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Embryonic Development of *Eucorydia yasumatsui* Asahina, with Special Reference to External Morphology (Insecta: Blattodea, Corydiidae)

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ABSTRACT As the first step in the comparative embryological study of Blattodea, with the aim of reconstructing the groundplan and phylogeny of Dictyoptera and Polyneoptera, the embryonic development of a corydiid was examined and described in detail using *Eucorydia yasumatsui*. Ten to 15 micropyles are localized on the ventral side of the egg, and aggregated symbiont bacterial "mycetomes" are found in the egg. The embryo is formed by the fusion of paired blastodermal regions, with higher cellular density on the ventral side of the egg. This type of embryo formation, regarded as one of the embryological autapomorphies of Polyneoptera, was first demonstrated for "Blattaria" in the present study. The embryo undergoes embryogenesis of the short germ band type, and elongates to its full length on the ventral side of the egg. The embryo undergoes katatrepsis and dorsal closure, and then finally, it acquires its definitive form, keeping its original position on the ventral side of the egg, with its anteroposterior axis never reversed throughout development. The information obtained was compared with that of previous studies on other insects. "Micropyles grouped on the ventral side of the egg" is thought to be a part of the groundplan of Dictyoptera, and "possession of bacteria in the form of mycetomes" to be an apomorphic groundplan of Blattodea. Corydiid embryos were revealed to perform blastokinesis of the "non-reversion type (N)", as reported in blaberoid cockroaches other than Corydiidae ("Ectobiidae," Blaberidae, etc.) and in Mantodea; the embryos of blattoid cockroaches (Blattidae and Cryptocercidae) and Isoptera undergo blastokinesis of the "reversion type (R)," in which the anteroposterior axis of the embryo is reversed during blastokinesis. Dictyopteran blastokinesis types can be summarized as "Mantodea (N) + Blattodea [= Blaberoidea (N) + Blattoidea (R) + Isoptera (R)].

KEY WORDS: Dictyoptera; Blattodea; comparative embryology; blastokinesis; micropyle; mycetome

INTRODUCTION

Insecta is the most speciose group of organisms, accounting for 75% of all known animal species. Its evolution has attracted much interest. In recent years, many researchers have attempted to reconstruct the phylogeny of the group, but some open questions remain (see Trautwein et al., 2012; Kjer et al., 2016). One of these issues concerns “Polyneoptera.” Polyneoptera is very important in elucidating the groundplan of insects, as they represent the basal lineage of the most diverse group Neoptera, which is characterized by the ability of their adults to fold back their wings. Polyneoptera is composed of 11 traditional orders: Plecoptera, Dermaptera, Embioptera, Phasmatodea, Orthoptera, Zoraptera, Grylloblattodea, Mantophasmatodea, Mantodea, “Blattaria,” and Isoptera. The relationships among these 11 polyneopteran groups are, however, highly controversial and far from consensus, with even the monophyly of the lineage questioned by some authors (see Kristensen, 1981, 1991; Grimaldi and Engel, 2005; Terry and Whiting, 2005; Klass, 2009). Phylogenetic difficulties with Polyneoptera may be due to fast diversification over a relatively short time span in the Carboniferous, and then the separate accumulation of specializations in members of each lineage over a very long period from the Carboniferous (see Grimaldi and Engel, 2005).

Recent research from various disciplines, such as comparative morphology, comparative embryology, and molecular phylogenetics, strongly suggests that Polyneoptera is most likely to be monophyletic (e.g., Yoshizawa, 2007, 2011; Ishiwata et al., 2011; Mashimo et al., 2014; Misof et al., 2014), whereas the relationships of the orders remain uncertain. Among Polyneoptera, the interordinal relationships remain highly controversial; however, Dictyoptera has been widely accepted as monophyletic based on various morphological studies (e.g., Kristensen, 1975, 1981, 1991; Klass,

2003; Beutel and Gorb, 2006; Klass and Meier, 2006; Beutel et al., 2014) and molecular phylogenetics (e.g., Maekawa et al., 1999; Wheeler et al., 2001; Kjer, 2004; Terry and Whiting, 2005; Kjer et al., 2006). Despite its suggested monophyly, the relationships of three constituents of Dictyoptera, i.e., Mantodea, "Blattaria," and Isoptera, have been disputed (see Deitz et al., 2003; Klass and Meier, 2006). In several older studies, such as Cleveland et al. (1934), in which the life history, brood care, and intestinal flagellate fauna of termites and cockroaches were examined and compared, Isoptera were suspected to be subordinate to "Blattaria" (cockroaches), which are considered as "highly derived eusocial cockroaches." Recent phylogenetic studies have established that termites belong within the "Blattaria" cockroaches, most likely as a sister to the subsocial Cryptocercidae. Thus, "Blattaria" is paraphyletic with regard to Isoptera, and the subordinate position of termites in "Blattaria" has been consistently confirmed (e.g., Lo et al., 2003; Klass and Meier, 2006; Djernæs et al., 2015). The monophyletic group comprising cockroaches and termites is called "Blattodea."

Dictyoptera, which previously was regarded as being composed of three orders, currently has been discussed in the framework of a "two-order-system," or the "Mantodea-Blattodea system," and the phylogenetic focus on Dictyoptera has been shifted to the interrelationships of several major blattodean lineages, i.e., 1) Cryptocercidae + Isoptera, 2) Blattidae, 3) Lamproblattidae, 4) Tryonicidae, 5) Corydiidae (= Polyphagidae), 6) Nocticolidae, and 7) Blaberoidea [including Blaberidae, and paraphyletic with regard to the former, "Ectobiidae" (= "Blattellidae")] (see Djernæs et al., 2012, 2015).

Comparative embryology is an important method for reconstructing the groundplan of a group and solving phylogenetic concerns. Many embryological studies have been conducted on "Blattaria;" however, most of them treat the derived families

containing pest species, for example, “Ectobiidae” and Blattidae (e.g., Wheeler, 1889; Heymons, 1895; Lenoir-Rousseaux and Lender, 1970; Tanaka, 1976), and the comparative embryological information covering major blattodean lineages described above needs further examination.

