

The European medicinal leech *Hirudo medicinalis* L.: Morphology and occurrence of an endangered species

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Abstract

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Although the European medicinal leech (*Hirudo medicinalis* L. 1758) is one of the best-known members of the Hirudinea due to its use in phlebotomy, this species has been confused with the Mediterranean taxon *H. verbana* Carena 1820. Here we describe the morphology of adult and juvenile *H. medicinalis* and document its genetic distance to *H. verbana*, using newly acquired mitochondrial DNA-sequence (cytochrome *c* oxidase subunit I, CO-I)-data from specimens collected in Germany. Our CO-I analysis shows that *H. medicinalis* and *H. verbana* differ by 9.4 %. Hence, the original *Hirudo*-population diverged ca. 10 million years ago so that today two geographically separated biospecies exist that co-occur in only a few natural habitats. We analyzed the behaviour of adult *H. medicinalis*, but could not find differences with respect to its sister taxon *H. verbana*. Finally, we summarize the occurrence of *H. medicinalis* in Central Europe and conclude that this once widely distributed freshwater species largely disappeared in many countries. We suggest that the loss of natural freshwater ecosystems, with flat, warm banks, and amphibians (frogs, newts and toads) as preferred host organisms for the juveniles, are largely responsible for the decline of *H. medicinalis* in Northern Europe.

Introduction

Among the currently ca. 14 000 accepted species of Annelida (segmented worms) found worldwide in freshwater, marine and terrestrial ecosystems, Linnaeus (1758) first described the two most well-known forms: the earthworm (*Lumbricus terrestris*) and the medicinal leech (*Hirudo medicinalis*). However, this Linnaean system of the “classis Vermes”, with the three orders “Intestina, Mollusca and Testacea”, was soon replaced by Lamarck’s more detailed account of classification of the “lower animals” (Vinarski 2014). In Vol. 5 of a series of monographs on the systematics of invertebrates, Lamarck (1818) introduced the class Hirudinea (leeches), and listed two European species, *Hirudo medicinalis*, and “*H. sanguisorba*”. While the first taxon, the type species of the class Hirudinea Lamarck, 1818 (sangue médicinale, i.e., the medicinal leech), is still accepted today as a valid species, Lamarck’s “*H. sanguisorba*” remains an enigma.

During subsequent decades, notably when the use of leeches in phlebotomy (bloodletting) became very popular throughout Europe (ca. 1850), numerous “varieties” of “*H. medicinalis*” were distinguished by naturalists as well as practitioners (Herter 1936, 1937).

In his classic monograph on leeches, Mann (1962) adopted this interpretation and wrote that *H. medicinalis* must be regarded as a highly variable species. Accordingly, Herter (1968) and Sawyer (1986), in their influential books, argued that all colour variants of European medicinal leeches should be assigned to the taxon *H. medicinalis* Linnaeus, 1758 (syn. *H. officinalis* Savigny, 1822; see also Hechtel and Sawyer 2002). However, based on detailed analyses of pigment patterns and DNA-sequences, it was documented that *H. officinalis* is not a “colour variant of Linnaeus’ type species”, but a separate taxon, the Mediterranean medicinal leech *H. verbana* Carena, 1820 (Nesemann and Neubert 1999; De Salle et al. 2005; Kutschera 2004, 2006, 2007, 2012a, 2012b; Trontelj et al. 2004; Siddall et al. 2007; Phillips and Siddall 2009; Elliott

and Kutschera 2011; Elliott and Dobson 2014). Utevsky and Trontelj (2005) provided a key to all known European species in the genus *Hirudo*, and Kutschera (2012a, 2012 b) summarized their geographical distribution.

In the present article, we describe the morphology of juvenile and adult *H. medicinalis*-individuals, add information on its evolutionary distance to its sister taxon *H. verbana*, and summarize observations on the behaviour, ecology and distribution of this endangered species.

Materials and methods

Adult and juvenile European medicinal leeches (*H. medicinalis*) (plus cocoons) were obtained from undisturbed habitats of eastern Germany (Elliott and Kutschera 2011), and specimens of Mediterranean medicinal leeches (*H. verbana*) were purchased from a commercial supplier (Sudak, Tr-59560 Murefte Tekirdag, Turkey) (Kutschera and Roth 2005). The leeches were kept in aqua-terraria (90 x 40 x 60 cm, depth of the pond water ca. 10 cm; temperature 22 to 26 °C), and observed/photographed alive. Specimens of *H. medicinalis* were killed by adding 80 % ethanol to the water, so that the animals were preserved in their non-contracted, natural shape, and photographed. Extraction of DNA from part of the posterior sucker, sequencing of a fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (CO-I), and phylogenetic analyses, based on newly acquired (and deposited) GenBank-data were performed as described (Kutschera et al. 2007, 2013; Wirschansky and Shain 2010).

