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DOI	http://dx.doi.org/10.12739/NWSA.2022.17.3.5A0170	
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**THE DISTRIBUTION, ECONOMIC IMPORTANCE AND STATUS OF MEDICINAL LEECH
(*Hirudo orientalis*) IN THE AZERBAIJAN**

ABSTRACT

Although medicinal leeches are used for many years in Azerbaijan for therapeutic purposes, relatively little is still known about their distribution, economic importance, and general status. The medicinal leech species of Azerbaijan was named *Hirudo medicinalis* or *H. medicinalisorientalis* and is still exported under the same names in CITES records. However, within the scope of the study, this medicinal leech species was found to be 100% similar to *H. orientalis* according to the COI mtDNA gene region. In this study, it was determined that a person could collect an average of 24.19±11.14 medicinal leeches per unit of time and there was 0.60±0.28 leech/m² medicinal leech per unit area in the wetlands of Lankaran-Astara region of Azerbaijan. The trade and culture of medicinal leeches in Azerbaijan is a sector that has started in recent years. The country's leech exports in 2021 exceeded the exports of all countries and have increased to around 1498500 units (~1500kg).

Keywords: Medicinal leech, *Hirudoorientalis*, Economic Importance, Distribution, Phylogeny, Azerbaijan

1. INTRODUCTION

Palaearctic medicinal leech *H.orientalis* belongs to the Hirudinidae family and based on available evidence, the native distribution of mentioned species ranges from mountainous areas in Transcaucasian countries, including Azerbaijan, Uzbekistan, and Iran [1 and 2]. As in many European countries, until the beginning of the 21st century, it was known that *H. medicinalis* was distributed as a medicinal leech in Azerbaijan. But, with the development of genetic science, new leech species have been discovered in this class, and changes in some known species names [3]. Based on these developments, a new medicinal leech species, *H. orientalis*, was identified in the Azerbaijan geography by Utevsky, Trontelj [4]. Molecular and phylogenetic analysis showed that *Hirudo* comprises at least six different species: *H. medicinalis* Linnaeus, 1758, *H. troctina* Johnson, 1816, *H. verbena* Carena, 1820, *H.nipponia* Whitman, 1886, *H. orientalis* Utevsky and Trontelj, 2005, *H.sulukii* Saglam et al., 2016. The biogeography of medicinal leeches in the Palaearctic region, has been revised with some recent research [2, 5, 6, 7, 8, 9, 10, 11 and 12]. *H. medicinalis* is distributed from Britain and southern Norway to the southern Urals and probably as far as the Altai Mountains; *H. verbena* is spread from Italy and Switzerland to Türkiye and Uzbekistan; *H. orientalis* is distributed in Central Asia, the Transcaucasian countries, and Iran; *H. troctina* occurs in north-western Africa and southern Iberia and finally *H. sulukii* is, so far, only has found from south-eastern Anatolia of Türkiye [5 and 9]. Leeches are not

How to Cite:

Farzali, S. and Sağlam, N., (2022). The Distribution, Economic Importance and Status of Medicinal Leech (*Hirudo orientalis*) in The Azerbaijan. Ecological Life Sciences, 17(3):112-123, DOI: 10.12739/NWSA.2022.17.3.5A0170.



only used live for direct treatment, but also as a source of drugs for the treatment of many diseases, especially cardiovascular and haematological diseases, thanks to the enzymes, hormones, antithrombotic, and other bioactive substances they produce in their bodies [13, 14, 15, 16, 17, 18, 19, 20, 21 and 22].

Populations in wetlands tend to weaken day by day due to the intensive use of medicinal leeches for medicinal purposes, environmental pollution, and the effects of global climate change. In some wetlands, it is no longer possible to find leeches [3, 23, 24, 25 and 26]. For this reason, medicinal leeches are on the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species, Annex-III list of the Bern Convention, Annex-II list of the Convention on International Trade in Endangered Plant and Animal Species (CITES) and the European Union Habitats Directive. Listed in Annex-V of (92/43/EEC) [27, 28, 29 and 30]. The endangered medicinal leech species, *H. orientalis* planned to be listed in the "Red Book" of the Republic of Azerbaijan, so it is not allowed to be hunted for another purpose. The assessment of the medicinal leech species and its inclusion in the Red Book is worthwhile step considering that. Despite excessive interest and researches to the medicinal leech in the world, scarce data are currently available about the distribution, biology and ecology of the medicinal leech *H. orientalis* in Azerbaijan [31, 32, 33 and 34]. Most of the known occurrence localities and species are based on records collected in the second half of the twentieth century [35 and 36].

