877		0000-0003-4569-5435	0000-0002-8118-9516		
Corresponding Author Naim Sağlam						

THE STUDY OF FRESHWATER LEECH SPECIES IN SOME WETLANDS OF TÜRKİYE

ABSTRACT

The leech samples were collected and identified in some wetlands of Türkiye between January 2009 and September 2016. During the study, leech samples were taken from nine wetlands. In the study, seven species belonging to Erpobdellidae (*Erpobdella octoculata, Erpobdella testacea*), Glossiphonidae (*Helobdella stagnalis, Placobdella costata*), Hirudinidae (*Hirudo sulukii, Hirudo verbana*) and Haemopidae (*Haemopis sanguisuga*) were determined. *E. octoculata* and *E. testacea* from Cip Dam Lake and Behramaz stream, from Durusu (Terkos) Lake and Kaledibi Marsh, and *H. verbana* from Iron *H. sanguisuga* Marsh and Sülüklü Lake are the first record. *H. sulukii* and *H. verbana* leech species are the most important medicinal leech species used in traditional and integrative medicine. Extensive international trade in *H. verbana* was identified as a major threat to natural populations, so it has been listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Keywords: Erpobdellidae, Glossiphonidae, Hirudinidae, Haemopidae, Türkiye

1. INTRODUCTION

Some leeches have a temporary parasitic life, sucking the blood of vertebrates (sanguivorous). They show several parasitic adaptations in their habits, habitat, and structure. Türkiye is very rich in wetlands. However, evaluating the people and the use of these wetlands is insufficient. The leech fauna of Türkiye has not been fully defined yet. This study determined that leech species consist of ecological and economic importance. Our country's wetlands have been identified with various species of leeches. Moreover, studies on leeches are still being continued. Geldiay identified medicinal leech (*Hirudo medicinalis*) in a comparative study of macro and micro fauna of Çubuk Dam Lake and Emir Lake [1]. Geldiay, Tareen determined to leech species such as *E. octoculata*, *E. testacea*, *H. stagnalis*, *H. medicinalis*, and Piscicola geometra in Gölcük Lake [2]. A new leech species of Erpobdellids was defined in a cave located in Konya-Çamlık Dalayman, and it was named Dina vignai [3]. Freshwater leeches,

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Haementeria costata, and Dina lineate were found in the Köyceğiz region by Kazancı, Reiner-Hartmut, Neubert, Izbırak [4].

Batracobdella euxina was described based on two specimens from Türkiye, the holotype from Malkara near Bursa and the paratype from a stream near Ankara by Neubert, Nesemann [5]. In basin of Tahtali Dam Lake was identified *E. octoculata*, *G. complanata*, *H. sanguisuga*, *Hemiclepsis marginata*, *Haementeria costata* and *H. stagnalis* [6]. Balık, Ustaoğlu, Sarı reported *H. stagnalis* and *E. octoculata* in some streams of the Northern Aegean region. Kasparek, Demirsoy, Akbulut, Akbulut, Çalışkan, Durmuş determined to distribution and population densities of medicinal leech in central and western Anatolia of Türkiye [7 and 8]. Demirsoy, Kasparek, Akbulut, Durmuş, Akbulut, Çalışkan [9] studied the Phenology of the medicinal leech, *H. medicinalis*, in north-western Türkiye.

P. costata and H. stagnalis were reported in the discharge channels of fish culture pools in the Cip Fisheries Research Station of the Fisheries Faculty, Firat University, Elazığ [10 and 11]. Ustaoğlu, Balık, Özbek, Sarı identified six leech species (D. lineata, E. octoculata, G. complanata, H. sanguisuga, H. stagnalis, and H. medicinalis) in Gediz basin [12]. Balık, Ustaoğlu, Sarı, Özdemir Mis, Aygen, Taşdemir, Yıldız, Topkara, Sömek, Özbek, İlhan identified D. lineata and H. verbena in Bozalan Lake (Menemen-Izmir) [13]. D. lineata, E. octoculata, H. stagnalis, H. marginata, H. medicinalis, H. verbana, G. complanata, P. costata and Theromyzon tessulatum were determined in the wetlands of the Western Black Sea region by Özbek, Sarı [14]. Sağlam, Dörücü, Özdemir, Şeker, Sarıeyyüpoğlu studied medicinal leeches' distribution and economic importance in Eastern Anatolia Region [15]. Six leech species (Nephelopsis obscura, H. stagnalis, Haemopis sanguisuga, D. lineata, G. complanata, and P. costata) were identified by Demiroğlu, Mısırlıoğlu in the wetlands of Eskisehir province [16]. However, a new medicinal leech species was identified and named H. sulukii near Adiyaman, Batman, and Gaziantep in Türkiye's South Eastern Anatolia Region [17]. This study aimed to examine the leech species composition and fauna in nine wetlands in various Türkiye regions.

