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On the occurrence of the Dragon leech *Hirudo troctina* Johnson, 1816 (Annelida, Hirudinea) in Sardinia (Italy)

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Abstract

In recent decades, medicinal leeches of the genus *Hirudo* have experienced a sharp decline throughout their distribution range due to their overexploitation for medical use and habitat alteration. Such a phenomenon is not fully understood because of the complex taxonomy of the genus, which remained unsettled until the beginning of the XXI century, when the implementation of DNA taxonomy allowed for a better understanding of the diversity of the genus and the distribution ranges of *Hirudo* species. Only the Mediterranean medicinal leech *Hirudo verbana* Carena, 1820 is currently reported to occur in Italy and its major islands, although records of other *Hirudo* species have been reported in the literature. In this study, we report the occurrence of the Dragon leech *Hirudo troctina* Johnson, 1816 in Sardinia and provide its molecular characterization. In addition, based on a review of the existing literature and museum collections, we provide an ample synopsis of the available evidence regarding the occurrence of the species on the island. *Hirudo troctina* proved to be widespread and abundant in Sardinia during the XIX century but became rarer and rarer in the XX and XXI centuries possibly due to its overexploitation for medicinal use and export in mainland Europe, the abandonment of traditional grazing practices, and the decline of wetland habitats and amphibian populations. Currently, the only recently validated occurrence sites of the species are the water bodies of the "Giara di Gesturi", a basaltic plateau in central-southern Sardinia. No corroborated evidence of the possible occurrence of other *Hirudo* species on the island was found. A better understanding of *Hirudo troctina* distribution in Sardinia will allow the amelioration of its protection and management practices, also under the provisions of the European "Habitats Directive", where the *H. medicinalis* species complex is listed in Annex V.

Keywords: *Hirudinidae*, *mitochondrial DNA*, *COI*, *12S rRNA*, *geographical distribution, checklist of the Italian fauna*

Introduction

The hematophagous leeches included in the Palearctic hirudinid genus *Hirudo* Linnaeus, 1758 are popularly known as "medicinal leeches" (Sket & Trontelj 2008; Phillips & Siddall 2009). Most XX century monographs and checklists dealing with *Hirudo* species listed the occurrence of the only *H. medicinalis* in Europe (e.g., Minelli 1979a; Sawyer 1986), which was considered a widespread and highly variable taxon as suggested by Moquin-Tandon (1846). However, thorough phylogenetic and taxonomical studies carried out in the last decades allowed to get a better understanding of the real diversity within the genus, which is now considered to include seven species: *Hirudo medicinalis* Linnaeus, 1758, *H. troctina* Johnson, 1816, *H. verbana* Carena, 1820, *H. nipponia* Whitman, 1886, *H. orientalis* Utevsky & Trontelj, 2005,

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H. sulukii Saglam et al., 2016, as well as two Eastern Asian species *H. nipponia* Whitman, 1886 and *H. tianjinensis* Liu, 2022, that appear to be less related to the Western Palearctic group (cf. Darabi-Darestani et al. 2018; Wang et al. 2022, and references therein). At least three *Hirudo* species are native to Europe and the circum-Mediterranean area: *Hirudo medicinalis* in central and northern Europe, *H. troctina* in the Maghreb and Iberian Peninsula, and *H. verbana* in southern Europe (Nesemann & Neubert 1999; Utevsky et al. 2010; Trontelj & Utevsky 2012), which is characterized by a noteworthy molecular structure (Arias et al. 2021).

In recent papers and synopses on Italian Hirudinea, the occurrence of *Hirudo medicinalis* only was reported in the country (for example, Minelli 1979a, 1979b, 1995; Margraf & Maas 1982; Bazzanti et al. 1996; Fontaneto et al. 1999; Sket 1999; Lapini et al. 2013; Osella & Pannunzio 2013) although the existence of "unresolved systematic problems" for the Italian populations ascribed to the genus *Hirudo* was explicitly mentioned by Minelli (1977, 2006) and Lapini et al. (2013). After the reinstatement of *Hirudo verbana* (Trontelj & Utevsky 2005, 2012), Minelli et al. (2016), Marrone and Canale (2019), Marrone et al. (2021a) and Vecchioni et al. (2024) tentatively ascribed to *Hirudo verbana* all the *Hirudo* populations reported for Italy, although stressing that the occurrence of native or introduced populations of other *Hirudo* species in the country could not be ruled out based on the scant literature and samples available. Thus, the identity of the *Hirudo* populations from Sardinia remained to be ascertained. Indeed, although the occurrence of the Dragon leech *Hirudo troctina* was reported for the island by several authors in the XIX and early XX centuries (that is, Ebrard 1857, pag. 37; Panceri 1875, pag. 39; Blanchard 1893, pag. 24; Blanchard 1894, pag. 41; Seurat 1922), some doubts were expressed about its real occurrence in Sardinia, and even about its status as *bona species* (Minelli 1977, 1979a). The taxonomic validity of *Hirudo troctina* was also questioned by Nesemann and Neubert (1999), until Hechtel and Sawyer (2002) confirmed it as a valid species and redescribed *H. troctina* based on specimens from North Africa, at the same time stressing that the reports of the species from Spain and Sardinia remained unsubstantiated, and thus needed to be validated.

The finding of a *Hirudo* population in southern Sardinia, and the consequent availability of recent samples to be studied, allowed to clarify the identity of the medicinal leeches occurring on the island and fostered the realization of a review aimed at better understanding the natural history of the taxon on the island.

Materials and methods

Bibliographical review & citizen science

An attempt at carrying out an exhaustive review of the papers citing the presence of medicinal leeches of the genus *Hirudo* in Sardinia was based on the datasets of the authors, including grey literature, combined with an extensive literature search through the databases of SCOPUS (www.scopus.com) and Google Scholar (https://scholar.google.it/) using the keywords "*Hirudo*", "medicinal leech", and "Sard*".

Moreover, social media and naturalistic platforms are nowadays widely used as valid integrative tools for wildlife studies, as they represent a rich source of valuable data (Haklay et al. 2021). The critical use of the web and citizen science is particularly useful for difficult cases for which the collection of accurate distributional data is pivotal, such as rare (Fontaine et al. 2022; Faraone et al. 2024) and alien (Faraone et al. 2017; Johnson et al. 2020) species. Therefore, websites and social media were screened for possible reports of *Hirudo* sp. in Sardinia through the search engine "Google Search" using the keywords "*Hirudo*", "medicinal leech", "sanguisuga", "Sardegna" and "Sardinia" (and different combinations of them), and through manual search on the databases of GBIF (www.gbif.org), iNaturalist (www.inaturalist.org), NaturaMediterraneo (www.naturamediterraneo.com), and on the Facebook group "Flora e fauna della Sardegna e non solo" (https://www.facebook.com/groups/206872009324117).

Museum collections

Through the courtesy of the curators (see the acknowledgements section), the invertebrate collections of the following Italian Museums have been screened for the possible presence of Sardinian *Hirudo* samples: "Museo Regionale di Scienze Naturali" (Torino, Italy), the Natural History Museum, University of Florence (Florence, Italy), the "Collezione Zoologica" of the University of Sassari (Sassari, Italy), the "Museo di Zoologia" of the University of Cagliari (Cagliari, Italy), and the "Museo di Zoologia 'Pietro Doderlein'" (Palermo, Italy).

