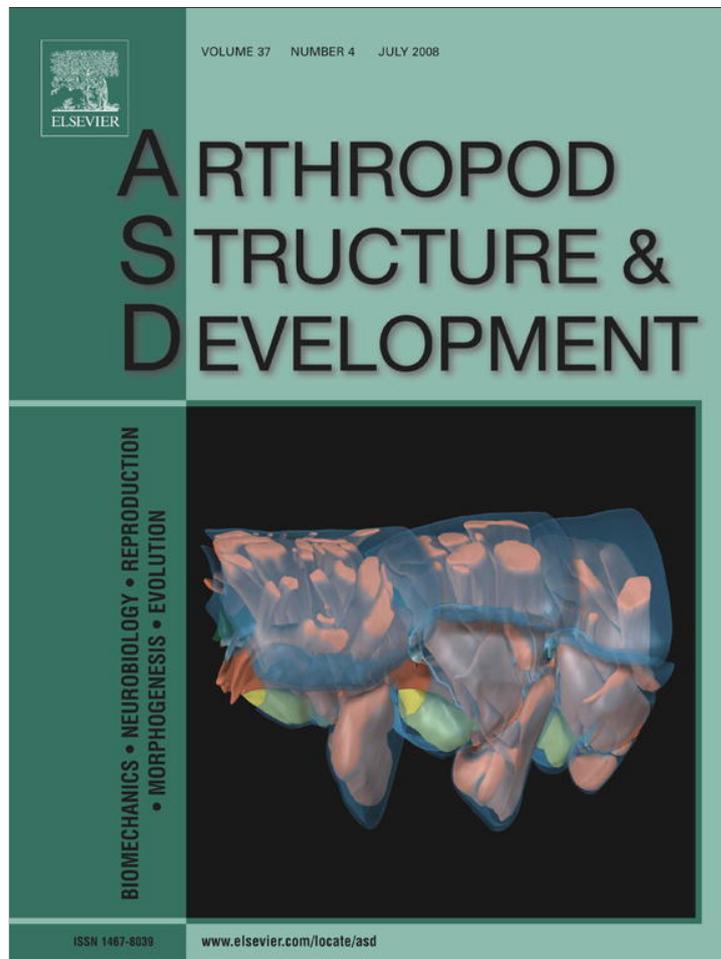


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# Skeletomuscular development of genital segments in the dragonfly *Anax imperator* (Odonata, Aeshnidae) during metamorphosis and its implications for the evolutionary morphology of the insect ovipositor<sup>☆</sup>

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## Abstract

The skeleton-muscular organisation of abdominal segments 7–9 in female *Anax imperator* L. (Anisoptera, Aeshnidae) was examined in the stages of ultimate larva, teneral imago, and mature imago, with special emphasis on the transformation of the muscle arrangement. The absence of certain muscles in the genital segments compared to the 7th pre-genital segment was noted on all studied stages. Reductions of certain muscles in adults compared to those in larvae are reported. Some of ovipositor's muscles appear already in larvae. Attachment sites of larval muscles are retained in freshly emerged females concurrently with integument transformations. This situation allows for precise determination of the borders of newly differentiated genital sclerites and, therefore, of the possible origin of certain ovipositor elements in odonates. All changes in the segmental sets of studied abdominal muscles during metamorphosis are tabulated, and displacements of muscles are documented and illustrated. Schematic figures illustrating homologies between the parts of larval and imaginal abdominal sclerites are provided. The origins of the components of the endophytic ovipositor in Odonata as well as their implications for the evolutionary morphology of the insect ovipositor are discussed.

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*Keywords:* Skeleton; Muscles; Integument transformation; Gonangulum; Dragonflies

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## 1. Introduction

The insect ovipositor was previously used for phylogeny reconstructions (e.g. Nénon et al., 1995; Aspöck et al., 2001; Klass, 2003) as well as for studies of reproductive strategies of animals and their biological traits as a whole (Howden, 1992; Le Ralec et al., 1996; Quicke et al., 1999; Rasnitsyn and Quicke, 2002). It was proposed that the evolution of Odonata was associated with the change (or multiple changes) of egg-laying strategies (e.g. St. Quentin, 1962; Bechly et al., 2001) and, therefore, that the morphological features of the

ovipositor may be good indicators of dragonflies' phylogeny (Matushkina, 2005).

The cutting ovipositor is considered to be a plesiomorphic state in odonates (Bechly et al., 2001). It exists in all zygopterans, anisozygopterans and in aeshnid anisopterans (Corbet, 1999). The females use it mainly for endophytic egg-laying, i.e. for egg deposition within plant tissues (St. Quentin, 1962). Such an endophytic ovipositor is a compound organ situated on the genital 8th and 9th abdominal segments. Its general construction was studied in several works (e.g. van der Weele, 1906; Snodgrass, 1935; Asahina, 1954; St. Quentin, 1962; Pfau, 1985, 1991; Srivastava and Srivastava, 1989; Matushkina and Gorb, 1997). The skeletal organisation of the endophytic ovipositor apparatus is similar in the representatives of all suborders of Odonata and represents: 1) the shaft of the ovipositor, including paired cutting 1st and 2nd valves suitable for substrate penetration; 2) paired large plates, the

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3rd valves which sheath the shaft of the ovipositor laterally in its resting position; 3) one basal plate of ovipositor; 4) paired elongated lateral sclerites beside the 3rd valves; and 5) one unpaired internal sclerite, connected by two membranous straps with the bases of the 2nd valves (Asahina, 1954; Pfau, 1985; Matushkina and Gorb, 1997). Also, the distal edges of the 3rd valves bear movable stick-like appendages, the styli, which have been identified as sensory structures (Matushkina and Gorb, 2002).

The muscle set of the endophytic ovipositor varies also a little (Asahina, 1954; Pfau, 1985; Matushkina and Gorb, 1997; Matushkina, 2004). The complete set of muscles comprises seven paired muscles and one unpaired muscle, as previously recorded in zygopterans (Matushkina and Gorb, 1997). However, in aeshnids the paired muscle is lost (Asahina, 1954; Pfau, 1991; Matushkina and Gorb, 1997).

The terminology of certain constituent parts of ovipositor in Odonata varies by authors and requires unification (Table 1), chiefly because of doubtful and vague interpretations of some parts. However, the origin of the ovipositor components may be relatively easily ascertained by tracing their development during ontogenesis (Machotin, 1929, 1953). In dragonflies, as is characteristic for most hemimetabolous insects, the rudiments of the ovipositor appear on larval stages and enlarge gradually instar-by-instar (van der Weele, 1906; Machotin, 1929; Asahina, 1954). The most dramatic reconstruction of genital segments occurs during the emergence of the imago. In this situation the sternum of the 9th abdominal segment fragments into a set of sclerites which then are included in the ovipositor (Machotin, 1929). The larval muscles are still temporarily retained by the freshly emerged imago (Whedon, 1929), so that it is possible to identify the borders of arising sclerites more precisely and to compare them with the parts of the larval segment wall.

