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The female cephalothorax of *Xenos vesparum* Rossi, 1793 (Strepsiptera: Xenidae)

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Abstract

The female cephalothorax of *Xenos vesparum* (Strepsiptera, Xenidae) is described and documented in detail. The female is enclosed by exuvia of the secondary and tertiary larval stages and forms a functional unit with them. Only the cephalothorax is protruding from the host's abdomen. The cephalothorax comprises the head and thorax, and the anterior half of the first abdominal segment. Adult females and the exuvia of the secondary larva display mandibles, vestigial antennae, a labral field, and a mouth opening. Vestiges of maxillae are also recognizable on the exuvia but almost completely reduced in the adult female. A birth opening is located between the head and prosternum of the exuvia of the secondary larva. A pair of spiracles is present in the posterolateral region of the cephalothorax. The musculature of the female cephalothorax is strongly reduced. Only muscles of the mandibles, foregut and a pair of longitudinal muscles are present. The nervous system is strongly flattened dorsoventrally. The brain is shifted to the prothoracic region together with the frontal ganglion. Well-developed optic nerves are present and vestiges of stemmata. The suboesophageal ganglion is fused with the thoracic and abdominal ganglia thus forming a compact undivided ganglionic mass. The dorsal vessel forms a ring-shaped structure around the brain. A valvula cardiaca is present between the posterior foregut and midgut. The midgut is strongly bloated and probably involved in inflating the cephalothorax during pheromone release of the female. The Nassonov's glands are located on the ventral side of the cephalothorax. Structural features of the females of *X. vesparum* are compared to conditions found in the head and thorax of the free-living females of Mengenillidae and cephalothoracic characters of *Stylops ovinae* (Stylopidae). The highly modified morphology of female Stylopidia is dicussed with respect to their permanent endoparasitism and also with their neotenous development.

Key words

Strepsiptera, *Xenos vesparum*, female, cephalothorax, morphology.

1. Introduction

Strepsiptera are one of the smallest groups of holometabolous insects, with presently about 600 described species (e.g. Pohl & Beutel 2013). It is an extremely specialized endoparasitic group characterized by numerous autapomorphies (e.g. Pohl & Beutel 2005, 2008). Its systematic position was one of the most strongly disputed issues in insect phylogenetics (e.g. Kristensen 1981: "the Strepsiptera problem"; Kjer et al. 2016) and a sistergroup relationship with Coleoptera was only confirmed recently by analyses of transcriptomes and genomes (Niehuis et al. 2012; Boussau et al. 2014; Peters et al. 2014; Misof et al. 2014).

The species treated in this study, *Xenos vesparum* Rossi, 1793, was the first described strepsipteran. It belongs to the Xenidae and the large clade Stylopidia, which contains more than 97% of the species of the order (Pohl & Beutel 2005). The most important autapomorphy of Stylopidia is the obligatory and permanent endoparasitism of the female (Pohl & Beutel 2008), in contrast to the free-living wingless females of the basal Mengenill-

idae (e.g. Kinzelbach 1971). Profound changes in the morphology and life cycle are correlated with the endoparasitic life style. Whereas a distinctly developed head and a thorax with functional legs are present in females of Mengenillidae, the legs are completely absent and the anterior body regions form a compact cephalothorax as a secondary tagma (Kinzelbach 1971; Pohl & Beutel 2008). In contrast to the winged adult males and females of Mengenillidae the females of Stylopidia remain permanently within the host's body with their sack-shaped abdomen. Only the sclerotized cephalothorax protrudes from the host's abdomen or gaster (e.g. Kathirithamby 1989). It bears the external orifice of the brood canal on the ventral side between the head and the prosternum which is connected with the birth organs in the abdomen (Kathirithamby 1989). This is the opening where the copulation takes place (e.g. Peinert et al. 2016) and where the first instar larvae are released (e.g. Kathirithamby 1989; Beani et al. 2005).

The postembryonic development of Stylopidia comprises three larval stages (Kathirithamby et al. 1984; Beani et al. 2005; Manfredini et al. 2007). The extremely miniaturized and agile first instar larvae enter the host, in the case of *X. vesparum* larvae of *Polistes dominula* (Christ, 1791) (Vespidae) (Manfredini et al. 2007). The secondary larvae emerging after the first moult are grub-shaped and are increasing to the final size of the female or the male puparium. After protruding the cephalothorax of the secondary larva the female moults to the tertiary larva and the adult. The old exuviae are not shed and adult females of Stylopidia are enclosed by three layers of cuticle, the exuviae of the secondary and tertiary larvae and the adult cuticle (Beani et al. 2005). The exuvia of the secondary larva is often referred to as puparium (e.g. Kinzelbach 1971). Whereas the female tertiary larval stage was referred to as pupa in older contributions (e.g. Lauterbach 1954), this designation was not used in most recent studies (e.g. Kathirithamby et al. 1984; Beani et al. 2005; Manfredini et al. 2007).

The morphology of larvae and males is relatively well known (e.g. Kinzelbach 1971; Pohl 2002; Pohl & Beutel 2004, 2005, 2008; Beutel et al. 2005; Osswald et al. 2010). In contrast, the knowledge of the female anatomy is still very fragmentary. Studies on the musculature, nervous and tracheal system (Kinzelbach 1971), on the ultrastructure of the Nassonov's glands (Dallai et al. 2004) and midgut (Giusti et al. 2007) of *X. vesparum* are available. Diploma theses on the thorax and head of *Eoxenos laboulbenei* De Peyerimhoff, 1919 (Mengenillidae) were presented by Müller (2009) and Marquart (2010). A study on the cephalothorax of *Stylops ovinae* Noskiewicz & Poluszyński, 1928 (Stylopidae) is the only comprehensive treatment of a cephalothorax based on modern techniques (Löwe et al. 2016).

Considering the scarcity of data, the major aim of this study was to document the cephalothoracic anatomy of *X. vesparum* in detail, using a broad spectrum of traditional and innovative techniques. The evolution of this character system in Stylopidia is evaluated under phylogenetic and functional aspects. Structural features probably involved in inflating the cephalothorax during pheromone release of the female described by Hrabar et al. (2014) for females of *X. peckii* Kirby, 1813 are discussed, and also possible neotenous effects leading to the highly modified morphology of the females (e.g. Kathirithamby 1989; Beani et al. 2005; Erezyilmaz et al. 2014; Kathirithamby et al. 2015; McMahon & Hayward 2016).

2. Material and methods

Material. The females of *X. vesparum* were collected within its host *P. dominula* by H. Pohl on the 16.vii.2014, in the vicinity of Mettenheim (Rheinland-Pfalz, Germany). Seventeen specimens were available for the present study.

Fixation. The females were extracted from the hosts, fixed in Dubosq-Brasil over night, and then transferred into 70% ethanol (n = 2). Specimens used for measurements, photomicrography and scanning electron microscopy (SEM) were directly fixed in 70% ethanol (n = 15).

Measurements. The width and length of the cephalothorax, and the total length of 12 females of *X. vesparum* were measured with a Zeiss Stemi SV11 stereomicroscope with a calibrated ocular micrometer.

Scanning electron microscopy. Three complete and one specimen with removed larval exuviae of the cephalothorax were transferred to 100% acetone over an ascending series of ethanol (70, 80, 90, 96, 100%). Critical point drying was carried out with liquid CO_2 in an Emitech K 850 Critical Point Dryer (Sample preparation division, Quorum Technologies Ltd., Ashford, England). They were glued on the tip of minute insect pins with super glue and then mounted on a rotatable specimen holder (Pohl 2010), sputter coated with gold with an Emitech K 500 (Sample preparation division, Quorum Technologies Ltd., Ashford, England). The Philips ESEM XL30 (Philips, Amsterdam, Netherlands) was equipped with Scandium FIVE software (Olympus, Münster, Germany). To increase the depth of field images with different focus were processed with Zerene Stacker (Zerene Systems LLC, Richland, USA). To investigate the fine structure of the cuticula, one specimen within its host was embedded in methacrylate and longitudinal sectioned with a microtome (s.b.). Methacrylate was then dissolved using Xylol. Xylol was gradually replaced by acetone and the specimen was critical point dried and subsequently examined using the SEM.

Microtome sectioning. The anterior third of the body of a virgin female and another one inseminated about five minutes before fixation were cut off with a razor blade,

dehydrated as described above and embedded in Araldit-CY 212 (Agar Scientific, Stansted/Essex, England). Both were sectioned with a thickness of 1 µm with a Microtom HM 360 (Microm, Walldorf, Germany) equipped with a diamond knife. Cross sections were made of the inseminated female and longitudinal sections of the other one. The sections were stained with toluidine blue and pyronine G (Waldeck GmbH & Co. KG/Division Chroma, Münster, Germany). The cross section series was documented with an Axioskop (Carl Zeiss AG, Oberkochen, Germany) with a 5×-objective (1.6×-post-enlargement) and a camera (PixeLink Capture Oem) equipped with PixeLink Capture OEM software (PixeLink, Ottawa Canada). To document some selected sections in higher resolution image stacks were taken with an Olympus dot. Slide microscope (BX51, software version 3.4, Olympus, Tokyo, Japan). Single images were exported with Fiji (Schindelin et al. 2012) or alternatively with ImageJ (Schneider et al. 2012) and combined with Zerene Stacker (Zerene Systems LLC, Richland, USA).

3D-Modelling. Alignment of images of cross sections was carried out with the Plugin Trak_EM2 (Cardona et al. 2012) of Fiji (Fiji Life-Line version, 22.xii.2015), initially with the rigid alignment option and then with elastic alignment (Saalfeld et al. 2012). Amira 6.0 and 6.1 (Visage Imaging GmbH, Berlin, Germany) was used for the reconstruction. Different structures were labelled as materials and exported with the plugin-Script "multiExport" (Arbeitsgruppe Frank Friedrich, Universität Hamburg). The exported .tiff image series were transformed into smoothed surfaces with Imaris 6.2.1 (Bitplane AG, Zürich, Switzerland) which were then transformed with Amira 6.1 into Wavefront-files (.obj). The size of the materials was reduced with Transform 2 Version 8.3.24 (Heiko Stark, Jena, Germany, URL: http://starkrats.de) for further processing. Final editing of the surfaces and images was done with Maya 2016 (Alias Wavefront, Toronto/ Ontario, Canada).

Photomicrography. To document the coloration of the exuvia of the secondary larval stage and the adult females two specimens were dried at the critical point, glued to the tip of a an minute insect pin and photographed with a Nikon D 90 and a 63 mm Zeiss Luminar macro lense, in combination with an adjustable extension bellows. The specimens were illuminated with two flashlights fitted with a transparent cylinder for soft light. Zerene Stacker (Zerene Systems LLC, Richland, USA) was used to combine stacks of images with different focus.

Image processing. All images were processed with Adobe Photoshop® CS5 (Adobe System Incorporated, San Jose, USA) and arranged as plates with this software. Adobe Illustrator® CS5 (Adobe Systems Incorporated, San Jose, USA) was used for the lettering of the plates.

Terminology. The terminology used for the females is based on Löwe et al. (2016) unless stated otherwise.

3. Results

3.1. General morphology of female

The female is enclosed by the exuviae of the secondary and tertiary larvae and forms a functional unit with them. The morphology is strongly modified. Legs are completely absent and other appendages or external structures are strongly simplified or also missing (Fig. 1). The ventral side of the body is opposed to the abdominal tergites of the host. The dorsal side of the cephalothorax rests on the tergite directly posterior to the protruding site, in stylopized *Polistes* with a single parasite at the fourth or fifth segment of the gaster.

The total length of the females is on average 5.8 mm (min. 5.1 mm, max. 7.2 mm, n = 12). The sclerotized cephalothorax protrudes from the host's gaster. It is a compact unit comprising the head, the thorax, and the anterior portion of abdominal segment I. The lateral region of the head is caudally prolonged, and a short cranially directed bulge is present on the anterolateral region of the first abdominal segment (Figs. 1A–D, 2). The paired spiracles open at the anterior region of this bulge of segment I on the posterior third of the cephalothorax and are the only openings of the tracheal system (Figs. 1A,C, 2B,C, 3A,C). Abdominal segment I is characterized by a constriction in its middle region, which marks the border between the exposed cephalothorax and the posterior part of the body. The posterior body comprises the posterior half of abdominal segment I and the remaining abdominal segments II–X. It is weakly sclerotized and sack-shaped. The brood canal is located on the ventral side of the cephalothorax and the abdomen (Fig. 1A,B).

The cephalothorax is dark brown to yellowish-brown (Fig. 1A–D), less strongly pigmented on the dorsal side and darker laterally and ventrally. The regions close and posterior to the spiracles are distinctly infuscated (Fig. 1C). The ventral side of the metathorax is light brown. The cuticle at the constriction of abdominal segment I (Fig. 1) is weakly sclerotized, soft and brownish. Only the region of the brood canal in the abdomen displays a greyish-brown coloration (Fig. 1A–C).

