Arch. Biol. Sci., Belgrade, 62 (2), 469-477, 2010 DOI:10.2298/ABS1002469B

MORPHOLOGICAL CHANGES OF THE OVIPOSITOR IN SPECIES OF CHELONINAE (HYMENOPTERA: BRACONIDAE) IN THE COURSE OF ADAPTATION TO EGG-LARVAL PARASITISM

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Abstract – Adaptation to the parasitic way of life in braconids has led to a number of consequences in the morphology of the ovipositor of females. As a derivative of the paired appendages of the eighth and ninth abdominal segments, the ovipositor is a complex morphological structure of gonapophysal origin whose muscular system makes possible highly complex movements during the act of egg-laying. In some groups of braconids, imaginal and egg-larval forms of parasitism have developed in the course of evolution. In adapting to these two forms of parasitism, the ovipositor underwent significant changes, while still remaining a successful structure for the paralyzation and laying of eggs in the host. This paper presents a survey of the ovipository apparatus structure in the species of the genera *Ascogaster* Wesmael, *Leptodrepana* Shaw, *Chelonus* Panzer, *Microchelonus* Szépligeti, and *Phanerotoma* Wesmael, and gives a review of the changes in structure of the ovipositor during adaptation to egg-larval parasitism.

Key words: Braconidae, Cheloninae, ovipositors, comparative morphology, parasitism

UDC 595.79:591.557.8

INTRODUCTION

Species of the subfamily Cheloninae are clearly recognizable by the nervature of the wings, structure of the ovipositors and structure of the metasome carapaces (Achterberg, 1976; Shaw, 1997). Braconids are primarily parasites of other insects. Only certain species have phytophagous larvae that develop in plant seeds (Marsh, 1991). Among braconids, the ectoparasitic species are considered to be more basal, and endoparasitic more derived. This traditionally accepted view has recently been questioned on the basis of results obtained using new methods of phylogenetic research (Dowton et al., 1998, 2002).

Adaptation to the parasitic way of life has led to a number of consequences in the morphology of the ovipositor of females (Le Ralec et al., 1996). Most braconids are adapted to laying their eggs in the larvae of other insects (Papp, 1974).

As a derivative of the paired appendages of the eighth and ninth abdominal segments, the ovipositor of braconids is a complex morphological structure of gonapophysal origin (Marlat, 1891; Snodgrass, 1931, 1933; Michener, 1944; Gustafson, 1950; Scudder, 1961). In the course of evolution coxites of the eighth and ninth segments were transformed into triangular and oblong plates (the first and second valvifers). According to Michener (1944), their gonapophyses give rise to the first and second valvulae. Matsuda (1976) maintained that the quadrate plates arose from tergites of the ninth abdominal segment. These are the largest and are joined to the third valvulae. The form of these plates varies to a fairly great extent within the family Braconidae.

The plates of the ovipositor of braconids are connected by numerous muscles that enable complex movements during the act of egg-laying (Alam, 1952, 1955; Venkatraman and Subba Rao, 1954; Quicke, 1995). The first valvulae (lancets) are separated throughout their entire length. Their proximal edges are joined to triangular plate ramuses. The second valvulae are fused together and

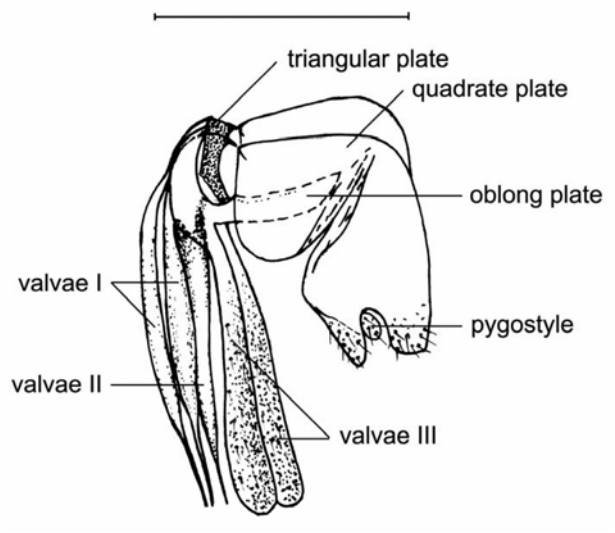

Fig. 1. *Ascogaster argentifrons*, female genitalia, lateral view (scale 0,5 mm)

