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ON THE APPENDAGE REGENERATION OF *EUPOLYBOTHRUS TRANSSYLVANICUS* (LATZEL) (CHILOPODA: LITHOBIIDAE)

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Abstract — We have analyzed and discussed the indirect evidence of regeneration of the forcipular telopodites and the walking legs (reduced in size or abnormal characteristics such as atypical morphology and a reduced number of articles) in wild populations of the lithobiomorph centipede *Eupolybothrus transsylvanicus* (Latzel). In our opinion, these morphological defects can be interpreted as a result of incomplete regeneration. In some cases, the full segmentation of the regenerating appendages will be completed later in ontogeny through an anamorphic schedule of post-embryonic segment addition.

Key words: Centipedes, regeneration, blastema, forcipular telopodite, walking leg, anamorphic development, Serbia.

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INTRODUCTION

Regeneration in Metazoa can be classified into two general groups according to the following criteria: regeneration which occurs in the absence of active cell proliferation, and regeneration which requires cell proliferation (Sánchez Alvarado, 2000). The first mode of regeneration (morphallaxis) involves the re-creation of missing body parts solely by the remodeling of pre-existing cells. An example of morphallactic regeneration is provided by hydra.

On the other hand, epimorphic regeneration requires cell proliferation and is subdivided into two broad categories: non-blastemal and blastemal-based regeneration. Non-blastemal regeneration occurs as a result of the transdifferentiation of the remaining tissue into the missing structure, limited dedifferentiation and proliferation of the surviving cells in the organ after injury or amputation, and by the proliferation and differentiation of stem cells already present in the damaged tissue. Examples of these are provided, respectively, by lens regeneration in urodele amphibians, and by liver and bone regeneration in humans.

Blastemal regeneration involves the formation of a specialized structure known as the regeneration blastema. This structure is similar in form and organization to the early embryonic limb buds produced during vertebrate embryogenesis. As in a limb bud, a regeneration blastema is made of two, well-defined compartments: a superficial sheet of cells of epithelial origin covering the full extent of the bud, and an underlying mass of cells of mesenchymal origin. Depending on the organism, the regeneration blastema may form either within hours or days after amputation or injury. The missing parts are regenerated by the eventual differentiation of the blastema. This mode of regeneration is common to planarians, gastropods, cephalopods, echinoderms, urochordates, and limb and tail regeneration in vertebrates (Sánchez Alvarado, 2000).

Arthropods, nematodes and the other molting animals do not regenerate any missing part of the main body axis, but this does not mean that they lack regenerative powers completely (Minelli, 2003). In many cases, the simplest explanation for the lack of regeneration is the lack of mitotic activity in the tissues, regions or stages in which the amputation occurred. This explanation, however, is not true for most ecdysozoans, where mitotic activity, although mainly confined to the (pre)molting phase, may last over most of the post-em-

bryonic life. The principal cause for the lack of regeneration of portions of the main body axis is possibly different — very early determination in the embryonic development of the anterior and posterior termini of the body, through expression of molecular markers that the animal is not able to re-express later in life, what would be necessary for the regeneration of missing parts of the main body axis.

There is general agreement (Eason, 1964; Lewis, 1981; Minelli et al., 2000; Maruzzo et al., 2005) that regeneration of lost appendages is widespread within centipedes, even if not present or documented in some groups. In *Scutigera* Lamarck, the regenerating leg is already complete as soon as it appears, after the first post-operative molt or a molt later, depending on the timing of the amputation within the intermolt period (Verhoeff, 1902-25; Cameron, 1926).

In *Lithobius* Leach, Verhoeff (1902-25) described the leg regeneration from the preferred breakage point as consisting at first of prefemur, femur, tibia and a tarsus of one article, and completely lacking setae, epidermal glands, muscles and tendons. With another molt the appendage becomes longer (about half the length of an undamaged leg) with a trochanter, a second tarsal article, a claw and its tendon. The musculature is also developing, although it is still gracile. Many sensory setae and epidermal glands appear as well, however, most of the spines are still missing. A further molt leads to a complete appendage that is slightly smaller than an undamaged one. Regeneration of legs is also possible from any level distal to the preferred breakage point. The mechanism does not seem to be the same in all species and stadiums: in *Bothropolys asperatus* (L. Koch), the regenerating legs have an incomplete number of segments if the damage occurred in a larva, and the full number of segments in a post-larval specimen. The antennae can regenerate too: the number of segments usually increases with subsequent molts. The number of segments shown after the first post-operative molt not only depends on the point of amputation, but also on the instar and the intermolt stage at the time of the operation (Verhoeff, 1902-1925; Scheffel, 1989; Weise, 1991).