Results

Morphology of adult *H. medicinalis*-individuals

Leeches are animals with an organization akin to that of earthworms, but having certain modifications associated with a predatory or parasitic mode of life. The limitation of the number of body segments facilitates a greater degree of agility than would be the case if the body was as long as that of most earthworms. The segments are each subdivided into a number of annuli, five in the Hirudinidae. There is some disagreement about the relationship between annulation and segmentation (Mann 1962). Externally, the annuli look much alike, and there is little indication of segmentation. Perhaps the best guide is the pattern of colouring, which often repeats itself once per segment. For example, a distinctive pattern separates *H. medicinalis* from its sister taxon *H. verbana* (Figs 1, 2). On the middle annulus of each segment are sensory papillae (Pap in Fig. 3A). These may be prominent, and are often marked by spots of light pigment. Papillae may also be present on other annuli of a segment. On the first few segments of the body, some of the sensory papillae are replaced by black-pigmented eye spots, five pairs of eyes arranged in a crescent in *Hirudo medicinalis* (four eyes are marked in Fig. 3B).



Figure 1. Photograph of living adult specimens of the European medicinal leech (*H. medicinalis* Linnaeus 1758) and the Mediterranean medicinal leech (*H. verbana* Carena 1820). The leeches, maintained in pond water, are depicted in dorsal view, with their disk-shaped posterior sucker attached to a petri dish.

The size of the suckers relative to the body varies according to the mode of life of the leech species and, in *H. medicinalis*, the anterior sucker is quite small. The buccal cavity is lined by muscular ridges surmounted by cuticular teeth, and the mouth is a wide aperture occupying the whole of the anterior sucker (Fig. 3C). Following the pharynx is a region of the alimentary canal, the crop, which is dilated for the storage of food. In the sanguivorous *H. medicinalis*, it is drawn out into lateral arms referred to as diverticula.

The clitellum is situated towards the anterior of the body (Fig. 4A). The male reproductive aperture is median and unpaired. There are two internal ducts leading to it but these unite to form a single genital atrium with one external gonopore and a ‘tube-like’ male copulatory organ (Fig. 4B). The female pore is likewise median and unpaired, and is posterior to the male pore.

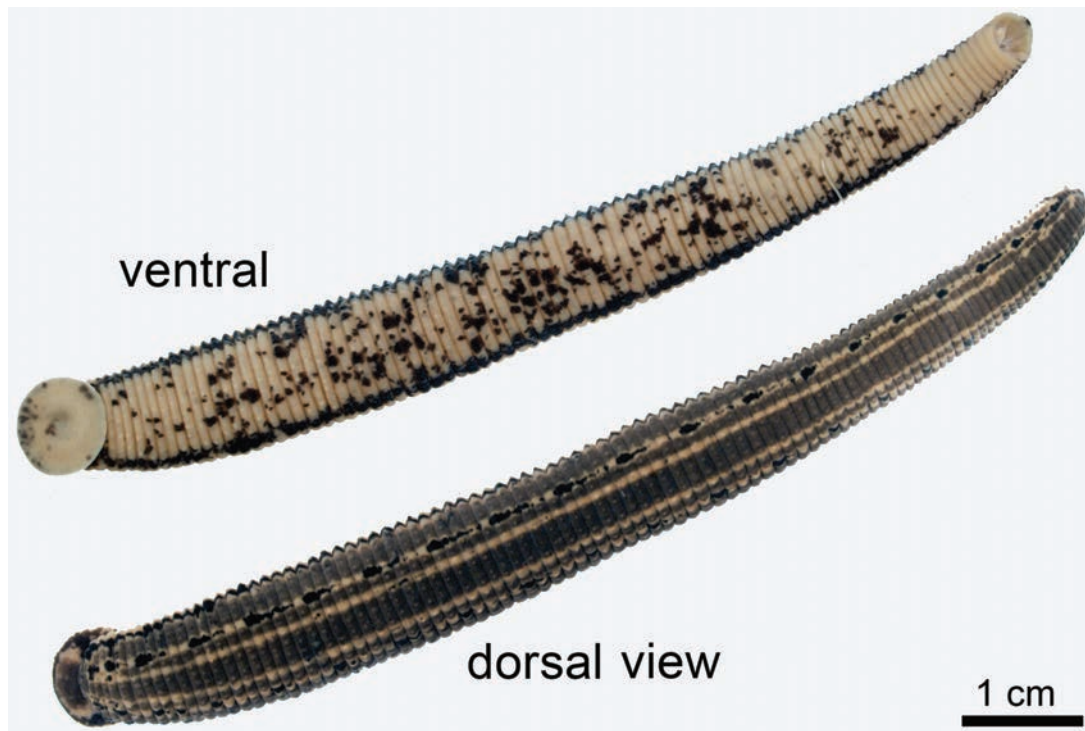


Figure 2. Dorsal and ventral views of a representative, alcohol-preserved specimen of *H. medicinalis* collected in eastern Germany. The species-specific pigment patterns are visible.