3.2. Cephalothorax of secondary larva

The cephalothorax of the secondary larva is at the same time the functional external layer of the female. It is 0.8–1.1 mm long (mean 1 mm, n = 12) and between 1.0–1.3 mm broad (mean 1.2 mm, n = 12). Its shape is compact and ovoid, slightly tapering anteriorly. In cross section it appears as a flattened ellipsoid (Fig. 10).

Head capsule. The head capsule is dorsoventrally strongly flattened and prognathous. Including the lateral extensions it is about 1/3 as long as the entire cephalothorax (Figs. 1B,C, 2A,C). It is distinctly separated from the prothorax by the brood canal opening and laterally by a suture (**sbhp** in Figs. 1B,C, 2D). Eyes or cephalic sutures

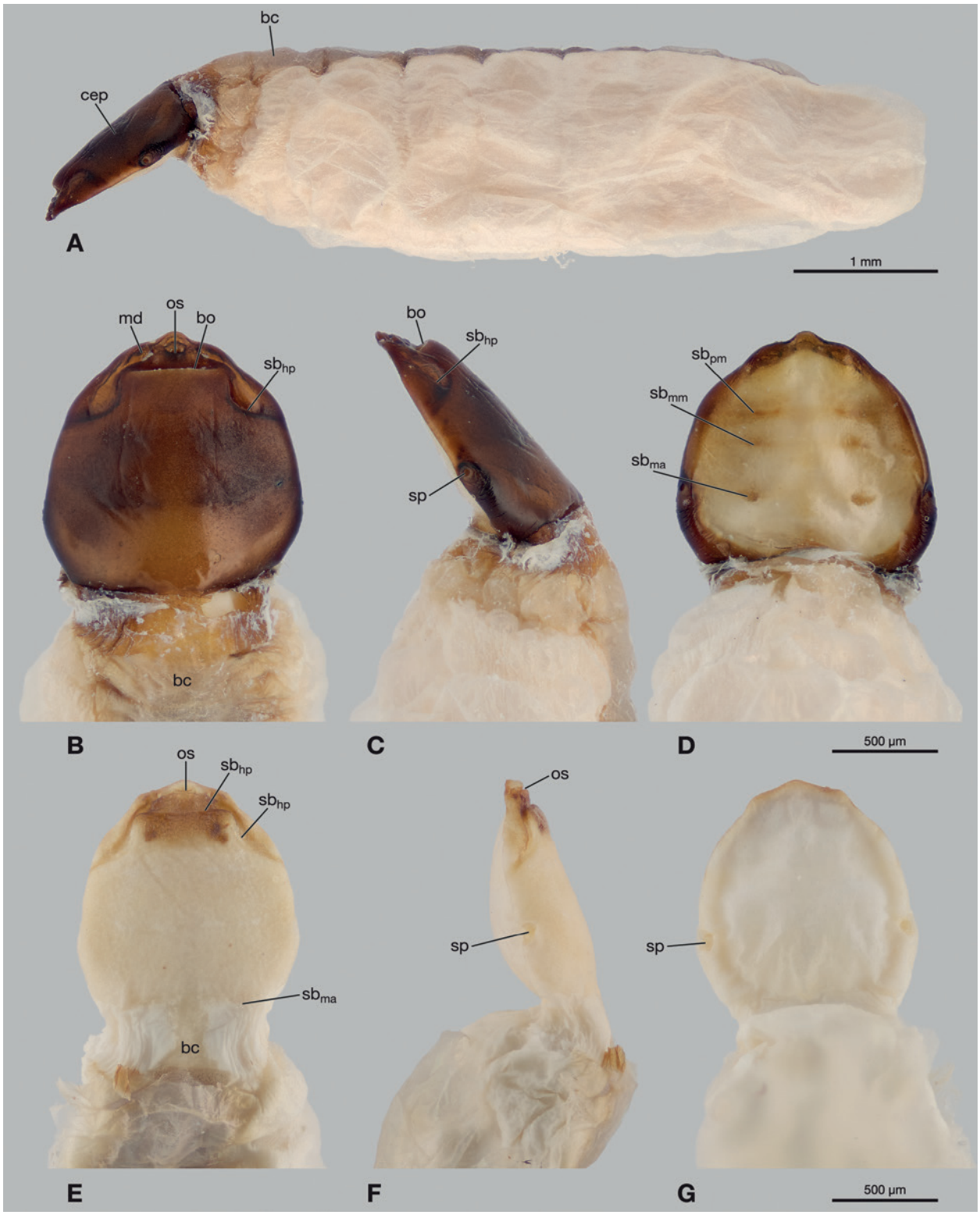

Fig. 1. *Xenos vesparum*, female, habitus, photomicrographs. **A**: lateral view, anterior is toward the left and ventral side (physiological dorsal side) facing upwards; **B** – **D**: cephalothorax and anterior abdomen (B, ventral; C, lateral; D, dorsal view); **E** – **G**: cephalothorax and anterior abdomen with larval euviae removed (B, ventral; C, lateral; D, dorsal view). — ***Abbreviations***: bc – brood canal, bo – birth opening, cep – cephalothorax, md – mandible, os – mouth opening, sb_{hp} – segmental border between head and prothorax, sb_{ma} – segmental border between metathorax and abdomen, sb_{mm} – segmental border between mesothorax and metathorax, sb_{pm} – segmental border between prothorax and mesothorax, sp – spiracle.

(strengthening ridges or cleavage lines) are missing. Several short setae arranged in two or three rows are inserted on the ventral side anterior to the oval field anterior to the mouth opening (Fig. 5B). On the anterior head capsule several sensilla are present (Fig. 5B).

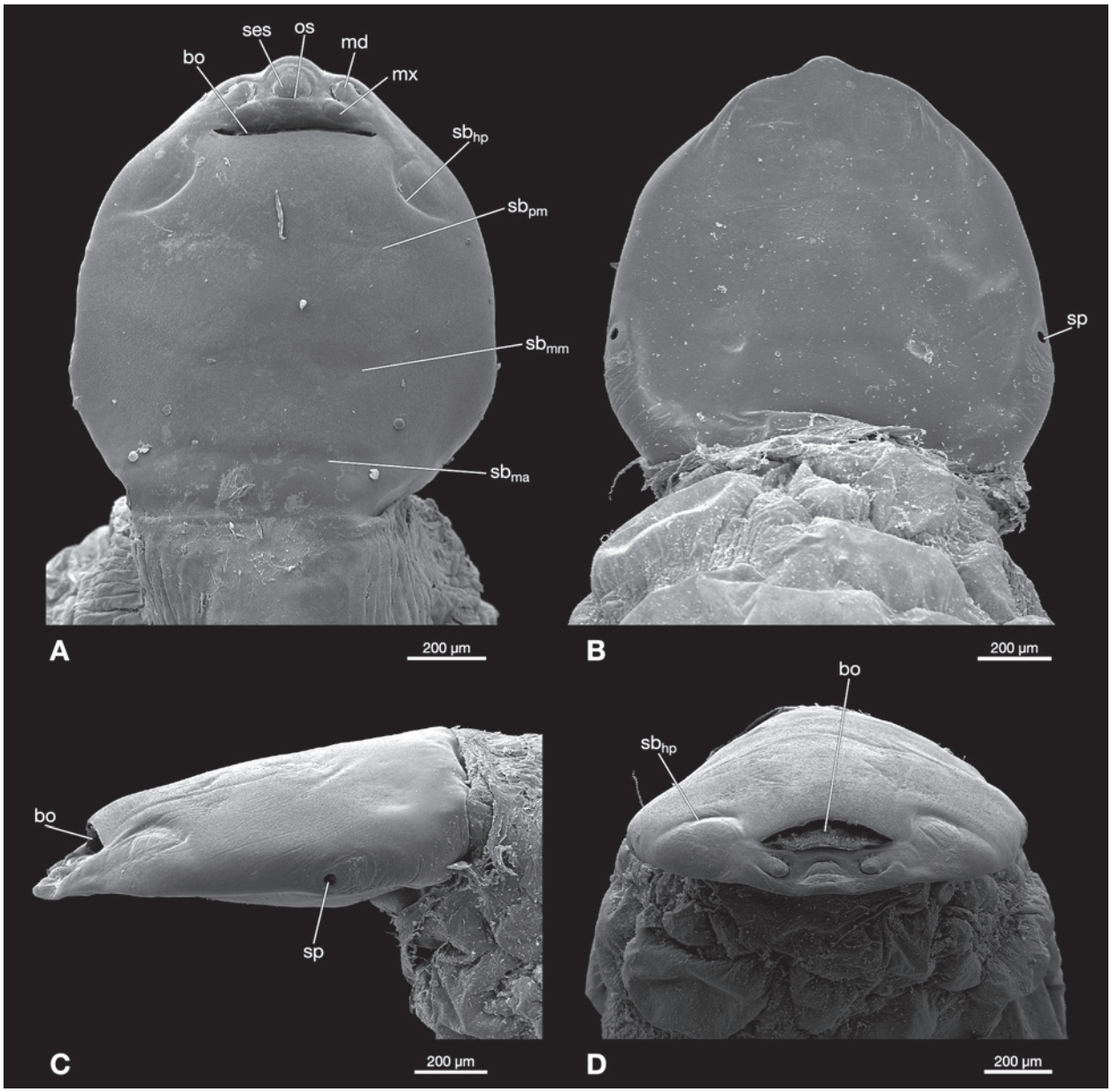

Fig. 2. *Xenos vesparum*, female, cephalothorax, SEM-micrographs. **A**: ventral; **B**: dorsal; **C**: lateral, ventral side (physiological dorsal side) facing upwards; **D**: frontal, ventral side facing upwards. — ***Abbreviations***: bo – birth opening, md – mandible, mx – vestige of maxilla, os – mouth opening, sb_{hp} – segmental border between head and prothorax, sb_{ma} – segmental border between metathorax and abdomen, sb_{mm} – segmental border between mesothorax and metathorax, sb_{pm} – segmental border between prothorax and mesothorax, ses – semicircular field possible of labral origin, sp – spiracle.

Antenna. Distinctly developed antennae are missing. Small, rounded plates (diameter ca. 3 µm) are present on the dorsal side of the head capsule (Fig. 3B) close to the lateral margin of the head on the level of the maxillary vestiges. The number of the plates is between six and nine and in addition an approximately triangular plate is present on one side.

Labrum. A distinctly developed labrum is missing. It is possible that a delimited oval field anterior to the mouth opening is of labral origin (**ses** in Figs. 4A, 5A).

Mandible. The mandibles are pointed towards each other anterad and forming an oblique angle (Figs. 4A,B, 5A,C). A serrate tooth is present distally and a bulge laterally near the tooth. The bulge bears 7–8 sensilla (Fig. 5C). The cuticle in the front half is sculptured, in contrast to the smooth region between the tooth and the ventral mandibular joint. The dorsal mandibular joint is absent. A strongly developed condyle forms the ventral joint articulating with the cephalic part of the cephalothorax in front of the maxilla (**co** in Fig. 4B).

Maxilla. Ovoid elevations caudomediad the mandibles are probably vestiges of maxillae (Figs. 4, 5A). In their middle region they bear a hollow, possibly representing vestigial palps (Fig. 4).

Labium and hypopharynx. The labium is not recognizable as a separate structure and probably fused with the head capsule (Figs. 4A, 5A). A hypopharynx is not present.