Gasimov, Likhodeeva first investigated the hydrofauna of the Soyukhbulag Stream, a tributary of the Kura River, and in this study, the first records of seven freshwater leech species for Azerbaijan, including *H. medicinalis*, were given [37]. In the Caucasus region, two types of medicinal leeches with different colour structures were determined by Kobakhidze [38] in Georgia. This medicinal leech, known as Georgian leech, and also mentioned by Lukin [39]. Later, Shevkunova, Kristman [40] was named mentioned species as *H. medicinalis orientalis*. Subsequently, with the use of molecular DNA methods on leeches, the name of this medicinal leech species was determined as *H. orientalis* [4]. Farzali, Sağlam [7] investigated the freshwater leech fauna for the first time in the eastern region of Azerbaijan and identified five species including *H. orientalis*. The lack of enough distribution data of *H. orientalis* in Azerbaijan is an important shortcoming. We present updated, validated new distribution localities and data in the Lankaran-Astara region of Azerbaijan to be used as baseline data in future research and monitoring studies with this study. In addition, it has been prepared in order to evaluate the economic importance of medicinal leech production, which has become increasingly widespread in Azerbaijan in recent years.

2. RESEARCH SIGNIFICANCE

This study investigates for the first time the economy of medicinal leeches, whose export and production have increased in Azerbaijan in recent years. In addition, molecular definitions of medicinal leech *H. orientalis*, which are currently collected from nature and produced and sold in Azerbaijan, were made.

Highlights:

- The precise identification of the medicinal leech *H. orientalis* produced in Azerbaijan and collected from natural environments was made according to the molecular COI mtDNA gene region.
- The status of the medicinal leech farms in Azerbaijan was evaluated. The population density of medicinal leeches in the natural waters of Azerbaijan was determined.



- The study reveals Azerbaijan's leech economy for the first time in a comprehensive manner.

3. MATERIALS AND METHODS

In this study, samples were taken by visiting the wetlands in the Lankaran-Astara region, which has the largest leech export. It was determined that *H. orientalis* was found in 15 wetlands in the Lankaran-Astara region during the field surveys between 2019-2022. The medical leech trade in Azerbaijan was determined by personnel interviews with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) database, the Ministry of Ecology, and leech producers. Lankaran-Astara economic geographical region located in the South-East part of the Azerbaijan Republic borders the Iran Islamic Republic in the South and on the Caspian Sea in the North; Jalilabad, Masalli, Yardimli, Astara, Lankaran, and Lerik are sub-regions of the territory [41]. The area of the region comprises 6.14 thousand km². The surface is mainly covered with mountainous (Talysh Mountains) and lowlands (Lankaran lowland). Total forest covers only 26% of the territory. From the biogeographical perspective, the Lankaran-Astara region is considered one of the most important areas that distinguished the biological diversity of Azerbaijan [42].

Collected leech samples were preserved in 70% ethanol after relaxation in weak ethanol in the field. Preserved specimens were transported in special containers to Firat University, Fisheries Faculty for identification. Morphological and molecular examination of the collected medical leech samples was carried out in order to make a definitive diagnosis. External and internal structures of preserved specimens were observed by stereomicroscope (Nikon SMZ NT-45). The leech samples were identified according to updated available keys [1, 43 and 44]. In order to determine the genus of the collected medicinal leech, the sequences of the COI mtDNA gene region of the leech samples brought from three different regions were obtained and phylogenetic analysis was performed. The phylogenetic tree was prepared by Maximum Likelihood analysis and a distance matrix was obtained. The phlogenetic tree of the collected samples was prepared together with other medicinal leeches. By contacting the Ministry of Ecology, leech farms and their production capacities and the annual sales quotas of leeches collected from the wild were discussed and evaluated. In addition, Azerbaijan's exports and imports of medicinal leeches within the scope of CITES in the last 20 years were screened (Table 2).

