Article

Medicinal leeches: historical use, ecology, genetics and conservation

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Abstract

After a short introduction to the classification of medicinal leeches, their historical use in phlebotomy (blood-letting) and contemporary use in neurobiology and medicine are summarised. Over-collecting of wild *Hirudo medicinalis* in Europe led to reduced populations and the need to import other species, especially the closely related *Hirudo verbana* from Turkey and, more recently, the Caribbean and Asian leech, *Hirudinaria manillensis*. The limited information on the quantitative ecology of European medicinal leeches is summarised next. They require warm-water ponds with a range of suitable hosts, especially amphibians, to survive and prosper. Medicinal leeches can persist with a low minimum viable population size, which may be typical of rare freshwater invertebrates in isolated habitats, especially species limited by high temperature requirements and specialised food sources. Phylogenetic relationships, using molecular methodology, show that there are at least two independent lineages of medicinal leeches with *Hirudo medicinalis*, *H. verbana* and *Hirudinaria manillensis* being closely related. The type species, *H. medicinalis*, was once abundant in Europe but is now rare and on the endangered list in several countries. Genetic studies have confirmed the erroneous marketing of *H. verbana* as *H. medicinalis*. It is highly probable that *H. verbana* has already escaped into the wild. Unlike *H. medicinalis*, *H. verbana* has no legal protection. We conclude that the major factor in the decline of medicinal leech populations has been the general loss of wetlands, especially eutrophic ponds and marshes throughout Europe. Destruction of these water bodies has also led to a decline in amphibians that are an important source of blood-meals for the leeches and are crucial for the survival of their juveniles. More quantitative information is required on *H. medicinalis*, and especially *H. verbana*, to facilitate their conservation and management, and to prevent them becoming extinct in the wild.

Keywords: *Hirudo medicinalis*; *Hirudo verbana*; *Hirudinaria manillensis*; phlebotomy (blood-letting); ecology; water temperature; minimum viable population size; phylogeny; conservation; endangered species.

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Introduction

Although there is general agreement on the position of leeches in the classification of invertebrates, opinions differ on some of the finer points of their placement. Leeches belong to the Phylum Annelida, the segmented worms. The name, introduced by Grube (1850), is derived from the Latin annelus or annellus (diminutive of anulus), meaning 'little ring' and referring to the external ringed appearance of the worms. Leeches are also placed in the Class Hirudinea (from Latin for leech, *Hirudo*) in the Sub-Phylum Clitellata, together with the Class Oligochaeta (earthworms and other detritus feeders). The name is derived from the Latin clitellum, meaning 'saddle' and referring to the broad, swollen ring that is clearly visible in mature earthworms. The function of the clitellum is to secrete a tubular mucous 'slime tube' that eventually forms a sealed capsule enclosing the eggs (sometimes called a 'cocoon'). Both oligochaetes and leeches have a segmented body plan and are hermaphrodites, i.e. they are bisexual with each mature individual producing both male and female gametes. Oligochaetes (= 'few chaetae') have a highly variable number of body segments (10 to 130 depending on the species) and usually have bristle-like chaetae which are moved by muscles and facilitate locomotion. Chaetae are absent in leeches which have a much more compact body than oligochaetes with a constant number of 33 segments, a greatly reduced body cavity, and suckers at both ends of the body. The fusion of segments 1 to 4 at the anterior end of the body has led to the fusion of ganglia to form a primitive brain and well-developed sense organs, whilst the large posterior sucker is formed from segments 25 to 33 (Sawyer, 1986a, b, c; Shain, 2009).

The classification of the Clitellata, a monophyletic group of ringed worms (Borda & Siddall 2004a, b; Struck et al., 2011), varies between authors. Some (e.g. Barnes et al., 2001) name them as a Class with Sub-Classes Oligochaeta, Branchiobdella (Order Branchiobdellida: minute ectoparasites of freshwater Crustacea), and Hirudinoidea. The latter Sub-Class is divided into three Orders: Acanthobdellida, Rhynchobdellida, Arhynchobdellida. There is only one living species in the primitive Acanthobdellida, namely *Acanthobdella peledina* Grube (1850). This rare ectoparasite of salmonid fish, especially brown trout (*Salmo trutta* L.) and grayling (*Thymallus thymallus* L.), occurs in Fennoscandia and northern Alaska/Eurasia. *A. peledina* is a 'living relic', with characters that are intermediate between extant oligochaetes and leeches (Kutschera & Epshtein, 2006). It has chaetae on the five anterior segments, no anterior sucker, a posterior sucker, and no chaetae on 90 % of the segments. The remaining two orders are usually placed in the Euhirudinea (true leeches) which is named as a Sub-Class of the Class Hirudinea by some authors (e.g. Sawyer, 1986a, b; Apakupakul et al., 1999; Kutschera & Wirtz, 2001).

All leeches are either predatory or parasitic carnivores, and their brain and sense organs combined with a flexible, muscular body enable them to actively pursue their prey. They have been described as 'worms with character' (Kutschera & Elliott, 2010). The Rhynchobdellida (from Greek *Rhynchus*, a bill) have a jawless pharynx and utilise an evertible proboscis to penetrate the skin of their hosts. They include the Families Glossiphoniidae and Piscicolidae, and one glossiphoniid species, *Haementeria* (= *Placobdella*) *costata* (Fr. Müller), was once used as a medicinal leech in the Crimea (Kowalevsky, 1900; Elliott & Mann, 1979). As their name indicates, the Arhynchobdellida lack a proboscis, and include the Families Erpobdellidae and Hirudinidae. In some classifications, the erpobdellids are placed in the Sub-Order Erpobdelliformes or the Pharyngobdellida, the latter referring to their weakly muscularised pharynx which lacks jaws or teeth. They suck in their invertebrate prey, which are usually swallowed whole (Elliott, 1973; Kutschera, 2003). In some classifications, the Hirudinidae, which include the type species *Hirudo medicinalis* L. 1758 and related taxa, are placed in the Sub-Order Hirudiniformes or the Gnathobdellida, the latter referring to their short muscular pharynx which usually has toothed jaws (Nesemann & Neubert, 1999; Borda & Siddall, 2004a, b).

The present review first summarises the occurrence and use of medicinal leeches by man from historical times to the present and then examines the limited information on their quantitative ecology. Finally, we consider genetic aspects and conservation

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problems in relation to the importation of other species of medicinal leeches into western Europe.

The European medicinal leech and its cousin

Two species in the Hirudinidae occurred naturally in the wild in most countries in western Europe, the now rare medicinal leech, *Hirudo medicinalis*, and the widely distributed, so-called horse leech, *Haemopis sanguisuga* L. 1758 (Fig. 1). In spite of its Latin name meaning blood-sucker, the blunt teeth of the latter species cannot pierce the skin of mammals. It devours a wide range of prey that includes earthworms, molluscs, insects, tadpoles, small or wounded fish and frogs, leeches of its own and other species, and almost any carrion (Elliott & Mann, 1979). Hoffman (1960) observed this species feeding on the bodies of toads, *Bufo bufo* L., that had been killed by the blood-sucking of *H. medicinalis*, that co-existed in the same habitat. The name horse is probably derived from the Anglo-Saxon word for false in the same sense as 'horse mackerel' and 'horse chestnut' with its bitter seed. The gatherers of medicinal leeches probably recognised the horse leech as a 'false' species that was of no use in phlebotomy (extracting blood from a vein; based on the Greek for vein, '*phlebos*').