In light of this background, we began a comparative embryological study of Blattodea. Corydiidae, for which embryological knowledge was totally lacking, is one of the most enigmatic blattodean lineages, being i) often regarded as the basalmost branch in Blattodea (e.g., McKittrick, 1964; Roth, 1991), ii) otherwise allied to Cryptocercidae + Isoptera (e.g., Djernæs et al., 2012), or iii) considered as paraphyletic with regard to Nocticolidae (e.g., Djernæs et al., 2015). Following our recent study (Fujita and Machida, 2014) on the postembryonic development of the Japanese corydiid, *Eucorydia yasumatsui* Asahina, 1971, which is endemic to the Yaeyama Islands of Okinawa Prefecture, in the present study we examine and describe the embryonic development of the same species, and compare the obtained information with that of other groups, with the goal of providing a new basis for contributing to the reconstruction of the groundplan and phylogeny of Dictyoptera and Polyneoptera.

MATERIALS AND METHODS

Adults and larvae of *Eucorydia yasumatsui* were collected from Komi, Iriomote Island, Taketomi and Mt. Yarabu-take, Ishigaki Island, Okinawa Prefecture, Japan. They were kept and reared in a plastic case (14 cm in diameter and 6.5 cm in height) with a moistened soil bottom at room temperature, and were fed compound food (grained food for goldfish : grained chlorella tablet : grained beer yeast tablet : grained balanced food = 9 : 3 : 3 : 1), in accordance with Fujita et al. (2011). Females deposited oothecae on the soil.

We collected several hundreds of oothecae, each of which contains about eight eggs. Eggs were taken from the deposited oothecae in Ephrussi–Beadle's physiological saline solution (0.75% NaCl + 0.035% KCl + 0.021% $CaCl_2$). The chorion was usually partially or fully torn off in this process. The eggs were fixed with Bouin's fixative (saturated aqueous picric acid solution : formalin : acetic acid = 15 : 5 : 1) for 24 h, or alternatively with Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde in 0.1 M HCl-sodium cacodylate buffer, pH 7.2) for 24 h. The eggs fixed with Bouin's fixative or with Karnovsky's fixative respectively were stored in 70% or 90% ethyl alcohol in 0.1 M HCl-sodium cacodylate buffer, pH 7.2 at 4°C. For eggs in which the amniotic pore was closed and the serosal cuticle had been secreted, the serosal cuticle was perforated using a fine tungsten needle prior to fixation, to facilitate penetration of the fixative. Fixing often produces aggregations of amniotic fluid on the embryo surface, which is often a significant obstacle for the external observation of embryos. The following methods were effective in solving this problem. First, we dissected out the embryos from living eggs using fine forceps and a needle. We placed them in Ephrussi–Beadle's solution, and then rinsed them with the same solution several times to wash away the amniotic fluid around the embryo prior to fixation. We also perforated the amnioserosal fold using a fine needle, and maintained the eggs in Ephrussi–Beadle's solution for 12 h so that the amniotic fluid washed out from the amniotic cavity, and then replaced the solution with saline.

Fixed eggs or embryos were stained using a DNA-specific fluorescent dye, DAPI [4',6-diamidino-2-phenylindole dihydrochloride, diluted to approximately 10 μg/mL with distilled water (DW)], for 24 h or several days. Lipids in the eggs often hinder the staining solution from infiltrating the materials. In such cases, it was more effective to soak the materials in acetone for several hours to remove the lipids. For this

purpose, the specimens were initially dehydrated in a graded series of ethyl alcohol, kept in acetone for a period, hydrated through the alcohol series in reverse, and finally transferred into the staining solution. Stained materials were observed using a fluorescence stereomicroscope (MZ FL III + FLUO COMBI, Leica, Heerbrugg, Switzerland), with UV-excitation at 360 nm.

A proportion of the fixed eggs or embryos were postfixed with 1% OsO_4 for 1 h. They were dehydrated through a graded series of ethyl alcohol, and dried using a critical point dryer (Samdri-PVT-3D, tousimis, Rockville, USA). The dried specimens were mounted on a stub, coated with gold in an ion sputter (Ion Sputter JFC-1100, JEOL, Tokyo, Japan), and observed using a scanning electron microscope (SEM) (SM-300, TOPCON, Tokyo, Japan) at an accelerating voltage of 15 kV.

In later stages, the embryonic cuticle often becomes a major obstacle for SEM observation of external features. The embryonic cuticle wrinkles up during sample processing, separating from the surface due to shrinkage caused by fixation. In such cases, low-vacuum SEM of non-coated specimens (Machida, 2000), or ordinary SEM of non-fixed specimens using the nano-suit method (Takaku et al., 2013), has substantial advantages. In the case of low-vacuum SEM, fixed embryos were dehydrated in a graded series of ethyl alcohol, dried using a critical point dryer, and then observed with a low-vacuum SEM (SM-300 Wet-4, TOPCON, Tokyo, Japan) with a pressure of 13 Pa, at an accelerating voltage of 15–20 kV. Using this method, the surface of non-coated embryos could be observed because the electron beam can transmit through the wrinkled embryonic cuticles. Low-vacuum SEM of non-coated specimens was also useful for observing micropyles, which are sometimes filled with a dried gelatinous substance. For SEM of non-fixed specimens using the nano-suit method (Takaku et al., 2013), embryos were soaked in 1% polyoxyethylene sorbitan monolaurate (Tween 20)

solution for 1 h, mounted on a stub, and observed using SEM under high vacuum with an accelerating voltage of 5 kV, according to the modification by Fujita et al. (2016). In this method, the embryonic cuticle does not wrinkle up because the embryos suffer from very little shrinkage.