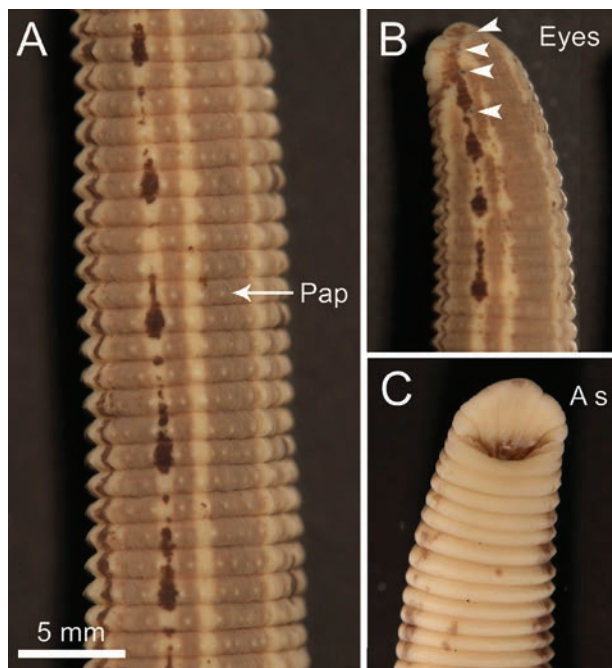


Figure 3. Details of the midbody, in dorsal view (A), the head (B) and the anterior sucker (ventral view) (C) of an adult, alcohol-preserved *H. medicinalis*. As = anterior sucker.

Cocoons and juvenile *H. medicinalis*

Mature medicinal leeches leave the water to deposit their cocoons in a moist place just above the water line on the shore or bank. The spongy cocoons (Fig. 5A) are laid

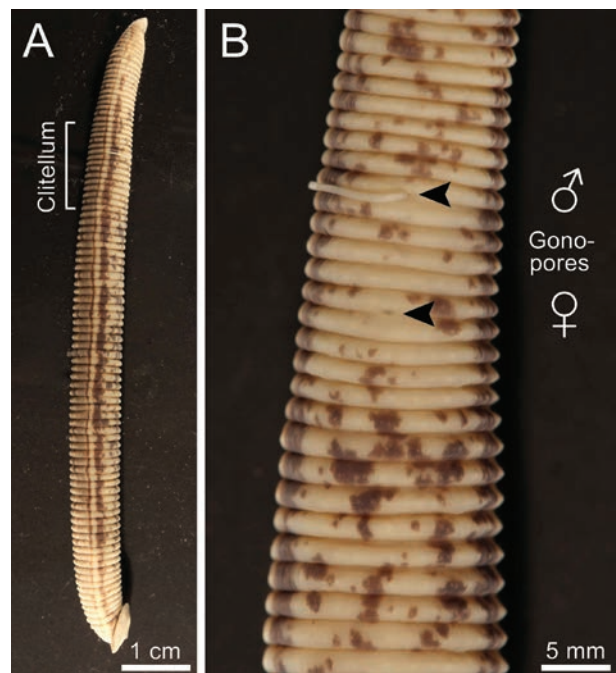


Figure 4. Lateral view of an adult, alcohol-preserved *H. medicinalis* (A) and position of the male (♂) and female (♀) gonopores on the ventral side (B), with the tube-like male copulatory organ outside of the body.

chiefly in July and August. Over one to 12 days, each mature leech will lay 1 to 8 cocoons with usually 12 to 16 eggs per cocoon; sometimes more, but with some infer-

