Habitat preferences and ventral color variability of *Hirudo medicinalis* (Clitellata: Hirudinida)

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**Abstract**

This study deals with the habitat preferences of medicinal leech *Hirudo medicinalis* Linnaeus, 1758 in the Czech Republic, in a highly agriculturally used and fragmented landscape, and provides the detailed information about the ventral coloration of this species. The study was conducted in 2010–2011 at 11 out of total 15 localities of *H. medicinalis* in the Czech Republic. At each locality a phytocenological sample of littoral and aquatic vegetation was recorded and physicochemical variables were measured. All captured leeches were measured and weighted, and a photo of ventral side was taken. Division of localities based on aquatic vegetation (TWINSPAN) formed groups of localities which corresponded to the population sizes of *H. medicinalis*. According to the abiotic factors localities with *H. medicinalis* were rich in carbon, poor in oxygen and situated in an area with warm climate. In this study four different ventral color patterns were described. During the ontogenetic development ventral side becomes darker, as indicated by the significant relationship between the percentage of dark color and leech size.

**Key words**: medicinal leech, suitable locality, aquatic vegetation, color pattern, ontogenetic development.

**Introduction**

*Hirudo medicinalis* Linnaeus, 1758 (Clitellata: Hirudinida) is a blood sucking ectoparasite distributed in the Western Palaearctic. Among the twenty European countries with records of *H. medicinalis*, the species was most frequently reported from the Netherlands, Poland and Latvia (Ayres & Comensaňa Iglesias 2008, Utevsky *et al.* 2010a, Kasparek *et al.* 2000). The population sizes within distribution area vary under the influence of local conditions, historical effects and anthropogenic interventions. Generally, typical biotope of *H. medicinalis* is a shallow standing water body with rich aquatic vegetation, muddy substratum, and occurrence of amphibians as hosts for juvenile leeches (Elliott & Tullet 1984, Sawyer 1986, Arnold 1993, Grosser 2006). Well developed littoral vegetation can also serve as a refugium for resting leeches (Zbikowski & Kobak 2007). Exact vegetation composition has been poorly described with most often recorded *Typha* sp. (Buczynski *et al.* 2011) or *Carex lasiocarpa*, *Juncus effusus*, *J. conglomeratus*, *Typha angustifolia*, *Phragmites australis*, *Alisma plantago-aquatica* and *Sagittaria sagittifolia* (Buczynski 2003). In several countries, such as northwestern Germany and Poland, medicinal leeches are relatively common in lakes and mires (Jueg 2009, Buczynski *et al.* 2008). In those cases where landscape is overall filled with water and composed of many marches and small water bodies, leeches can inhabit untypical, often man-made habitats: channels, reservoirs, polluted or strongly eutrophic water bodies in towns (Buczynski *et al.* 2011).
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2008, Koperski 2009) due to mass effect (Townsend 1989). Little is known about the chemistry of waters inhabited by H. medicinalis; in scattered sources pH of 6.9–8 and conductivity of 110–1392 µS.cm⁻¹ was documented (Maquet 1985, Arnold 1993). Current distribution of H. medicinalis has been severely affected by the intensive usage for medicinal purposes during the 19th century. These influences together with the extensive destruction of natural wetlands by drainage and flood-control management lead in some countries into present status of this species as critically endangered, e.g. in the Czech Republic (Schenková & Košel 2005).