Moreover, the collections of the Natural History Museum (London, United Kingdom), the Museum für Naturkunde (Berlin, Germany), the Naturmuseum Senckenberg (Frankfurt am Main, Germany), and the Muséum National d'Histoire Naturelle (Paris, France) were referred through their online interfaces (https://data.nhm.ac.uk/dataset/, http://zmb.sesam.senckenberg.de, https://

search.senckenberg.de/aquila-public-search/search, and https://science.mnhn.fr/institution/mnhn/search?lang=en_US, respectively).

Field surveys and identification

Several *Hirudo* individuals were occasionally observed by FM and FS on 13th March 2008 in a temporary pond on the basaltic plateau known as "Giara di Gesturi" (Sardinia, Italy). After this occasional observation, dedicated samplings for leeches were carried out on the "Giara di Gesturi" on 12nd March 2010 and 9th May 2022 (Figure S1). Moreover, attention was paid about the possible occurrence of medicinal leeches in the frame of extensive sampling surveys performed in Sardinian water bodies in 2008 (March), 2010 (March), 2018 (March), 2019 (June, September, and December), and 2021 (March and May) (cf. Marrone et al. 2021b). In the visited sites, taking advantage of *Hirudo*'s behavior of actively swimming towards water disturbance, water was troubled to attract and collect animals. Collected animals were provisionally identified based on their dorsal and ventral color pattern according to Hechtel and Sawyer (2002) and Trontelj and Utevsky (2005).

Total genomic DNA was extracted starting from *Hirudo* collected specimens' tissue using the BIORON GmbH "Ron's Tissue DNA Mini Kit" following the protocol provided by the manufacturer. The extracted DNA was amplified by polymerase chain reaction (PCR). A fragment of the mitochondrial markers cytochrome oxidase subunit 1 (*COI*) and 12S rDNA (*12S*) were chosen for amplification via PCR. The primer pair "LCO1490" and "HCO2198" (Folmer et al. 1994) was used to amplify a fragment of the *COI*; the primers "12S" designed by Trontelj and Utevsky (2005) were used for the amplification of the *12S rRNA*.

The PCR for the *COI* was carried out with 35 thermal cycles on a 25 µl reaction volume containing 19.9 µL double-distilled water, 2.5 µL Buffer 10X including 25 mM $MgCl_2$ solution, 0.4 µL dNTPs (10 mM of each), 0.4 µL of each primer (10 µM), 0.4 µL BIORON DFS-Taq DNA Polymerase 5 U/µL, and 1 µL of DNA template, for a total volume of 25 µL. The thermal cycle consisted of an initial 5 min denaturation phase at 94°C, followed by 35 cycles with denaturation at 94°C for 40 s, annealing at 45°C for 45 s, and extension at 72°C for 1 min, followed by 5 min at 72°C for a final extension step. The composition of the PCR mix for the *12S* included 19.2 µl of distilled water, 2.5 µl of Buffer 10X (including 25 mM of $MgCl_2$), 0.4 µl of dNTPs (10 mM each), 0.5 µl of each primer (10 µM), 0.4 µl of Taq polymerase (5 U/µl), and 1.5 µl of DNA template, for a total volume of 25 µl. The thermal cycle consisted of an initial denaturation phase at 94°C, with a duration of 5 min. This is followed by 30 denaturation cycles (94°C, 45 s), annealing (46°C, 45 s), and extension (72°C, 1 min), plus a final extension cycle of 5 min at 72°C. Afterwards, 5 µl of each PCR product was used to perform electrophoresis on 2% agarose gel, with a voltage of 90 V, for 25 min. The outcome of the electrophoresis was verified using a UV transilluminator. Only PCR products that showed a clear and single band of the expected length for each marker used were purified using the Exo-SAP-IT kit (Affymetrix USB). Sequencing was conducted by Macrogen Europe through an ABI 3130×L sequencer (Applied Biosystems). The same primers used for the PCR were used for the direct sequencing of the PCR products. The quality of the resulting chromatograms was verified by measuring their "Phred score" value (Richterich 1998). Among these, only the sequences that showed continuous readings of high-quality bases (QV > 20) were kept. The software Chromas v. 2.6.2 (Technelysium, Pty. Ltd. 1998, Queensland, Australia) was used for chromatogram analysis. All novel *Hirudo* mtDNA sequences were deposited in the public database GenBank (Accession Number, A.N.: PP754720-PP754721 for *COI*; PP754725-PP754726 for *12S*). Aiming to compare the novel produced *COI* and *12S Hirudo* sequences with those publicly available for other *Hirudo* species, 40 *Hirudo* spp. sequences (21 for *COI* and 19 for *12S*) were downloaded from GenBank to constitute the "*COI* dataset" and "*12S* dataset" (see Table S1 for their A.N.). All sequences were aligned with the software MEGA11 (Tamura et al. 2021) using the ClustalW method (Thompson et al. 1994). Moreover, novel COI sequences were translated to aminoacids to check for the possible presence of frameshifts or stop codons, which would indicate the presence of pseudogenes or sequencing errors.

The molecular identification of the studied specimens and reconstruction of the phylogenetic relationships among taxa was performed, for both datasets, based on the Bayesian inference of phylogeny (BI) using the software package MrBayes v. 3.2.6 (Ronquist et al. 2012). The best evolutionary model was selected among those analysed by MrBayes using Bayesian model choice criteria (nst = mixed, rates = invgamma). Two independent Markov Chain Monte Carlo analyses were carried out with 1 million generations each (temperature: 0.2; default priors). Trees and parameter values

were sampled every 100 generations, resulting in 10,000 trees for each analysis. The convergence of chains was reached in the analyses (Effective Sample Size, ESS > 200 in all the analyses). The initial 25% of trees were discarded as "burn-in". Posterior probability values were used as nodes support.

Results

Bibliographical review

The bibliographical review showed that, in the XIX century, Sardinia had a flourishing trade exporting medicinal leeches in Europe. Angius (2006) reported the abundance of medicinal leeches in Sardinia, mentioning several places where they were actively collected and commercially exploited throughout the island. Panceri (1875, pag. 39) mentioned that *Hirudo* leeches were exported in astonishingly numbers from Sardinia to France ("*E' ben noto come l'isola di Sardegna facesse, un tempo, importante esportazione di quest'anellide, che veniva spedito in Francia specialmente. Dal 1827 al 1836 la Sardegna mandò in Francia 5,038,000 sanguisughe, superando di molto le cifre di quelle provenienti dalla Svizzera, dall'Ungheria, dalla Grecia e dall'Algeria*"), where non-native populations of the species are also known to have established in Brittany although seem to be now disappeared (Delphy 1935; Lecaplain & Noel 2019). The importance of medicinal leech trade in Sardinia was also confirmed by Asunis (2014), who reported on a single trader from Cagliari, who bought 30.000 wild-caught medicinal leeches to be exported in 1828 only. Such a trade, although likely scaled down, also survived at the beginning of the XX century, if the writer Grazia Deledda mentions the job of "*pescatore di sanguisughe*" in her book "*Dopo il divorzio*", set in Sardinia (Deledda 1902).