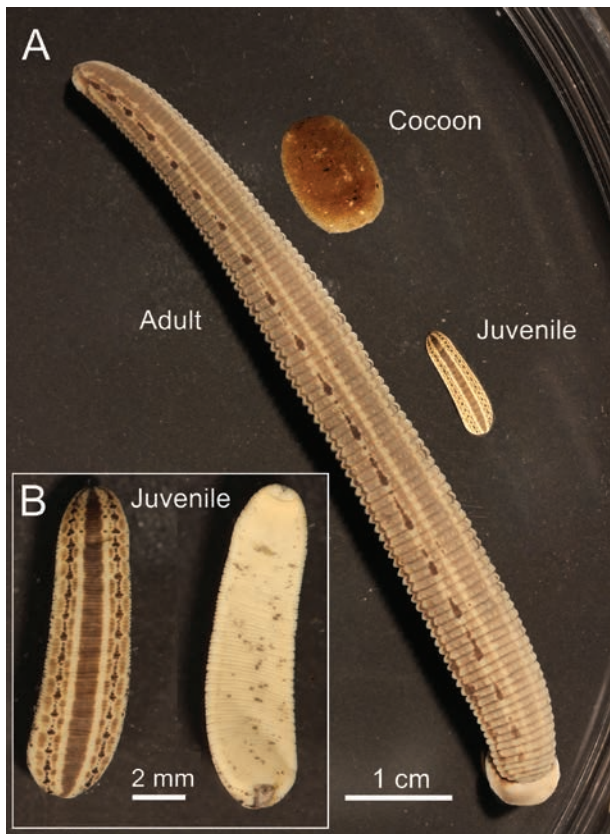


Figure 5. Adult and juvenile alcohol-preserved *H. medicinalis*, and a cocoon in dorsal view (A). The *Inset* shows the characteristic pigment pattern of a newly hatched individual in dorsal and ventral view, respectively (B).

tile eggs. In the laboratory, each adult laid 1 to 7 cocoons with 3 to 30 eggs per cocoon, and produced 2 broods per year under optimum conditions. Hatching time varied from 4 to 10 weeks, depending upon the temperature, and the live mass of each newly-hatched leeches (length: 8–12 mm) varied from 12 to 60 mg.

The markings of the juveniles are very similar to those of the adults except there is less pigment on the ventral surface (Figs 5, 6). Hatchlings can survive for up to 100 days without feeding, but fed leeches in the laboratory attained a live mass of 0.5 to 0.6 g at the end of their first year, about 1.4 g in their second, and about 2.4 g in their third year. Similar results were obtained for *H. verbana* (Kutschera and Roth 2006). Although there is a paucity of field information, it is generally agreed that *H. medicinalis* and *H. verbana* take at least two years to reach the breeding stage in the wild, and slow-growing leeches may not breed until they are three or four years old.

Behaviour of *H. medicinalis* vs. *H. verbana* and hyperparasitism

Living, adult individuals of *H. medicinalis* and its sister species *H. verbana* were maintained in aqua-terraria. Despite the fact that the species were clearly distinguishable based on their pigment patterns on both the dorsal and ventral sides of their body (Fig. 1), qualitative observation

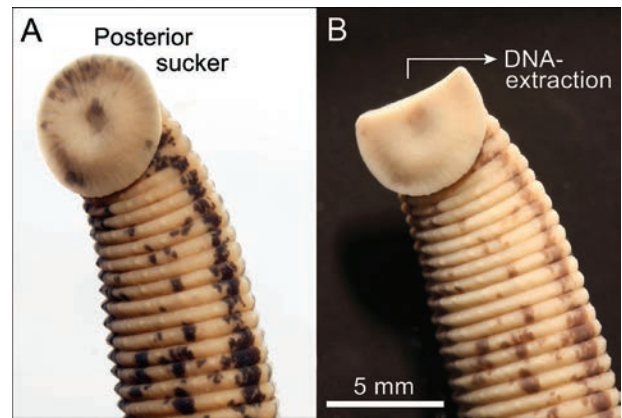


Figure 6. Intact (A) and fragmented (B) posterior sucker of an adult alcohol-preserved *H. medicinalis*. The disk-shaped sucker is largely composed of muscle tissue containing numerous mitochondria. DNA-extractions for mt-sequence analysis (fragments of the gene CO-I) were performed from this part of the body that is not contaminated with the gut content of the blood-sucking annelid.

of their behavioural patterns revealed no differences. For a large part of the year when water temperatures are low, medicinal leeches are quiescent and remain buried in the mud or under submerged objects at the edge of the pond. As water temperature increases, the leeches become very responsive to water disturbance caused by a potential host, and swim towards the source of blood. Laboratory experiments showed that 86 % and 95 % of unfed leeches responded to low-amplitude surface waves (about 1 mm high) by swimming, whilst only ca. 60 % of fed leeches displayed a reaction. The neurophysiology of this detection of water motion was described in detail by Friesen (1981).

Laboratory experiments have also shown that when a medicinal leech is near a mammalian host, such as the skin of a human, it uses heat detection, the optimum response occurring at 33 to 40 °C (Dickinson and Lent 1984), and also chemosensory stimuli (Elliott 1986), both receptors being located in the anterior end of the leech (Fig. 3B). The leech explores the outer cell layer of the host for a suitable feeding site, then pierces the skin with its three jaws armed with numerous sharp teeth, and finally sucks the blood of its host. We also observed that, in the wild and in the laboratory, *H. medicinalis* suck blood from amphibians, such as the edible frog (Fig. 7).

