Embryonic Development of Zoraptera with Special Reference to External Morphology, and Its Phylogenetic Implications (Insecta)

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ABSTRACT The embryonic development of *Zorotypus caudelli* Karny (Zoraptera) is described with the main focus on its external features. A small heart-shaped embryo is formed on the dorsal side of the egg by the fusion of paired blastoderm regions with higher cellular density. The orientation of its anteroposterior axis is opposed to that of the egg. This unusual condition shows the potential autapomorphy of Zoraptera. The embryo extends along the egg surface and after reaching its full length, it migrates into the yolk. After developing there for a period of time, it reappears on the surface, accompanied by a reversion of its antero-posterior axis, finally taking its position on the ventral side of the egg. The definitive dorsal closure completes, and the prelarva hatches after perforating the chorion with very long egg tooth formed on the embryonic cuticle. Embryological data suggest the placement of Zoraptera among the "lower neopteran" or polyneopteran lineage: features supporting this are embryo formation by the fusion of paired regions with higher cellular density and blastokinesis accompanied by full elongation of the embryo on the egg surface. The extraordinarily long egg tooth has potential synapomorphy with Embioptera or Eukinolabia (= Embioptera + Phasmatodea). Together with the results from our previous studies on the egg structure, male reproductive system and spermatozoa, the close affinity of Zoraptera with Eukinolabia appears likely, that is, a clade Zoraptera + (Embioptera + Phasmatodea). J. Morphol. 275:295–312, 2014. © 2013 Wiley Periodicals, Inc.

KEY WORDS: Zorotypidae; Eukinolabia; Polyneoptera; comparative embryology; blastokinesis; egg tooth

INTRODUCTION

Zoraptera are small, inconspicuous insects (less than 4 mm) with cryptic habits. They live in subcortical spaces in decaying logs in tropical and subtropical zones. The order is one of the smallest in terms of species diversity and was understudied for a long time. So far, only 39 species have been described (Mashimo et al., 2013), all classified in the single genus *Zorotypus* Silvestri (Engel and Grimaldi, 2000). However, the true diversity of these cryptic insects is apparently insufficiently explored (Mashimo et al., 2013).

The systematic position of Zoraptera is one of the most controversial and persistent problems in higher level phylogeny of insects. More than 10 different phylogenetic hypotheses have been proposed since their discovery 100 years ago (Silvestri, 1913) and its placement in neopteran insects remains an open question (Engel and Grimaldi, 2002; Beutel and Weide, 2005; Yoshizawa, 2007, 2011; Ishiwata et al., 2011). The term "Zoraptera problem" was coined by Beutel and Weide (2005) to highlight this controversial phylogenetic status, analogous to the "Strepsiptera problem" earlier introduced by Kristensen (1991), but unlike Strepsiptera, which have recently been identified as a sister group of monophyletic Coleoptera (Niehuis et al., 2012), Zoraptera remain a systematic enigma, even in the "age of phylogenomics" (B. Misof, pers. comm.). Groups that have recently been proposed as sister group candidates are Paraneoptera, or Acercaria (to avoid confusion, in this study we use the term "Acercaria" instead of Paraneoptera, which often includes Zoraptera; Hennig, 1969; Kristensen, 1975; Beutel and Weide, 2005; Beutel and Gorb, 2006), Holometabola (Rasnitsyn,

1998), Eumetabola (Acercaria + Holometabola, Beutel and Gorb, 2001), Dermaptera (Carpenter and Wheeler, 1999; Jarvis et al., 2005; Terry and Whiting, 2005), Dictyoptera (Boudreaux, 1979; Wheeler et al., 2001; Yoshizawa and Johnson, 2005; Ishiwata et al., 2011; Wang et al., 2013), Embioptera (Minet and Bourgoin, 1986; Engel and Grimaldi, 2000, 2002; Grimaldi and Engel, 2005; Yoshizawa, 2007, 2011) and Eukinolabia (Dallai et al., 2011; Mashimo et al., 2011).

In the last decade, the investigation of Zoraptera has greatly accelerated, with different approaches and a focus on different character systems. The skeleto-muscular system of the head was studied by Beutel and Weide (2005), the thoracic skeleto-muscular system by Friedrich and Beutel (2008), wing venation by Kukalová-Peck and Peck (1993), wing base structures by Yoshizawa (2007, 2011), the postabdomen by Hünefeld (2007), the reproductive systems by Dallai et al. (2011, 2012a,b), the egg structure by Mashimo et al. (2011), fossil species by Engel and Grimaldi (2002), and mating by Dallai et al. (2013). Despite greatly increased knowledge about Zoraptera, the embryonic development remains completely unknown, an apparently serious gap in the growing body of evidence and a major impediment to attempts to place the group phylogenetically. Consequently, we addressed the embryology of Zoraptera, with detailed documentation of the egg structure of *Zorotypus caudelli* Karny (Zorotypidae) as the first step of our project (Mashimo et al., 2011). In this study, the embryonic development of *Z. caudelli* is described, focusing on externally recognizable features. Organogenesis will be treated in a follow-up study, aiming at complete documentation of the ontogenesis of this enigmatic group of insects.

MATERIALS AND METHODS

Zorotypus caudelli adults and nymphs were collected from under the bark of decaying logs in Ul Gombak (Selangor, Peninsular Malaysia). They were kept in plastic cases (15 × 8 × 3 cm^3) with a layer of moist soil at room temperature (ca. 22–24°C), and fed with dry yeast, powdered dried *Bombyx* pupae (commercially sold fishing bait) and live springtails (*Folsomia* sp.). Collected eggs were transferred to other plastic cases containing wet tissue paper and incubated at 25°C for rearing.

Eggs were cleaned with a soft brush in commercial bleach (Seven premium kitchen bleach) for 30 sec, and rinsed in distilled water. The eggs were soaked in Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde 0.1 mol l^{-1} HCl-sodium cacodylate buffer solution, pH 7.2 [SCB]) for 1 min, punctured with a fine needle and fixed for 1 h. After making a small opening in the chorion with sharpened forceps, the eggs were further fixed with the same mixture at 4°C for 24 h and then stored in SCB at 4°C.

For detailed observations of external features of embryos, they were dissected out of living eggs with fine forceps and a razor blade in Ephrussi-Beadle's solution (0.75% NaCl, 0.035% KCl, 0.021% CaCl$_2$) containing detergent (0.1% Triton X-100), rinsed in new solution, and then fixed with Karnovsky's fixative for 12 h. Fixed embryos were also stored in SCB at 4°C.

Fixed eggs and embryos were stained with DAPI solution (4', 6-diamidino-2-phenylindole dihydrochloride, diluted about 10 μg/ l^{-1} with SCB) for several days and 20–30 min, respectively. Specimens stained with DAPI were observed with a fluorescence stereomicroscope (MZ FL III, Leica, Heerbrugg, Switzerland) under UV light excitation at 360 nm. Some fixed embryos, stained with 1% Delafield's hematoxylin, were observed with a biological microscope (Optiphot-2, Nikon, Tokyo, Japan) equipped with a long working distance objective (ELWD 20X, Nikon, Tokyo, Japan). Drawings were made using a camera lucida.

Fixed specimens were dehydrated in a graded ethanol series and embedded in methacrylate resin (Technovit 7100, Külzer, Wehrheim, Germany), as described by Machida et al. (1994a,b). Semithin sectioning was performed at a thickness of 2 μm using a semithin microtome (H-1500, Bio-Rad, Hercules, California) equipped with a tungsten carbide knife (Superhard Knife, Meiwafosis, Tokyo, Japan). Sections were stained with 0.5% Delafield's hematoxylin for 12 h, 0.5% eosin gelblich or eosin bläulich for 1 h, and 0.5% fast green FCF 80% ethanol solution for 1 min.

For scanning electron microscopy (SEM), some embryos fixed with Karnovsky's fixative were postfixed with 1% OsO$_4$ for 1 h. Fixed embryos were dehydrated in a graded ethanol series, dried with a critical point dryer (Samdri-PVT-3D, tousimis, Rockville, Maryland), coated with gold, and then observed with a SEM (SM-300, TOPCON, Tokyo, Japan) at 15 kV. The embryonic cuticle secreted over the entire surface of the embryo is often swollen at later developmental stages and separated from the embryo or wrinkled. In coated specimens this impedes accurate observation of the surface of the embryo in the usual high-vacuum SEM mode (Machida, 2000b). Consequently, some embryos were observed without coating using a low-vacuum SEM (SM-300 Wet-4, TOPCON, Tokyo, Japan) at 13 Pa at 15–30 kV.

RESULTS

The egg period of *Zorotypus caudelli* was about 40 days under incubation at 28°C. Based on the changes in external embryonic features, this period was divided into 12 stages, expressed as a percentage of total developmental time (DT), with 0% at oviposition and 100% at hatching (Bentley et al., 1979; Table 1).

Orientation of egg

The eggs of *Zorotypus caudelli* are elliptic, with a length of about 0.6-mm long and a diameter of about 0.3 mm. The surface shows a honeycomb pattern (Mashimo et al., 2011). Newly laid eggs are pale but assume yellowish coloration in a few days (Fig. 1A,B). The eggs are deposited on substrates such as bark or in galleries formed in rotting wood. Mashimo et al. (2011) previously designated the side of the egg facing the substrate as ventral and the opposite side bearing a pair of micropyles as dorsal. In the revised designation of axes (Fig. 1A), we followed the conventional concept (see e.g., Wheeler, 1893), which is based on the position of the embryo just before hatching. Consequently, the side of the egg facing the substrate is considered dorsal, the side with the micropyles as ventral, the slightly narrowed end

TABLE 1. Major developmental events in each stage
in Zorotypus caudelli Karny

Stage 12-15% DT	Formation of embryo
Stage 15-20% DT	Differentiation of protocephalon and protocorm, formation of amnioserosal fold (anatrepsis)
Stage 20-22% DT	Commencement of segmentation, differentiation of cephalic and thoracic segments
Stage 22-25% DT	Formation of appendages in the differentiated segments, formation of stomodaeum, commencement of caudal flexure
Stage 25-28% DT	Differentiation of clypeolabrum, segmentation of abdomen
Stage 28-30% DT	Elongation and articulation of cephalic and thoracic appendages, immersion of embryo into the yolk
Stage 30-40% DT	Completion of segmentation, formation of abdominal appendages and proctodaeum
Stage 40-50% DT	Differentiation of clypeus and labrum, formations of spiracles and sternal apophyses.
Stage 50-60% DT	Development of egg tooth, completion of appendicular articulation
Stage 60-65% DT	Regression of amnioserosal fold (katatrepsis), progressive provisional dorsal closure
Stage 65-80% DT	Formation of head capsule, secretion of embryonic cuticle
Stage 80-100% DT	Completion of definitive dorsal closure, acquisition of definitive form by embryo, secretion of larval cuticle

as anterior, and the slightly broadened end as posterior (Figs. 1A,B, 2L, 3L).