Sardinian medicinal leeches were ascribed by XIX century scientists to the Dragon leech *Hirudo troctina*, a species described by Johnson (1816) based on commercial individuals obtained from pharmacies in England (cf. Nesemann & Neubert 1999; Hechtel & Sawyer 2002). Whereas Ebrard (1857) and Panceri (1875) reported the occurrence of the species in Sardinia without providing detailed information on its distribution and without depositing specimens in public or private collections, Blanchard (1893, pag. 24; Blanchard 1894, pagg. 41–42) had the opportunity to study some large-sized *H. troctina* individuals collected in Sardinia by F. De Filippi and stored in the Museum of Turin (Italy). In the course of the XX century, no significant mentions of medicinal leeches are available for Sardinia apart for a generic statement by Seurat (1922) and the occasional reports of a species identified as *Hirudo medicinalis* by Margraf and Maas (1982) for a flooded meadow near "Pauli Ala de Mengianu" and by Burmeister et al. (1987) for the pond "Pauli Maiori", both located on the "Giara di Gesturi", where the word "*pauli*" indicates in Sardinian language large but usually shallow temporary or permanent ponds. Accordingly, Minelli (1977) expressed some doubts about the identity of the Sardinian medicinal leeches, and later reported the occurrence of *Hirudo medicinalis* in Sardinia (Minelli et al. 1995). However, lacking further reports of *Hirudo* species on the island, medicinal leeches were not reported as present in Sardinia in subsequent papers (Minelli 2006; Utevsky et al. 2010; Trontelj & Utevsky 2012). Conversely, based on samples collected by Stoch and Marrone in 2008 in the Giara di Gesturi, Minelli et al. (2016) reported *H. verbana* for Sardinia, a datum then reported also in Marrone et al. (2021a). Finally, the occurrence of *Hirudo verbana* in Sardinia was also reported in the standard data form of the N2K site ITB011102 "Catena del Marghine e del Goceano" (https://download.mase.gov.it/Natura2000/Trasmissione%20CE_dicembre2022/schede_mappe/Sardegna/ZSC_schede/Site_ITB011102.pdf).

Museum collections

No *Hirudo* specimens are currently stored at the "Museo di Zoologia" of the University of Cagliari and in the "Collezione Zoologica" of the University of Sassari, both located in Sardinia, nor in "Museo di Zoologia Pietro Doderlein" of the University of Palermo, Sicily. Conversely, two *Hirudo troctina* specimens collected in Sardinia are stored at the Sezione di Zoologia of the "Museo Regionale di Scienze Naturali" in Turin, Italy (Catalogue number 53; see Figure 1). Unfortunately, no accurate information about the collection locality is included in the label; however, the name of the collector is reported, confirming that these are the *Hirudo troctina* specimens collected by De Filippi and studied by Blanchard (1893). Moreover, in the Annelida collection of the "Museo di Storia Naturale - La Specola" of the University of Florence (Italy) there are two *Hirudo* samples collected in Sardinia, which are currently labelled as *Hirudo medicinalis*. These are: two specimens collected in 1877 in a "*palude presso Oristano*" ("a swamp near Oristano") (MZUF AN/591, Figures 2(a,b,c)), and four specimens collected on April 28, 1984, in "Giara di Gesturi" by Benedetto Lanza (MZUF AN/1000,

Figure 1. *Hirudo troctina* collected in an unknown locality in Sardinia by De Filippi, now stored at the "Museo Regionale di Scienze Naturali" (Torino, Italy).

Figures 2(d,e,f)). Both samples could be ascribed to *H. troctina* based on the dorsal and ventral color pattern. Interestingly, the original label of sample MZUF AN/591 reads "*Sanguisuga troctina*".

A *Hirudo troctina* sample from Sardinia ("*Tirso bei Oristano*") collected on 25 April 1914 by O. Krause is currently stored at Naturmuseum Senckenberg (Frankfurt am Main, Germany), with the Catalogue number 504 within the collection "Clitellata – SMF".

No *Hirudo* specimens from Sardinia are available in the invertebrate collections of the Natural History Museum (London, United Kingdom), the Museum für Naturkunde (Berlin, Germany), and the Muséum National d'Histoire Naturelle (Paris, France).

Citizen science

Only few evidence of the occurrence of *Hirudo troctina* in the wild is available online. On 6th January 2024, a picture of a *Hirudo troctina* individual taken on 13 July 2019 in Giara di Gesturi by Giacomo Assandri was published on iNaturalist (giacomo_assandri 2019, Figure 3), and then incorporated in the GBIF database. The provided coordinates (coordinates: 39.762940 N, 8.950911 E) point to a stream originating from a nearby spring and reservoir, close to "Pauli S'Ala de Mengianu". In the picture, the *H. troctina* individual swims within a swarm of *Gambusia* sp., possibly *Gambusia holbrooki* Girard, 1859 (cf. Orrù et al. 2010), thus attesting the permanent hydroperiod of the water body or, at least, its connection to a permanent water body.

Field activities

Despite the significant sampling effort we spent throughout the island, the occurrence of leeches belonging to the genus *Hirudo* was observed only in

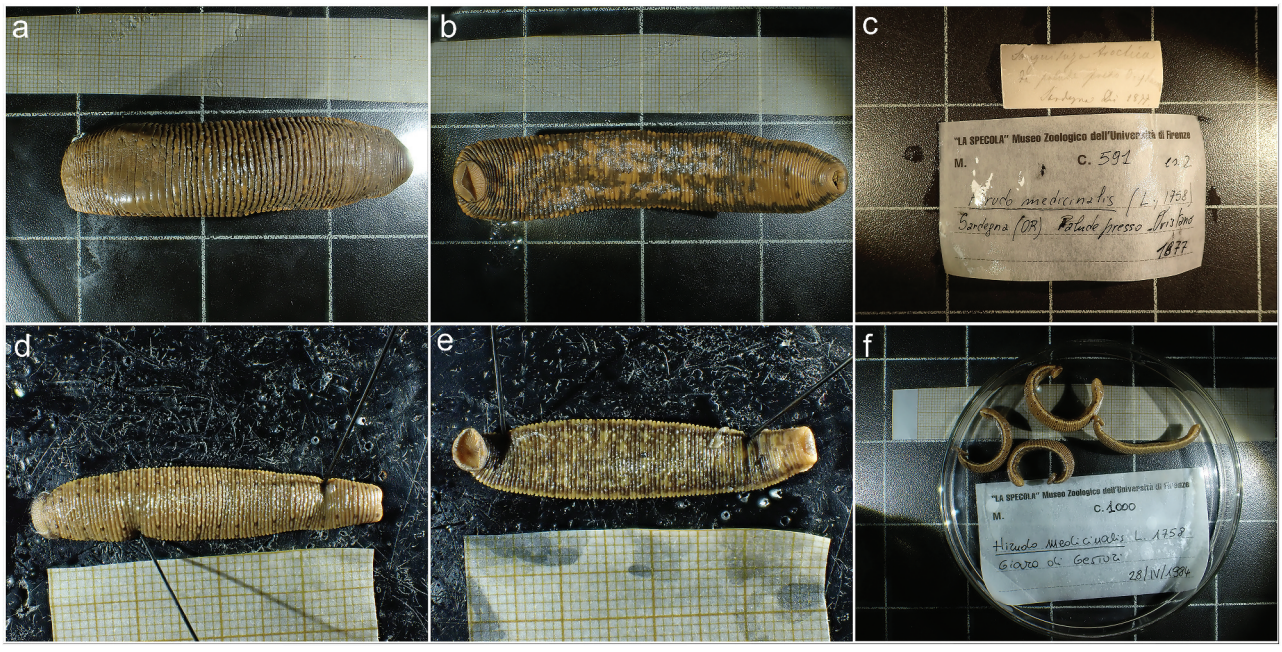

Figure 2. *Hirudo troctina* from Sardinia. (a, b, c): *H. troctina* collected in a swamp near Oristano in 1877. The original label reads "*Sanguisuga troctina*", a more modern one reads "*Hirudo medicinalis*"; (d, e, f): *H. troctina* collected on "Giara di Gesturi" on 28 April 1984. The label reads "*Hirudo medicinalis*". Samples stored at "Museo di Storia Naturale - La Specola" (Florence, Italy).