Regeneration of the legs has never been documented through experiment in Scolopendromorpha. Indirect evidence of regeneration of the forcipule and last pair of legs (reduced size and incomplete armature of spines) has been noted (Verhoeff, 1940; Lewis, 1968). No comparable data are available for the remaining legs. As for the antennae, the species with a fixed number of antennomeres can only increase the length, but not the number of the segments after amputation. True regeneration, with an increasing number of segments, seems only possible in those species that usually add a few antennomeres during post-embryonic development. In *Scolopendra* Linnaeus, Lewis (1968) observed antennae composed of a few proximal antennomeres of expected size followed by a variable number of very short ones, and interpreted those distal articles as regenerated. In this group, the regeneration of antennomeres is far from accurate, sometimes leading to atypically high numbers. Finally, no evidence is available for the occurrence of regeneration in craterostigmomorph and geophilomorph appendages (Lewis, 2000; Minelli et al., 2000).

In the present study we have mainly limited our attention to the natural occurrence of regeneration of the forcipular telopodites and the walking legs in the lithobiomorph centipede *Eupolybothrus transsylvanicus* (Latzel). As far as information is available, we have also paid attention to the post-embryonic developmental schedules. The discovery of regenerated specimens was reported without detailed morphological and developmental analysis by Mitić (2005).

MATERIAL AND METHODS

E. transsylvanicus is widely distributed in Serbia, Romania, Croatia, Bosnia and Herzegovina, Montenegro, Albania, Bulgaria, and Greece (Latzel, 1882; Matic, 1966; Kos, 1992; Stoev, 1997, 2002; Mitić, 2001, 2002, 2005; Zapparoli, 2002; Mitić and Tomić, 2008), occurring both in natural sites and close to human

settlements. The centipede material analyzed in this study was collected from four sites in Serbia during the period 2001-2007. The first site was in a mixed oak forest in Košutnjak (Belgrade); the others were Čarapićev Brest (village of Beli Potok, Mt. Avala), Rapajlovača (village of Rošci, Mt. Kablar), and Dubočica (village of Miliće, Mt. Radočelo).

The number of analyzed specimens was 1186, distributed in the following post-larval stadia: first post-larval stadium (PL1), six specimens (or 0.51%); second post-larval stadium (PL2), 44 specimens (or 3.71%); third post-larval stadium (PL3), 38 specimens (or 3.20%); fourth postlarval stadium (PL4), 101 specimens (or 8.52%); fifth post-larval stadium (PL5), 373 specimens (or 31.45%); and adult, 624 specimens (or 52.61%). The sample was assigned to an age group using the criteria described by Andersson (1978), Kos (1997), and Mitić and Tomić (2008).

The animals with regenerated appendages were examined using light microscopy and standard methods for clearing, dissection and temporary mounting of the lithobiomorph centipedes (Lewis, 1964). The observed regenerations are shown on Figs. 1-19. These illustrations were drawn using an Ergaval binocular microscope from Carl Zeiss with objective magnification of 3.2x with the aid of a demonstration screen with 10x magnification. All specimens belong to the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade.

RESULTS AND DISCUSSION

Of the total number of field-caught specimens of *E. transsylvanicus*, 22 adults (3.5%) and one juvenile had regenerated appendages which can be recognized by their smaller size or abnormal characteristics, such as atypical morphology of the forcipular telopodites and atypical number or arrangement of spines on the walking legs. Here we present a closer analysis of the 19 individual abnormalities recorded.

PL5 (Fig. 1). An interesting example of regeneration is evident in this juvenile specimen. The regenerated forcipular telopodite is unsegmented and considerably reduced in size, probably formed at the first molt after an injury. The setae are absent.

Male (Fig. 2). The morphology of the femuroid, the tibia, the tarsus and the apical claw is atypical and the size of the regenerated telopodite is reduced. Moreover, the setae are absent in the basal segment.

Male (Fig. 3). In the analyzed centipede, the femuroid and the tibia of the regenerated forcipular telopodite are not fully demarcated. The entire appendage is reduced in size, and the setae are reduced in number.

Male (Fig. 4). In this case, we observed partial telopodite regeneration in which the segments distal to the femuroid have been lost and regenerated as indicated by their small size and absent setae in the tarsus and the apical claw.