The pre-genital muscles in larva and imago of some dragonflies have been examined by earlier authors (Whedon, 1918, 1929; Asahina, 1954; St. Quentin, 1962). Whedon (1929) made a detailed description of the phased reduction of pre-genital muscles in *Anax junius* Brauer, 1865 (Anisoptera, Aeshnidae) during its metamorphosis. These modifications were proposed to be caused by changes of odonatan respiration and locomotion. Also, Asahina (1954) discussed the identity of larval and imaginal musculature in Odonata and provided a review of this topic. Unfortunately, information about muscular equipment of female genital segments at the larval adult female stages was restricted to the sketchy drawings of *A. junius* by Whedon (1929, pl. 23, 27), while skeletal and muscular reorganization of the genital segments during the metamorphosis had not been studied up to now.

The main purposes of this study were to: 1) discern differences in the musculature of the female pre-genital and genital segments in the final larval instar; 2) compare the muscle sets of genital segments in the final larval instar and imago; 3) determine the consequences of the origin of ovipositor muscles in metamorphosis; 4) trace possible transformations of integumentary structures of the genital segments from larva to imago using attachment sites of muscles as markers; and 5) discuss

Table 1  
Nomenclature of skeletal parts of ovipositor in Odonata

This paper	van der Weele, 1906; St. Quentin, 1962	Machotin, 1929*	Asahina, 1954	Pfau, 1985, 1991	Srivastava and Srivastava, 1989	Klass, 2003
Name	Origin					
Basal plate of ovipositor	Gonocoxite of 8th segment + anterior part of gonangulum		Basivalvula	Valvifer 1 (partly)	First valvifers + gonangula	Ante-laterocoxa IX (part of gonangulum)**
Elongated sclerite	Lateral part of gonangulum	Coxa 8th segment	Valvifer	Valvifer 1 (partly)	Laterotergite	Post-laterocoxa IX (part of gonangulum)
1st Valve	Gonapophyses of 8th segment	Valves of 8th segment	Ventral valvula	Valvulae 1	First valvulae	Gonapophysis VIII
2nd Valve	Gonapophyses of 9th segment	Valves of 9th segment	Inner valvula	Valvulae 2	Second valvulae	Gonapophysis IX
3rd Valve	Gonocoxite of 9th segment	Coxa of 9th segment = lateral valves	Lateral valvula	Valvifer 2 + valvulae 3	Second valvifers (lateral valves)	Coxa IX
Internal sclerite	Intervalvula posterior = median part of 9th sternite	Part of extended bases of valves of 9th segment (en)	Posterior intervalvula	—	—	***
Poststernite of 9th segment	Posterior margin of 9th sternite	Intervalvula superior (iws)	Poststernite 9	—	—	—
Stylus	Gonostylus of 9th segment	Stylus	Stylus	Stylus	Terminal style	Stylus IX

\* Translated from Russian with addition of some Latinized names, used by the author.

\*\* Author reports in the text that the gonangulum of Odonata is divided into two sclerites, *ante-* and *post-laterocoxa IX*. Based on this statement, the anterior part of gonangulum (*ante-laterocoxa IX*) might be included into the basal plate of the ovipositor (Klass, 2003: 176–177). Unfortunately, the cited article lacks drawings, explaining this notion.

\*\*\* Designated on drawings, but not named.

the possible homologies in the skeleton of the ovipositor between odonates and some other insects.

## 2. Materials and methods

Penultimate (F-1) and ultimate (F-0) instar larvae, and mature adults of females of *Anax imperator* Leach, 1815 (Anisoptera, Aeshnidae) were collected during 1997–1999 from a shallow bay of the Desna River near Khotjanivka village (vicinity of the Kiev city, Central Ukraine, 50°34'55N, 30°33'51E). When collected, some of the F-0 and all the F-1 larvae were placed individually in plastic containers (15 × 10 × 5 cm) with river water, kept under near room temperature (20 °C), and exposed to the sun throughout the day, in order to rear teneral imagines. Larvae were fed with oligochaete worms, and water was changed once per day. Abdominal fragments of the reared teneral females were fixed in Bouin's solution in the stage of the newly emerged imago after the first flight. Other specimens were stored in Bouin's solution either whole (larvae) or in dissected condition (adults) immediately after capture.

### 2.1. Preparation techniques

Terminal segments were washed in 70% ethanol, then dissected in the median and frontal planes. The musculature was examined in water under a stereomicroscope using manual dissections and subsequent layer-by-layer reconstruction of muscles. Subsequently, cuticle parts were macerated in 10% KOH and examined in glycerol under the stereomicroscope. The skeleto-muscular system was reconstructed by series of drawings. Overall, 17 specimens were dissected, including 6 F-0 larvae, 6 teneral females, and 5 mature females.

### 2.2. Description remarks and nomenclature

Whedon (1929) reported that the complete set of muscles of an abdominal segment includes five principal groups of paired muscles, classified according to their attachment sites to: 1) the intersegmental tergal muscles, which originate on their respective tergite and are inserted to the next tergite; 2) the intersegmental sternal muscles connecting the respective sternite with the next one; 3) the intersegmental tergo-pleural muscles connecting the respective tergite with the next pleurite; 4) the intersegmental sterno-pleural muscles connecting the respective sternite with the pleurite of the next segment; and 5) segmental tergo-pleural muscles connecting the tergite and the pleurite within a segment. All these groups of muscles were found in the studied insects (Table 2), and I follow Whedon's classification in the subsequent descriptions.

The following nomenclature compilations were used: Corbet (2002) for larval stadia, Whedon (1929) for muscles, Asahina (1954) for the skeleton of non-genital segments, and Matushkina and Gorb (1997) for the skeleto-muscular organisation of the ovipositor.

### 2.3. Abbreviations

#### 2.3.1. Skeleton

*Lam*, basal plate of ovipositor (*lamina valvarum*); *V1*, first valves of ovipositor (*valvulae 1*); *V2*, second valves of ovipositor (*valvulae 2*); *V3*, third valves of ovipositor (*valvulae 3*); *aAp*, anterior apophysis of *V3*; *pAp*, posterior apophysis of *V3*; *St*, stylus; *piv*, internal sclerite, or posterior intervalvula; *pst*, poststernite of 9th segment; *rV1*, *rV2*, *r piv*, *rSt*, larval precursors of *V1*, *V2*, *piv* and *St*, respectively.