However, other leech species will sometimes feed on *H. medicinalis*. Young *Glossiphonia complanata* that were co-cultivated with medicinal leeches frequently obtain their first meal by feeding on the body of *H. medicinalis*. In a quantitative study in a tarn (= pond) in Northwest England, *H. medicinalis* were found to be carrying all sizes of *Helobdella stagnalis* that were feeding on the host. The proboscis was inserted deep into the body wall of the host and the anterior portion of the body contracted regularly as fluid was extracted from the host, i.e., hyperparasitism was documented unequivocally. *H. stagnalis* did not kill its host or produce any obvious reactions. Similar observations were reported for *H. verbana* (Kutschera et al. 2010).



Figure 7. Two adult, free-living *H. medicinalis* in the process of sucking blood from an edible frog (*Rana esculenta* L.). The amphibians usually survive these attacks (adapted from Manzke and Winkler 2012).

Phylogenetic analysis, divergence time and geographic distribution

In order to verify the taxonomic status of *H. medicinalis* from Germany (Figs 1 to 7), the DNA-barcoding approach was employed (De Salle et al. 2005). About half of the posterior sucker of ethanol-fixed individuals was excised and used for DNA-extractions (Fig. 6A, B). We obtained 625 base pair (bp)-sequences of the mitochondrial gene CO-I and compared our newly acquired data with those deposited in GenBank, a comprehensive database that contains publicly available nucleotide sequences (Benson et al. 2013). Our “German” sequences were found to be 100 % identical with two deposited CO-I-data for *H. medicinalis*, GenBank-Nos. AY786458 and HQ333519, obtained from individuals collected in France and Sweden, respectively (the latter corresponds to the Swedish Neotype of *H. medicinalis*, SMNH Type-8027; Kvist et al. 2010). Hence, populations of

H. medicinalis in these three European countries display identical genetic signatures with respect to the CO-I-barcoding technique, and hence clearly represent one widely distributed biospecies.

Based on CO-I-sequences acquired in our laboratory for *H. verbana* and other leech species (Kutschera 2010, 2011; Kutschera et al. 2007, 2013), supplemented by additional GenBank CO-I-data, the results shown in Table 1 were obtained. The type species *H. medicinalis* and its sister taxon *H. verbana* differ by ca. 9.4 % with respect to CO-I-sequences. This distance indicates that *H. medicinalis* and *H. verbana* diverged ca. 10 million years ago (Wirchansky and Shain 2010), presumably via geographic isolation of one ancestral parent species, so that today two biospecies occur that occupy the Northern and Southern parts of Europe, respectively, with little overlap of the populations (Fig. 8A, B). The genetic distance of all the other leech species listed in Table 1 is ca. 17 to 26 %, documenting much larger divergence times.

Table 1. Genetic distance between the type species of the Hirudinea, *Hirudo medicinalis* L. 1758, and other leeches, based on mitochondrial DNA-sequence data. The GenBank Accession Numbers for the mt-gene cytochrome *c* oxidase subunit I (CO-I) are added. AF = Africa, AS = Asia, EU = Europe, US = United States.

Taxon	Locality	GenBank Acc.-No. CO-I	Identity (%)
<i>Hirudo medicinalis</i>	Sweden, EU	HQ333519	100
<i>Hirudo verbana</i>	Turkey, EU/AS	EF125043	90.6
<i>Haemopsis sanguisuga</i>	Sweden, EU	AF462021	83.3
<i>Hirudinaria mallinensis</i>	Malaysia, AS	AY425449	82.4
<i>Erpobdella octoculata</i>	Germany, EU	AF003274	75.0
<i>Trocheta intermedia</i>	Germany, EU	DQ009669	74.1
<i>Glossiphonia complanata</i>	Germany, EU	AF003277	77.7
<i>Helobdella californica</i>	California/US	HQ686307	73.5
<i>Malagabdella fallax</i>	Madagascar, AF	EF125044	79.5
<i>Xerobdella lecomtei</i>	Austria, EU	EF125040	76.0

Discussion

The historical use, ecology, genetics and conservation of medicinal leeches was recently summarized (Elliott and Kutschera 2011; Elliott and Dobson 2014), and the present discussion is based in part on these extensive reviews. As noted in the Introduction, *Hirudo medicinalis* was once abundant in Northern Europe, from Ireland in the west to the Ural Mountains in the east, and from Southern

Scandinavia to the countries bordering the Mediterranean (Elliott and Tullett 1984, 1986, 1992), where the Southern species *H. verbana* occurs (Fig. 8A, B). It is now rare throughout Western Europe, and endangered in many countries (Wells and Coombes 1987; Utevsky et al. 2008, 2010; Kovalenko and Utevsky 2012; Petrauskienė et al. 2011; Westendorff et al. 2008; Sawyer 2013a, 2013b; Trontelj and Utevsky 2005, 2012).