Male (Fig. 5). As in the previous specimen, we have very small forcipular segments distal to the femuroid, but the setae are absent in all of them.

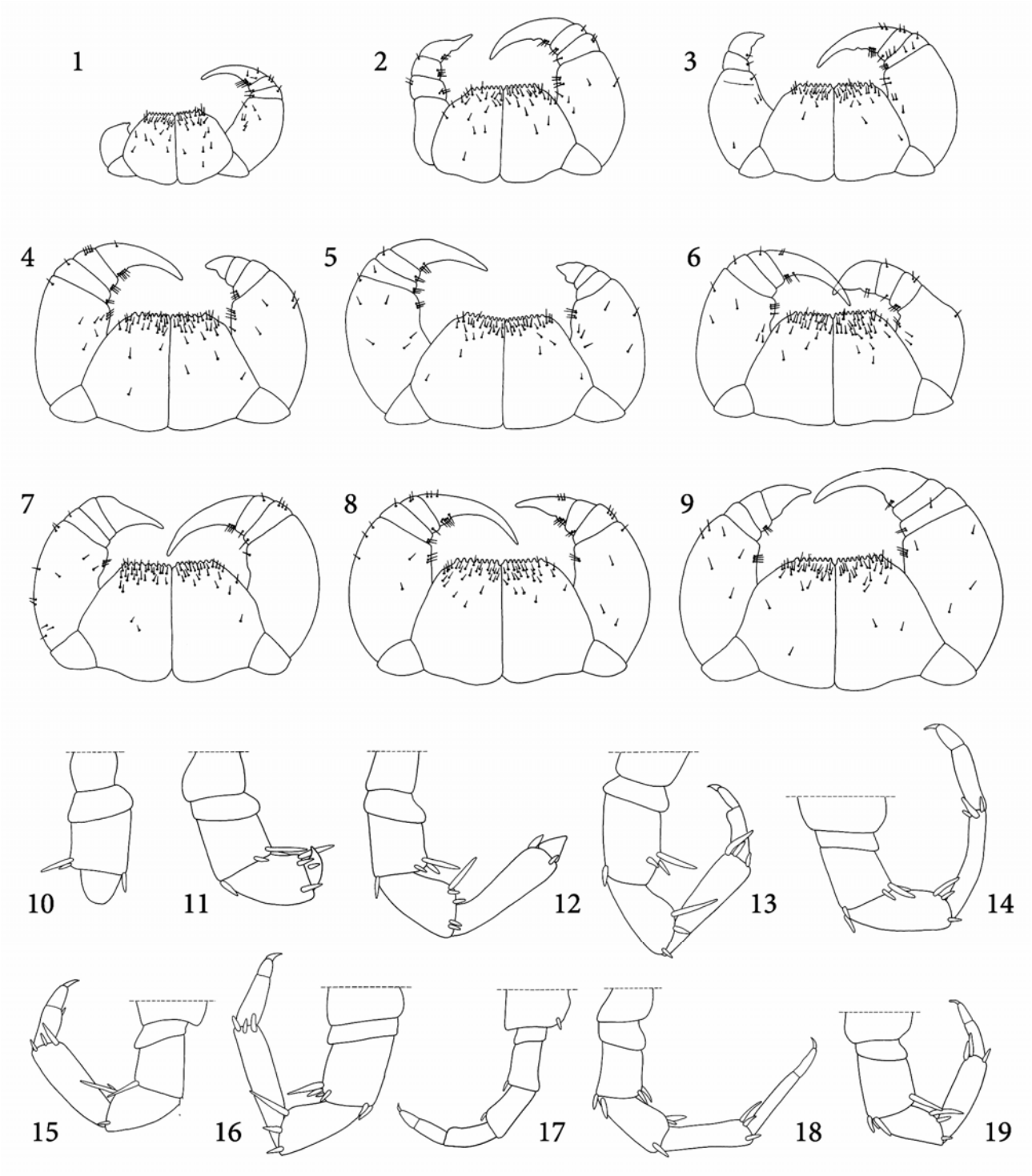
Male (Fig. 6). The regenerated forcipular telopodite is rather atypical in shape and reduced in size. In spite of this pronounced abnormality, the number of setae is reduced only in the apical claw.

Female (Fig. 7). In this adult, almost all segments of the regenerated telopodite are of normal length except for the apical claw. The setae are reduced in number in the tibia, and absent in the tarsus and the terminal segment.

