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New distributional data for the Mediterranean medicinal leech *Hirudo verbana* Carena, 1820 (Hirudinea, Hirudinidae) in Italy, with a note on its feeding on amphibians

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### **SUMMARY**

Scarce data are currently available about the distribution of the Mediterranean medicinal leech *Hirudo verbana* in Italy, and most of the known occurrence localities are based on records collected in the nineteenth and the first half of the twentieth century, which were not confirmed in the last decades, mostly due to a lack of surveys. Accordingly, the available knowledge on the distribution of the species is far from being updated and representative, although a significant negative trend of *H. verbana* throughout the country is supposed. The lack of sound distribution data is a significant shortfall, which hinders the implementation of the monitoring of the species as required by the Article 17 of the "Habitats Directive" on the species of Union concern. To provide recent, validated distributional data for the Mediterranean medicinal leech in Italy to be used as baseline data for further surveys and monitoring, we present herein a set of unpublished observations collected in the last decades in peninsular Italy, Sicily, and Sardinia. Moreover, we report observation of *H. verbana* feeding on amphibians, a feeding habit to date poorly documented for the Mediterranean medicinal leech. Based on both published and novel data, *H. verbana* is characterised by a large but fragmented distribution in Italy. Therefore, dedicated monitoring programs and the collection of validated occasional observations are highly desirable to get a clearer picture of the real distribution of the species.

### **INTRODUCTION**

The Mediterranean medicinal leech Hirudo verbana Carena, 1820 is the only Italian leech included in the European "Habitats Directive" (EU Directive 92/43/CEE), where it is listed in Annex 5 sub Hirudo medicinalis Linnaeus. 1758 (i.e., the European medicinal leech). In fact, different medicinal leech species were lumped under this binomen when the "Habitats Directive" came into force, until recent morphological and molecular evidence unequivocally proved that they are distinct species (Trontelj and Utevsky 2012; Saglam et al. 2016). Based on available evidence, the native distribution of Hirudo verbana ranges from Spain to Turkey, including eastern European steppe landscapes, the Caucasus and Uzbekistan, and the populations of the species are subdivided into three major clades, i.e.,

Iberian, Western and Eastern ones, which can be explained by distinct postglacial colonisations from separate refugia (Trontelj and Utevsky 2012; Arias et al. 2021).

In Italy, *Hirudo verbana* has a scattered distribution, and only few records of the species were reported in the last decades, often *sub Hirudo medicinalis* (Bazzanti et al. 1996; Fontaneto et al. 1999; Minelli 2006; Utevsky et al. 2010; Sorgi et al. 2011; Lapini et al. 2013; Osella and Pannunzio 2013; Marrone and Canale 2019). The vast majority of the known occurrence localities derive from observations carried out in the nineteenth and the first half of the twentieth century (see references in Minelli 1979, 2006). The scarcity of recent records and the lack of confirmation for most of the historical occurrence localities is generally ascribed to "a significantly (or rather,

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dramatically) negative trend" of the species throughout the country (Minelli 2006), although the lack of targeted surveys of *Hirudo verbana* in Italy prevented obtaining an adequate picture of its current distribution, status, and trend.

For this reason, we report herein a collection of unpublished occurrence data for *Hirudo verbana* in Italy, with the aim of providing reliable and geo-referenced baseline data to be used for future studies and as a reference for the monitoring of the species under the provisions of Article 17 of the "Habitats Directive". Moreover, the observation of feeding behaviour on amphibians is here reported and documented.

### MATERIALS AND METHODS

The occurrence of the species was occasionally registered in the frame of sampling activities

carried out in lentic and lotic water bodies across the Italian peninsula, Sardinia, and Sicily (Fig. 1, Table 1). Moreover, the two records available on the social network iNaturalist (www.inaturalist.org) at 10 March 2021 were considered reliable and were included in the dataset. Observed *Hirudo verbana* individuals were identified and photographed *in situ*. The identification of the collected leeches was based on the most updated available identification keys (Neubert and Nesemann 1999; Trontelj and Utevsky 2005; Kutschera 2012).

The new localities of the species were plotted on a map including also its published localities based on Bazzanti et al. (1996), Fontaneto et al. (1999), Minelli (2006), Utvesky et al. (2010), Sorgi et al. (2011), Lapini et al. (2013), Osella and Pannunzio (2013) and Marrone and Canale (2019).