2. RESEARCH SIGNIFICANCE

This study investigates leech species in wetlands in different regions of Türkiye. It defines some leech species distributed in the Central Black Sea, Marmara, Eastern Anatolia, and Southeast Anatolia regions.

Highlights:

- Seven different leech species from nine different localities were identified in this study.
- Some identified leech species are shown from new localities.
- Information on the habitats of leech species is given.

3. MATERIAL AND METHODS

Leeches were collected from nine wetlands distributed all over the country between January 2009 and September 2016: Karagöl (37°59'35" N-38°48'52"E) (Gerger-Adıyaman), Büyük Sülüklü Lake (38°53'05" N-40°35'25"E) (Bingöl), İron Marsh (38°37'12" N-42°1'48"E) (Bitlis), Cip Dam Lake (38°40'30" N-39°03'56"E) and Behramaz stream (38°30'11" N-39°30'24"E) (Sivrice, Elazığ), Durusu (Terkos) Lake (41°20'24" N-28°34'22"E) (İstanbul), Simenit Lake (41°17'51" N-36°55'40"E) and Akgöl (41°16'55" N-36°56'37"E) (Terme-Samsun), Kaledibi Marsh (38°30'17" N-43°19'56"E) (Van) (Figure 1).

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(e.g., hard objects such as stone or rock) immersed in the freshwater. The leech specimens were transported to the laboratory in plastic and glass jars, where they were carefully examined and identified. The sampled leeches were examined alive and then killed. Some leech samples have been fragmented and sectioned to be more accurately diagnosed. The following method, given by Klemm and Mann, was used to relax and fix leech specimens [18 and 19]. Specimens were narcotized in a 70% solution of ethanol and added slowly to the container of water containing the leeches. There was added gradually, increasing the concentration, until all movement stopped or the specimens no longer responded to probing. Depending on the size and number being narcotized, the leeches completely relaxed in a 15 to 30-minute period. After the relaxation of leech samples, they were fixed in 4% formalin, preserved in 70% ethanol, and examined under a dissecting microscope. The collected leeches were identified according to Govedich, Bain, Moser, Gelder, Davies, Brinkhurst, Elliott, Mann, Klemm, Sağlam, and Sawyer [18, 20, 21, 22 and 23].



Figure 1. Localities where leech species are collected in Türkiye 1:Karagöl Lake; 2:Tarbasan Lake; 3:Cip Dam Lake; 4:Behramaz Stream; 5:Durusu (Terkos) Lake; 6:Simenit Lake; 7:Akgöl Lake; 8:Kaledibi Marsh; 9:İron Marsh

4. RESULTS AND DISCUSSION

The present study recorded seven leech species belonging to four families. Two of six leech species belonging to the family Erpobdellidae, Glossiphonidae, and Hirudinidae were defined, and one species belonging to the family Haemopidae was determined.

- Family: Erpobdellidae Blanchard, 1849.
- Genus: Erpobdella Blainville, 1918.
- Species: Erpobdella octoculata (Linnaeus, 1758).
- Synonyms: Hirudo octoculata Linnaeus, 1758; Herpobdella octoculata Linnaeus, 1758; Nephelis atomaria Blanchard, 1893.
- **Description:** The leech specimens are collected from Cip Dam Lake and Behramaz stream (Elazığ, Türkiye) (Figure 1, Table 1). E. octoculata is the first record for these two wetlands of Türkiye. *E. octoculata* has four pairs of eyes, two labial pairs opening forward, and two buccal pairs opening posterolaterally. The mid-body segments of leech are five annuli (b₁, b₂, a₂, b₅, b₆). The color of living leeches is red to dark brown. The leech has black pigment on the dorsal surface. The oral and caudal



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disk-shaped attachment organ (sucker) discs are small. The body length of *E. octoculata* is 37.0-67.0mm. Male gonopore large, female gonopore small, separated by 2.5 annuli.