Low densities and fluctuations of populations of H. medicinalis in the Czech Republic resulted in the regular monitoring of this species, during which unusual color variability was recorded. Color pattern of dorsal and ventral part of the body is the basic identification feature of four Western Palaearctic species (Utevsky et al. 2010a). Dorsal pattern of these species is similar with orange or reddish stripes interrupted by black dots on olive green background (Utevsky & Trontelj 2005). Three of four congeners (H. medicinalis, H. orintalis Utevsky and Trontelj, 2005 and H. troctina Johnson, 1816) can be distinguished by thickness of stripes and shape of dark spots, which are not easy distinguishable features. Better diagnostic features are on ventral side, where only H. verbana Carena, 1820 has unicolored yellow-greenish ventral part with two black marginal stripes. H. medicinalis and H. troctina have ventral part with irregularly arranged and sized black dots on yellow or greenish background with dark marginal stripes, which are straight in H. medicinalis and zigzag-shaped in H. troctina. H. orientalis is unique by possessing metameric pairs of light-colored markings on black background (Utevsky & Trontelj 2005). Greatest distribution range overlap has been recorded for H. medicinalis and H. verbana (Utevsky et al. 2010a, Kasparek et al. 2000), but due to a trade with medicinal leeches, introduction of non-native species can be expected. A single Hirudo species, which was recorded in the Czech Republic so far, H. medicinalis, typically has three pairs of narrow longitudinal orange or reddish stripes with elongated metameric black dots on dorsal side. Each of the stripes possesses particular variability and their mutual combinations were analyzed several times (Moquin-Tandon 1846, Kaczmarek 1968, Utevsky et al. 2010b). Ventral color is composed of dark irregular spots on bright yellow background, and varies from small number of small dark dots to predominance of dark color with occasional yellow spots (Lukin 1976, Neubert & Nessemann 1999). On the contrary to the frequent interest in dorsal pattern, only a single recent paper from Ukraine deals also with the polymorphism of ventral patterns (Utevsky et al. 2010b) and describes five color patterns of dark spots distribution on ventral side (Fig. 1) (Utevsky et al. 2010b). The coloration is connected with geographic distribution and should be therefore used in geographical studies or microevolution processes investigation (Lukin 1976, Utevsky et al. 2010b).

Recently, populations of H. medicinalis are in many central European countries scattered and restricted to small refugia within agriculturally utilized landscape. The aim of this study was therefore to analyze physicochemical and biological conditions for successful development and reproduction of this species. For the improvement of H. medicinalis identification detailed evaluation of ventral color variability and its relationship to leech individual development were analyzed.

Figure 1. Ventral color patterns of Ukrainian Hirudo medicinalis (Utevsky et al. 2010b).

Material and Methods

Leeches were sampled at 11 out of total 15 localities of H. medicinalis in the Czech Republic (Fig. 2). At each locality two collectors in fisherman boots were disturbing water for one hour to attract leeches, which were caught by a strainer while swimming or picked up from the boots. Subsequently, the maximal length and weight of each specimen were recorded. After measurements, each leech was put into the small aquarium and the photo of its ventral part was taken.

At each locality a phytocenological sample on 10 m long and 3 m wide area of littoral vegetation was recorded using Braun-Blanquet abundance scale (Van Der Maarel 1979), basic physical-chemical variables such as water pH, water conductivity and oxygen volume were measured in situ, other chemical
variables (organic (TOC), inorganic (IC) and total carbon (TC) content, total nitrogen content (TN), Fe, \( \text{NH}_4^+ \), \( \text{NO}_3^- \) and \( \text{PO}_4^{3-} \) ions) were analyzed from water samples in laboratory (Tab. 1).

**Figure 2.** Distribution of *Hirudo medicinalis* in the Czech Republic. 15 localities are represented only by 10 points in the map because of their close distance.