Stage 12–15% developmental time

Paired lateral regions with higher cellular density form on the dorsal side of the blastoderm close to the equator, only slightly posterior to the middle region (Fig. 1D). These areas migrate medially and fuse into a small heart-shaped embryo at the equator of the dorsal side of the egg (Figs. 3A, 4A). The anterior end of the embryo faces toward the posterior pole of the egg: the anteroposterior axes of the embryo and egg are reversed (Figs. 2A, 3A). Secondary yolk cells are observed to be segregated from the serosa (Fig. 1C).

Stage 15–20% developmental time

The embryo extends along the dorsal surface of the egg, and the anterior protocephalon and posterior protocorm differentiate (Figs. 2B, 3B, 4B). The amnion starts to emerge from the embryonic margin. It forms the amnioserosal fold (Fig. 4B,C), which extends over the ventral surface of the embryo. The margins fuse with each other above the central area of the protocephalon, thus completing anatrepsis. Further elongation of the embryo follows (Fig. 4D).
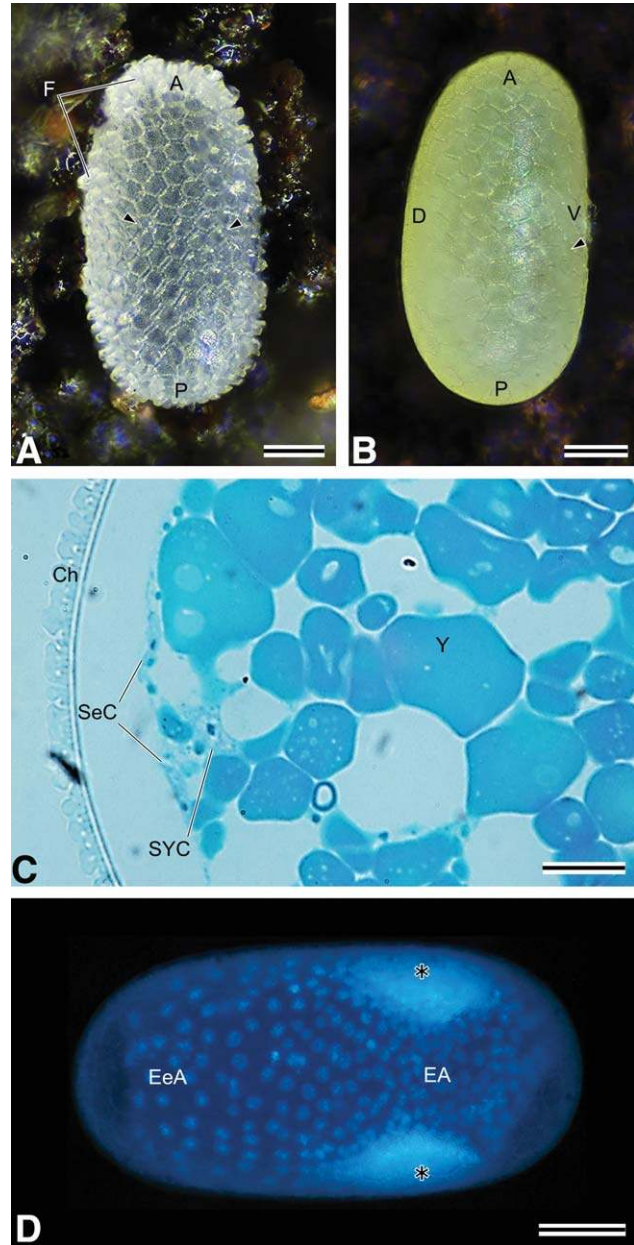
Fig. 1. Eggs of *Zorotypus caudelli*. **A**: A newly laid egg, ventral view. **B**: An egg after cleaning in bleach, lateral view. Extrinsic material such as the fringe was dissolved and removed. **C**: Cross section of an egg at 12–15% DT. A secondary yolk cell is observed to be just segregated. **D**: An egg at 12% DT, dorsal view, anterior to the left, fluorescence microscopy. Asterisks show paired regions with higher cellular density. Scale bars in A,B,D = 100 μm; in C = 20 μm. A, anterior pole; Ch, chorion; D, dorsal side; EA, embryonic area; EeA, extraembryonic area; F, fringe; P, posterior pole; SeC, serosal cell; SYC, secondary yolk cell; V, ventral side; Y, yolk. Arrowheads show micropyles.

Stage 20–22% developmental time

The protocephalon is enlarged laterally and a distinct head lobe differentiates. Segmentation starts almost simultaneously from the antennal segment to the prospective metathorax (Figs. 3C,
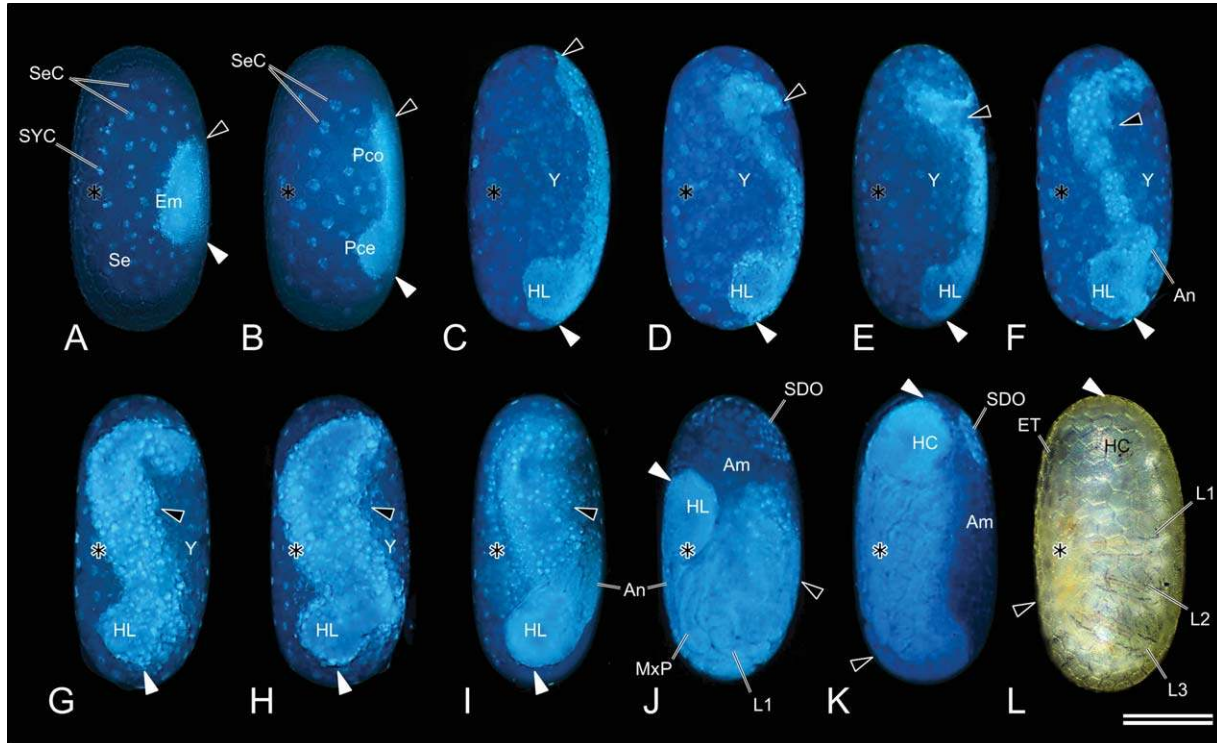
Fig. 2. Embryonic development of *Zorotypus caudelli*, lateral view of eggs, anterior to the top, ventral to the left, fluorescence microscopy other than L. **A**: 12–15% DT. The serosal cells and secondary yolk cells which were segregated from the formers are clearly distinguished in size of nuclei: the nuclei of the secondary yolk cells are more compact than those of serosal cells. **B**: 15–20% DT. **C**: 20–22% DT. **D**: 22–25% DT. **E**: 25–28% DT. **F**: 28–30% DT. **G**: 30–40% DT. **H**: 40–50% DT. **I**: 50–60% DT. **J**: 60–65% DT. **K**: 65–80% DT. **L**: 80–100% DT. Scale bar = 200 μm. Am, amnion; An, antenna; ET, egg tooth; HC, head capsule; HL, head lobe; L1-3, pro-, meso- and metathoracic legs; MxP, maxillary palp; Pce, protocephalon; Pco, protocorm; SDO, secondary dorsal organ; Se, serosa; SeC, serosal cell; SYC, secondary yolk cell; Y, yolk. White and black arrowheads show cephalic and caudal ends of the embryo, respectively. Asterisks show the position of micropyles.

4E). The neural groove appears along the median line (Fig. 4E).

Stage 22–25% developmental time

In the antennal, mandibular, maxillary, labial, and thoracic segments, the appendages differentiate as lateral swellings (Figs. 3D, 4F). The prospective mandibles are considerably smaller than the other appendages (Figs. 3D, 4F). No appendicular structure develops in the intercalary segment throughout embryonic development. Segmentation proceeds to the first abdominal segment. The neural groove becomes distinct. At its anterior end, the stomodaeum differentiates as a shallow pit (Fig. 4F). The caudal end of the embryo elongates and starts to bend ventrally (Figs. 2D, 4G). The serosal cuticle starts to be secreted beneath the chorion (cf. Fig. 8A).

Stage 25–28% developmental time

The prospective antennae, maxillae, labium, and legs elongate, whereas the mandibles remain short (Figs. 3E, 4H). The anlage of the clypeolabrum develops as a median swelling anterior to the sto-

modaeum (Figs. 3E, 4H). Segmentation proceeds posteriorly, reaching abdominal segment III (Fig. 4I), and appendages develop in the newly differentiated segments. Caudal flexure is increased (Figs. 2E, 4I).

