

# Postembryonic development of the ground louse *Zorotypus caudelli* Karny (Insecta: Zoraptera: Zorotypidae)

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## Abstract

Based on captive breeding, the postembryonic development of the ground louse *Zorotypus caudelli* Karny, 1927 (Zoraptera, Zorotypidae) was examined and described in detail. The number of nymphal instars in *Z. caudelli* is five. During the second molt (2<sup>nd</sup> to 3<sup>rd</sup> instar), the number of antennomeres increases from eight to nine by subdivision of the basal flagellomere (meriston). Apterous and winged forms differentiate in the 4<sup>th</sup> nymphal instar. In the 4<sup>th</sup> instar of the winged form, small wing pads and small ocular spots appear. In the 5<sup>th</sup> instar, the wing pads elongate and the ocular spots are widened, and three ocelli are differentiated. Wing dimorphism may be a phenomenon independent of crowding. The two sexes closely resemble each other as in other zorapteran species, and sexual dimorphism does not appear until the final (5<sup>th</sup>) nymphal instar: in the 5<sup>th</sup> instar of males, setae increase in number on the 9<sup>th</sup> and 10+11<sup>th</sup> abdominal terga, and a small posteromedian swelling appears on the 10+11<sup>th</sup> abdominal tergum, the precursor of the mating hook. Key to nymphal instars of *Zorotypus caudelli* was given. The formation of thoracic pleural sclerites was examined and reevaluated.

## Key words

Zoraptera, postembryonic development, life history, nymphal morphology, wing dimorphism, antennal development.

## 1. Introduction

Zoraptera are small, inconspicuous insects and one of the least diverse neopteran orders. They are widely distributed in tropical and subtropical regions. Thirty-nine extant and nine fossil species are described, but their diversity remains underexplored (ENGEL 2008; MASHIMO et al. 2013). The systematic position of Zoraptera is one of the most controversial problems in insect phylogeny (e.g., ENGEL & GRIMALDI 2002; Yoshizawa 2007, 2011;

KLASS 2009; ISHIWATA et al. 2011; MASHIMO et al. 2014). The term ‘Zoraptera problem’ was coined to underline the controversial phylogenetic status of this enigmatic insect order by BEUTEL & WEIDE (2005), modeled after the ‘Strepsiptera problem’ proposed by KRISTENSEN (1981). Cladistic analyses using morphological data sets suggested a close affinity between Zoraptera and Acercaria or Eumetabola (=Acercaria+Holometabola), respectively

(BEUTEL & GORB 2001, 2006; BLANKE et al. 2012). Recent morphological and embryological studies and phylogenetic analyses based on molecular data support the placement of Zoraptera within Polyneoptera (GRIMALDI & ENGEL 2005; ISHIWATA et al. 2011; YOSHIKAWA 2011; LETSCH et al. 2012; MASHIMO et al. 2014), but the precise position remains unclear.

The study of Zoraptera has been neglected for a long time. Even though investigation of the group has markedly intensified in the last decade (skeletal-muscular system of the head: BEUTEL & WEIDE 2005; wing base structures: YOSHIKAWA 2007, 2011; skeletal-muscular system of the thorax: FRIEDRICH & BEUTEL 2008; postabdomen: HÜNEFELD 2007; reproductive system: DALLAI et al. 2011, 2012a,b, 2014; egg structure and embryonic development: MASHIMO et al. 2011, 2014), the biology is still very insufficiently known. Several studies on the life history (GURNEY 1938; RIEGEL & EYTALIS 1974; SHETLAR 1978) are available. However, the descriptions are fragmentary and the documentation of details insufficient.

Mating behavior is relatively well studied and three types of mating have been hitherto reported (SHETLAR 1978; CHOE 1994a,b, 1995, 1997; MASHIMO et al. 2011; DALLAI et al. 2013). In contrast to this, the life history and postembryonic development are largely unknown. GURNEY (1938) provided some information on food preferences and habitat, and VALENTIN (1986) reported grooming behavior. Embryonic development was recently described by MASHIMO et al. (2014). However, as the number of nymphal instars has only been suggested to be four or five (RIEGEL & EYTALIS 1974; SHETLAR 1974, 1978), the postembryonic stages remain very insufficiently known. The aim of the present study is to provide more detailed information on postembryonic development using *Zorotypus caudelli*, which was successfully reared in the lab. The number of nymphal instars was assessed based on detailed observations. Detailed documentation of the external morphology of all immature stages of the species is provided.

## 2. Material and methods

Adults and nymphs of *Zorotypus caudelli* were collected under the bark of decaying logs in Ul Gombak (Selangor, Peninsular Malaysia). They were kept in plastic cases (15 cm × 8 cm × 3 cm) with a bottom layer of moist soil at room temperature (ca. 26–28°C), and fed on dry yeast and powdered dried *Bombyx* pupae (commercially sold fishing bait).

In order to identify nymphal stages and to assess their duration, more than 100 individuals of presumptive 1<sup>st</sup> and 2<sup>nd</sup> instars were kept separately in plastic cases (3.6 cm × 3.6 cm × 1.4 cm) with a bottom layer of moist soil at 26°C. We surveyed morphological changes under a stereomicroscope Olympus SZ61 every day.

Some of the nymphs were anesthetized using CO<sub>2</sub>, fixed with FAA fixative (ethyl alcohol:formalin:acetic acid=15:5:1) for 10 h and stored in 80% ethanol. The following measurements were taken: (1) antennal length, (2) head width, (3) length and (4) width of pronotum, length (5) and width (6) of profemur, (7) protibial length, (8) length and (9) width of mesofemur, (10) mesotibial length, (11) length and (12) width of metafemur, and (13) metatibial length.

Living specimens and specimens slide-mounted in Euparal were photographed with a NIKON Digital Sight DS-Fi2 camera, under a stereomicroscope Leica MZ12 and a biological microscope Nikon Optiphot-2, respectively. For scanning electron microscopy (SEM), fixed specimens were dehydrated in a graded ethanol series, dried with a critical point dryer Tousimis Samdri-PVT-3D, coated with gold, and then observed with a scanning electron microscope TOPCON SM-300 at 15 kV.

For the sclerites we use the terminology of SNODGRASS (1935) and MATSUDA (1970). In the interpretation of highly modified prothoracic sclerites we also refer to FRIEDRICH & BEUTEL (2008) (see 4.5.).

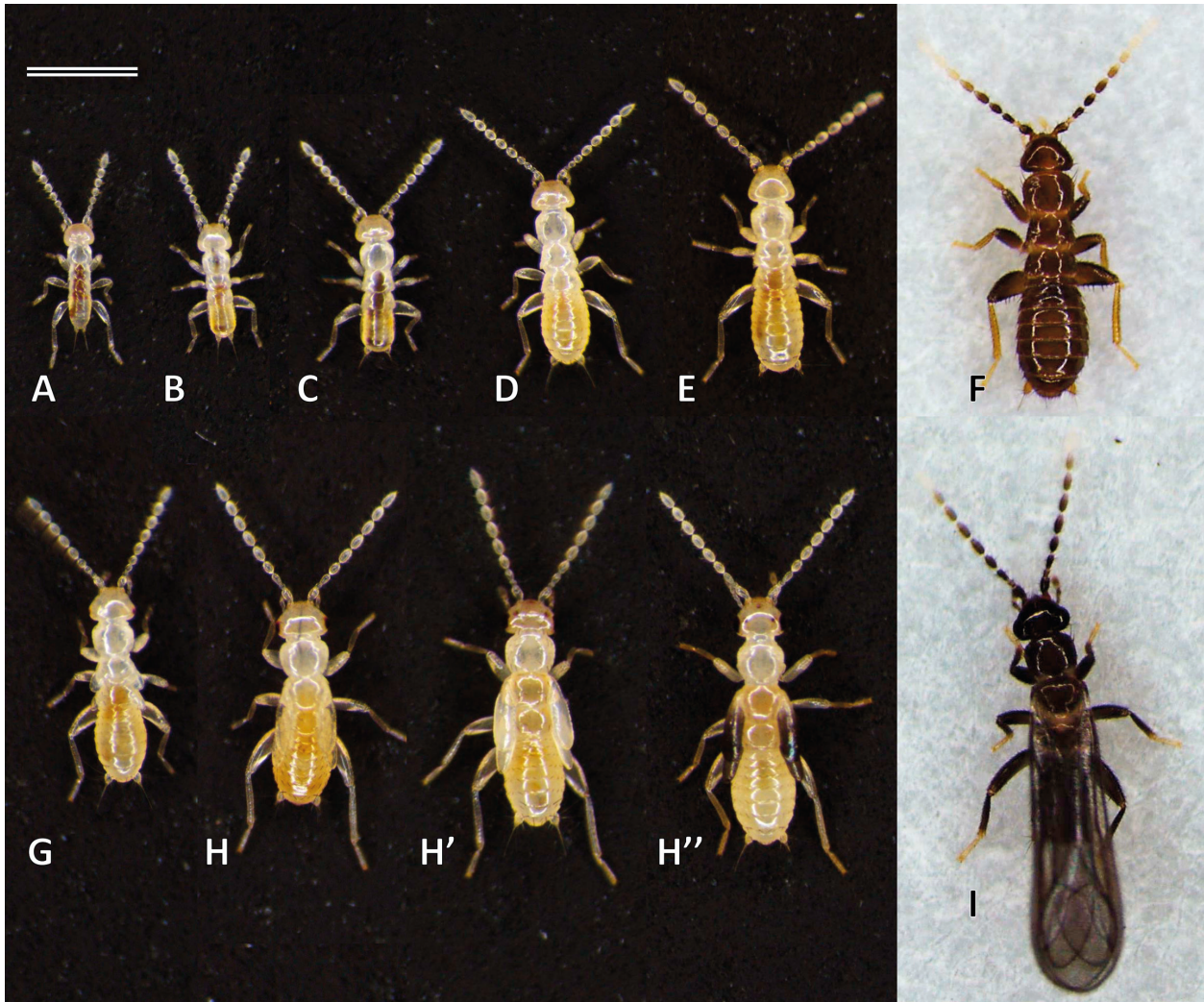
## 3. Results

### 3.1. Determination of the number of nymphal instars

Daily checking of exuviae and of the appearance of the chaetotaxy of the next instar of separately reared individuals (Fig. 2A) revealed five nymphal instars in *Zorotypus caudelli* (Fig. 1). Some of the separately reared individuals of each instar were fixed. Samples of identified instars including fixed individuals were used for morphological observations and measurements (Tables 1, 2, Figs. 1–9).

