

## The taxonomic status of dark-pigmented medicinal leeches of the genus *Hirudo* (Hirudinea: Hirudinidae)

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With 2 figures

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**Schlagwörter:** *Hirudo*, Hirudinea, Medizinische Blutegel, Taxonomie

The leech *Hirudo verbana* was described in 1820 by H. Carena based on individuals collected in the Lacus Verbanus (Lago Maggiore, Italy). Large populations of this colourful species, obtained from leech gatherers in Turkey, are maintained in artificial ponds in the ZAUG Blutegelzucht in Biebertal, Germany. Among some 1000 individuals, black-pigmented leeches were regularly found. Based on several criteria, these black medicinal leeches are described here as a colour variant of *Hirudo verbana*, var. *nigra*. The nominate species of this taxon is the coloured form *Hirudo verbana*. Hybrids between these varieties occur, but apparently at very low abundance.

### 1 Introduction

Leeches of the genus *Hirudo* have historically been used for medicinal purposes, mainly to remove "bad blood" from the patient. Medieval medicinal manuscripts that were found in several European countries illustrate the use of leeches for the treatment of a variety of disorders. This unspecific therapeutic use of medicinal leeches reached a height between 1820 and 1840. As a result, leeches were so scarce in France that about 4,5 million animals were imported in the year 1833 (Herter 1968, Sawyer 1986). The demand for these blood-sucking annelids became so great that medicinal leeches almost became extinct in several European countries. From some ponds, experienced leech gatherers could harvest as many as 2500 individuals per day so that the natural supply soon became exhausted. After the establishment of leech farms, the commercial leech trade was re-vitalized in Germany and France around the year 1900 (Herter 1968, Sawyer 1986).

In two recent reports I have shown that most medicinal leeches that are kept in European farms and sold to physicians are *Hirudo verbana*, a species described by Carena in 1820. The "true" medicinal leech, *Hirudo medicinalis* L., 1758, is only rarely traded; this endangered species can be distinguished from its relative *H. verbana* by its unique colour pattern (Kutschera 2004, 2006).

At the ZAUG leech farm in Biebertal (Germany), large populations of *H. verbana* are bred in artificial ponds (Kutschera & Roth 2006 a, b). Some black

leeches were regularly found among the 1000 or so *H. verbana*-individuals. In this contribution I describe these dark-pigmented medicinal leeches as a colour variant of the taxon *H. verbana*.

## 2 Materials and Methods

The leeches were purchased from a commercial supplier in Turkey. All animals were collected in a natural reserve close to the town of Samsun (Turkey), imported to Germany and then released in artificial ponds. Dark-pigmented *Hirudo*-individuals were collected and cultivated in aquaria (depth of the water ca. 5 cm). Over a period of two years, 16 black leeches were found in the *H. verbana* populations in Biebertal. Feeding experiments were carried out as follows. A dying gold fish (*Carassius carassius* spp. *auratus*), obtained from a local dealer, was added to the aquarium where the hungry leeches were kept. The subsequent feeding episode was documented with a digital camera.

For extraction of tissue pigments, adult leeches were killed by immersion in 20 % ethanol. The dead specimens (body mass ca. 1,4 g) were extracted with 5 ml of 70 % ethanol for 48 h (25 °C, in darkness). Thereafter, the green-brown solution was removed, diluted 1:10 with 70 % ethanol and analyzed with a spectrophotometer (Uvikon 931, Kontron Instruments, Neufahrn, Germany). For comparison, adult specimens of two dark-pigmented leeches, *Haemopsis sanguisuga* and *Trocheta haskonis*, were treated and analyzed as described above. All experiments were repeated at least three times with similar results. Representative observations and data are depicted in the figures 1 and 2.

