Arch Biol Sci. 2016;68(2):445-450 DOI:10.2298/ABS1508021281

ANAMORPHIC DEVELOPMENT OF *APFELBECKIA INSCULPTA* (L. KOCH, 1867) (DIPLOPODA: CALLIPODIDA: SCHIZOPETALIDAE)

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Received: August 2, 2015; **Revised**: September 3, 2015; **Accepted**: September 16, 2015; **Published online**: November 3; 2015

Abstract: An overview of the anamorphic development of *Apfelbeckia insculpta* is provided. As in other myriapods and arthropods, post-embryonic period of the life cycle includes different stages that are separated by molts. Based on an earlier description of post-embryogenesis of *A. insculpta* and on our data, we describe ten stadia that occur after juveniles of our focal species hatch from the egg. Each molt is accompanied by the addition of podous and apodous pleurotergites, leg-pairs and ocelli. Thus, the numbers of these structures can be used as reliable criteria for the separation of post-embryonic stadia in *A. insculpta*. Adulthood is reached through teloanamorphosis, i.e., with the ninth and last molt individuals become sexually mature and achieve adulthood. Sexes can be distinguished from stadium VIII onward.

Key words: *Apfelbeckia insculpta*; post-embryonic development; teloanamorphosis

INTRODUCTION

Most millipedes can be described as multi-segmented and multi-legged animals. However, newly hatched millipedes usually have a significantly lower number of segments and legs than adults and the final, or adult number of these structures is acquired during the post-embryonic phase of the life cycle. In its broadest sense, post-embryogenesis can be defined as any period after embryogenesis and it usually includes extensive growth, patterning, morphogenesis and maintenance of the adult form [1].

Overall, arthropod post-embryonic development is classed with respect to the number of segments added after hatching [2]. In epimorphosis, animals are hatched with the adult number of segments and no addition of new body segments occurs at the time of molting [3,4], whereas in anamorphosis, the adult number of segments is acquired through a series of molts that are accompanied by the addition of new segments [2-4].

All millipedes develop by anamorphosis during their post-embryonic development [5]. Three general modes of anamorphosis are recognized in Diplopoda: euanamorphosis, hemianamorphosis, and teloanamorphosis [4-6]. In euanamorphosis (recognized in Julida and Colobognatha), animals molt throughout life and every molt is accompanied by the addition of new segments. Hemianamorphosis (observed in Pentazonia and Penicilliata) is characterized by two periods – 'anamorphic', which includes molts that are accompanied by the addition of new segments, and 'epimorphic', which implies molts without the addition of new segments. Finally, teloanamorphosis (typical for Chordeumatida and Polydesmida) is a mode of post-embryonic development where molts and the addition of new segments stop at the adult stadium [4-7]. Only a few studies on the post-embryonic development of Callipodida have been made (see [5] for summary on data concerning callipodidan post-embryogenesis). Based on these data, adulthood in most Callipodida is achieved through teloanamorphosis, while in some species hemianamorphosis may be present [8].

The callipodidan oligotypic genus *Apfelbeckia* Verhoeff, 1896 is an endemic taxon mostly distrib-

uted in caves and other underground habitats of the Dinaric area of the Balkan Peninsula and in Greece. Among members of the genus, *Apfelbeckia insculpta* (L. Koch, 1867) has the largest distributional range and can be found in Serbia, Montenegro, Bosnia and Herzegovina, Croatia and Albania [9].

Lang [10] was the first to describe the post-embryonic development of this species. It is his paper that forms the basis for our knowledge on this period of life of *A. insculpta*. In general, Lang's description included eight stadia that can be separated by the number of segments (podous and apodous), by the number of leg-pairs and by the organization of ocellar field (number of rows of ocelli and number of ocelli). However, as Enghoff et al. [5] noticed, some uncertainties remain in Lang's description of postembryonic development of *A. insculpta* (e.g., 'eye formulae' indicate that he missed the second stadium and that there is one more stadium than the described eight stadia). In order to refine Lang's work, to determine precisely the different post-embryonic stadia and to confirm the mode of anamorphosis in *A. insculpta*, we have analyzed a large number of specimens collected from different localities across most of its distributional range.