For the observation of micropyles, chorions were cleaned with an ultrasonic cleaner, then soaked in 0.5% sodium hypochlorite solution for 10 min, mounted with Heinz medium (10 g polyvinyl alcohol + 80 ml DW + 35 ml lactic acid + 10 ml glyceline + 25 ml phenol + 20 g chloral hydrate), and examined using a differential interference contrast microscope (DM6000B, Leica, Wetzlar, Germany).

A proportion of the fixed eggs or embryos were dehydrated in a graded series of ethyl alcohol, and embedded in a methacrylate resin (Technovit 7100, Külzer, Wehrheim, Germany), in accordance with Machida et al. (1994a, b). The eggs/embryos were processed into 3 μm thick serial sections using a semi-thin microtome (H-1500, Bio-Rad, Hercules, California, USA) equipped with a tungsten carbide knife (Superhard Knife, Meiwafosis, Tokyo, Japan). Sections were stained with 1% Delafield's hematoxylin for 24 h at 60°C, 0.5% eosin G for 1 h, and 0.5% fast green FCF ethyl alcohol solution for 1 min. We observed the stained sections using a biological microscope (Optiphot-2, Nikon, Tokyo, Japan).

For transmission electron microscopy (TEM), the eggs or embryos fixed in Karnovsky's fixative were postfixed with 1% OsO_4 for 2 h. They were embedded in a water-miscible epoxy resin (Quetol-651, Nisshin EM, Tokyo, Japan), and processed into 80 nm thick sections using an ultramicrotome (MT-XL, RMC, Tucson, USA) equipped with a diamond knife (Histoknife Wet 8 mm, Drukker, Cuijk, Netherlands). These sections were observed using a TEM (HT-7700, Hitachi, Tokyo, Japan) at 80 kV.

RESULTS

The egg period of *Eucorydia yasumatsui* was 64.1 ± 11.9 days (n = 184) at room temperature (18–24°C) (Fujita et al., 2011). Based on the changes in external embryonic features, embryonic development is divided into Stages 1 through 12, which are also expressed as a percentage of total developmental time, with 0% at oviposition and 100% at hatching (Bentley et al., 1979).

Eggs

Females of *Eucorydia yasumatsui* produce five to 15 oothecae during their lifetime, each of which contain 8.1 ± 1.3 eggs (n = 20), with a minimum of five and a maximum of 10 eggs. The eggs are ellipsoidal, about 2.1 mm in length and 0.8 mm in width, yellowish-white in color, with the inside being visible through the translucent brownish chorion (Fig. 1A,B). The dorsal side of the egg, facing the oothecal wall, is convex, and its ventral side, on which the embryo is formed, is almost straight or slightly concave (Fig. 1B). As described by Fujita and Machida (in press), 10–15 micropyles, with funnel-shaped entrances, are localized in the posterior region of the ventral side of the egg (arrowheads in Fig. 1C,D).

It is known that cockroaches harbor symbiotic bacteria of the genus *Blattabacterium* in their fat bodies, that the bacteria are transmitted vertically to offspring via transovarial transmission, and that they appear as a massive form called the “mycetome (strictly “bacteriome”)” inside the egg (e.g., Gier, 1936; Sabree et al., 2009). The mycetomes of *Eucorydia yasumatsui* first begin their residencies at the anterior and posterior poles of the eggs (Fig. 5A–C), which include numerous microorganisms (Fig. 5D). The mycetomes keep their bipolar positioning in the early developmental stages, i.e., up to the commencement of protocorm elongation in late

Stage 3 (Figs. 3A, 7A–C, 8A–C). The primary yolk nuclei, which are directly derived from cleavage nuclei remaining in the yolk that have not migrated to the egg periphery, gather to and enter the mycetomes (Fig. 2D). Late in Stage 3, when the protocorm starts to elongate, the posterior mycetome leaves the posterior pole of the egg (in Fig. 4B, the posterior mycetome is hidden from view by the posterior region of the elongating embryo). Subsequently, in Stage 4, the anterior mycetome leaves the anterior pole of the egg, and both mycetomes begin to migrate toward the center of the egg (Fig. 6A). Consequently, in Stage 5, the two mycetomes approach each other (Fig. 6B), and finally in Stage 6, they fuse with each other at the center of the egg (Fig. 6C). The unified mycetome maintains its position near the center of the yolk until Stage 9, when katatrepsis occurs (Fig. 6D). Once katatrepsis is complete, the mycetome breaks down, and liberated bacteria migrate to and nest in mesodermal cellular masses, that later on differentiate into the fat bodies (Fig. 6E,F).

Stage 1: 0–8% developmental time

The first and second maturation divisions occur in the cytoplasmic (maturation) island, which is at the equator of the ventral side of the egg (Fig. 2A). The female pronucleus migrates inward from the cytoplasmic island to the posterior region of the yolk, and there, 1 day after oviposition, conjugates with the male pronucleus (Fig. 2B). The resultant synkaryon begins dividing mitotically (Fig. 2C); cleavage is of the superficial type. The nuclei divide synchronously until the seventh cleavage, and synchrony is lost during the course of the eighth cleavage, 2 days after oviposition. At this stage, most of the cleavage nuclei arrive at the egg periphery to form the syncytial blastoderm, with a higher nuclear density in the posterior region of the egg. A small proportion of the cleavage nuclei, about 10, remain in the yolk. These become the

primary yolk nuclei, which gather to the anterior and posterior mycetomes (Fig. 2D). Due to the migration and proliferation of cleavage nuclei, a unicellular layer or the cellularized blastoderm is completed (Figs. 7A, 8A). The newly formed blastoderm already shows a regional difference in cellular density, being higher in the posterior (Fig. 3A), leading to a differentiation of the embryonic and extraembryonic areas, i.e., the posterior half of the blastoderm with higher cellular density represents the presumptive embryonic area. A few nuclei are found to be segregated and liberated from the blastoderm into the yolk, representing the secondary yolk nuclei (cf. Fig. 14A,B).