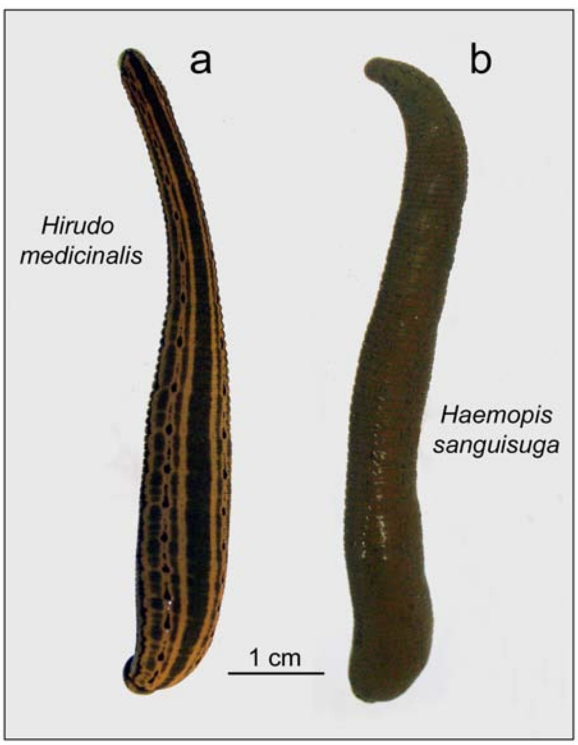

Fig. 1. Adult specimens of the medicinal leech *Hirudo medicinalis*, a blood feeder, and the so-called horse leech, *Haemopis sanguisuga*, a predator and scavenger. The living specimens depicted here were collected in undisturbed habitats of eastern Germany.

In some nature reserves of eastern Germany, populations of both species still exist today (Fig. 1). However, there are no systematic observations on the interactions between these species. Thus, we have little information as to the comparative ecology of the medicinal leech and its cousin in central Europe. In a recent report, Wirchansky & Shain (2010) documented the monophyly of North American haemopids and terrestrialism of these leeches. However, the question concerning a possible interaction or co-occurrence of *Haemopis* species and the North American medicinal leech (*Macrobdella decora* Say 1824) was not addressed.

A short history of phlebotomy

When Carl Linnaeus (1707-1778) first named *Hirudo medicinalis* in 1758, he was probably describing adult, ca. 10 cm long specimens obtained from a local apothecary in Sweden. Medicinal leeches have been used for phlebotomy (blood-letting) since several centuries BC and there are records from ancient Egypt, Rome and Greece (Hartnett, 1972; Payton, 1981; Sawyer, 1981; Elliott & Tullett, 1992). A Greek, Nicander of Colophon (ca. 130 BC), recorded their use in medicine, and in medieval times, classic works advising their use were often Latin translations of Arabic writings, the latter often derived from Greek texts. An early medical treatise, published in France in 1570 and translated into English in about 1630, mentions the identification and application of leeches. Leeching was one of several remedies used to restore the balance of the four humours (blood, phlegm, choler, melancholy). It was also one of the many forms of counter-irritation, a treatment in which something was applied to irritate the skin or gut and thereby to counteract the effects of a disease. Medicine had a hierarchical structure in the sixteenth and seventeenth centuries with physicians at the top, then surgeons, barber surgeons, and finally apothecaries at the bottom. The latter, with some surgeons, were chiefly responsible for blood-letting because physicians found it demeaning to be in close contact with their patients!

For inexplicable reasons, phlebotomy became very fashionable in the eighteenth and nineteenth centuries, especially in France (Fig. 2). Several treatises on medicinal leeches were published and those in English and French, respectively, were by Johnson (1816, 1825) and by Moquin-Tandon (1846). The consumption of *H. medicinalis* in France was enormous. For example, the annual use in one Paris hospital between 1830 and 1838 varied from 132 700 to 178 812 leeches (Herter, 1968; Sawyer, 1979). Most French hospitals used lower numbers but these still varied between 5000 and 60 000 per year from 1820 to 1850. An import duty of 1 franc per 1000 leeches commenced on 7 March 1817 and records were kept of the number of leeches imported into France. From 1827 to 1844, these varied from 15 224 671 to 57 491 000 per year (Sawyer, 1981). The imported leeches came chiefly from the Turkish Empire, Britain, Germany and Spain. There were a few brave physicians who questioned their use. The eminent French physician, Pierre Charles Alexandre Louis (1787-1872), was one of the earliest to assess statistically the value of various therapies, and he concluded that blood-letting was harmful rather than beneficial (Louis, 1836). This demand for medicinal leeches was not restricted to Europe. *H. medicinalis* does not occur naturally in North America, and large numbers were imported into the United States from Europe in the eighteenth and nineteenth centuries (Hagy, 1991). Several attempts were made to rear this species in the United States but all were unsuccessful (Hessel, 1881, 1884).

Fig. 2. The use of European medicinal leeches (*H. medicinalis*) for the treatment of obesity, ca. 1750. At that time, this annelid species was still abundant in European freshwater ecosystems.

The enormous demand for medicinal leeches in Europe led to a reduction in their numbers in the wild and several countries forbade the export of leeches. William Wordsworth (1770-1850), in his poem 'Resolution and Independence' written in 1802, described a meeting with an old man gathering H. medicinalis near Grasmere in northwest England. The leech-gatherer laments that once he could collect leeches everywhere, but now they were more difficult to find. Over-collecting is frequently blamed for the decline in numbers of H. medicinalis throughout Europe. However, their decreasing use for medical purposes may have contributed to their decline. When they were used for blood-letting, they were kept in special jars that were often ornate and can still be seen in some pharmacies (Fig. 3). Leech jars were produced in a variety of sizes and shapes, and are highly collectible today (Lothian, 1959). Once the leeches were used, they were frequently discarded into the nearest ditch or pond. This constant re-introduction of mature individuals probably helped to sustain the wild population and ensured a regular supply of leeches in country districts.

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Leech imports and confusions

As medicinal leeches became more difficult to find in the 19th century, the indigenous supply was supplemented by importations from abroad. For example, an Australian species, *Richardsonianus australis* (Bosisto) (= *H. quinquestriata* Schmarda), was imported into England and at least one unwanted batch was dumped in the River Thames (Richardson, 1970). Even greater numbers of the 'trout leech' were imported into Britain from western North Africa and the Iberian Peninsula. This leech is sometimes regarded as a variety '*tesselata*' of *H. medicinalis*, but is usually recognised as a valid species, *H. troctina* Johnson (Sawyer, 1986a, b, c). The common name is due to the large orange or reddish spots with black centres on the dorsal surface which thus has a pattern similar to that on a brown trout (Elliott, 1994).

Another method to combat the decline of the supply of wild leeches was 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 per year (Herter, 1968). Leech farms still exist today (for instance, in Biebertal, Germany; see Roth, 2002) but unfortunately they often rely on imported leeches from southeastern Europe and Turkey as detailed below. These imports are often not *H. medicinalis*, but the closely-related species, *H. verbana* Carena 1820, which has been confused with the 'true' medicinal leech (Kutschera, 2004, 2006, 2007a, b) (Fig. 4). This species 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*) (see, for instance, the title of the article of Zapkuviene, 1972a). The implications of this introduction will be discussed in more detail in later sections of this Review Article.