MATERIALS AND METHODS

We examined the specimens of *Apfelbeckia insculpta* deposited in the collection of Institute of Zoology, University of Belgrade – Faculty of Biology. Analysis of post-embryonic development included 1214 individuals belonging to 50 populations from Serbia, Montenegro, Bosnia and Herzegovina and Croatia (Appendix, Fig. 1). Most of the material was collected by hand, while some samples were from pit-fall traps. All samples are preserved in 70% ethanol.

To ascertain the stadium of each individual, we relied on counts of the podous and apodous pleurotergites, leg-pairs, rows of ocelli and number of ocelli. These characters were used for the separation of different stadia in previous studies of the post-embryonic development of Callipodida [5 and references therein]. Although Callipodida (and other nematophorans) have free sternites, we have adopted terms such are 'podous pleurotergite' or 'apodous pleurotergite', as in previous literature [5]. Additionally, body length, body width, and body height were measured. The latter two characters were measured at midbody (maximal distances between left to right side (body width) and ventral to dorsal side of pleurotergite (body height)). All specimens were processed using a Carl Zeiss Stemi 2000-c binocular stereomicroscope with an AxioCam MRc camera. Measurements were performed using Axiovision software (ver. 4.2 for Windows, Carl Zeiss, Münich, Germany).

RESULTS

Stadia I and II were not observed in this study. Lang [10] provided a description of stadium I, while his description of stadium II actually corresponds with stadium III. Descriptions of stadium III and the stadia onward are presented in the following text.

Stadium III: At this stadium, animals measure 7.30-7.95 mm and have a yellowish body and legs (Fig. 2). Body width and height are 1.21-1.34 mm and 1.43-1.65 mm, respectively (Table 1). Each individual has 10 podous and 4 apodous pleurotergites (10+4+T), as well as 17 leg-pairs. Number of ocelli is 3 and they are arranged in two rows (1+2) in all specimens (Table 1, Fig. 3).

Stadium IV: Individuals at this stadium show the same color pattern as in the previous one and are 9.35-10.92 mm long, 1.40-1.65 mm wide and 1.83-2.05 mm high (Table 1). All specimens have 14 podous pleurotergites and 6-8 apodous pleurotergites, so 3 segmental formulae are recognized at stadium IV (Table 2). Number of leg-pairs in all samples is 25. Ocellar field consists of 6 ocelli arranged in 3 rows (1+2+3) (Table 1, Fig. 3).

Stadium V: At stadium V, the body dimensions of juveniles are 14.78-20.80 mm (body length), 1.85-2.40 mm (body width) and 2.15-2.97 mm (body height) (Table 1). Body color is yellowish with some darker brown patches at the posterior end (Fig. 2). Millipedes

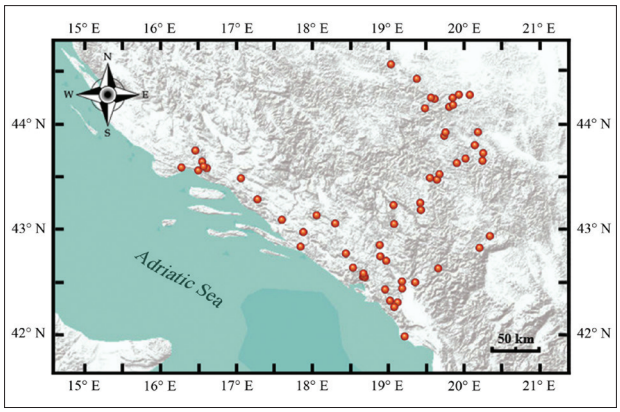

Fig. 1. Populations of *A. insculpta* examined in this study.