Stage 28–30% developmental time

The embryo, which has been greatly elongated on the egg surface during the preceding stages, is immersed in parallel with the egg surface into the central yolk mass (Figs. 2F, 3F). The clypeolabrum develops above the stomodaeum (Figs. 2F, 4J). The length of the antennae increases. They turn toward the median line and divide into the scapus, pedicellus, and flagellum (Fig. 4J). The mandibles remain smaller than the other appendages (Fig. 4J). The appendages of the maxillary, labial, and thoracic segments elongate and divide into two subcomponents, the proximal coxopodite and the distal telopodite (Fig. 4J,K). In the mesal regions of the maxillary and labial coxopodites, prospective endite lobes appear as swellings (Fig. 4J). Segmentation proceeds posteriorly and reaches abdominal segment VI. Appendages develop on each
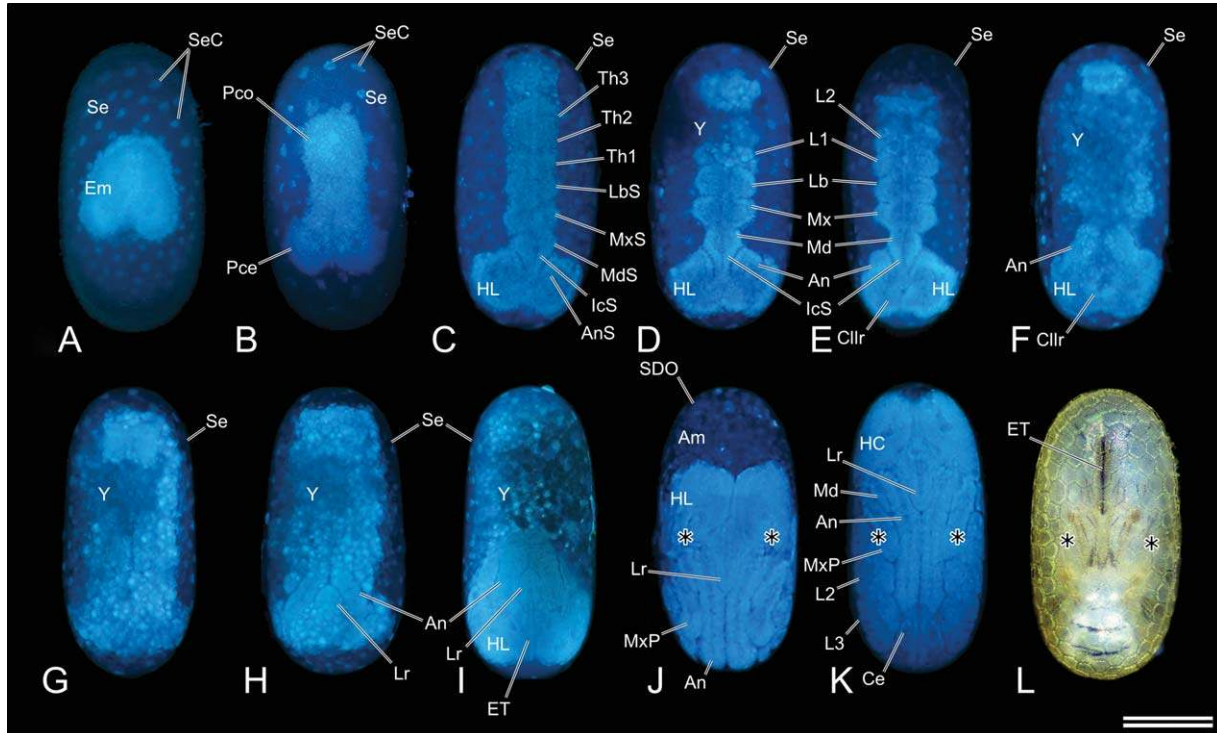
Fig. 3. Embryonic development of *Zorotypus caudelli*, ventral view to the embryo: A–I, dorsal view to the egg; J–L, ventral view to the egg, anterior to the top, fluorescence microscopy other than L. **A**: 12–15% DT. **B**: 15–20% DT. **C**: 20–22% DT. **D**: 22–25% DT. **E**: 25–28% DT. **F**: 28–30% DT. **G**: 30–40% DT. **H**: 40–50% DT. **I**: 50–60% DT. **J**: 60–65% DT. **K**: 65–80% DT. **L**: 80–100% DT. Scale bar = 200 μm. Am, amnion; An, antenna; AnS, antennal segment; Ce, cercus; Cllr, clypeolabrum; Em, embryo; ET, egg tooth; HC, head capsule; HL, head lobe; IcS, intercalary segment; Lb, labium; LbS, labial segment; Lr, labrum; L1-3, pro-, meso- and metathoracic legs; Md, mandible; MdS, mandibular segment; Mx, maxilla; MxP, maxillary palp; MxS, maxillary segment; Pce, protocephalon; Pco, protocorm; SDO, secondary dorsal organ; Se, serosa; SeC, serosal cell; Th1-3, pro-, meso- and metathoracic segments; Y, yolk. Asterisks show the position of micropyles.

abdominal segment (Fig. 4K). The abdomen begins to curve to the ventral side (Figs. 2F, 4K).

Stage 30–40% developmental time

The embryo is immersed more deeply into the central yolk mass and as a consequence the visibility of its details decreases (Figs. 2G, 3G). The antennal flagellum subdivides into four segments (Fig. 5A). The differentiation of the maxillae and labium continues: their endites enlarge and elongation of the telopodites, that is, the palps, continues. The maxillary palp divides into five segments and the labial palp into three (Fig. 5A). Elongation of the thoracic telopodites also continues and they divide into the trochanter, femur, tibia, tarsus, and pretarsus (Fig. 5B). The abdominal segments VII–XI differentiate and appendages develop on each as slight swellings (Fig. 5C,D). The segmental appendages of abdominal segment I or the pleuropodia differentiate into coxopodites and telopodites (Fig. 5C), whereas those of abdominal segments II–X remain undivided. Cerci differentiate as distinct paired appendages of abdominal segment XI (Fig. 5C). The proctodaeum with its Y-shaped opening invaginates between the cerci

(Fig. 5E). The ventral curvature of the abdomen becomes more distinct and the embryo assumes an S-shaped body form (Figs. 2G, 5B). The thickness of abdominal segments VII–X increases compared to the anterior abdominal segments (Fig. 5D).

Stage 40–50% developmental time

The embryo migrates still deeper into the yolk mass (Figs. 2H, 3H). The formation of the clypeolabrum continues. It divides into the clypeus and labrum, and a lateral external rim divides the former into the anteclypeus and postclypeus (Fig. 5F). The coxopodites of the gnathal and thoracic appendages divide into two parts, the proximal subcoxae and the distal coxae (Fig. 5G). The endites of maxilla and labium differentiate into two parts, the mesal lacinia and lateral galea, and the mesal glossa and lateral paraglossa, respectively (Fig. 5F). The labial appendages of both sides begin to migrate toward the median line (Fig. 5F) and are hardly visible from the lateral view, as shown in Figure 5G. The thoracic appendages assume a mesal orientation (Fig. 5F, cf. Fig. 5A). The cerci subdivide into two segments, a proximal coxopodite and a distal telopodite (Fig.
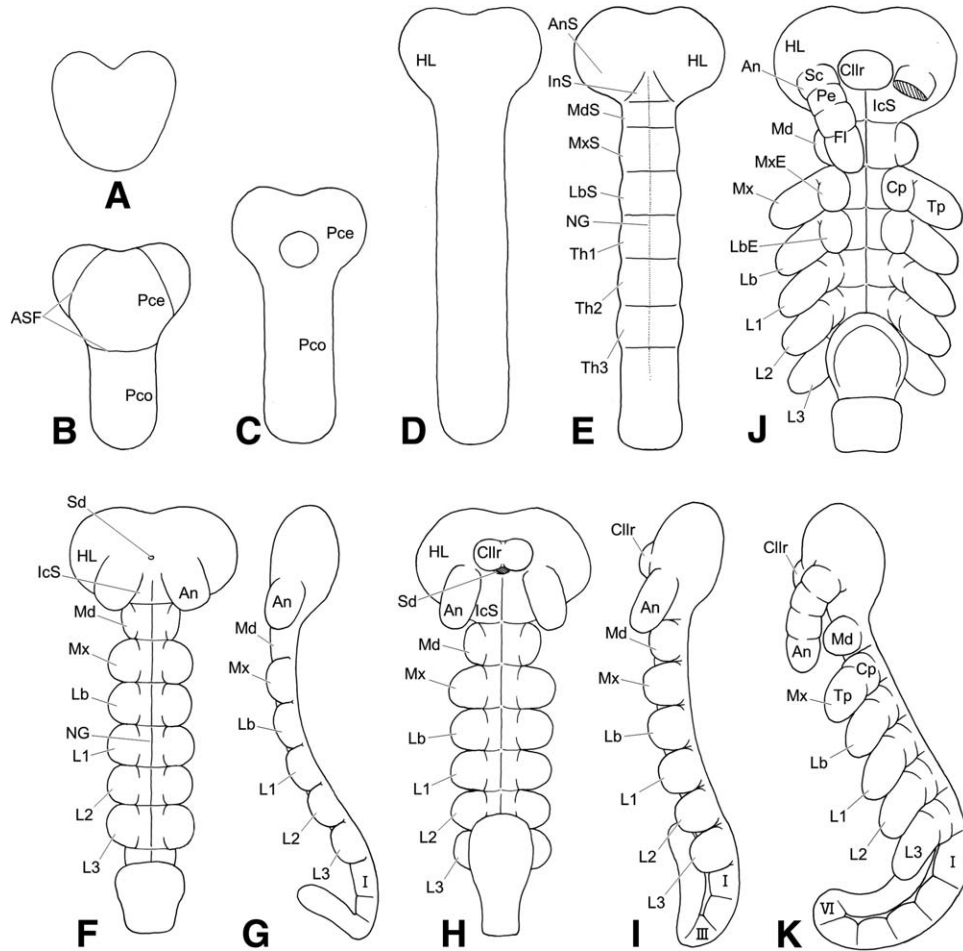
Fig. 4. External features of embryos of *Zorotypus caudelli*, I. **A**: 12–15% DT, ventral view. **B,C,D**: Early (B), middle (C), and late (D) Stage 15–20% DT, ventral view. **E**: 20–22% DT. **F,G**: 22–25% DT, ventral (F) and lateral (G) views. **H,I**: 25–28% DT, ventral (H) and lateral (I) views. **J,K**: 28–30% DT, ventral (J) and lateral (K) views. An, antenna; AnS, antennal segment; ASF, amnioserosal fold; Cllr, clypeolabrum; Cp, coxopodite; Fl, flagellum; HL, head lobe; IcS, intercalary segment; Lb, labium; LbE, labial endite; LbP, labial palp; LbS, labial segment; L1-3, pro-, meso- and metathoracic legs; Md, mandible; MdS, mandibular segment; Mx, maxilla; MxE, maxillary endite; MxP, maxillary palp; MxS; maxillary segment; NG, neural groove; Pce, protocephalon; Pco, protocorm; Pe, pedicellus; Sc, scapus; Sd, stomodaeum; Th1-3, pro-, meso- and metathoracic segments; Tp, telopodite; I, III, VI, first, third and sixth abdominal segments.