Figure 3. *Hirudo troctina* from a stream close to Pauli S'Ala de Mengianu (23 July 2019; photo by G. Assandri).

"Pauli Bartili" (coord.: 39.765556 N, 8.976112 E) and "Pauli Oromeo" (coord.: 39.745083 N 8.996662 E), two temporary ponds on the "Giara di Gesturi", a basaltic plateau located in the southern central part of the island. Active individuals were observed in the temporary ponds of the "Giara di Gesturi" on 13 March 2008, 12 March 2010, and 9 May 2022 (Figures 4(a–d), S1). Based on the dorsal and ventral color pattern, all the observed individuals could be identified as *Hirudo troctina*. Two individuals collected in "Pauli Bartili" (13 March 2008) and "Pauli Oromeo" (5 May 2022) are now stored in the Annelida Collection of the "Museo di Storia Naturale - La Specola" of the University of Florence (Italy) with the catalogue numbers MZUF AN/6929 and MZUF AN/6930.

Molecular identification

The inferred phylogenetic trees, based on a 617 and 447 base-pair long fragments (*COI* and *12S* datasets, respectively), included both novel and published *Hirudo* sequences and were rooted on the haemadipsid *Haemadipsa yanyuanensis* Liu & Song, 1977 (*COI* A.N., MZ820660; *12S* A.N., OQ076770).

In good accordance with the preliminary identification based on the color pattern of the collected specimens, the *COI* and *12S* sequences of the two investigated individuals collected in 2022 in "Pauli Oromeo" (Giara di Gesturi) cluster within the *Hirudo troctina* clade, along with *Hirudo* sequences from Tunisia and Morocco (see Figure 5). The Sardinian sequences were sister to the Tunisian sequences and diverged for an uncorrected "*p*" distance of 0.05% from the

Figure 4. *Hirudo troctina* from Sardinia. (a, b): dorsal and ventral view of *H. troctina* from Pauli Bartili (13 March 2008); (c, d): dorsal and ventral view of *H. troctina* from Pauli Oromeo (5 May 2022).

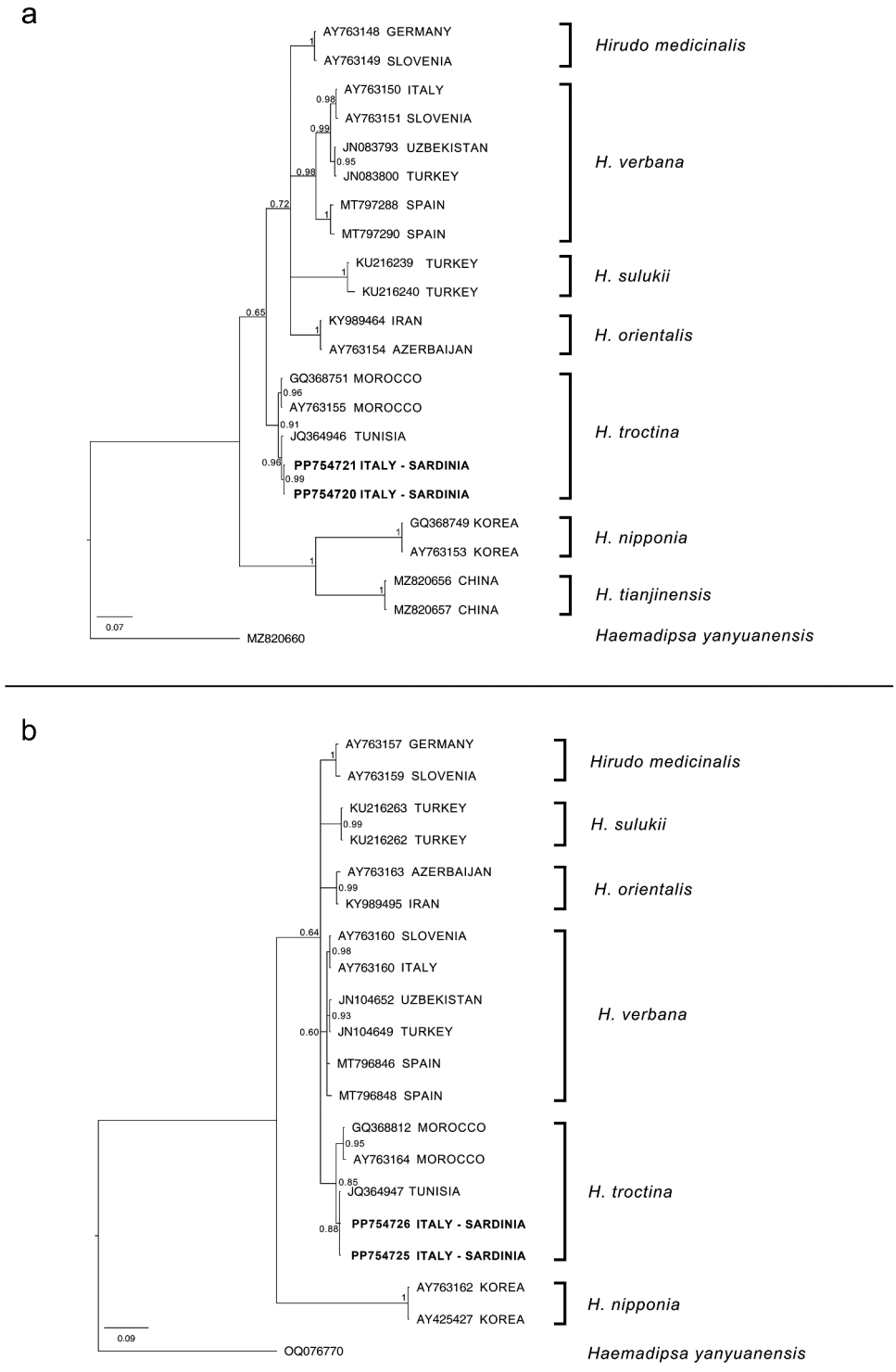

Figure 5. A: Bayesian phylogram (50% majority rule consensus tree) for *Hirudo* spp. based on the 617-bp fragment of the mitochondrial *COI* gene. *Haemadipsa yanyuanensis* (A.N., MZ820660) was used as outgroup to root the tree. Posterior probabilities values are reported as node statistical support. Square brackets group the samples according to the current taxonomy of the genus. Our novel sequences are reported in bold. B. Bayesian phylogram (50% majority rule consensus tree) for *Hirudo* spp. based on the 447-bp fragment of the mitochondrial *12S ribosomal RNA* gene. *Haemadipsa yanyuanensis* (A.N., OQ076770) was used as outgroup to root the tree. Posterior probabilities values are reported as node statistical support. Square brackets group the samples according to the current taxonomy of the genus. Novel sequences are reported in bold.