Stage 2: 8–10% developmental time

With the progressive proliferation of cells, and their concentration at the posterior ventral side of the egg, a pair of areas with a higher cellular density differentiates (asterisks in Fig. 3B). These areas migrate medially, and then condense to the ventroposterior region of the egg, assuming a V-shape, due to lower cellular density in the median longitudinal region of its anterior half (Fig. 3C), and with further condensation, they finally fuse into a heart-shaped embryo (Figs. 7B, 8B). The extraembryonic area is now called the “serosa.” In the newly formed embryo, the inner layer, or the mesoderm, starts to segregate (Fig. 14A).

Stage 3: 10–13% developmental time

The embryo extends on the ventral surface of the egg, and the anterior protocephalon and posterior protocorm differentiate (Figs. 7C, 8C). Production of amnion begins from the embryonic margin, leading to the formation of the amnioserosal fold, which is more progressive towards the posterior, and anatrepsis (see DISCUSSION, Embryonic Development, Blastokinesis) starts (Fig. 14B, inset). Late in this stage, the amnioserosal

folds fuse around the central area of the protocephalon, that is, the amniotic pore is closed (Fig. 8C). The inner layer or mesoderm, which was produced on the ectoderm in the protocorm (Fig. 14A), extends anteriorly to supply the mesoderm to the protocephalic region (Fig. 14B).

The protocorm elongates posteriorly, and simultaneously, the embryo starts to migrate anteriorly. The embryo takes its position in the middle of the ventral surface of the egg (Fig. 4A,B). The protocephalon enlarges laterally to become the head lobes, with the posterolateral regions occupied by the newly differentiated antennal segment (Fig. 4A). At the center of the head lobe, the stomodaeum appears as a shallow depression (Fig. 4A,B). Until the closure of amniotic pore, both anterior and posterior mycetomes keep their original, bipolar positions. Late in this stage, however, the posterior mycetome starts to migrate towards the anterior, so that it is blocked by the embryo, and can no longer be observed from the ventral view (Fig. 4B).

Stage 4: 13–17% developmental time

Segmentation begins in Stage 4, and the intercalary segment, and the gnathal and thoracic segments differentiate almost simultaneously (Figs. 7D, 8D, 9A). The mesoderm is also segmented in the differentiated segments (Fig. 14C). The neural groove appears along the median ventral line. Subsequent to the posterior mycetome, the anterior mycetome begins to migrate towards the center of the egg.

Stage 5: 17–27% developmental time

The embryo further elongates posteriorly, along the ventral surface of the egg, with the position of the cephalic end almost unchanged (Figs. 7E, 8E). In the mandibular, maxillary, labial, and thoracic segments, the appendages differentiate,

directing laterally (Fig. 9B,C), but not in the intercalary segment.

Late in this stage, segmentation proceeds to the fifth abdominal segment, and the caudal end of the embryo slightly bends ventrally (Figs. 7E, 8E, 9C, 12A). The proctodaeum appears (Fig. 9C). Associated with the formation of the proctodaeum, the differentiation of the 10th and 11th abdominal segments and the telson (anal lobes) precedes that of the sixth to ninth abdominal segments (Fig. 9C). An unpaired clypeolabrum forms, just anterior to the stomodaeum. Mandibular appendages are less developed and shorter than others (Fig. 9C). In the earlier differentiated, i.e., first to third abdominal segments, appendages develop (Fig. 9C). The lateral region, dorsal to the developing appendage, begins to extend in the gnathal and thoracic segments, initiating the formation of the tergal region (Fig. 12A). Coelomic sacs develop in the gnathal, thoracic, and four anterior abdominal segments (Fig. 14D).

The anterior and posterior mycetomes further migrate toward the center of the egg and come close to each other (Fig. 6B).

Stage 6: 27–38% developmental time

The embryo continues to elongate posteriorly, with the position of the cephalic end almost unchanged, and the posterior abdomen is folded and immersed into the yolk (Figs. 7F, 8F). The posterior region of the abdomen undergoes segmentation, and 11 abdominal segments are completed (Figs. 9D, 12B).

The stomodaeum continues to deepen and its ectodermal wall thickens (Fig. 14E). Apically, the clypeolabrum assumes a weakly bilobed form (Fig. 9D). Formation of the tergum begins in the segments of the anterior half of the abdomen (Fig. 12B). Two endites develop in both the maxillary and labial appendages, the medial lacinia and lateral galea, and the medial glossa and lateral paraglossa, respectively. The appendage

formation proceeds to the fifth abdominal segment, and the appendages of the first abdominal segment are the pleuropodia (Fig. 9D). The proctodaeum deepens, and a supraanal lobe and a pair of subanal lobes form around the proctodaeum (Figs. 9D, 12B).

The anterior and posterior mycetomes fuse with each other at the center of the egg (Fig. 6C).

Stage 7: 38–39% developmental time

The embryo increases in size almost without changing its position (Figs. 7G, 8G). The posterior abdomen, which is directed ventrally in the previous stage (Fig. 8F), changes its direction to anterior (Fig. 8G).

The clypeolabrum divides into the proximal clypeus and distal labrum; the latter is characterized by its bilobed appearance (Fig. 10A). In the antennae, the scapus and pedicellus can be distinguished. The flagellum is subdivided into three or four annuli (Fig. 12C). Maxillary, labial, and thoracic appendages, and the pleuropodia on the first abdominal segment, divide into the proximal coxopodite and distal telopodite (Figs. 12C). In the maxillary and labial appendages, the telopodites develop into palps, and their endites are enlarged (Figs. 10A, 12C). The thoracic telopodites divide into trochanter, femur, and tibia + tarsus (Fig. 10A). Formation of the appendages and terga proceeds to the 11th abdominal segment (Fig. 12C). While the second to eighth, and the 10th abdominal appendages do not develop further, the ninth and 11th abdominal appendages differentiate into the styli and cerci, respectively (Fig. 12C).