#### 2.3.2. Muscles

*dva*, m. dorsoventralis anterior; *dvm*, m. dorsoventralis medialis; *dvo*, m. dorsoventralis obliquus; *dvo'*, secondary m. dorsoventralis obliquus; *dvp*, m. dorsoventralis posterior; *itls*, m. longitudinalis sternalis internus; *lpsp*, m. longitudinalis sternopleuralis; *mndv*, m. dorsoventralis minor; *pls*, m. lateralis sternalis primus; *plt*, m. lateralis tergalis primus; *plt'*, secondary m. lateralis tergalis primus; *qls*, m. lateralis sternalis quartus; *qlt*, m. lateralis tergalis quartus; *qnl*, m. lateralis tergalis quintus; *sls*, m. lateralis sternalis secundus; *slt*, m. lateralis tergalis secundus; *slt'*, secondary m. lateralis tergalis secundus; *sxlt*, m. lateralis tergalis sextus; *tls*, m. lateralis sternalis tertius; *tl*, m. lateralis tergalis tertius; M1–M8, muscles of the ovipositor.

## 3. Results

### 3.1. Ultimate larva (F-0) (Fig. 1)

The muscular system of the 7th pre-genital segment includes 18 muscle pairs, which represent all groups of muscles mentioned above.

Two large internal (nearer to the alimentary tract) muscles – *plt*, *slt*, and four external (nearer to the segment wall) muscles – *tl*, *qlt*, *qnl*, *sxlt* belong to the intersegmental tergal muscles. The first two attach to the anterior part of the 7th tergite and the widened anterior carina of the 8th tergite. Their insertions partly overlap, but they are easily discernible by different directions of their muscle fibers. The *plt* itself is subdivided into two layers with different directions of fibers. The external muscles pass from the middle part of the 7th tergite to anterior carinae of the 8th tergite. The *tl* and *qlt* are relatively narrow. They have the identical attachment sites on the 8th tergite, but these sites are different on the 7th tergite. The larger muscle *sxlt* originates beneath the origins of muscles *tl* and *qlt*. The *qnl* is the largest of all external tergal muscles, and is situated more ventrally than *sxlt*.

The intersegmental sternal muscles are represented by five muscles: two larger internal *pls* and *sls* connecting the anterior carinae of the 7th and 8th sternites, three smaller external muscles inserted on the anterior carina of the 8th sternite, originating from the middle of the 7th sternite (*itls*, *tls*) and from its lateral edge (*qls*).

Two intersegmental tergo-pleural muscles, *dvo* and *dvo'*, originate dorso-anteriorly from the first pleural apophysis of

Table 2  
Reorganization of the muscles sets of 7th–9th abdominal segments in female *Anax imperator* during metamorphosis

Groups of muscles	F-0 larva			Teneral female			Mature female		
	Abdominal segments								
	VII	VIII	IX	VII	VIII	IX	VII	VIII	IX
mm. intersegmentales tergali	<i>plt</i>	<i>plt</i>	<i>plt</i>	<i>plt</i>	<i>plt</i>	<i>plt, plt'</i>	—	—	<i>plt'?</i>
	<i>slt</i>	<i>slt</i>	—	<i>slt</i>	<i>slt</i>	—	—	—	—
	<i>tlt</i>	<i>tlt</i>	—	<i>tlt</i>	<i>tlt</i>	—	<i>tlt</i>	<i>tlt</i>	—
	<i>qlt</i>	<i>qlt</i>	—	<i>qlt</i>	<i>qlt</i>	—	<i>qlt</i>	<i>qlt</i>	—
	<i>qnlt</i>	<i>qnlt</i>	<i>qnlt</i>	<i>qnlt</i>	<i>qnlt</i>	<i>qnlt</i>	<i>qnlt</i>	<i>qnlt</i>	<i>qnlt</i> (M3)
	<i>sxlt</i>	<i>sxlt</i>	—	<i>sxlt</i>	<i>sxlt</i>	—	—	—	—
mm. intersegmentales sternali	<i>pls</i>	<i>pls</i>	}?	<i>pls</i>	<i>pls</i>	}?	<i>pls</i>	—	—
	<i>sls</i>	<i>sls</i>	—	<i>sls</i>	<i>sls</i>	—	—	—	—
	<i>itls</i>	—	—	<i>itls</i>	—	—	—	—	—
	<i>tls</i>	—	—	<i>tls</i>	—	—	—	—	—
	<i>qls</i>	—	—	<i>qls</i>	—	—	—	—	—
m. intersegmentalis sternopleuralis	<i>lpsp</i>	—	—	<i>lpsp</i>	—	—	—	—	
mm. intersegmentales tergopleurali	<i>dvo'</i>	<i>dvo'</i>	<i>dvo'</i>	<i>dvo'</i>	<i>dvo'</i>	<i>dvo'</i>	<i>dvo'</i>	—	<i>dvo'</i>
	<i>dvo</i>	—	<i>dvo</i>	<i>dvo</i>	—	—	—	—	—
mm. segmentales tergopleurali	<i>dva</i>	<i>dva</i>	<i>dva</i>	<i>dva</i>	<i>dva</i>	<i>dva</i>	<i>dva</i>	<i>dva</i>	—
	<i>dvm</i>	<i>dvm</i>	<i>dvm</i>	<i>dvm</i>	<i>dvm</i>	<i>dvm</i>	<i>dvm</i>	<i>dvm</i>	—
	<i>dvp</i>	<i>dvp</i>	<i>dvp</i>	<i>dvp</i>	<i>dvp</i>	<i>dvp</i>	<i>dvp</i>	<i>dvp</i>	—
	<i>mndv*</i>	<i>mndv</i>	<i>mndv</i>	—	—	—	—	—	—
Muscles of ovipositor	—	—	M4	—	M1	M4	—	M1	M4
			M5			M5			M5.1, M5.2
		—	M7		M2	M7		M2	M7
			—			M8			M8

\* *Musculus dorsoventralis minor*, *mndv*, was described for the first time. Muscles with signs of degeneration are marked by italics. Question-marks indicate uncertain homologies, the explanations of which are given in the text.

the 8th segment and attach to the anterior carina (*dvo*) and middle part (*dvo'*) of the 7th tergite.

The single intersegmental sterno-pleural muscle (*lpsp*) runs from the anterior carina of the 7th sternite to the second pleural apophysis of the 8th segment.

The group of segmental tergo-pleural muscles contains three large (*dva*, *dvm*, *dvp*), and one small (*mndv*) muscles. The *dvm* and *dvp* are the largest of this group. Their ends (on the central part of the pleurite and hind middle part of the tergite) are situated closely, and so seem as one entire muscle subdivided with tracheae into two parts. The muscles *dva* and *mndv* connect the anterior edge of the pleurite and the thickened anterior carina of the tergite.

The muscular set of the 8th genital segment is incomplete compared with the 7th segment because of partial reconstruction of the sternum into basal parts of VI.