### 3.2. Duration of nymphal instars

To assess the duration of each instar, more than 100 nymphs of the 1<sup>st</sup> or 2<sup>nd</sup> instar were kept separately in plastic cases. We could determine the duration of the 3<sup>rd</sup> to 5<sup>th</sup> nymphal instars, but not of the 1<sup>st</sup> and 2<sup>nd</sup> nymphal instars due to the high mortality of the 1<sup>st</sup> nymphal instar. Therefore, we repeated the experiment rearing nymphs hatching on the same date in a group of only 10–20 individuals. As the mortality of the 1<sup>st</sup> instar nymphs was distinctly lowered, the duration of the first two instars could be determined.



**Fig. 1.** *Zorotypus caudelli* Karny, 1927: nymphs and adults. **A:** 1<sup>st</sup> instar. **B:** 2<sup>nd</sup> instar. **C:** 3<sup>rd</sup> instar. **D:** 4<sup>th</sup> instar, apterous form. **E:** 5<sup>th</sup> instar, apterous form. **F:** Adult female, apterous form. **G:** 4<sup>th</sup> instar, winged form. **H, H', H'':** Early (H), middle (H') and late (H'') 5<sup>th</sup> instar, winged form. **I:** Adult male, winged form. – Scale bar = 1 mm.

The duration of each instar was as follows (also summarized in Table 1): 1<sup>st</sup> instar,  $14.98 \pm 2.82$  days ( $n=46$ ); 2<sup>nd</sup> instar,  $13.48 \pm 5.74$  days ( $n=21$ ); 3<sup>rd</sup> instar,  $12.12 \pm 3.07$  days ( $n=33$ ); 4<sup>th</sup> instar (apterous form),  $14.58 \pm 3.66$  days ( $n=12$ ); 4<sup>th</sup> instar (winged form),  $17.51 \pm 5.33$  days ( $n=35$ ); 5<sup>th</sup> instar (apterous form),  $16.44 \pm 3.10$  days ( $n=18$ ); 5<sup>th</sup> instar (winged form),  $24.88 \pm 4.64$  days ( $n=32$ ). The adults continued living for several months but exact records are not available.

### 3.3. Morphological features of nymphs

**Instar I** (Figs. 1A, 3, 8A). Head orthognathous, subtriangular (Fig. 3A). Antenna 8-segmented (Fig. 3B); antennomere 1 (scapus) elongate, approximately twice as long as wide; antennomere 2 (pedicellus) and antennomere 3 (1<sup>st</sup> flagellomere) small, spherical, half as long as scapus (Fig. 3B'); antennomeres 4–7 spherical; antennomere

8 large, 1.5 times as long as scapus, with pointed apex (Fig. 3B). Pronotum subrectangular; meso- and metathoracic nota trapezoidal, slightly widening posteriorly; lateral margin of each thoracic notum less developed (Fig. 3A,D). Propleurite with anterior, intermediate and posterior sclerites; anterior sclerite slender, intermediate sclerite small and subrectangular; triangular trochantin located anterior to procoxa (Fig. 3C); dorsal parts of anterior and intermediate propleural sclerites fused, but separated by a non-uphove, less sclerotized region from posterior sclerite (Fig. 3C); propleural suture represented by invagination line of propleural apophysis along anteroventral margin of posterior sclerite. Meso- and metathoracic pleurites divided into anterior episternum and posterior epimeron by a pleural suture between the lateral notal margin and pleuro-coxal joint. Sutures separating anepisternum, katepisternum and preepisternum, or anepimeron and katepimeron not recognizable (Fig. 3D). Preepisternum represented by region anterior to trochantin according to FRIEDRICH & BEUTEL (2008). Subtriangular trochantins located anterior to meso- and

**Table 1.** Duration of nymphal instars of *Zorotypus caudelli*.

Instar	I	II	III	Apterous IV	Winged IV	Apterous V	Winged V
Specimens examined	46	21	33	12	35	18	32
Average duration [days]	14.98 ± 2.82	13.48 ± 5.74	12.12 ± 3.07	14.58 ± 3.66	17.51 ± 5.33	16.44 ± 3.10	24.88 ± 4.64
Maximum duration [days]	20	25	19	24	30	24	34
Minimum duration [days]	11	8	8	10	11	11	17

**Table 2.** Measurements of *Zorotypus caudelli* nymphs of each instar.

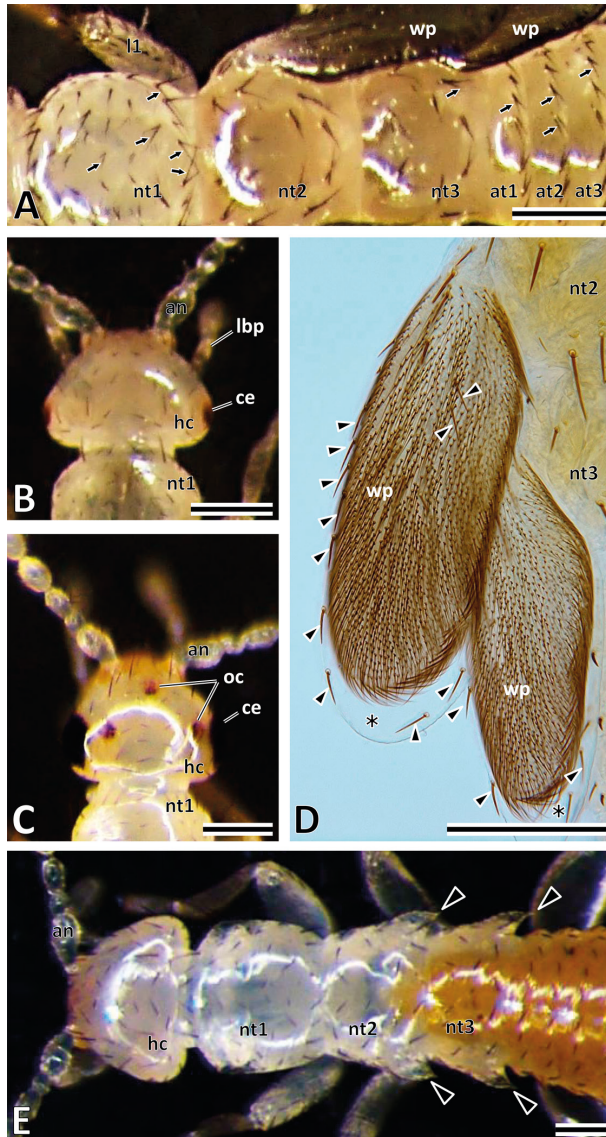
Instar	Larva					Adult
	I	II	III	IV	V	VI
Specimens examined	5	5	5	5	5	9
Antennal length [mm]	0.64 ± 0.02	0.77 ± 0.02	0.82 ± 0.03	0.93 ± 0.06	1.16 ± 0.01	1.35 ± 0.08
Head width [µm]	260 ± 8	318 ± 7	336 ± 4	388 ± 19	436 ± 23	455 ± 32
Pronotum length [µm]	170 ± 8	198 ± 10	244 ± 10	294 ± 19	320 ± 13	355 ± 15
Pronotum width [µm]	216 ± 10	252 ± 7	274 ± 14	318 ± 25	376 ± 27	405 ± 40
Profemur length [µm]	209 ± 9	242 ± 11	270 ± 8	321 ± 11	372 ± 22	441 ± 23
Profemur width [µm]	88 ± 6	109 ± 9	120 ± 8	136 ± 8	161 ± 16	181 ± 12
Protibia length [µm]	190 ± 6	233 ± 11	253 ± 2	298 ± 6	357 ± 14	404 ± 30
Mesofemur length [µm]	180 ± 10	233 ± 14	264 ± 8	308 ± 10	368 ± 35	428 ± 23
Mesofemur width [µm]	69 ± 3	92 ± 6	98 ± 4	112 ± 7	140 ± 12	143 ± 4
Mesotibia length [µm]	182 ± 11	218 ± 6	253 ± 16	302 ± 11	339 ± 15	396 ± 13
Metafemur length [µm]	227 ± 10	292 ± 10	327 ± 14	395 ± 16	485 ± 15	566 ± 37
Metafemur width [µm]	83 ± 5	113 ± 8	129 ± 8	158 ± 9	208 ± 12	214 ± 12
Metatibia length [µm]	257 ± 6	315 ± 10	341 ± 14	425 ± 12	509 ± 18	575 ± 21

metacoxa (Fig. 3D). Small sclerite (black star in Fig. 3D) with spiracle located anterior to anepisternum of mesothorax; sclerite bearing metathoracic spiracle (white star in Fig. 3D), which is hidden and not discernible from lateral, smaller than that in mesothorax. Femur of each leg relatively slender (Fig. 8A). Profemur wider than mesofemur. Bristles arranged as a comb inserted on ventral side of distal half of protibia. Metafemur longer than pronotum and mesofemora, respectively, approximately 3.5 times longer than wide, slightly swollen proximally (Fig. 8A). Abdominal terga I–VIII uniformly sclerotized, with a row of setae along posterior margin (Fig. 3A,E). Tergum IX short, with a medial pair of setae (Fig. 3F). Tergum X subtriangular, with a pair of thin setae (Fig. 3F). Tergum XI short and less sclerotized. Pairs of spiracles located on less sclerotized pleurites of segments I–VIII (Fig. 3E). Spiracles of segment I more dorsal. Abdominal sterna I and II less sclerotized (Fig. 3G). Sterna III–VII partly less sclerotized (arrows in Fig. 3G), each with a pair of setae in restricted sclerotized region. Sternum VIII wide and approximately twice as long as other abdominal sterna. Sternum IX shorter than sternum VIII. Sternum X not recognizable externally (see MASHIMO et al. 2014). The ventral side of segment XI occupied by coxopodites of cerci (asterisks in Fig. 3F, cf. MASHIMO et al. 2014). Cerci one-segmented, approximately conical, with one long apical seta, several moderately long subapical setae, and some very long and fine setae (Fig. 3A,F). Cerci almost adjacent, posteriorly directed, with their surface covered with numerous minute cuticular spicules (Fig. 3F).