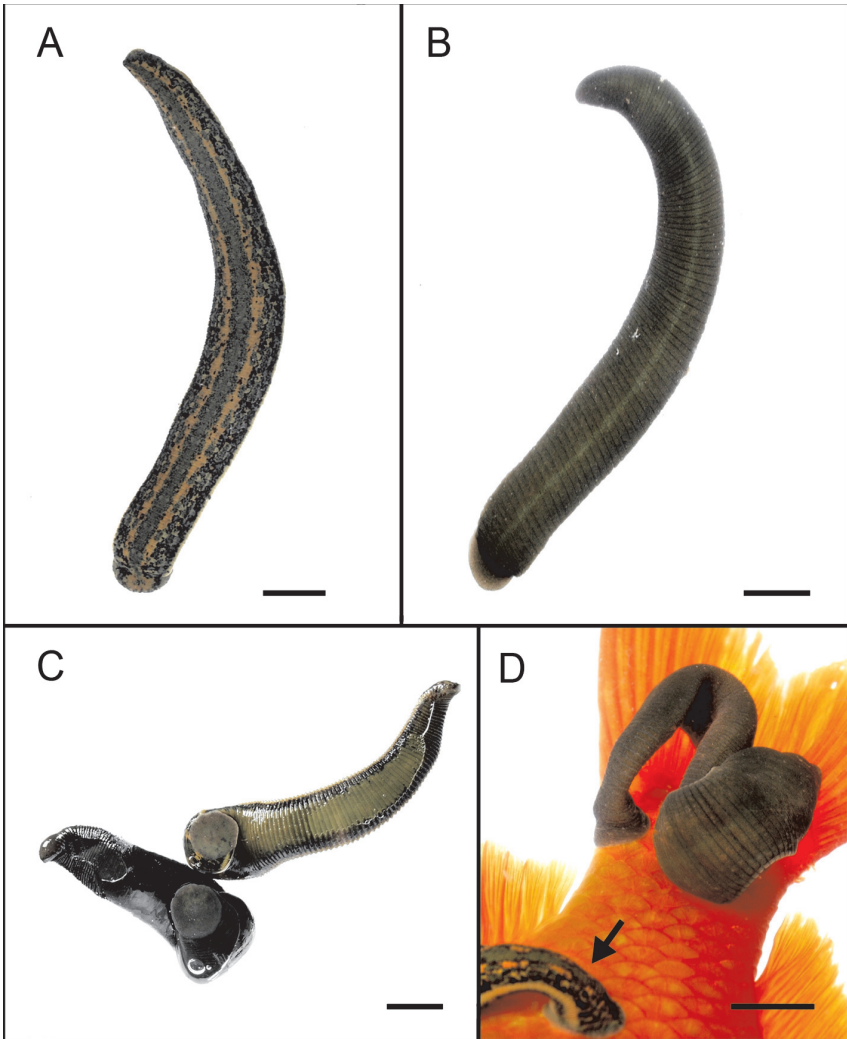
## 3 Results

A representative specimen of *Hirudo verbana* (Carena 1820) is depicted in figure 1A. Adult leeches of this taxon show the following characteristics: length at rest 80–100 mm, width 10–12 mm, fully extended up to 140 mm long; body mass  $1,41 \pm 0,18$  g ( $n = 6$ ). Dorsal side: basal colour green-yellow with a red/orange-pigmented longitudinal reticulum. Ventral side: yellow-green with no (or very few) dark spots and a pair of black marginal stripes (Fig. 1C). This typical form was described by Carena (1820) as the coloured taxon *Hirudo verbana* (nominat species).

Within these *H. verbana*-populations individual leeches were found that are dark pigmented (Fig. 1B) and characterized as follows. Length of adult individuals at rest 82–105 mm, width 10–12 mm, fully extended up to 143 mm long; body mass:  $1,42 \pm 0,24$  g ( $n = 6$ ). Dorsal side: dark grey-black with one green-grey median longitudinal stripe. Ventral side: uniformly dark-grey-black with no spots (Fig. 1C). This taxon is described here as the dark-pigmented colour variant *Hirudo verbana* "var. *nigra*".

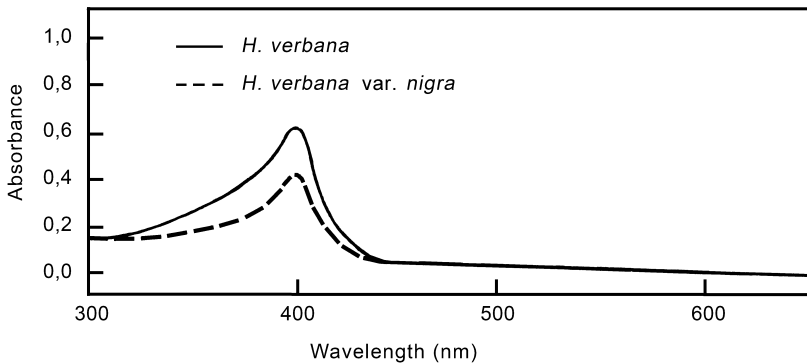
Individuals of both varieties rapidly attack dying fish (Fig. 1D) when hungry and suck blood from the skin of humans (results not shown).