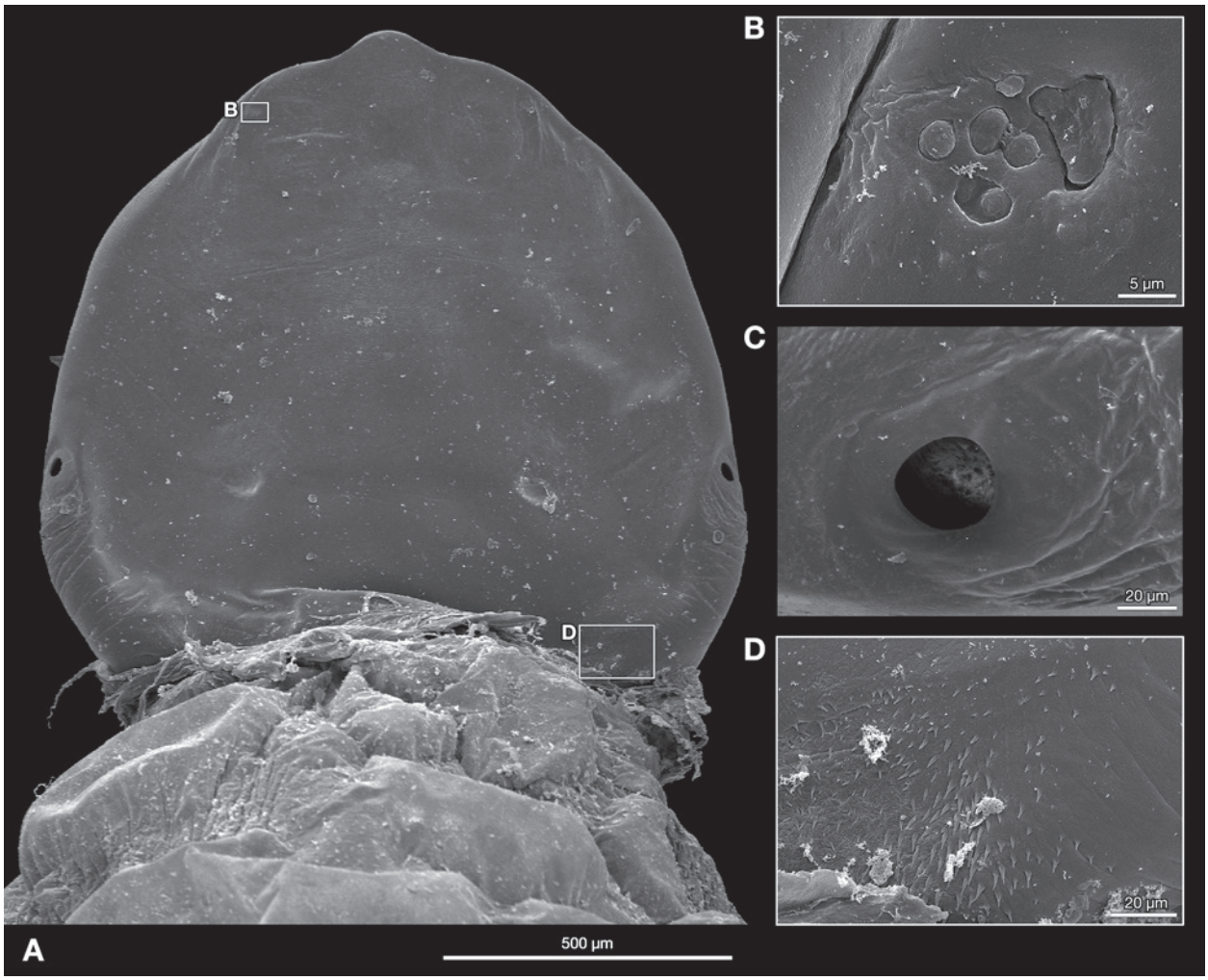

Fig. 3. *Xenos vesparum*, female, cephalothorax, SEM-micrographs. **A**: dorsal; **B**: vestige of antenna; **C**: spiracle; **D**: cuticular spines.

Mouth opening. The mouth opening is a narrow transverse cleft between the mandibles (**os** in Figs. 4A, 5A).
Salivarium. A salivarium is not developed.
Birth opening. The birth opening is a narrow cleft at the border between the head and prosternal region (**bo** in Figs. 1B,C, 2A,C,D, 4A, 5A). The width of the birth opening extends to the insertion of the mandibles laterally (Figs. 4A, 5A).
Thorax and abdominal segment I. The segments of the thorax are completely fused with each other and with the first abdominal segment. It reaches its greatest width at the level of the spiracles. The segmental borders between pro- and mesothorax, meso- and metathorax and metathorax and abdominal segment I are indistinct on the dorsal side (Figs. 1D, 2B). On the ventral side the segmental borders are variably pronounced in the three studied specimens. In one specimen the border between pro- and mesothorax is indistinct, the border between meso- and metathorax is marked by a mesal furrow. In the other two specimens the segmental borders between the thoracic segments and abdominal segment I are marked by mesal furrows (Fig. 2A). On the dorsal side the segmental borders between head and prothorax, pro- and mesothorax and meso- and metathorax are marked by paired, lateromedian strongly pigmented areas (Fig. 1D). Tergites, pleurites and sternites of the segments are fused. Vestiges of legs are missing. A constriction in the middle region of abdominal segment I is the border between the cephalothorax and the main part of the abdomen (Figs. 1B–D, 2A,B). The cephalothorax is bent dorsad towards the host, thus forming a shallow angle with the abdomen (Figs. 1A,C, 2C). Posterolaterally directed cuticular spines are present on the middle region of abdominal segment I (Fig. 3D). The cuticle displays a reticulate surface sculpture on large parts of the ventral side of the cephalothorax (Fig. 4A).
Spiracles. The paired, approximately circular spiracles are located in the dorsolateral region of the posterior third of the cephalothorax. The cuticle around the openings forms a distinct ring-shaped structure but is only slightly elevated (**sp** in Figs. 2B,C, 3A,C). The spiracles have a laterocranial orientation.

3.3. Cephalothorax of adult female

The cephalothorax of the adult female is in shape and proportions very similar to the cephalothorax of the secondary larval stage (Figs. 1E–G, 6).

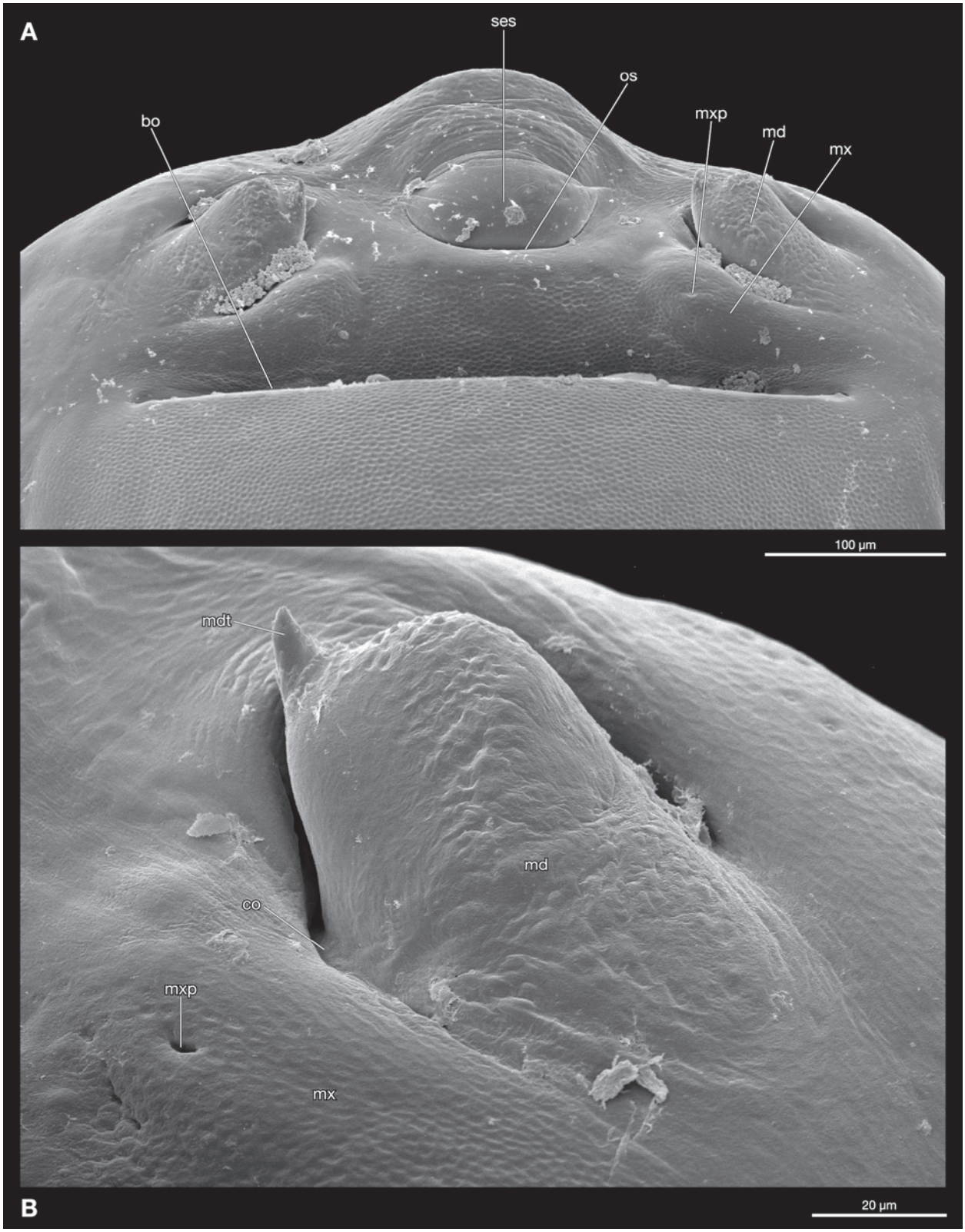

Fig. 4. *Xenos vesparum*, female, cephalothorax, ventral view, SEM-micrographs. **A**: anterior region of the cephalothorax, head and prothorax; **B**: left mandible and vestige of the maxilla. — ***Abbreviations***: bo – birth opening, co – condylus, md – mandible, mdt – mandibular tooth, mx – vestige of maxilla, mxp – vestige of maxillary palp, os – mouth opening, ses – semicircular field possible of labral origin.

Head capsule. The general morphology of the head capsule differs slightly from that of the last larval stage. The cuticular surface structure differs distinctly from that of the ventral side of the thoracic region. A dense vestiture of microtrichia is present on the prothorax but lacking on the distinctly sculptured cuticle of the head (Figs. 6A, 9). Several short setae are inserted on the dorsal side of the head capsule. An externally visible segmental bor-

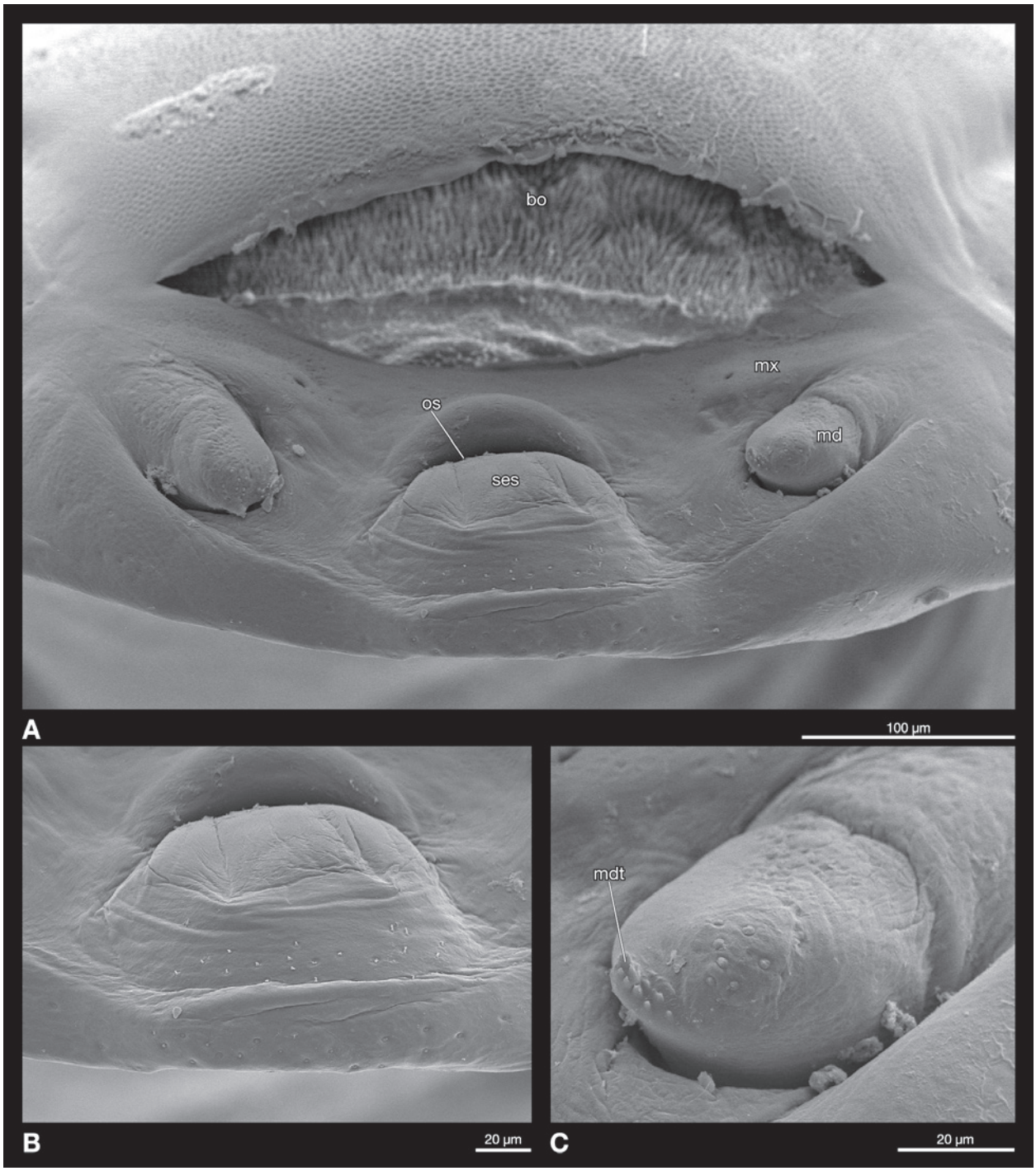

Fig. 5. *Xenos vesparum*, female, cephalothorax, frontal view, ventral side (physiological dorsal side) facing upwards, SEM-micrographs. **A**: anterior region of cephalothorax, head and prothorax; **B**: anterior head region with field of labral origin; **C**: right mandible. — ***Abbreviations***: bo – birth opening, md – mandible, mdt – mandibular tooth, mx – vestige of maxilla, os – mouth opening, ses – semicircular field possible of labral origin.

der between head and prothorax is missing on the dorsal side. On the ventral side the head is separated from the prothorax by a distinct edge in its middle region, marking the birth opening, and laterally by a suture (**sb_{hp}** in Fig. 6A).