Fig. 3. An English leech jar for the cultivation of *H. medicinalis*, ca. 1880 (Photo: T.L. Furnass).

Use of medicinal leeches by man

The contemporary use of medicinal leeches includes not only medical purposes but also as a model organism in neurobiology (e.g. Nicholls & van Essen, 1974; Muller et al., 1981; Leake, 1983). Medicinal leeches have exceptionally large nerve cells (neurones) and supporting glial cells that are ideal for studies of the interrelationships between the two cell types and between paired neurones. If nerve connections are damaged deliberately, neurones in one ganglion will re-connect physically and functionally with their original target cells in the adjacent ganglion, so that chemical and electrical synapses are regenerated. A single neurotransmitter, serotonin, orchestrates many aspects of feeding behaviour (Lent & Dickinson, 1987, 1988). These studies may facilitate an explanation of how neurochemicals control behaviour in other animals, including the human species.

Medicinal leeches are used to drain a haematoma (a collection of partially clotted blood) from a wound, the most obvious examples being a 'black eye', a 'cauliflower ear', 'gum boils' and 'minor ulcers' (Bunker, 1981; Roth, 2002; Michalsen & Roth, 2006). The use of leeches to reduce a periorbital haematoma is not new and was described by Oristasius in 330 AD (Johnson, 1816). Medicinal leeches are also used to remove post-operative

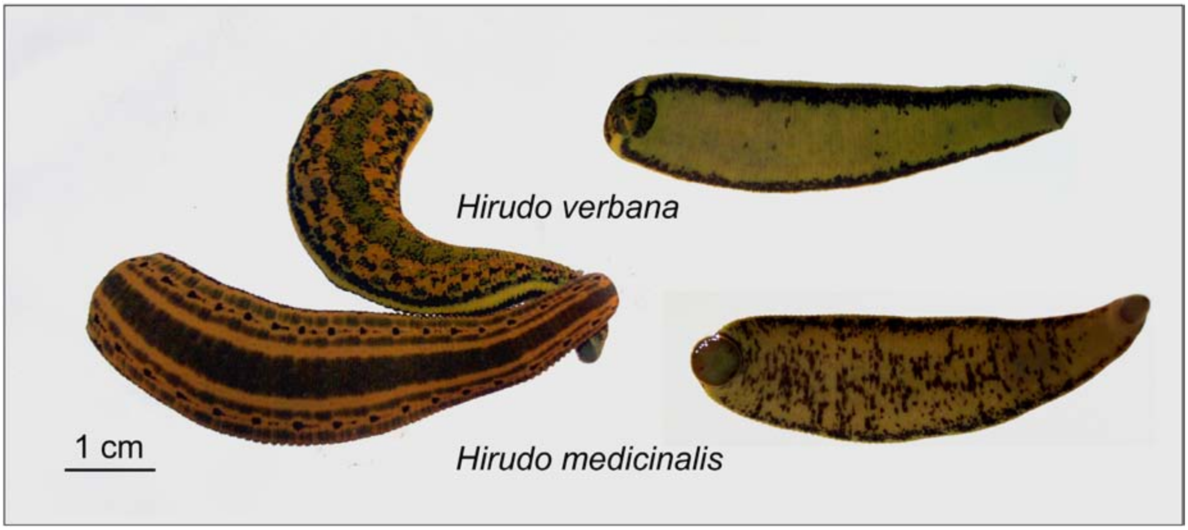

Fig. 4. The markings on live adults of *Hirudo medicinalis* and *H. verbana* in dorsal and ventral views (left and right, respectively).

occlusions to enhance the success of tissue transplants and the surgical joining of amputated appendages such as fingers and ears (Henriot et al., 1990). Necrosis (cell death), sometimes followed by gangrene, can occur as a result of an inadequate blood supply. The removal of stagnant blood by the leech stimulates blood flow into the wound and capillary growth. However, a 'pseudomonad' bacterium, *Aeromonas hydrophila* (Stanier) (= *Pseudomonas hirudinis* Büsing), is a normal symbiote in the leech gut and produces enzymes that facilitate the digestion of blood meals (Whitlock et al., 1983; Laufer et al., 2008). This organism is pathogenic to humans and has been associated with diarrhoea, infections occurring after injuries in contaminated water, and infection in immuno-compromised patients. It has also infected wounds and led to the rejection of skin grafts (e.g. Dickson et al., 1984; Mercer et al., 1987; Tissot-Guerraz et al., 1987; Watt, 1987; Snower et al., 1989). Therefore, the infection risks associated with the medicinal leech must be balanced against the benefits from their use in surgery (Roth, 2002; Michalsen & Roth, 2006).

The saliva of *H. medicinalis* contains hirudin, the most powerful natural anti-coagulant known. Until heparin was discovered, it was the only means to prevent blood clotting. Markwardt (1985) provides a useful review of the pharmacology of hirudin. It was first discovered by Haycraft in 1884, but it was not until the 1950s that it was isolated and described chemically by Markwardt. It is an acidic polypeptide with a molecular mass of about 9000, and acts as a selective thrombin inhibitor. The reduction of a thrombus (blood clot) within a blood vessel by the action of hirudin (Wallis, 1988) was probably the reason why some treatments with leeches in past centuries were beneficial, but those applying the leeches did not know the correct explanation for this result! The extraction of hirudin from whole *H. medicinalis* necessitates the destruction of large numbers of leeches and at least 12 000 kg of leeches are used for this purpose in Europe each year (Wells & Coombes, 1987). Several major pharmaceutical companies now market hirudin-based products. Therefore, a welcome development is the cloning and expression of a recombinant gene for hirudin in yeast and bacteria (Wallis, 1988). The large-scale production of bacterial hirudin would save the lives of large numbers of medicinal leeches. More recently discovered substances, produced by medicinal leeches, are potentially as important as hirudin. These include histamine (which causes the vasodilation of blood vessels in the host and hence increases the blood flow while the leech feeds), hyaluronidase (a 'spreading factor' that causes breakdown of host tissues, thereby increasing permeability), and bdellin and eglin (both proteolytic inhibitors that are the basis of the anti-inflammatory response following a leech

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bite) (Wells & Coombes, 1987; Henriot et al., 1990). It is often claimed that the saliva also contains an anaesthetic and thus the leech bite is painless. There is no reliable evidence for this and those bitten by a medicinal leech have discovered that it is far from causing no pain! It should be noted that throughout Asia the hirudinid leech *Hirudinaria manillensis* (Lesson, 1842) is used, as detailed below. Adult specimens of this exotic species can reach a body length of up to 18 cm (Kutschera & Roth, 2006b).

Ecology of European medicinal leeches

The early literature on the ecology of *Hirudo medicinalis* has been reviewed in four books and is now summarised here (Mann, 1962; Herter, 1968; Elliott & Mann, 1979; Sawyer, 1986a, b, c). There was surprisingly little quantitative information on medicinal leeches in the wild and most of the numerical values were from laboratory studies. The typical habitat is a eutrophic pond with a muddy substratum, littoral vegetation, and a high summer temperature. It should also be a breeding site for frogs, toads and newts. Although *H. medicinalis* and *H. verbana* (Fig. 4) are often reported as feeding almost exclusively on the blood of mammals (cattle, horses, deer, humans), they will also feed on the blood of fish, water birds, and especially amphibians, both the adults and their larvae. 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 (Fig. 5). Early studies in the laboratory showed that each leech can take two to five times its own mass in one blood meal that is digested slowly over several months. For example, a leech of 128 mg dry mass sucked in 640 mg dry mass of blood in one meal and then took about 200 days to digest this meal. As the leech lived for another 100 days without food, this one meal supported the leech for almost a year (Elliott & Mann, 1979). However, there was no evidence to suggest that a similar feeding pattern occurred in the wild.