likewise proximally joined to the triangular plates. When functional, the first and second valvulae form a canal through which braconids eject secretions from the acidic and basic glands into the host, after which they lay their eggs through the same canal on, or in, the body of the host. In some of the braconids that parasitize concealed hosts (such as ones that are hidden under tree bark or in plant tissue), these valvulae also serve as the means of boring into the substrate, which is a highly complex and laborious task. The third pair of valvulae are free of each other. They serve as support for the first and second valvulae during egg-laying in some species of braconids, while in others they are simply carriers of the sensitive hairs that help to locate the host. In size and form, they vary to a fairly great extent within the family Braconidae.

Significant changes in the structure of the ovipositor of braconids were also dictated by changes in the structure of the metasoma (abdomen). It is a characteristic of the Braconidae that their second and third metasomal tergites are immovably fused. The remaining segments in most species are free. However, there is a very well-expressed trend among braconids toward the creation of a carapace (shield) that arises through the partial or complete fusion of the first three metasomal tergites, while the rest undergo desclerotization and significant reduction (Tobias and Dudarenko, 1974). This phenomenon is most pronounced among species of the subfamilies Cheloninae, Sigalphinae, and Caliptinae (Brachistinae).

MATERIAL AND METHODS

The selected material, consisting of dry collection specimens, was first held for two days in moist sand in order to relax it. Dissection was then carried out using dissecting needles and fine forceps. The extracted ovipositors were cooked for several minutes in a 10% KOH solution, which removed all muscle. The ovipositors free of muscles and grease were passed through a series of alcohols (65, 85, and 100%) for dehydration. Following dehydration, the ovipositors were held for several minutes in xylol and later mounted in Canada balsam on a microscope slide. The preparations were then dried.

RESULTS

All the changes in the structure of the valvulae and genital plates are most pronounced in the species of the genera *Ascogaster* Wesmael, 1835 and *Leptodrepana* Shaw, somewhat less pronounced within the genera *Chelonus* Panzer and *Microchelonus* Szépligeti, and least pronounced (but with different degrees of expression) in the species of the genus *Phanerotoma* Wesmael.

The studied species of the genus *Ascogaster* Wesmael, 1835 [*A. argentifrons* Provancher, 1886 (Fig. 1), *A. borealis* Shaw, 1983 (Fig. 2), *A. quadridentata* Wesmael, 1835, *A. impatientis* Shaw, 1983, *A. marshi* Shaw, 1983, *A. rufa* Muesebeck and Walkley, 1951, and *A. provancheri* Dalla Torre, 1898] have T-shaped triangular plates, their dorsal side being elongated, while the ventral side is in the form of a rod. Compared to other species of braconids, the oblong plates are very short. They are characterized by a narrow apex, short body, and significantly larger posterior dorsal extensions that differ in form and length. In the species *Ascogaster quadridentata* and *A. argentifrons* (Fig. 1), they are even

Fig. 2. *Ascogaster borealis*, female genitalia, ventral view (scale 0,5 mm)

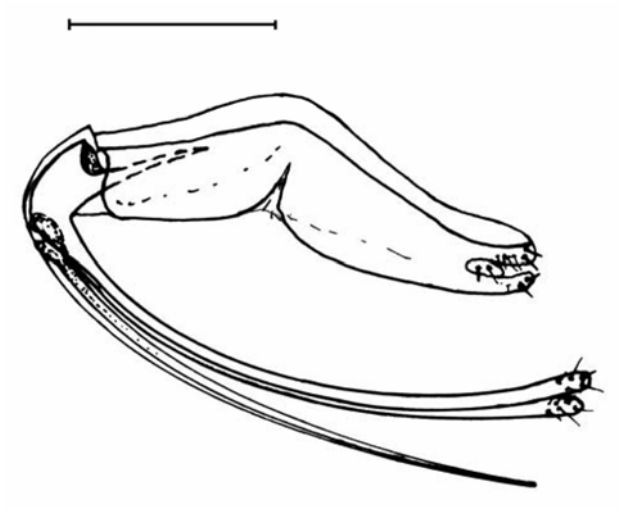

Fig. 3. *Leptodrepana flaviceps*, female genitalia, lateral view. Scale 0.5 mm.

longer, their tips bending toward the dorsal side. The species *Ascogaster impatientis* is characterized by the longest extensions, which taper apically.

