

# New records of leeches of the genus *Limnatis* (Hirudinea, Praobdellidae) from the South Caucasus and Central Asia: phylogenetic relationships of Eurasian and African populations

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Utevsky, S., Mabrouki, Y., Taybi, A. F., Huseynov, M., Manafov, A., Morhun, H., Shahina, O., Utevsky, G., Khomenko, A., Utevsky, A., 2022. New records of leeches of the genus *Limnatis* (Hirudinea, Praobdellidae) from the South Caucasus and Central Asia: phylogenetic relationships of Eurasian and African populations. *Animal Biodiversity and Conservation*, 45.1: 43–52, Doi: <https://doi.org/10.32800/abc.2022.45.0043>

## Abstract

*New records of leeches of the genus Limnatis (Hirudinea, Praobdellidae) from the South Caucasus and Central Asia: phylogenetic relationships of Eurasian and African populations.* Leeches of the genus *Limnatis* Moquin–Tandon, 1827 infest mucous membranes of various mammals, including humans and domestic ungulates. The type species of the genus *L. nilotica* (Savigny, 1822) was initially thought to occur throughout the Western Palaearctic, from North Africa to the Middle East and Central Asia. It was later found that *L. paluda* (Tennent, 1859) is a widespread Western Asian species. However, the South Caucasus and vast areas of Central Asia have not been explored sufficiently in terms of leeches of the genus *Limnatis*. We recorded *L. paluda* from Azerbaijan and Uzbekistan for the first time. We also carried out the first molecular characterisation of *L. nilotica* herein. We found a deep genetic differentiation (8%) between the Western Asian *L. paluda* and North African (Moroccan) *L. nilotica* based on their COI sequences. This finding corroborates a previous morphology–based hypothesis on their separate species assignments. The low genetic diversity of *L. paluda* is explained by the recent colonisation of arid landscapes of Western Asia.

Key words: Annelida, *Limnatis paluda*, *Limnatis nilotica*, COI, Genetic diversity

## Resumen

*Nuevos registros de sanguijuelas del género Limnatis (Hirudinea, Praobdellidae) en el Cáucaso meridional y Asia central: relaciones filogenéticas de las poblaciones eurasiáticas y africanas.* Las sanguijuelas del género *Limnatis* Moquin–Tandon, 1827 infestan las mucosas de varios mamíferos, incluidos los seres humanos y los ungulados domésticos. Se creía que la especie tipo del género, *L. nilotica* (Savigny, 1822), estaba presente en todo el paleártico occidental, desde África del norte hasta Oriente Medio y Asia central. Posteriormente, se observó que *L. paluda* (Tennent, 1859) es un especie ampliamente distribuida en Asia occidental. Sin embargo, no se han hecho estudios suficientes sobre el género *Limnatis* en la zona del Cáucaso meridional ni en buena parte de Asia central. Registramos *L. paluda* por primera vez en Azerbaiyán y Uzbekistán. La primera caracterización molecular de *L. nilotica* tuvo lugar allí. Las secuencias del gen citocromo oxidasa (COI) permitieron constatar que existe una profunda diferenciación genética (del 8%) entre *L. paluda*, de Asia occidental, y *L. nilotica*, de África del norte (Marruecos). Ello corrobora la hipótesis basada en la morfología que se asignaba especies diferentes. La escasa diversidad genética de *L. paluda* se explica por la reciente colonización de territorios áridos de Asia occidental.

Palabras clave: Anélidos, *Limnatis paluda*, *Limnatis nilotica*, COI, Diversidad genética

*Received: 02 VII 21; Conditional acceptance: 15 X 21; Final acceptance: 25 XI 21*

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## Introduction

The genus *Limnatis* Moquin–Tandon, 1827 comprises bloodsucking leeches that occur in the south–western Palaearctic. They infest mucous membranes of various organs such as the pharynx, nasopharynx, oesophagus, larynx, trachea, bronchial tubes and female genital organs in humans and domestic mammals, including horses, cattle, camels, deer, and dogs (Kaburaki, 1921; Moore, 1927; Almallah, 1968; Arenas et al., 1993; Boye and Joshi, 1994; Al–Ani and Al–Shareefi, 1995; Aġin et al., 2008; Bahmani et al., 2012, 2014; Negm–Eldin et al., 2013; Rajaei et al., 2014; Raele et al., 2015). Moreover, leeches of the genus *Limnatis* can also be parasitic on amphibians (Lukin, 1976). The members of this genus are therefore important in terms of medicine, veterinary science, and parasitology. Taking global climate change into account, it is clear that they can pose a potential invasive threat by shifting their ranges, as has already happened with many species (Parmesan and Yohe, 2003).

According to the current view on the classification, the genus contains three species, *Limnatis bacescui* Manoleli, 1972, *Limnatis nilotica* (Savigny, 1822) and *Limnatis paluda* (Tennent, 1859) (Nakano et al., 2015). The type species of the genus, *L. nilotica*, was first described by the French zoologist Jules Cesar Savigny from Egypt under the name *Bdella nilotica* (Savigny, 1822). Later, in 1827, Moquin–Tandon re-named Savigny's *Bdella nilotica* to *Limnatis nilotica* in his seminal monograph (Moquin–Tandon, 1827). The second species is *Limnatis bacescui* Manoleli, 1972 from Romania (South–Eastern Europe), where it was described and is currently known only from its type locality (Manoleli, 1972). The third member of the genus, *L. paluda*, was described by Tennent (1859) as *Haemopsis paludum* from Sri Lanka (= Ceylon). Its taxonomic status was revised by Moore (1927) so that the species was transferred to the genus *Limnatis* and its name was changed to *L. paluda* (Tennent, 1859).