Antenna. Missing like in the larvae. Small, partly indistinct cuticular plates (1 – 5) similar to those of the secondary larva are present dorsolaterally, approximately on the level of the mandibular base (Fig. 6B).

Labrum. An approximately oval field anterior to the mouth opening is probably a vestigial labrum, like in the secondary larvae. Its surface is smooth in contrast to the uneven cuticular surface anterior to it (**ses** in Fig. 9).

Mandible. The mandibles of the females are visible as processes at the same sites as in the secondary larvae. They are embedded in the enclosing larval cuticle and are hardly protruding from the concavity they are located in. Their surface is smooth. Mandibular joints are missing (Fig. 9).

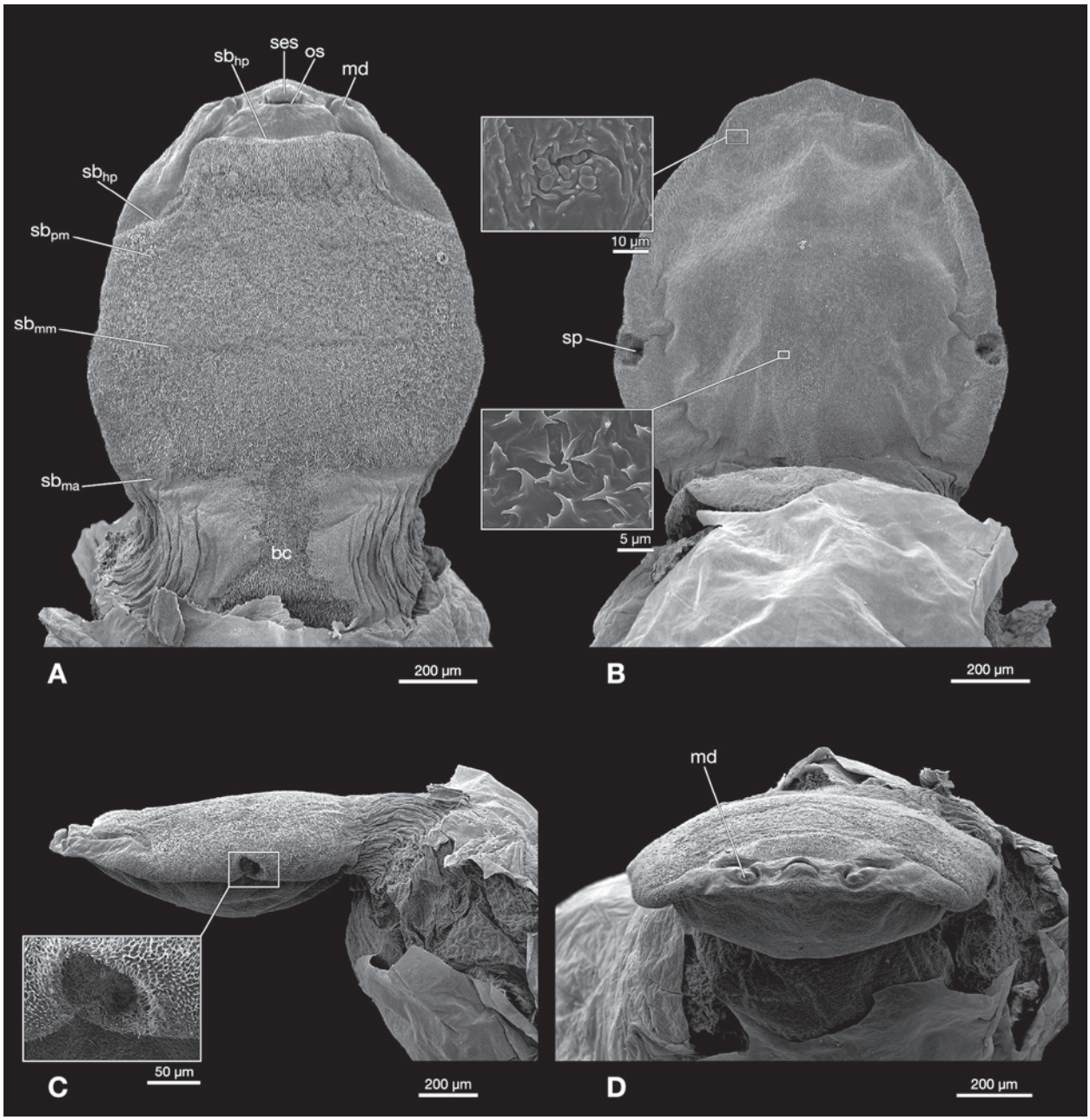

Fig. 6. *Xenos vesparum*, female, larval exuviae removed, cephalothorax, SEM-micrographs. **A**: ventral; **B**: dorsal, vestige of antenna (top) and cuticular structure (bottom) shown in box; **C**: lateral, ventral side directed upwards, detail of spiracle shown in box; **D**: frontal, ventral side directed upwards. — ***Abbreviations***: bc – brood canal, md – mandible, os – mouth opening, sb_{hp} – segmental border between head and prothorax, sb_{ma} – segmental border between metathorax and abdomen, sb_{mm} – segmental border between mesothorax and metathorax, sb_{pm} – segmental border between prothorax and mesothorax, ses – semicircular field possible of labral origin, sp – spiracle.

Maxilla. An indistinct elevation is recognizable caudomediad the mandible, corresponding with the distinct convexity of the last larval exuvia. In contrast to the larval maxilla no countersunk structures are recognizable (Fig. 9).

Labium and hypopharynx. A labium is not recognizable. A uniform, smooth approximately trapeziform field is present posterior to the mouth opening (Fig. 9). The hypopharynx is missing.

Mouth opening. The mouth opening is a narrow, straight transverse cleft posterior to the vestigial labrum (**os** in Fig. 9).

Salivarium. A salivarium is not developed.

Thorax and abdominal segment I. Like in the secondary larva the head, thorax and abdominal segment I are fused (Figs. 1E–G, 6). Almost the entire cephalothorax has a lighter, beige coloration. Only the ventral head surface and the anterior prosternal region are distinctly darker. On both sides of the prosternum are two dark brown spots (Fig. 1E). Segmental borders are not recognizable on the dorsal side (Fig. 6B). The entire surface of the ventral side of the thoracic region is densely covered with microtrichia (Figs. 6A, 7A). The ventral segmental borders are visible as shallow furrows. Nodose bulges are

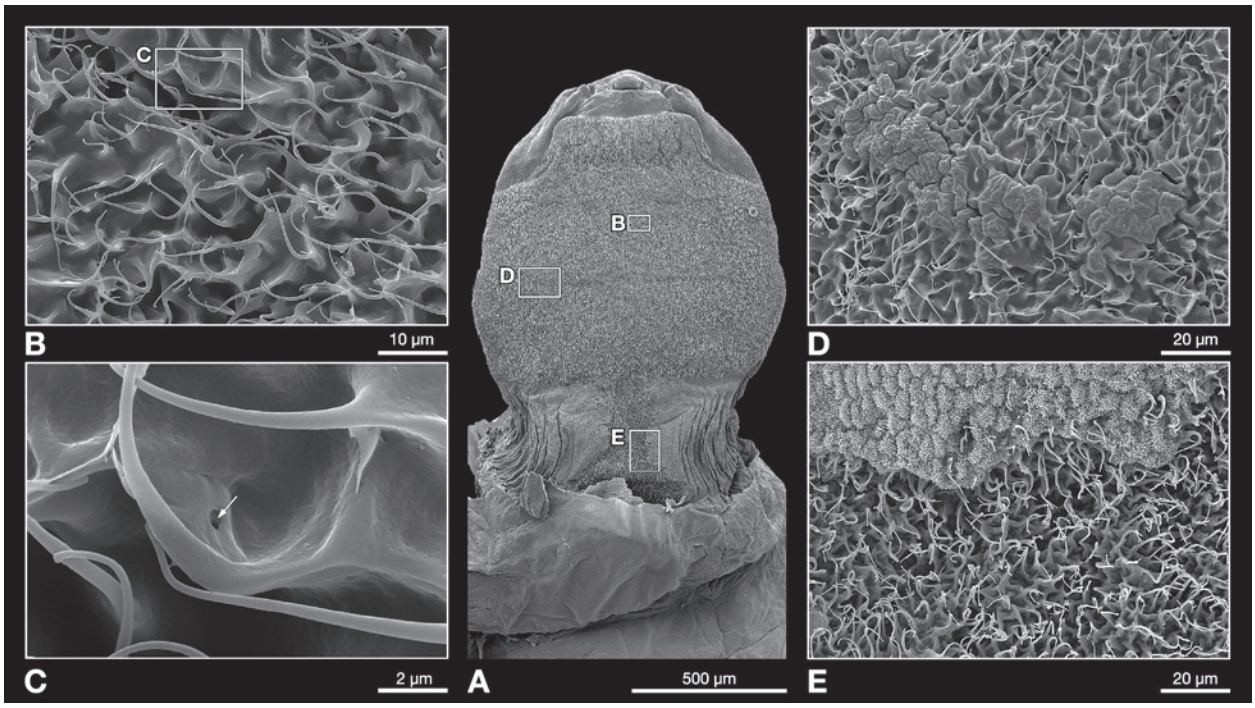

Fig. 7. *Xenos vesparum*, female, larval exuviae removed, cephalothorax with details of the surface structures, ventral view, SEM-micrographs. **A**: overview; **B**: cuticular structure with microtrichia; **C**: opening of Nassonov's glands (arrow) and bases of microtrichia; **D**: cuticular structure at segmental border between meso- and metathorax; **E**: cuticular structure of abdominal segment I, brood canal.

present in the border regions of the pro- and mesothorax and meso- and metathorax, respectively (Fig. 7D). The prothorax can be easily distinguished from the head by the different cuticular surface structure (see above). Microtrichia are missing in the cephalic region and on abdominal segment I (Fig. 6A), with the exception of its middle region (region of the brood canal). In the region of the I. abdominal segment the cuticular structure is nodose (Fig. 7A,E). Thin microtrichia are placed on extended sockets, taper strongly distad and are about 10 µm long (Fig. 7B). Openings of the Nassonov's glands are present at the bases of several microtrichia (Fig. 7C). The vestiture of microtrichia is distinctly less dense on the dorsal side and the individual hairs are much shorter (ca. 4 µm) compared to those of the ventral side (Fig. 6B insert). They are also placed on extended sockets, in some cases several on a single base.

Spiracles. The spiracular openings are also approximately circular but widened mesad on the dorsal side. Numerous microtrichia similar to those of the ventral thoracic surface are present at the margin and in the interior (Fig. 6C, insert). An additional pouch-like cavity is present laterad the spiracular opening.

3.4. Internal morphology

Adult cuticle, larval exuviae and epidermis. The adult cuticle is only slightly sclerotized. Anterior to the birth opening and in the anterior region of the brood canal it is ca. 8–10 µm thick ventrally and dorsally ca. 2–3 µm. In the remaining cephalothoracic areas between the brain and the constriction of abdominal segment I the cuticle is ca. 5 µm thick on the ventral and ca. 2 µm on the dorsal side. The weakly sclerotized cuticle of the abdomen has a thickness of only ca. 2–3 µm. A cleft is recognizable between it and overlaying cuticular layers in most areas (Fig. 10). It is distinctly extended in the anterior area of the brood canal (**bc** in Fig. 10B–F). The cuticular layers are more tightly adjacent to each other in the lateral region of the cephalothorax (Fig. 10). The exuvia of the third larval stage is very thin (ca. 0.2 µm) and appears corrugated in cross section. It fits very tightly with the exuvia of the secondary larval stage in the region of the brood canal (Fig. 8). The exuvia of the secondary larva is strongly sclerotized and ca. 12–13 µm thick on the ventral side and dorsally about 4 µm. In the lateral mandibular region it is ca. 20 µm thick, and in the lateral region of the anterior brood canal ca. 35 µm (Fig. 10). Its thickness decreases slightly on the dorsal side towards the caudal part of the cephalothorax. At the constriction of abdominal segment I it is about 4 µm thick on the ventral side and dorsally about 3 µm. The epidermis at the anterior region of the brood canal is about 13 µm thick ventrally and dorsally about 4 µm. In the remaining regions of the cephalothorax it is ca. 4 µm thick ventrally and ca. 2.5 µm on the dorsal side. The nuclei of the epidermal cells in the anterior region of the brood canal in front of the Nassonov's glands are shifted into extensions of the cells, which protrude into the body lumen (Fig. 11).

Fat body. Most parts of the lumen of the cephalothorax are densely packed with fat body cells between the organs, but only few of them are present anterior to the birth opening (Figs. 10, 11). The cells have a diameter of ca. 20 µm.