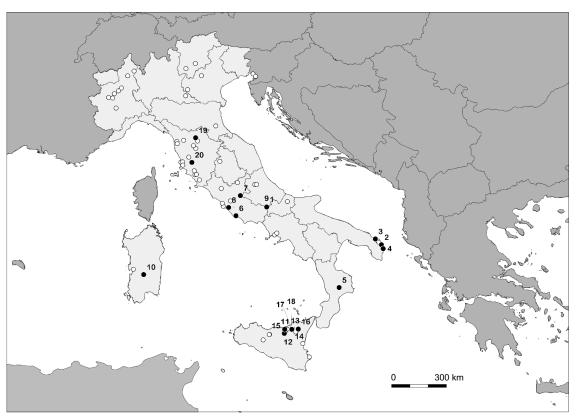


Figure 1. Known and novel locations of occurrence of *Hirudo verbana* in Italy. Black dots represent novel sites reported in this paper, with numbers referring to Table 1 rows. White dots refer to published records (Bazzanti et al. 1996; Fontaneto et al. 1999; Minelli 2006; Utvesky et al. 2010; Sorgi et al. 2011; Lapini et al. 2013; Osella and Pannunzio 2013; Marrone and Canale 2019). Some dots may overlap due to the map scale.

Table 1. List of the novel occurrence localities of *Hirudo verbana* in Italy. Geographical decimal coordinates are reported according to the WGS84 datum.

Site	Region	Locality	Site name	Latitude N	Longitude E	Elevation (m a.s.l.)	Year	Source
1	Abruzzo	Alfedena (AQ)	Stagno di Campitelli	41.69888	13.98611	1448	2013- 2018	A. Iannarelli
2	Apulia	Melendugno (LE)	Canale Brunese	40.26583	18.42775	12	2001	D. Ferreri
3	Apulia	Torre Chianca (LE)	Canale di bonifica	40.45944	18.21389	1	2004	G. Alfonso
4	Apulia	Otranto (LE)	Fiume Idro	40.14252	18.47915	5	2009	D. Ferreri
5	Calabria	Taverna (CS)	Tirivolo, valle del Torrente Crocchio	39.10002	16.62170	1556	2012- 2014	P. Brandmayr & A. Mazzei
6	Latium	Nettuno (RM)	Stagno nel Bosco di Foglino	41.47195	12.72706	30	2005	F. Stoch
7	Latium	Percile (RM)	Lagustelli di Percile	42.07877	12.94201	720	2019	V. Moretti
8	Latium	Rome (RM)	Stagno a Decima Malafede	41.73284	12.44584	82	2021	R. Novaga
9	Molise	Pizzone (IS)	Pozza nella Valle Fiorita	41.69143	13.98444	1395	2018	A. Iannarelli
10	Sardinia	Gesturi (SU)	Paule Bartili, Giara di Gesturi	39.745083	8.996662	582	2008, 2010	F. Marrone & F. Stoch
11	Sicily	Cesarò (ME)	Biviere di Cesarò	37.95324	14.71469	1280	1980	P. Brandmayr
12	Sicily	Nicosia (EN)	Laghetto Campanito	37.83187	14.39072	1258	1985	R. Gerecke
13	Sicily	Cesarò (ME)	Pozza presso il Lago Maulazzo	37.94318	14.67469	1430	2016	E. Schifani & M. Pecoraro
14	Sicily	Cesarò (ME)	Stagno di Sollazzo Verde	37.95061	14.68527	1397	2016	S. Costa
15	Sicily	Caronia (ME)	Lago Zilio	37.95288	14.41408	1072	2017	G. Farina
16	Sicily	Randazzo (CT)	Stagno di c.da Baiardo	37.93694	14.93388	1099	2018	R. Barbagallo
17	Sicily	Alcara Li Fusi (ME)	Stagno di p.lla Maulazzo	37.93578	14.67370	1480	2021	F. Marrone & L Vecchioni
18	Sicily	Cesarò (ME)	Stagno Pappanu	37.94158	14.68367	1559	2021	F. Marrone & L Vecchioni
19	Tuscany	La Chiusa (FI)	Torrente Marina	43.88306	11.17917	85	2010	G. Mazza
20	Tuscany	Montieri (GR)	Laghetto di Montieri	43.13791	10.99550	630	2012	G. Bruni, M. Menchetti & E. Mori