All intersegmental tergal muscles (*plt*, *slt*, *tlt*, *qlt*, *qnlt*, *sxlt*) as well as all segmental tergo-pleural muscles (*dva*, *dvm*, *dvp*, *mndv*) are identically located and sized compared with the 7th segment. The largest of abdominal muscles *plt* and *slt* connect the thickened margins of this tergite and posterior tergites. Other groups lose some muscles. In particular, only two external intersegmental sternal muscles (*pls*, *sls*), which are difficult to distinguish one from another, and one oblique intersegmental tergo-pleural muscle (*dvo'*) remains in this segment.

The main peculiarity of the muscle composition of the 9th segment is the presence of muscles controlling components of the anal pyramid. Additionally, the annular one-piece 10th segment lacks a separated sternite and tergite.

The intersegmental tergal muscle *slt* retains its attachment points, whereas the muscle *qnlt* is inserted into the processus of the anterior carina of 10th segment. None of the other intersegmental tergal muscles (*plt*, *tlt*, *qlt*, *sxlt*), nor any intersegmental sternal (*pls*, *sls*, *tls*, *qls*), or intersegmental sterno-pleural (*lpsp*) muscles were found. The intersegmental tergo-pleural muscles (*dvo*, *dvo'*) and segmental tergo-pleural (*dva*, *dvm*, *dvp*, *mndv*) are present. Their insertions are situated on the sternite rather than on pleurites of the 9th segment, but homonomy with pleural musculature of 8th segment raises no doubt because of topographic similarity.

Also, some ovipositor muscles were found in the 9th segment. The paired *M4* passes from antero-lateral parts of the 9th tergite to the bases of V2's precursors. The paired *M5* originates on the 9th tergite close to *M4* and inserts into the middle of the 9th sternite between the attachment sites of *dvm* and *dvp*. The unpaired transverse muscle *M7* connects the symmetrical parts of segment wall near the insertions of the right and left *M5*.

### 3.2. Teneral female (Figs. 2 and 3)

Most muscles are retained in the 7th pre-genital segment. Pleurites are membranous and their muscles are attached to the lateral carinae of the sternite. The latter is narrowed and impressed, so the segmental tergo-pleural muscles (*dva*, *dvm*, *dvp*) seem to be contracted, and the intersegmental tergal muscles (*plt*, *slt*, *tlt*, *qlt*, *qnlt*, *sxlt*), as well as intersegmental tergo-pleural muscles (*dvo*, *dvo'*) originate more ventrally compared to those in the F-0 larva. The intersegmental tergal

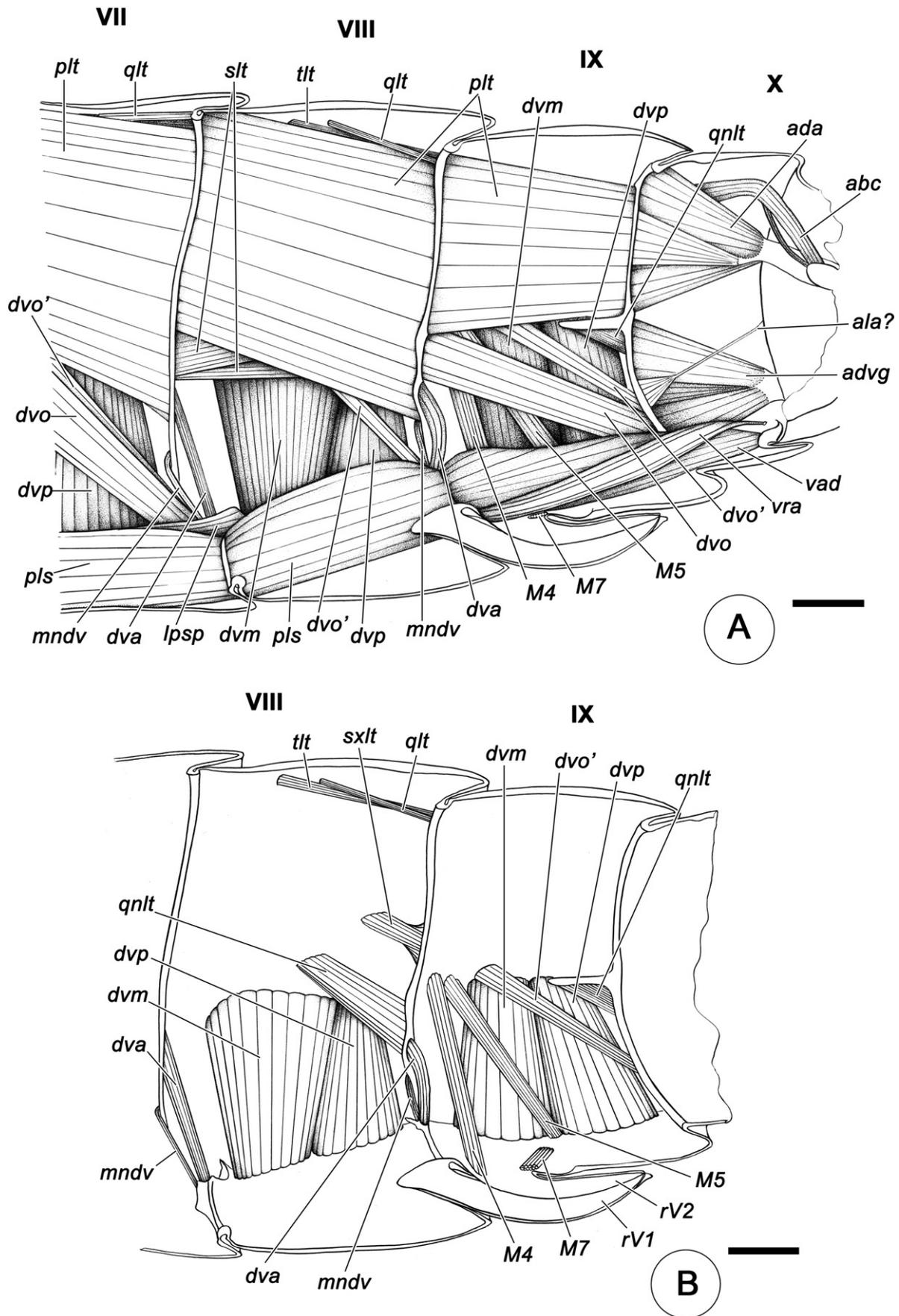


Fig. 1. Musculature of the terminal segments in the final instar larva of *Anax imperator*: (A) internal layer, and (B) external layer. Scale bars = 1 mm. *rV1*, *rV2*, larval precursors of first and second valves, respectively.