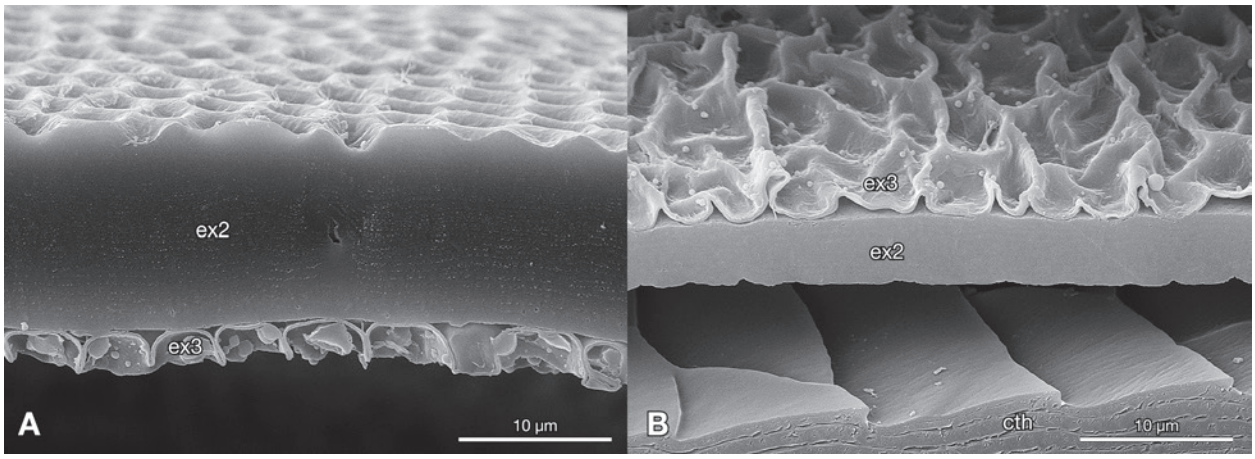

Fig. 8. *Xenos vesparum*, female, section through cuticular layers in the region of the brood canal, ventral side (physiological dorsal side) facing upwards, SEM-micrographs. **A**: ventral side; **B**: dorsal side. — ***Abbreviations***: cth – host cuticle, ex2 – exuvia of the secondary larval stage, ex3 – exuvia of third larval stage.

Tracheal system. The paired main tracheal branches originate at the spiracles and enter the abdomen closely below the adult cuticle. Within the abdomen side branches split off, leading to the cephalothorax (**tr_s** in Figs. 12, 13). They extend anterad mesad the main branches and form an anastomosis in the prothoracic region, shortly anterior to the border between the foregut and midgut (**ana** in Figs. 12, 13). Smaller tracheae originating from the anastomoses are connected to the organs of the cephalothorax (not shown in reconstruction).

Dorsal vessel. In the thoracic region the dorsal vessel is confined to the narrow space between the body wall and the inflated midgut (**dv** in Figs. 10, 12, 13). In the abdomen it is more distant from the unsclerotized external cuticle (Fig. 10K). It is largely straight, with weak curvatures restricted to the regions of the prothorax and abdominal segment I (Fig. 12A,C). It is slightly inclined ventrad over its entire length (Fig. 13B). It splits dorsoventrally in the prothoracic region posterior to the tracheal anastomoses (Fig. 10E–H). The dorsal and ventral branches form a loop around the tracheal arc and brain (**dvr** in Fig. 13B). After reconnecting anterior to the brain they narrow towards their blind ending terminal part at the level of the frontal ganglion (Fig. 12C).

Nervous system. The brain and suboesophageal ganglion are shifted from the head to the thoracic region. The brain lies within the prothorax and is tilted posteriorly. The circumoesophageal connectives link it with the suboesophageal ganglion, which lies in the region of the meso- and metathorax and is fused with the thoracic ganglia (Figs. 12, 13). Two pairs of nerves originating from the brain anteriorly extend anterolaterad towards the mandibular musculature (Fig. 12A,C). The frontal connectives link the frontal ganglion with the brain and both are closely adjacent posteriorly (Fig. 12A,C). Well-developed optic nerves originating laterally from the brain innervate strongly pigmented cells (Figs. 10E, 12A,C). Three pairs of nerves originate from the complex formed by the suboesophageal ganglion and thoracic ganglia. The first anteriorly directed pair originates close to the area of junction of the circumoesophageal connectives. The two following pairs extend anterolaterally and posterolaterally, respectively. An abdominal nerve is present posterior to the fused ganglia of the ventral nerve cord (**ne_{ab}** in Fig. 12D). From the abdominal nerve one thin nerve arises on the left side and extends into the abdomen. All elements of the nervous system are strongly flattened, especially the frontal ganglion (Fig. 13).

Musculature. Most parts of the cephalothoracic musculature are distinctly reduced. The adductor and abductor of the mandibles are strongly developed. The left adductor is slightly smaller than its right counterpart in the specimen examined and only the tendon of the right abductor is recognizable (Fig. 12). The left abductor is distinctly larger than the adductor. The musculature of the foregut is also well developed. Transverse and longitudinal muscles are present on the dorsal and ventral side of the pharynx. The dorsal layer, which is distinctly more voluminous, begins shortly after the functional mouth opening and ends shortly before the midgut. The ventral layer is distinctly shorter and partly interrupted, extending approximately over the length of the brain and frontal ganglion (Fig. 12). Additionally several pairs of pharyngeal dilators are present. Three nearly horizontal muscles are inserted on the posterior foregut region. The anterior and posterior ones originate dorsally. The largest one between these two has a more ventral origin and passes between the circumoesophageal connectives and the brain (Figs. 12, 13). Six additional dilators insert dorsally on the anterior pharyngeal region, in the examined specimen three on the right side, two on the left, and one medially (Fig. 12A,C). Two thin longitudinal muscles are present in the region of the pro- and mesothorax (Fig. 12). Dorsoventral muscle fibres are recognizable in the lateral region of abdominal segment I directly below of the epidermis (Fig. 10K) and also close to the spiracles.

Nassonov's glands. Nassonov's glands are located in a layer below of the ventral body wall. Numerous small globular units are connected with the body surface by a duct (**ng** in Figs. 10, 11, 12A,B, 13A). The Nassonov's

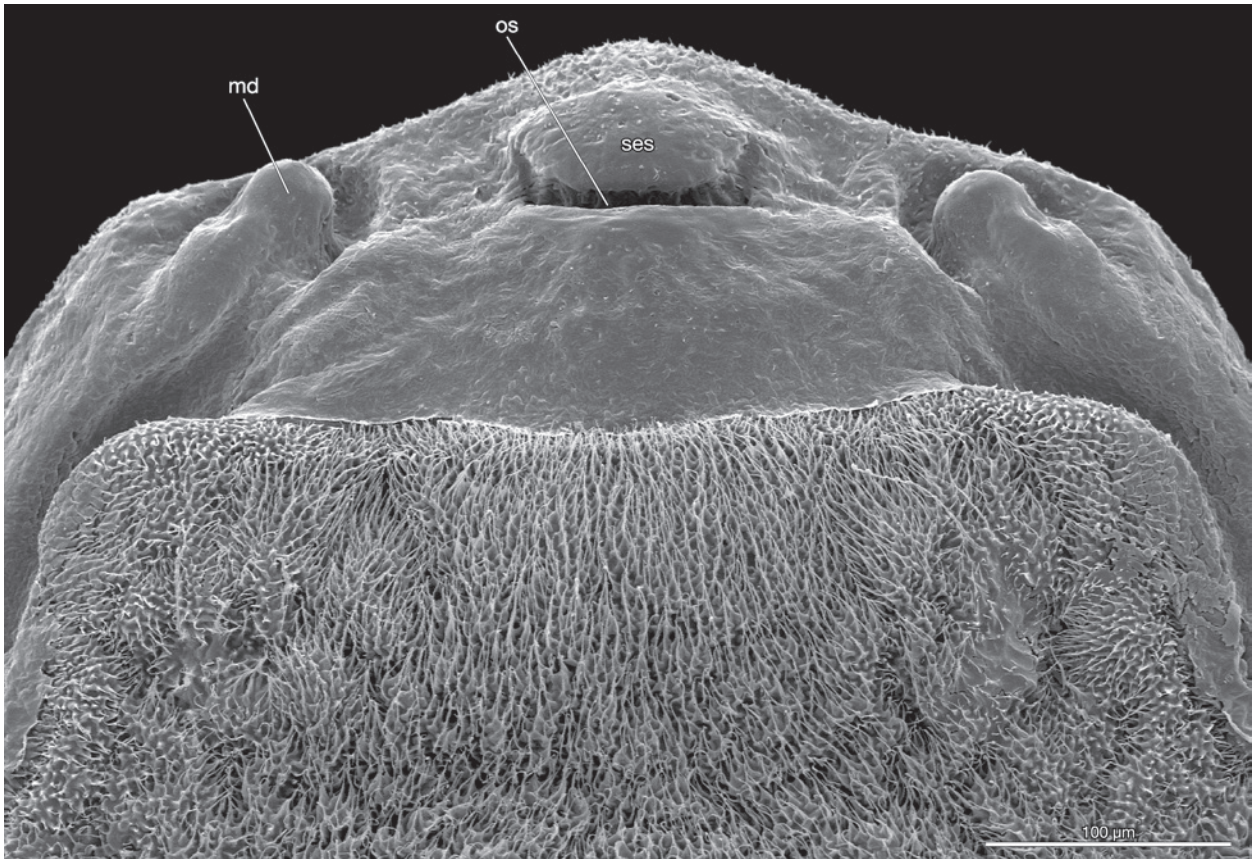

Fig. 9. *Xenos vesparum*, female, larval exuviae removed, head and prosternal region, ventral view, SEM-micrograph. — ***Abbreviations***: md – mandible, os – mouth opening, ses – semicircular field possibly of labral origin.

glands reach from the brain to the posterior end of the metathorax (Figs. 11, 12A,B).

Digestive tract. The functional mouth opening and the foregut and midgut are located in the cephalothorax. Pharynx and oesophagus cannot be distinguished morphologically. Salivary glands and salivary ducts are missing. The cuticle of the secondary larval stage is retained in the mouth opening and anterior foregut, resulting in a very narrow lumen (Fig. 11). The mouth opening is not completely closed. The foregut is slightly bent dorsad in the prothorax and is connected with the midgut in the posterior part of this segment in the central body region (Figs. 11, 12, 13A). A valvula cardiaca separates both parts of the digestive tract (**vc** in Figs. 10H, 11). The midgut is strongly enlarged and occupies the largest part of the cephalothoracic lumen (Figs. 11, 12, 13A). It extends straight through this anterior body region and forms another slight dorsal loop in the abdomen (Fig. 13A). The midgut cells have a granular, gland-like structure. They appear inflated around the nuclei but otherwise the midgut epithelium appears unusually flat (Figs. 10I–K, 11).

4. Discussion

The results of this study are mainly compared with features of the cephalothorax of *S. ovinae* (Stylopidae) (LÖWE et al. 2016) and the morphology of the head (MARQUART 2010) and thorax (MÜLLER 2009) of *Eoxenos laboulbenei* (Mengenillidae). The formation of the cephalothorax is part of secondary tagmosis closely linked with the endoparasitic life style (e.g. POHL & BEUTEL 2005; KATHIRITHAMBY et al. 2015). The far-reaching fusion of the primary tagmata and body segments increases the stability of the body part extruded from the host (POHL & BEUTEL 2008). Many cephalic and thoracic structures are distinctly or completely reduced, including the compound eyes, antennae, mouthparts, legs and wings, obviously correlated with endoparasitism (e.g. KINZELBACH 1971; KATHIRITHAMBY 1989; POHL & BEUTEL 2005). At the same time, specialized structures involved in the reproduction have evolved, like the brood canal or birth organs (e.g. KINZELBACH 1971; PEINERT et al. 2016). The cephalothorax contains the anterior parts of the circulatory system and digestive tract, the spiracles and the anterior part of the tracheal system, Nassonov's glands, and a large part of the central nervous system. The large part of the abdomen located in the host contains a large proportion of the eggs and in the case of *X. vesparum* four or five birth organs (KINZELBACH 1971; four according to BEANI et al. 2005).