**Statistical analysis**

Based on the composition of littoral vegetation, localities of medicinal leeches were divided by modified TWINSPIAN analysis (Roleček *et al.* 2009) in JUICE program (Tichý 2002) on presence-absence data. Diagnostic species for each group were those exceeding fidelity value of 55. To analyze physical and chemical variables, 23 localities from another leech study (Kubová & Schenková 2014) in the Czech Republic were added. Data from those localities were collected by the same methods as in the present study. The GPS position was directly measured in the field and used to obtain the altitude and the climatic conditions (i.e. average annual air temperature, average annual precipitation and area data) based on Tolasz (2007), in the ArcGIS 8.3 program (ESRI 2003). Principal Components Analysis (PCA) was run on the extended data set: environmental variables of localities with *H. medicinalis* and additional 23 localities with only other Hirudinida species. All 17 explanatory variables were subjected to the PCA on the correlation matrix (centered and standardized) to reveal the main gradients of environmental variation and to show the relations among variables. The data were log-transformed as \( Y = \log (n+1) \). Multiple regression of physical-chemical variables with ordination axes, i.e. variables fitting, was calculated and tested by 999 permutations in R software (version 3.0.2, R Core Team, 2013), program package „vegan“ (Oksanen *et al.* 2011).

Photos of 44 medicinal leeches’ ventral coloration were analyzed in program NIS-ELEMENTS D 3.2. Because the ventral coloration of head and caudal parts differed from the rest of leech belly, middle part of the body was used for the analysis. Photo of 10 annuli area between 10 and 20 body sommite entered the program, which computed percentage of dark color. Relation between leeches length (substitute variable for leech age) and percentage of ventral dark color was explained by linear regression model in R software (version 3.0.2, R Core Team, 2013), where angular transformation of color data was used.
Table 1. Physical-chemical variables measured at studied localities. Localities legend: 1 Černá jezera temporary pool, 2 Božíká pond, 3 Kutnar pond, 4 Zámecký pond, 5 Rybníček pond, 6 Písečný pond, 7 Sekulská Morava dead river arm, 8 Moravičany pool, 9 Pastvisko wetland, 10 Ostrovec pond, 11 Zahradníkovo rameno dead river arm.

| Localities | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|------------|---|---|---|---|---|---|---|---|---|----|----|
| NH$_4$+ (mg.L$^{-1}$) | - | 0.24 | 1.35 | 0.39 | 0.90 | 0.71 | 1.62 | 1.29 | 0.85 | 1.70 | 0.43 |
| NO$_3$- (mg.L$^{-1}$) | - | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.52 | 0.00 | 0.00 |
| PO$_4$3- (mg.L$^{-1}$) | - | 0.10 | 0.00 | 0.08 | 0.04 | 0.13 | 0.84 | 0.08 | 1.27 | 0.12 | 0.12 |
| Total carbon content (mg.L$^{-1}$) | - | 90.36 | 60.07 | 40.54 | 58.69 | 109.70 | 89.65 | 59.09 | 89.48 | 55.25 | 63.36 |
| Inorganic carbon content (mg.L$^{-1}$) | - | 50.88 | 46.14 | 25.55 | 19.33 | 76.39 | 63.04 | 25.15 | 47.54 | 8.09 | 37.43 |
| Total organic carbon content (mg.L$^{-1}$) | - | 39.48 | 13.93 | 14.99 | 39.36 | 33.31 | 26.61 | 33.94 | 41.94 | 47.16 | 25.93 |
| Total nitrogen content (mg.L$^{-1}$) | - | 0.90 | 0.69 | 1.03 | 2.02 | 1.59 | 2.24 | 2.38 | 1.04 | 2.25 | 0.54 |
| Fe (mg.L$^{-1}$) | - | 0.00 | 0.04 | 0.04 | 0.14 | 0.07 | 0.00 | 0.22 | 0.11 | 0.34 | 0.00 |
| Conductivity (µS.cm$^{-1}$) | 649.00 | 713.00 | 462.00 | 557.00 | 324.00 | 921.00 | 559.00 | 181.50 | 666.00 | 97.10 | 433.00 |
| O$_2$ volume (mg.L$^{-1}$) | 0.80 | 3.22 | 4.48 | 3.80 | 12.24 | 3.67 | 0.42 | 2.01 | 3.10 | 7.01 | 1.39 |
| O$_2$ saturation (%) | 11.60 | 27.40 | 103.40 | 141.30 | 158.60 | 45.50 | 6.50 | 26.30 | 101.50 | 90.60 | 52.10 |
| pH | 7.32 | 8.13 | 8.35 | 8.60 | 7.38 | 8.03 | 7.16 | 6.39 | 6.29 | 6.41 | 7.33 |