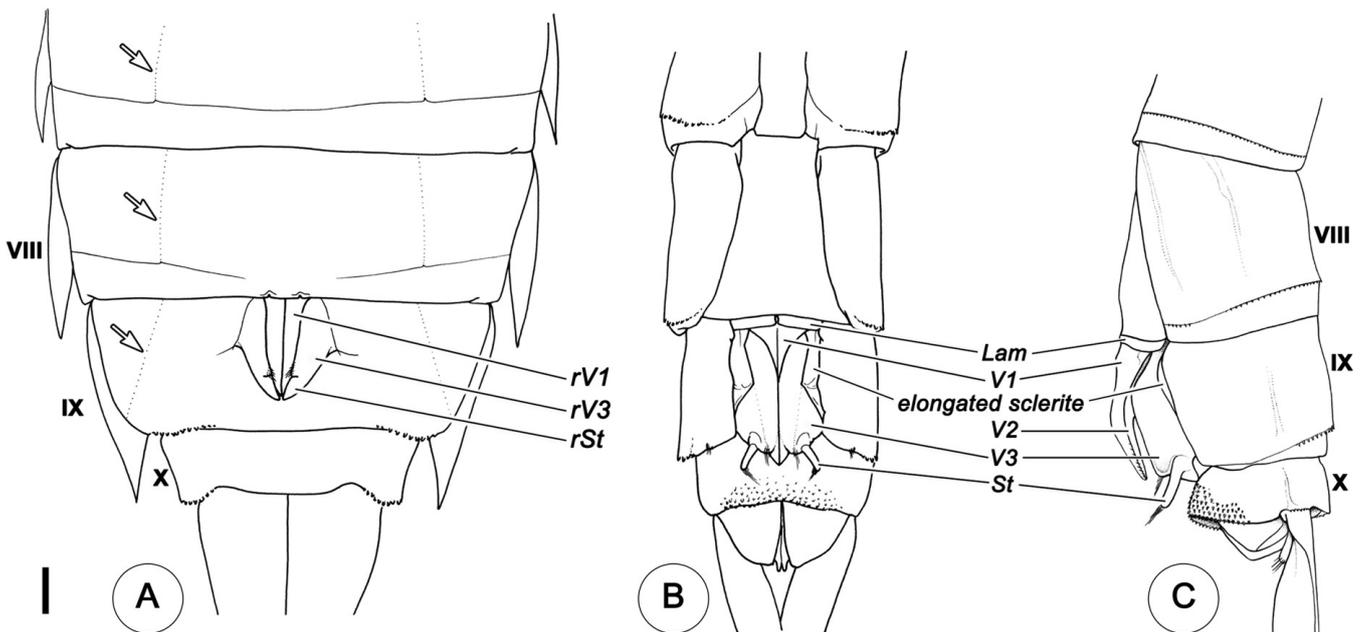


Fig. 2. The terminal segments of the final instar larva (A) and adult female (B, C) of *Anax imperator*, external ventral (A, B) and lateral (C) views. White arrows mark the sterno-pleural suture in the larva. Scale bar = 1 mm. *Lam*, basal plate of ovipositor (*lamina valvarum*); *V1*, first valves of ovipositor (*valvulae 1*); *V2*, second valves of ovipositor (*valvulae 2*); *V3*, third valves of ovipositor (*valvulae 3*); *St*, stylet; *rV1*, *rV2*, *rSt*, larval precursors of *V1*, *V2* and *St*, respectively.