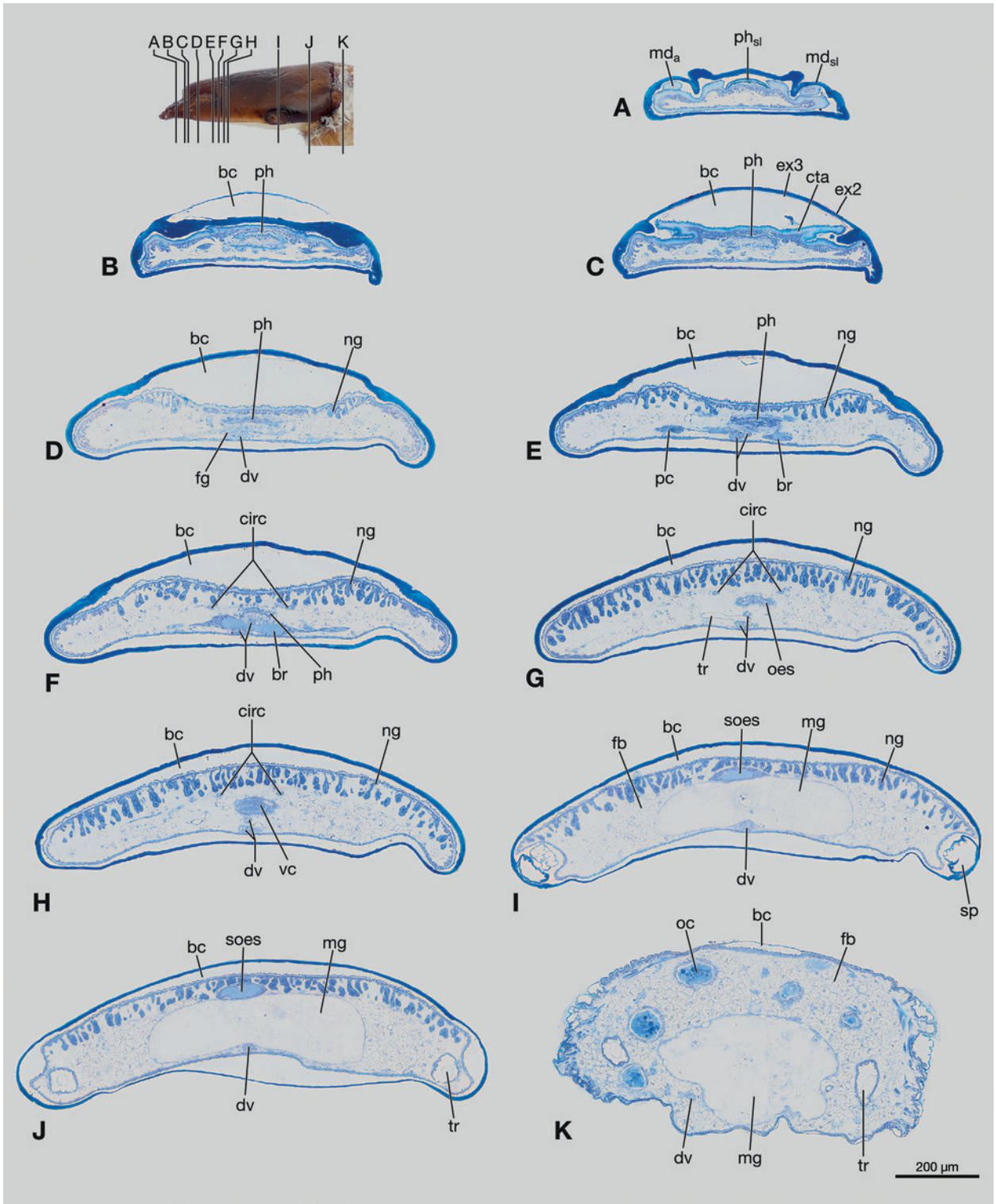

Fig. 10. *Xenos vesparum*, female, cross sections of female cephalothorax, position of sections indicated in photomicrograph, ventral side (physiological dorsal side) facing upwards. — ***Abbreviations***: bc – brood canal, br – brain, circ – circumoesophageal connective, cta – cuticle of adult female, dv – dorsal vessel, ex2 – exuvia of the secondary larval stage, ex3 – exuvia of third larval stage, fb – fat body, fg – frontal ganglion, md_a – adult mandible, md_{sl} – mandible of secondary larval stage, mg – midgut, ng – Nassonov's gland, oc – egg cell, oes – oesophagus, pc – pigmented cells, ph – pharynx, ph_{sl} – pharynx of secondary larval stage, soes – suboesophageal ganglion-complex, sp – spiracle, tr – trachea, vc – valvula cardiaca.

4.1. Secondary larval stage

The homology of the cephalothorax was repeatedly discussed. According to different authors it is composed of the head and thorax (e.g. Lauterbach 1954), head and prothorax (Hrabar et al. 2014; Kathirithamby et al. 2015), or head, thorax and at least a part of abdominal segment I (Kinzelbach 1971; Pohl & Beutel 2005, 2008;

LÖWE et al. 2016). Recognizable segmental borders and different cuticular surface structures in females of *X. vesparum* clearly support that the cephalothorax comprises the head, the thorax and the anterior part of abdominal segment I. The constriction of abdominal segment I possibly prevents the cephalothorax from sliding back into the host's body lumen. Cuticular spines on the anterior region of the dorsal side of abdominal segment I have probably the same function. In contrast to the constriction they are apparently missing in *S. ovinae* (LÖWE et al. 2016). The cephalothorax of *X. vesparum* is flattened like that of *S. ovinae* (LÖWE et al. 2016) and bent dorsad along its lateral margins. It is conceivable that this stabilizes its position between the tergites of the host. The head of the last larval stage of *E. laboulbenei* is also flattened compared to the head of the last larval stage of the males (MARQUART 2010), but less distinctly than the anterior cephalic region of stylopidian females.

The narrow functional mouth opening is a vestige retained from the secondary larval stage. As the female does not consume food, the mouth opening has no function in this context (GIUSTI et al. 2007), as it is also the case in all other groups of Strepsiptera (POHL & BEUTEL 2005; LÖWE et al. 2016). Food uptake of female Mengenillidae would be possible on principle, but does not take place (H. Pohl unpubl. observations).

The birth opening at the border between head and prosternum is an autapomorphy of Stylopiformia (POHL & BEUTEL 2005, 2008). With the exception of *Stylops*, it is used for copulating (PEINERT et al. 2016) and in all Stylopiformia for the release of the mobile first instar larvae. In the examinend virgin specimen of *X. vesparum* a thin brood canal membrane is present covering the birth opening (**bcm** in Fig. 11). Several authors reported that this membrane is perforated by the male penis during copulation, however KATHRITHAMBY et al. (2015) hypothetize that the membrane is ruptured during superextrusion of the female's cephalothorax during mate signalling. The mode of the copulation in Stylopidia was long disputed, as reliable observations were scarce and different modes in different taxa could not be excluded (e.g. BEANI et al. 2005; PEINERT et al. 2016). BEANI et al. (2005) discussed copulation via the brood canal for *X. vesparum*, but did not exclude traumatic insemination. PEINERT et al. (2016) could show that traumatic insemination definitely occurs in *S. ovinae*, with a cephalothoracic invagination only occurring in the genus *Stylops*.

The reduction of antennae, maxillae, labium, labrum, compound eyes and legs is obviously linked with endoparasitism (POHL & BEUTEL 2005). In contrast to *S. ovinae* (LÖWE et al. 2016) assemblages of circular fields occur in *X. vesparum* on the dorsal side and they likely represent vestiges of the antennae. KINZELBACH (1971) and POHL & BEUTEL (2005) also described vestiges of the antennae for Stylopidia. Elevations caudomesad the mandibles are probably vestiges of the maxillae and the knob-shaped structure are likely vestiges of the maxillary palps. *S. ovinae* is lacking comparable structures (LÖWE et al. 2016).

A circular field anterior to the mouth opening of *S. ovinae* was interpreted as everted pharyngeal roof of the last larval stage ("nach außen gedrücktes Pharynxdach (Epipharynx?) …") by LAUTERBACH (1954). In the present study a labral origin is assumed as already discussed by LÖWE et al. (2016). The only movable cephalic appendages are the mandibles. According to LAUTERBACH (1954) they are moved sideways during the penetration of the host's abdominal body wall. This is made possible by the presence of antagonistic muscles and the flexible condition of the articulatory membrane as long as the female stays within the host or shortly thereafter (e.g. KATHIRITHAMBY 2000). Shortly after the emergence from the host the entire cephalothorax is sclerotized and the mandibles accordingly immobilized. Mandibles functioning in a similar way and also equipped with a tooth are also present in *S. ovinae* (LÖWE et al. 2016). In contrast, the mandibles of free-living females of Mengenillidae are curved mesad and interact with each other like in most other groups of insects (KINZELBACH 1971).

4.2. Adult female

Females of Stylopidia are enclosed by three layers of cuticle. The adult female forms a functional unit with the larval exuviae according to LAUTERBACH (1954), KINZELBACH (1971), POHL & BEUTEL (2005), LÖWE et al. (2016) and others. These authors referred to the layers as cuticle of the adult female, pupal cuticle and cuticle of the second larval stage or puparium (e.g. KINZELBACH 1971). The homology of these cuticular layers of *Elenchus tenuicornis* Kirby, 1815 (Elenchidae) was interpreted by KATHIRITHAMBY et al. (1984) as exuviae of the secondary and tertiary larval stages, and of a fourth larval stage, which becomes sexually mature. As the exuviae are generally not shed in Stylopidia ("apolysis without ecdysis": KATHIRITHAMBY et al. 1984) the females remain enclosed within them. Identical conditions were described for *X. vesparum* by MANFREDINI et al. (2007) and the presence of the three layers could also be demonstrated in the present study. MANFREDINI et al. (2007) assumed that the enclosing exuviae enable the females to circumvent the immune system of the host.

The additional two cuticular layers of adult females of Stylopidia clearly distinguish them from females of the basal Mengenillidae, which usually shed the larval and pupal exuviae (e.g. KINZELBACH 1971; POHL & BEUTEL 2005; MÜLLER 2009; MARQUART 2010). This impedes a direct comparison of the morphology. However, it has to be noted that some females of Mengenillidae remain in their puparia and reproduce parthenogenetically (POHL & BEUTEL 2005, 2008). However, the pupal exuvia is always discarded (H. Pohl unpubl. observations). In the following the adult female of *X. vesparum* is compared with that of *E. laboulbenei*, like the exuvia of the last larval stage with that of *S. ovinae*.

Anterior to the birth opening a deep invagination is present in *S. ovinae*, which was termed by PEINERT et al.

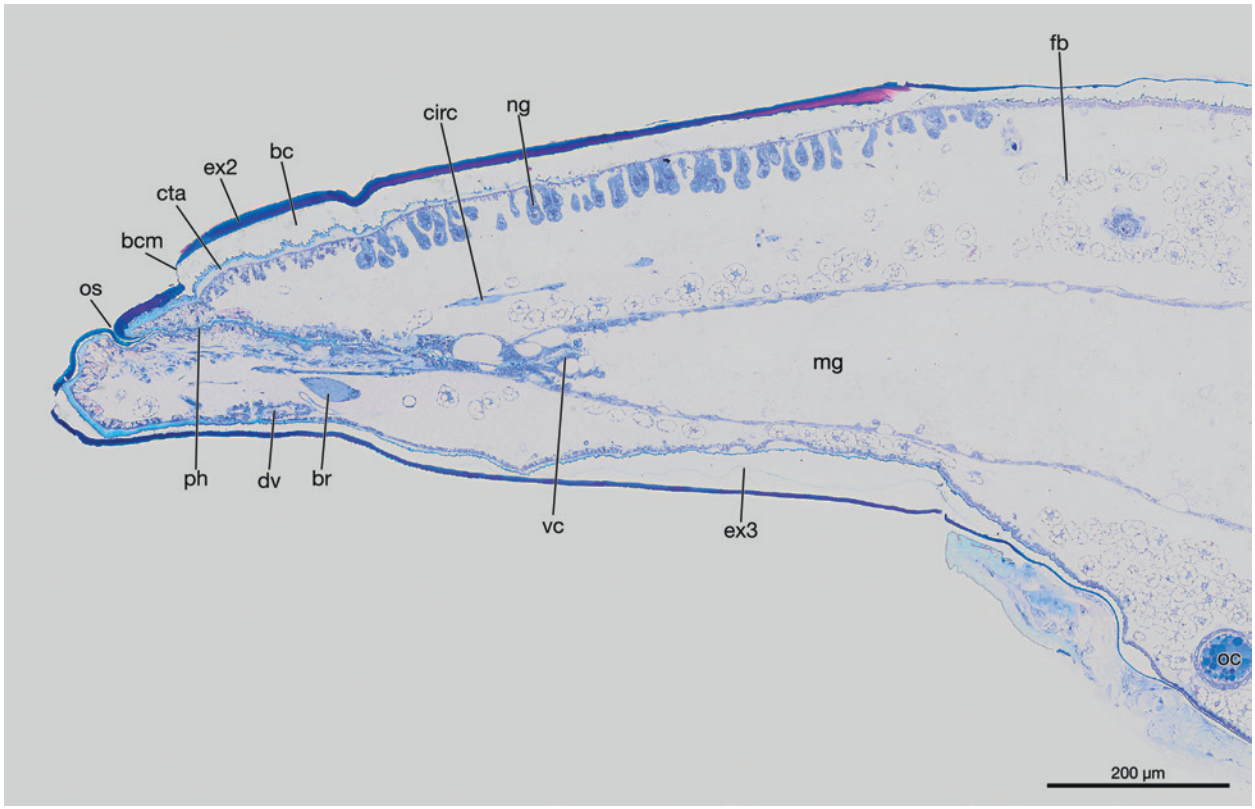

Fig. 11. *Xenos vesparum*, female, slightly semilateral sagittal section of female cephalothorax, ventral side (physiological dorsal side) facing upwards. — ***Abbreviations***: bc – brood canal, bcm – brood canal membrane – br – brain, circ – circumoesophageal connective, cta – cuticle of adult female, dv – dorsal vessel, ex2 – exuvia of the secondary larval stage, ex3 – exuvia of third larval stage, fb – fat body, mg – midgut, ng – Nassonov's gland, oc – egg cell, os – mouth opening, ph – pharynx, vc – valvula cardiaca.