**Instar II** (Figs. 1B, 4, 8B, 9A). Antenna composed of eight antennomeres (Fig. 4B); antennomere 3 ovoid, approximately twice as long as antennomere 2 (Fig. 4B'). Thoracic paranota slightly developed laterally (Fig. 4A). Metafemur swollen proximally and three times longer than wide (Fig. 8B). Abdominal terga extended laterally, being fused with spiracle-bearing pleurites to form tergopleurites (Fig. 4C); in the posterior abdominal segments, sclerotization of the tergopleurites yet to complete (cf. asterisk in Fig. 4C). Sternum II slightly sclerotized (asterisk in Fig. 9A), but sterna III–VII uniformly sclerotized with two pairs of setae (Fig. 9A). Sterna VIII–IX become longer and shorter, respectively (Fig. 9A). Cerci slightly apart from each other (Fig. 4D).

**Instar III** (Figs. 1C, 5, 8C, 9B). Antenna composed of eight antennomeres (Fig. 5B); antennomere 3 constricted in the middle, this being a sign of subdivision of “meriston”; antennomeres 4–8 slightly elongated (Fig. 5B,B'). A few short setae newly differentiated lateral to antennal bases (Fig. 5A). One small seta added to each of meso- and metaepimeron (Fig. 5C). Metafemur further swollen as shown in Figure 8C. In the posterior abdominal segments, sclerotization of the tergopleurites completed (Fig. 5D). Abdominal tergum X extended posteriorly, assuming hemicircular (Fig. 5E). Abdominal sterna as shown in Figure 9B.

**Instar IV** (Figs. 1D,G, 2B, 6, 8D, 9C). Antenna 9 segmented by subdivision of antennomere 3 (Fig. 6B); an-

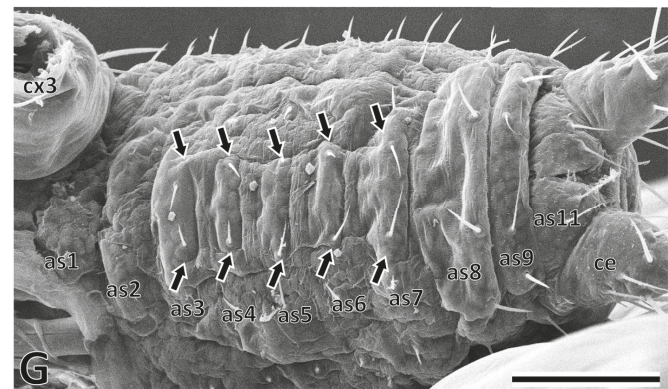
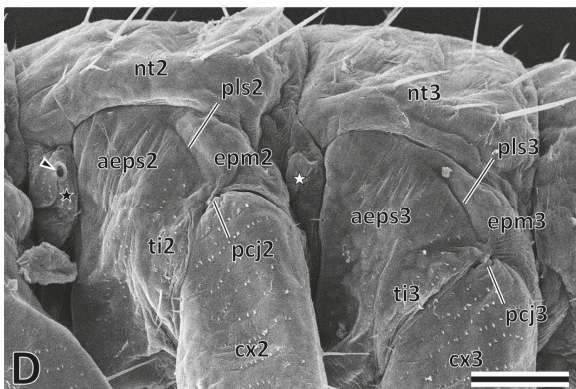
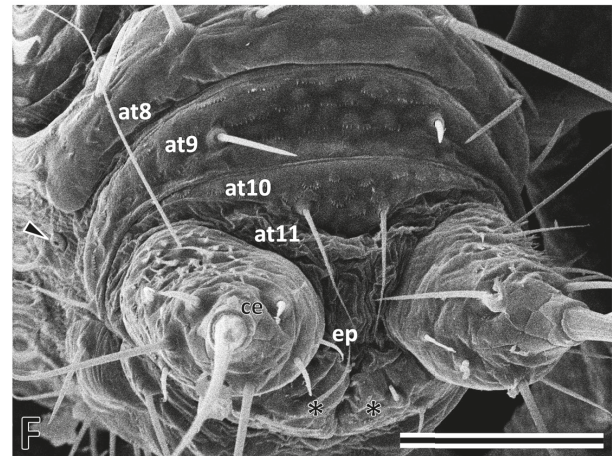
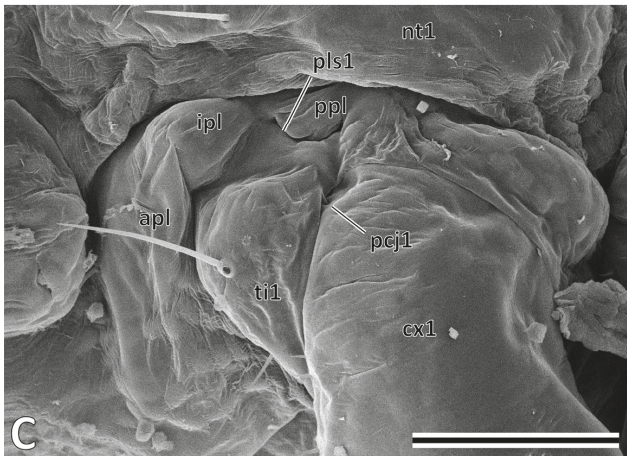
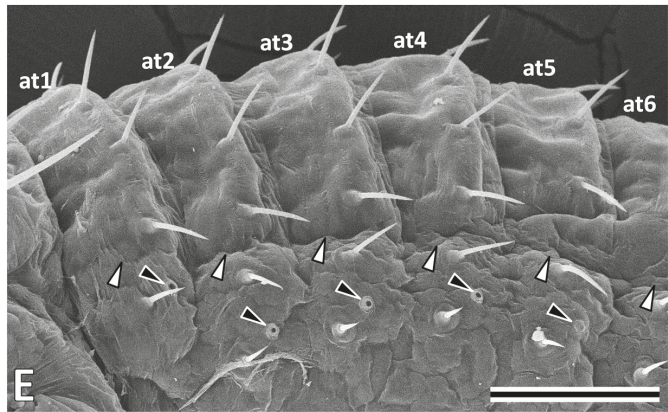
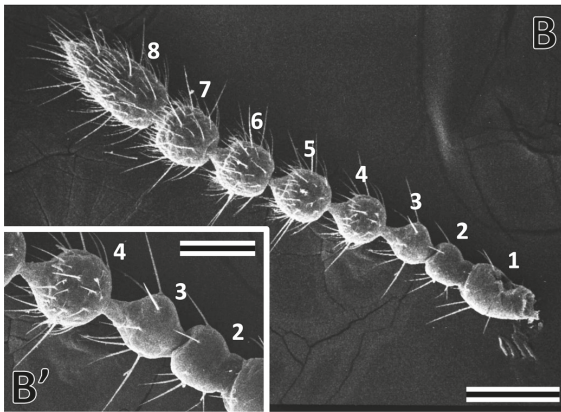
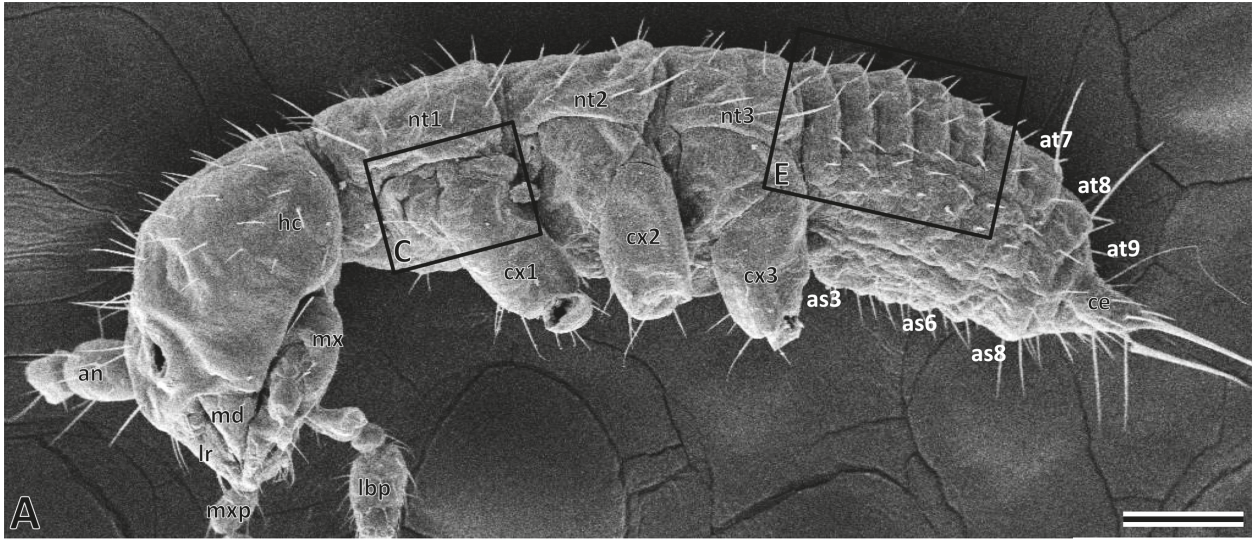