Male (Fig. 8). The regenerated appendage is normal with the exception of the size of the apical claw.

Male (Fig. 9). The regenerated telopodite has normal morphology but it is reduced in size, and the setae are absent in the second and the fourth segment.

Male (Fig. 10). In this specimen, leg VI was probably formed at the first molt after an injury and



Figures 1-19.

consists of the coxa, the trochanter, the prefemur, and the regeneration blastema. The spines of the prefemur are well-developed.

Male (Fig. 11). As in the previous case, leg VI was probably formed at the first molt after an injury and is composed of the coxa, the trochanter, the prefemur, the femur, and the regeneration blastema. The leg spines are well-developed.

Male (Fig. 12). In this centipede, leg III consists of the coxa, the trochanter, the prefemur, the femur, the tibia, and the regeneration blastema. The spines of the femur and the tibia have begun to develop.

Female (Fig. 13). Leg VIII has atypical spinulation on the prefemur, as well as abnormal morphology and reduced size of the segments distal to the tibia.

Male (Fig. 14). In leg VI all segments distal to the femur have atypical size and proportion.

Female (Fig. 15). In this specimen, the segments of leg IX have an atypical proportion distal to the femur, and one spine on the edge of the tarsus which is normally spineless.

Female (Fig. 16). Leg VII has an abnormal spine arrangement on the prefemur and an atypical proportion distal to the femur.

Male (Fig. 17). In this specimen the size of the segments and the number of spines of the regenerated leg XIV are reduced.

Male (Fig. 18). The regenerated part of leg IV, distal to the tibia, shows atypical proportion.

Female (Fig. 19). As in the previous case, the regenerated articles of leg III (distal to the tibia) are out of proportion.

The analysis of the defective appendages in wild populations of the centipede *E. transsylvanicus* gives indirect support to the hypotheses (Minelli et al., 2000; Maruzzo et al., 2005) that these animals can regenerate a full appendage (both forcipular telopodites and walking legs). In our opinion, the appendage abnormalities described above can be interpreted as the result of incomplete regeneration. During this process, growth and differentiation of the epidermis, nerves, muscles and tracheae are to some extent mutually independent, thereby sometimes failing to reproduce their usual developmental interactions, with obvious consequences on the reconstruction of the lost part of the appendage (Maruzzo et al., 2005). An alternative hypothesis to explain the origin of the defective appendages in the analyzed species could be defective embryonic or post-embryonic development without any traumatic removal of the original appendage or a part of it.

From a developmental perspective, a comparison between the developmental schedules and regeneration processes in centipedes suggests intriguing relationships. Among arthropods the segmentation of the main body axis can follow two different modes. In epimorphic development all body segments are already present at the end of embryonic development, whereas in anamorphic development juveniles hatch with an incomplete complement of segments. In the latter case, the final adult number of segments is reached later in ontogeny through a specific schedule of post-embryonic segment addition (Enghoff et al., 1993). Similarly, full segmentation of the developing appendages can be complete at their first appearance, or only later in ontogeny. In the regeneration of appendages, the definitive number of segments in the regenerate (sometimes lower than the full number) is often complete within the first post-operative molt. Sometimes the number of segments increases according to an “anamorphic” schedule.

In our case, the regenerating appendage probably develops gradually with each succeeding molt. Consequently, if the injury occurs at an early stage of development an almost perfect appendage may develop, but at later stages it will be stunted and deformed. It is very important to bear in mind all these possibilities when recording the important systematic characteristics of lithobiomorphs, such as the number of antennal articles and the number and position of the leg spines.

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РЕГЕНЕРАЦИЈА ЕКСТРЕМИТЕТА КОД ВРСТЕ *EUPOLYBOTHRUS TRANSSYLVANICUS* (LATZEL) (CHILOPODA: LITHOBIIDAE)

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У овом раду су анализирани индиректни докази регенерације максилопеда и ногу за ходање у природним популацијама литобиоморфне врсте *Eupolybothrus transsylvanicus*. Регистроване аномалије обухватају редуковану величину или абнормалне карактеристике, као што су атипична морфологија и редукован број чланака. Сматрамо да је некомплетна регенерација узрок ових морфолошких дефеката. У неким случајевима, пуна сегментација регенерисаних екстремитета ће бити достигнута касније у онтогенији, кроз анаморфни модел додавања сегмената.