Results

Littoral and aquatic vegetation

According to littoral and aquatic vegetation, medicinal leeches inhabited diverse localities in the Czech Republic. They included water bodies with high plant species richness and well developed vegetation cover, as well as very poor localities with low vegetation cover. TWINSPAN analysis based on aquatic vegetation divided localities into three groups (Fig. 3), which corresponded to population sizes of _H. medicinalis_ recorded there.

Group 1: Diagnostic species were _Potamogeton pusillus_ agg., _Urtica dioica_, _Spirodela polyrhiza_, _Ranunculus sceleratus_, _Potamogeton pectinatus_, _Persicaria minor_, _Persicaria lapathifolia_, _Callitriche hamulata_, _Acer campestre_, and _Bidens frondosa_. This group included two dead arms of the Morava River. Localities were poor in littoral vegetation but extremely rich in aquatic vegetation _Lemna minor_ and _Spirodela polyrhiza_. According to our observations this type of localities was very suitable for _H. medicinalis_, with regular records almost every year.

Group 2: Diagnostic species were _Potamogeton lucens_, _Carex riparia_, _Equisetum arvense_, _Ceratophyllum demersum_. This group included four ponds and one temporary pool, mostly with low plant species richness. All localities were poor in littoral vegetation but extremely rich in aquatic vegetation _Lemna minor_ and _Spirodela polyrhiza_. According to our observations this type of localities was very suitable for _H. medicinalis_, with regular records almost every year.

Group 3: Diagnostic species were _Juncus effusus_, _Glyceria fluitans_, _Poa palustris_, _Myosotis palustris_ agg., _Utricularia australis_, _Scutellaria galericulata_, _Ricciocarpus natans_, _Alnus glutinosa_, _Agrostis stolonifera_. All water bodies in this group were similar in gentle shores and very high plant species richness. These localities seem to be very suitable for _H. medicinalis_, which was proved by our previous observations, too. Leeches at these localities were recorded regularly during every control between 2005 and 2011. The
most stable medicinal leech population in the Czech Republic is present in the Pastvisko wetland which belongs to this group.

![Figure 3](image)

**Figure 3.** Groups of localities divided by TWINSPAN, based on littoral and aquatic vegetation data.

**Physical-chemical conditions**
In the PCA analysis on extended dataset of leech localities (with and without *H. medicinalis*) the first axis accounted for 32%, the second for 21%, the third for 9% of total variability. Among 17 tested variables, 10 were significant (Tab. 2). Localities with *H. medicinalis* were clustered in one quadrant of the ordination space of the first and the second axes, which showed their mutual similarity. High amount of all forms of carbon (TC, IC and TOC), high conductivity, temperature and low altitude and oxygen concentration were typical for *H. medicinalis* localities (Fig. 4).

**Table 2.** Coefficients of determination of 10 significant variables from PCA analysis. Significance was proved at p = 0.001.

| variable                              | coefficient of determination |
|---------------------------------------|-----------------------------|
| Altitude                              | 0.88                        |
| Average annual air temperature        | 0.86                        |
| Total carbon content                  | 0.84                        |
| Total organic carbon content          | 0.70                        |
| Oxygen volume                         | 0.77                        |
| Average annual precipitation          | 0.74                        |
| Oxygen saturation                     | 0.72                        |
| Conductivity                          | 0.70                        |
| pH                                    | 0.66                        |
| Inorganic carbon content              | 0.48                        |

**Ventral coloration**
Four different color patterns (Fig. 5) were distinguished at ventral body part of 52 individuals from seven localities. The light pattern (I) was predominantly light with small black dots on yellow background. Black marginal stripes were sometimes present. The marbled pattern (II) had evenly distributed yellow and black color, approximately 50:50. Black color at the edges of the body sometimes created marginal stripes.
similarly as in the light pattern. The transitional pattern (III) was characteristic for marginally accumulated yellow color forming running dots on black belly. The dark pattern (IV) was predominantly dark, with only two marginal lines of yellow dots.