Traditionally, praobdellid leeches, found both in North Africa and Western Asia, were identified as *L. nilotica*. Representatives of this species had been recorded for Kazakhstan and Central Asia (Lukin, 1976), and southern Iran (Grosser and Pešić, 2006). Subsequently, Phillips and Siddall (2009) and Nakano et al. (2015) found that the leeches of the genus *Limnatis* of Israel, Afghanistan and Kazakhstan should be assigned to *L. paluda*.

Despite the long history of previous studies, vast areas of Central Asia and the Caucasus have not been explored sufficiently in terms of their leeches of the genus *Limnatis*. There are no records based on molecular data concerning the species identity of those leeches in the South Caucasus and Central Asian countries except in Kazakhstan (Nakano et al., 2015), long known as regions of the *Limnatis* range (Lukin, 1976). Furthermore, North African leeches of the genus *Limnatis* have never been characterised based on their DNA sequences. The differentiation between the Western Asian *L. paluda* and the North

African *L. nilotica* has relied on morphological and geographical considerations (Moore, 1938). For this reason we aimed to identify leeches of the genus *Limnatis* collected in Uzbekistan (Central Asia) and Azerbaijan (the South Caucasus) and to clarify taxonomic and phylogenetic relationships between North African and Western Asian leech populations of the genus *Limnatis* using both morphological and molecular characters.

## Material and methods

### Sample collection

Leeches were collected during field trips in Uzbekistan, Azerbaijan, and Morocco (table 1). These samples were anesthetized in 10% ethanol, fixed, and preserved in 96% ethanol for further examination using both morphological and molecular methods. The specimens are stored in the collection of invertebrate animals at the Department of Zoology and Animal Ecology, V. N. Karazin Kharkiv National University.

### Morphological examination

Identification was carried out using a stereomicroscope Konus Crystal–45. Photo documentation was done using a USB HDCE–50B camera. We relied on Moore (1938) to find morphological features distinguishing *L. nilotica* and *L. paluda*.

### DNA extraction, amplification and sequencing

Using molecular methods we analysed four specimens of *L. paluda* collected in Uzbekistan, one specimen from Azerbaijan and one from Morocco assigned to *L. nilotica*. A small piece of tissue from the posterior part of the body was taken for DNA extraction. Genomic DNA was isolated using a GeneElute Mammalian Genomic DNA Minprep Kits.

The mitochondrial cytochrome *c* oxidase subunit I (CO1) fragment was chosen as a standard animal DNA barcode gene region (Hebert et al., 2003) and amplified using following primers (Folmer et al., 1994): LCO1490, 5'–GGTCAACAAATCATA–AAGATATTGG–3' (forward) and HCO2198, 5'–TA–AACTTCAGGGTGACCAAAAAATCA–3' (reverse) by applying 5 cycles of 30 s at 94°C, 1 min 30 s at 45°C and 1 min at 72°C, 35 cycles of 30 s at 94°C, 45 s at 51°C and 1 min at 72°C, and 1 cycle of 5 min at 72°C after an initial 3 min denaturation step at 94°C. Alternatively, another PCR protocol (Utevsky et al., 2021) was implemented.

PCR products were cleaned using two enzymes, Exonuclease I and Shrimp alkaline phosphatase (SAP) (Fermentas, Thermo Fisher Scientific, USA). Exonuclease I (0.2 µl) and SAP (1 µl) were added to 10 µl of the PCR product. The mixture was then incubated for 45 min at 37°C followed by 15 min incubation at 80°C. The cleaned PCR product was then sequenced in both directions by MacroGen

Table 1. Information on specimens examined: COI, COI sequence code; N, number of specimens.

Tabla 1. Información sobre los ejemplares examinados: COI, código secuencias COI; N, número de especímenes.

Species	Date	COI	N	Locality	Collector
<i>L. paluda</i>	15 VI 2005	LN	19	A mountain stream, Urgut District, Samarqand Region, Uzbekistan	A. Abdullaev
<i>L. paluda</i>	7 VI 2007	m20, m21	4	Biological Station (University of Samarqand), Samarqand Region, Uzbekistan	S. Utevsky
<i>L. paluda</i>	VIII 2017	T9	4	Boysun District, Surxondaryo Region, Uzbekistan	M. Asrorov
<i>L. paluda</i>	9 VIII 2016	T10	1	41°01'19.48" N, 48°41'45.50" E, a warm spring, Hashi, Quba District, Azerbaijan	A. Manafov
<i>L. nilotica</i>	22 IX 2019	U59	4	33°58'01.4"N, 3°02'19.0"W, Ain Tafrent spring, Debdou, Morocco	Y. Mabrouki

Inc. (the Netherlands) using the same primers as at the amplification stage. The chromatograms of sequences were processed in ChromasPro 1.32 (Technelysium Pty., Queensland, Australia). The length of the newly generated COI sequences was 650–661 bp.