Tunisian and 1.3% from the Moroccan *COI* sequences. Conversely, the Sardinian and Tunisian *12S* sequences were identical, whereas the uncorrected "*p*" distance between the Sardinian and Moroccan *12S* sequences was of 1.6%. Unfortunately, no Algerian or Iberian sequences are currently available for this species, so that a comparison with these populations is currently unfeasible.

Discussion

The Dragon Leech *Hirudo troctina* was long considered of doubtful taxonomical validity (e.g., Minelli 1979b; Nesemann & Neubert 1999) but it was later revalidated by Hechtel and Sawyer (2002), who established a neotype of the species from the Moyen Atlas, in Morocco. The species, originally described based on commercial individuals from pharmacies in England (Johnson 1816) and later also found in pharmacies in France (Moquin-Tandon 1827 *sub Sanguisuga interrupta*), was then reported to occur throughout the Maghreb and Iberian Peninsula (Hechtel & Sawyer 2002; Utevsky et al. 2010; Mabrouki et al. 2019; Arias et al. 2021, and references therein). Conversely, despite reports published in the XIX century (see above), its occurrence in Sardinia and Libya was considered doubtful or unsubstantiated (cf. Minelli 1977, 1979a; Hechtel & Sawyer 2002), so the species was not included in the Italian fauna in the recent checklists (Minelli et al. 1995; Minelli 2006). However, the color pattern of all the Sardinian *Hirudo* individuals we could examine, and the molecular identification based on both mtDNA *COI* and *12S* sequences of the specimens from Giara di Gesturi, concordantly and unambiguously allowed to identify the Sardinian medicinal leeches as *Hirudo troctina*. The present work thus attests to the long-term occurrence of the species in Sardinia and confirms the reports of the species on the island reported in the literature of the XIX century. The list of known occurrence localities of the Dragon leech in Sardinia is reported in Table I. More than a century after its last mention in the country (Seurat 1922), *Hirudo troctina* is thus confirmed to be part of the Italian fauna. Accordingly, the Italian fauna includes at least two *Hirudo* taxa, i.e., *Hirudo verbana*, which is widespread in Sicily and mainland Italy (Marrone et al. 2021a), and *Hirudo troctina*, currently known only from Sardinia. However, the possible presence of native or translocated populations of *Hirudo medicinalis* in northern Italy cannot at present be ruled out, and further surveys aimed at characterizing the *Hirudo* populations of the Alpine regions and the Po Valley is needed.

The only Sardinian population of the species that could be genetically characterized to date, i.e., the one from Pauli Oromeo on the Giara di Gesturi, shows

Table I. Synopsis of all the existing records of *Hirudo troctina* in Sardinia.

Location	Date	Source	Notes
"Sardinia"	n.a.	Ebrard (1857); Panceri (1875), Seurat (1922)	No samples nor accurate locality data available.
"Sardinia"	n.a.	Blanchard (1893)	Collected by F. De Filippi, now stored at Museum of Turin (Italy), Catalogue number 53
Swamp near Oristano	1877	"Museo di Storia Naturale - La Specola", MZUF AN/591.	The label reads: "*Sanguisuga troctina*, Palude presso Oristano"
River Tirso, near Oristano	25 April 1914	Naturmuseum Senckenberg (Frankfurt am Main, Germany), "Clitellata – SMF504"	The label reads: „Tirso, bei Oristano". Collected by O. Krause
Giara di Gesturi	28 April 1984	Museo di Storia Naturale "La Specola" (MZUF AN/1000)	Collected by B. Lanza
Giara di Gesturi, flooded meadow near "Pauli Ala de Mengianu"	"winter and spring"	Margraf and Maas (1982)	Reported *sub Hirudo medicinalis*
Giara di Gesturi, "Pauli Maiori"	n.a.	Burmeister et al. (1987)	Reported *sub Hirudo medicinalis* ssp.
Giara di Gesturi, "Pauli Bartili"	13 March 2008	Present Work	Geographical coordinates: 39.765556 N, 8.976112 E
Giara di Gesturi, "Pauli Oromeo"	12 March 2010; 9 May 2022	Present Work	Geographical coordinates: 39.745083 N 8.996662 E
Giara di Gesturi, "Pauli S'Ala de Mengianu"	13 July 2019	https://www.inaturalist.org/observations/195921339. Accessed on 27/03/2024	Geographical coordinates: 39.762940 N, 8.950911 E
N2K site ITB011102 "Catena del Marghine e del Goceano"	n.a.	N2K Standard form	Reported *sub Hirudo verbana*

a close similarity with a *H. troctina* population from Tunisia, which is in accordance with the widespread presence in Sardinia of animal species originating from North Africa (e.g., Cosson et al. 2005; Carranza et al. 2008; Fritz et al. 2009; Planas et al. 2013; Faraone et al. 2020). However, in light of the paucity of molecular sequences for the Maghrebian populations of the species and the complete lack of molecular data on the *H. troctina* populations inhabiting the Iberian Peninsula, this issue is still open. Thus, it is currently difficult to ascertain whether the arrival of *H. troctina* in Sardinia is of natural or anthropogenic origin, as suggested by Blanchard (1893). The latter is a plausible origin, given that leeches have been widely used by humans in the Mediterranean basin since at least 1500 BC and are often translocated for medicinal purposes (Wells et al. 1993; Whitaker et al. 2004; Papavramidou & Christopoulou-Aletra 2009). Furthermore, Sardinia has historically been both under the influence of Maghrebi and Iberian people (Gabriele 2014), and the man-mediated origin in historical times of a large part of the fauna that the island shares with the Maghreb has been genetically supported (Giovannotti et al. 2007; Fritz et al. 2009; Mattucci et al. 2013; Faraone et al. 2020). However, in our case, lacking sound evidence and a more complete picture of the genetic diversity of the species throughout its distribution range, we propose to apply the precautionary principle and consider *Hirudo troctina* as a native species in Sardinia until proven otherwise. Moreover, a clear signature of human impact on the geographical distribution of medicinal leeches has not been detected in a previous phylogeographic study (Trontelj & Utevsky 2012), which agrees with the possible native origin of *H. troctina* in Sardinia.

The first validated evidence ascribable to the species attests to its widespread presence in Sardinia at least from the beginning of the XIX century, thus excluding the hypothesis of a recent introduction of the species on the island. No evidence of past or present occurrence of other *Hirudo* species on the island could be retrieved, so the reports on the Giara di Gesturi of *Hirudo medicinalis* (Margraf & Maas 1982; Burmeister et al. 1987) or *H. verbana* (Minelli et al. 2016; Marrone et al. 2021a) are rather to be ascribed to *Hirudo troctina*, as confirmed by the museum samples stored at La Specola, our novel samples, and the single available citizen science report. Although the occurrence of *H. verbana* in Sardinia could be expected, *H. medicinalis* does not inhabit Mediterranean landscapes and should be assigned to representatives of the fauna of the more northerly European arboreal zone (Utevsky et al. 2010).