**Fig. 2.** *Zorotypus caudelli* Karny, 1927: 4<sup>th</sup> and 5<sup>th</sup> instar. **A:** Thorax and abdomen, late 5<sup>th</sup> instar, winged form. Chaetotaxy of next instar or adult visible through cuticle: setae of next instar appear faintly as part of “seemingly bifurcated” setae (arrows). **B,C:** Heads of 4<sup>th</sup> (B) and 5<sup>th</sup> instar (C), winged form. **D:** Wing pads of late 5<sup>th</sup> instar. Asterisks and arrowheads show the 5<sup>th</sup> instar cuticle and its setae, respectively. **E:** Head and thorax of 5<sup>th</sup> instar. Arrowheads indicate posterolateral projections of pterothoracic nota. – Abbreviations: an=antenna; at1–3=abdominal terga I–III; ce=compound eye; hc=head capsule; l1=proleg; lbp=labial palp; nt1–3=pro-, meso- and metathoracic nota; oc=ocellus; wp=wing pad. – Scale bars=200  $\mu$ m.

tennomere 4 newly differentiated and subequal to the length of antennomere 3, with several small setae in the subapical region (Fig. 6B’); antennomeres 5–8 slightly elongated (Fig. 6B). Apterous and winged forms distinguishable (Fig. 1D,G). In winged form, prospective compound eyes appeared as small black spots at the posterolateral corners of the head (Figs. 1G, 2B), although the cuticle around the ocular black spots showed no change (Fig. 6A vs. C). Cephalic chaetotaxy, irrespective

of apterous or winged, basically the same as that of the previous instar (Fig. 6A). Small wing pads differentiated at the posterolateral corners of the pterothoracic nota in the winged form (Figs. 1G, 6D). One small seta added in the posterior area of the meso- and metaepisternum (Fig. 6D). Metafemur as shown in Figure 8D. Abdominal tergum XI is finally sclerotized, and terga X and XI cannot be distinguished with each other, both together assuming trapezoid (Fig. 6E): MASHIMO et al. (2013) erroneously interpreted tergum IX in the present study as a mixture of short tergum IX with anterior trapezoidal expansion and moderate-lengthened tergum X, but following the postembryonic changes of the posterior abdominal terga, it was revealed that tergum IX remains independent of other structures without fusion with anything, but that tergum X appears to instead fuse with tergum XI. One additional pair of setae on tergum X+XI (Fig. 6E). Setae of the posterior row in each of sterna III–VII added (Fig. 9C). A few pairs of setae newly differentiated on sternum VIII (Fig. 9C).

**Instar V** (Figs. 1E,H,H’,H’’, 2A,C–E, 7A–H, 8E, 9D). Antenna composed of nine antennomeres (Fig. 7B); antennomeres 3 and 4 elongate, 1.5 times longer than antennomere 2 (Fig. 7B’); numerous short setae on the subapical region of antennomere 4 (Fig. 7B’). In the winged form, the ocular black spots on the posterolateral corners of the head widened (Figs. 1H, 2C), but the cuticle over the ocular spots still showed no change (Fig. 7C). Toward emergence, black spots more extensive and intensive, and three prospective ocelli visible between the compound eyes (Figs. 1H’, 2C). In the heads of both apterous and winged forms, a few additional setae added lateral to the antennal bases (Fig. 7A,C). In the winged form, transparent and thin wing pads of pterothoraces enlarged, and those of metathorax reaching segment IV (Figs. 1H, 7F); in the middle of 5<sup>th</sup> instar, wing pads thickened and whitish (Fig. 1H’); in the late of 5<sup>th</sup> instar, wing pads turning blackish due to numerous imaginal short setae being formed and darkened beneath the nymphal cuticle (Figs. 1H’’, 2D). Very rarely, nymphs with smaller ocular spots are found, of which the posterolateral corners of pterothoraces slightly protrude (Fig. 2E). They become adults with a similar body color to apterous adults and with conspicuous black ocular spots, and they have a pair of sclerotized projections at the posterolateral corners of pterothoracic nota. Pleural regions of the pterothoracic segments of the apterous and winged forms, as shown in Fig. 7D and E, respectively. Metafemur as shown in Fig. 8E, which is basically the same as the definitive form of adults (Fig. 8F). A few setae newly added in the lateral region of tergum in each of abdominal segments I–VII (Fig. 7A). Two different patterns in chaetotaxy of terga IX and X+XI are recognized (Fig. 7G,H). Under SEM, a small posteromedial swelling could be found on tergum X+XI in some nymphs (white arrow in Fig. 7G): individuals with this projection are masculine, so the difference in the chaetotaxy in terga IX and X+XI is a sexual diagnosis. A few setae added anterior to the posterior row



of setae on sterna IV–VII (Fig. 9D). Sternum VIII enlarged, with several short setae added (Fig. 9D).

### 3.4. Key to nymphal instars of *Zorotypus caudelli*

- 1 Wing pads and prospective compound eyes present ..... **2**
- Wing pads and prospective compound eyes absent ..... **3**
- 2 Small wing pads, small black ocular pigment at the posterolateral corners of the head, antennomere 3 subequal to 2 ..... **4<sup>th</sup> instar of winged form**
- Long wing pads, prospective compound eyes present as large black spots, occasionally with prospective ocelli, antennomere 3 about twice as long as antennomere 2 ..... **5<sup>th</sup> instar of winged form**
- 3 Nine antennomeres ..... **4**
- Eight antennomeres ..... **5**
- 4 Antennomere 3 subequal to 2 ..... **4<sup>th</sup> instar of apterous form**
- Antennomere 3 about twice as long as 2 ..... **5<sup>th</sup> instar of apterous form**
- 5 Antennomere 3 constricted in middle, meso- and metathoracic notum angular ..... **3<sup>rd</sup> instar**
- Antennomere 3 not constricted, meso- and metathoracic notum rounded ..... **6**
- 6 Antennomere 3 oval, cerci slightly separated ..... **2<sup>nd</sup> instar**
- Antennomere 3 spherical, cerci almost adjacent ..... **1<sup>st</sup> instar**

## 4. Discussion

### 4.1. Identification of nymphal instars

Even though several studies on the life history of Zoraptera are available, the total number of nymphal in-

stars has remained ambiguous (RIEGEL & EYTALIS 1974; SHETLAR 1974, 1978; RIEGEL 1987), with estimations of either four or five stages based on different measurements (RIEGEL & EYTALIS 1974; SHETLAR 1974; RIEGEL 1987):

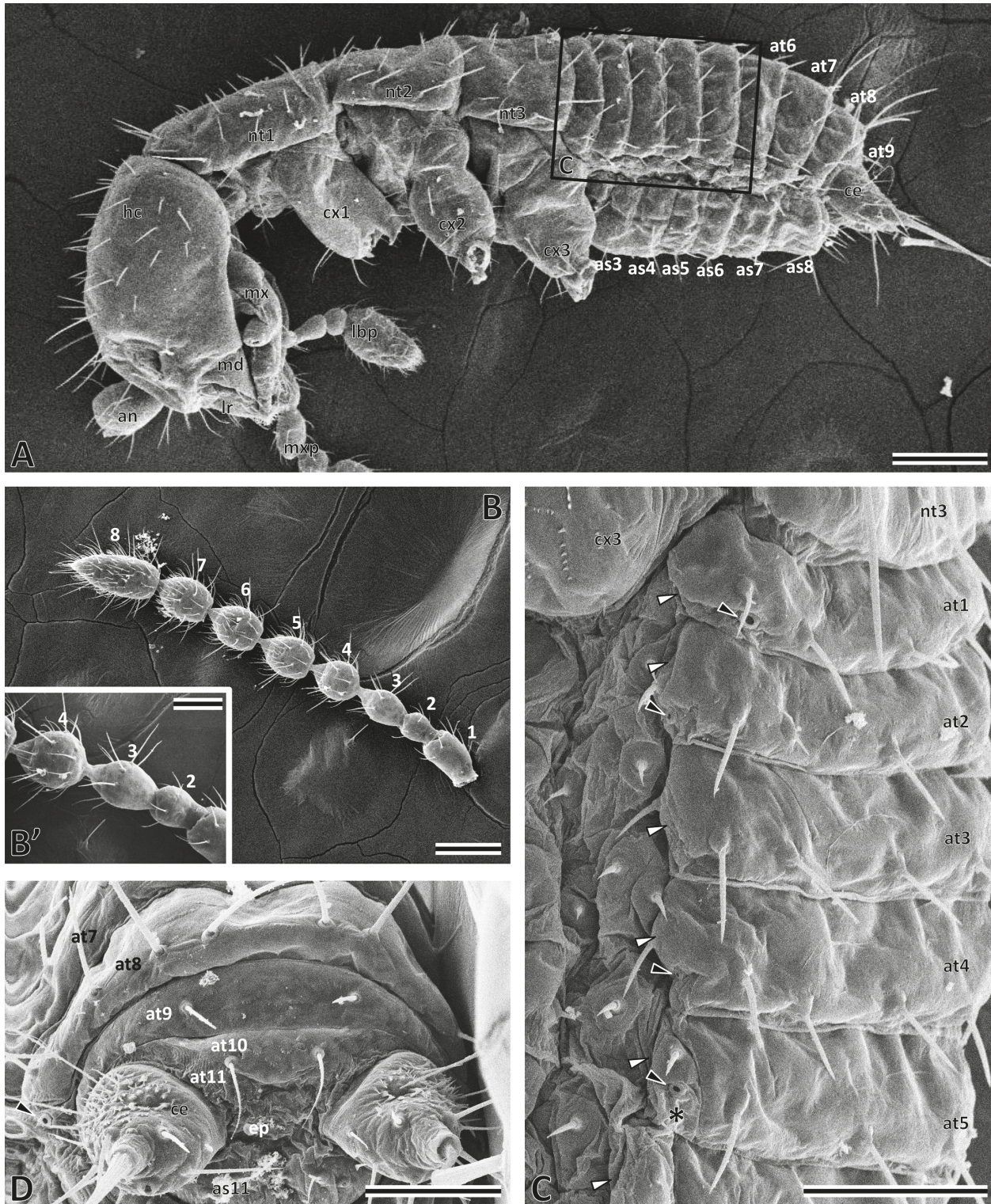
SHETLAR (1974, 1978) suggested the number of five nymphal instars using the head width of *Zorotypus hubbardi* Caudell, 1918, which is widely distributed in North America. In contrast, RIEGEL & EYTALIS (1974) estimated four instars for the same species based on the length of the prothorax, profemur, metafemur and metatibia. Previous studies apparently contained insufficient measurement data, and SHETLAR (1974) failed to present significant differences for the identification of instars (SHETLAR 1974: tables 1, 2, graphs 1, 2).