Figure 4. 1st and 2nd axes of PCA for significant variables; □ localities with *Hirudo medicinalis*, ○ localities without *Hirudo medicinalis*. Level of chemical-physical variables is marked by size and darkness of symbols (large and dark symbol means high value of variable).

Figure 5. Ventral color patterns of *Hirudo medicinalis* in the Czech Republic.
Positive correlations between percentage of the ventral dark color and leeches’ body length, and body weight, respectively, were significant (Spearman correlation coefficient $r_s=0.72$; $p<0.001$, and $r_s=0.71$, $p<0.001$, respectively). However, assessment of the ontogenetic development based on leech weight can be inaccurate, because small leeches can increase their weight after feeding several times (Dickinson & Lent 1984, Sawyer 1986) and be thus heavier than the oldest leeches. We therefore used the leeches’ length for the further analysis. Relationship between percentage of ventral dark color and body length was described by model of linear regression ($F_{42}=41.3$; $r^2=0.5$; $p<0.001$) (Fig. 6). The results showed that the ventral part of leeches is getting darker during their lifetime. Simultaneously, their ventral color pattern changes, too (Fig. 7). Small leeches (up to 7.5 cm) had relatively variable ventral color. This length category was the only one with the light pattern, on the other side, we found juvenile specimen with marbled and transitional pattern as well. Dark pattern started to appear on some of the bigger leeches (8–10.5 cm), and from the length of 11 cm, more than half of specimens bore this pattern. The last category was typical with low color variability; only the marbled and the dark pattern were observed, expected transitional being absent.
Discussion

The importance of shallow water bodies rich in aquatic vegetation for successful development of *H. medicinalis* populations (Elliott & Tullet 1984, Neubert & Nessemann 1999, Ausden *et al.* 2002) was confirmed by our results. Hostility of localities without vegetation cover can be caused by low densities of amphibians, which represent essential source of blood for juvenile leeches’ feeding (Mann 1955, Sawyer 1986). Frequently mentioned character of localities as “small” water bodies (e.g. Kutschera & Elliott 2014) does not refer to their area but water depth, which is often lower in smaller water bodies thus enabling the growth of macrophytes; there is no reason why leeches would not prefer large shallow wetlands with well developed littoral and aquatic vegetation. Unfortunately, without local protection, such localities are disappearing from agricultural landscape in Central Europe. For the conservation purposes there is a crucial need for precise specification of suitable localities.

Division of localities according to the vegetation corresponded with the frequency of leech records within particular groups. Although the vegetation composition did not influence medicinal leech directly, it reflected suitability of particular habitats. Dead river arms (group 1), so called parapotamon (Ward & Stanford 1995), are important localities with respect to potential spreading of the leeches into surrounding aquatic habitats, as they are interconnected with the main channel during floods. On the other hand, cocoons deposited on banks above the water level can possibly be washed away during floods. Fortunately, floods come usually during early spring in south Moravia, and therefore they do not disturb leech reproduction in May. Diagnostic vegetation species of this group have broad ecological valences. Massive occurrence of pleustophytes *Lemma minor* and *Spirodela polyrhiza* is dependent on the relatively stable water level (Chytrý 2011) which is crucial also for *H. medicinalis* occurrence. These pleustophytes cover the whole water surface, thus limiting primary production in water. As a result of poor oxygen conditions fish assemblages are highly reduced there. In contrast to fish species, *H. medicinalis* is well adapted to oxygen deficiency (Mann 1955, Buczynski *et al.* 2008) as dorsoventral undulation helps it to intake oxygen. Mass of pleustophytes also prevented water birds to see prey, so the predation pressure at leeches was low.