In all of the examined species, the quadrate plates are broad and very weakly sclerotized. They are downwardly bent in the region of the posterior dorsal crest, a condition that is a trait of all representatives of the Cheloninae. Their dorsal edge is more strongly sclerotized and on its anterior margin it is differentiated into small finger-like extensions. Pygostyles in the examined species are well-developed and covered with long sparse hairs.

The first and second valvulae are short, broad and apically needle-like. The tips of the valvulae I are obliquely truncated, while the valvulae II basally form a fairly large bulbus. They have no differentiations of any kind on their tips. The third pair of valvulae is just as long as the first and second pairs, since they arise from the anterior parts of the oblong plates. They are basally constricted, expand apically, and carry sparse hairs.

Three species of the genus *Leptodrepana* Shaw, 1983 were examined: *L. flaviceps* (Ashmead, 1889) (Fig. 3), *L. opuntiae* Shaw, 1983, and *L. oriens* Shaw, 1983 (Fig. 4). They are characterized by having rectangular and dorsoventrally elongated triangular plates. The oblong plates are short and with large anterior apices. Their posterior dorsal extensions are very long and pointed. The joint invagination is broad and with sparse sensilla. The quadrate plates are very long and broad. They are bent in the middle, and their dorsal edges are in the form of a more strongly sclerotized narrow band. The pygostyles are large, irregularly shaped, and overgrown with sparse hairs.

The first and second valvulae are somewhat longer than the complex of genital plates. They are basally expanded, but apically needle-like. The third valvulae are of the same length as the preceding ones, very narrow, and with several hairs only on each of their tips.

The examined species of the genus *Chelonus* Panzer, 1806 were *C. aciculatus* McComb, 1968, *C. annulipes* Wesmael, 1835, *C. carinatus* Provancher, 1881, *C. muesbecki* McComb, 1962, *C. narayani* Subba Rao, 1955 (Fig. 6), *C. pectinophorae* Cush-

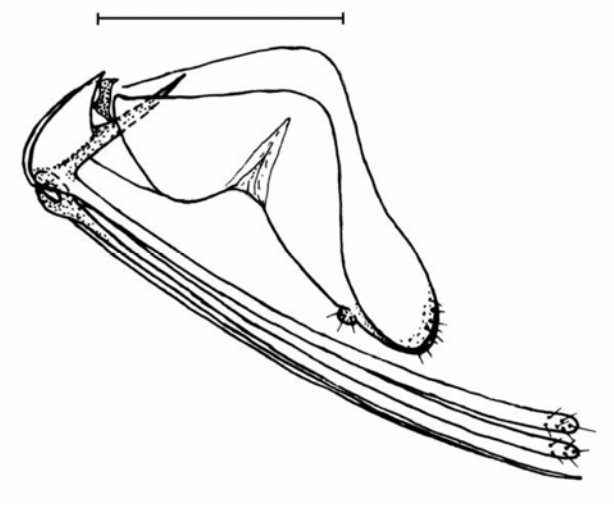

Fig. 4. *Leptodrepana oriens*, female genitalia, lateral view. Scale 0.5 mm.

man, 1931, *C. sericeus* (Say, 1824) (Fig. 5), and *C. texanus* Cresson, 1872.

In all of the examined species the triangular plates are narrow and dorsally differentiated into two finger-like extensions. The oblong plates have short anterior apices whose bases give rise to the third valvulae. The posterior dorsal extensions are large and variable in form and size. They are somewhat shorter and wider in *Chelonus carinatus*, *C. sericeus* (Fig. 5), and *C. annulipes*, while in *C. narayani* (Fig. 6), *C. texanus*, and *C. pectinophorae* they are of the same length as in the preceding species, but pointed. The joint invagination is spacious and with sparse sensilla. The quadrate plates are broad and broken at the level of the posterior dorsal crests. The pygostyles are of irregular form and have sparse hairs.