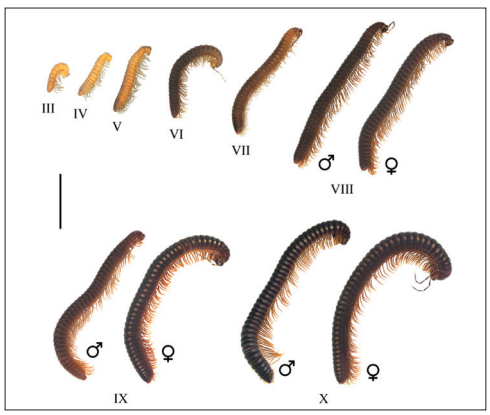

Fig. 2. Post-embryonic stadia of *A. insculpta* observed in this study. From stadium VIII and after, males and females can be clearly distinguished. Scale line = 10 mm.

have 20 or 21 podous and 5-7 apodous segments (Table 1). Five segmental formulae are observed at this stadium (Table 2). All specimens have either 37 or 39 leg-pairs. Total number of ocelli is 10 and they are organized in 4 rows (1+2+3+4) (Table 1, Fig. 3).

Stadium VI: Animals measure 24.10-30.15 mm in length, 2.18-3.00 mm in width and 2.45-3.62 mm in height (Table 1). Color pattern is dark brown with some lighter brown patches at the anterior end of the body (Fig. 2). In addition, yellowish or lighter brown spots below the level of the ozopore can be seen on the lateral side of the animal. Specimens in our study show 33-35 podous and 4-7 apodous pleurotergites (Table 1). Four segmental formulae are found at this stadium (Table 2). This is the first stadium with discrepancies between number of podous pleurotergites and number of leg-pairs. Namely, specimens at this stadium have 27 or 28 podous pleurotergites, so one would expect the animals to have 51 or 53 pairs of walking legs. However, beside these numbers of leg-pairs, we observed specimens with 52 leg-pairs. The ocellar field has 5 rows with 15 ocelli (1+2+3+4+5) (Table 1, Fig. 3).

Stadium VII: At stadium VII, juveniles measure 31.23-52.18 mm (body length), 2.48-3.90 mm (body width), and 2.80-4.11 mm (body height) (Table 1). Body color as in stadium VI. Individuals at this stadium have 33-35 podous and 4-7 apodous pleurotergites. Number of leg-pairs is 63-65, or 67 (Table 1). This is the stadium with the highest number of segmental formulae (Table 2). All animals at this stadium have 21 ocelli arranged in 6 rows (1+2+3+4+5+6) (Table 1, Fig. 3).

Table 1. Quantitative characters of different post-embryonic stadia of *A. insculpta*. The table summarizes data from all populations. Abbreviations: PTs = pleurotergites.

Stadium	Number of podous PTs	Number of apodous PTs	Number of leg-pairs	Number of rows of ocelli	Number of ocelli	Body length	Body width	Body height
III	10	4	17	2	3	7.30-7.95	1.21-1.34	1.43-1.65
IV	14	6-8	25	3	6	9.35-10.92	1.40-1.65	1.83-2.05
V	20, 21	5-7	37, 39	4	10	14.78-20.80	1.85-2.40	2.15-2.97
VI	27, 28	5-7	51-53	5	15	24.10-30.15	2.18-3.00	2.45-3.62
VII	33-35	4-7	63-65, 67	6	21	31.23-52.18	2.48-3.90	2.80-4.11
VIII	39-43	3, 4	75-77, 79, 81-83	7	28	40.52-84.88	2.61-4.28	3.01-4.95
IX	43-47	1	83, 85, 87-92	8	34-36	52.08-90.15	2.80-4.53	3.18-5.28
X	44-48	1	84-94	9	40-45	67.40-105.68	2.94-4.70	3.33-5.47