The unified mycetome maintains its position in the central region of the egg.

Stage 8: 39–44% developmental time

Almost without changing its position, the embryo grows further (Figs. 7H, 8H). A definitive dorsal closure occurs in the posterior abdomen. Head lobes enlarge and curve up (Figs. 10B, 12D). Paired swellings appear on the head lobes, representing the development of cerebral ganglia (asterisks in Figs. 10B, 12D). The ventral marginal area of the head gnathal region, or the subgena, extends ventrally and covers the base of the mandible (Fig. 12D). The antennal flagellum subdivides into eight annuli (Fig. 10B). Labial appendages of both sides start to migrate medially (Figs. 10B, 12D). The thoracic coxopodites are demarcated into two regions: the proximal subcoxa and the distal coxa (Figs. 10B, 12D). In the thoracic telopodites, the trochanter, femur, tibia, tarsus with two tarsomeres, and pretarsus are distinguishable (Fig. 10B). A small ectodermal invagination appears on the lateral side of each thoracic femur (white arrowheads in Fig. 10B). In the anterolateral region of the developing terga, for each of the second thoracic to eighth abdominal segments, a pair of tracheal invaginations, or spiracles, appears (black arrowheads in Figs. 10B, 12D). The 11th abdominal segment is reduced, and its boundary with the 10th abdominal segment is obscured (Figs. 10B, 12D).

The mycetome maintains its position at the center of the egg.

Stage 9: 44–48% developmental time

The amnioserosal fold tears and katatrepsis occurs (Figs. 7I, 8I). Simultaneously, the embryo contracts and descends posteriorly, with its abdomen slightly bent backward. The serosal cells, which have covered the egg, are condensed toward the anterodorsal region of the egg (Figs. 7I, 8I). With progressive condensation and the withdrawal of serosal cells, the amnion replaces the serosa, and finally spreads over the dorsal yolk as a provisional dorsal closure, and the embryo carries the yolk on its back (Figs. 7I, 8I).

The head capsule is complete due to the dorsal fusion of the head lobe and gnathal terga (Figs. 10C, 13A). Labrum loses its bilobed appearance and becomes apically rounded (Fig. 10C). The thoracic terga begin to extend ventrally to cover the thoracic subcoxae, which have been clearly demarcated from the coxae (Fig. 13A). The styli in the ninth abdominal segment, and the cerci in the 11th, elongate further (Figs. 10C, 13A). The ventral region, formed by the fusion of the 10th and 11th abdominal segments, swells substantially (Fig. 10C).

The mycetome maintains its position at the center of the egg (Fig. 6D).

Stage 10: 48–50% developmental time

Serosal cells continue to condense anterodorsally (Figs. 7J, 8J). The embryo, which contracted and descended posteriorly at the commencement of katatrepsis in the previous stage (Fig. 7I), elongates anteriorly as its growth progresses (Fig. 7J). The definitive dorsal closure proceeds, replacing the provisional dorsal closure, or the amnion (Fig. 8J).

The flagellum divides into nine segments (Fig. 11A). Compound eyes appear behind the antennae (Fig. 13B). Mandibles flatten anteroposteriorly, and the incisors differentiate at their distal ends (asterisks in Fig. 15). The maxillary palp divides into four segments, while the labial palp divides into three segments. The hypopharynx enlarges with its distal end bilobed (Fig. 15). Thoracic legs elongate further and differentiate, and the four tarsomeres, and the pretarsus, with paired ungues, are distinguishable (Fig. 11A). Cerci and styli elongate further (Figs. 11A, 13B). The abdominal segments VIII and IX are compressed due to caudal flexion and swelling of the “10th abdominal segment” (Fig. 11A). Subanal lobes enlarge (Fig. 11A).

The mycetome disintegrates and liberated bacteria migrate to, and nest in, the

cellular mesodermal masses (Fig. 6E,F), which are beginning to differentiate into fat bodies.

Stage 11: 50–52% developmental time

The secondary dorsal organ formation completes at the anterior pole of the egg, just anterior to the head (Figs. 7K, 8K, 11B, 13C), but soon begins to degenerate, and finally sinks into the developing midgut. The embryonic cuticle is secreted.

The antennal flagellum subdivides into 10 segments (Fig. 11B). Compound eyes become pigmented. Thoracic legs develop further and acquire their definitive organization, with the tarsus dividing into five. In the first abdominal appendages, or pleuropodia, the globular telopodal region still exists (Fig. 13C), but it soon degenerates during this stage. Cerci and styli further elongate, assuming a long cone-shape (Fig. 11B).

Stage 12: 52–100% developmental time

Further embryonic growth occurs, in which the head finally reaches the anterior end of the egg (Figs. 7L, 8L), and acquires its definitive configuration (Fig. 11C). The definitive dorsal closure is complete. Beneath the embryonic cuticle, the larval cuticle is secreted, with setation visible on its surface (Fig. 16B,C). At this point, an observation is made of a row of pointed, tiny egg teeth forming on the embryonic cuticle along the median line of the vertex (Fig. 16A); this is the first finding of egg teeth in cockroaches. The embryonic cuticle enveloping the stylus elongates because of the formation of long setae at the tip of the stylus (Fig. 16D), seemingly as the stylus itself elongates. The flagellum subdivides into 11 segments. The compound eyes become well-defined (Figs. 8L, 16B) and dark brown in color. The tips of mandibular teeth are sclerotized (Fig.

16C). Thoracic legs elongate further, and the paired claws on their tips become distinct. The large swelling on the ventral side of the 10th abdominal segment is atrophied; it is retracted and concealed behind the ninth abdominal segment (Fig. 14F).

The episternum and epimeron are identified as the sclerites lying anterior and posterior, respectively, to the oblique pleural suture that forms a strong ridge inside (Fig. 16B; cf. Fig. 17C). The episternum extends medially and occupies the upper side of the coxa, with the trochantin intervening between them. The subcoxal elements medial to the coxae of both sides largely extend on the ventral side, fusing with each other to form the basisternum, and carry the paired sternal apophyses on their posterior margin (Fig. 16C).