(2016) and Löwe et al. (2016) as "cephalothoracic invagination". In this area the cuticle and epidermis are ventral strongly thickened, probably to reduce the costs of the traumatic insemination in *Stylops* (Peinert et al. 2016). An invagination is missing in *X. vesparum*. However in the anterior region of the brood canal the cuticle and epidermis is ca. twice as thick as in posterior regions. Possibly the dark spots in this area (see above) are penetration sites of the penis and mating scars as in *Stylops*.

The fissure-shaped functional mouth opening of *X. vesparum* is similar to that of *S. ovinae* (Löwe et al. 2016). In contrast, in *E. laboulbenei* a functional mouth opening with a mouthfield sclerite comparable to that of the males is present (Marquart 2010). In adult females of all three taxa no food uptake takes place through the mouth opening. Kathirithamby (2000) described the brood canal of female Myrmecolacidae as "apron", a structure enabling them to take up food substrate from the host's body. This can be definitely excluded in the case of for *X. vesparum* based on the studies of Giusti et al. (2007). The well-developed fat body of *X. vesparum* suggests that sufficient reserve substances are accumulated to supply the developing embryos (e.g. Lauterbach 1954).

The mandibles of the adult females lack a tooth in contrast to the secondary larvae and like in *S. ovinae* (Löwe et al. 2016) they have no function (Kinzelbach 1971). The mandibles of female Mengenillidae are used when they hatch from the puparium (H. Pohl unpubl. observations; cited from Löwe et al. 2016). A labral field is missing in adult females of *S. ovinae* (Löwe et al. 2016), whereas it is present in adult females of *X. vesparum*. A labrum is recognizable in *E. laboulbenei*, but only as a shallow convexity and not as a separate sclerite (Marquart 2010). In contrast to *X. vesparum* a bipartite labium fused with the head capsule is present in *S. ovinae*. The labium of *E. laboulbenei* is completely reduced like in *Xenos* (Marquart 2010).

Antennae are completely missing in *S. ovinae* (Löwe et al. 2016) in contrast to the presence of rounded vestiges in *X. vesparum*. The antennae of females of Mengenillidae are well developed (e.g. Kinzelbach 1971; Pohl & Beutel 2005; Marquart 2010). Vestiges of the maxilla as they are recognizable on the exuvia of secondary larva of *X. vesparum* are only recognizable as very indistinct convexities caudomediad the mandibles in the adult females. Comparatively well-developed maxillae with a one-segmented palp are present in females of Mengenillidae (Marquart 2010; Pohl et al. 2012), and strongly simplified, lobe-like structures in *S. ovinae* (Löwe et al. 2016). In contrast to Kinzelbach (1971: fig. 164B) vestiges of legs could not be identified in *X. vesparum*. The presence of functional legs in free-living females of Mengenillidae (Müller 2009) shows that this is a groundplan condition of Strepsiptera.

The paired spiracles in the posterior third of the cephalothorax are the only external openings of the female

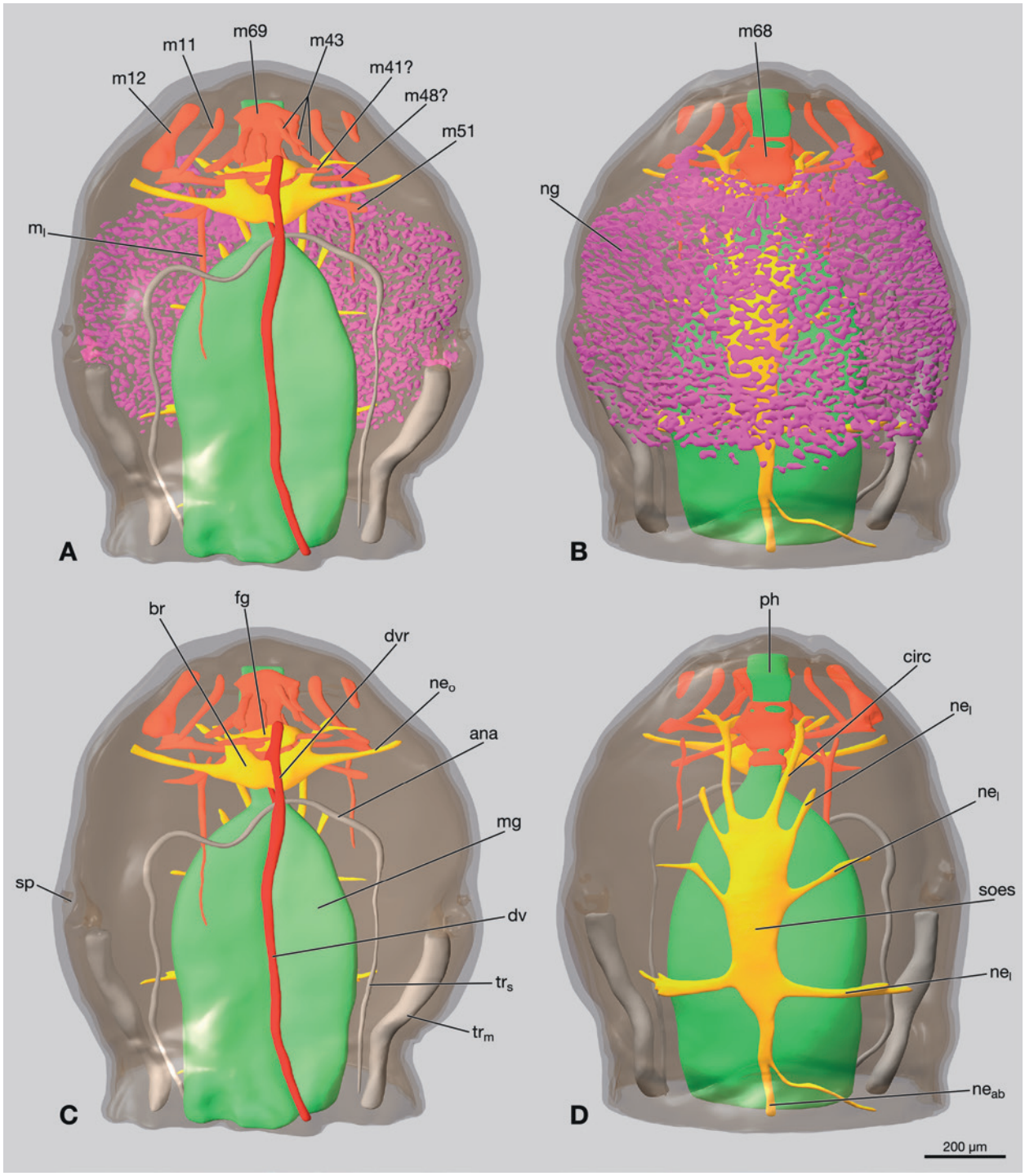

Fig. 12. *Xenos vesparum*, female, 3D-reconstructions; **A** and **C** dorsal view, **B** and **D** ventral view. — ***Colours***: transparent brown, exuvia of secondary larval stage and adult cuticle; pink, Nassonov's glands; grey, tracheal system; red, dorsal vessel; yellow, nervous system; green, digestive tract; orange, musculature. — ***Abbreviations***: ana – anastomosis of side branch of tracheal system, br – brain, circ – circumoesophageal connective, dv – dorsal vessel, dvr – ring of dorsal vessel, fg – frontal ganglion, m11 – Musculus craniomandibularis internus, m12 – M. craniomandibularis externus, m41? – M. frontohypopharyngealis (or m45 – M. frontobuccalis anterior), m43 – M. clypeopalatalis, m48? – M. tentoriobuccalis anterior (or m50 – M. tentoriobuccalis posterior), m51 – M. verticopharyngealis, m68 – M. anularis stomodaei, m69 – M. longitudinalis stomodaei, m_l – longitudinal muscle, mg – midgut, ne_{ab} – nervus abdominalis, ne_l – leg nerve, ne_o – nervus opticus, ng – Nassonov's glands, ph – pharynx, soes – suboesophageal ganglion complex, sp – spiracle, tr_m – main tracheal stem, tr_s – side branch of tracheal system. — An interactive PDF of this figure can be found as Electronic Supplement File 1.

tracheal system. Also in all other females of Stylopidia with the exception of *Callipharixenos* (Callipharixenidae) only one pair of functional spiracular openings is present in abdominal segment I (KINZELBACH 1971; LÖWE et al. 2016). In Callipharixenidae functional spiracles are present in the metathorax and abdominal segment I, in Mengenillidae in the abdominal segments I–VII (KINZELBACH 1971).

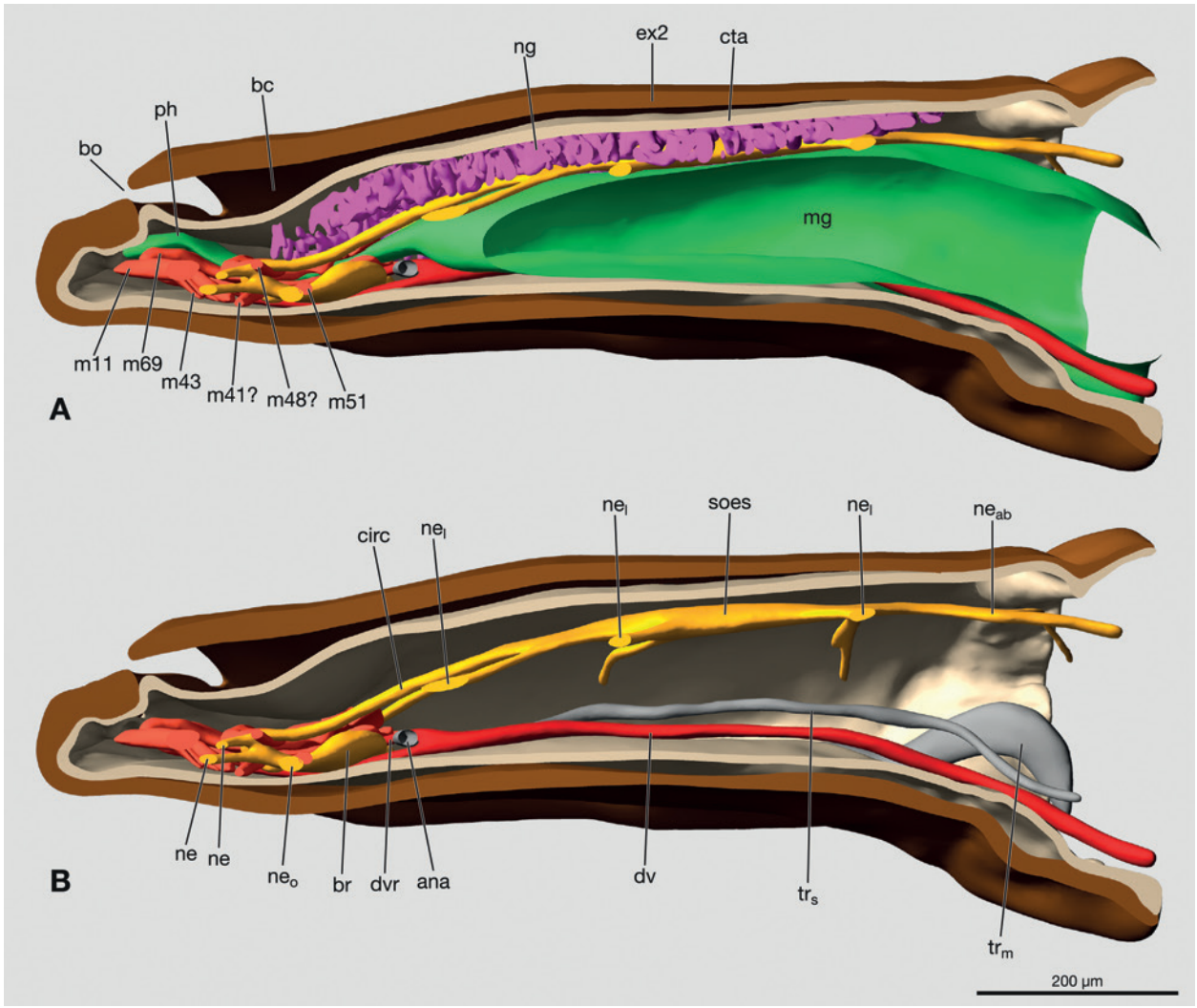

Fig. 13. *Xenos vesparum*, female, 3D-reconstructions, sagittal view, anterior is toward the left and ventral side facing upwards; **A**: with complete interior structures; **B**: Nassonov's glands and gut removed. — ***Colours***: dark brown, exuvia of secondary larval stage; light brown, adult cuticle; pink, Nassonov's glands; grey, tracheal system; red, dorsal vessel; yellow, nervous system; green, digestive tract; orange, musculature. — ***Abbreviations***: ana – anastomosis of side branch of tracheal system, bc – brood canal, bo – birth opening, br – brain, circ – circumoesophageal connective, cta – cuticle of adult female, dv – dorsal vessel, dvr – ring of dorsal vessel, ex2 – exuvia of the secondary larval stage, m11 – Musculus craniomandibularis internus, m41? – M. frontohypopharyngealis (or m45 – M. frontobuccalis anterior), m43 – M. clypeopalatalis, m48? – M. tentoriobuccalis anterior (or m50 – M. tentoriobuccalis posterior), m51 – M. verticopharyngealis, m69 – M. longitudinalis stomodaei, mg – midgut, ne – nerve, ne_{ab} – nervus abdominalis, ne_l – leg nerve, ne_o – nervus opticus, ng – Nassonov's glands, ph – pharynx, soes – suboesophageal ganglion complex, tr_m – main tracheal stem, tr_s – side branch of tracheal system.