The first and second valvulae are short, of the same length as the complex of genital plates, and slightly longer only in the species *Chelonus muesbecki* and *C. sericeus* (Fig. 5). The valvulae are basally wide and taper gradually toward their tips, which have needle-like ends. The third valvulae are of the same length as the first and second pairs, considerably wider than them, and with sparse hairs only on their tips.

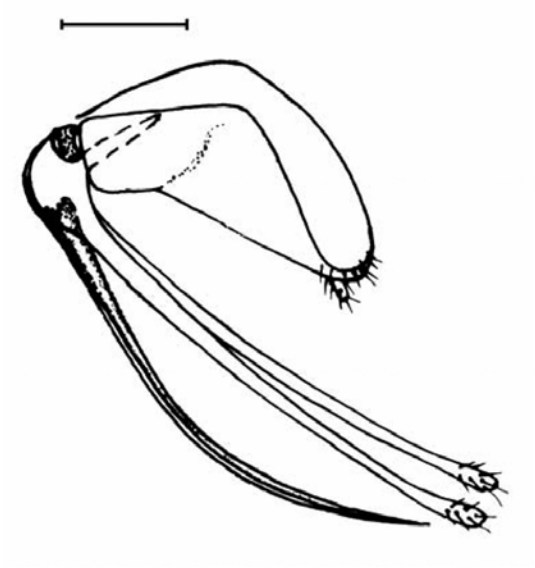

Fig. 5. *Chelonus sericeus*, female genitalia, lateral view. Scale 0.5 mm.

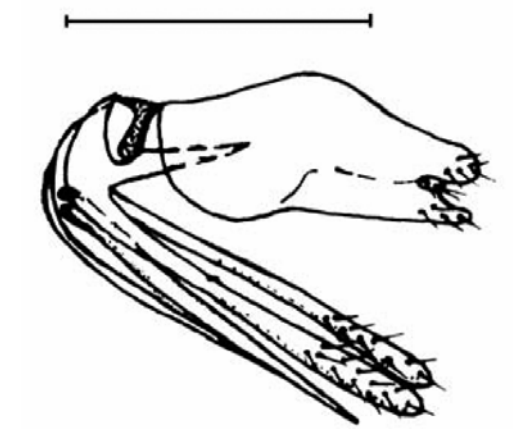

Fig. 6. *Chelonus narayani*, female genitalia, lateral view. Scale 0.5 mm.

Of the genus *Microchelonus* Szépligeti, 1908 we examined the species *M. blackburni* Cameron, 1886 (Fig. 7) and *M. heliopae* Gupta, 1955 (Fig. 8).

The first and second valvulae are of the same length as the complex of genital plates, very broad for more than half of their length, and apically needle-like. The third valvulae are basally some-

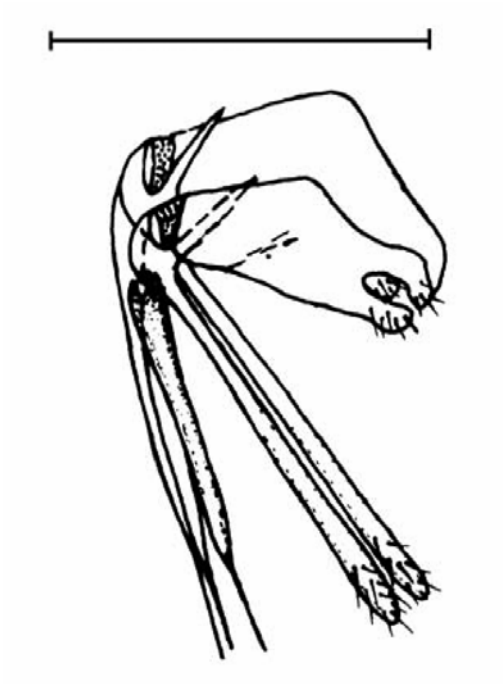

Fig. 7. *Microchelonus blackburni*, female genitalia, lateral view. Scale 0.5 mm.