For tiny insects such as zorapterans, it may be very difficult to designate significant differences in measurements, and herewith to determine the number of instars. Therefore, measurement cannot be a crucial clue for the identification of instar numbers of Zoraptera. The qualitative and quantitative data presented here unambiguously show that postembryonic development comprises five nymphal instars in *Zorotypus caudelli*, a number also directly confirmed by observing the ecdyses. The number of nymphal instars is the same as that estimated for *Z. hubbardi* by SHETLAR (1974, 1978) with his data set.

Nymphal instars can be identified by the characteristics of the antennomeres, thoracic nota, and wing pads (see key). Further postembryological observations such as those provided here are required for more species of Zoraptera to test whether the total number of five nymphal instars belongs to the groundplan of the order. This is tentatively suggested by the very similar size of the eggs (ca. 0.6–0.7 mm) and adults (ca. 2 mm) in *Z. hubbardi*, *Z. caudelli*, and other zorapteran species (cf. SILVESTRI 1946; CHOE 1989; MASHIMO et al. 2011; DALLAI et al. 2012b).

HEMING (2003) summarized the number of nymphal instars in insects, which was supplemented by MINELLI & FUSCO (2013). The nymphal instar number largely varies within each order in Neoptera, with “13 to 34” in Plecoptera and “three to 14” in Blattodea as extreme examples (MINELLI & FUSCO 2013). In addition to the nymphal instar number revealed in Zoraptera, “five” instars sporadically appear in several remote lineages of Neoptera, such as Mantophasmatodea, some each of Phasmatodea, Dic-

← **Fig. 3.** *Zorotypus caudelli* Karny, 1927: scanning electron micrographs (SEMs) of 1<sup>st</sup> instar. **A:** Body, lateral view. **B,B’:** Left antenna (B), antennomeres 2–4 (B’). **C:** Prothorax, lateral view. **D:** Meso- and metathorax, lateral view. Black and white stars indicate small sclerites anterior to meso- and metathoracic anepisterna, respectively. **E,F,G:** Abdomen, lateral (E), caudal (F) and ventral (G) views. Asterisks and arrows indicate coxopodites of segment XI and lateral margin of small sclerotized regions of sterna III–VII, respectively. White and black arrowheads indicate lateral margins of abdominal terga and spiracles, respectively. – Abbreviations: aeps2, 3 = meso- and metathoracic anepisterna; an = antenna; apl = anterior propleurite; as1–11 = abdominal sterna I–XI; at1–11 = terga I–XI; ce = cercus; cx1–3 = pro-, meso- and metacoxa; ep = epiproct; epm2, 3 = meso- and metathoracic epimera; hc = head capsule; lbp = labial palp; lr = labrum; md = mandible; ipl = intermediate propleurite; mx = maxilla; mxp = maxillary palp; nt1–3 = pro-, meso- and metathoracic nota; pcj1–3 = pro-, meso- and metathoracic pleuro-coxal joints; pls1–3 = pro-, meso- and metathoracic pleural sutures; ppl = posterior propleurite; ti1–3 = pro-, meso- and metatrochantines; 1–8 = antennomeres 1–8. – Scale bars = A,B,E,G: 100 µm; B’,C,D,F: 50 µm.

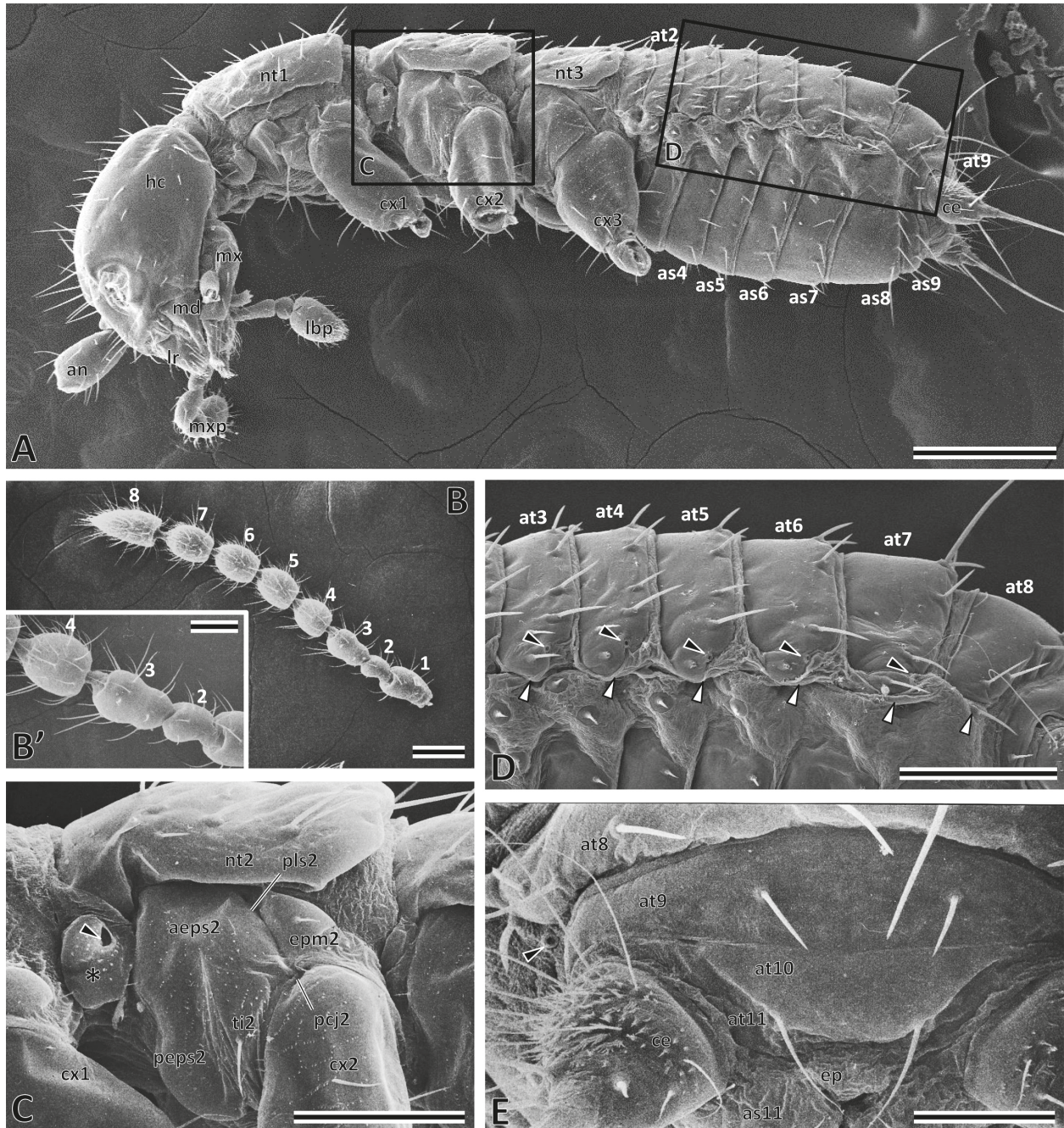


**Fig. 4.** *Zorotypus caudelli* Karny, 1927: SEMs of 2<sup>nd</sup> instar. **A:** Body, lateral view. **B, B':** Left antenna (**B**) and antennomeres 2–4 (**B'**). **C, D:** Abdomen, lateral (**C**) and caudal (**D**) views. White and black arrowheads indicate lateral margins of each abdominal tergopleurite and spiracles, respectively. Asterisk indicates unsclerotized area of the tergopleurite. – Abbreviations: an=antenna; as3–8, 11=abdominal sterna III–VIII, XI; at1–11=abdominal terga I–XI; ce=cercus; cx1–3=pro-, meso- and metacoxa; ep=epiproct; hc=head capsule; lbp=labial palp; lr=labrum; md=mandible; mx=maxilla; mxp=maxillary palp; nt1–3=pro-, meso- and metathoracic nota; 1–8=antennomeres 1–8. – Scale bars=A, B, 100 µm; B', C, D, 50 µm.

tyoptera, Orthoptera and Hemiptera, and some holometabolous orders, and we could not find any phylogenetic

signals for discussing the position of Zoraptera from the present findings.