Medicinal leeches were certainly abundant and widespread throughout Sardinia in the XIX century (e.g., Panceri 1875; Angius 2006) and were the object of massive collections to be exported to European countries, where a strong decline of wild populations of medicinal leeches had already occurred (Whitaker et al. 2004). Medicinal leech collection was so widespread that some laws had to be enforced to regulate their collection and trade in Sardinia (Asunis 2014). Such a massive exploitation, along with the abandonment of traditional grazing practices and the decline of wetland habitats and amphibian populations, apparently led to a collapse of the Sardinian populations of the species, so that in the XX century extremely scarce observations are available for *Hirudo troctina*, all of which referring to the population inhabiting the "Giara di Gesturi" (i.e., Margraf & Maas 1982; Burmeister et al. 1987; and specimens collected in 1984 by B. Lanza and now stored at the "Museo di Storia Naturale - La Specola" of the University of Florence (Italy)), with the single exception of the sample collected near Oristano in 1914 and now stored at the Naturmuseum Senckenberg (Frankfurt am Main, Germany) (Table I). In the XXI century, our novel observations and the single one available on the social networks (giacomo_assandri 2019), further confirmed the occurrence of the species on Giara di Gesturi, but no other occurrence localities are currently known for the island, albeit its water bodies have been widely investigated in the last two decades (e.g., Buffagni 2021; Marrone et al. 2021b; Austoni et al. 2023). Based on currently available data, the only validated current occurrence locality of *Hirudo troctina* in Sardinia (and the whole Italy) is thus the basaltic plateau "Giara di Gesturi". If the Sardinian *H. troctina* populations will be confirmed as native or parautochthonous by further studies, their monitoring and a rigorous protection will be desirable, along with the realization of further surveys aimed at finding other populations possibly surviving in Sardinia. Moreover, they are included in the Annex V of the "Habitats Directive" (Council Directive 92/43/EEC) *sub Hirudo medicinalis*.

The ecology of *Hirudo troctina* is to date poorly known, and the scant information available is apparently contradictory. In Sardinia, the species was observed in both temporary and permanent water bodies located on the Giara di Gesturi, co-occurring with the mosquitofish *Gambusia* in at least one site. As already suggested by Margraf and Maas (1982), its occurrence in temporary ponds is likely due to its opportunistic colonization

from a source locality characterized by permanent hydroperiod. According to Arias et al. (2021), *Hirudo troctina* in Spain is mostly linked with flowing waters, whereas the sympatrically co-occurring *H. verbana* prefers lentic water bodies. Conversely, Ben Ahmed et al. (2015) and Mabrouki et al. (2019) reported that this species prefers standing water bodies in Tunisia and Morocco. As for other *Hirudo* species, little is known also about the feeding behavior of the species, which is reported to feed on fish by Johnson (1816) and assumed to feed on large vertebrates by Burmeister et al. (1987). In Maghreb, *Hirudo troctina* has often been observed feeding on amphibians (Ben Hassine & Escoriza 2014; Merabet et al. 2017; Merabet & Karar 2021). On the Giara di Gesturi, the amphibians *Hyla sarda* (Betta, 1853) and *Bufotes balearicus* (Boettger, 1880) breed in the water bodies inhabited by *H. troctina*, which are also used as watering points by wild boars and large herds of horses and goats (Margraf & Maas 1982); both amphibians and mammals are thus potentially available as hosts or prey for the medicinal leeches. Molecular genomic metabarcoding of bloodmeals of wild-caught *H. troctina* individuals is required to obtain some information about the diet and prey items of the species in Sardinia (cf. Williams et al. 2020), an important information for its correct management and protection.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data used for this work are available from the corresponding author on reasonable request.

Supplementary material

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References

Angius V. 2006. 1833-1856. Città e villaggi della Sardegna dell'Ottocento. (A cura di Luciano Carta). Bibliotheca Sarda Grandi Opere n° 5. Ilisso, Nuoro, Italy.

Arias A, Surugiu V, Carballeira R, Popa OP, Popa LO, Utevsky S. 2021. Unravelling the extent of diversity within the Iberian medicinal leeches (Hirudinea: *Hirudo*) using molecules and morphology. Biology 10(4):315. DOI: 10.3390/biology10040315.

Asunis A. 2014. Elmas - Paese di Sardegna. Logus Mondi Interattivi, Italy. EAN: 9788898062454

Austoni M, Boggero A, Kamburska L, Lugliè A, Marchetto A, Oggioni A, Padedda BM, Volta P, Zaupa S. 2023. Biological survey of lakes and reservoirs from Sardinia and Piedmont (Italy), a georeferenced dataset from the project LIFE INHABIT. Journal of Limnology 82(s1):2114. DOI: 10.4081/jlimnol.2023.2114.

Bazzanti M, Baldoni S, Seminara M. 1996. Invertebrate macrofauna of a temporary pond in central Italy: Composition, community parameters and temporal succession. Archiv für Hydrobiologie 137(1):77–94. DOI: 10.1127/archiv-hydrobiol/137/1996/77.

Ben Ahmed R, Romdhane Y, Tekaya S. 2015. Checklist and distribution of marine and freshwater leeches (Annelida, Clitellata, Hirudinea) in Tunisia with identification key. Ecologica Montenegrina 2(1):3–19. DOI: 10.37828/em.2015.2.1.

Ben Hassine J, Escoriza D. 2014. *Bufo spinosus* in Tunisia: New data on occurrence, parasitism and tadpole morphology. Herpetological Bulletin 127:22–32.

Blanchard R. 1893. Révision de Hirudinées du Musée de Turin. Bollettino Dei Musei di Zoologia Ed Anatomia Comparata

della R. Università di Torino. 8(145):1–32. DOI: 10.5962/bhl.part.27223.

Blanchard R. 1894. Hirudinées de l'Italie continentale et insulaire. Bollettino dei musei di zoologia ed anatomia comparata della R. Università di Torino 9(192):1–84. DOI: 10.5962/bhl.part.8048.

Buffagni A. 2021. The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers. Freshwater Biology 66(1):1–203. DOI: 10.1111/fwb.13596.

Burmeister E-G, Dettner K, Holmen M. 1987. Die Hydradephaga Sardiniens (Insecta, Coleoptera: Gyrinidae, Haliplidae, Noteridae, Hygrobiidae, Dytiscidae). Spixiana 10 (2):157–185.

Carranza S, Arnold EN, Geniez P, Roca J, Mateo JA. 2008. Radiation, multiple dispersal and parallelism in the skinks, *chalcides* and *sphenops* (Squamata: Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara Desert. Molecular Phylogenetics and Evolution 46(3):1071–1094. DOI: 10.1016/j.ympev.2007.11.018.

Cosson JF, Hutterer R, Libois R, Sarà M, Taberlet P, Vogel P. 2005. Phylogeographical footprints of the Strait of Gibraltar and quaternary climatic fluctuations in the western Mediterranean: A case study with the greater white-toothed shrew, *Crocidura russula* (Mammalia: Soricidae). Molecular Ecology 14(4):1151–1162. DOI: 10.1111/j.1365-294X.2005.02476.x.

Darabi-Darestani K, Sari A, Sarafrazi A, Utevsky S. 2018. Entrapped by the uneven central and middle eastern terrains: Genetic status of populations of *Hirudo orientalis* (Annelida, Clitellata, Hirudinida) with a phylogenetic review of the genus *Hirudo*. Molecular Phylogenetics and Evolution 121:52–60. DOI: 10.1016/j.ympev.2017.12.024.

Deledda G. 1902. Dopo il divorzio. Torino-Roma, Italy: Casa editrice Nazionale Roux eViarengo.