#### Phylogenetic analysis

To reveal phylogenetic relationships of the Caucasian, Central Asian and North African leeches of the genus *Limnatis*, all available COI sequences of leeches assigned to that genus, sequences of Nearctic and Neotropic praobdellid leeches plus members of other families of Hirudiniiformes and the erpobdellid leech *Trocheta danastrica* Stschegolew, 1938 were chosen for analysis and downloaded from GenBank (table 2).

The COI sequences were unambiguously aligned using MUSCLE algorithm in MEGA X. The final dataset contained a total of 1,302 positions. The alignment was checked for stop codons by translating it to amino acids using MEGA X (Kumar et al., 2018). Best-fit models of molecular evolution were determined for each codon position under the Bayesian information criterion using KAKUSAN4 (Tanabe, 2011): HKY85 with gamma distribution (+G) for the first codon position and GTR+G for the second and third positions. Phylogenetic relationships were assessed by Bayesian inference using MrBayes v3.2.7a (Ronquist and Huelsenbeck, 2003) as implemented in the CIPRES Science Gateway (Miller et al., 2010, accessible at [www.phylo.org](http://www.phylo.org)). Searches were performed in two

parallel runs with eight chains each for ten million generations, sampled every 100th generation. After the first 25% of the sampled trees were discarded, the final topologies were consented following the 50% majority rule.

In addition, using MEGA X, we calculated the number of uncorrected base differences per site (based on p-distances) between species-level clades of the genus *Limnatis*.

## Results

### Morphology

The specimens collected in Azerbaijan and Uzbekistan were identified as *L. paluda* based on their morphological characters. The leeches range from 12.6 to 95.4 mm in length. The specimen from Azerbaijan is 48.9 mm in length. The body is flattened, indistinctly separated into the trachelosome and the urosome. Some of the specimens have a well-defined clitellum. The oral sucker is confluent with the trachelosome. The caudal sucker is wide and constitutes 0.86 of the maximum width of the urosome. The oral sucker has a sulcus on its inner surface. The anus is inconspicuous. Mid-body segments are five-annulated. The gonopores are separated by five annuli. The leeches have five pairs of eyes that line up, creating a parabolic arc pattern. Papillae are weakly developed. Live leeches are greenish dorsally with no dark pattern. Lateral margins of the body are orange. The venter is bluish black (fig. 1A, 1B).

Table 2. Collection sites and sequence accession data for COI sequences analysed: GenBank, GenBank accession number and specimen code. (Species names are presented according to this study).

Tabla 2. Lugares de recolección y datos sobre las accesiones de las secuencias COI analizadas: GenBank, número de acceso y código de la muestra. (Los nombres de las especies se indican de acuerdo con este estudio).

Taxon	GenBank	Country	Reference
<i>Limnatis nilotica</i> (Savigny, 1822)	MZ318072, U59	Morocco	This study
<i>Limnatis</i> sp.	GQ368754	Namibia	Phillips and Siddall (2009)
<i>Limnatis</i> sp.	AY763152	Croatia	Trontelj and Utevsky (2005)
<i>Limnatis paluda</i> (Tennent, 1859)	KY989474	Iran	Darabi-Darestani et al. (2021)
<i>L. paluda</i>	KY989473	Iran	Darabi-Darestani et al. (2021)
<i>L. paluda</i>	KY989472	Iran	Darabi-Darestani et al. (2021)
<i>L. paluda</i>	KY989471	Iran	Darabi-Darestani et al. (2021)
<i>L. paluda</i>	GQ368755	Afghanistan	Phillips and Siddall (2009)
<i>L. paluda</i>	AB981656	Kazakhstan	Nakano et al. (2015)
<i>L. paluda</i>	AB981654	Kazakhstan	Nakano et al. (2015)
<i>L. paluda</i>	AY425452	Israel	Borda and Siddall (2004)
<i>L. paluda</i>	MZ318071, T9	Uzbekistan	This study
<i>L. paluda</i>	MZ318070, T10	Azerbaijan	This study
<i>L. paluda</i>	MZ318067, LN	Uzbekistan	This study
<i>L. paluda</i>	MZ318068, m20	Uzbekistan	This study
<i>L. paluda</i>	MZ318069, m21	Uzbekistan	This study
<i>Limnobia mexicana</i> Blanchard, 1893	GQ368758	Mexico	Phillips and Siddall (2009)
<i>L. mexicana</i>	GQ368756	Mexico	Phillips and Siddall (2009)
<i>L. mexicana</i>	GQ368757	Mexico	Phillips and Siddall (2009)
<i>L. mexicana</i>	GQ368759	Mexico	Phillips and Siddall (2009)
<i>Myxobdella sinanensis</i> Oka, 1925	LC192132	Japan	Nakano et al. (2017)
<i>M. annandalei</i> Oka, 1917	GU394014	India	Phillips et al. (2010)
<i>Pintobdella chiapasensis</i> (Caballero, 1957)	GU394015	Mexico	Phillips et al. (2010)
<i>Tyrannobdella rex</i> Phillips et al., 2010	GU394016	Peru	Phillips et al. (2010)
<i>Hirudo orientalis</i> Utevsky and Trontelj, 2005	EF405599	Uzbekistan	Utevsky et al. (2007)
<i>Hirudo nipponia</i> Whitman, 1886	AY763153	Korea	Trontelj and Utevsky (2005)
<i>Whitmania laevis</i> (Baird, 1869)	KT693113	India	Chatterjee et al. (2017)
<i>Aliolimnatis michaelsoni</i> (Augener, 1936)	GQ368738	Guinea-Bissau	Phillips and Siddall (2009)
<i>Hirudinaria manillensis</i> (Lesson, 1842)	AY425449	Puerto Rico	Borda and Siddall (2004)
<i>Haemopsis sanguisuga</i> (Linnaeus, 1758)	AF462021	Sweden	Siddall (2002)
<i>Semiscolex similis</i> Ocegüera-Figueroa, 2005	AY425457	Bolivia	Borda and Siddall (2004)
<i>Patagoniobdella fraternal</i> Ringuelet, 1976	AY425459	Chile	Borda and Siddall (2004)
<i>Oxyptychus braziliensis</i> (Pinto, 1920)	AY425455	Brazil	Borda and Siddall (2004)
<i>Macrobodella decora</i> (Say, 1824)	MH672573	North America	Müller et al. (2019)
<i>M. decora</i>	EU100095	USA	Borda et al. (2008)
<i>Philobdella gracilis</i> Moore, 1901	DQ097218	USA	Phillips and Siddall (2005)
<i>Philobdella floridana</i> (Verrill, 1874)	DQ097219	USA	Phillips and Siddall (2005)
<i>Haemadipsa sylvestris</i> Blanchard, 1894	AF003266	Vietnam	Siddall and Bureson (1998)
<i>Trocheta danastrica</i> Stschesgolew, 1938	MT013043	Ukraine	Khomenko et al. (2020)