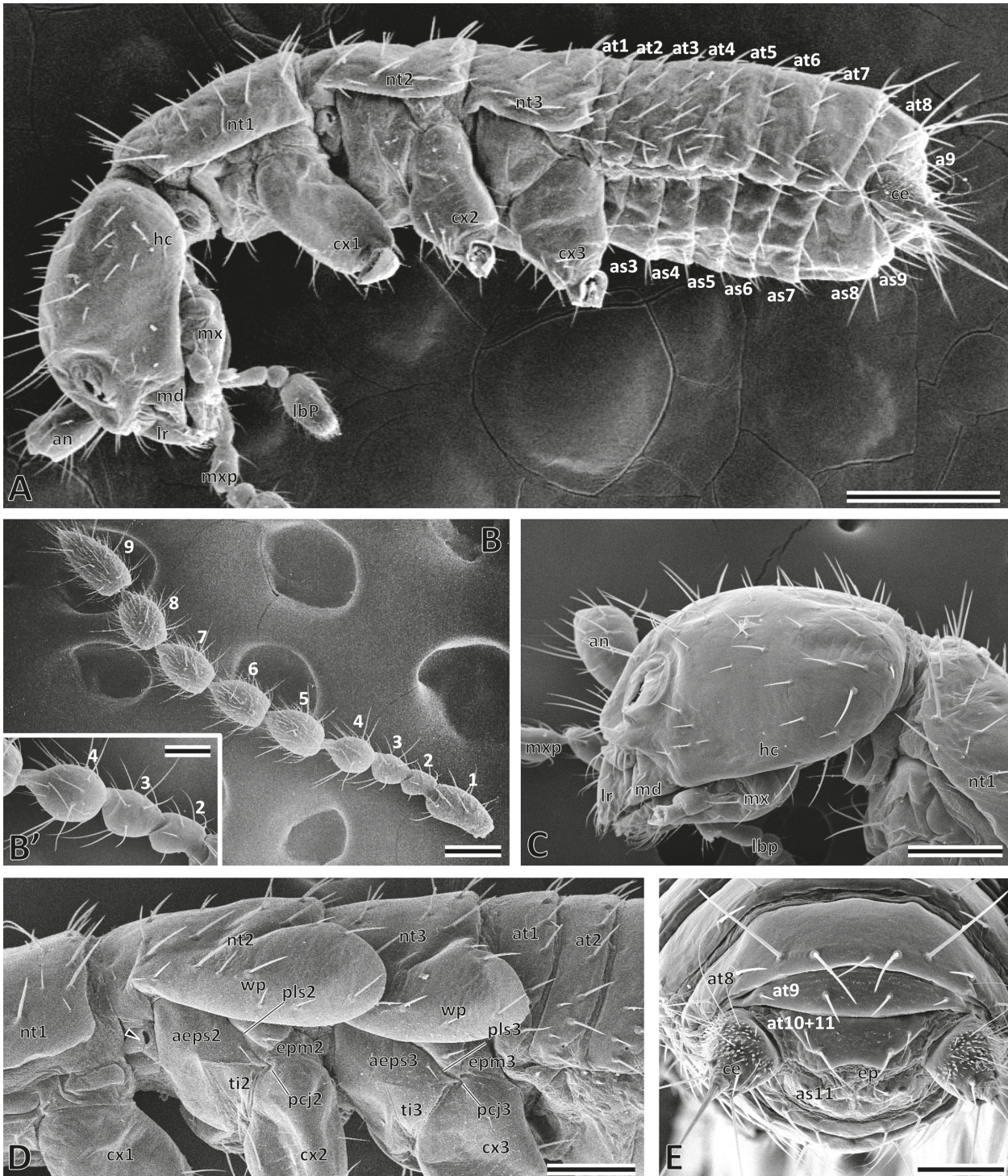
**Fig. 5.** *Zorotypus caudelli* Karny, 1927: SEMs of the 3<sup>rd</sup> instar. **A:** Body, lateral view. **B,B':** Left antenna (**B**), antennomeres 2–4 (**B'**). **C:** Mesothorax, lateral view. **D,E:** Abdomen, lateral (**D**) and caudal (**E**) views. White and black arrowheads indicate lateral margins of abdominal tergum and spiracles, respectively. Asterisk indicates small sclerotized region anterior to mesothoracic anepisternum. – Abbreviations: an=antenna; aeps2=mesothoracic anepisternum; as4–8, 11=abdominal sterna IV–VIII, XI; at2–11=abdominal terga II–XI; ce=cercus; cx1–3=pro-, meso- and metacoxa; ep=epiproct; epm2=mesothoracic epimeron; hc=head capsule; lbp=labial palp; lr=labrum; md=mandible; mx=maxilla; mxp=maxillary palp; nt1–3=pro-, meso- and metathoracic nota; pcj2=mesothoracic pleuro-coxal joint; peps2=mesothoracic preepisternum; pls2=mesothoracic pleural suture; ti2=mesotrochantin; 1–8=antennomeres 1–8. – Scale bars=A: 200 µm; B,C,D: 100 µm; B',E: 50 µm.

#### 4.2. Wing dimorphism

Wing dimorphism is a relatively common feature in insects (ROSS 1986; SIMPSON et al. 2011). It is often found in gregarious species and undoubtedly evolved independently in different lineages. In a typical case, the apterous

(brachypterous) form with higher reproductive capacity appears under stable and favorable environmental conditions. Deteriorating local environmental conditions trigger the appearance of winged forms with the ability to move to new habitats (ROSS 1986; SIMPSON et al. 2011).

Zoraptera were initially described as wingless (SILVESTRI 1913). However, the scientific name given to the



**Fig. 6.** *Zorotypus caudelli* Kary, 1927: SEMs of 4<sup>th</sup> instar. **A:** Body of apterous form, lateral view. **B,B':** Left antenna (**B**) and antennomeres 2–4 (**B'**). **C:** Head of winged form, lateral view. Black ocular spot visible under light microscope (e.g., Fig. 2B), surface structure of cuticle around it not yet recognizable (cf. **C**). **D:** Meso- and metathorax of winged form, lateral view. Arrowhead indicates spiracle. **E:** Abdomen, caudal view. – Abbreviations: aeps2, 3 = meso- and metathoracic anepisterna; an = antenna; as3–9, 11 = abdominal sterna III–IX, XI; at1–11 = abdominal terga I–XI; ce = cercus; cx1–3 = pro-, meso- and metacoxa; ep = epiproct; epm2, 3 = meso- and metathoracic epimera; hc = head capsule; lbp = labial palp; lr = labrum; md = mandible; mx = maxilla; mxp = maxillary palp; nt1–3 = pro-, meso- and metathoracic nota; pcj2, 3 = meso- and metathoracic pleuro-coxal joints; pls2, 3 = meso- and metathoracic pleural sutures; ti2, 3 = meso- and metatrochantines; wp = wing pad; 1–9 = antennomeres 1–9. – Scale bars = A: 200 µm; B,C,D,E: 100 µm; B': 50 µm.

order (“purely apterous”, Greek: zoros=pure, strong; aptera=apterous) is a misnomer, since CAUDELL (1920) found that winged forms occur. Until now, morphs have

been reported for many zorapteran species, and wing and eye dimorphism is considered one of the potential autapomorphies of Zoraptera (FRIEDRICH & BEUTEL 2008).

In Zoraptera, developed compound eyes and three ocelli are present in the winged form, but absent in the apterous form. In *Zorotypus caudelli*, the morphological differences between apterous and winged forms become distinct from the 4<sup>th</sup> nymphal instar. In the 4<sup>th</sup> instar of the winged form, small wing pads and small ocular spots appear. In the 5<sup>th</sup> instar of the winged form, wing pads elongate and ocular spots are widened, and soon, three ocelli appear. The mechanism of wing dimorphism in Zoraptera has not been examined in detail. We have only fragmentary information on zorapteran wing dimorphism from breeding by SHETLAR (1974, 1978). He could not identify the key factor controlling wing dimorphism, but mentioned that, “Crowding does not seem to have an effect on production of winged individuals,” since no difference in the numbers of the winged form was found in laboratory colonies of different densities ranging from 10 to 50 individuals; however, the details of rearing experiments and the occurrence rate of the winged form were not mentioned. In the present study, we reared around 150 individuals separately, most of which became the winged form (data not shown). This may support SHETLAR (1974, 1978) that crowding is not always a key factor controlling wing dimorphism in Zoraptera. However, the young nymphs examined in the present study were derived from eggs laid by females reared in a high density of 100–200 individuals in a case of 15 cm × 8 cm × 3 cm, and the possible effect of crowding could not be completely rejected. Although we obtained relatively many winged males in the present study, it was reported that winged males are very rare in the field, and that the majority of the winged form is female (GURNEY 1938; SHETLAR 1974; GRIMALDI & ENGEL 2005). SHETLAR (1974, 1978) suggested that the production of the winged form may be not sex determined, but sex influenced or sex related. To understand the mechanism of wing dimorphism in Zoraptera, culture experiments over several generations are needed. We observed that very rarely in *Z. caudelli* the 5<sup>th</sup> instar nymphs appear with ill-developed ocular spots and wing pads roughly comparable to those of the 4<sup>th</sup> instar (Fig. 2E). These wing pads persist in the adults as small sclerotized projections at the posterolateral corners of pterothoracic nota. A similar report was published by SHETLAR (1978). These cases of ill-developed wings may provide a hint to clarify the key factor controlling wing dimorphism in Zoraptera.