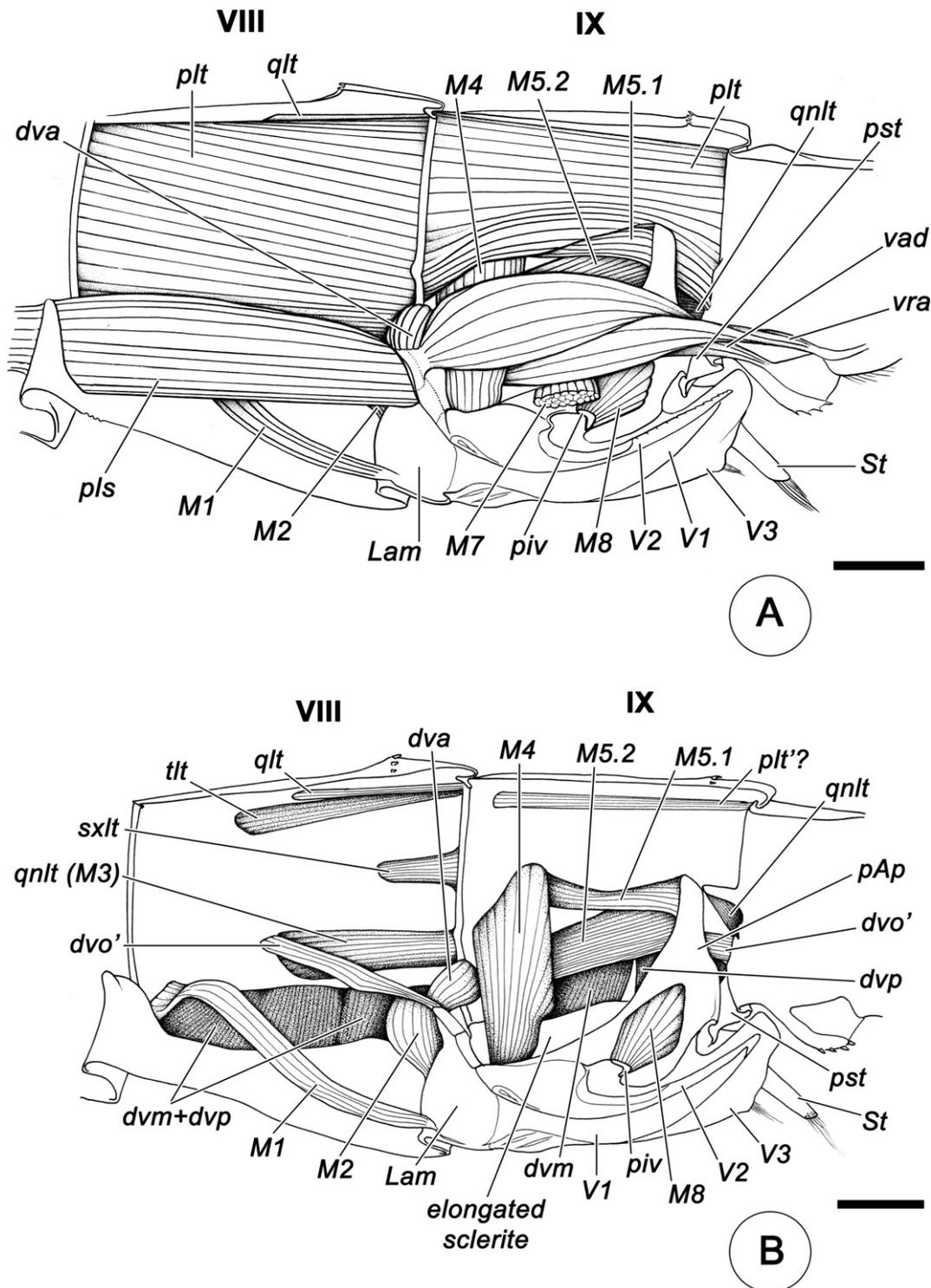


Fig. 3. Musculature of genital segments in a teneral female of *Anax imperator*: (A) internal layer, and (B) external layer. Scale bars = 1 mm. *Lam*, basal plate of ovipositor (*lamina valvarum*); *V1*, first valves of ovipositor (*valvulae 1*); *V2*, second valves of ovipositor (*valvulae 2*); *V3*, third valves of ovipositor (*valvulae 3*); *pAp*, posterior apophysis of *V3*; *St*, stylus; *piv*, internal sclerite or posterior intervalvula; *pst*, poststernite of 9th segment.

muscles *plt*, *slt*, *sxt* exhibit some signs of degeneration — they are loose and obviously thinner than in F-0. The segmental tergo-pleural muscle *mndv* was not found. The rest of muscles (*pls*, *sls*, *tls*, *qls*, *lpls*) were found in unchanged condition.

The sternites of the genital segments undergo more diverse modifications (Fig. 2). The 8th sternite is folded transversely

in the posterior region, originating in the central part of the basal plate of the ovipositor (*Lam*). The anterior edge of the latter was straight and transverse and served as attachment points of two paired ovipositor muscles *M1* and *M2*. *M1* runs anterior from the medial part of *Lam* and ends on the 8th tergite near its ventral carina; *M2* begins on the lateral

edge of *Lam* and inserts on the posterior carina of the 8th tergite. The pleurites of the 8th segment are also not sclerotized.

The 9th sternite transforms into a set of sclerites connected via membranous cuticle. The major part of the rest of the 9th sternite is divided into two large symmetrical plates, the third valves (*V3*), connected with each other by thin cuticle in the middle. Each *V3* possess one stick-like endoskeletal outgrowth, the *posterior apophysis*, serving as the insertion point of the paired *M5* muscle of the ovipositor. A small rigid part of the integument, surrounded by thin cuticle, is retracted inside the sternite between both *V3*. It originates from the internal sclerite (*piv*) bearing the ends of the paired ovipositor muscle *M8*. The narrow hindmost margin of the 9th sternite is fused with the corresponding tergite, originating on *poststernite 9*.

The symmetrical antero-lateral parts of the larval 9th sternite undergo the most considerable reshaping. Each of them folds transversely in the zone of its anterior corner, becoming here partly desclerotized, and divided into two pieces (Fig. 5). The first, more anterior piece, situated laterally to *V2*'s rudiments and bearing the ends of the muscles *dva*, *dvo'* and *vad*, are stuck to the posterior edges of main part of *Lam*. It forms two articulations — the condylar one with *V1* ventrally and the uniaxial one with the anterior carina of 9th tergite dorsally. Thus this paired piece of larval 9th sternite is the origin of the symmetrical movable lateral parts of *Lam*. The second piece, which includes the lateral carina of the larval 9th sternite and bears the attachment points of the muscles *dvm*, *dvp*

and *vra*, originates the elongated sclerite. The anterior end of the elongated sclerite fuses with the anterior carina of the 9th tergite, whereas its posterior end articulates via a condyle with *V3* just under the *posterior apophysis*.

Other muscles in the genital segments retain their locations. *M4* and *M7* are larger, compared to those in F-0 larva. *M5* is still entire or becomes divided into two fascicles in some specimens. Some muscle fibers of the intersegmental tergal muscle, *plt*, in the 9th segment begin anterior to the rest of the muscle (*plt'*). Certain muscles (in the 8th segment: *plt*, *slt*, *sxlt*, *dvo'*; in 9th segment: *slt*, *dva*, *dvm*, *dvp*) are degenerated to variable extents in different specimens studied. The segmental tergo-pleural muscle, *mandv*, as well as the intersegmental tergo-pleural muscle, *dvo*, were not found.

### 3.3. Mature female (Fig. 4)

The imaginal musculature of the 7th segment comprises eight paired muscles: *tlt*, *qlt*, *qnlt*, *pls*, *dvo'*, *dva*, *dvm*, *dvp*. The latter two seem to be united, but their borders are marked somewhat by tracheae branches. All muscles are identically located as in the freshly emerged imago.

Part of the intersegmental tergal (*tlt*, *qlt*, *qnlt*) and tergo-pleural (*dva*, *dvm*, *dvp*) musculature is retained in the 8th segment. Compared to the teneral imago the *tlt* and *qlt* are larger, whereas *qnlt* retains its size and shape. The *dvm* is reduced and coalescent with *dvp*. *Lam* forms two lateral apophyses bearing