Large numbers of *H. medicinalis* were obtained from the wild in the 18th and early 19th centuries, and towards the end of this period, they were already scarce in many countries. This demand for medicinal leeches was not restricted to Europe. *Hirudo medicinalis* does not occur naturally in North America, and large numbers were imported from Europe into the United States in the 18th and 19th centuries. Several attempts were made to rear this species in the US, without positive results (Elliott and Kutschera 2011). As medicinal leeches became more difficult to find in the 19th century, the indigenous supply was supplemented by importations of other species of medicinal leeches from outside Western Europe. There was also the development of ‘leech farms’, especially in France and Germany. As late as 1890, a leech farm near Hildesheim in Germany was breeding between three and four million individuals per year (Herter 1968). Leech farms still exist today but, unfortunately, they often rely on imported leeches from Southeastern Europe and Turkey. These imports are often not *H. medicinalis*, but the closely-related

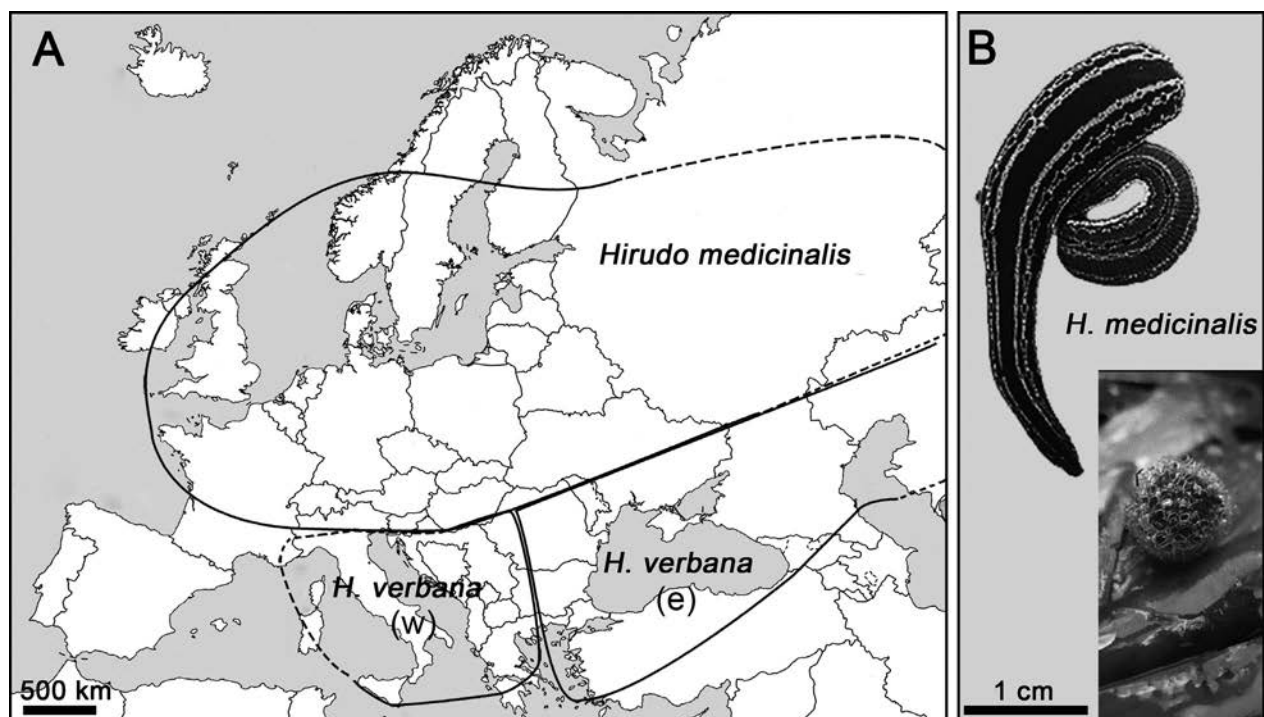


Figure 8. Geographical distribution of *Hirudo medicinalis* and *H. verbana*, based on data published in 2012 (A). In the species *H. verbana*, a western (w) and an eastern (e) phylogroup has been identified. Occurrence of medicinal leeches in the nest of aquatic birds (B). The photograph shows adult, living specimens of *H. medicinalis* (with cocoon, see Inset) collected from a nest of a water bird (western marsh harrier, *Circus aeruginosus*) in Poland (adapted from Kovalenko and Utevsky 2012 [A] and Buczyński et al. 2014 [B], respectively).

species, *H. verbana*, which has been confused with the ‘true’ medicinal leech (Michalsen and Roth 2006).