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 disturbance. Two laboratory experiments showed that 86 % and 95 % of unfed leeches responded to low-amplitude surface waves (about 1 mm high) by swimming, whilst only 60 % of fed leeches responded (Young et al., 1981). 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, it uses heat detection, the optimum response occurring at 33 to 40 °C (Dickinson & Lent, 1984), and also chemosensory stimuli (Elliott, 1986), both receptors being located in the anterior end of the leech. The leech explores the skin of the host for a suitable feeding site, then pierces the skin with its three jaws armed with numerous sharp teeth,

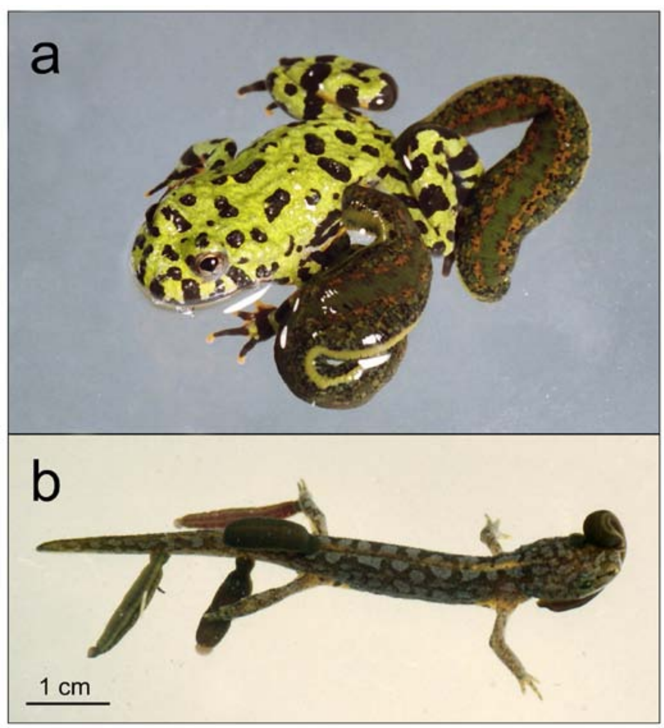

Fig. 5. The medicinal leech *Hirudo verbana* as an exoparasite on amphibians: (a) two adult leeches attacking a fire-bellied toad (*Bombina orientalis* Boulenger 1890); (b) juvenile leeches sucking on the body of a half-grown newt (*Triturus alpestris* Laurenti 1768).

and finally sucks the blood of its host. Satiated leeches are sometimes attacked by unfed leeches in the laboratory, but it is not known if similar behaviour occurs in the wild (Kutschera & Roth, 2005). However, other leech species will sometimes feed on *H. medicinalis*. Young *Glossiphonia complanata* (L.) frequently obtain their first meal by feeding on other leeches, including *H. medicinalis* (references in Elliott & Mann, 1979). In a quantitative study in a tarn (= pond) in northwest England, samples of *H. medicinalis* were taken every two weeks from March to November in four years (Tullett & Elliott, 1986). The total catch of medicinal leeches was 1296 of which 196 (15 %) were carrying adult individuals of the glossiphonid leech, *Helobdella stagnalis* (L.). All sizes of *H. stagnalis*, which are 5 to 10 mm in length, were found on the hosts which were up to 10 cm long, and some *H. stagnalis* taken in June were carrying eggs. When the leeches were brought back to the laboratory, it was obvious that *H. stagnalis* was feeding on its 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 (Tullett & Elliott, 1986). Similar observations were made on the sister species *H. verbana*. In laboratory experiments, adult hungry *H. stagnalis* rapidly attached to the skin of *H. verbana*-individuals and were carried around for several weeks. However, the attached *H. stagnalis* did not take up blood from the well-fed *H. verbana* so that only the first stage towards hyperparasitism was recorded in this study (Kutschera et al., 2010).

Reproduction and population growth

The earlier literature reviews agree that mating occurs via a tube-like male copulatory organ of these hermaphrodites in summer and that adequate feeding is required for successful reproduction (Fig. 6a). Sperm are stored and there can be a delay of one to nine months between

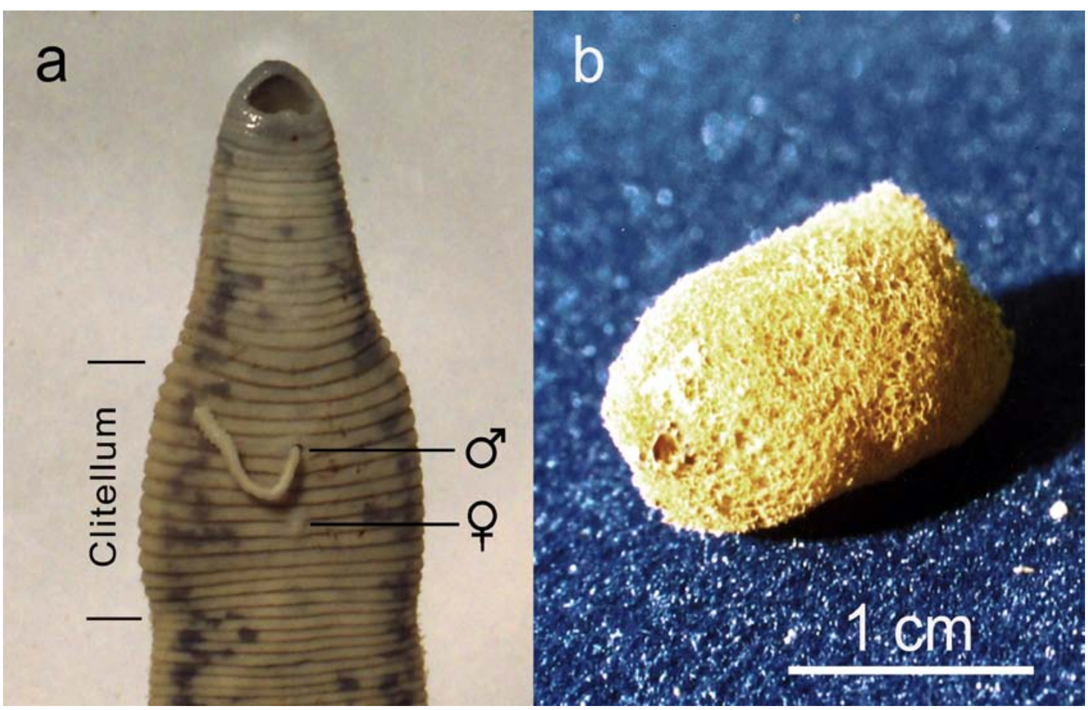

Fig. 6. (a) Ventral side of the head and clitellar region of an adult, alcohol-preserved *Hirudo verbana*. The male (♂) and female (♀) gonopores are visible, with the tube-like male copulatory organ outside of the body. (b) Spongy cocoon of *H. verbana*, deposited by the leech on land. The hole at the end of the berry-like egg capsule, through which the juvenile leeches hatched, is visible.