4.3. Internal morphology

Internal structures and organs of *X. vesparum* are strongly reduced in correlation with endoparasitism (e.g. Pohl & Beutel 2005, 2008). Endoskeletal structures are completely absent. Vestiges of a tentorium were described by Kinzelbach (1971), but were not found in *X. vesparum* and are also absent in *S. ovinae* (Löwe et al. 2016). As it is also missing in *E. laboulbenei* (Marquart 2010) and all male strepsipterans (Pohl & Beutel 2005) the reduction of the cephalic endoskeleton has apparently occured early in the evolution of Strepsiptera.

The strongly developed fat body of *X. vesparum* almost completely fills out the space between the other cephalothoracic organs. It is distinctly less voluminous in *S. ovinae* (Löwe et al. 2016: fig. 7). This is certainly not due to a different age of the examined females. One female of *X. vesparum* was fixed only few minutes after the copula and the other was virgin as the studied females of *S. ovinae* (Löwe et al. 2016). The different number of fat body cells is probably correlated with a different life cycle. Females of *X. vesparum* remain in their host after the insemination in July or August until they release the first instar larvae in June or July of the following year (H. Pohl unpubl. observations). Sufficient nutrients are required for this prolonged period. In contrast, the embryonic development starts immediately after the insemination in *S. ovinae* and the first instar larvae are released at the end of March or the beginning of April (Fraulob et al. 2015).

Kinzelbach (1971) already described the principle features of the tracheal system of *Xenos*, with posteri-

Table 1. Terminology and homologization of the cephalic musculature of the female of *Xenos vesparum*. Homologization with muscles of female of *Stylops* (LÖWE et al. 2016), females of Stylopidia (KINZELBACH 1971) and generalized insect cephalic musculature (v. KÉLER 1963; WIPFLER et al. 2011).

Name	Abbrev.	Origin	Insertion	LÖWE et al. 2016 *Stylops ovinae* (Stylopidae)	KINZELBACH 1971 Stylopidia	v. KÉLER 1963	WIPFLER et al. 2011
M. craniomandibularis internus	m11	head capsule of female, segmental border between head and prothorax	mandibular base	m11	3′	11	0md1
M. craniomandibularis externus	m12	close to m11	mandibular base, close to m11	m12	4′	12	0md2
M. frontohypopharyngalis (or M. frontobuccalis anterior)	m41 (or m45)	dorsal head capsule laterad frontal ganglion	pharynx at anatomical mouth opening	–	–	41 (45)	0hy1 (0bu2)
M. clypeopalatalis	m43	dorsal head capsule anterior to anatomical mouth opening	pharynx anterior to anato-mical mouth opening	m43	6′	43	0ci1
M. tentoriobuccalis anterior (or M. tentoriobuccalis posterior)	m48 (or m50)	ventral head capsule close to posterior margin	pharynx posterior to anato-mical mouth opening	m48	7′	48 (50)	0bu5
M. verticopharyngalis	m51	dorsal head capsule posterior to brain	pharynx at level of brain	–	–	51	0ph1
M. transversalis buccae	m67	pharynx anterior to brood canal	pharynx at level of brain	–	–	67	0hy9
M. anularis stomodaei	m68	pharynx anterior to brood canal	pharynx at level of brain	m68	8′	68	0st1
M. longitudinalis stomodaei	m69	pharynx anterior to brood canal	pharynx at level of brain	m69	9′	69	0st2

orly directed main trunks in the cephalothorax, branches extending from the abdomen into the cephalothorax, and anastomoses close to the foregut-midgut border. A similar condition is found in *S. ovinae*, however with the anastomoses distinctly shifted posteriorly to the caudal region of the cephalothorax. As the configuration is also similar in the first instar larvae, KINZELBACH (1971) assumed pedomorphic effects on the tracheal system. The tracheal system is also simplified in *E. laboulbenei*. Paired main trunks extend along the gut. A smaller paired branch splits off in the metathorax and extends towards the mesothoracic region (MÜLLER 2009).

The dorsoventral split of the dorsal vessel in the anterior region is an unusual feature, hitherto not observed in female strepsipterans. The loop formed by the two branches encloses the tracheal anastomosis and the brain in *X. vesparum*. An improved sustenance of the brain is a plausible explanation for this unusual condition. A ring of the dorsal vessel is also present in *S. ovinae* in a similar position. However, it encircles the pharyngeal dilators and possibly results in an improved supply of these muscles (LÖWE et al. 2016). Ring-shaped elements of the circulatory system are completely missing in *E. laboulbenei* (MARQUART 2010).

A shift of the brain into the prothorax does also occur in *S. ovinae* (LÖWE et al. 2016), and it is located within the head capsule in *E. laboulbenei* (MARQUART 2010). However, the nervous system is also characterized by simplifications and fusions in Mengenillidae. In all three species compared here the suboesophageal ganglion is fused with the thoracic and abdominal ganglia. This compact ganglionic mass is possibly retained from the extremely miniaturized first instar larvae, which are characterized by an extremely compacted nervous system: all elements of the central nervous system form a compact mass in the middle region of the body (BEUTEL et al. 2005). The dorsally tilted brain of *X. vesparum* is possibly also an effect of miniaturization, but this condition is not found in *S. ovinae* despite of a similar size and shape (LAUTERBACH 1954; LÖWE et al. 2016). The optic nerves originate far posteriorly and innervate vestiges of the stemmata. Eyes and optic nerves are completely missing in *S. ovinae* (LÖWE et al. 2016). Two additional nerves originating from the brain could be identified in *X. vesparum*. The posterior one is probably the antennal nerve. The anterior one is likely linked with the pharyngeal musculature, but this could not be verified. Similar anterior nerves of *S. ovinae* are seemingly associated with these muscles (LÖWE et al. 2016), even though LAUTERBACH (1954) addressed them as antennal nerves. The six nerves of the fused thoracic ganglionic complex were interpreted as retained leg nerves by LÖWE et al. (2016), based on the absence of any other nerves in *E. laboulbenei* (MÜLLER 2009). We follow this interpretation also in the case of *X. vesparum*.

The musculature of the cephalothorax is strongly reduced. Most muscles are located in the anterior head region, like the complex pharyngeal musculature or the strongly developed mandibular muscles (Table 1). Thoracic transversal muscle bundles were missing in *X. vesparum* in contrast to KINZELBACH (1971). Some dorsoventral fibres close to the spiracles are possibly involved in the regulation of the width of the spiracular openings. Similar muscular conditions were observed in *S. ovinae*

(Löwe et al. 2016), except for the absence of the spiracular muscles. Strongly developed mandibular adductors and abductors were also found in this species, but none of them is as degenerated as in *X. vesparum*. The pharyngeal dilators of *S. ovinae* are dorsoventrally oriented, whereas some of them are almost horizontally arranged in *X. vesparum*. This is possibly correlated with the different course of the dorsal vessels, which form no ring around the dilators in *X. vesparum* in contrast to *S. ovinae*. The muscle set of *E. laboulbenei* is distinctly more complex, with 13 muscles of the head (Marquart 2010) and 51 of the thorax (Müller 2009). The muscles missing in Stylopidia are mainly linked to the mouthparts and legs (Müller 2009; Marquart 2010). Their reduction in Stylopidia is obviously correlated with structural and functional simplifications resulting from endoparasitism. In contrast, muscles involved in penetrating the host's body wall and food uptake during the larval development are retained. The degeneration of the mandibular muscles in the females (and asymmetry in the specimen we examined) is not surprising, as they have apparently no function in the adult stage. A similar case was described for ant queens (e.g. Keller et al. 2014).

Nassonov's glands are voluminous structures in the thoracic region of the cephalothorax. They produce a sexual pheromone. The precise chemical structure was described for *S. ovinae* and *X. peckii*. In the former it is (*9R*)-*3,5-syn*-3,5,9-trimethyldodecanal (Cvačka et al. 2012; Tolasch et al. 2012) and in the latter (7*E*,11*E*)-3,5,9,11-tetramethyltridecadienal (Hrabar et al. 2015). The elements of the glands of *X. vesparum* are large, pear-shaped cells, which form a layer below the ventral body wall. The arrangement differs distinctly from the condition in *S. ovinae*, where the glands fill out large parts of the lumen of the cephalothorax (Löwe et al. 2016). Like in the present study Löwe et al. (2016) could identify pores in the cuticle associated with the glands. Similar perforations continuous with the ducts of the glands were described by Dallai et al. (2004). Interestingly, pores are absent in the exuviae of the secondary larval stages of *X. vesparum*, but present in those of *S. ovinae* (Löwe et al. 2016). It is likely that the pheromone is released through the birth opening in the former because the brood canal membrane is ruptured during superextrusion of the female's cephalothorax during mate signalling (see above). Lauterbach (1954) suggested diffusion through the exuvia of the secondary larval stage. This was confirmed for *S. ovinae* by Löwe et al. (2016), who demonstrated the presence of pores in the exuvia of the secondary larvae.

The digestive tract has no function in the adult female and degenerates with increasing age (Giusti et al. 2007). Like in *S. ovinae* (Löwe et al. 2016) the pharynx and oesophagus cannot be distinguished morphologically. Kinzelbach (1971) described a valvula cardiaca as a valve between the foregut and midgut. This is conforming to our observations and it is also present in *E. laboulbenei* (Müller 2009), whereas it is missing in *S. ovinae* (Löwe et al. 2016). The midgut of *X. vesparum* is strongly bloated, resembling the air-filled "balloon gut" of males of the extant strepsipteran groups (Pohl & Beutel 2005). This condition is absent in *S. ovinae* (Löwe et al. 2016), but it also occurs in *E. laboulbenei* (Müller 2009). Giusti et al. (2007) also described an inflated midgut for *X. vesparum*, occupying a large part of the lumen of the cephalothorax. It is conceivable that the balloon gut of *X. vesparum* is functionally linked with the signaling behaviour of unfertilized female of the species (or genus) (Hrabar et al. 2014). The cephalothorax is slowly inflated, lifted from the host's tergite and elevated in the free air. This behaviour was also observed in some females of *X. vesparum* (H. Pohl unpubl. observations). Hrabar et al. (2014) assumed that these movements are made possible by a change of hemolymph pressure. In contrast, the inflation of the cephalothorax is probably a combined effect of bloating the midgut by increasing the hemolymph pressure in the abdomen of the female.

4.4. Neoteny of the female

Females of Strepsiptera, especially those of Stylopidia, were addressed as pedomorphic or neotenous by different authors (e.g. Kinzelbach 1971; Kathirithamby 1989; Beani et al. 2005; Pohl & Beutel 2005, 2008). McMahon & Hayward (2016) assigned them to a type II paedomorphosis (or neoteny) development: no acceleration of the development of the germ line takes place relative to the somatic development, but the somatic development is stopped before the pupation and the development of the germ line takes its normal course. In the case of the stylopidian females the only distinct difference between adults and larval stages is the presence or absence of functional birth organs and Nassonov's glands. A typical holometabolan metamorphosis does not take place. Erezyilmaz et al. (2014) could show that that an increased expression of the juvenile hormone-effector broad (br), which usually enhances the transition between the larval and pupal stages in holometabolous insects, does not occur in females of *X. vesparum*. The present study also supports the suggested neoteny in female Stylopidia: no distinct metamorphosis was observed in *X. vesparum*. The overall external morphology of adult females and the last larval exuvia is similar. Mandibles, labral field and antennal vestiges differ only slightly, only the maxillary vestiges are not distinctly developed in the adult. Löwe et al. (2016) observed more differences in *S. ovinae* even though the general similarity is only slightly affected: a bipartite labium and simple maxillae are present in adult females but missing in the secondary larva. However, it should be noted that the number of molts in males and females are identical in contrast to many Diaspididae (Sternorrhyncha) (Strümpel 2005). The exuvia of the third larval stage of the females is therefore homologous to the pupal exuvia of the males in Stylopidia.

Neotenous development also occurs in several groups of Elateroidea (Coleoptera, Polyphaga), where the last larval stage is followed by a morphologically similar wingless female. There is one major advantage of neo-

tenous development: distinctly more energy can be invested in the reproduction, as it is not needed for a complete metamorphosis. This is also arguably linked with a switch to a parasitic life style, allowing for instance to make optimal use of the host's resources (MCMAHON & HAYWARD 2016).

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File 1: richter&al-xenoscephalothorax-asp2017-electronicsupplement1.pdf – *Xenos vesparum*, female cephalothorax, interactive PDF.