- **Habitat:** *E. octoculata* generally live in stagnant waters and slack coastal areas of rivers in Türkiye.
- Species: Erpobdella testacea (Savigny, 1822).
- Synonyms: Nephelis testacea (Savigny, 1822).
- Description: E. testacea specimens are collected from Cip Dam Lake and Behramaz stream (Elazığ, Türkiye) (Figure 1, Table 1). This species is the first record for these wetlands of Türkiye. The color of live leeches is a uniform red-brown appearance. The dorsal and ventral surfaces are uncolored. This leech has midbody segments that are Fiveannulate (b₁, b₂, a₂, b₅, b₆). The leech has eight eyes, two labial pairs opening forward, and two buccal pairs opening posterolaterally. The caudal and anterior sucker discs are small. Body length 31.0-54.0mm. The male gonopore is larger than the female gonopore and is separated by four annuli. The male and female genital openings are in grooves XII b1/b2 and XII b6/ XII b1, respectively.
- **Habitat:** The procurable *E. testacea* in Türkiye is generally in stagnant waters and slack coastal areas of rivers.
- o Family: Haemopidae Richardson, 1969.
- o Genus: Haemopis Savigny, 1822.
- o Species: Haemopis sanguisuga (Linnaeus, 1758).
- o **Synonyms:** Hirudo sanguisuga Linnaeus, 1758.
- o **Description:** *H. sanguisuga* specimens are collected from Durusu (Terkos) Lake, Kaledibi Marsh (Figure 1, Table 1). H. sanguisuga is the first record for these two wetlands of Türkiye. The dorsal surface of the leech has uneven small dark spots, and the dorsal surface usually has a pair of dark obliquely railway-like markings on each side located longitudinally. The ventral surface of the leech is usually light yellowish colored with large, irregularly scattered, and dark-colored spots. H. sanguisuga has a larger anus. There are three jaws with rows of blunt teeth incapable of piercing the human skin in the mouth of the leech. The eyes are five pairs on II, III, IV al, V al, and VI a2, forming a parabolic arc. Five annuli and conspicuous separate male and female genital pores. The leech has a very long penis. The male genital hole XI b5/b6 is located in the furrow, and the female genital hole XII b5/b6 is in the furrow. The body length of H. sanguisuga is 84.0-172.0mm, maximum body width of 9.0-12.0mm. The mid-body segments of leech are five annuli (b1, b2, a2, b5, b6).
- Habitat: H. sanguisuga is a predator on small invertebrates, vertebrates, and terrestrial gastropods. This leech prefers to live in slow-flowing parts of rivers and lakes.
- ✓ Family: Hirudinidae Whitman, 1886.
- ✓ Genus : Hirudo Linnaeus, 1758.
- ✓ Species: Hirudo sulukii Saglam, Saunders, Lang and Shain 2016.
- ✓ Synonyms: H. sulukii was first detected by Sağlam et al., (2016) in the South Eastern. Anatolia region of Türkiye. This leech species has no synonyms available.
- ✓ Description: The leech specimens are collected from Karagöl Lake (Figure 1, Table 1). The dorsal side of the leech is variably olive green, and the body surface is covered with numerous small sensory papillae. *H. sulukii* has two orange paramarginal stripes, broad and encompassing black, segmentally-arranged united ellipsoid and elongated spots, dorsal lateral margins of

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the body with yellow stripes encompassing zigzagged black longitudinal. There are small irregular dark markings on the ventral surface of these leech species, or sometimes they are absent. This leech has massive epididymis. The evident caudal sucker disc of *H. sulukii* is large but does not exceed the maximum body width. The eyes of this leech are arranged as five pairs of parabolic arcs form on II, III, IVa1, Va1, and VIa2. The male genital pore is larger than the female genital pore and conspicuous, and five annuli separate both genital pores. Male and female pores are located in the furrow XIb5/b6 and XIIb5/b6, respectively. Body length 57.0-102.0mm, maximum body width 8.0-13.0mm, the diameter of caudal sucker 6.0-10.2mm. The leech segments I-III, IV-V, VI-VII, VIII, and IX, have one, two, three, four, and five (b1, b2, a2, b5, and b6) annuli, respectively.