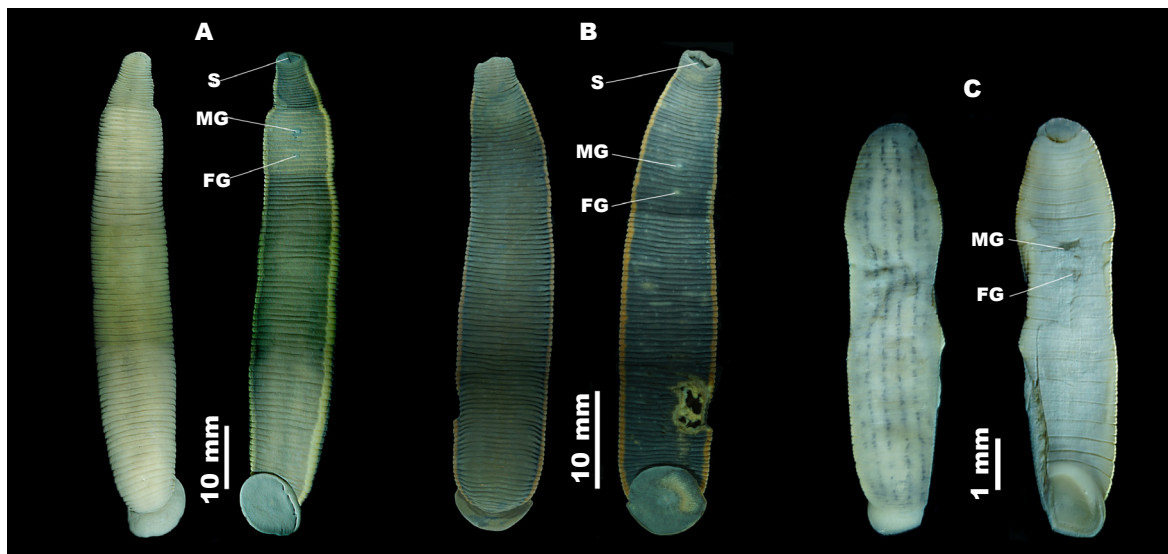


Fig. 1. External characters of leeches of the genus *Limnatis*: A, *Limnatis paluda* from Uzbekistan (Samarqand Region); B, *Limnatis paluda* from Azerbaijan; C, *Limnatis nilotica* from Morocco: FG, female gonopore; MG, male gonopore; S, sulcus. (Dorsal sides are depicted on the left and ventral sides, on the right for each specimen).

Fig. 1. Caracteres externos de las sanguijuelas del género *Limnatis*: A, *Limnatis paluda* procedente de Uzbekistán (región de Samarcanda), barra de escala de 10 mm; B, *Limnatis paluda* procedente de Azerbaiyán; C, *Limnatis nilotica* procedente de Marruecos: FG, gonoporo femenino; MG, gonoporo masculino; S, surco. (La imagen izquierda de cada ejemplar muestra la vista dorsal y la imagen derecha, la vista ventral).

The Moroccan leeches, which were assigned to *L. nilotica*, are 7.7–13.0 mm in length. The posterior sucker constitutes 0.76 of the maximum body width. The dorsal coloration pattern includes six longitudinal rows of black dots and short lines. Pigmentation was largely bleached due to preservation in ethanol. The specimens do not have a well discernible sulcus. The gonopores are separated by five annuli (fig. 1C).

#### Phylogeny and genetic differentiation

Phylogenetic analyses show the family Praobdellidae is a well-supported monophyletic group with a posterior probability of 1.00. The genus *Limnatis* is also a well-supported clade. All Middle Eastern, Caucasian and Central Asian samples joined a clade with a posterior probability of 0.78. This monophyletic group matches *L. paluda*. The phylogenetic structure of the clade is simple and shallow. The Namibian *Limnatis* sp. is sister to the clade of *L. paluda* with a posterior probability of 0.98. This group of Namibian and Western Asian leeches is sister to the clade consisting of the Moroccan *L. nilotica* and Croatian *Limnatis* sp. The latter clade is supported by a posterior probability of 0.62 (fig. 2).