H. verbana was first described from Lago Maggiore in Northeast Italy (Latin: *Lacus Verbanus*) by Carena (1820) and later regarded as a sub-species of the European medicinal leech (*H. medicinalis* ssp. *officinalis*). Living, adult *H. medicinalis* and *H. verbana* (as well as the juveniles) have very distinct markings (Figs 1, 5). These two closely related leech species, which diverged ca. 10 million years ago from a common ancestor, were confused or both labelled as “*Hirudo medicinalis*”, until a close examination of their morphology, combined with breeding studies and DNA-sequencing experiments, yielded unequivocal proof that they are distinct, reproductively isolated taxa (Nesemann and Neubert 1999; Trontelj et al. 2004; De Salle et al. 2005; Kutschera 2004; 2006, 2007, 2012a, 2012b; Siddall et al. 2007; Phillips and Siddall 2009).

Earlier reviews of the literature on the ecology of *Hirudo medicinalis* showed that there was surprisingly little quantitative information on medicinal leeches in the wild and most of the numerical values were from laboratory studies (Mann 1962; Herter 1968; Elliott and Mann 1979; Sawyer 1986; Petrauskienė et al. 2011). Representative data, compiled from Davies and McLoughlin (1996), are summarized in Table 2. The typical natural habitat is a eutrophic pond with a muddy substratum, littoral vegetation, and a high summer temperature. It should also be a breeding site for amphibians (frogs, toads and newts). Although *H. medicinalis* and *H. verbana* are often reported as feeding almost exclusively on the blood of mammals (cattle, horses, deer, humans), they will also suck the blood of fish, water birds, and especially amphibians, both the adults and their larvae. Hoffman (1960) observed adult leeches feeding on toads (*Bufo bufo* L.) in April when the latter returned to ponds to breed. The leeches attacked both male and female toads, often when the male was grasping the female in the ‘nuptial embrace’. Large numbers of toads died from loss of blood and their corpses provided food for another leech species, *Haemopsis sanguisuga*. Tadpoles as well as juvenile newts are especially important for young medicinal leeches that are unable to pierce mammalian skin for the first two feedings. We corroborated Hoffman’s (1960) findings that were extended by Benecke (2009), Manzke and Winkler (2012) and Winkler and Manzke (2014) (Fig. 7). These authors provided, together with Jueg (2009), evidence for the occurrence of as yet undiscovered relict-populations of *H. medicinalis* in Eastern Germany.

Six decades ago, laboratory studies showed that the preferred temperature of *H. medicinalis* in a gradient of 7 to 43 °C was 21 °C (Kaiser 1954), a value midway between the 50 % and 90 % active leeches, as documented in a more recent study (Elliott 2008). Optimum temperature ranges for growth (22 to 25 °C) and breeding (25.5 to 27.5 °C) in the laboratory were similar to predicted values for maximum activity in the field. At 39 to 43.5 °C, the upper lethal range is remarkably high for this species

Table 2. Life history variables and reproductive success (i.e., number of offspring per individual and life time) of *H. medicinalis*, cultivated under sub-optimal laboratory conditions (20 °C). The animals were subsisted on mammalian (bovine) blood (n = 30) (adapted from Davies and McLoughlin 1996).

Parameter	Range	Mean (\pm SE)
Time (years) from hatching to death	1.3–2.3	2 \pm 0.1
Cocoons produced/individual	2–41	12 \pm 5
Hatchlings/cocoon	0–14	4 \pm 1
Offspring produced/individual	13–97	45 \pm 13

(Kaiser 1954). These high temperature requirements have important implications for the survival of *H. medicinalis* in the wild: the leeches were unable to reproduce and survive in many water bodies simply because of the low water temperatures (Elliott and Tullett 1986).

A number of explanations have been proposed for the loss of many populations of *H. medicinalis* in Northern Europe, and these should all be considered in combination. Extensive over-collecting for blood-letting in the nineteenth century is frequently blamed, but used leeches were regularly discarded into the nearest pond or stream and thus may have enabled the survival of this species in the countryside. Contemporary collecting for experimental biology, medical use and pharmaceutical needs is probably a serious threat because the leeches are destroyed, often in large numbers (Shain 2009). Although leech farms offer an obvious solution, this only works if the commercial suppliers actually rear leeches, rather than importing them and thereby reducing populations in the wild (Michalsen and Roth 2006).

A reduction in the availability of suitable vertebrate hosts is another possible reason for the decline in countries where troughs are now used instead of ponds for the watering of cattle and horses. Changes in land use not only caused the loss of ponds but also isolation of the remaining freshwater ecosystems, even to wild animals such as deer, and this may have contributed to a reduction in blood meals from this source. However, there are still many parts of Europe where wild animals such as deer are plentiful, and therefore the almost complete absence of *H. medicinalis* in these areas is not due to a lack of mammalian hosts.

