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TRANSITIONAL MORPHOLOGY IN HYBRIDS OF *HIRUDO VERBANA* AND *H. ORIENTALIS* (CLITELLATA, HIRUDINIDA)

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Transitional Morphology in Hybrids of *Hirudo verbana* and *H. orientalis* (Clitellata, Hirudinida). Kovalenko M. V., Utevsky S. Yu. — The aim of this research was to test the ability of hybridizing *Hirudo orientalis* S. Utevsky et Trontelj, 2005 and *Hirudo verbana* Carena, 1820. We obtained offspring from both species. The color pattern of all the hybrids was uniform and quite different from both the parents. The venter was similar to *H. verbana*. The dorsum was very similar to the color pattern of *Hirudo medicinalis* Linnaeus, 1758 that was not involved to the experiment. The coloration pattern of the hybrids, which is very similar to that of *H. medicinalis* suggests the hybrid origin of this species.

Key words: *Hirudo*, medicinal leech, hybrid, speciation.

Промежуточные морфологические признаки у гибридов *Hirudo verbana* и *H. orientalis* (Clitellata, Hirudinida). Коваленко М. В., Утевский С. Ю. — Целью данной работы было исследовать способность медицинских пиявок *Hirudo orientalis* S. Utevsky et Trontelj, 2005 и *Hirudo verbana* Carena, 1820 к гибридизации. В результате эксперимента мы получили потомство от обоих видов. Окраска всех гибридов была однообразна и отличалась от окраски обоих родительских видов. Окраска вентральной стороны тела сходна с *H. verbana*. Окраска дорсальной стороны наиболее сходна с таковой у вида *Hirudo medicinalis* Linnaeus, 1758, который не участвовал в эксперименте. Значительное сходство окраски гибридов и *H. medicinalis* наводит на мысль о гибридном происхождении этого вида.

Ключевые слова: *Hirudo*, медицинская пиявка, гибрид, видообразование.

Introduction

According to the recent studies (Siddall et al., 2007; Utevsky, Trontelj, 2005; Utevsky S. et al., 2010; Trontelj, Utevsky, 2005, 2012) the genus *Hirudo* includes at least four species of medicinal leeches; three of them, *Hirudo medicinalis* Linnaeus, 1758, *H. verbana*, Carena, 1820 and *H. orientalis* S. Utevsky et Trontelj, 2005, were considered as a single species *H. medicinalis* for centuries (Moquin-Tandon, 1846; Lukin, 1976). In addition to studies of genetic differentiation, anatomy and color pattern, the qualitative and quantitative comparison of saliva compounds (Baskova et al., 2008; Hilderbrandt, Lemke, 2011) and caryological analysis (Utevsky et al., 2009) were carried out for these three species. The caryological study discovered the difference in chromosome numbers of the related species of medicinal leeches. This fact suggests some obstacle in the interspecific hybridization and yielding viable hybrids that could be caused by possible reproductive isolation. Although the hybridization in laboratory has been already attempted (Petrauskiene et al., 2009), the previous authors have concentrated on quantitative traits of hybrid offspring and reproductive biology. Moreover, the earlier experiments were carried out with adult leeches, being kept isolated for only a month before the copulation. The confidence of that the leeches have not been fertilized previously with conspecific individuals seems not to be absolute, as medicinal leeches may be able to preserve viable sperm in their reproductive system after copulation for many months (unpublished data).

Therefore, we carried out an interspecific crossing of medicinal leeches using only those individuals that definitely have never copulated within the species before. For this research two species of medicinal leeches *H. verbana* and *H. medicinalis* were applied.

Material and methods

Two species of medicinal leeches *H. verbana* and *H. orientalis* were involved into the crossbreeding experiment. Individuals of the former species were reared in captivity and originate from a lake in the Kharkiv Region, Ukraine. Individuals of *H. orientalis* originate from a pharmacy in Kharkiv, though it is known that they

came from Azerbaijan. Juveniles of both species were kept separately from their siblings to prevent copulation within each species at the early stages of the sexual maturity. For this, couples of *H. verbana* plus *H. orientalis* were combined since hatching from the cocoons and placed into 3L jars. After five feedings at the age of nine months, which was considered to be the age of sexual maturity, they were kept singly during a month before breeding. After that leeches were fed with porcine blood in sausage enclosures at 37 °C and placed in pairs for another month for copulation. Then leeches were placed into containers with moist peat for laying cocoons.

Nikon Coolpix 5400 camera was applied for photographing color patterns.

Results and discussion

Six interspecific pairs (12 individuals) were used in the experiment. Only two leeches laid cocoons: one individual of *H. verbana* laid two cocoons and *H. orientalis* — two cocoons. Both the maternal individuals were from different pairs. All other leeches appeared fruitless. Although, one more individual had swollen clitellum that usually is a sign of a leech being ready to lay cocoons, but it failed.