#### RESULTS AND DISCUSSION

Hirudo verbana was found in 20 natural and semi-natural water bodies where the occurrence of the species was previously unknown. Moreover, H. verbana was observed in the pond named Rekikenca (Colludrozza, Trieste), where the species was considered locally extinct by Sket (1999, sub Hirudo medicinalis). The occurrence of Hirudo verbana in two localities in the central Apennines (i.e., sites 1 and 9, Table 1) and in one locality in Calabria (site 5, Table 1) are particularly noteworthy since they are located far away from any published record of the species (Fig. 1). In addition to these validated observations, further occurrence localities of *H. verbana* in Calabria ("Monte Caloria", WWF Calabria 2015) and in

Lombardy ("Valli del Mincio", Rigoni 2010) are reported *sub H. medicinalis*; however, considering that the real presence and identity of the reported species could not be verified, these sites are not included in Table 1.

In accordance with the known broad ecological adaptability of the species, *H. verbana* was observed in different types of water bodies, from temporary ponds to permanent lakes, lateral pools of small rivers, and artificial canals, located at altitudes ranging from 1 to 1,559 m a.s.l. in different microclimates and habitats. The apparent absence of well-defined ecological preferences of the species prevents the application of species distribution statistical modelling for defining the areas of its potential presence.

Only a few of the water bodies which are apparently suitable for the presence of the species are occupied by *H. verbana*; this is in agreement with the observation that sporadic, non-deterministic occurrences appear to be characteristic to the species of the genus *Hirudo* (Utevsky et al. 2010). Lacking dedicated surveys aimed at the census of Hirudinea in Italy, it is utmost likely that the currently known distribution of the species is still largely incomplete and far from being exhaustive. In this situation, the collection and publication of validated occasional observations of *H. verbana* is a useful approach for trying to get a map of the real distribution of the species.

Hirudo verbana was observed feeding on amphibians both in Sicily (site 14, Table 1), where it was observed attacking a tadpole of Hyla intermedia Boulenger, 1882, and in Abruzzo (site 1, Table 1), where it was observed feeding on H. intermedia tadpoles, and adult individuals of Lissotriton vulgaris meridionalis (Boulenger, 1882) and Triturus carnifex Laurenti, 1768 at several dates (Fig. 2). Although other *Hirudo* species are known to regularly feed on amphibians (e.g., Neubert and Nesemann 1999; Merilä and Sterner 2002), such observations are noteworthy for H. verbana, which is currently considered to use ungulates as the main food resource (Kovalenko and Utevsky 2015), and its habit to feed on amphibians was to date documented only on Bufo bufo (Linnaeus, 1758) (Fontaneto et al. 1999). This feeding behaviour suggests that the presence of ungulates is not a necessary prerequisite for habitat selection and species distribution.



Figure 2. A: *H. verbana* feeding on *Hyla intermedia* (Site 14, August 25<sup>th</sup> 2016, photo by S. Costa); B: *H. verbana* feeding on *Hyla intermedia* (site 1, August 4<sup>th</sup> 2013, photo by A. Iannarelli); C: *H. verbana* feeding on *Lissotriton vulgaris* (Site 1, April 24<sup>th</sup> 2018, photo by A. Iannarelli); D: *H. verbana* feeding on *Triturus carnifex* (Site 1, May 19<sup>th</sup> 2013, photo by A. Iannarelli).

Based on currently available data, H. verbana is characterised by a large but fragmented distribution area in Italy (Fig. 1). Moreover, the occurrence of native or introduced populations of H. medicinalis in Italy cannot be ruled out based on the scant literature. An assessment of the identity of the extant populations from the sites reported in the previous literature sub H. medicinalis is highly recommended. The scattered distribution in isolated water bodies, if confirmed by further evidence, might likely negatively affect the long-term persistence of Italian medicinal leeches, exposing them to the risk of local extinction. The implementation of a monitoring plan based on standardized sampling surveys and the collection of occasional, but validated, observations is highly desirable, also for the purpose of fulfilling the requirements of the Article 17 of the "Habitats Directive" on the species of Union concern. Eventually, as already suggested by Utevsky et al. (2010), the assessment of the species and its inclusion in the IUCN Red List of Threatened Species (https://www.iucnredlist.org/) is worthwhile, considering that only Hirudo medicinalis is included up to now as NT - Nearly Threatened (Utevsky et al. 2014).

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