4. RESULTS AND DISCUSSION

4.1. Morphological Structure of *H orientalis*

Family : Hirudinidae Whitman, 1886
Genus : *Hirudo* Linnaeus, 1758
Species : *Hirudo orientalis* Utevsky, Trontelj [4].
Synonym : *Hirudo medicinalis*, *Hirudo medicinalis orientalis*

Among the collected samples, the longest one was found in the Yardimli (93mm, Arus v.) and the shortest one in the Lerik (35mm) region. The identifier body colour of the Palaearctic medicinal leech species *H. orientalis* is grass green. It has thin, orange-colored paramedian stripes on the dorsal. In the paramarginal dorsal lines, black quadrangular or rounded dots are located in a certain symmetrical layout from anterior to posterior. The ventral coloration pattern of *H. orientalis* is metamericly arranged, with diffusely dark pigmentation and regular structure (Figure 1).



Figure 1. View of dorsal (A) and ventral (B) of *H. orientalis*

Five pairs of eyes are located in a parabolic position on the annuli of II, III, IV a1, V a1 and VI a2 in the anterior-dorsal of the body. The male gonopore is larger than the female gonopore and is located ventral the body. The gonopore are separated from each other by five annuli. Male and female pores are located in the grooves of the XI b5/b6 and XII b5/b6 annuli, respectively (Figure 2). These descriptive characters distinguished *H. orientalis* from its five (*H. medicinalis*, *H. nipponia*, *H. sulukii*, *H. troctina*, *H. verbana*) closest relatives [4 and 9].

4.2. Molecular Identification

Medicinal leech samples collected from wetlands in Azerbaijan were found to be 100% similar to *H. orientalis* according to the distance matrix based on the COI mtDNA gene region made with other *Hirudo* species. COI mtDNA sequences of obtained Azerbaijan *H. orientalis* samples were uploaded to the Genbank database with access numbers ON098204, ON098205, and ON098206. According to our study results, it was determined that this medicinal leech species differed molecularly by 9% with *H. medicinalis*, 11% with *H. verbana*, ~11% with *H. troctina*, 14% with *H. sulukii*, and ~29% with *H. nipponia* (Table 1). It is seen that *H. orientalis* a separate basal branch and is clearly distinguished from other species of *Hirudo*, according to the Maximum Likelihood phylogenetic tree made between the *H. orientalis* DNA sequences obtained and the sequences of other *Hirudo* species in the GenBank (Figure 2). These findings were completely similar to the studies of Utevsky, Trontelj who described the *H. orientalis* species in Azerbaijan. In addition, the genetic distances revealed in other studies conducted on *Hirudo* species overlap with the data of this study [4, 9, 11, 45 and 46].

Table 1. Distance matrix of other *Hirudo* species with Azerbaijani samples

	1	2	3	4	5	6	8	10	12	14	15
ON098206-FSM24	-										
ON098205-FSM22	0.00	-									
ON098204-OR1	0.00	0.00	-								
MG271836- <i>H.orientalis</i>	0.00	0.00	0.00	-							
KX215709- <i>H. orientalis</i>	0.00	0.00	0.00	0.00	-						
MN314118- <i>H. orientalis</i>	0.00	0.00	0.00	0.00	0.00	-					
HQ333519- <i>H. medicinalis</i>	0.09	0.09	0.09	0.09	0.09	0.09	-				
KU216244- <i>H. verbana</i>	0.11	0.11	0.11	0.11	0.11	0.11	0.11	-			
KU216241- <i>H. sulukii</i>	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.15	-		
MZ820662- <i>H. nipponia</i>	0.30	0.30	0.30	0.29	0.29	0.28	0.29	0.29	0.27	-	
JQ364946- <i>H. troctina</i>	0.11	0,11	0,11	0,10	0,10	0,10	0,10	0,13	0,15	0,27	-