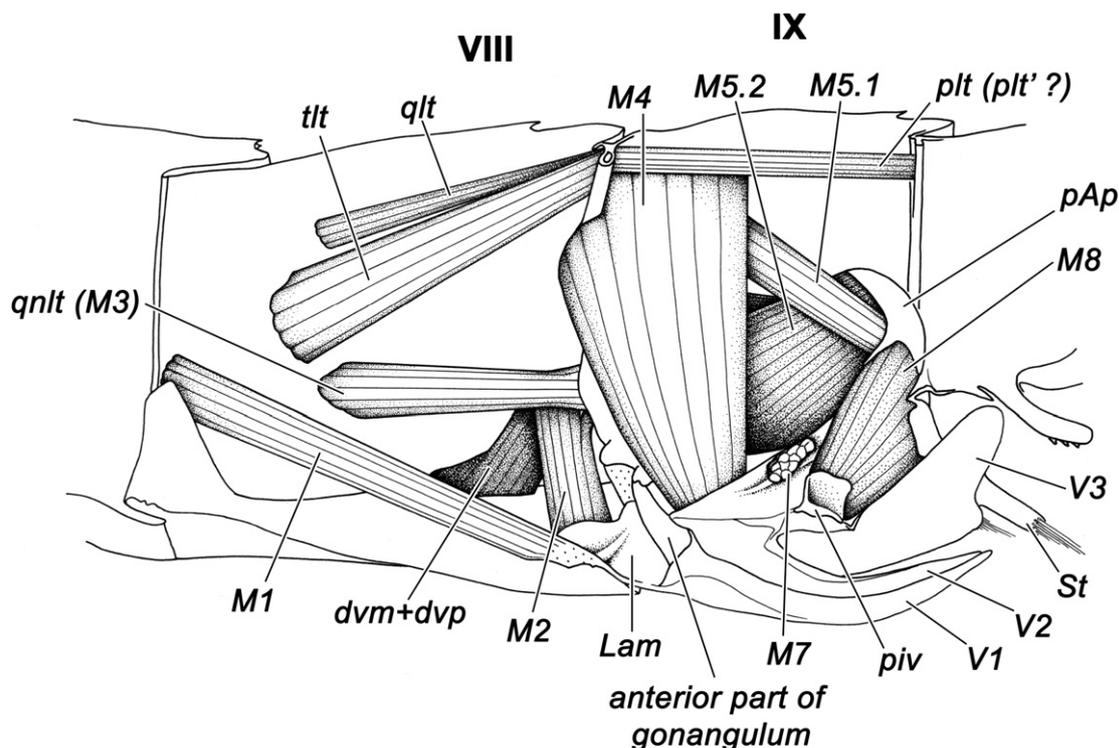


Fig. 4. Musculature of genital segments in the mature female of *Anax imperator*. Scale bar = 1 mm. *Lam*, basal plate of ovipositor (*lamina valvarum*); *V1*, first valves of ovipositor (*valvulae 1*); *V2*, second valves of ovipositor (*valvulae 2*); *V3*, third valves of ovipositor (*valvulae 3*); *pAp*, posterior apophysis of *V3*; *St*, stylus; *piv*, internal sclerite, or posterior intervalvula.



from one part of the larval *plt*. Muscles of the ovipositor, situated in the 9th segment (*M5*, *M4*, *M7*, *M8*), are distinctly enlarged compared with those in the teneral imago. The anterior apophyses of the *V3* develop underneath the ends of the muscle *M4*.

#### 4. Discussion

##### 4.1. Peculiarities of the musculature of genital segments in the metamorphosing female

Female genital segments in *A. imperator* are characterised by incomplete muscle sets as compared to the 7th pre-genital segment on all studied stages, and the musculature of the 9th segment is always poorer than that of the 8th segment (see Table 2). Mostly those muscles were reduced that had attachments on the sternum of pre-genital segments. Thus, the ultimate larva partly loses intersegmental sternal and sternopleural muscles in the 8th segment, whereas in the 9th segment they are completely absent. As stated above, these reductions seem to be associated with the development of some ovipositor parts in both genital segments, as well as with the attachment of two large paired muscles of the anal pyramid (*vad*, *vra* according to Asahina, 1954: pl. 44, Fig. A8) to the anterior carina of 9th sternite. These muscles pass to the ventral margins of the paraprocts through the 9th and 10th segments, and their contraction adducts the paraprocts. Both genital segments of the imago lose the sternal musculature completely.

The musculature associated with the tergites appears to be more distinctly differentiated. The system of intersegmental tergal and segmental tergo-pleural muscles is identical in both the 7th and 8th segments and in both the ultimate larva and the imago. Moreover, the homonymous intersegmental tergal muscles in these two segments disappear during emergence (*plt*, *slt* and *sxlt*). In contrast, four of the six intersegmental tergal muscles and five of six tergo-pleural muscles are absent in the 9th segment of the final instar larva. The tergite musculature of the 9th segment of an adult insect is the remnant of the larval muscles *plt* and *dvo*'. This situation is essentially different in more anterior segments, where the intersegmental tergal muscles *plt* disappear during maturation. Asahina called the *plt* a "larval muscle" (Asahina, 1954: Table 9), assuming its reduction in imagines of all odonates studied. Our observations have shown that the larval muscle *plt* in the 9th segment was transformed to the adult *plt*.

We have found new small paired intersegmental muscles, named *musculus dorsoventralis minus*, *mndv*, in the 7th–9th segments of the F-0 larva. They connect, on each side, the anterior apophysis of the sternite with the anterior carina of the tergite near the *dva*, and completely disappear in adults.

##### 4.2. Origin of basal parts and muscles of the ovipositor

The general changes of the abdominal skeleton in odonates during metamorphosis are the transformation of the pleural sclerites into the pleural membrane, the enlargement of the tergite and the narrowing of the sternite (Asahina, 1954). As was

expected, the reshaping of the genital segments is more complex. The 8th sternite of the final instar larva is folded transversally, so that the internal edge of the fold forms the anterior margin of *Lam*. This margin is slightly curved and bears insertions of muscles *M1* and *M2* in the teneral female, whereas the paired apophysis of *Lam*, bearing the end of *M2*, developed later during the female maturation.

The venter of the larval 9th segment consists of one central and two lateral plates. The largest central part bears the ends of both sternal and pleural muscles, and hereby resembles the fused sternite and the largest pleural part of the anterior segments (Fig. 5). The lateral part loses attachments of the muscles like the small antero-lateral pleural parts of the anterior segments. The lateral plates become membranous in the imago, while the central one is divided into a set of sclerites that form the origin of some basal elements of the ovipositor's valves. The anterolateral edge of the larval 9th sternite is divided into two unequally shaped plates (Fig. 5C). The first, more anterior part becomes fused via an elastic cuticle with the middle part of *Lam*, originating from the 8th segment and, therefore, is included in the imaginal *Lam*. This plate forms two articulations of *Lam* – medially with the first valve and laterally with the 9th tergite. The second plate gives rise to the *elongated sclerite* from the lateral carina of the larval 9th sternite. Its anterior end becomes fused immovably to the anterior carina of the 9th tergite and forms the dorsal surface of the uniaxial "9th tergite-*Lam*" articulation, whereas the posterior end of the *elongated sclerite* forms a condylar articulation with the *V3*. Hence, the *elongated sclerite* allows the *V3* to move relative to the 9th tergite. During emergence of the female, the outer part of the *V3* extends ventrad and distad, covering the cutting valves of the ovipositor (*V1* + *V2*). The central part of the larval 9th sternite, with the attachment sites of *M4* and *M5*, transforms into the endoskeleton margins of the *V3*. The most medial part of the larval 9th sternite invaginates deeply inside the segment and forms an imaginal internal sclerite that later serves as the attachment zone of the paired ovipositor muscles, *M8*. The posterior carina of the 9th sternite fuses with the 9th tergite and gives rise to the imaginal *poststernite 9*, following Asahina's terminology (1954). The rest of the larval 9th sternite is not sclerotised in the imago.

The ultimate larva acquires some of the ovipositor muscles, namely the paired *M4*, *M5* and the unpaired *M7*. The muscle *M8* and the posterior endoskeletal apophyses of the *V3*, which are situated underneath the ends of the muscle *M5*, appear in the emerged imago. Enlargement of all muscles, appearance of the anterior apophyses of *V3* and divergence of *M5* (in most specimens) occurred during maturation of the female.