#### 4.3. Sexual dimorphism

Adults of Zoraptera show no distinct difference in size between the sexes. The genitalia are very different in the two sexes (e.g. GURNEY 1938) but are not exposed and were not included in our study. In most species, the non-genital parts of the abdominal terminalia show only subtle differences between sexes: in males of *Zorotypus caudelli*, eight pairs of setae are arranged on the

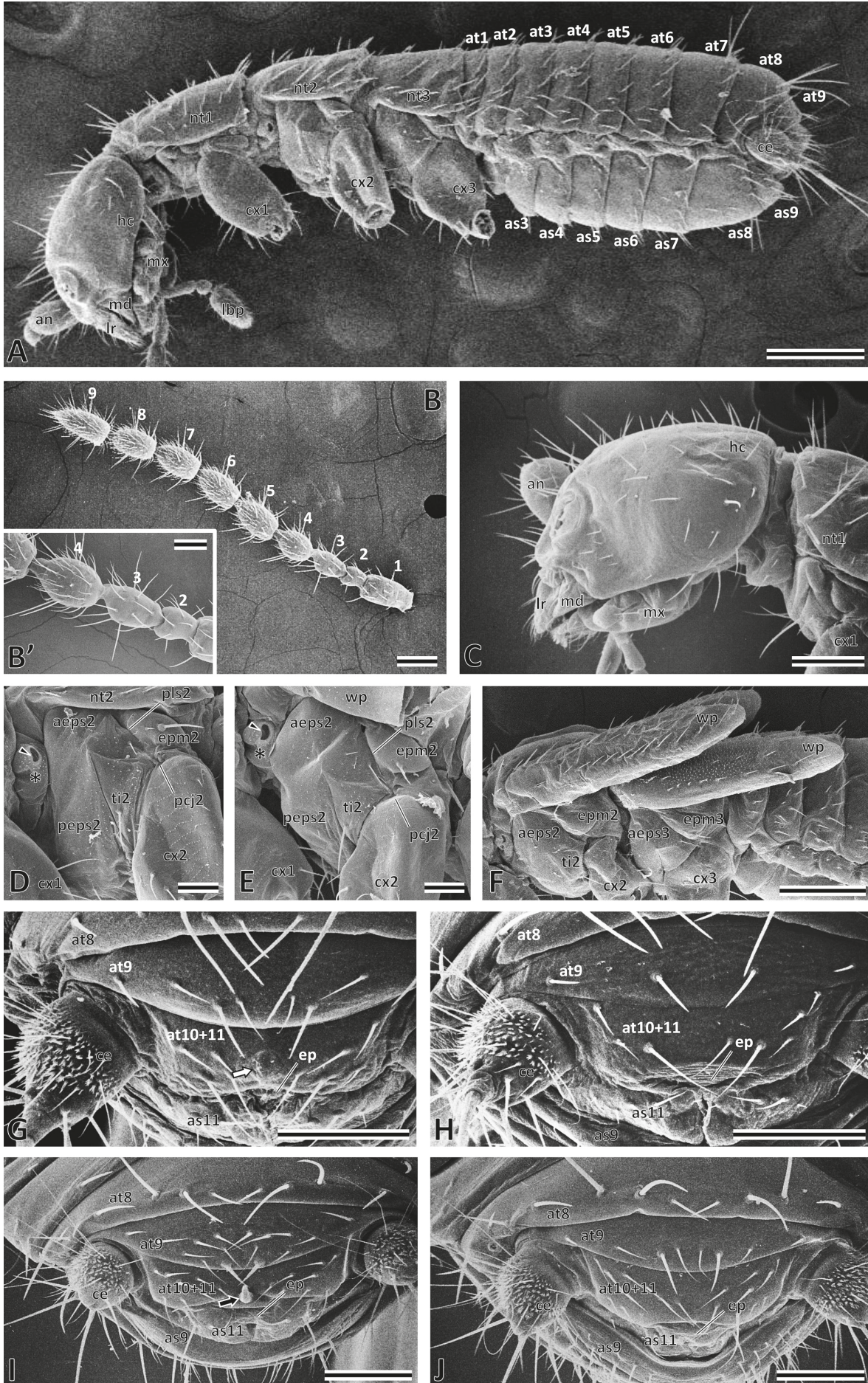
abdominal tergum IX, and tergum X+XI (see 3.3. Instar V) is represented by a pair of lateral triangular sclerites (hemitergites) and a small upcurved mating hook (Fig. 7I); meanwhile, in the female, only few pairs of setae are present on tergum IX, and tergum X+XI is uniformly sclerotized (Fig. 7J); sterna VIII and IX of females are broader and shorter than those of the males (cf. MASHIMO et al. 2013).

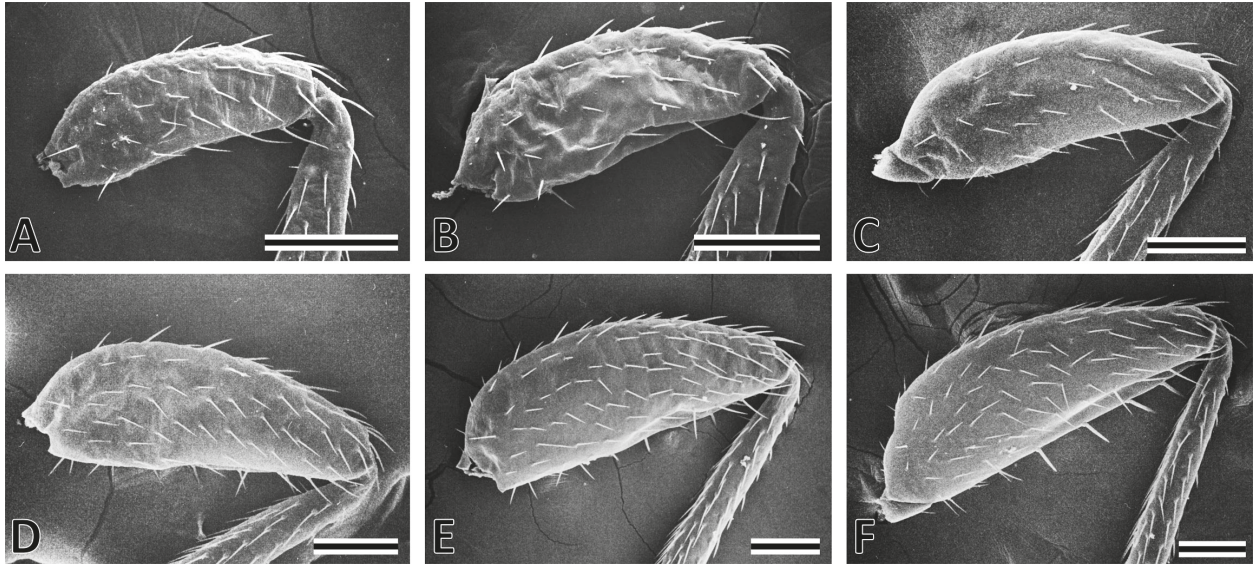
Sexual dimorphism does not appear until the final 5<sup>th</sup> instar. In the prospective male nymph shown in Fig. 7G, four pairs of setae are arranged on tergum IX, and a small posteromedian swelling is present on tergum X+XI (white arrow in Fig. 7G): the mating hook may be formed in the territory of XI, because it takes its position in the posteriormost region of tergum X+XI. In the prospective female 5<sup>th</sup> instar nymph, two pairs of setae are arranged on tergum IX, and no swelling is present on tergum X+XI (Fig. 7H). The posteromedian swelling on the tergum X+XI of the prospective male 5<sup>th</sup> instar likely corresponds with the mating hook (black arrow in Fig. 7I). As the swelling is only visible in SEM micrographs, the postabdominal chaetotaxy is the only available diagnostic feature for light microscopic sexing of nymphal stages of *Z. caudelli*.

#### 4.4. Antennal development

In polyneopteran groups, new flagellomeres are added by division of flagellomeres, and there are three kinds of source flagellomeres: (1) The basalmost flagellomere (meriston) always contributes. (2) The second flagellomere (derived from meriston in the preceding round of division) can also contribute (then called meristal annulus). In cases where (2) applies, (3) the third and further distal flagellomeres (derived from meriston in the preceding-but-one round of division, and from the meristal annulus in the preceding round) can additionally contribute. Division occurs only according to (1) in type 1, according to (1) and (2) in type 2, and according to (1), (2), and (3) in type 3 of flagellomere development, i.e. the types are distinguished by the longevity of the potential to divide in “daughter flagellomeres” of the meriston. A further difference lies in the number of flagellomeres formed per molt by the various sources (1)–(3).

In *Zorotypus caudelli*, the annular addition occurs only once when molting from the 3<sup>rd</sup> to 4<sup>th</sup> instar. The 1<sup>st</sup> flagellomere of the 3<sup>rd</sup> instar (meriston) becomes constricted in the middle and then divides into two during molting, increasing the number of antennomeres to nine. This mode of antennal development may be categorized into the first, simplest mode. This pattern is also known in Isoptera (FULLER 1920), Blattaria, Plecoptera (QADRI 1938) and Dermaptera (DAVIES 1966; SHIMIZU & MACHIDA 2011). However, Zoraptera are a special case with only one single antennomere added, which distinguishes it from other potentially related groups. In addition, as





**Fig. 8.** *Zorotypus caudelli* Karny, 1927: SEMs of left metafemora, anterior view. **A:** 1<sup>st</sup> instar. **B:** 2<sup>nd</sup> instar. **C:** 3<sup>rd</sup> instar. **D:** 4<sup>th</sup> instar. **E:** 5<sup>th</sup> instar. **F:** Adult. – Scale bars = 100  $\mu$ m.

HOCKMAN et al. (2009) discussed, the intraordinal variation of antennal development limits the phylogenetic value of this characteristic. Furthermore, the antennal development of some polyneopteran groups such as Embioptera and Phasmatodea has not been examined yet. Reconstructing the ancestral mode of antennal growth in Polyneoptera and Pterygota requires more data covering all major groups.