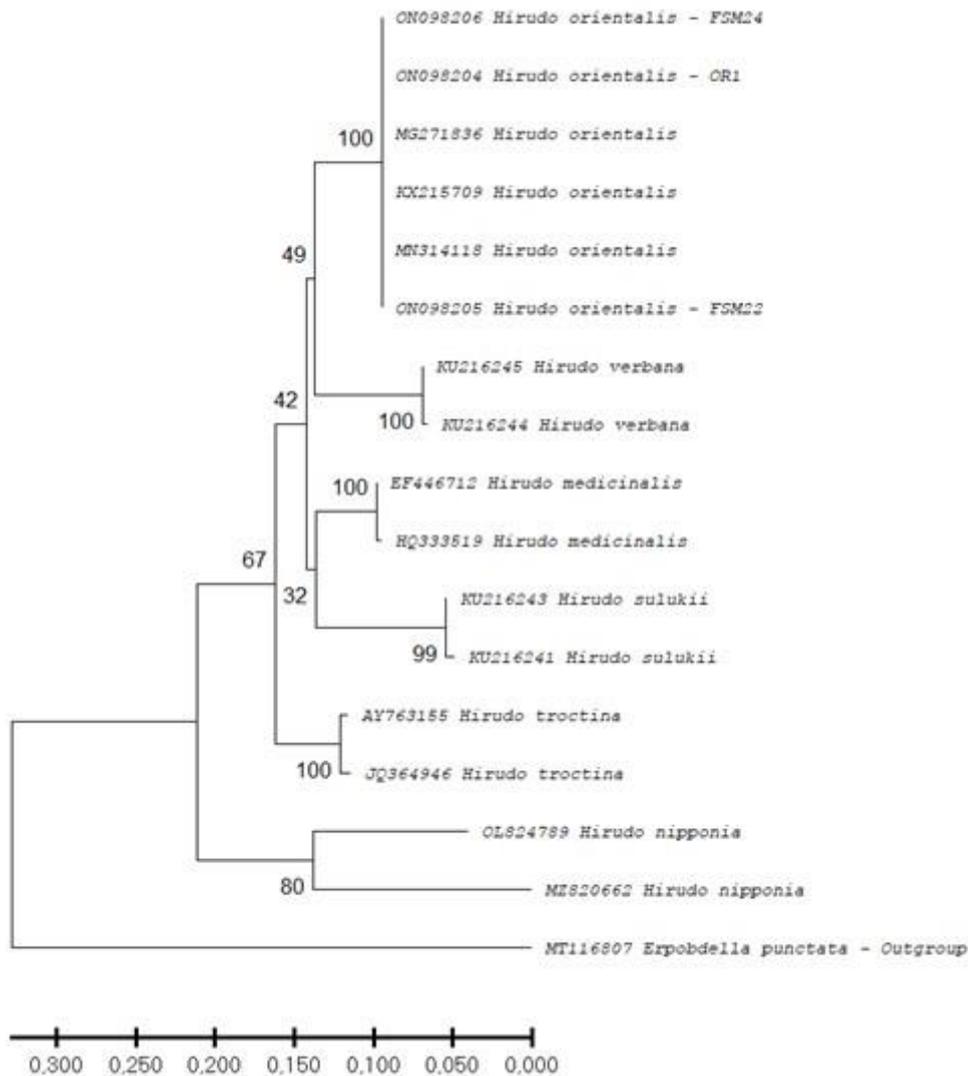


Figure 2. Maximum Likelihood analysis of COI mtDNA haplotypes (598 total positions) of medicinal leech, *H. orientalis*

4.3. Distribution of *H. orientalis*

Habitat: Just like in other parts of the world leech fauna of Azerbaijan dominates freshwater species. This species of medicinal leech was observed in different types of water bodies; prefers temporary ponds, gulf, and small and mesotrophic freshwater lakes in high mountainous areas in Lankaran-Astara region. Mentioned leech occur in practically all suitable habitats and the only region where *H. orientalis* were found to be absent is that of the large river deltas and in dam lakes. The typical habitat where *H. orientalis* were collected is a eutrophic pond with a muddy substratum, emergent vegetation is represented by broad leaf cattail (*Typhalatifolia*), rush (*Juncus* sp.) and common duck weed (*Lemna minor*). It is noteworthy that marsh frogs *Pelophylax ridibundus*, Talysh toad *Bufo eichwaldi* and water snakes were very common in the *H. orientalis* habitat. *H. orientalis* reported to feed exclusively on the blood of mammals, water birds, fish, adult or larvae of amphibians in nature [24].

The abundant species of medicinal leech in wetlands of Azerbaijan is only *H. orientalis* and was found in 15 natural water bodies where the investigation of the species was previously not done. A survey of all



the major potential habitats in Lankaran-Astara region show that *H. orientalis* widely distributed over the country. The presence of *H. orientalis* in two different localities (Lerik, Yardimli) in the Lankaran-Astara region is noteworthy because it gives different regions from those in other publications related to the species. The currently known distribution of the species is still largely incomplete and far from detailed, as there are no specific studies to determine the density of *H. orientalis* in the region. This research will help to create a map of distribution and density of *H. orientalis* in Azerbaijan.