- ✓ Habitat: H. sulukii prefers wetlands with the reeds and swamp character. This leech species have been identified in wetlands in the region where the highest annual temperature difference is in Türkiye.
- > Species: Hirudo verbana Carena, 1820.
- > Synonyms: H. verbana has no synonyms available.
- > Description: The leech specimens are collected from Sülüklü Lake, İron Marsh, Durusu Lake, Simenit Lake, and Akgöl Lake (Figure 1, Table 1). H. verbana is the first record for Sülüklü Lake and Iron Marsh of Türkiye. H. verbana has broad, scattered paramedian stripes with pale orange coloring. The ventral part of H. verbana is a uniform color from greenish to yellow, with two black bands on the lateral part. The body surface is slightly rough due to numerous small sensory papillae. The body length of H. verbana is 57.0-121.0mm, maximum body width 8.5-11.3mm, and diameter of caudal sucker 7.0-9.1mm. The caudal sucker of the leech is larger than the anterior sucker but not exceeds the maximum body width. The eyes of this leech have located as five pairs of parabolic arcs form on II, III, IVa1, Val, and VIa2, like in other Hirudo species. The male pore is larger than the female pore and conspicuous, separated by five annuli. Male and female pores are located in the furrow XIb5/b6 and XIIb5/b6, respectively. This leech has an epididymis, which is not much larger than the ejaculatory bulb, and a long penis. There are three jaws in the mouth of the leech. The anus is large and conspicuous and located in the furrow between the last annulus of the leech and the caudal sucker. Body length 57.0-121.0mm, maximum body width 8.5-11.3mm, the diameter of caudal sucker 7.0-9.1mm. Segments I, II and III have uniannulate, IV and V have biannulate, VI and VII have triannulate, VIII has four annuli and mid-body segments are five annuli (b1, b2, a2, b5, b6).
- Habitat: The habitat of this leech is the eutrophic wetlands with a muddy substratum and littoral vegetation in Türkiye and other countries of the world.
- Family: Glossiphoniidae Vaillant, 1890.
- ✤ Genus : Helobdella Blanchard, 1896.
- Species: Helobdella stagnalis (Linnaeus, 1758).
- Synonyms: Hirudo bioculata Bergmann, 1757; Hirudo stagnalis Linnaeus, 1758; Hirudo bioculata Müller, 1774; Hirudo pulligera Daudin, 1800; Hirudo circulans Sowerby, 1806; Helluo (Hirudo) bioculatus Oken, 1815; Glossiphonia perata Johnson, 1816; Glossopora punctata Johnson, 1817; Erpobdella bioculata de



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Blainville, 1818; Clepsine bioculata Savigny, 1822; Glossopora bioculata Fleming, 1822; Clepsine sowerbyi Moquin-Tandon, 1827; (Glossobdella) pulligera de Blainville, 1827; Hirudo *bioculata* de Blainville, 1828; Erpobdella Glossobdella stagnalis Templeton, 1836; Clepsina stagnalis de Filippi, 1837; Glossiphonia circulans Moquin-Tandon, 1846; Glosopora circularis Johnston, 1846; Glossiphonia bioculata Moquin-Tandon, Glossipora bioculata Thompson, 1856; Clepsine 1846; filippi Polonio, 1863; Clepsine modesta Verrill, 1872; Clepsine submodesta Nicholson, 1873; Clepsine viridissima Picaglia, 1877; bioculata Levinsen, 1883; Clepsine Glossiphonia modesta Vaillant, 1890; Glossosiphonia stagnalis Blanchard, 1894; Glossiphonia scutifera Young, 1894; Helobdella stagnalis Blanchard, 1896; Helobdella bioculata Bayer, 1898; Glossiphonia *stagnalis* Moore, 1898; Glossiphonia *stagnalis* Castle, 1900; Glossiphonia (Helobdella) stagnalis Moore, 1922; Glossopora punctata Johnson, 1925: Helobdella *stagnalis* Andre, 1930; Bakedebdella gibbosa Sciacchitana, 1939; Erpobdella stagnalis Oliver, 1958; Helobdella stagnalis Moore, 1952