#### 4.5. Homology of thoracic sclerites

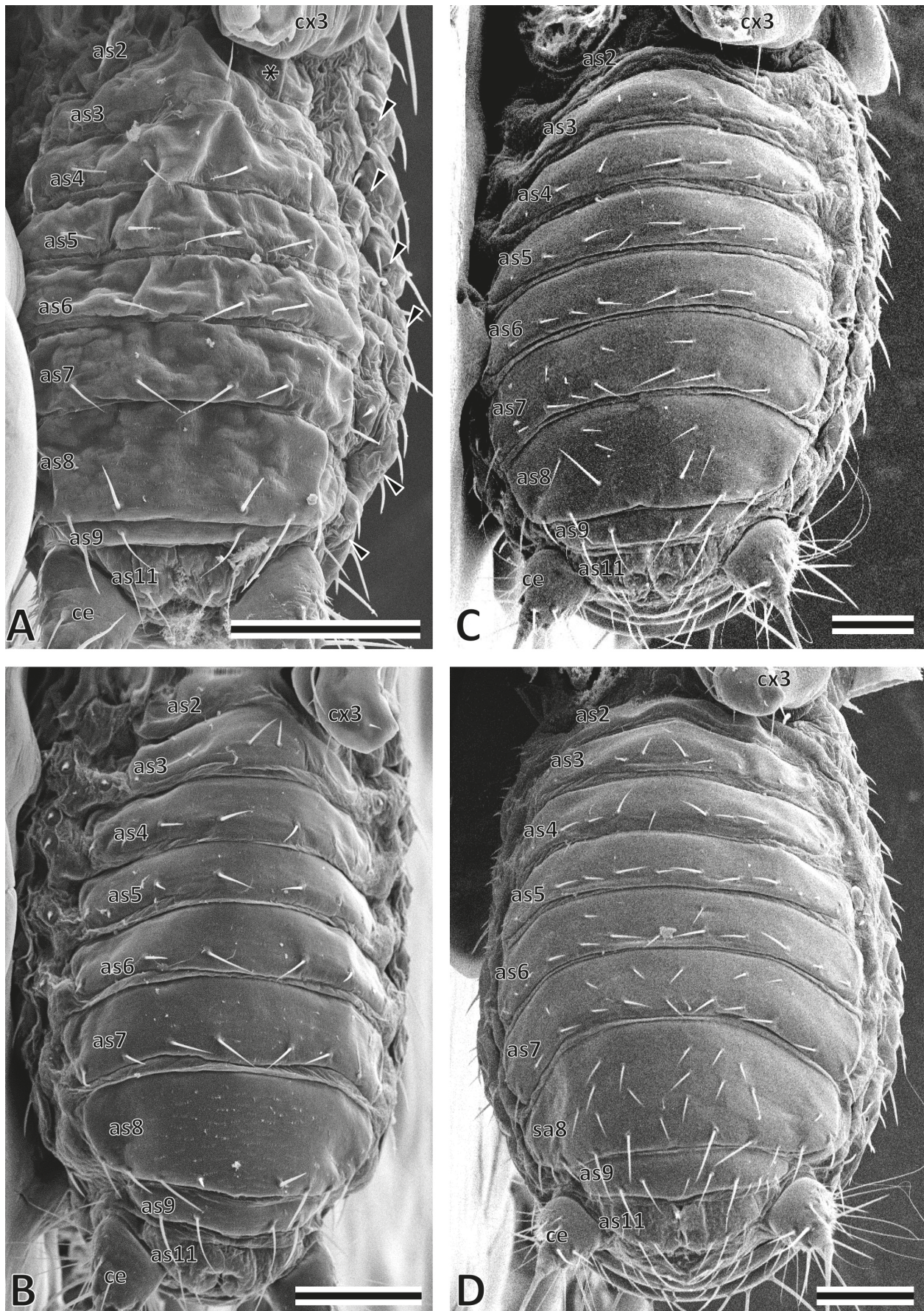
The thoracic exoskeleton of Zoraptera was investigated by CRAMPTON (1920, 1926), DELAMARE-DEBOUTTEVILLE (1947), RASNITSYN (1998) and FRIEDRICH & BEUTEL (2008). As in other pterygote insects, the zorapteran prothorax is uniform between winged and apterous forms, both regarding adults and nymphs. In contrast, the exoskeleton of the prothorax and pterothoracic segments differs considerably (CRAMPTON 1920, 1926; FRIEDRICH & BEUTEL 2008). The prothoracic pleural sclerites are simple and less differentiated, mainly due to lacking flight

function. The homology of zorapteran prothoracic pleural sclerites with those of other pterygote insects is problematic, as is the serial homology between prothoracic and pterothoracic sclerites (FRIEDRICH & BEUTEL 2008).

In previous studies, the posterior propleural sclerite has been interpreted as an element corresponding with the pterothoracic epimeron (CRAMPTON 1920, 1926; MATSUDA 1970; FRIEDRICH & BEUTEL 2008). Our results confirm this interpretation, based on the relative position of these structures to the pleural sutures (cf. Fig. 3C vs. D). FRIEDRICH & BEUTEL (2008) examined the skeleto-muscular systems of *Zorotypus hubbardi* and *Zorotypus weidneri* New, 1978 and designated several muscular features supporting this serial homology. DELAMARE-DEBOUTTEVILLE (1947) described a paracoxal suture in the prothoracic pleuron in *Zorotypus guineensis* Silvestri, 1913 (cf. MATSUDA 1970). Similarly to CRAMPTON (1920, 1926) and FRIEDRICH & BEUTEL (2008), we could not identify this structure.

The characterization of the anterior and intermediate propleural sclerites of Zoraptera remains controversial. The anterior sclerite has been variously termed, i.e., the lateropleurite (CRAMPTON 1920; DELAMARE-DEBOUTTEVILLE 1947), precoxale (CRAMPTON 1926), episternum/

← **Fig. 7.** *Zorotypus caudelli* Karny, 1927: SEMs of the 5<sup>th</sup> instar nymphs and adults. **A:** Body of apterous form of 5<sup>th</sup> instar, lateral view. **B,B':** Left antenna (B) and antennomeres 2–4 (B') of 5<sup>th</sup> instar. **C:** Head of 5<sup>th</sup> instar of winged form, lateral view. **D:** Mesothorax of 5<sup>th</sup> instar of apterous form, lateral view. **E:** Mesothorax of 5<sup>th</sup> instar of winged form, wing pad removed, lateral view. **F:** Meso- and metathorax of 5<sup>th</sup> instar of winged form, lateral view. **G–J:** Abdomen, caudal view. Prospective male (G) and female (H) 5<sup>th</sup> instar, male (I) and female (J) adults. White and black arrows indicate the posteromedian swelling and mating hook on abdominal terga X and XI, respectively. Arrowheads indicate spiracles. Asterisks show small sclerites anterior to the mesothoracic anepisternum. – Abbreviations: aeps2, 3 = meso- and metathoracic anepisterna; an = antenna; as3–9, 11 = abdominal sterna III–IX, XI; at1–11 = abdominal terga I–XI; ce = cercus; cx1–3 = pro-, meso- and metacoxa; ep = epiproct; epm2, 3 = meso- and metathoracic epimera; hc = head capsule; lbp = labial palp; lr = labrum; md = mandible; mx = maxilla; nt1–3 = pro-, meso- and metathoracic nota; pcj2 = mesothoracic pleuro-coxal joint; peps2 = mesothoracic preepisternum; pls2 = mesothoracic pleural suture; ti2 = mesotrochantin; wp = wing pad; 1–9 = antennomeres 1–9. – Scale bars = A, F: 200  $\mu$ m; B, C, G, H, I, J: 100  $\mu$ m; B', D, E: 50  $\mu$ m.



**Fig. 9.** *Zorotypus caudelli* Karny, 1927: SEMs of abdomen, ventral view. **A:** 2<sup>nd</sup> instar nymph. Black arrowheads show spiracles. Asterisk shows a part of the sclerotized region of the abdominal sternum II. **B:** 3<sup>rd</sup> instar nymph. **C:** 4<sup>th</sup> instar nymph. **D:** 5<sup>th</sup> instar nymph. – Abbreviations: as2–11=abdominal sterna II–XI; ce=cercus; cx3=metacoxa. – Scale bars=100  $\mu$ m.

anapleurum (the proximal part representing the preepisternum) (MATSUDA 1970), or preepisternum+anterior anepisternum (FRIEDRICH & BEUTEL 2008). The intermediate propleural sclerite was referred to as the episternum (CRAMPTON 1926; DELAMARE-DEBOUTTEVILLE 1947) or posterior anepisternum (FRIEDRICH & BEUTEL 2008). We found that the anterior and intermediate sclerites of *Zorotypus caudelli* stay connected in their dorsal regions throughout postembryonic development, whereas their ventral separation deepens gradually and becomes more distinct. CRAMPTON (1920, 1926) and FRIEDRICH & BEUTEL (2008) depicted the anterior and intermediate sclerites as separated, but the present study confirmed that they are dorsally connected, as suggested by DELAMARE-DEBOUTTEVILLE (1947). Based on their position in relation to the ventral notch and trochantin (see Fig. 2C), it is conceivable that they represent the preepisternum and anaepisternum, respectively, even though they are not clearly demarcated by a separating structure such as a suture or ridge.

In contrast to the prothorax, there seem to be few controversial issues concerning the meso- and metapleural sclerites. However, we found hitherto undescribed small sclerites with spiracles located anterior to the meso- and metathoracic anepisterna (cf. asterisks in Figs. 5C, 7D,E). UCHIFUNE & MACHIDA (2005) traced the formation of thoracic eusternal and pleural sclerites in the grylloblattid *Galloisiana yuasai* Asahina, 1959, and discussed the origins of thoracic sclerites. According to their interpretation that spiracles are associated with the preepisternum, we may have to re-characterize the meso- and metathoracic pleurites, especially focusing on the origin of the preepisternum.

#### 4.6. Concluding remarks

Most studies on the postembryonic development of insects focus on the morphology of specific structures such as antennae and genitalia (e.g., QADRI 1938, 1940; NAGASHIMA 1991; HOCKMAN et al. 2009). The available data set of nymphal characteristics is insufficient to clarify the placement of Zoraptera among the polyneopteran lineages. This may be partly due to the lack of postembryonic data for members of the polyneopteran orders. Extensive and detailed documentation of nymphal development and morphology covering the entire polyneopteran lineages is greatly desirable.

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