5H). Paired tracheal pits or spiracles invaginate in the meso- and metathoracic regions and also in abdominal segments I–VIII (Fig. 5G,H,I). A pair of ectodermal invaginations forms at the mesal bases of the thoracic appendages, developing into sternal apophyses, that is, furcae (Fig. 8A). Abdominal equivalents of these apophyses could not be observed throughout embryonic development. The size of the stomodaeum and proctodaeum increases distinctly during this stage (Fig. 5G,I)

Stage 50–60% developmental time

During this stage, the embryo develops within the yolk mass (Figs. 2I, 3I). In the anterior head region, the precursor of the egg tooth appears as a very long median longitudinal ridge (Fig. 5J). A pair of shallow longitudinal depressions appears anterior to the antennal bases (arrows in Fig.

5J,K). Microtome sections reveal that the formation of these concavities is related to strong inflation of the adjacent protocerebral lobes, probably between lobes 1 and 2. The mandibles become flattened anteroposteriorly, and their teeth differentiate on the distal side (Fig. 5J). The hypopharynx appears as a single swelling between the mandibles (Fig. 5J). The thoracic appendages fold, with each femur overlapping the coxa and trochanter, and the tarsi subdivide into two segments (Fig. 5K). In the pleuropodia, the telopodite region collapses into the coxopodite (Fig. 5L). In the posterior abdomen the definitive dorsal closure proceeds from the posterior (Fig. 5M).

Stage 60–65% developmental time

The amnioserosal fold ruptures near the gnathal region, and katatrepsis occurs, involving marked
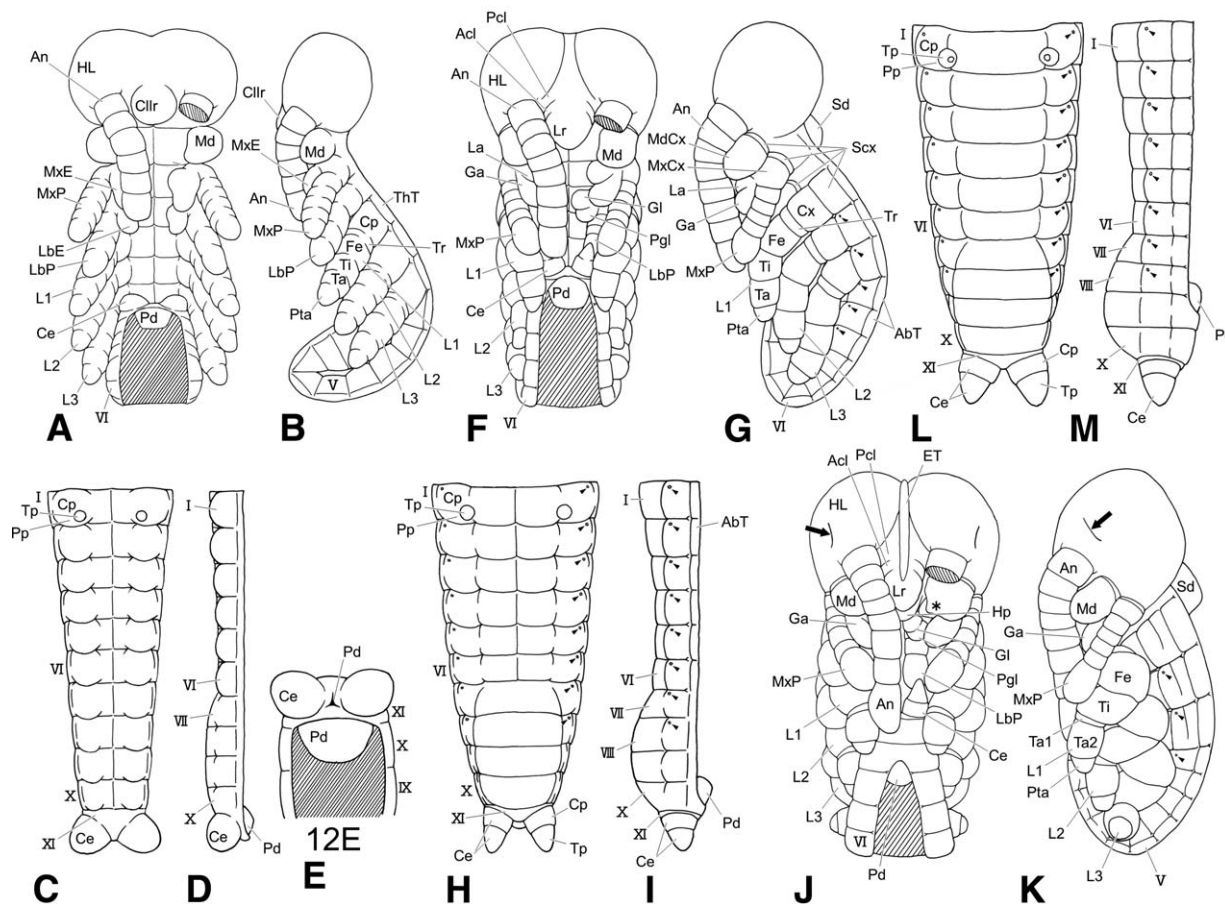
Fig. 5. External features of embryos of *Zorotypus caudelli*, II. **A,B**: Embryo at 30–40% DT, ventral (A) and lateral (B) views. **C,D,E**: Abdomen at 30–40% DT, ventral (C), lateral (D) and caudal (E) views. **F,G**: Embryo at 40–50% DT, ventral (F) and lateral (G) views. **H,I**: Abdomen at 40–50% DT, ventral (H) and lateral (I) views. **J,K**: Embryo at 50–60% DT, ventral (J) and lateral (K) views. **L,M**: Abdomen at 50–60% DT, ventral (L) and lateral (M) views. For arrows, see the text. AbT, abdominal tergum; Acl, anteclypeus; An, antenna; Ce, cercus; Cllr, clypeolabrum; Cp, coxopodite; Cx, coxa; ET, egg tooth; Fe, femur; Ga, galea; Gl, glossa; HL, head lobe; Hp, hypopharynx; La, lacinia; LbE, labial endite; LbP, labial palp; Lr, labrum; L1-3, pro-, meso- and metathoracic legs; Md, mandible; MdCx, mandibular coxa; MxCx, maxillary coxa; MxE, maxillary endite; MxP, maxillary palp; Pcl, postclypeus; Pd, proctodaeum; Pgl, paraglossa; Pp, pleuropodium; Pta, pretarsus; Scx, subcoxa; Sd, stomodaeum; Ta, tarsus; Ta1, 2, first and second tarsomere; ThT, thoracic tergum; Ti, tibia; Tp, telopodite; Tr, trochanter; I, V–XI, first and fifth to 11th abdominal segments. Arrowheads and asterisk show the spiracles and mandibular teeth, respectively.

movement of the embryo. After being deeply immersed within the yolk mass in the previous stages, the embryo reappears on the egg surface. The head follows the movement of the amnion around the posterior pole, then along the ventral surface of the egg toward the anterior pole (Figs. 2J, 3J, 6A,B). Accordingly, the anteroposterior axis of the embryo reverses to correspond with that of the egg. Serosal cells move toward the anterodoral region of the egg and form the secondary dorsal organ there (Figs. 2J, 6B). With the progressive condensation and withdrawal of serosal cells, the amnion replaces the serosa and spreads over the dorsal yolk as the provisional dorsal closure (Figs. 2J, 6B).

Stage 65–80% developmental time

The embryo, which has undergone katatrepsis, takes its position on the ventral side of the egg with its abdomen flexed (Figs. 2K, 3K). The head lobes extend dorsally and fuse to form the head capsule. The cerci develop as conical structures (Fig. 6E,F). The definitive dorsal closure proceeds toward the posterior thoracic region and anterior abdomen, replacing the provisional dorsal closure or the amnion (Figs. 2K, 6D). The secondary dorsal organ starts degenerating and sinks into the developing midgut. The embryonic cuticle is secreted, and the long blade-like egg tooth forms along the median line of the anterior head capsule (Fig. 6C,D).