The experimental cross breeding yielded offspring from both species: eight specimens hatched from the cocoons of *H. orientalis* female and 6 specimens from *H. verbana* female. The number of hatchlings per cocoon appeared different from the number of hatchlings for intraspecific breeding: 4–6 individuals in hybrid cocoons comparatively to 10–15 (Lukin, 1976) or 8–10 (Petrauskiene et al., 2010) in non-hybrid cocoons. However, this difference is not statistically significant due to the small data set.

Concerning other characters of the obtained hybrids, the color pattern of offspring appeared the main and the most significant confirmation of their hybridous origin.

The venter of all the young leeches was unicolor olive greenish with only seldom few dark dots and two dark longitudinal stripes laterally (fig. 1, *a*; 4, *b*), which are always discernible. This coloration pattern is typical to *H. verbana* (fig. 2, *b*) (Nesemann, Neubert, 1999), although in this case it was a character of both 1 ♀ *H. verbana* and 1 ♀ *H. orientalis* hubrid offspring. The ventral pattern typical for *H. orientalis* (fig. 3, *b*) was absent in all hybrids.

The dorsum of *H. verbana* is green with a pair of diffused broad paramedian orange

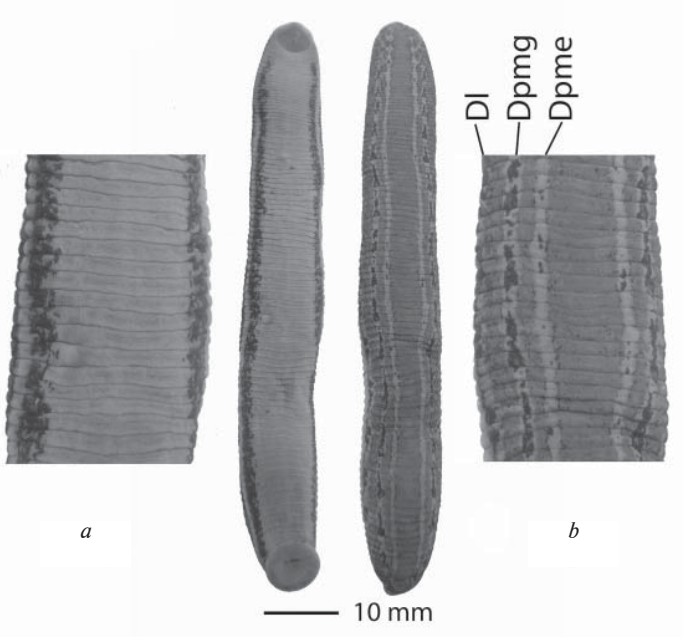

Fig. 1. Coloration of hybrids from a crossing of *H. orientalis* × *H. verbana*: *a* — venter; *b* — dorsum (Dpme, Dpmg, Dl — paramedial, paramarginal and lateral stripes).

Рис. 1. Окраска гибридов при скрещивании *H. orientalis* × *H. verbana*: *a* — вентральная сторона, *b* — дорсальная сторона (Dpme, Dpmg, Dl — парамедиальная, парамаргинальная и латеральная полосы).

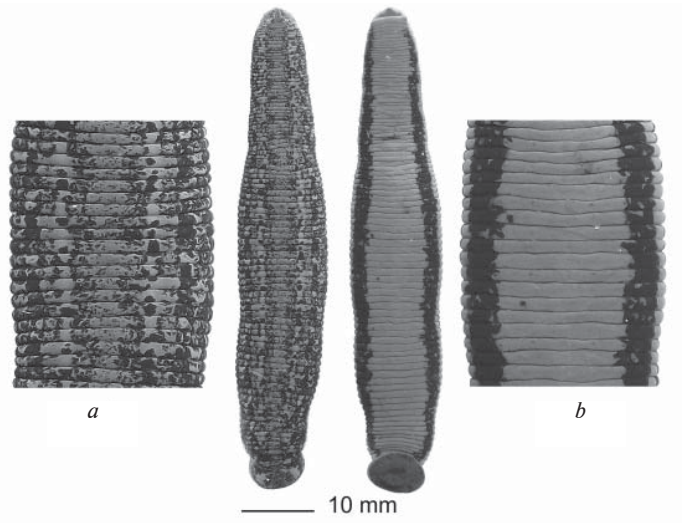

Fig. 2. Coloration of *H. verbana*: *a* — dorsum; *b* — venter.

Рис. 2. Окраска *H. verbana: a* — дорсальная сторона; *b* — вентральная сторона.

stripes that possess seldom black dots. Paramarginal stripes coalesce with paramedian stripes forming a network pattern. Paramarginal black spots are oval, diffused, segmentally arranged or merged. Lateral margins of the body are with diffused black stripes of irregular width (fig. 2, *a*). The dorsum of *H. orientalis* is grass green with three pairs of yellow or orange longitudinal stripes. Two paramedian stripes are thin with regular black dots. Paramarginal stripes are broad encompassing black segmentally arranged quadrangular or roundish spots (fig. 3, *a*). Lateral margins of the body are with yellow stripes that have regular black roundish or quadrangular spots located at the level of marginal spots (Utevsky, Trontelj, 2005).