The number of base differences per site from averaging sequence pairs within the clade of *L. paluda* is as low as  $0.0030 \pm 0.0012$ . Uncorrected distances between the Western Asian *L. paluda*, Balkan *Limnatis* sp., North

African *Limnatis* sp. and South African *Limnatis* sp. exceed 0.06 (table 3), suggesting species-level differences between those populations.

#### Discussion

Morphological examination of the leeches of the genus *Limnatis* suggests the specimens collected in Azerbaijan and Uzbekistan should be assigned to the Middle Eastern and Central Asian *L. paluda*. This identification is based on the morphological features as follows: in contrast to the North African *L. nilotica*, Central Asian and South Caucasian leeches of the genus *Limnatis* are characterised by the monotonous green coloration with no dark dots or lines on the dorsum. The dorsal coloration pattern consisting of black stripes, lines and dots is characteristic of the North African *L. nilotica* (Moore, 1938). The Moroccan specimens have the typical coloration of *L. nilotica*.

The phylogenetic analysis corroborated the morphological identification and revealed a deep differentiation between North African and Western Asian leeches of the genus *Limnatis*. Molecular characterisation of the North African *Limnatis*, which is currently assigned to *L. nilotica* in the strict sense, was performed herein for the first time. All Middle Eastern, Caucasian, Central Asian and Afghan leeches joined the clade of *L. paluda* (fig. 2). This

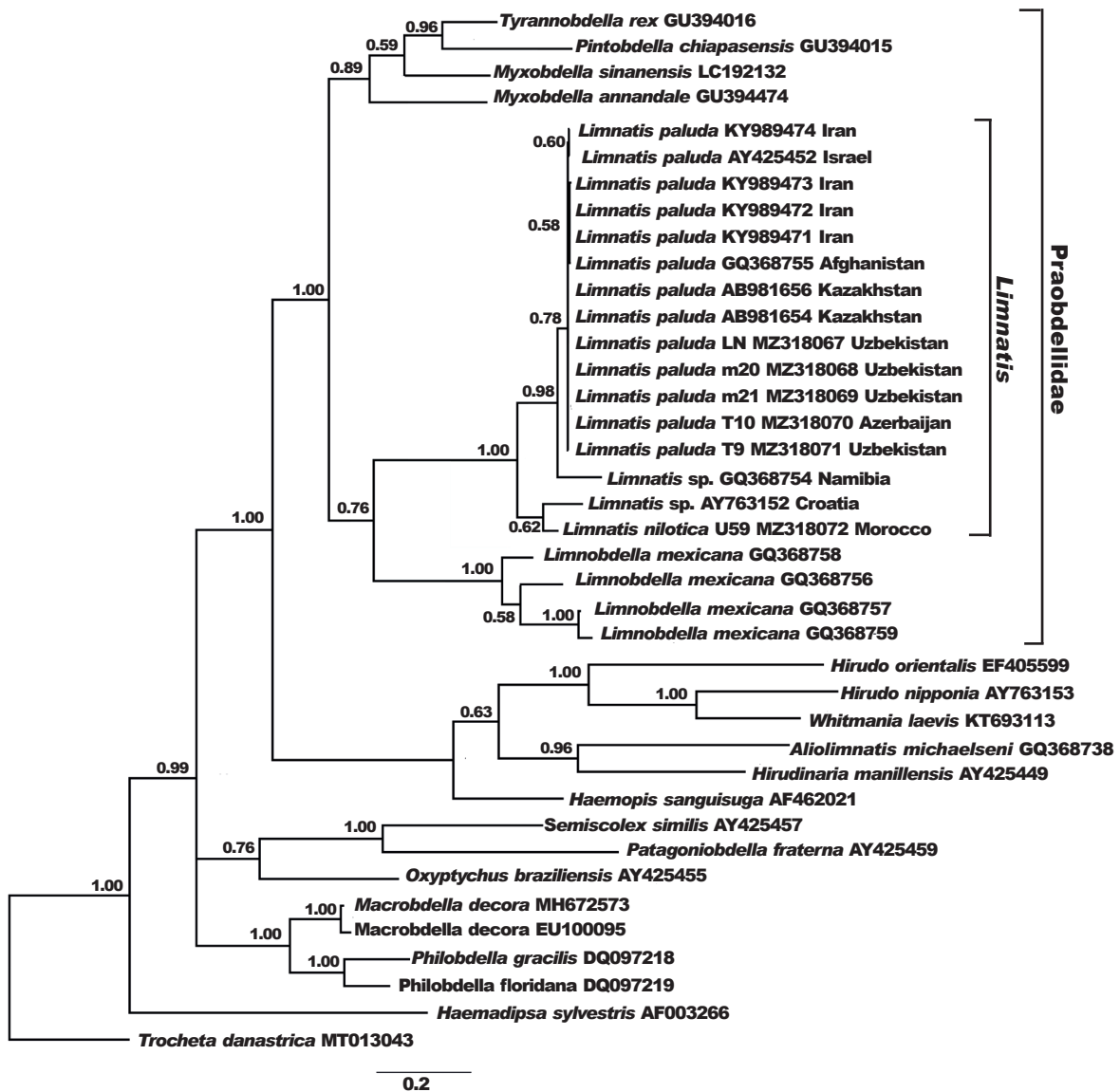


Fig. 2. Bayesian phylogenetic tree of arhynchobdellid taxa, including the genus *Limnatis* and other members of the family Praobdellidae, based on *COI* sequences. Posterior probabilities are shown for clades. The tree is rooted at *Trocheta danastrica*.