Stage 85–100% developmental time

Definitive dorsal closure is completed (Figs. 2L, 6H). The larval cuticle is secreted beneath the embryonic cuticle, with the setae inserted into its surface (Figs. 2L, 3L). The egg tooth is sclerotized
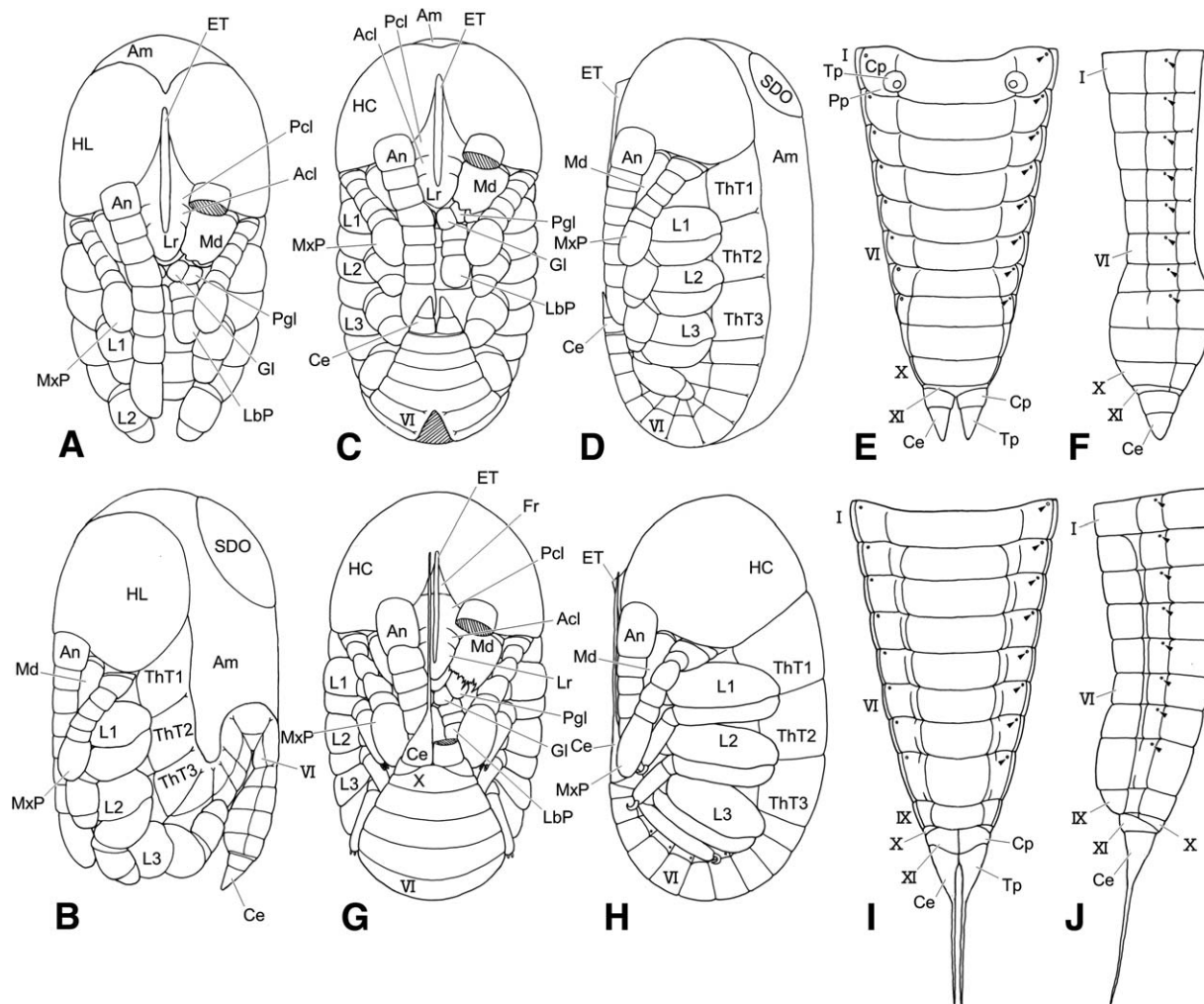
Fig. 6. External features of embryos of *Zorotypus caudelli*, III. **A,B**: Embryo at 60–65% DT, ventral (A) and lateral (B) views. **C,D**: Embryo at 65–80% DT, ventral (C) and lateral (D) views. **E,F**: Abdomen, ventral (E) and lateral (F) views. **G,H**: Embryo at 80–100% DT, ventral (G) and lateral (H) views. **I,J**: Abdomen at 80–100% DT, ventral (I) and lateral (J) views. Acl, anteclypeus; Am, amnion; An, antenna; Ce, cercus; Cp, coxopodite; ET, egg tooth; Fr, frons; Gl, glossa; HC, head capsule; HL, head lobe; LbP, labial palp; Lr, labrum; L1-3, pro-, meso- and metathoracic legs; Md, mandible; MxP, maxillary palp; Pcl, postclypeus; Pgl, paraglossa; Pp, pleuropodium; Pta, pretarsus; SDO, secondary dorsal organ; ThT1-3, pro-, meso- and metathoracic terga; Tp, telopodite; I, VI, IX–XI, first, sixth and ninth to 11th abdominal segments. Arrowheads show the spiracles.

and strongly pigmented (Fig. 3L). The egg tooth appears to attain the labral territory (Fig. 7A), but a sagittal section reveals that it only protrudes above the proximal part of the labrum (Fig. 7C). SEM observations of embryos with the embryonic cuticle removed clarify the boundaries between the frons and postclypeus (epistomal suture) and between the ante- and postclypeus (Fig. 7B): the origin of the egg tooth lies in the territory from the frons to the anteclypeus (Fig. 6G). Compound eyes, which develop only in winged forms, are formed in the postembryonic stage. The tips of mandibular teeth become sclerotized and pigmented. The differentiation of thoracic appendages is completed and they acquire their definitive form (Fig. 6H), including the pair of pretarsal claws (Fig. 6H). In each thoracic segment, the sternal

apophyses on both sides are shifted toward the median line and fuse to form the furcae (Fig. 8B). Friedrich and Beutel (2008) reported thoracic spinae. However, they are poorly developed in adults of *Zorotypus hubbardi* and *Zorotypus weidneri*. Throughout the embryonic development of *Zorotypus caudelli* mesal ectodermal invaginations representing prospective spinae do not develop. It is conceivable that they emerge during postembryonic development. The strongly retracted abdominal sternum X is hardly visible externally (Figs. 6I,J, 7D). A sagittal section shows that it is in fact invaginated between sterna IX and XI and concealed beneath the former (Fig. 7E). The coxopodites of cerci extend and almost completely occupy the ventral side of abdominal segment IX (Fig. 6I). Later in this stage, a long and strong
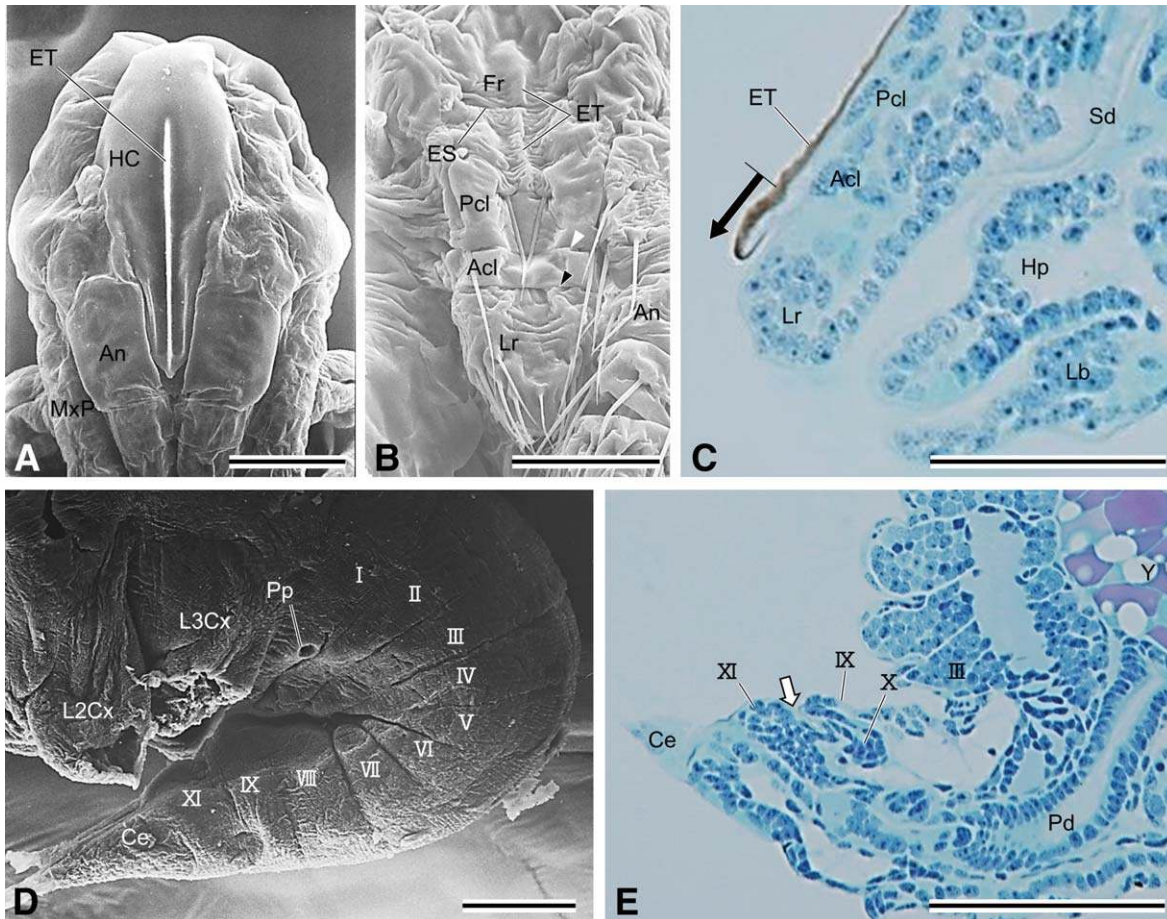
Fig. 7. Embryos of *Zorotypus caudelli*. **A**: Head at 80–100% DT, anterior view, SEM. **B**: An enlargement of head with embryonic cuticle removed at 80–100% DT, anterior view, SEM. White and black arrowheads show boundary between ante- and postclypeus and that between anteclypeus and labrum (epistomal suture), respectively. **C**: Sagittal section of head at 80–100% DT. Black arrow shows protrusion of the egg tooth over the proximal part of labrum. **D**: Abdomen at 80–100% DT, lateral view, SEM. **E**: Sagittal section of abdomen at 80–100% DT. White arrow shows the invagination of 10th abdominal sternum between ninth and 11th sterna. Scale bars in A,D,E = 100 μm; in B,C = 50 μm. Acl, anteclypeus; An, antenna; Ce, cercus; ES, epistomal suture; ET, egg tooth; Fr, frons; HC, head capsule; Hp, hypopharynx; Lb, labium; Lr, labrum; L2, 3Cx, meso- and metacoxa; MxP, maxillary palp; Pcl, postclypeus; Pd, proctodaeum; Pp, pleuropodium; Sd, stomodaeum; Y, yolk; I–XI, first to 11th abdominal segments.

seta forms at the tip of the cercus (Fig. 6I,J): from externally, the cerci appear elongated.

Hatching: 100% developmental time

The embryo has acquired its definitive shape when the prelarva (= prolarva) medially severs the chorion with the egg tooth and hatches. The head emerges first, followed by the thorax and abdomen. Peristaltic movements are involved in the process. The distal parts of the thoracic legs remain within the egg, whereas the proximal region is exposed. The former function as anchors for the prelarva to enable it to shed the embryonic cuticle (Fig. 8C,D). Emerging from the egg and the embryonic cuticle, the prelarva becomes the first instar larva. Shortly after hatching it starts to move actively. The embryonic cuticle with the egg tooth visible as a dark structure is left on the egg surface (Fig. 8E).