Delphy J [in Perrier R]. 1935. La Faune de la France en tableaux synoptiques illustrés. Vers et némathelminthes. Librairie Delagrave éditeur, Paris, France, tome IB: 1–179.

Ebrard E. 1857. Nouvelle monographie des Sangsues médicinales. Paris, France: Legare Street Press. pp 494.

Faraone FP, Giacalone G, Canale DE, D'Angelo S, Favaccio G, Garozzo V, Giancontieri GL, Isgrò C, Melfi R, Morello B, Navarria F, Russo G, Tinnirello V, Torre A, Torre D, Torre G, Urso G, Vinci P, Zizzo MG, Marrone F. 2017. Tracking the invasion of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Decapoda Cambaridae) in Sicily: A "citizen science" approach. Biogeographia - the Journal of Integrative Biogeography 32:25–29. DOI: 10.21426/B632135512.

Faraone FP, Melfi R, Di Nicola MR, Giacalone G, Lo Valvo M. 2020. Phylogenetic relationships of the Italian populations of horseshoe whip snake *Hemorrhois hippocrepis* (Serpentes, Colubridae). Acta Herpetologica 15(2):129–135. DOI: 10.13128/a_h-9058.

Faraone FP, Vecchioni L, Goldenberg J, Giacalone G, Muscarella C, Signorello G, Battista A, Nicolosi G, Marrone F. 2024. Distribution and natural history of *Plutonium zwierleini* (Chilopoda: Scolopendromorpha) in Sicily (Italy). European Zoological Journal 91(1):305–317. DOI: 10.1080/24750263.2024.2324118.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5):294–299.

Fontaine A, Simard A, Brunet N, Elliott KH. 2022. The scientific contributions of citizen science applied to rare or threatened animals. Conservation Biology 36(6):e13976. DOI: 10.1111/cobi.13976.

Fontaneto D, Guidali F, Scali S. 1999. Parasitism and necrophagy of two leech species on *Bufo bufo*. Current studies in herpetology. In: Miaud C, Guyétant R, editors. Proceedings of the 9th ordinary general meeting of the societas europaea herpetologica, 25-29 August 1998. Chambéry, France: Societas Europaea Herpetologica. pp. 121–124.

Fritz U, Harris DJ, Fahd S, Rouag R, Martínez EG, Casalduero AG, Široký P, Kalboussi M, Jdeidi TB, Hundsdörfer AK. 2009. Mitochondrial phylogeography of *Testudo graeca* in the Western Mediterranean: Old complex divergence in North Africa and recent arrival in Europe. Amphibia-Reptilia 30(1):63–80. DOI: 10.1163/156853809787392702.

Gabriele N 2014. A History of Sardinia. Università degli Studi di Cagliari. Italy.giacomo_assandri. 2019. iNaturalist observation. Available: https://www.inaturalist.org/observations/195921339. Accessed Mar 2024 27

giacomo_assandri. 2019. https://www.inaturalist.org/observations/195921339. Accessed Mar 2024 27.

Giovannotti M, Nisi Cerioni P, Kalboussi M, Aprea G, Caputo V. 2007. Phylogeographic inferences from the mtDNA variation of the Three-toed skink, *Chalcides chalcides* (Reptilia: Scincidae). Journal of Experimental Zoology: Part B, Molecular and Developmental Evolution 308B (3):297–307. DOI: 10.1002/jez.b.21149.

Haklay MM, Dörler D, Heigl F, Manzoni M, Hecker S, Vohland K. 2021. What is citizen science? The challenges of definition. In: Vohland K, Land-Zandstra A, Ceccaroni L, Lemmens R, Perelló J, Ponti M, Samson R, Wagenknecht K, editors. The science of citizen science. Amsterdam: Springer. pp. 13–33.

Hechtel FOP, Sawyer RT. 2002. Toward a taxonomic revision of the medicinal leech *Hirudo medicinalis* Linnaeus, 1758 (Hirudinea: Hirudinidae): Re-description of *Hirudo troctina* Johnson, 1816 from North Africa. Journal of Natural History 36(11):1269–1289. DOI: 10.1080/00222930110048945.

Johnson BA, Mader AD, Dasgupta R, Kumar P. 2020. Citizen science and invasive alien species: An analysis of citizen science initiatives using information and communications technology (ICT) to collect invasive alien species observations. Global Ecology and Conservation 21:e00812. DOI: 10.1016/j.gecco.2019.e00812.

Johnson JR. 1816. A treatise on the medicinal leech, including its medical and natural history, with a description of its anatomical structure: Also remarks upon the diseases, preservation, and management of leeches. The Medico-chirurgical journal and review 2(12):481–96.

Lapini L, Dorigo L, Glerean P, Giovannelli MM. 2013. Status di alcune specie protette dalla Direttiva Habitat 92/43/CEE nel Friuli-Venezia Giulia (Invertebrati, Anfibi, Rettili, Mammiferi). Gortania 35:61–139.

Lecaplain B, Noel F 2019. Les sangsues d'eau douce du Nord-Ouest de la France (Annelida – Hirudinida): Normandie, Bretagne, Pays de la Loire. *Recherche, récolte et identification*. (GRETIA, UMS PatriNat AFB-CNRS-MNHN).

Mabrouki Y, Ben Ahmed R, Fouzi Taybi A, Rueda J. 2019. An annotated checklist of the leech (Annelida: Hirudinida) species of the Moulouya River basin, Morocco, with several new distribution records and a historical overview. African

Zoology 54(4):199–214. DOI: 10.1080/15627020.2019.1671218.

Margraf J, Maas B. 1982. Zur Ökologie der temporären Süßwasserflachseen des Tafelbergs "Giara di Gesturi" auf Sardinien. Spixiana 5(1):69–99.

Marrone F, Alfonso G, Barbagallo R, Brandmayr P, Bruni G, Costa S, Farina G, Gerecke R, Iannarelli A, Mazza G, Mazzei A, Menchetti M, Moretti V, Mori E, Novaga R, Pecoraro M, Schifani E, Stoch F, Vecchioni L. 2021a. New distributional data for the Mediterranean medicinal leech *Hirudo verbana* Carena, 1820 (Hirudinea, Hirudinidae) in Italy, with a note on its feeding on amphibians. Biogeographia – the Journal of Integrative Biogeography 36: a007. DOI: 10.21426/B636052446.

Marrone F, Alfonso G, Cottarelli V, Botta MM, Koepp C, Stoch F. 2021b. An updated checklist and biogeography of the Sardinian large branchiopods, with a focus on Spinicaudata (Crustacea, Branchiopoda). Biogeographia – the Journal of Integrative Biogeography 36:a010. DOI: 10.21426/B636054480.

Marrone F, Canale DE. 2019. Occurrence, distribution and bibliography of the medicinal leech *Hirudo verbana* Carena, 1820 (Hirudinea, Hirudinidae) in Sicily (Italy). Biogeographia – the Journal of Integrative Biogeography 34:33–38. DOI: 10.21426/B634143807.

Mattucci F, Oliveira R, Bizzarri L, Vercillo F, Anile S, Ragni B, Lapini L, Sforzi A, Alves PC, Lyons LA, Randi E. 2013. Genetic structure of wildcat (*Felis silvestris*) populations in Italy. Ecology and Evolution 3(8):2443–2458. DOI: 10.1002/ece3.569.