Fig. 2. Árbol filogenético bayesiano de los taxones de arrincobdélidos, incluidos el género *Limnatis* y otros miembros de la familia de los praobdélidos, basado en las secuencias de *COI*. Se muestra la probabilidad posterior de los clados. El árbol está enraizado en *Trocheta danastrica*.

corroborates independent species statuses of the North African and Western Asian populations, both of which had been assigned by some classical authors (Lukin, 1976) to *L. nilotica*. The Namibian *Limnatis* is sister to the *L. paluda* clade and belongs to an unidentified species, which may represent the little-known taxonomical diversity of the genus *Limnatis* in Africa (Moore, 1938). The Moroccan and Croatian leeches are in sister relationships. While the Moroccan specimen should be assigned to *L. nilotica*, the

Croatian leech is another unidentified species. The Moroccan–Croatian clade is sister to the clade of *L. paluda* plus the Namibian leech. The phylogenetic relationships imply that the taxonomy of the genus *Limnatis* is far from complete. Further studies of Southern European and South African populations of the genus *Limnatis* (fig. 3) are needed for proper identification. Moreover, the conspecific relationships of Sri Lankan and Western Asian leeches, which are currently assigned to *L. paluda* (Moore, 1927;

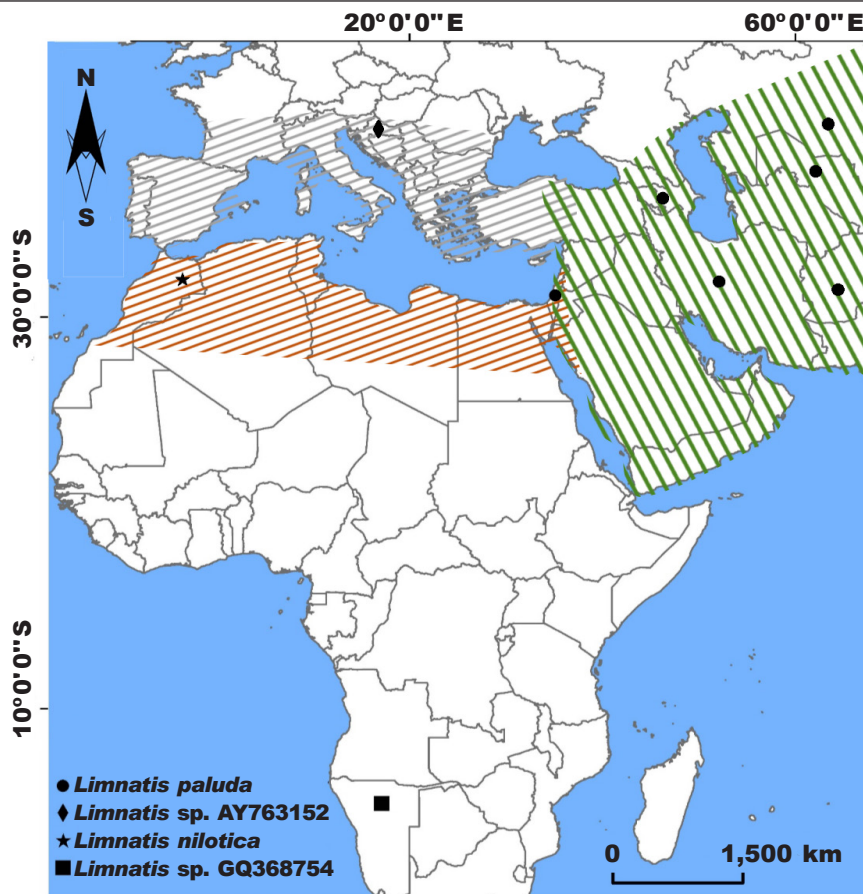


Fig. 3. Geographical distribution of leeches of the genus *Limnatis*: red, *L. nilotica*; green, *L. paluda*; grey, South European *Limnatis* sp.

Fig. 3. Distribución geográfica de las sanguijuelas del género *Limnatis*: en rojo, *L. nilotica*; en verde, *L. paluda*; en gris, *Limnatis* sp. del sur de Europa.

Phillips and Siddall, 2009; Nakano et al., 2015), should be substantiated using fresh specimens and molecular data. Biogeographically, the occurrence of the same leech species in Sri Lanka and in Western Asia appears questionable.

Despite the vast range of *L. paluda*, the species was found to have low genetic diversity and a shallow phylogenetic structure. This may be explained by the recent colonisation of arid landscapes in Western Asia. The range expansion could be attributed to the

Table 3. Estimates of evolutionary divergence based on *p*-distances between *COI* sequences of leeches of the genus *Limnatis* and their standard errors.

Tabla 3. Estimación de la divergencia evolutiva a partir de la distancia entre las secuencias (*p*-distance) de *COI* de las sanguijuelas del género *Limnatis* y su error estándar.