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copulation and cocoon deposition. The spongy cocoons (Fig. 6b) are laid chiefly in July and August in a damp place just above the water line on the shore or bank. Over 1 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 infertile eggs. In the laboratory, Zapkuviene (1972a, b) found that each adult *H. medicinalis* 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 leech varied from 12 to 60 mg. Hatchlings can survive for about 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 year, and about 2.4 g in their third year. Similar results were obtained for *H. verbana* (Kutschera & Roth, 2006a). Although there was a paucity of information in the field, the literature reviews generally agree 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.

As medicinal leeches can grow only if they have fed, and feed only if they can find hosts, it was important to determine the major environmental factors which affected their swimming activity towards potential hosts. This was the objective of a quantitative field study on the effects of temperature and atmospheric pressure on the swimming activity of *H. medicinalis* in a Lake District tarn in northwest England (Elliott & Tullett, 1986). Atmospheric pressure was included because the older literature suggested that the best time to collect medicinal leeches was just before a thunderstorm when they came to the water surface. Leeches kept in leech jars in the 18th and 19th centuries were also reported to be more active when atmospheric pressure decreased. This use of medicinal leeches as 'living barometers' was seen in the 'Merryweather Barometer' which was exhibited as the 'Tempest Prognosticator' at the London Great Exhibition of 1851, and relied on active leeches ringing a bell when low pressure and bad weather was imminent (Browne, 1946). Unfortunately, atmospheric pressure at the time of sampling and its rate of change over 24 h preceding sampling had no significant effect on catches of swimming leeches in the tarn, not even on the residuals of catches after the effect of temperature had been removed (Elliott & Tullett, 1986). Even in the 18th and 19th centuries, some workers doubted the 'barometer theory' and suggested that the leeches were responding to other factors such as changes in temperature, oxygen concentration or relative humidity.

Medicinal leeches (total = 841) were caught by Elliott & Tullett (1986) in samples taken every two weeks from March/April to October/November over three years. In marked contrast to atmospheric pressure, water temperature was found to be the major factor affecting leech activity in the tarn. A mathematical model was developed to describe this relationship and was used to predict the percentage of active leeches at different temperatures (Fig. 7). Mean temperatures for 10 %, 50 % and 90 % active leeches were 11.9 °C, 19.0 °C and 22.9 °C, respectively. Earlier laboratory studies showed that the preferred temperature in a gradient of 7 to 43 °C was 21 °C (Kaiser, 1954), a value midway between the 50 % and 90 % active values. Optimum temperature ranges for growth (22 to 25 °C) and breeding (25.5 to 27.5 °C) in the laboratory (Zapkuviene, 1972a, b) were similar to predicted values for maximum activity in the field (Fig. 7). The upper lethal range is remarkably high for this species at 39 to 43.5 °C (Kaiser, 1954). These high temperature requirements have important implications for the survival of *H. medicinalis*. The leeches could not survive in many water bodies simply because of the low water temperatures (Elliott & Tullett, 1986).

Minimum viable population sizes in wild leech populations

The small population of *H. medicinalis* in the Lake District tarn is probably typical of most sites in Britain, the exception being the metapopulation in disused gravel pits at Dungeness, Kent, which was studied in detail for a PhD thesis (Wilkin, 1987) and produced four excellent publications (Wilkin, 1989; Wilkin & Scofield, 1990, 1991a, b). The leeches in that population were hot branded in 1984 and 1985 for a capture-mark-release-recapture method to estimate the population as being in excess of

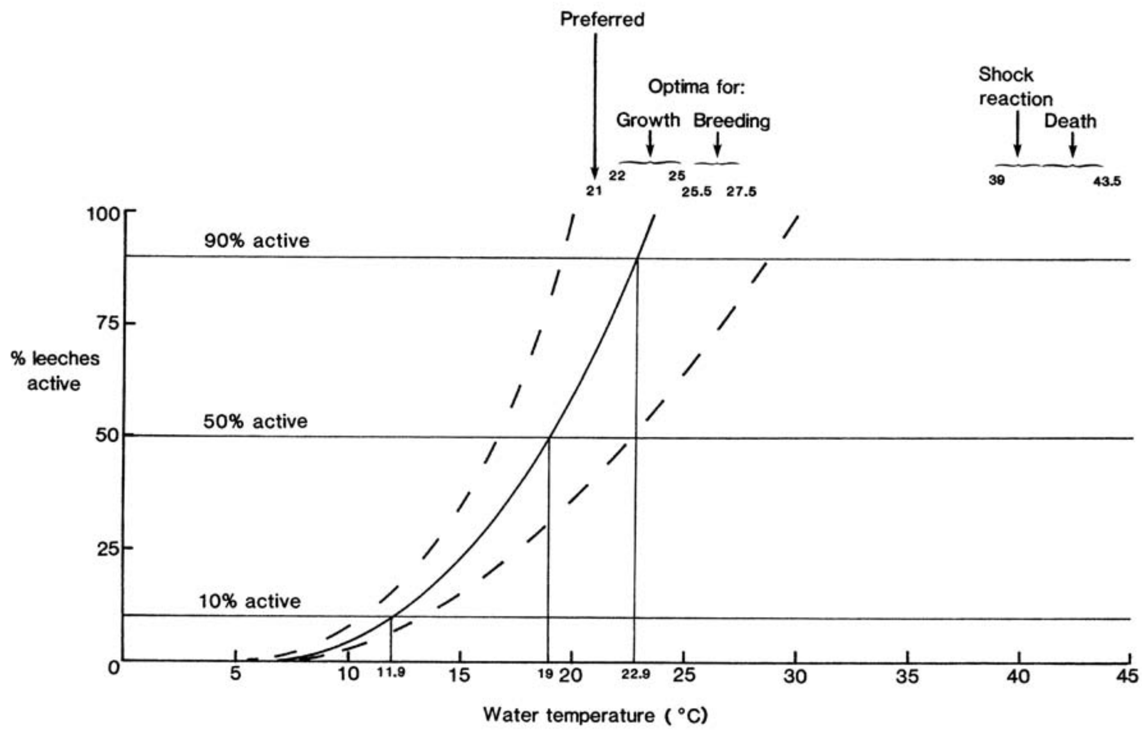

Fig. 7. Relationship between the percentage of medicinal leeches (*H. medicinalis*) actively swimming and the water temperature; curvilinear regression line given by the equation in Elliott & Tullett (1986) (broken lines indicate 95 % CL for the curve); preferred temperature and ranges for optimum growth, breeding, shock reaction and death are provided for comparison. From Elliott & Tullett (1986).

10 000 (0.112 leeches m^{-2}). This must have been the largest population of medicinal leeches in Britain during that time. The population was dominated by small leeches and, as sexually mature leeches were rare, few offspring were produced. A serological technique on the blood-meals of the leeches showed that they fed chiefly on amphibians, with smaller contributions from birds and fish, and almost none from mammals. The marsh frog, *Rana ridibunda* Pall., was the most important host. Although antisera were not prepared against blood sera of smooth newts, *Triturus vulgaris* L., many dead newts with leech bites were found. Therefore, this species was also an important host (see Fig. 5b). Perhaps the most surprising conclusion from these analyses was that the leech population was sustained almost entirely by non-mammalian hosts. Leeches from the population were kept in the laboratory for growth studies, using bovine blood as food. Weight gains were much greater than those shown in the wild. The most detailed study of growth and reproduction in the laboratory was that of Davies & McLoughlin (1996). Their leeches were also fed on bovine blood and took only about 9.5 months to reach maturity compared with field estimates of 2 to 4 years in other studies (Elliott & Mann, 1979; Elliott & Tullett, 1986; Sawyer, 1986a, b).