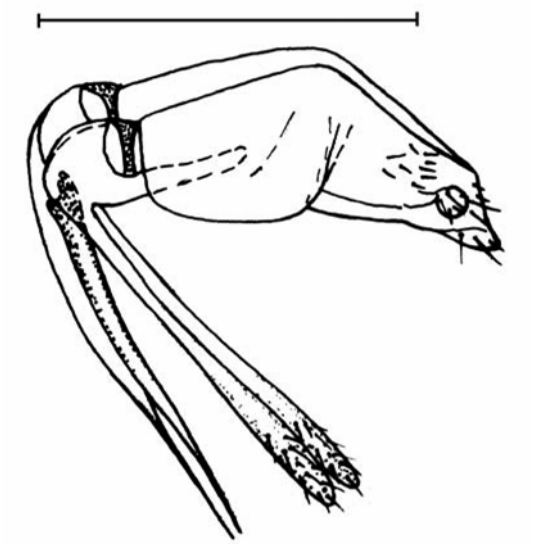

Fig. 8. *Microchelonus heliopae*, female genitalia, lateral view. Scale 0.5 mm.

what constricted, then expand toward their tips, on which sparse hairs are present.

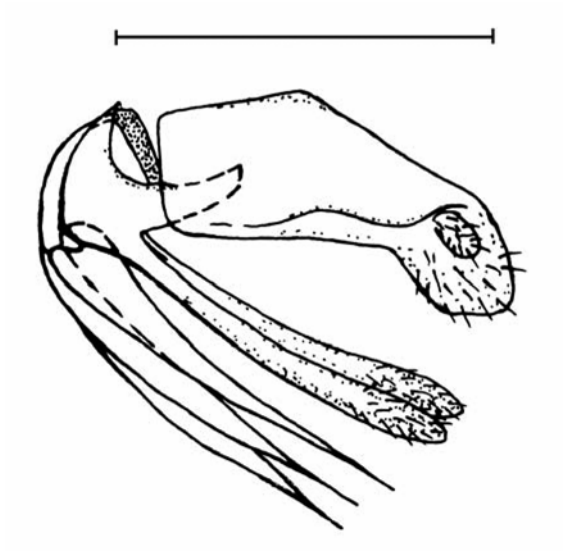

Fig. 9. *Phanerotoma fasciata*, female genitalia, lateral view. Scale 0.5 mm.

The triangular plates are very narrow and dorsoventrally elongated. The oblong plates are short, with short anterior apices. The dorsal extensions are long and rectangular in *Microchelonus heliopae* (Fig. 8), but pointed in *M. blackburni* (Fig. 7). The quadrate plates are wide and broken in back. The pygostyles are round and covered with sparse hairs.

The examined species of *Phanerotoma* Wesmael, 1838 were *P. grapholithae* Muesebeck, 1933, *P. fasciata* Provancher, 1881 (Fig. 9), *P. franklini* Gahan, 1917, *P. hawaiiensis* Ashmead, 1901 (Fig. 10), *P. longicauda* Walley, 1951 (Fig. 11), and *P. zeteki* Cushman, 1914 (Fig. 12).

The triangular plates are dorsoventrally elongated and somewhat expanded dorsally. The oblong plates are very short, with narrow anterior apices. The posterior dorsal expansions are clearly developed and fairly long. The quadrate plates are broad and broken in back, except in *Phanerotoma longicauda* (Fig. 11), where they are considerably narrower, straight, and almost completely membranous. The pygostyles are large and covered with sparse hairs.

The first and second valvulae are of the same length as the complex of genital plates, except in the

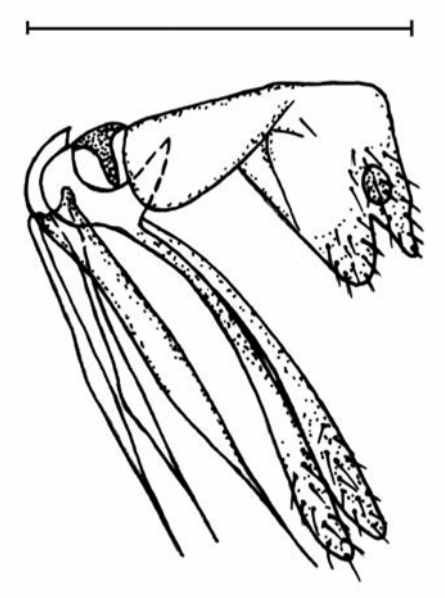

Fig. 10. *Phanerotoma hawaiiensis*, female genitalia, lateral view. Scale 0.5 mm.