	<i>Limnatis paluda</i>	<i>Limnatis</i> sp. Namibia	<i>Limnatis</i> sp. Croatia
<i>Limnatis</i> sp. Namibia	0.0723 ± 0.0107		
<i>Limnatis</i> sp. Croatia	0.0947 ± 0.0115	0.1192 ± 0.0137	
<i>Limnatis nilotica</i> Morocco	0.0795 ± 0.0103	0.1028 ± 0.0126	0.0628 ± 0.0093



parasitism of these leeches on their ungulate hosts that appear to be able to transmit their parasites over long distances (Nakano et al., 2015). The eastern medicinal leech *Hirudo orientalis* Utevsky and Trontelj, 2005 is another instance of the rapid colonization of that area, which caused comparable genetic consequences (Trontelj and Utevsky, 2012). Migrations of nomads and their livestock throughout vast territories of the Middle East, Caucasus and Central Asia or other human activities in that area (as was discussed in Nakano et al., 2015) could contribute to shaping the genetic structure of *L. paluda*. Obviously, more studies are needed to clarify the evolutionary history and to elaborate a robust classification of the leeches of the genus *Limnatis*.

### Acknowledgements

Our sincere thanks to all the people who helped us in the field and collected leech specimens. Abdumalik Abdullaev kindly shared his experience in leech habitats and localities in Uzbekistan. Madamin Asrorov sampled other leeches from Uzbekistan.

### References

- Ağın, H., Ayhan, F. Y., Gülfidan, G., Cevik, D., Derebaşı, H., 2008. Severe anemia due to the pharyngeal leech *Limnatis nilotica* in a child. *Turkiye Parazitoloji Dergisi*, 32: 247–248.
- Al-Ani, F. K., Al-Shareefi, M. R., 1995. Observation on medical leech (*Limnatis nilotica*) in camel in Iraq. *Journal of Camel Practice and Research*, 2(2): 145.
- Almallah, Z., 1968. Internal Hirudiniasis in Man with *Limnatis nilotica*, in Iraq. *The Journal of Parasitology*, 54: 637–638.
- Arenas, A., Moreno, T., Maldonado, A., Becerra, C., Tarradas, M. C., 1993. Leech (*Limnatis nilotica*) parasitism in wild red deer (*Cervus elaphus*) in west Sierra Morena (Spain). *Erkrankungen der Zootiere*, 35: 209–211.
- Bahmani, M., Eftekhari, Z., Mohsezzadeghan, A., Ghotbian, F., Alighazi, N., 2012. Leech (*Limnatis nilotica*) causing respiratory distress in a pregnant cow in Ilam province in Iran. *Comparative Clinical Pathology*, 21: 501–503.
- Bahmani, M., Karamati, S. A., Anari, M., Rahimirad, A., Asadzadeh, J., Kheiri, A., Hajjiglozadeh, G., Ghotbian, F., Bahmani, F., 2014. Case report of oral cavity infestation in a 3-year old jackass with *Limnatis nilotica* from Ilam province, west of Iran. *Asian Pacific Journal of Tropical Disease*, 4: 210–212.
- Borda, E., Oceguera-Figueroa, A., Siddall, M. E., 2008. On the classification, evolution and biogeography of terrestrial haemadipsoid leeches (Hirudinida: Arhynchobdellida: Hirudiniformes). *Molecular Phylogenetics and Evolution*, 46: 142–154.
- Borda, E., Siddall, M. E., 2004. Arhynchobdellida (Annelida: Oligochaeta: Hirudinida): phylogenetic relationships and evolution. *Molecular Phylogenetics and Evolution*, 30: 213–225.
- Boye, E. S., Joshi, D. C., 1994. Occurrence of the leech *Limnatis paluda* as a respiratory parasite in man: case report from Saudi Arabia. *The Journal of Tropical Medicine and Hygiene*, 97: 18–20.
- Chatterjee, N., Dhar, B., Bhattacharya, D., Deori, S., Doley, J., Bam, J., Das, P. J., Bera, A. K., Deb, S. M., Devi, N. N., Paul, R., Malvika, S., Ghosh, S. K., 2017. Genetic assessment of leech species from yak (*Bos grunniens*) in the tract of Northeast India. *Mitochondrial DNA Part A*, 29: 73–81.
- Darabi-Darestani, K., Sari, A., Khomenko, A., Kvist, S., Utevsky, S., 2021. DNA barcoding of Iranian leeches (Annelida: Clitellata: Hirudinida). *Journal of Zoological Systematics and Evolutionary Research*, 59: 1438–1452.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: 294–299.
- Grosser, C., Pešić, V., 2006. On the diversity of Iranian leeches (Annelida: Hirudinea). *Archives of Biological Sciences*, 58: 21–24.
- Hebert, P. D., Cywinska, A., Ball, S. L., deWaard, J. R., 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270: 313–321.
- Kaburaki, T., 1921. Note on the leech *Limnatis nilotica*. *Records of the Indian Museum*, 18: 213–214.
- Khomenko, A., Utevsky, S., Utevsky, A., Trontelj, P., 2020. Unrecognized diversity of *Trocheta* species (Hirudinea: Erpobdellidae): resolving a century-old taxonomic problem in Crimean leeches, *Systematics and Biodiversity*, 182: 129–141.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547–1549.
- Lukin, E. I., 1976. *Leeches of fresh and saline waters. Fauna of the USSR. Leeches*. Nauka, Leningrad. [In Russian]
- Manoleli, D., 1972. A new species of leech *Limnatis bacescui* sp. nov. (Hirudinoidea: Hirudinidae). *Revue Roumaine de Biologie, Série de Zoologie*, 17(4): 237–239.
- Miller, M. A., Pfeiffer, W., Schwartz, T., 2010, November. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop*: 1–8. IEEE, New Orleans.
- Moore, J. P., 1927. *Arhynchobdellæ*. In: *The Fauna of British India, including Ceylon and Burma. Hirudinea* (W. A. Harding, J. P. Moore, Eds.). Taylor and Francis, London.
- 1938. Additions to our knowledge of African leeches. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 90: 297–360.
- Moquin-Tandon, A., 1827. *Monographie de la Famille des Hirudinées*. Maison de Commerce, Montpellier.
- Müller, C., Lukas, P., Lemke, S., Hildebrandt, J. P., 2019. Hirudin and decorsins of the North American medicinal leech *Macrobdella decora*: gene structure reveals homology to hirudins and hirudin-like