DISCUSSION
Appendage Formation

Our observations confirm that, as in other insects, the development of the maxillae and labium differs distinctly from that of the mandibles, even though these appendages are apparently serial homologues belonging to the fourth, fifth, and sixth head segments, respectively (Machida, 2000a; Uchifune and Machida, 2005). The former divide into two major subelements, whereas the anlage of the mandible neither shows distinct elongation nor division throughout embryonic development. Serial homology suggests that the proximal and distal parts of the developing maxillae and labium are equivalent to the coxopodite and telopodite of the thoracic appendages, whereas the mandible is only represented by the coxopodite, as suggested in other hexapods (cf. Machida, 2000a; Uchifune and Machida, 2005).
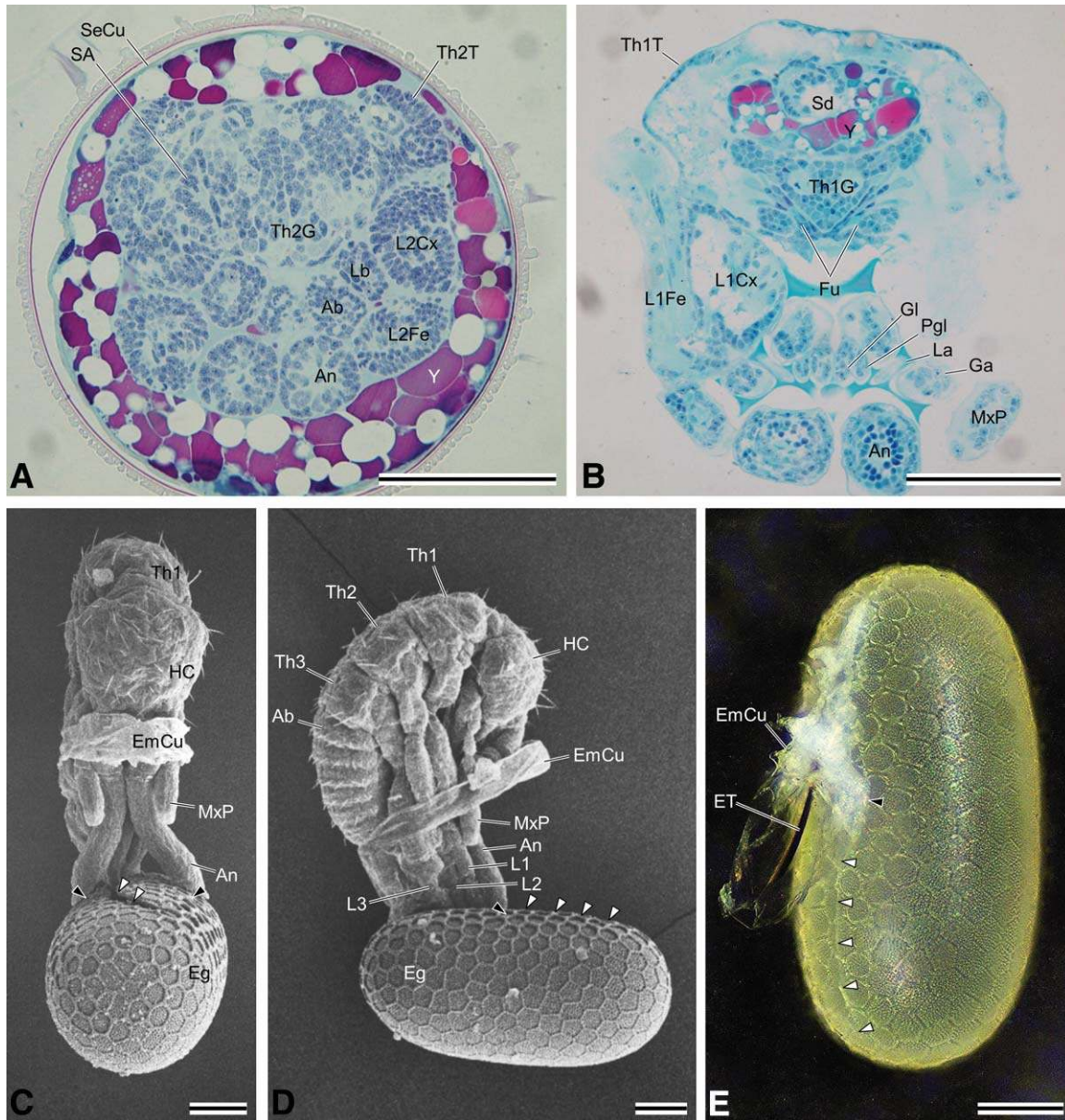
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Fig. 8. Eggs and larva of *Zorotypus caudelli*. **A**: Cross section of an egg at 40–50% DT. **B**: Cross section of an egg with chorion and serosal cuticle removed at 80–100% DT. **C,D**: Hatching, posterior (C) and lateral (D) views, SEM. The egg was cleaned in advance with bleach to remove extrinsic material such as the fringe. **E**: Egg exuvia, lateroventral view. The egg was cleaned as in C and D. White and black arrowheads show a split line in the chorion for hatching and the position of micropyles, respectively. Scale bars = 100 μm. Ab, abdomen; An, antenna; Eg, egg; EmCu, embryonic cuticle; ET, egg tooth; Fu, furca; Ga, galea; Gl, glossa; HC, head capsule; La, lacinia; Lb, labium; L1-3, pro-, meso- and metathoracic legs; L1, 2Cx, pro- and mesocoxa; L1, 2Fe, pro- and mesofemur; MxP, maxillary palp; Pgl, paraglossa; SA, sternal apophysis; Sd, stomodaeum; SeCu, serosal cuticle; Th1-3, pro-, meso- and metathoracic segments; Th1, 2G, pro- and mesothoracic ganglia; Th1, 2T, pro- and mesothoracic terga; Y, yolk.

At 40–50% DT, the maxillary and labial coxopodites subdivide into proximal and distal parts, that is, the stipes and cardo in the maxilla, and the prementum and postmentum in the labium. These proximal and distal parts of the coxopodites of the mouthparts may be serially homologous to the thoracic subcoxa and coxa, respectively. The maxillary and labial telopodites differentiate into segmented elements, that is, the palps. Likewise,

at 40–50% DT the mandibles subdivide into two parts, similar to other gnathal and thoracic coxopodites, as described by Machida (2000a) for developing appendages of archaeognathan embryos. Machida identified the proximal and distal parts of archaeognathan mandibles as the mandibular subcoxa and coxa, respectively, and the mandibular parts in the embryo of *Zorotypus caudelli* apparently correspond with these subelements.

Generally, insect mandibles are approximately the same size as the other segmental appendages in their early stage of development. In Zoraptera, however, they appear as distinctly smaller swellings (Fig. 3D). This unusually small size is a potential autapomorphy of the order.

Egg Teeth

In the apterygote orders, an egg tooth occurs only in zygentomans, which suggests that it is absent in the groundplan of Hexapoda. In zygentomans, it is formed by the larval cuticle and persists during the first instar stage (Konopová and Zrzavý, 2005). In contrast to this, the egg teeth of most pterygotes including zorapterans are formed by the prelarval embryonic cuticle and are consequently absent after hatching.

The pterygote egg tooth is usually formed as a short longitudinal ridge or a small pointed projection (Sikes and Wigglesworth, 1931; Kishimoto and Ando, 1985; Uchifune and Machida, 2005; Shimizu, 2013). The extremely elongate condition distinguishes Zoraptera from other pterygote orders with the notable exception of Embioptera (Jintsu, 2010). In *Aposthonia japonica*, a Japanese embiopteran species, a robust longitudinal egg tooth covers the entire length of the frons. The strong degree of elongation could be considered as a potential synapomorphy of both orders. However, the evolution of egg teeth in Pterygota is presently not well understood. They can be present or absent or occur in entirely different body regions, as it is for instance the case in Coleoptera (e.g., Beutel, 1997).

Formation of the Embryo and Blastokinesis

In *Zorotypus caudelli*, a small heart-shaped embryo is formed. It gradually grows, with segments subsequently added from anterior to posterior. Thus, the embryo of *Z. caudelli* can be categorized as belonging to the short germ band type (cf. Krause, 1939; Sander, 1984). Two alternative varieties of this category occur in Insecta (= Ectognatha). In most groups of the lower neopteran insects, or Polyneoptera, the embryo is formed by a pair of blastoderm regions with higher cellular density (Bedford, 1970; Uchifune and Machida, 2002, 2005; Jintsu, 2010; Shimizu, 2013). In other groups, the cells near the posterior pole concentrate and proliferate to form the embryo. The latter type is known in Palaeoptera and Acercaria (Goss, 1952; Ando, 1962; Heming, 1979; Haga, 1985; Tojo and Machida, 1997, 1998), but also in the apterygote ectognathan orders Archaeognatha (Machida et al., 1990) and Zygentoma (Masumoto and Machida, 2006). This strongly suggests that this type of embryo formation belongs to the groundplan of Ectognatha and

Pterygota, whereas the former may be regarded as potential autapomorphy of Polyneoptera, which is still strongly disputed as a clade (e.g., Kristensen, 1995). This study revealed that this developmental feature also occurs in Zoraptera.

It is noteworthy that the early embryo forms on the dorsal side in Zoraptera, with its anteroposterior axis diametrically opposed to that of the egg. In a typical case, the insect embryo forms on the ventral side with its anteroposterior axis corresponding with the orientation of the egg. However, it is also known that the position of the embryo can vary considerably, from around the equator to close to the posterior pole on the ventral side of the egg, even within a single order (Cobben, 1968; Warne, 1972). One explanation could be that the unusual position in Zoraptera just lies within this wide range in insects. Another possible interpretation is that the unusual position is due to "precocious migration of the embryo." It is conceivable that the migration of blastoderm cells toward the posterior region, which is the driving factor in the formation of the embryo, is accelerated in Zoraptera, leading finally to placement on the dorsal side of the egg with reversed orientation. In the embryonic development of the immersed type in hemimetabolous insects (see Johannsen and Butt, 1941; Anderson, 1972; Heming, 2003), progressive elongation along the egg surface also results in a shift of the embryo from the ventral to the dorsal surface, with a reversed anteroposterior axis. In the case of Zoraptera, the unusual position of the early embryo might be caused by the unusually early start of cell migration, leading to "precocious migration of embryo to the dorsal side of the egg." To our knowledge, the position of the early zorapteran embryo is unique and shows the potential autapomorphy of the order.