4.4. Medicinal leech Cultivation in Azerbaijan

The species of the genus *Hirudo* are threatened but still used worldwide in human treatment. In addition, under the legislation of the FDA leeches should be taken from leech farms for use of human in the context of alternative and complementary medicine regulations. In this respect, medical leech breeding has gained great importance. The first foundations of medicinal leech cultivation in Azerbaijan gained momentum with the conference on medicinal leech breeding given at the conference held by the Institute of Zoology of Azerbaijan National Academy of Sciences (ANAS) by Sağlam and Sağlam [47 and 48]. In the continuation of this symposium, leech breeding was started and carried out by us for the first time in a laboratory condition at ANAS Zoology Institute in 2017. One of these medicinal leech farms was established in the city of Gabala with the contributions of Sağlam after the conference [47]. This leech farm is one of the first leech farms of Azerbaijan. Current time there are 11 leech breeding farms in Azerbaijan. These farms are located in Absheron, Masally, Gabala, Sheki, Sabirabad and Bilesuvar regions. In these farms 100 thousand medicinal leeches were produced in 2020 year, 800 thousand in 2021 year. In 1934, during peak usage, wetlands of Lankaran, Astara, Masally district hunted 1.5million medicinal leeches for medicinal purposes. In recent years, over-collecting, habitat drainage and loss of wetlands resulting in the decline of medicinal leech populations in Lankaran regions. In 1962, a leech farm near Yanardag in Baku, was breeding one million per year. The leeches of this farm were obtained from the Gizilagacgulf [49].

4.5. Medical Leech Trade and Economy in Azerbaijan

Extensive international trade in medicinal leeches was identified as a major threat to natural populations, so that it has been listed in Appendix II of CITES first *H. medicinalis* in the 1987, and then *H. verbena* [30]. All leech species in the *Hirudo* genus were started to subsequently followed by CITES. Countries signing this agreement decided to place a quota on the collection and exportation of medicinal leeches. In Azerbaijan the export quota of *H. orientalis* are applied by Ministry of Ecology and Natural Resources. The export quotas of the medicinal leech *H. orientalis*, which are collected from wildlife and allowed to be exported, are given below (Table 2).

Table 2. Quota amounts for export of wild leeches by Azerbaijan Ministry of Ecology (2020-2022)

Years	Leech species	Quota (Kg)	Trade term	Source
2020	<i>H. orientalis</i>	36	Live	Wildlife
2021	<i>H. orientalis</i>	36	Live	Wildlife
2022	<i>H. orientalis</i>	36	Live	Wildlife

Trade of wildlife species is highly lucrative but difficult to quantify. This international trade is regulated under the Convention on International Trade in Endangered Plant and Animal Species (CITES) to which the European Union and some other countries are parties [29 and



30]. Azerbaijan has started international trade with medicinal leeches that it has extensively in the last three years from leech farms. The selling prices in Azerbaijan of each medicinal leech, *H. orientalis* for the export are 0.14-0.20USD and 1.4-1.5 Euros. However, the selling price of this product in the domestic market is 0.4-0.7 AZN. Each medicinal leech can find buyers in Europe and the USA in the range of 4.60-7.0€ and 14.50-16.50\$, respectively [50]. It is seen that the prices of medicinal leeches are far below the world averages in Azerbaijan.

Azerbaijan is one of the most important of country commercial harvesting with medicinal leech in Transcaucasia. Azerbaijan imported leeches from Russia between 2010 and 2012 and did not engage in any international leech trade activity for six years between 2013-2018. In 2019, it started to export leeches in an increasing trend every year, together with the medical leech farming sector that is developing in the country. While exporting medical leeches to only one country in 2019, the number of countries it exports to has increased to six countries a year later, and to 10 countries in 2021. Having imported medicinal leeches from Russia for three consecutive years, Azerbaijan has started to export medicinal leeches to Russia in the last two years. The country's leech exports in 2021 exceeded the exports of all countries and increased to around 1498500 units (~1500kg) (Table 2). In 2021, only two countries' medical leech export records are visible. These two countries are Azerbaijan and Turkiye. Turkiye's medical leech exports were 449.5 kg and 1000 pieces in 2021. 13.5kg of this amount and 1000 of them were exported as culture leeches. It has become the first country in the world, surpassing the export figures of Turkiye, which has been the leading country in the leech trade for many years. Azerbaijan's export amount in 2021 was more than 3 times of Turkiye's exports in the same year [51]. Although there is an annual quota of 36 kg for the export of medicinal leeches from wildlife area in Azerbaijan, it is possible to see that this quota has never been used and that Azerbaijan has exported the leeches produced in farms in recent years (Table 2 and 3).