In the study of the *H. medicinalis* population in a Lake District tarn, it soon became obvious that an accurate estimate of the total population and the number of mature adults was important to provide an estimate of the minimum viable population size for a rare and endangered species. Therefore, using a catch-removal method, the total population was estimated every two years (1986, 1988, 1990, 1992), both by maximum likelihood and regression methods (Elliott, 2008). All leeches were weighed alive and size groups were separated by polymodal frequency analysis. A small sample of the blood meal in each leech gut was taken before the leeches

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were returned to the tarn, and was used to estimate the proportion of mammalian and non-mammalian blood in the meals. Both methods of estimating population size produced similar values, increasing confidence in the estimates. Values for the total population in June and July varied among years from 248 to 288 individuals, the maximum value being only 16 % higher than the minimum. Values for the number of mature leeches varied from 48 to 58 (19 to 20 % of the total population), and this was an estimate of the effective population size. The population was much lower than the approximate estimate in excess of 10 000 medicinal leeches in the gravel pits and drainage ditches near Dungeness (Wilkin, 1989; Wilkin & Scofield, 1991a). However, the density of the latter metapopulation was estimated as 0.112 leeches m^{-2}. The surface area of the Lake District tarn was 2546 m^2, and therefore the densities equivalent to the values for total population were 0.10 to 0.11 leeches m^{-2}; values remarkably close to that for the larger metapopulation. The structure of the two populations differed markedly. The larger metapopulation was dominated by small leeches with few mature individuals, whereas the small population in the tarn was composed of only 14 to 17 % small leeches with mature leeches forming 19 to 20 % of the population. One obvious reason for this discrepancy could be the scarcity of mammalian hosts at Dungeness compared with the tarn.

There were four size groups in the Lake District tarn. The largest mature leeches (live weight >5 g) in group IV formed only 1 % of the population, and the smallest (0.02 to 0.5 g) in group I formed only 14 to 17 %. Most leeches were in two overlapping groups of immature (64 to 67 % of the population) and mature (18 %) leeches with size ranges of 0.4 to 3.4 g and 2.5 to 5 g, respectively. The percentage of leeches in each size group was very consistent among years. Blood meals were found in 38 to 44 % of the leeches in group I, 45 to 50 % in group II, 70 to 75 % in group III, and 100 % in group IV, but mammalian blood was present only in larger mature leeches (>3.5 g). The mature leeches in the largest group III were probably about three years old, most being >3 g, a threshold value which agrees with that for the Dungeness leeches (Wilkin, 1989). The largest leech weighed 7.1 g, similar to the largest individuals in the Dungeness metapopulation (Wilkin & Scofield, 1991a). However, under optimum conditions in the laboratory, medicinal leeches can grow up to 29 g in only 300 days (Wilkin & Scofield, 1991b), and 23 g in 322 days (Davies & McLoughlin, 1996). It is not known if medicinal leeches ever attain such large sizes in the wild.

Medicinal leeches were first detected in the Lake District tarn in 1980 and were still present in 2010, so the population has persisted for at least 30 years. Compared with minimum viable population sizes for other species, including many endangered species, values for this medicinal leech population are extremely low, but may be typical of some rare freshwater invertebrates in isolated habitats, especially species limited by high temperature requirements and specialised food sources (Elliott, 2008).

Medicinal leeches from Germany, the *Lacus Verbanus* and Asia

As noted earlier, many of the commercially available medicinal leeches are not *H. medicinalis*, but the sister taxon, *H. verbana*. These two closely related leech species (Fig. 4) 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 & Neubert, 1999; Kutschera, 2004, 2006, 2007a, b; Siddall et al., 2007). The latter leeches originate from a commercial supplier in Turkey and information on their ecology in the wild is largely lacking. Cocoon deposition and cluster formation of the juveniles have been described for captive *H. verbana* (Kutschera & Roth, 2006a). Cocoons (Fig. 6b) were laid in dark, damp places in moist soil, never in water, as also in *H. medicinalis*. Within a few hours, the spongy cocoon membrane hardened so that buoyant, sturdy egg capsules were formed with enclosed albumen as food for the developing young (usually 8 to 12 per cocoon). Hatching occurred about 4 weeks after cocoon deposition and the hatchlings rapidly entered the water. Up to 50 juveniles formed dense clusters that lasted up to 20 days, notably in the cold. Clustering also occurred in adult leeches at temperatures below 10 °C. The significance of this clustering behaviour is not known but it may offer individual leeches protection from low temperatures. Some specimens of *H. verbana* are black-pigmented and co-exist with the normal coloured form (Kutschera, 2007b). These 'black *Hirudos*' were described by Moquin-Tandon (1846) as a sub-species of the European medicinal leech (*H. medicinalis* var. *nigrescens*). However, DNA-sequence analyses unequivocally revealed that these dark-pigmented leeches, which look like *Haemopis sanguisuga* individuals (Fig. 1), are over-pigmented varieties of *H. verbana*. Since these enigmatic 'black medicinal leeches' have repeatedly been described in the literature they were denoted as the taxon *H. verbana* 'var. *nigra*' (Kutschera, 2007b).

Satiated *H. verbana* in captivity (described wrongly as *H. medicinalis*) were attacked by hungry conspecifics (Kutschera & Roth, 2005), as also noted earlier for *H. medicinalis* (Sawyer, 1986a, b). As *H. verbana* could escape into the wild and may already have done so (Grosser, 2004), there is clearly a need for more information on its ecology so that it can be compared with *H. medicinalis*.

In 2005, a batch of leeches collected in freshwater ponds on the northern coast of the Dominican Republic was imported to Germany and maintained in freshwater aquaria at the commercial leech farm in Biebertal and the Institute of Biology of the University of Kassel (Germany). Based on morphological observations and DNA-sequence data (determination of part of the mitochondrial gene cytochrome c oxidase subunit I, CO-I), these imported leeches were identified as *Hirudinaria manillensis* (Fig. 8). Some individuals of this species reach an enormous body length and therefore have been described as 'buffalo leeches' (Kutschera & Roth, 2006b). In their natural habitat, these large blood-sucking leeches were found to be attached to the belly and feet of cows, where they caused bleeding wounds and hence severely impaired the vitality of their hosts. Moreover, it was reported that humans are regularly attacked by *H. manillensis*, an annelid that is therefore also known as the 'Asian cattle leech' (A. Knecht, personal communication).

Fig. 8. The Asian medicinal leech *Hirudinaria manillensis*, adult specimen in two positions.

Sawyer et al. (1998) have shown that the widespread 'Caribbean leech' is identical with *H. manillensis*, a conclusion corroborated more recently by Phillips & Siddall (2009). Since these tropical warm-water annelids have been used in India and neighbouring countries of South-East Asia for medicinal purposes, *H. manillensis* should be named the 'Asian medicinal leech' (Sawyer et al., 1998; Kutschera & Roth, 2006b). In many local leech farms throughout Asia, *H. manillensis* populations are bred and large quantities of leeches sold to practitioners (for instance, by companies such as Agro Medic Enterprise in Penang, Malaysia). Thus, it is justified to conclude that this giant leech (Fig. 8) is the Asian counterpart to the two *Hirudo* species of Europe depicted in Fig. 4.