The embryo of *Z. caudelli* thus differentiated on the dorsal side of the egg develops there into its full elongation, undergoing embryogenesis of the short germ band type. The embryo then migrates in parallel with the egg surface deep in the yolk and develops for a short period. Katatrepsis then occurs, and the embryo appears again on to the egg surface, accompanied by the reversion of its anteroposterior axis, finally taking its position on the ventral side of the egg. As has been mentioned and discussed thus far, the formation of the embryo and blastokinesis of *Z. caudelli* (Zoraptera; Fig. 9D) may be characterized as follows: 1) the formation of an embryo by the fusion of paired blastoderm regions with higher cellular density, 2) differentiation of the embryo on the dorsal side of the egg, 3) embryogenesis of the short germ band type, 4) full elongation of the embryo on the egg surface, 5) immersion of the embryo into the yolk after its full elongation, and 6) katatrepsis accompanied by the reversion of the embryo's anteroposterior axis.
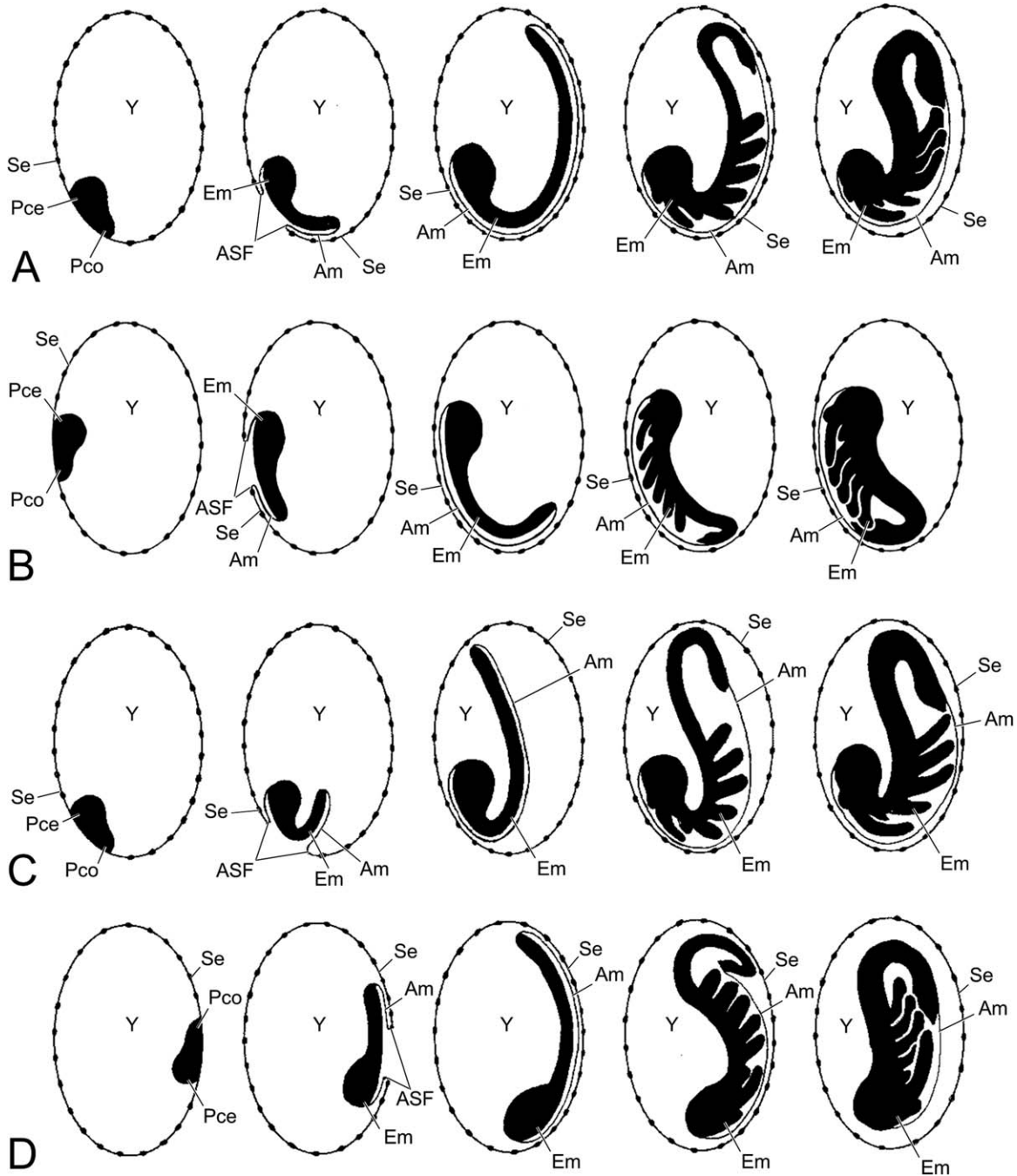
Fig. 9. Diagrammatic representations showing blastokinesis during the prekatatrepsis period in hemimetabolous insects, lateral view, anterior to the top, ventral to the left. **A**: Polyneoptera, immersed type. **B**: Polyneoptera, superficial type. **C**: Acercaria and Palaeoptera. **D**: Zoraptera. Am, amnion; ASF, amnioserosal fold; Em, embryo; Pce, protocephalon; Pco, protocorm; Se, serosa; Y, yolk.

Phylogenetic Implications of Comparative Embryology

Polyneopteran versus acercarian affinities of Zoraptera? As already pointed out in the introduction, the systematic position of Zoraptera is apparently one of the few remaining enigmas in insect phylogeny (e.g., Hennig, 1969; Kristensen, 1975; Beutel and Gorb, 2001, 2006; Beutel and Weide, 2005; Yoshizawa, 2007, 2011; Ishiwata et al., 2011; Trautwein et al., 2012; Blanke et al., 2012). Recent morphological and molecular studies tentatively support their placement in Polyneoptera (Engel and Grimaldi, 2000, 2002; Yoshizawa and Johnson, 2005; Yoshizawa, 2007, 2011; Ishiwata et al., 2011). However, even the monophyly of this lineage is a long debated problem (e.g., Boudreaux, 1979; Kristensen, 1991; Kjer, 2004; Grimaldi and Engel, 2005; Kjer et al., 2006; Misof

et al., 2007; Klass, 2009; Ishiwata et al., 2011; Yoshizawa, 2011) and the neutral term "lower Neoptera" is often used (e.g., Kristensen, 1981, 1995), although we use the term "Polyneoptera" in this study. The presence of euplantulae (Minet and Bourgoin, 1986; Beutel and Gorb, 2001) and a fan-like anal lobe of the hindwing (Grimaldi and Engel, 2005; Beutel and Gorb, 2006) have been proposed as autapomorphies of Polyneoptera. However, these features are not present in all polyneopterans, and both are missing in Zoraptera (Minet and Bourgoin, 1986; Grimaldi and Engel, 2005; Yoshizawa, 2011). So far, the most conclusive evidence has been provided by Yoshizawa (2011), who proposed four apomorphies of the wing base in support of Polyneoptera, including Zoraptera.

The main alternative hypothesis, the "Paraneoptera concept" with Zoraptera as a sister group of Acercaria (e.g., Hennig, 1969; Beutel and Weide, 2005), has gained no support in more recent studies and morphological arguments were discussed critically by Yoshizawa (2007). Nevertheless, with the present knowledge, this option cannot be ruled out with certainty.

In insect comparative embryology, it is well known that Polyneoptera and Acercaria show a profound contrast in the process of the embryo's migration into the yolk (Fig. 9A,C). Blastokinesis has been examined in all polyneopteran orders (Plecoptera: Miller, 1940; Kishimoto and Ando, 1985; Dermaptera: Heymons, 1895; Shimizu, 2013; Orthoptera: Roonwal, 1937; Warne, 1972; Grylloblattodea: Uchifune and Machida, 2005; Mantophasmatodea: Machida et al., 2004; Phasmatodea: Thomas, 1936; Embioptera: Kershaw, 1914; Mantodea: Hagan, 1917; Blattodea: Wheeler, 1889; Heymons, 1895; Ando, 1971; Isoptera: Knower, 1900; Striebel, 1960), although the data are fragmentary in some cases. In Polyneoptera, two distinctly different varieties of blastokinesis were distinguished by Anderson (1972), the immersed type and the superficial type. The first is found in Plecoptera, Grylloblattodea, Mantophasmatodea, Embioptera, Isoptera, and Blattoidea. In these groups, the embryo is formed on the ventral side of the egg and covered with the amnioserosal fold (Fig. 9A). It extends and moves along the dorsal egg surface and migrates into the yolk after reaching its full elongation. The second type occurs in Dermaptera, Phasmatodea, Mantodea, and Blaberoidea. The embryo is also formed on the ventral side and is covered by the amnioserosal fold (Fig. 9B), but without a shift to the dorsal side of the egg and without immersion into the yolk. The embryo maintains its original superficial position on the ventral side and reaches its full length there.

Blastokinesis was also described for members of all acercarian orders, although with a clear bias toward Hemiptera (Psocoptera: Goss, 1952, 1953; Phthiraptera: Schölzel, 1937; Thysanoptera: Heming, 1979; Haga, 1985; Moritz, 1988; Hemiptera: Butt, 1949; Cobben, 1968; Heming and Huebner, 1994). A small embryo forms on the ventral side. It gradually elongates and migrates into the yolk from its rear. This is accompanied by the formation of the amnioserosal fold. At the end of the process the embryo is deeply immersed in the yolk mass (Fig. 9C).

Blastokinesis in the two palaeopteran orders strongly resembles what is described for acercarian groups (Ephemeroptera: Tojo and Machida, 1997, 1998; Odonata: Ando, 1962), with a very similar pattern of embryo formation, elongation and migration into the yolk. The phylogenetic pattern of Palaeoptera outside of Neoptera (outgroups), and Acercaria as a monophyletic neopteran subunit clearly shows that the palaeopteran-acercarian type is a groundplan feature of Pterygota and of Neoptera. The immersed and superficial types occurring in polyneopterans seem to differ greatly, but both share a marked common feature. That is, full elongation of the embryo occurs on the egg surface in these groups (Fig. 9A,B), in contrast to Palaeoptera and Acercaria (Fig. 9C), in which this process occurs in the yolk, keeping step with the formation of the amnioserosal fold and the embryo's immersion in the yolk. This feature may be an autapomorphy of Polyneoptera, which are not supported by a single nonhomoplastic morphological feature at present (see above), and has not unequivocally confirmed by molecular data (Letsch et al., 2012).