Hatching and first instar larva

The entire keel of the ootheca splits apart, and the prelarvae hatch from the ootheca synchronously. The prelarvae, covered by a thin, transparent embryonic cuticle, rupture the chorion, and emerge from the seam along the midline of the keel by peristaltic movement (see Fujita and Machida, 2014, Fig. 6A,B). Subsequently, they shed the embryonic cuticle and become first instar larvae (nymphs), which soon leave the ootheca. A few hours after hatching, a larval body becomes dorsoventrally flattened, and the coloration begins to change from white to brown. The mandible is enlarged substantially, with the teeth becoming sclerotized and darkly pigmented. The endites of the maxilla are well developed. The lacinia is pigmented and two well-sclerotized teeth are present on its apex. The galea bears numerous spinules on its apex (Fig. 17B). The hypopharynx is covered with numerous setae (Fig. 17B). The distal and proximal parts of the coxopodites of the labial appendages of both sides are in close contact or fused with each other to form the prementum and postmentum, respectively (Fig. 17A). The

paranotal regions of thoracic terga extend laterally. The thoracic legs are subdivided into coxa, trochanter, femur, tibia, and a tarsus with five tarsomeres and a pretarsus with ungues (Fig. 17C,D). The sternum and pleuron are well-sclerotized into distinct sclerites (Fig. 17E). In the abdomen, 10 terga and nine sterna are distinguished, the latter of which is smaller in number because the11th sternum has been reduced, and the 10th has retracted beneath the ninth (cf. Fig. 14F).

DISCUSSION

Egg Structures

Micropyles. Micropyles are small pores on the chorion for the entry of spermatozoa into the egg. Several studies on the micropyles of cockroaches have shown that 10 to several dozen of these small pores are localized and grouped on the ventral side of the egg: e.g., 80–100 micropyles in *Blatta orientalis* (= *Periplaneta orientalis*) (Blattidae) (Kadyi, 1879), 20–30 in *Blattella germanica* (“Ectobiidae”) (Wheeler, 1889; Fujita, 2016), and 10–15 in *Eucorydia yasumatsui* (Corydiidae) (Fujita and Machida, in press). Thipaksorn and Machida (unpublished) investigated the embryogenesis of an enigmatic, wood-feeding cockroach, *Cryptocercus punctulatus* (Cryptocercidae), and found a dozen micropyles localized on the ventral side of the egg. Thus, it may be safely asserted that “micropyles grouped on the egg’s ventral side” is a common feature to cockroaches.

Cockroaches, or the “Blattaria,” are currently regarded as a paraphyletic taxon, with termites (Isoptera) nested within the group, and cockroaches and termites together constitute a monophyletic taxon, Blattodea. As for the micropyles of Isoptera, it was reported that they are grouped on the “*dorsal side*” of the eggs (Mukerji, 1970). The distributions of micropyles of cockroaches and termites seem to be reversed with regard

to the dorsoventral axis. Herein, we must acknowledge that isopteran embryos undergo a 180° rotation around the anteroposterior axis of the egg during development (Striebel, 1960). Isopteran micropyles are originally situated on the side where the embryo forms, which is the "*ventral side*" of the egg, as in cockroaches. Hence, we can assume that micropyles on the "ventral side" of the egg are a part of the groundplan of Blattodea (= "Blattaria" + Isoptera).

The micropyles of Blattodea and Mantodea, the dictyopteran sistergroup of Blattodea, seem to differ substantially in their localization and arrangement. For Mantodea, there is only one report on micropyles by Iwaikawa and Ogi, (1982), stating that the eggs of *Tenodera aridifolia* have a single micropyle at the center of the anterior pole of the egg, enclosed by a circular arrangement of several other micropyles. However, there are some points to be re-examined in their description, other than the distribution. For example, according to their report, the mantodean micropyles have an extraordinarily wide opening of about 4 µm. A careful re-examination of the egg structure of mantodeans would be desirable. Recently, our research group has begun a study on the micropyles of *Metallyticus splendidus* as an "ancestral" mantodean (Metallyticidae) (M. Fukui, pers. comm.), and we have re-examined the same species studied by Iwaikawa and Ogi (1982), *T. aridifolia*, as an advanced representative of the family Mantidae (Machida, unpublished). As a result, we have found several dozen small pores, identifiable as micropyles, localized on the ventral side of the egg in both species, similar to our observations in Blattodea (Fukui and Kobayashi, in press). Thus, we conclude that "micropyles grouped on the ventral side of the egg" are a part of the groundplan of the Dictyoptera, and taking into consideration that such a distribution has not been found in other insects, this is likely an apomorphic groundplan feature of this lineage.

Mycetomes. Cockroaches are known to store surplus nitrogen in the form of uric acid in the adipocytes in fat bodies, and to recycle this nitrogen source into vitamins and/or amino acids with the aid of the symbiotic *Blattabacterium* in the mycetocytes of the fat bodies (Sabree et al., 2009). The endosymbiotic *Blattabacterium* are transmitted vertically from the mycetocytes (strictly "bacteriocytes") to the offspring, via transovarial transmission (Sacchi et al., 1988; Lambiase et al., 1997). *Periplaneta* spp., *Blatta orientalis* and *Eurycotis floridana* (Blattidae); *Parcoblatta* spp. and *Blattella germanica* ("Ectobiidae"); and *Cryptocercus puncutulatus* (Cryptocercidae), all of these are known to have the "mycetome(s)," that often assume(s) the shape of a ball in oocytes and eggs (Gier, 1936; Sacchi et al., 1996).