Phylogenetic relationships of medicinal leeches and related taxa

As described in the *Introduction*, the classical systematics of leeches has been deduced based on morphological and anatomical traits, and a broad agreement has been reached concerning the general classification of these 'annelids with character'. We distinguish between three major groups: the Gnathobdellida, parasites/predators characterised by a short pharynx with more or less toothed jaws (e.g.

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Hirudinidae); the Pharyngobdellida, predators with a long, musculous pharynx (e.g. Erpobdellidae); and the Rhynchobdellida, parasites that use an evertible proboscis to suck body fluids from their prey (e.g., Glossiphoniidae) (Mann, 1962; Herter, 1968; Elliott & Mann, 1979; Sawyer 1986a, b; Kutschera & Wirtz, 2001). However, leech systematics, as all other branches of taxonomy, were revolutionised about three decades ago following the establishment of techniques for DNA-sequencing and comparative data analysis. The neighbour-joining tree shown in Fig. 9, which is based on mitochondrial cytochrome *c* oxidase subunit I (CO-I) gene sequences (ca. 600 base pairs in length) and published GenBank data, corroborates this morphology-based classification. The methods for DNA extraction, gene amplification, sequencing, editing and data analysis were described previously (Pfeiffer et al., 2004; Kutschera et al., 2007).

Six European species plus the Asian medicinal leech were analysed with respect to their DNA-based taxonomy. As Fig. 9 shows, the closest relative of the type-species *Hirudo medicinalis* is *H. verbana* (Fig. 4). The sister taxon to these European medicinal leeches is the tropical blood sucker *Hirudinaria manillensis*. Two other members of the Gnathobdellida, *Haemopis sanguisuga* (Fig. 1) and *Limnatis nilotica* Savigny 1822 were the next relatives of these three medicinal leeches. It should be noted that the blood-sucking Nile leech (*L. nilotica*) has been classified as a member of the Hirudinidae (Herter, 1968). This species, which occurs in countries around the Mediterranean, with a distribution as far east as Iran and Tadjikistan, and as far west as the Azores (Orevi et al., 2000), is a voracious blood feeder, which inhabits water holes or ponds, where it attacks drinking animals and humans. The leeches enter the mouth or nostrils and suck blood from the mucous membranes of the pharynx, larynx or nostrils, often causing the death of their victims (Herter, 1968; Orevi et al., 2000).

Finally, our DNA-based phylogenetic tree (Fig. 9) shows that the Pharyngobdellida and the Rhynchobdellida, represented here by the type species *Erpobdella octoculata* L. 1758 (Erpobdellidae) and *Glossiphonia complanata* L. 1758 (Glossiphoniidae), form separate clades. In summary, our molecular data are in accordance with the morphology-based systematics of freshwater leeches (Mann, 1962; Herter, 1968; Elliott & Mann, 1979; Sawyer, 1986a, b; Kutschera & Wirtz, 2001).

More comprehensive molecular analyses of the phylogenetic (evolutionary) relationships of the Hirudinidae, inclusive of the blood-sucking tropical

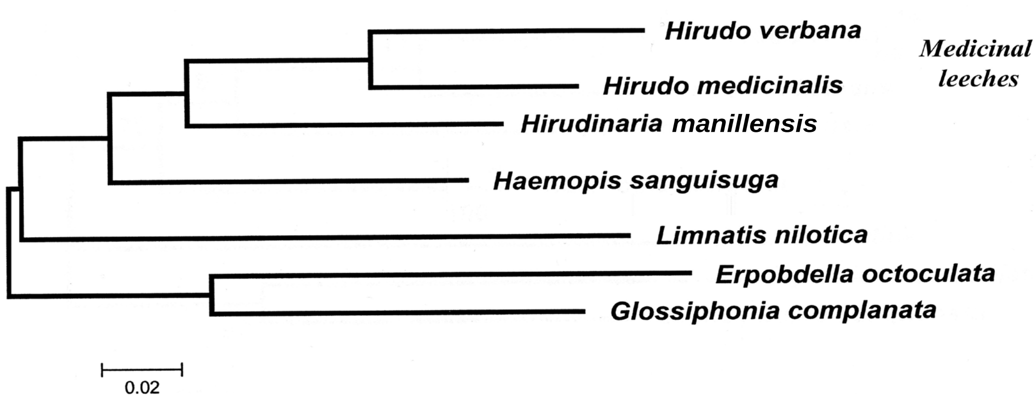

Fig. 9. Phylogenetic relationships of seven annelid species, inclusive of the three medicinal leeches discussed in this article (*Hirudo medicinalis, H. verbana, Hirudinaria manillensis*). The diagram shows a neighbour-joining analysis obtained from newly acquired CO-I (cytochrome *c* oxidase subunit I) DNA sequences and data deposited in the GenBank.

land leeches (Haemadipsidae), to which the new species *Tyranobdella rex* sp. n. belongs (Phillips et al., 2010), have been published by M.E. Siddall and co-workers (Apakupakul et al., 1999; Borda & Siddall, 2004a, b, 2010; Phillips & Siddall, 2009; Siddall & Borda, 2003; Siddall et al., 2007). In their most complete DNA-based taxonomic analysis, which is in general accordance with our phylogenetic tree (Fig. 9), Phillips & Siddall (2009) document that the family Hirudinidae should only include members of the genera *Hirudo*, *Haemopis*, *Hirudinaria*, *Asiaticobdella*, *Goddardobdella*, *Aliolimnatis* and *Whitmania*. A second, independent clade, to which the blood-sucking Nile leech (*Limnatis nilotica*) belongs, includes the North American medicinal leech *Macrobdella decora* Say 1824, a species not discussed in this article. Hence, there are at least two independent lineages of medicinal leeches, and we must conclude that the divergence in the evolutionary history of these freshwater annelids occurred hundreds of millions of years ago (Phillips & Siddall, 2009).

Genetics and conservation of medicinal leeches

The type species of the Hirudinea, *Hirudo medicinalis* was once abundant in Europe, from Ireland in the west to the Ural mountains in the east, and from southern Scandinavia to the countries bordering the Mediterranean (Johnson, 1816, 1825; Moquin-Tandon, 1846; Herter, 1968; Sawyer, 1986b; Elliott & Tullett, 1984, 1992). Three decades ago, Sawyer (1981) suggested that this species was now extinct in most countries in western Europe. However, this conclusion was rather premature, and more detailed studies have shown that medicinal leeches are still present in at least 26 European countries, but there are no recent records for Portugal and European Turkey (Elliott & Tullett, 1984; Wells et al., 1984; Wells & Coombes, 1987). It is now rare throughout western Europe, extinct in Ireland, threatened in at least 15 countries (Austria, Belgium, Britain, Bulgaria, Czech and Slovak Republics, Denmark, Finland, France, Germany, Italy, Luxembourg, Poland, Romania, Spain), not threatened in three (Hungary, Norway, Sweden), and of unknown status in the rest (Albania, Greece, Netherlands, Portugal, Russia, Switzerland, Turkey, countries forming the former Yugoslavia) (Utevsky et al., 2008; Westendorff et al., 2008) (Fig. 10).

Fig. 10. The habitat of a population of *Hirudo medicinalis* in eastern Germany (semi-permanent kettle hole close to Müncheberg in the county Märkisch-Oderland) (adapted from Westendorff et al., 2008).