- Description: H. stagnalis specimens are collected from Cip Dam Lake (Table 1, Figure 1). The color of the leech body is composed of mixed creamy-yellow or transparent aureate. The body of the leech is flat and short. The body length is 7.0-18.0mm, with a width of 2.2-5.1mm. The dorsal surface of the leech is convex, ventral somewhat concave. This leech has a nuchal plate on the dorsal surface of segment VII (between annuli 12/13). Somites I and II are uniannulate, III and IV are biannulate and mid-body segments are triannulate (a1, a2, a3). The leech has one pair of eyes. The caudal sucker of the leech is smaller than the body's width. One annulus separates male and female gonopore pores in furrows XII a1/a2 and XII a2/a3, respectively. The baby leeches shelter the rootstock leech on its abdomen.
- Habitat: The specimens of *H. stagnalis* were collected under stones and similar other hard objects, especially in stagnant and slowly or moderately flowing streams.
- Genus: Placobdella Blanchard, 1893.
- Species: Placobdella costata (Fr. Müller, 1846).
- Synonyms: Placobdella roszkowskii Oka, 1932; Clepsine costata F Müller 1846; Glossiphonia catenigera Moquin-Tandon 1846; Haementeria costata De Filippi 1849; Clepsine affinis Diesing 1850; Placobdella raboti Blanchard 1893; Placobdella guernei Blanchard 1894; Placobdella catenigera Blanchard 1894; Haementeria nusbaumi Gedroyc 1913.
- Description: P. costata specimens are collected from Cip Dam Lake (Figure 1, Table 1). The leech's body is flat, and the color is olive-green and brownish. The dorsal surface has six longitudinal rows of yellow spots. The body length is 17.0-32.0mm, with a width of 6.9-14.0mm. Somites I and II are uniannulate, III and IV are biannulate, and mid-body segments are triannulate (a1, a2, a3). The mouth pore is located near the anterior rim of the oral sucker. Two annuli separate the male and female gonopore, pores situated in furrows XI a2/a3 and XII a1/a2, respectively.
- Habitat: P. costata was collected under stones and other hard objects in stagnant and moderate running water.



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Table 1. List of freshwater leeches in the studied wetlands of Türkiye					
Wetlands	Provinces	Leech species	Density of Leeches (leech/m²)	Coordinates	
Karagöl Lake	Adıyaman	Hirudo sulukii	1.60	37°59'35"N 38°48'52"E	
Sülüklü Lake	Bingöl	Hirudo verbana	1.15	38°53'05"N 40°35'25"E	
Cip Dam Lake	Elazığ	Erpobdella octoculata Erpobdella testacea Helobdella stagnalis Placobdella costata	0.60 1.65 1.25 0.85	38°40'30"N 39°03'56"E	
Behramaz Stream	Elazığ	Erpobdella octoculata Erpobdella testacea	0.55 0.65	38°30'11"N 39°30'24"E	
Durusu (Terkos) Lake	İstanbul	Haemopis sanguisuga Hirudo verbana	1.73 1.22	41°20'24"N 28°34'22"E	
Simenit Lake	Samsun	Hirudo verbana	1.20	41°17'51"N 36°55'40"E	
Akgöl Lake	Samsun	Hirudo verbana	1.15	41°16'55"N 36°56'37"E	
Kaledibi Marsh	Van	Haemopis sanguisuga	0.85	38°30'06"N 43°20'04"E	
Iron Marsh	Bitlis	Hirudo verbana	2.64	38°37'12"N 42°1'48"E	

Many leeches feed exclusively on the blood of vertebrates, and some of these species, including *H. medicinalis* and *Haementeria* ghilianii and H. sulukii, are specifically adapted to feeding on mammals [17 and 23]. These bloodsucking leech species have special bioactive useful substances in their saliva that prevent blood clotting. In this study, the blood-sucker leech species H. verbana was found in Büyük Sülüklü Lake, Iron Marsh, Durusu Lake, Simenit Lake, and Akgöl Lake. However, H. sulukii was defined only in Karagöl Lake (Adıyaman, Türkiye). Although only H. medicinalis was found in Akgöl by Kasparek, Demirsoy, Akbulut, Akbulut, Çalışkan, Durmuş, medicinal leeches were identified as H. verbana species in this lake [8]. Hirudo species, known as medicinal leeches, have played an important role in traditional and modern medicine. However, extensive international trade of this leech species was identified as a major threat to natural populations. It has been listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Countries signing this agreement decided to place a quota on the collection and exportation of these leeches [24 and 25]. The export quota of medicinal leeches was reduced by 1000kg every year from 2011 to 2014. It was realized as 2000 kg between 2014-2021 and was reduced to 1500kg in 2022. The quota for leech exports has not been filled except in 2005. The leech export quota was exported 100% in the 2005 year. The amount of leech export and the rate decreased every year. Only a portion of 21.8% (436kg) of the quota (2000kg) permitted by the Ministry of Agriculture and Rural Affairs was able to be exported in 2021 [26]. There are still problems with the medicinal leech species of Türkiye. Therefore, comprehensive and well-planned studies on Türkiye's medicinal leeches are needed.