The present study revealed that a corydiid, *Eucorydia yasumatsui*, also develops mycetomes. These appear first at the anterior and posterior poles of the eggs, then migrate towards the center of the egg, and finally fuse with each other in the middle of development. The fused mycetome then disintegrates after katatrepsis, and the liberated bacteria migrate to, and nest in, cellular masses of the mesoderm, which later differentiate into fat body elements. Mycetomes were reported previously for Blattidae, "Ectobiidae," and Cryptocercidae, and are reported now also in Corydiidae, which is currently regarded as representing one of the major clades of Blattodea (see INTRODUCTION) (e.g., McKittrick, 1964; Roth, 1991). In Isoptera, however, the mycetomes have not been confirmed, but *Blattabacterium* endosymbionts were reportedly residing in the fat bodies of *Mastotermes darwiniensis* from Mastotermitidae, the sistergroup of all other termites (Jucci, 1952). In summary, it is more parsimonious to consider the possession of endosymbiotic *Blattabacterium* in the form of mycetomes as an apomorphic groundplan feature of Blattodea; the loss of mycetomes may be regarded as an apomorphic groundplan of Isoptera.

Embryonic Development

Important features of the embryonic development of *Eucorydia yasumatsui* include the following: i) the embryo is formed by the fusion of paired blastoderm regions with higher cellular density; ii) the embryo undergoes embryogenesis of the short germ band type, with segments sequentially differentiated toward the posterior; iii) the embryo elongates to its full length on the egg surface; and iv) katatrepsis occurs, the dorsal closure proceeds, and finally the embryo acquires its definitive form, with the embryo keeping its original position on the egg's ventral side, and its anteroposterior axis not reversed.

Feature no. 2, the "embryogenesis of the short germ band type," is commonly found in Polyneoptera (e.g., Krause, 1939; Schwalm, 1988; Plecoptera: Kishimoto and Ando, 1985; Dermaptera: Shimizu, 2013; Embioptera: Jintsu, 2010; Phasmatodea: Anderson, 1972a; Orthoptera: Nelsen, 1934; Zoraptera: Mashimo et al., 2014; Grylloblattodea: Uchifune and Machida, 2005; Mantophasmatodea: Machida et al., 2004; Mantodea: Görg, 1959; Isoptera: Mukerji, 1970). In *Eucorydia yasumatsui*, a small heart-shaped embryo with no sign of segmentation forms, then with the successive differentiation of segments from anterior to posterior, the germ band elongates. This is a typical short germ band type of embryogenesis, and is also found in other cockroaches: e.g., *Blatta orientalis* (Heymons, 1895), *Periplaneta americana* (Lenoir-Rousseaux and Lender, 1970) (Blattidae), *Blattella germanica* (Wheeler, 1889) ("Ectobiidae"), *Blabera craniifer* (Bullière, 1969) (Blaberidae), and *Diploptera punctata* (Stay and Coop, 1973) (Blaberidae). This type of development is predominant in "lower insects," including apterygote entognathans and Palaeoptera (e.g., Ando, 1962; Tojo and Machida, 1997; Nakagaki et al., 2015), which indicates that this is a

plesiomorphic trait in cockroaches and Polyneoptera, and a groundplan feature of this lineage.

Concerning feature no. 3, “elongation of the embryo,” Mashimo et al. (2014) compared the manner of embryo elongation in non-holometabolan Pterygota, and distinguished two types. In Palaeoptera and Acercaria, the embryo elongates, keeping step with the immersion into the yolk. In contrast, in Polyneoptera, the full elongation of the embryo occurs on the egg surface. For the groups in which the embryo sinks into the yolk, the immersion of the embryo takes place after its full elongation on the egg surface. The embryo’s elongation type, shared by Palaeoptera and Acercaria, is obviously a groundplan feature of Pterygota and Neoptera. This implies that the pattern found in Polyneoptera is an autapomorphy of this group. The elongation of the embryo to its full length on the egg surface in *Eucorydia yasumatsui* is a condition also found in other cockroaches (e.g., Wheeler, 1889; Heymons, 1895; Bullière, 1969; Lenoir-Rousseaux and Lender, 1970; Stay and Coop, 1973).

Features no. 1, “formation of the embryo,” and no. 4, “blastokinesis,” are discussed in separate sections.

Formation of the embryo. Mashimo et al. (2014) compared the manners of embryo formation in non-holometabolan insects. Their embryogenesis is of the short germ band type, and two categories can be distinguished. In Polyneoptera, the embryo is formed by a pair of blastoderm regions with higher cellular density (e.g., Dermaptera: Shimizu, 2013; Embioptera: Jintsu, 2010; Phasmatodea: Bedford, 1970; Orthoptera: Miyawaki et al., 2004; Nakamura et al., 2010; Zoraptera: Mashimo et al., 2014; Grylloblattodea: Uchifune and Machida, 2005). In Palaeoptera and Acercaria (e.g., Ephemeroptera: Tojo and Machida, 1997, 1998; Odonata: Ando, 1962; Psocodea: Goss, 1952; Thysanoptera: Heming, 1979; Haga, 1985), cells near the posterior pole

concentrate in one area and proliferate to form the embryo. This type is also found in the apterygote ectognathan orders Archaeognatha (Machida et al., 1990) and Zygentoma (Masumoto and Machida, 2006), clearly suggesting that this is a plesiomorphic condition belonging to the groundplan of the Ectognatha and Pterygota. Consequently, the alternative type is a potential autapomorphy of Polyneoptera.

Despite the statement of Mashimo et al. (2014), the embryo formation in Blattodea was not well understood so far, mainly due to a lack of precise and detailed observations (Wheeler, 1889; Heymons, 1895). Using fluorescence microscopy with DAPI, a very sensitive DNA-specific dye, the observations of embryo formation in *Eucorydia yasumatsui* revealed that the paired lateral areas of the blastoderm with higher cellular density migrate medially, and then, further condense to fuse into a small heart-shaped embryo. This corroborates Mashimo et al. (2014) who postulated that embryo formation with the fusion of paired areas of higher cellular density is a potential autapomorphy of Polyneoptera.