The dorsal pattern of the hybrids is composed of reddish-orange longitudinal stripes and black spots (fig. 1, *b*). The reddish-orange paramedian and paramarginal longitudinal stripes are narrow in contrast to *H. verbana* and similar to *H. orientalis* (fig. 1, *a*; 4, *a*). Paramedian stripes are with seldom black dots or without them (fig. 1, *a*; 4, *a*). Paramarginal stripes are with elongated narrow black spots that are different from both

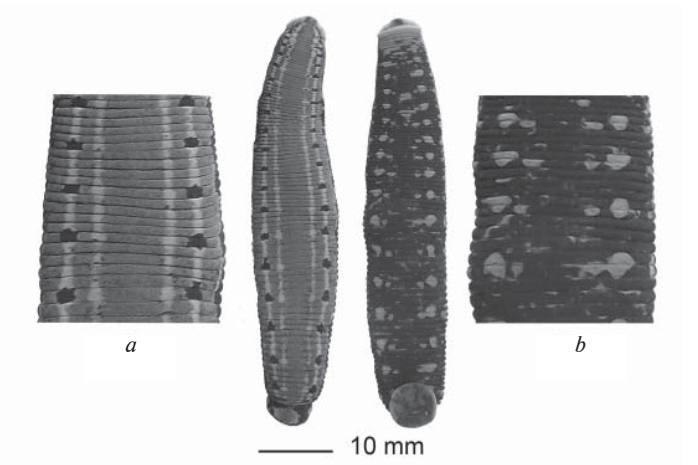

Fig. 3. Coloration of *H. orientalis*: *a* — dorsum, *b* — venter.

Рис. 3. Окраска *H. orientalis: a* — дорсальная сторона; *b* — вентральная сторона.

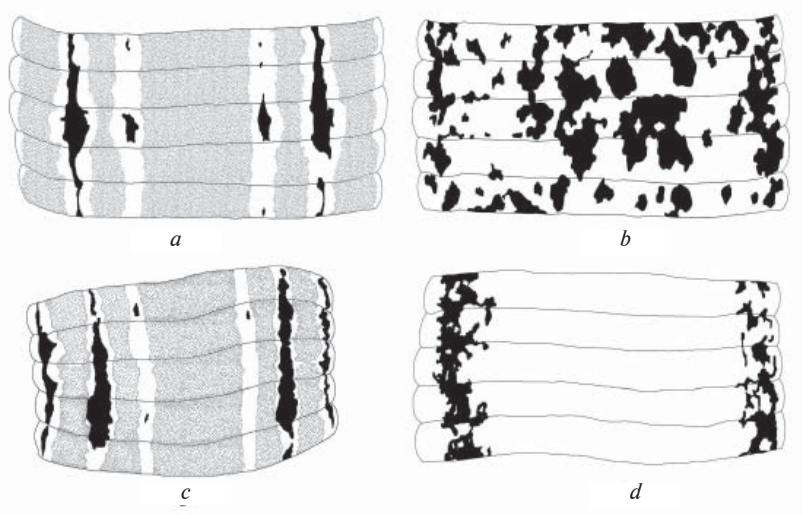

Fig. 4. Color pattern comparison of *H. medicinalis* and hybrids from a crossing of *H. orientalis* × *H. verbana* (midbody segment): *a* — hybrid, dorsal view; *b* — hybrid, ventral view; *c* — *H. medicinalis*, dorsal view; *d* — *H. medicinalis*, ventral view.

Рис. 4. Сравнение образцов окраски *H. medicinalis* и гибридов между *H. orientalis* × *H. verbana* (сегмент середины тела): *a* — гибрид, вид с дорсальной стороны; *b* — гибрид, вид с вентральной стороны; *c* — *H. medicinalis*, вид с дорсальной стороны; *d* — *H. medicinalis*, вид с вентральной стороны.

the parental species. The lateral margins of body have reddish-orange longitudinal stripes with black elongated diffused spots on them. Spots are merged into the entire stripes with seldom sites of disconnection. The shape of longitudinal stripes is more similar to *H. medicinalis* (Utevsky A. et al., 2010) (fig. 4, *c*) than to those of their parents. The coloration pattern of hybrids, which is very similar to that of *H. medicinalis* suggests the hybrid origin of this species. The hybrid origin of some invertebrate species has already been revealed and seems to be not uncommon (Mavárez, 2006). However, this has not been ever recorded for leeches and the possibility of the hybrid origin of *H. medicinalis* (fig. 4, *c, d*) should be proved using diverse methods of genetic analysis.

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