Above, we enumerated six features characterizing the embryogenesis of Zoraptera. Among these features, the first, fourth, and fifth features are especially significant in discussing the zorapteran affiliation. Namely, the first feature, "formation" as already discussed and the fourth, "full elongation of the embryo on the egg surface" as mentioned just above can be proposed as potential autapomorphies of Polyneoptera, including Zoraptera. The fifth feature, "immersion of the embryo into the yolk after its full elongation" should be also noticed, being typical of polyneopteran blastokinesis of the immersed type. Consequently, embryological data strongly suggest the placement of Zoraptera among the Polyneoptera.

Blastokinesis of Zoraptera clearly belongs to the immersed polyneopteran type, which associates Zoraptera with the polyneopteran orders showing this developmental pattern. However, blastokinesis varies strongly, in some cases even within one order (Ando, 1962; Cobben, 1968; Masumoto, 2006), especially in Blattodea (Wheeler, 1889; Heymons, 1895; Lenoir-Rousseaux and Lender, 1970; Tanaka, 1976). Solid evolutionary interpretation of the types of blastokinesis occurring in Polyneoptera will require careful screening of this developmental process in each order. It is possible that

characteristics linked to blastokinesis will be found to be strongly affected by homoplasy, at least in some of the orders.

Affinities of Zoraptera within Polyneoptera
Closer affinity between Zoraptera and Dermaptera has been suggested based on morphological and molecular data sets (Carpenter and Wheeler, 1999; Terry and Whiting, 2005), but this is in contrast to the developmental features discussed above (immersed versus superficial type). Inherent problems with the direct optimization (POY) used in molecular analyses (Carpenter and Wheeler, 1999; Jarvis et al., 2005; Terry and Whiting, 2005) were pointed out by Simmons (2004), Kjer et al. (2007), Morgan and Kelchner (2010), Yoshizawa (2010), and Simmons et al. (2011), and it was shown in an empirical study (Ogden and Rosenberg, 2007) that POY performs less well than other approaches. In addition, Yoshizawa (2010) pointed out that the specific 18S rRNA sequence was erroneously assigned to Zoraptera (*Zorotypus hubbardi*) by Terry and Whiting (2005) as a result of contamination. This was shown by BLAST search analysis, which assigned this sequence to the dermapteran genus *Tagalina*.

A sister group relationship between Zoraptera and Dictyoptera has been suggested based on morphological characteristics, molecular data, and combined evidence (Silvestri, 1913; Caudell, 1918; Crampton, 1920; Weidner, 1969, 1970; Boudreaux, 1979; Wheeler et al., 2001; Yoshizawa and Johnson, 2005; Ishiwata et al., 2011; Wang et al., 2013). Four morphological characteristics, that is, a disc-shaped pronotum, a forward-slanting pleural suture, ill-developed indirect flight muscles, and posteriorly directed coxa, have been suggested as potential synapomorphies (Boudreaux, 1979; Wheeler et al., 2001). However, Beutel and Weide (2005) and Friedrich and Beutel (2008) pointed out that the indirect flight muscles are well developed in winged forms, that the other three arguments are greatly weakened by superficial character definition, and that they are obviously either subject to homoplasy or are plesiomorphic. A forward-slanting pleural suture for instance is found in most if not all groups of pterygote insects. Analyses of 18S (Yoshizawa and Johnson, 2005), 28S RNA (Wang et al., 2013) and three protein-coding genes (Ishiwata et al., 2011) suggested a close affinity between Zoraptera and Dictyoptera. However, Yoshizawa and Johnson (2005) pointed out that this result might be affected by the unusual characteristics of these genes, such as a markedly accelerated substitution rate, resulting in very long branches, modifications of the secondary structure, and long insertions. According to Ishiwata et al. (2011), the close affinity between Zoraptera and Dictyoptera suggested by sequences of protein-coding genes (*DPD1, RPB1, RPB2*) has only low support in maximum likelihood analyses,

even though it appears well supported by Bayesian analysis.

A clade Zoraptera + Embioptera ("Mystroptera": Rafael and Engel, 2006) is suggested by several potential synapomorphies, including a reduced number of tarsomeres, paddle-shaped wings, a metafemur with a unique musculature, wing base structures, and ecology-related characteristics such as wing dimorphism and a gregarious lifestyle (Minet and Bourgoin, 1986; Engel and Grimaldi, 2000, 2002; Yoshizawa, 2007, 2011). This study revealed a marked embryological feature shared by embryos of members of both orders, that is, an extraordinarily long egg tooth. As an alternative scenario, a sister group relationship between Embioptera and Phasmatodea has been suggested based on morphological and molecular evidence (Rähle, 1970; Tilgner, 2002; Kjer, 2004; Terry and Whiting, 2005; Bradler, 2009; Ishiwata et al., 2011; Wipfler et al., 2011; Friedemann et al., 2012), and the name "Eukinolabia" was proposed for this clade by Terry and Whiting (2005). A clade comprising Zoraptera, Embioptera, and Phasmatodea was first suggested based on wing base structures by Yoshizawa (2007), but after performing formal cladistic analysis, he suggested the close affinity between Phasmatodea and Orthoptera (Yoshizawa, 2011). A monophyletic unit, Zoraptera + Embioptera + Phasmatodea, was again tentatively supported by the results of recent studies on egg structures. Mashimo et al. (2011) described a pair of micropyles only occurring in eggs of Zoraptera and Timematodea (see also Jintsu et al., 2010), the latter generally placed as the basalmost branch of Phasmatodea (e.g., Friedemann et al., 2012). A pair of micropyles is located very closely, as if they could be a single micropyle in Euphasmatodea (Godeke and Pijnacker, 1984). Consequently, this was suggested as potential groundplan apomorphy of a clade comprising Zoraptera + Eukinolabia. Dallai et al. (2011, 2012b) provided a detailed description of the male reproductive system and sperm ultrastructure, and also suggested the close affinity of Zoraptera and Eukinolabia based on two apomorphic characteristics, 17 protofilaments comprising accessory tubules of axonomes, and L-shaped electron-dense lamellae accompanying microtubular triplets in the centriole adjunct.

The interpretation of the elongated egg tooth occurring in Zoraptera remains ambiguous in the scenario with Zoraptera as the sister group of Eukinolabia. Egg teeth were considered to be absent in Phasmatodea, which possess tough egg shells and an operculum (Thomas, 1936; Bedford, 1970). This suggests that the specialized structures of Zoraptera and Embioptera have either evolved independently or egg teeth are secondarily absent in phasmatodean embryos.

The prelarvae of Zoraptera use an egg tooth to penetrate the chorion of the egg, which lacks an
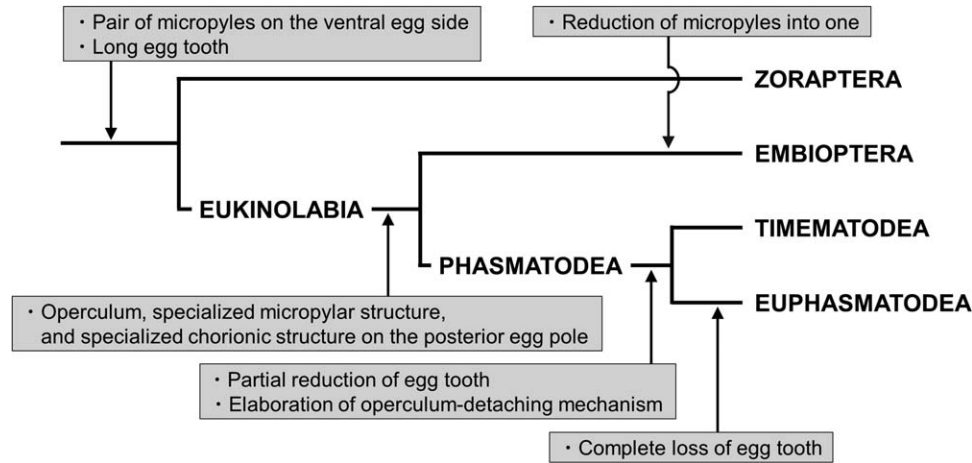
Fig. 10. Proposed affinities of Zoraptera and Eukinolabia based on comparative embryological evidence (see Discussion).

operculum (Fig. 8C,D). In contrast, an operculum is used for hatching by the prelarvae of Embioptera and also of Phasmatodea. It is noteworthy that an egg tooth is preserved in embryos of the former group but apparently does not interact with the chorion. The operculum-detaching mechanism is less elaborate in eggs of Embioptera than those of Phasmatodea, which lack a perforating device. The phasmatodean egg is characterized by a distinct detachment line between the operculum and the egg body (Hinton, 1981; Jintsu et al., 2010), whereas in embiopteran eggs a less well-defined spongy zone of weakness forms an opening mechanism (Jintsu and Machida, 2009; Jintsu, 2010). It is conceivable that a longer evolutionary pathway led to the typical condition of the phasmatodean operculum and opening mechanism, along with increasing reduction of the primarily present egg tooth. This interpretation is tentatively supported by an interesting finding in timematodean eggs. We identified a discontinuous and ill-defined but long egg tooth in the frontal region of the prelarvae of *Timema monikensis* (Y. Uchifune-Jintsu and R. Machida, pers. obs.). This suggests that an elongated egg tooth is groundplan apomorphy of the Zoraptera-Eukinolabia clade, with partial secondary reduction in Phasmatodea (groundplan) and complete loss as autapomorphy of Euphasmatodea. That Phasmatodea is more closely related with Embioptera is clearly supported by several derived features of the egg: 1) a detachable operculum, 2) a specialized micropylar structure on the ventral side of the egg, that is, micropylar plate or tube, 3) a small number of micropyles (one or two) associated with the specialized micropylar structure, and 4) a specialized chorionic structure at the posterior pole of the egg, that is, a polar mound or projection. An evolutionary scenario for the egg tooth and egg structures is shown in Figure 10.

The results we present here shed new light on the phylogeny of polyneopteran lineages and especially on the problematic systematic placement of Zoraptera. Different follow-up investigations are required to rigorously test the phylogenetic arguments and evolutionary interpretations presented: 1) study of the embryonic development of more zorapteran (and embiopteran) species, 2) detailed documentation of organogenesis, 3) formal characteristic analysis using available morphological data sets (e.g., Beutel and Gorb, 2006), and 4) reconstruction of characteristic evolution based on trees resulting from transcriptomes analysed in the 1KITE project (see www.1KITE.org/).

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