Coloured and black "varieties" of *H. verbana* are characterized by a dark green-grey body pigment (or mixture of pigments) that can be extracted with 70 %



**Fig. 1: Adult individuals of typical (coloured) *Hirudo verbana* (A) and dark-pigmented *H. verbana* "var. nigra" (B), dorsal view. The same leeches are shown in ventral view, *H. verbana* "var. nigra" left; *H. verbana* right (C). Two adult individuals of *H. verbana* "var. nigra" and a *H. verbana* (arrow) feeding on a dying gold fish (D). Bars = 1 cm**

ethanol at room temperature. The absorbance spectra of tissue extracts show a single peak with a maximum at a wavelength of 400 nm (Fig. 2). The pigment concentration in extracts of *H. verbana* "var. *nigra*" is about two times higher compared with that of the coloured form (*H. verbana*). Since tissue pigments are of considerable taxonomic significance (Needham 1966) these data (Fig. 2) document the close phylogenetic relationship between the "varieties" investigated here. As a control, tissue pigments, extracted from two other dark grey-black coloured leech species, as described above, were analyzed (*Haemopsis sanguisuga* and *Trocheta baskonis*). No absorbance maximum at 400 nm was measured in these samples. A large and broad peak occurred around at 250–300 nm (results not shown).



**Fig. 2: Absorbance spectra of tissue pigments extracted from an adult *H. verbana* and a *H. verbana* "var. *nigra*"-individual, respectively. The pigments were extracted with 70 % ethanol and diluted 1 : 10 before the measurements were carried out**

Within the *H. verbana*-populations (more than 500 adult leeches, thousands of juveniles), the coloured form "var. *verbana*" accounted for >99 % of the individuals. Only 1 to 5 black individuals were found among ca. 1000 adult leeches, i.e., this dark variant represents less than 1 % of the population. Over the past year, several individuals were discovered that are intermediates between the taxon *H. verbana* (coloured form) and the "var. *nigra*". These rare leeches have a dark-grey pigmented dorsal side with a faint colour pattern that is characteristic of "var. *verbana*". On the ventral side of their body the dark marginal stripes (Fig. 1C) are broader than in the typical *H. verbana*-individual and the number of dark spots is larger (results not shown).

#### 4 Discussion

In this contribution the black "colour variant" of the medicinal leech *Hirudo verbana* is described as *H. verbana* "var. *nigra*". This conclusion is based on the

following facts: 1. both taxa have about the same body size and -mass; 2. they occupy the same habitats (ponds in Turkey; the exact locations are not known); 3. both leech-"varieties" feed on the same host organisms with indistinguishable intensities (fish, humans etc.); 4. they possess the same tissue pigments, but at different concentrations, and 5. some intermediate colour variants (i.e. dark-grey *H. verbana*-individuals) have been found. This last observation indicates that individuals of these colour varieties occasionally copulate and produce viable young. Hence, the apparent "sub-populations" are not reproductively isolated units but can exchange genes.

In addition, a DNA analysis yielded identical 660 bp sequences for both taxa (DNA-barcoding, analysis of the mitochondrial gene cytochrome *c* oxidase subunit I) (unpublished results). These molecular data indicate that the coloured and black leeches (Fig. 1A-C) belong to the same biospecies, *Hirudo verbana* (see Pfeiffer et al. 2004).

According to Mayr and Ashlock (1991) the subspecies is the rank immediately subordinate to a species. It is defined as an "aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of that species". This definition does not apply to the dark-pigmented *H. verbana*-individuals described here, because occasionally single black individuals were discovered in the same population (i.e., the ponds of the leech farm). Although the term "variety" is no longer used by animal taxonomists (Mayr and Ashlock 1991), we use this word here in the sense of "over-pigmented colour variant". Like albinos (white individuals that lack certain tissue pigments), our "var. *nigra*" are pigment variants that look like a "separate species", but are members of the same reproductive community (biospecies) as their coloured conspecifics.