Table 3. Import and export situation of Azerbaijan for medicinal leech. BY, Belarus; CH, Switzerland; DE, Germany; IL, Israel; KG, Kyrgyzstan; KZ, Kazakhstan; MA, Morocco; RU, Russian Federation; TR, Turkiye; UA, Ukraine; UZ, Uzbekistan [51]

Years	Leech Species	Export	Import	Trade Term	Source	Exporter/Importer Countries
		(pieces/year)				
2010	<i>H. medicinalis</i>	-	3280	Live	Culture	RU
2011	<i>H. medicinalis</i>	-	3280	Live	Culture	RU
2012	<i>H. medicinalis</i>	-	6780	Live	Culture	RU
2019	<i>H. medicinalis</i>	55000	-	Live	Culture	UZ
2020	<i>H. medicinalis</i>	303000	-	Live	Culture	BY, IL, KZ, MA, RU, UZ
2021	<i>H. medicinalis</i>	1498500				BY, CH, DE, IL, KG, KZ, RU, TR, UA, UZ

It was determined that a person can collect an average of 24.19±11.14 medicinal leeches per unit time in this field study carried out in the Lankaran-Astara region of Azerbaijan. The amount of this medicinal leech per unit area was calculated as 0.60±0.28leech/m² (Table 4). The presence of the medicinal leech, *H. orientalis* in the wetlands of the region has been revealed by the study and it has been seen that the leeches in these areas have the potential to meet the rootstock needs in Azerbaijan's leech cultivation.



Table 4. List of localities of *H.orientalis* in Lankaran-Astara region, Azerbaijan

Province	Locality	Coordinates	Leech/person/hour	Leech/m ²
Astara	1. Baku-Alat-Astara-Iran roadway, Gamishlichay	38°38'16.44"N 48°49'5.44"E	18	0,45
		38°38'20.95"N 48°48'0.86"E	15	0,38
Jalilabad	2. Gamishligol vil.	39°08'31.1"N 48°23'22.8"E	30	0,75
Lankaran	3. Sinovly vil. Wetland-1.	38°49'15.2"N 48°42'44.7"E	21	0,53
	4. Sinovly vil. Wetland-2	38°49'9.24"N 48°42'28.7"E	12	0,30
Lerik	5. Sors vil.	38°51'1.11"N 48°23'23.2"E	30	0,75
	6. Yardimli - Lerik boundary, wetland-1	38°52'1.16"N 48°23'1.38"E	12	0,30
	7. Yardimli - Lerik boundary, wetland-2	38°52'9.98"N 48°23'9.81"E	15	0,38
	8. Yardimli - Lerik boundary, wetland-3	38°52'11.91"N 48°23'4.41"E	15	0,38
	9. Yardimli - Lerik boundary, wetland-4	38°52'9.1"N 48°23'1.10"E	9	0,23
Masalli	10. Gizilagaj gulf	39°11'48.44"N 49°2'11.25"E	45	1,13
Yardimli	11. Golkhamar Pond	38°56'6.14"N 48°15'5.16"E	30	0,75
	12. Osnakaran vil.	38°52'25.79"N 48°14'51.68"E	27	0,68
	13. Zalig Bil 2	38°51'59.51"N 48°14'58.01"E	30	0,75
	14. Bilne vil.	38°52'59.70"N 48°18'29.11"E	36	0,90
	15. Arus vil.	38°58'22.11"N 48°19'12.89"E	42	1,05
Mean±SE			24.19±11.14	0,60±0.28

5. CONCLUSION

The study focused on the identification of medicinal leech species in natural environments, determination of its density in wetlands, and revealing its place in the economy of Azerbaijan. This research is the first research that reveals the medical leech economy of Azerbaijan. Here, we shown, that leeches marketed as *H. medicinalis* are actually *H. orientalis*. Until the last three years, Azerbaijan has not exported medical leeches and has even sought to procure the leeches needs from Russia for years. Another method to struggle with the decline in wild leeches is the development of leech farms. Therefore, increasing leech breeding will provide important contributions both to the protection of medicinal leeches in natural environments and to the economy of Azerbaijan. Monitoring the natural environments and creating protection action plans for the species can only be achieved by knowing the leech distribution and density. DNA analyses is important to developing for robust data in species identification.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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 ethical committee permission and/or legal-special permission.



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