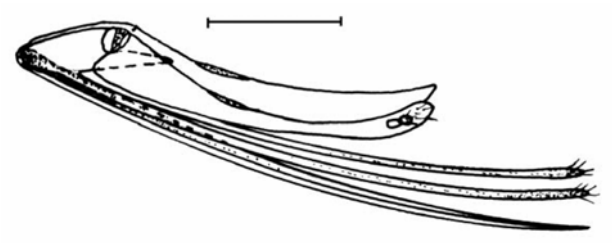

Fig. 11. *Phanerotoma longicauda*, female genitalia, lateral view. Scale 1 mm.

case of *Phanerotoma longicauda* (Fig. 11), in which they are considerably longer. These valvae in *Phanerotoma fasciata* (Fig. 9) and *P. zeteki* (Fig. 12) are very wide in their first two thirds, but needle-like apically. The third valvae in *Phanerotoma fasciata* (Fig. 9) and *P. zeteki* (Fig. 12) are somewhat constricted basally, then expand toward their tips, while in *P. longicauda* (Fig. 11) they are very narrow along their entire length. They have sparse hairs on their tips. It is evident that *Phanerotoma longicauda* (Fig. 11) in many characteristics of its ovipositor structure differs significantly from the other species of this genus.

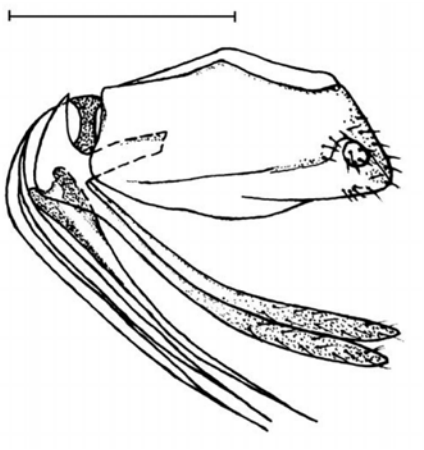

Fig. 12. *Phanerotoma zeteki*, female genitalia, lateral view. Scale 0.5 mm.

DISCUSSION

The Cheloninae are solitary endoparasites whose females lay their eggs in the eggs or young larvae of different species of Lepidoptera. Differentiation of the carapace (metasomal shield) and adaptation to egg-larval parasitism in representatives of the subfamily Cheloninae have dictated a number of changes in the structure of the valvulae and genital plates of the ovipositor. The act of laying eggs in the eggs of the host is considerably easier and simpler than laying eggs in actively mobile stages (larvae or imagoes). Because the eggs of the host are static and small, the ovipositor of the parasite underwent changes in the course of its evolution. These changes are very pronounced in the structure of valvulae I and II, which are wide in their basal and middle parts, but apically differentiated into micropipette-like structures that serve as a precision instrument. The third valvulae in the members of this subfamily lost the function of providing support for the preceding valvulae in the act of egg-laying and retained only the function of sensory organ. This resulted in the shortening of the oblong plates and insertion of the third valvulae immediately after the first and second ones.