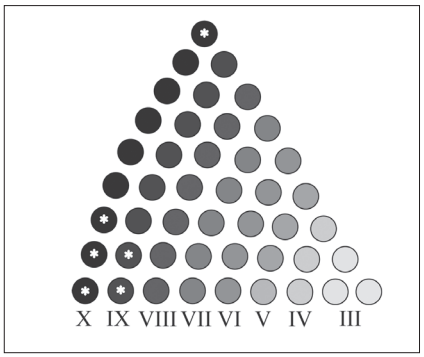

Fig. 3. Diagrammatic representation of the ocellar field of *A. insculpta*. Different shades indicate how ocellar field grows during post-embryonic development. Asterisks denote ocelli that are missing in some animals. These ocelli are given in parentheses in the Results section.

Stadium VIII: Body measurements are 40.52-84.88 mm (body length), 2.61-4.28 mm (body width), and 3.01-4.95 mm (body height) (Table 1). From this stadium onward, animals have the typical adult color pattern – a dark brown body with a lateral row of yellowish spots below the level of the ozopore (Fig. 2). Specimens in our sample have 39-43 podous pleurotergites and 3 or 4 apodous pleurotergites (Table 1). Six segmental formulae are recognized within this stadium (Table 2). Number of leg pairs is presented in Table 1. Ocellar field is constituted of 7 rows with a total of 28 ocelli (1+2+3+4+5+6+7) (Fig. 3). This is the first stadium where animals can be sexed.

Stadium IX: Animals at stadium are 52.08-90.15 mm in length, 2.80-4.53 mm in width, and 3.18-5.28 mm in height (Table 1). Number of podous pleurotergites is 43-47, while all specimens possess one apodous pleurotergite (Table 1). Five segmental formulae are observed (Table 2). Number of leg pairs is 83, 85 or 87-92. The ocellar field has gained one more row of ocelli, resulting in 8 rows of ocelli with total of 34-36 ocelli [1+2+3+4+5+6+7+6(2)]. Some ocelli may be missing in some animals (Table 1, Fig. 3).

Stadium X: This is the final (adult) stadium. At this stadium, animals measure 67.40-105.68 mm (body length), 2.94-4.70 mm (body width) and 3.33-5.47 mm (body height) (Table 1). Compared with stadium IX, adults have one more podous pleurotergite (44-48) and one apodous pleurotergite. The number of leg-pairs is 84-94 (Table 1). During the final molt, the addition of one more row of ocelli occurs, so the adult ocellar field has 40-45 ocelli arranged in 9 rows (1+2+3+4+5+6+7+6(2)+5(4)). Up to 4 ocelli may be absent from this row in some animals (Fig. 3). With the final molt, both males and females become sexually mature with the final development of gonopods and vulvae.

DISCUSSION

Through observation of field-collected material, we have analyzed the anamorphic development of *Apfelbeckia insculpta*. Based on the literature [10] and on our data, we propose that the post-embryonic period of life of *A. insculpta* is composed of ten stadia. Up to stadium X (or adult stadium), each molt results in an addition of podous and apodous pleurotergites, as well

Table 2. Segmental formulae during post-embryonic development of *A. insculpta*. The first number in the formula indicates the number of podous pleurotergites; the following indicates the number of apodous pleurotergites, while T is for telson. Number of individuals of each stadium and with each pleurotergal formula is given in parentheses after the formula. Asterisks mark formula that does not have a corresponding formula in the previous stadium. The principal post-embryonic pathway is indicated with bold types.