The taxa *H. verbana* (coloured) and *H. verbana* "var. *nigra*" (black) occur in the same ponds in Turkey from which they were caught by commercial leech gatherers. It is not known whether the coloured and black *Hirudo*-individuals inhabit different regions within these ponds. Moreover, we do not know whether or not the dark pigmented leeches have a better chance of survival in the wild (protection from certain predators, due to their "soil-like" colour). Investigations of free-living *Hirudo verbana*-populations are necessary to further explore the reasons why black leeches regularly occur at low abundance in these aquatic habitats.

Elliott & Tullett (1992) investigated natural relict populations of *H. medicinalis* in England, but did not report the occurrence of black individuals. Since no dark-pigmented "true" medicinal leeches are described in the literature (Herter 1968, Sawyer 1968, Nesemann & Neubert 1999) it is likely that this unique colour variant only occurs in the sister taxon *H. verbana*, the neglected annelid

from Lacus Verbanus (Kutschera 2004, 2006, Trontelj et al. 2004). Finally, it should be noted that Moquin-Tandon (1846, p. 331) mentioned the taxon "*H. medicinalis* var. *nigrescens*" in his classical monograph. It is likely that these dark-pigmented leeches are identical to the taxon *H. verbana* "var. *nigra*" described here. However, proof for this assumption is currently lacking.

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### References

- Carena, H. (1820): Monographie du genre *Hirudo* ou description des espèces de sangesues qui se trouvent ou qui sont en usage en piémont, avec des observations sur la génération, et sur d'autres points de l'histoire naturelle de quelques unes de ces espèces.- *Memorie della Reale Accademia delle Scienze de Torino* 25: 273-316, Turin
- Elliott, J. M. & P. A. Tullett (1992): The medicinal leech.- *Biologist* 39: 153-158, London
- Herter, K. (1968): Der medizinische Blutegel und seine Verwandten.- *Neue Brehm-Bücherei* Nr. 381, 172 pp. (A. Ziemsen Verlag), Lutherstadt Wittenberg
- Kutschera, U. (2004): Species concepts: leeches versus bacteria.- *Lauterbornia* 52: 171-175, Dinkelscherben
- Kutschera, U. (2006): The infamous blood suckers from Lacus Verbanus.- *Lauterbornia* 56: 1-4, Dinkelscherben
- Kutschera, U. & M. Roth (2006 a) Cocoon deposition and cluster formation in populations of the leech *Hirudo verbana* (Hirudinea: Hirudinidae).- *Lauterbornia* 56: 5 - 8, Dinkelscherben
- Kutschera, U. & M. Roth (2006 b) Notes on the ecology of the Asian medicinal leech *Hirudinaria manillensis* (Hirudinea: Hirudinidae).- *Lauterbornia* 56: 9-13, Dinkelscherben
- Mayr, E. & P. D. Ashlock (1991): *Principles of Systematic Zoology*. 2. Ed.- 475 pp. (McGraw-Hill, Inc.) New York
- Moquin-Tandon, A. (1846) *Monographie de la famille des Hirudinées*. 2. ed.- 448 pp., Paris
- Needham, A. E. (1966): The tissue-pigments of some fresh-water leeches.- *Comparative Biochemistry & Physiology* 18: 427-461, Oxford
- Nesemann, H. & E. Neubert (1999): Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea. In: Schwoerbel, J. & P. Zwick (eds.): *Süßwasserfauna von Mitteleuropa* 6/2, 178 pp. (Spektrum), Heidelberg
- Pfeiffer, I., B. Brenig & U. Kutschera (2004) The occurrence of an Australian leech species (genus *Helobdella*) in German freshwater habitats as revealed by mitochondrial DNA sequences.- *Molecular Phylogenetics and Evolution* 33: 214-29, San Diego
- Sawyer, R. T. (1986): *Leech Biology and Behaviour*. 3 Vols.- 1065 pp. (Clarendon Press), Oxford.
- Trontelj, P., M. Sotler & R. Verovnik (2004): Genetic differentiation between two species of the medicinal leech, *Hirudo medicinalis* and the neglected *H. verbana*, based on random-amplified polymorphic DNA.- *Parasitology Research* 94: 118-124, Berlin

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