The taxon *H. medicinalis* is included in their red data list of threatened species by the International Union for the Conservation of Nature and Natural Resources (Groombridge, 1994; IUCN, 2006). Several countries now include this species in their red and endangered list, e.g. in Britain it is in the 'British Red Data Books' and is protected under Schedule 5 of the Wildlife and Countryside

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Act (Bratton & Elliott, 1991). However, as noted earlier, some of the distribution records may be not for *H. medicinalis*, but for the closely-related *H. verbana* that was imported to augment populations in leech farms and has then escaped into the wild (Grosser, 2004; Kutschera, 2004, 2006, 2007a, b; Siddall et al., 2007). There are no records of *H. verbana* occurring in the wild in Britain.

Nesemann & Neubert (1999) re-established the species status of *H. verbana* and divided the distribution of the European medicinal leech into the northwestern *H. medicinalis* and the southeastern *H. verbana*. However, a more recent taxonomic revision by Hechtel & Sawyer (2002) concluded that *H. medicinalis* is distinct from the North African *H. troctina*, but that all varieties of medicinal leeches in Europe belong to one species, *H. medicinalis*. This conclusion has been refuted by recent molecular taxonomy. Random-amplified polymorphic DNA markers revealed a clear separation between four populations of *H. medicinalis* in Slovenia and four populations of *H. verbana* in Slovenia, Macedonia and Croatia (Trontelj et al., 2004). Further phylogenetic analyses, using nuclear and two mitochondrial gene sequences, indicated that the genus *Hirudo* was monophyletic with five species: *H. nipponia* Whitman from Japan, *H. troctina* from Morocco, Algeria and Tunisia, *H. medicinalis* from western and central Europe, the Ukraine and Lithuania, *H. verbana* from the eastern Mediterranean, Balkans, Moldova, Ukraine, Russia, Turkey and Armenia, and a new species, *H. orientalis* sp. n., from Trans-Caucasia, Iran and Uzbekistan (Trontelj & Utevsky, 2005). Utevsky & Trontelj (2005) describe *H. orientalis* in detail and provide a key to the five species of *Hirudo*. Such a key is important because it is possible that medicinal leeches imported into leech farms in western Europe are not *H. medicinalis*. This conclusion is supported by a recent study, using mitochondrial sequences and nuclear microsatellites, on medicinal leeches from 13 wild *H. medicinalis* sites and 13 wild *H. verbana* sites as well as commercially available leeches from four suppliers (Siddall et al., 2007). This study confirmed the erroneous marketing of *H. verbana* as *H. medicinalis*, including those sold by suppliers in Britain and the USA. It is also probable that studies on the ecology (Demirsoy et al., 2001), distribution and status (Kasparek et al., 2000) of the medicinal leech in Turkey, that cited *H. medicinalis*, were actually referring to *H. verbana*. As leech farms in western Europe obtain a large portion of their stock from Turkey, this practice should cease because it allows the introduction of an exotic species that could eventually escape into the wild. In eastern Germany, small *H. verbana* populations have been discovered that may have descended from leeches dumped into ponds and streams after use in hospitals (C. Grosser, personal communication). However, unlike *H. medicinalis*, *H. verbana* and *H. orientalis* have no legal protection in the wild in countries where they occur naturally. This situation should be remedied in the near future before populations of these two species follow the sad demise of many populations of *H. medicinalis*.

Several explanations have been proposed for the loss of many populations of *H. medicinalis* in western Europe and these should all be considered by those responsible for the conservation and management of this endangered species. Over-collecting for blood-letting in the 19th century is frequently blamed but, as mentioned earlier, used leeches were frequently discarded into the nearest ditch or pond and thus probably facilitated 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. Although leech farms offer an obvious solution, this only works if the farms actually rear leeches, rather than importing them and thereby reducing populations in the wild.

A reduction in the availability of suitable 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 ponds, 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, including Britain, where wild animals such as deer are plentiful, and therefore the absence of medicinal leeches in these areas is not due to a lack of mammalian hosts. Davies & McLoughlin (1996) proposed the plausible hypothesis that the declining abundance of field populations of the 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 the slow-growing leeches from Dungeness (Wilkin, 1989). 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 feeding on mammalian blood (Wilkin & Scofield, 1990). In a Lake District tarn, only the larger mature leeches (>3.5 g) fed on mammalian blood, the proportion of mature leeches feeding on mammals varying from 19 to 26 % among years (Elliott, 2008). The chief sources of blood for all leeches in the tarn were probably palmate newts, frogs, toads and their tadpoles. Therefore, the slow growth of the leeches could be partially due to 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 looped its way back to the water. Satiated leeches were never found and it was concluded that the leeches were feeding a little and often rather than to satiation (Elliott & Tullett, 1992). A similar conclusion was reached for the population at Dungeness (Wilkin, 1989).

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 & McLoughlin, 1996). 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 (Fig. 7). Water temperature 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 the medicinal leech impose limitations on its distribution and occurrence. Therefore, the absence of this 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.

Historical evidence indicates that *H. medicinalis* was once abundant in Lake District tarns in northwest England at the beginning of the 19th century. There was an extensive trade in leeches and the Lake District was one of the most famous collecting areas (Elliott & Tullett, 1984). About 50 to 100 years ago, the depth and volume of many tarns were increased so that they could be used as a source of clean drinking water and/or as fish-ponds suitable for brown trout, *Salmo trutta*. The temperature requirements of this species are much lower than those of the medicinal leech; the upper lethal range is 25 to 30 °C and growth occurs within 4 to 19 °C (Elliott, 1994). It is obvious that trout could not survive at the higher temperatures required for the survival of *H. medicinalis* (Fig. 7), and also that medicinal leeches could not survive in a trout-pond. Therefore, a man-made change of habitat could be responsible for the demise of some populations of *H. medicinalis*.

Concluding remarks

In this article we have summarised the occurrence, use, ecology and taxonomic status of the commercially available medicinal leeches *H. medicinalis*, *H. verbana* and *Hirudinaria manillensis*. It should be noted that we have not discussed the other European species of the genus *Hirudo* (*H. orientalis* and *H. troctina*), since they are rarely used and comparatively little is known about their ecology (Utevsky et al., 2008). With respect to the two most important European *Hirudo*-species (Fig. 4), we conclude that the major factor in the decline of these annelids must be the general loss of wetlands, especially eutrophic ponds and marshes throughout Europe, and the isolation of the remaining ponds by changes in land use (Fig. 10). Destruction of these water bodies has also led to a decline in amphibians. Adults and larvae of newts, frogs and toads are an important source of blood-meals for both leech species and are crucial for the survival of the juveniles (Fig. 5). Therefore, conservation of *H. medicinalis* and *H. verbana* requires the protection and maintenance of suitable habitats, not only for the leeches but also for their amphibian hosts. Although the typical habitat has been described in general terms, little is known about the

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exact requirements for the survival of *H. medicinalis* and *H. verbana*. Recent work on the diversity of suitable hosts and the high temperature requirements has been summarised in this review, but little comparable information exists on other aspects of the ecology of *H. medicinalis*. Even less is known about the ecological requirements of the closely-related sister species *H. verbana*. Such information is essential for the conservation and management of both leech species to prevent them becoming extinct in the wild. As Barnosky et al. (2011) have recently documented in detail, continued losses of species in the 'endangered' category could lead to the 'sixth mass extinction' on Earth within the next few centuries, and medicinal leeches could be one of the first victims of this human-made environmental catastrophe.

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