Stadium III				**10+4+T (5)**				
Stadium IV			14+6+T (5)	**14+7+T (21)**	14+8+T (2)			
Stadium V		20+7+T (3)	21+6+T (8)	**21+7+T (22)**	22+5+T (1)	22+6+T (2)		
Stadium VI		27+7+T (6)	28+5+T (2)	**28+6+T (32)**	28+7+T (10)			
Stadium VII	33+7+T (1)	34+5+T (4)	34+6+T (22)	**34+7+T (58)**	35+4+T (8)	35+5+T (12)	35+6+T (27)	35+7+T (4)
Stadium VIII	39+4+T (1)	40+4+T (10)	41+3+T (30)	**41+4+T (83)**	42+4+T (66)	43+4+T (2)*		
Stadium IX		43+1+T (1)	44+1+T (31)	**45+1+T (118)**	46+1+T (79)	47+1+T (36)		
Stadium X		44+1+T (4)	45+1+T (47)	**46+1+T (202)**	47+1+T (156)	48+1+T (15)		

as an increasing number of ocelli. The presence of one apodous pleurotergite in adults suggests that the mode of post-embryonic development in our subject species is teloanamorphosis. This mode of post-embryonic development is present in most Callipodida [5].

It is interesting to note that the post-embryonic development of *A. insculpta* is remarkable for its high number of segmental formulae. One segmental formula was recorded only at stadium III. The next stadium already shows three different segmental formulae (though all three formulae differ in the number of apodous pleurotergites, which in turn become podous pleurotergites in stadium V – see Table 2). In general, the number of apodous pleurotergites in the preceding stadium correlates directly with the number podous pleurotergites in the following stadium. The only exception to this pattern is one segmental formula (43+4+T) observed in two individuals at stadium VIII. We think that this is simply because we did not have an animal with a "predecessor formula" at stadium VII. Otherwise, stadium VII is the stadium with the highest number of segmental formulae. Such a high variation in the segmental formulae observed in the later post-embryonic stadia can be ascribed to the fact that the material in this study was collected from different populations (see Appendix, Fig. 1). The high degree of interpopulation differences in segmental formulae has already been documented in some millipedes [11,12] and our results fit in this pattern.

Enghoff et al. [5] noted that in most millipedes with free sternites, the correlation between tergites and sternites does not follow a consistent rule, i.e., discrepancies in the numbers of dorsal and ventral segmental structures in millipedes are possible. This means that, for example, the number of leg-pairs does not correlate with the number of pleurotergites. Indeed, one of the peculiarities that we recorded when analyzing the different post-embryonic stadia of *A. insculpta* is the mismatch between the number of pleurotergites and the number of leg-pairs. Up to stadium V, the number of pleurotergites and leg-pairs correlates directly. However, at stadium V, juveniles had 51, 52 or 53 pairs of walking legs and 27 or 28 podous pleurotergites. Even and odd numbers of leg-pairs were recorded in all upcoming stadia. A similar condition was observed in other callipodidan millipedes for which data are available (Tables 20-23 in [5]), so such a pattern seems to be usual in the Callipodida. An explanation for the discrepancy in numbers of dorsal and ventral segmental structures in millipedes was given in papers about the expression of segmentation genes in *Glomeris marginata* (Villers, 1798) [13-16].

CONCLUSIONS

Apfelbeckia insculpta undergoes teloanamorphosis during the post-embryonic phase of life. During this process, millipedes molt nine times and with the ninth and final molt, individuals achieve adulthood (stadium X). Each molt is accompanied by the addition of podous and apodous pleurotergites, leg-pairs and ocelli. Such a schedule offers easily delimitation of the different post-embryonic stadia.

Acknowledgments: We would like to thank two anonymous reviewers for their comments that greatly improved the manuscript. This study was supported by the Serbian Ministry of Education, Science and Technological Development (Grant No. 173038).

Authors' contributions: BSI participated in the collection of specimens, design of the study, carried out all measurements and counts of characters used in this study, drafted and wrote the manuscript. VTT and BMM participated in specimen collection and study design. LRL and BMM participated in the writing of the manuscript.

Conflict of interest disclosure: The authors declare that they have no competing interests.

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SUPPLEMENTARY MATERIAL (Available online)

APPENDIX

Available at: http://serbiosoc.org.rs/arch/files/SupplementaryInformationAppendix.pdf