- factors of Eurasian medicinal leeches. *Journal of Parasitology*, 105: 423–431.
- Nakano, T., Dujsebayaeva, T., Nishikawa, K., 2015. First record of *Limnatis paluda* (Hirudinida, Arhynchobdellida, Praobdellidae) from Kazakhstan, with comments on genetic diversity of *Limnatis* leeches. *Biodiversity Data Journal*, 3: e5004.
- Nakano, T., Tomikawa, K., Sakono, T., Yoshikawa, N., 2017. Praobdellidae (Hirudinida: Arhynchobdellida) is not specific only to the mucous–membrane after all: discovery of a praobdellid leech feeding on the Japanese freshwater crab *Geothelphusa dehaani*. *Parasitology International*, 66: 210–213.
- Negm–Eldin, M. M., Abd-raba, A. M., Benamer, H. E., 2013. First record, population ecology and biology of the leech *Limnatis nilotica* in the Green Mountain, Libya. *Travaux de l'Institut Scientifique*, 49: 37–42.
- Parmesan, C., Yohe, G., 2013. A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421: 37–42.
- Phillips, A. J., Arauco–Brown, R., Ocegüera–Figueroa, A., Gomez, G. P., Beltrán M., Lai, Y. T., Siddall, M. E., 2010. *Tyrannobdella rex* n. gen. n. sp. and the evolutionary origins of mucosal leech infestations. *Plos One*, 5: e10057.
- Phillips, A. J., Siddall, M. E., 2005. Phylogeny of the New World medicinal leech family Macrobdellidae (Oligochaeta: Hirudinida: Arhynchobdellida). *Zoologica Scripta*, 34: 559–564.
- 2009. Poly–paraphyly of Hirudinidae: many lineages of medicinal leeches. *BMC Evolutionary Biology*, 9(1): 246.
- Raele, D. A., Galante, D., Cafiero, M. A., 2015. Oral hiru–diniasis in a stray dog, first report in Italy. *The Journal of Veterinary Medical Science*, 77(10): 1315–1317.
- Rajaei, S. M., Khorram, H., Ansari Mood, M., Mashhadi Rafie, S., Williams, D. L. 2014. Oral infestation with leech *Limnatis nilotica* in two mixed–breed dogs. *Journal of Small Animal Practice*, 55: 648–651.
- Ronquist, F., Huelsenbeck, J. P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574.
- Savigny, M. J. C., 1822. Système des Annelides, principalement de celles des côtes de l'Égypte et de la Syrie, offrant les caractères tant distinctifs que naturels des ordres, familles et genres avec la description des espèces. In: *Description de l'Égypte, ou recueil des observations et des recherches qui ont été faites en Égypte pendant l'expédition de l'armée française*: 128 (M. J. C. Savigny, Eds.). Imprimerie Impériale, Paris.
- Siddall, M. E., 2002. Phylogeny of the leech family Erpobdellidae (Hirudinida: Oligochaeta). *Invertebrate Systematics*, 16: 1–6.
- Siddall, M. E., Bureson, E. M., 1998. Phylogeny of leeches (Hirudinea) based on mitochondrial cytochrome c oxidase subunit I. *Molecular Phylogenetics and Evolution*, 9: 156–162.
- Tanabe, A. S., 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources*, 11: 914–921.
- Tennent, J. E., 1859. *Ceylon: An Account of the Island, Physical, Historical, and Topographical with Notices of its Natural History, Antiquities and Productions*. 1st edition, volume 1. Longman, Green, Longman, and Roberts, London.
- Trontelj, P., Utevsky, S. Y., 2005. Celebrity with a neglected taxonomy: molecular systematics of the medicinal leech (genus *Hirudo*). *Molecular Phylogenetics and Evolution*, 34: 616–624.
- 2012. Phylogeny and phylogeography of medicinal leeches (genus *Hirudo*): fast dispersal and shallow genetic structure. *Molecular Phylogenetics and Evolution*, 63: 475–485.
- Utevsky, A., Solod, R., Utevsky, S., 2021. A new deep–sea fish leech of the bipolar genus *Pterobdellina* stat. rev. (Hirudinea: Piscicolidae) parasitic on the Antarctic toothfish *Dissostichus mawsoni* (Perciformes: Nototheniidae). *Marine Biodiversity*, 51: 15.
- Utevsky, S. Y., Utevsky, A. Y., Schiaparelli, S., Trontelj, P., 2007. Molecular phylogeny of pontobdelline leeches and their place in the descent of fish leeches (Hirudinea, Piscicolidae). *Zoologica Scripta*, 36: 271–280.