Merabet K, Dahmana A, Karar M, Moali A. 2017. First report of lech predation on *Pleurodeles nebulosus* (Guichenot, 1850) in Kabylia. Algeria. Boletín de la Asociación Herpetológica Española 28(1):1–9.

Merabet K, Karar M. 2021. First cases of predation of *Bufo spinosus* by two leech species in Algeria. The Herpetological Bulletin 156(156, Summer 2021):38–39. DOI: 10.33256/hb156.3839.

Minelli A. 1977. Guide per il riconoscimento delle specie animali delle acque interne italiane. 1. Irudinei (Hirudinea). Verona, Italy: Consiglio Nazionale delle Ricerche AQ/1/2. p. 43.

Minelli A 1979a. Fauna d'Italia 15: Hirudinea. Calderini, Bologna, Italy, 152 pp.

Minelli A. 1979b. Sanguisughe d'Italia. Catalogo orientativo e considerazioni biogeografiche. Lavori della Società Italiana di Biogeografia NS 7:279–313. DOI: 10.21426/B66110106.

Minelli A. 2006. Annelida Hirudinea. In: Ruffo S, Stoch F, editors. Checklist and distribution of the Italian fauna. Vol. 17. Verona, Italy: Memorie del Museo Civico di Storia Naturale di Verona, 2.Serie, Sezione Scienze della Vita, with data on CD-ROM. pp. 77–78.

Minelli A, Omodeo P, Rota E, Sambugar B. 1995. Annelida Clitellata, Aphanoneura. In: Minelli A, Ruffo S, La Posta S, editors. Checklist delle specie della fauna italiana. Bologna, Italy: Calderini. pp. 20.

Minelli A, Rovelli V, Zapparoli M, Bologna MA. 2016. *Hirudo verbana* Carena, 1820 (Sanguisuga). In: Stoch F, Genovesi P, editors. Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. Roma, Italy: ISPRA, Serie Manuali e linee guida, 141/2016. pp 14–15.

Moquin-Tandon A. 1827. Monographie de la Famille des Hirudinées. Montpellier, France: Maison de Commerce.

Moquin-Tandon A. 1846. Monographie de la Famille des Hirudineés. Paris, France: J.-B. Bailliére.

Nesemann H, Neubert E 1999. Annelida, Clitellata. Süsswasserfauna von Mitteleuropa 6/2. Spektrum Akademischer Verlag, Heidelberg, 178 pp.

Orrù F, Deiana M, Cau A. 2010. Introduction and distribution of alien freshwater fishes on the island of Sardinia (Italy): An assessment on the basis of existing data sources. Journal of Applied Ichthyology 26(Suppl. 2):46–52. DOI: 10.1111/j.1439-0426.2010.01501.x.

Osella G, Pannunzio G. 2013. Macrobenthos dei laghetti del Gran Sasso d'Italia. Quaderni del Museo di Storia Naturale di Ferrara 1:57–67.

Panceri P. 1875. Catalogo degli Anellidi, Gefirei e Turbellarie d'Italia. Atti della Società Italiana di Scienze Naturali 18:1–53.

Papavramidou N, Christopoulou-Aletra H. 2009. Medicinal use of leeches in the texts of ancient Greek, Romanand early Byzantine writers. Internal Medicine Journal 39(9):624–627. DOI: 10.1111/j.1445-5994.2009.01965.x.

Phillips AJ, Siddall ME. 2009. Poly-paraphyly of Hirudinidae: Many lineages of medicinal leeches. BMC Evolutionary Biology 9(1):246. DOI: 10.1186/1471-2148-9-246.

Planas E, Fernández-Montraveta C, Ribera C. 2013. Molecular systematics of the wolf spider genus *Lycosa* (Araneae: Lycosidae) in the Western Mediterranean Basin. Molecular Phylogenetics and Evolution 67(2):414–428. DOI: 10.1016/j.ympev.2013.02.006.

Richterich P. 1998. Estimation of errors in "raw" DNA sequences: A validation study. Genome Research 8 (3):251–259. DOI: 10.1101/gr.8.3.251.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3):539–542. DOI: 10.1093/sysbio/sys029.

Sawyer RT. 1986. Leech biology and behaviour. Oxford: Oxford University Press.

Seurat LG. 1922. Faune des eaux continentales de la Berbérie. Bulletin de la Société d'Histoire Naturelle de l'Afrique du Nord 13:45–140.

Sket B. 1999. The freshwater macroinvertebrates of friuli-venezia giulia (northeastern Italy): Leeches (Hirudinea). Gortania – Atti Museo Friulano di Storia Naturale 21:119–132.

Sket B, Trontelj P. 2008. Global diversity of leeches (Hirudinea) in freshwater. Hydrobiologia 595(1):129–137. DOI: 10.1007/s10750-007-9010-8.

Tamura K, Stecher G, Kumar S, Battistuzzi FU. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7):3022–3027. DOI: 10.1093/molbev/msab120.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22):4673–4680. DOI: 10.1093/nar/22.22.4673.

Trontelj P, Utevsky SY. 2005. Celebrity with a neglected taxonomy: Molecular systematics of the medicinal leech (genus *Hirudo*). Molecular Phylogenetics and Evolution 34 (3):616–624. DOI: 10.1016/j.ympev.2004.10.012.

Trontelj P, Utevsky SY. 2012. Phylogeny and phylogeography of medicinal leeches (genus *Hirudo*): Fast dispersal and shallow genetic structure. Molecular Phylogenetics and Evolution 63 (2):475–485. DOI: 10.1016/j.ympev.2012.01.022.

Utevsky S, Zagmajster M, Atemasov A, Zinenko O, Utevska O, Utevsky A, Trontelj P. 2010. Distribution and status of medicinal leeches (genus *Hirudo*) in the Western Palaearctic: Anthropogenic, ecological, or historical effects? Aquatic Conservation: Marine & Freshwater Ecosystems 20 (2):198–210. DOI: 10.1002/aqc.1071.

Vecchioni L, Bellucci D, Novaga R, Faraone FP, Utevsky S, Marrone F. 2024. Further evidence of the southern Mediterranean medicinal leech *Hirudo verbana* (Annelida, Hirudinea) feeding on fish, with a review of the use of fish hosts by Hirudo spp. Limnetica 44(1):000–000. DOI: 10.23818/limn.44.07.

Wang H, Meng F-M, Jin S-J, Gao J-W, Tong X-R, Liu Z-C. 2022. A new species of medicinal leech in the genus *hirudo* linnaeus, 1758 (hirudiniformes, hirudinidae) from Tianjin City, China. ZooKeys 1095:83–96. DOI: 10.3897/zookeys.1095.74071.

Wells MD, Manktelow RT, Boyd JB, Bowen V. 1993. The medical leech: An old treatment revisited. Microsurgery 14 (3):183–186. DOI: 10.1002/micr.1920140309.

Whitaker IS, Rao J, Izadi D, Butler PE. 2004. *Hirudo medicinalis*: Ancient origins of leeches, and trends in the use of medicinal leeches throughout history. The British Journal of Oral & Maxillofacial Surgery 42(2):133–137. DOI: 10.1016/S0266-4356(03)00242-0.

Williams KM, Barkdull M, Fahmy M, Hekkala E, Siddall ME, Kvist S. 2020. Caught red handed: iDNA points to wild source for CITES-protected contraband leeches. European Journal of Wildlife Research 66(5):80. DOI: 10.1007/s10344-020-01419-5.