Davies and McLoughlin (1996) proposed the plausible hypothesis that the declining abundance of field populations of the European medicinal leech could be the result of lower available energy for growth, reflecting leeches now feeding predominantly on amphibian blood of lower energetic value than mammalian blood. This conclusion was supported by slow-growing wild populations of leeches from Dungeness, UK. A serological test was positive for 128 blood meals and showed that most leeches were feeding on amphibian blood with smaller numbers feeding on fish and birds, and only one leech sucking mammalian blood (Wilkin and Scofield 1990, 1991a, 1991b). In a Lake District tarn, only the larger mature leeches (>3.5 g) had fed on mammalian blood, and the proportion of ma-

ture leeches feeding on mammals varied from 19 to 26 % among years (Elliott 2008). The most important sources of blood for all leeches in the tarn were probably amphibians, such as newts, frogs, toads and their tadpoles (Fig. 7). Therefore, the slow growth of the leeches could be partially caused by the scarcity of mammalian blood in their diet. Leeches were observed feeding on horses that had waded into the tarn. They never fed to satiation, as seen in the laboratory when offered bovine blood in a sausage skin. Soon after a horse left the water, the leech detached and rapidly crawled back into the water. In wild populations, satiated leeches were never found and it was concluded that the annelids were feeding a little and often, rather than to satiation. A similar conclusion was reached for the population at Dungeness (Wilkin and Scofield 1990, 1991a, 1991b).

Water temperature will also affect the growth of *H. medicinalis*. Fast-growing leeches that attained maturity after only 289 days were kept at a constant 20 °C (Davies and McLoughlin 1996) (Table 2). This is just above the threshold temperature of 19 °C for most leeches to be swimming and searching for a host in a Lake District tarn (Elliott and Tullett 1986). Water temperature in the tarn exceeded this value on only 100 to 120 days from April to September and was thus a limiting factor for feeding and growth. The high temperature requirements of medicinal leeches (*H. medicinalis* and *H. verbana*) impose limitations on their distribution and occurrence. Therefore, the absence of these species from many water bodies may be due partially to the relatively high temperatures required for swimming activity, feeding, growth and breeding, as well as the scarcity of mammalian hosts. It should be noted that there has been a loss of many small, shallow ponds throughout Western Europe, and these are often the ideal habitat for medicinal leeches, especially if they contain amphibian species and water birds. In South-eastern Poland, Buczyński et al. (2014) found adult, breeding *H. medicinalis*-individuals, inclusive of cocoons, in the nests of water birds (Fig. 8B). This finding is in accordance with earlier observations of Herter (1936), who suggested that medicinal leeches may feed on nestlings of aquatic birds, notably when they fall into the water and attract hungry leeches (Young et al. 1981). Buczyński et al. (2014) suggest that nests may be important secondary habitats for medicinal leeches, but more observations in aquatic ecosystems, where natural leech populations occur, are necessary to support this hypothesis. These observations indicate that man-made losses of habitat could be also responsible for the demise of some populations of *H. medicinalis* (Grosser 2004; Elliott and Kutschera 2011; Elliott and Dobson 2014; Utevsky et al. 2008, 2010; Kovalenko and Utevsky 2012).

Finally, we want to point out that, although the distinctive features between *H. medicinalis* and *H. verbana* are obvious (Fig. 1) and have been described repeatedly in the literature (Nesemann and Neubert 1999; Trontelj et al. 2004; De Salle et al 2005; Kutschera 2004, 2006, 2007, 2012a, 2012b; Siddall et al. 2007; Phillips and Siddall 2009; Elliott and Kutschera 2011), *Hirudo medicinalis* is still confused with *H. verbana* and other leech species. For instance, Reece et al. (2011) depicted an adult

H. verbana, but labelled the individual as “*H. medicinalis*”. In March 2012, the company *Leeches U.S.A. Ltd.* (Westbury, NY) sold “*Hirudo medicinalis*” for a research project to be conducted in the Weisblat-lab at the University of California (Berkeley). However, an inspection of the first author of this article (U. K.) revealed that all the specimens used for behavioural studies were unequivocally *H. verbana* Carena 1820 (see De Salle et al. 2005 for a DNA barcoding-identification for these mis-labelled medicinal leeches). Starr et al. (2011), Sartor et al. (2013) and Russel et al. (2014) depicted leech species, and described the individual as “*H. medicinalis*”, but in reality the taxa were the distantly related *Hirudinaria mallinensis* and *Malagabdella* sp., respectively (see Table 1).

Hence, despite the fact that the European medicinal leech is, in addition to the taxonomically diverse earthworm *Lumbricus terrestris* (James et al. 2010), one of the most popular known annelids, the unique phenotype of this beautiful “annelid with character” is still widely unknown, even among biologists.

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The frogs shown in Fig. 7 are not *R. esculenta*, but *Rana temporaria* L.