Table 1. List of examined Cheloninae species with specified figure numbers

Genus name	Species name	Figures
Ascogaster Wesmael 1835	*argentifrons* Provancher 1886	1
	borealis Shaw 1983	2
	quadridentata Wesmael 1835	
	impatientis Shaw 1983	
	marshi Shaw 1983	
	rufa Muesebeck and Walkley 1951	
	provancheri Dalla Torre 1898	
Leptodrepana Shaw 1983	*flavicepes* Ashmead 1889	
	opuntiae Shaw 1983	
	oriens Shaw 1983	3
Chelonus Panzer 1806	*aciculatus* McComb 1968	
	annulipes Wesmael 1835	
	carinatus Provancher 1881	
	muesbecki McComb 1962	
	narayani Subba Rao 1955	5
	pectinophorae Cushman 1931	
	sericeus (Say 1824)	4
	texanus Cresson 1872	
Microchelonus Szépligeti 1908	*blackburni* Cameron 1886	
	heliopae Gupta 1955	6
Phanerotoma Wesmael 1838	*grapholithae* Muesebeck 1933	
	fasciata Provancher 1881	7
	franklini Gahan 1917	
	hawaiiensis Ashmead 1901	
	longicauda Walley 1951	8

A common feature of all of the examined species is that the triangular plates have lost their triangular shape and are transformed into very narrow and dorsoventrally elongated structures. This change has also resulted in the reduction of the muscular system. Almost all species have short oblong plates, while the size of the posterior dorsal extensions exhibits significant variation that is specific for each genus, so that this trait can serve as a differential characteristic in the absence of others. The given extensions are large and broad in the species of the genus *Ascogaster*, somewhat shorter in species of the genus *Phanerotoma* and certain species of the genus *Chelonus*, and distinctly shorter and needle-like in others. It is very typical that the quadrate plates are weakly sclerotized and bent in the middle, except in the species *Phanerotoma longicauda* (Fig. 11). Such a structure of the quadrate plates is a consequence of the differentiation of the metasomal shield, which arose from the fusion and strong sclerotization of the first three metasomal tergites, while the rest remained desclerotized and withdrawn under the shield. All changes in the shape and degree of sclerotization of the genital plates resulted in the modification of the muscular system of the ovipositor, which in the majority of braconids is highly complicated and so well developed that it makes possible the very complex movements of the valvulae and genital plates in the act of laying eggs in the host.

CONCLUSION

All the structural characteristics of the ovipositor and metasome indicate that the species of the subfamily Cheloninae are a very specialized group that developed in the direction of egg-larval parasitism, which (together with the development of imaginal parasitism in the subfamily Euphorinae) is considered to be the highest form of the evolution of the endoparasitism within the family Braconidae. The entire ovipositor complex (including the genital plates and all three valvulae) is extremely desclerotized, which is in correlation with the strong sclerotization of the metasomal shield. The easy act of laying eggs in the eggs and young larvae of Lepidoptera dictated a significant reduction of the triangular and oblong plates. The quadrate plates are well-developed, but are extremely desclerotized and

in the majority of species are broken in back. Valvulae I and II are without apical teeth and are differentiated into precision instruments enabling eggs to be laid in the eggs of Lepidoptera. Valvulae III have lost the function of providing support for valvulae I and II and have retained only a sensory and protective function.

Acknowledgment - This study was supported by the Serbian Ministry of Science and Technological Development (Grant No. 143053).

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МОРФОЛОШКЕ ПРОМЕНЕ ЛЕГАЛИЦА КОД ВРСТА ПОДФАМИЛИЈЕ CHELONINAE (HYMENOPTERA: BRACONIDAE) ТОКОМ АДАПТАЦИЈЕ НА ЈАЈНО-ЛАРВАЛНИ ПАРАЗИТИЗАМ

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Прилагођавање на паразитски начин живота код браконида имало је низ последица на морфологију легалица женки. Као дериват парних наставака осмог и деветог абдоминалног сегмента, легалица представља комплексну морфолошку структуру гонапофизног порекла, чији мишићни систем омогућава изузетно комплексне покрете током акта полагања јаја. Ларвални паразитизам код браконида се сматра примарним, док се имагинални и јајно-ларвални паразитизам развијају током њихове еволуције. Прилагођавањем на ова два облика паразитизма легалица је трпела битне промене, остајући и даље ефикасна структура за парализовање и полагање јаја у домаћина. Овај рад даје упоредни преглед овипозиторних структура код врста родова *Ascogaster* Wesmael, *Leptodrepana* Shaw, *Chelonus* Panzer, *Microchelonus* Szépligeti и *Phanerotoma* Wesmael, као и приказ промена у грађи легалице током адаптација на јајно-ларвални паразитизам.