Group 2 with diagnostic species typical for eutrophic waters with high amount of nutrients and muddy bottom (Chytrý 2011) did not provide so friendly conditions for medicinal leeches as localities in group 1. Except of the temporary pool, all ponds had steep shores, which were inconvenient for leeches. There is a huge difference between bank and littoral humidity, so the cocoons and hatchlings can have problems to survive at dry conditions. High human activities in ponds, including fishing, resulted into irregular and low leech records within this group. Two localities were less suitable because of their instability – Černá jezera is a temporary pool, and Ježírko Kutnar is a small pond absolutely separated from the main river channel and unfortunately affected by unsuitable management (mud removing during winter).

Group 3 with diagnostic species of wetlands, wet meadows, mires and fens, which tolerate regular inundation (Chytrý 2011), included well preserved localities with high species diversity, gradual littoral and sufficient amount of amphibians. These parameters render localities of group 3 as suitable for leeches. Dense vegetation cover is a source of high organic carbon, which was generally found as typical feature of *H. medicinalis* localities (Fig. 4). One locality is geographically separated from others by 88.3 km distance as a relic of former medicinal leech distribution. Cover of pleustophytes similar to dead river arms of group 1 and difficult accessibility by humans, because this locality lies in the heart of the protected area, are probably the reasons for successfully surviving *H. medicinalis* there.

Analysis of the ventral color pattern revealed four different color patterns (Fig. 5), not mentioned in the identification keys (e.g. Neubert & Nessemann 1999, Sawyer 1986). Quite surprisingly we found that one of the four observed patterns – the dark pattern – was almost indistinguishable from the ventral color pattern of *Hirudo orientalis* as also Utevsky *et al.* 2010b reported. The reason for such similarity can be explained with their close phylogenetic position on the tree of the genus *Hirudo*, where they are sister groups and their parapatric occurrence was recently found, too (Trontelj & Utevsky 2012). These species are even able to breed and have live hybrid offspring in the laboratory conditions, but decrease of fecundity suggests some reproductive barriers (Petrusek et al. 2009). Similar color variability as in our specimens was observed in juvenile leeches of *H. orientalis* from Iran, where the same four color patterns as in our study were observed in young *H. orientalis*; typical ventral color of this species appeared in adult individuals (Masoumeh Malek, pers. communication). Four color patterns which we found are similar to those of *H. medicinalis* from Ukraine (Utevsky *et al.* 2008), except that the presence of dark marginal stripes at the light color pattern, which was regular in Ukraine and only occasional in our specimens. The only difference against Ukraine was
the presence of fifth color pattern with the reduction of yellow marginal dots and prevalence of dark color which was not recorded in specimens from the Czech Republic. For the first time we documented that the percentage of dark color significantly increases along the ontogenetic development of leeches (Fig. 6). This trend can be explained by accumulation of metabolites from blood processing during lifetime, which was also observed in another hematophagous species (Sawyer 1986, Capinera 2008). The smallest leech size category showed greatest color variability, which should be a good anti-predation strategy, because smallest leeches have broader spectrum of enemies. Similar color polymorphism occurring solely in juveniles was observed in other invertebrates, such as crabs (Krause-Nehring et al. 2010).

To conclude, in central European countries habitats of *H. medicinalis* are specific localities with well-developed vegetation which deserve special protection. Agricultural use of the landscape disturbs historical interconnection between localities and remaining suitable habitats start to act as islands in unsuitable landscape matrix, with the increasing risk for endangered species. As concerned ventral color of *H. medicinalis* and *H. orientalis*, their similarity is higher, than it is reported in identification keys (e.g. Neubert & Nessemann 1999, Sawyer 1986) and all four color patterns found in our study should be included there.

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