There are also leech species that do not suck blood. Many of these species have become predaceous from a hematophagous ancestry, which has occurred independently several times in the Hirudinea. These leeches eat aquatic insects, snail worms, and other invertebrates [23]. Predatory leeches either suck fluids with a proboscis (Glossiphoniidae) or have a suctorial mouth (*Erpobdellidae, Salifidae*, and *Haemopidae*) [20]. In this study, five predator leech species, including two Erpobdellids leeches (*E. octoculata, E. testacea*), two Glossiphonids leeches (*H. stagnalis, P. costata*) and one Haemopids



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leech (H. sanguisuga) were found. However, some papers reported Glossiphonids leech species H. stagnalis and P. costata as a parasite. H. stagnalis is an aquatic predator which prefers to feed on freshwater arthropods, annelids, and snails [23]. H. stagnalis has been reported as a parasite on fish on the poikilothermic vertebrates, on amphibians, Ambystoma tigrinum, and Rana temporaria, and in pericardial and mantle cavities and inner organs of the zebra mussel (Dreissena bugensis) [27, 28, 29, 30, 31, and 32]. P. costata was described on freshwater turtles as a temporary ectoparasite [23, 33, and 34]. By contrast, we found H. stagnalis and P. costata on the underside of stones, rocks, and other solid substrates in the shallow water zone [11 and 35].

The predatory leech species *E. octoculata* and *E. testacea* are widely distributed from European freshwater habitats and have been extensively investigated [21, 23, 36, and 37]. The life cycle of these two leech species in lentic and lotic waters is completed in one year [38 and 39], rarely in two-three years [40 and 41]. In our study, these two leech species were defined in stagnant and running freshwater habitats and collected in the two different wetlands of Elazığ, Türkiye.

The so-called horse leech, *H. sanguisuga* occurs naturally in the wild in most countries in western Europe and the widely distributed. It devours a wide range of prey, including earthworms, mollusks, insects, tadpoles, small or wounded fish and frogs, leeches of its own and other species, and almost any carrion [21, 42, and 43]. Wirchansky and Shain [44] demonstrated the monophyly of North American Haemopids and terrestrial of these leeches.

All of these seven leech species identified in our study have been previously determined in a variety of wetlands of Türkiye. The morphological characteristics of identified leeches are similar to the results of previous studies [2, 6, 7, 10, 11, 12, 14, 15, 16, 45, and 46].

5. CONCLUSIONS

This study determined seven leech species belonging to Erpobdellidae (E. octoculata, E. testacea), Glossiphonidae (H. stagnalis, P. costata), Hirudinidae (Hirudo sulukii, Hirudo verbana) and Haemopidae (H. sanguisuga) in different nine wetland of Türkiye. In this study, E octoculata, E. testacea, H. sanguisuga, and H. verbana were recorded for the first time in some lakes of Türkiye. H. verbana and H. sulukii are leech species that can be used for medical purposes and have economic importance. H. verbana is sold annually at 2000 kg with quota according to CITES from Türkiye to abroad. The rearing of this species is recently done on commercial farms in Türkiye. Determination of leech species and localities in natural environments is important in determining rootstock medicinal leeches for breeding.

CONFLICT OF INTEREST

The authors have no conflicts of interest to be disclosed.

FINANCIAL DISCLOSURE

The authors declare that this study has received no financial support.

DECLARATION OF ETHICAL STANDARDS

The authors of this article declare that the materials and methods used in this study do not require an ethical committee. Research permission was obtained from the Ministry of Agriculture and



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