The weakly pigmented, semi-translucent wall of the 9th segment of the newly emerged female allowed the observation of the alternating contraction of the large muscles *M4* and *M5* during emergence. They work antagonistically. Contraction of *M4* simultaneously causes the retraction of the anterior parts of the *V3* and the extension of their posterior parts. The following contraction of *M5* acts conversely. Thus, the ovipositor muscles *M4* and *M5* probably participate in

the peculiar reshaping of the 9th segment, which seems to be distinctly swollen, as well as in the positioning of some newly developed basal sclerites of the ovipositor. These functions may cause the surprisingly early development of the imaginal ovipositor muscles in a larva.

#### 4.3. Suggested homology of some ovipositor components in odonates and some other insect groups

The primary ovipositor of insects is believed to comprise two paired genital appendages of the 8th and 9th segments – the *gonapophyses* – articulated with the corresponding segments through two paired plates, *gonocoxites*, or *valvifers* (Snodgrass, 1935). The 9th sternite disappears to a greater or lesser extent, so that one or two unpaired sclerites, found in some Pterygota and named the *intervalvulae*, are actually its rudiments (Snodgrass, 1935; Scudder, 1957).

Whereas the homologies of the gonapophyses with the cutting valves in Odonata raised no doubts, the origins of other elements of the ovipositor were very vague (Table 1). For instance, the 3rd valves were considered as the gonapophyses laterales or the lateral valvula (Machotin, 1929; Asahina, 1954), as the 3rd valves jointed with the 2nd valviferae (Pfau, 1985), or as the 2nd valviferae itself (Srivastava and Srivastava, 1989). The elongated sclerite was regarded as having originated from the 8th segment (part of *first valvifera* in Pfau, 1985, 1991), from the 9th tergite (*laterotergite* in Srivastava and Srivastava, 1989), from the 9th sternite (*Basalstück* in van der Weele, 1906 and St. Quentin, 1962), or from different subcoxal elements of the 9th segment (*subcoxa* of 9th segment in Machotin, 1929, *post-laterocoxa IX* in Klass, 2003). The internal sclerite was considered as a derivative of the gonapophyses of the 9th segment (Machotin, 1929) or as the rest of the reduced 9th sternite (*posterior intervalvula* in Asahina, 1954). Also, the homologies between the parts of the 1st valviferae in odonates and other insects were not fully established.

The use of the muscles as developmental markers as proposed in this study allowed us to ascertain the origins of the above-mentioned basal parts of the ovipositor. The 3rd valves are entire plates that developed from the larval sternum of the 9th segment without any visible signs of fusion with other sclerites and can be homologised with gonocoxites of the 9th segment. Their lateral borders are marked by attachments of ovipositor muscles *M4*, *M7* and *M5*. The internal sclerite originates from the center of the larval 9th sternum and, therefore, belongs topographically to the *intervalvula posterior* sensu Asahina (1954).

According to Scudder's (1957, 1961) groundplan of the ovipositor of insects, both the first and the third valves (represented by gonapophyses of the 8th segment and gonocoxites of the 9th segment, respectively) are fixed to the same point on the 9th tergite through a triangular sclerite originating from the 9th segment and named the *gonangulum*. The presence of the latter had been considered an autapomorphy of the Zygentoma and Pterygota for a long time (e.g. Hennig, 1969), until Klass (2003) suggested that the formation of the

one-piece gonangulum is due to a fusion of two “gonangular” sclerites. This author indicated the presence of the two-piece gonangulum (LC9, following terminology of Klass, 2003) in Archaeognatha (Klass, 2003 using data of Bitsch, 1974), in some basal earwigs from the family Pygidicranidae, and in the damselfly *Calopteryx splendens* Harris, 1782 (Klass, 2008). Results of this study support Klass' statement of the two-piece gonangulum in Odonata. The gonangulum of *Anax imperator* represents actually two separate plates. The lateral part is named the elongated sclerite. Its position on the larval sternum of the 9th segment was indicated by the attachment of the muscle of the paraprot (*vra*) (Fig. 5D). The second, anterior part, is included into the basal plate of the ovipositor. Its border was marked with the ends of several larval muscles (*pls*, *vad*, *dvo*, *dva*) (Fig. 5D). The functions of the two gonangular parts in an adult female are different as it was previously mentioned, but jointly they perform the typical gonangular function – to join movable valves originating from both genital segments to the 9th tergite.

Interestingly, the one-piece gonangulum is believed to be apparently inherent to the most ancient dragonfly, the extinct megalopteran ‘protodonate’ *Erasipteroides valentini* Brauckmann, 1985 (Bechly et al., 2001). Its ovipositor was very long and simplified in its construction: only the ovipositor itself was developed actually, whereas the 3rd valves were strongly reduced. Reconstruction of the kinematics of the ovipositor in *Erasipteroides valentini* is a complicated task, because we know nothing about the mobility of the articulations, and, therefore, about the function of the gonangulum.

## 5. Conclusions

1. Female genital segments of *Anax imperator* are characterised by incomplete muscle sets as compared with that of the 7th pre-genital segment on all studied stages, and the musculature of the 9th segment is always poorer than that of the 8th segment. Most larval muscles are absent in adults. The reduction involves principally sternal rather than tergal musculature.
2. A newly described small intersegmental muscle, named *musculus dorsoventralis minus*, *mndv*, was found in the 7th and 8th segments of the F-0 larva. It connects the anterior apophysis of the sternite with the anterior carina of the tergite and completely disappears in the metamorphosing imago.
3. Some imaginal ovipositor muscles, namely the paired *M4*, *M5* and the unpaired *M7*, were already present in the F-0 larva. Their modifications (enlargement of all muscles, divergence of *M5*) and the presence of the remaining ovipositor muscles was found in adults. It was assumed that the unusually early development of some imaginal muscles (*M4*, *M5*) may be caused by their participation in the reshaping of the abdominal wall during the metamorphosis.
4. The 9th sternite transforms into a set of sclerites during the last molt of the female larva and provides the origin of some basal elements of the odonate ovipositor.

Unlike in most other Pterygota, the *gonangulum* in Odonata, originating from the anterolateral edge of the larval 9th sternite, is not a one-piece sclerite, but consists of two plates that are unequal in shape and function. The first, anterior part is fused with gonocoxite 8 and articulates with the first valve to the 9th tergite. The second, lateral part attaches the third valve to the 9th tergite. Except in Odonata, such bipartition of the gonangulum was found only in a basal dermapteran, *Tagalina burri* (Klass, 2003), among the Pterygota.

The middle part of the 9th sternite in the larval female is deeply invaginated inside the segment and forms an imaginal internal sclerite, the *intervalvula posterior*. From the central part of the larval 9th sternite, the symmetrical endoskeleton part of the 3rd valves of the ovipositor originates. The rest of the larval 9th sternite, except for the narrow cross of the hindmost carina, becomes unsclerotised in the imago.

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