Blastokinesis. In the present study, we define the terms concerning the blastokinesis as follows. Insect embryos immerse in the yolk in the early stage of development, due to the formation of the amnioserosal folds. Then the embryos elongate and take their position definitive in the pre-katatrepsis period. The descending process of embryo from the commencement of the amnioserosal fold formation up to this, is the “anatrepsis.” Usually in non-holometabolan Pterygota, the turnover of embryo is involved in the anatrepsis. After anatrepsis, the embryos develop for a period by the time katatrepsis occurs, maintaining this positioning: this phase is the “intertrepsis.” Then the withdrawal of amnioserosal fold occurs, which leads to the embryo’s reappearance on the egg surface: this ascending process is the “katatrepsis.” Usually in non-holometabolan Pterygota, drastic reversion of embryo’s axis is involved

in the katatrepsis. These processes or movements in embryonic development are collectively the “blastokinesis.”

Comprehensive surveys throughout the insect orders have revealed that the manner of blastokinesis is conservative, and blastokinesis has been used as one of the most useful comparative embryological features in phylogenetic arguments (cf. Johannsen and Butt, 1941; Ando, 1970; Anderson, 1972a, b; Schwalm, 1988; Ando and Kobayashi, 1996; Heming, 2003). However, we have a critical issue concerning the blastokinesis of cockroaches. From the cockroaches, or the single order “Blattaria,” two profoundly different types of blastokinesis have been reported. In one of them, a reversion of the anteroposterior axis of the embryo occurs; this “reversion type” (Fig. 18A) was observed in *Periplaneta americana* and *Blatta orientalis*, (Blattidae, Blattinae) (e.g., Heymons, 1895; Lenoir-Rousseaux and Lender, 1970). In the other, blastokinesis is not accompanied by reversion of the embryo’s axis; this “non-reversion type” (Fig. 18B) is known from *Blattella germanica* (“Ectobiidae,” Blattellinae) (Wheeler, 1889; Tanaka, 1976), *Blabera craniifer* (Blaberidae, Blaberinae) (Bullière, 1969), and *Diploptera punctata* (Blaberidae, Diplopterinae) (Stay and Coop, 1973). No satisfactory explanation has been given on this issue.

The present study revealed that embryos of *Eucroydia yasumatsui* develop in their original position on the ventral side of the egg without changing their anteroposterior axis throughout development. Blastokinesis in Corydiidae is categorized as the “non-reversion type,” as in “Ectobiidae” and Blaberidae. In an embryological study of Nocticolidae, one of the most controversial taxa in blattodean phylogeny (Djernæs et al., 2012, 2015), we revealed that blastokinesis is of the “non-reversion type” [data presented at 7th Dresden Meeting on Insect Phylogeny, 2015; see Fujita and Machida (2015)]. The embryos of Cryptocercidae, probably the sistertaxon of Isoptera

(e.g., Inward et al., 2007; Klass, 2009), perform blastokinesis of the “reversion type” [Thipaksorn and Machida’s unpublished data, presented at 7th Dresden Meeting on Insect Phylogeny, 2015, see Fujita and Machida (2015)]. On the basis of the newly available data, blastokinesis in cockroaches can be summarized as the “reversion type” in Blattidae and Cryptocercidae, and the “non-reversion type” in the “Ectobiidae,” Blaberidae, Corydiidae, and Nocticolidae.

A phylogenetic pattern for “Blaberoidea [= Blaberidae + Blattellidae (= Ectobiidae) + Polyphagidae (= Corydiidae) + Nocticolidae] + Blattoidea (= Blattidae + Cryptocercidae)” was suggested, based on both morphological traits (genitalia, musculature, proventriculus) and biological information (reproductive biology, oviposition behavior) (McKittrick, 1964; McKittrick and Mackerras, 1965; Roth, 1967, 1970, 1988, 1991). Although this “two-suborder system of Blattaria” was challenged by recent works based on morphology and/or DNA sequences (e.g., Klass and Meier, 2006; Lo et al., 2003, 2007; Inward et al., 2007; Djernæs et al., 2012, 2015), it is apparently reflected by the blastokinesis types reviewed above: i.e., the “reversion type” in the Blattoidea and “non-reversion type” in the Blaberoidea. Broadening focus over Dictyoptera, several studies on Isopteran families are available: Kalotermitidae (Striebel, 1960), Termopsidae (Striebel, 1960; Mukerji, 1970), Rhinotermitidae (Hu and Xu, 2005), and Termitidae (Knower, 1900). The embryos of all of these families perform blastokinesis of the “reversion type.” As for another dictyopteran order, Mantodea, several studies on the Mantidae family (Hagan, 1917; Görg, 1959) have described blastokinesis of the “non-reversion type.” Thus, in the framework of the “two-order (Mantodea-Blattodea) system of Dictyoptera,” we can summarize the blastokinesis types as: Dictyoptera = Mantodea (N) + Blattodea [= Blaberoidea (N) + Blattoidea (R) + Isoptera (R)], with “R” and “N” indicating “reversion type” and “non-reversion type,”

respectively.

The embryos of Pterygota, excluding Holometabola, almost exclusively perform blastokinesis of the “reversion type”: e.g., in general, Krause (1939), Schwalm (1988), Heming (2003) and Panfilio (2008); in Palaeoptera, Ephemeroptera: Tojo and Machida (1997); Odonata: Ando (1962); in Polyneoptera, Plecoptera: Kishimoto and Ando (1985); Dermaptera: Shimizu (2013); Embioptera: Jintsu (2010); Phasmatodea: Anderson (1972a); Orthoptera: Nelsen (1934); Zoraptera: Mashimo et al. (2014); Grylloblattodea: Uchifune and Machida (2005); Mantophasmatodea: Machida et al. (2004); Isoptera: Mukerji (1970); in Acercaria, Psocodea: Goss (1952); Thysanoptera: Heming (1979); Hemiptera: Cobben (1968). Therefore, the blastokinesis of the “reversion type” is regarded as plesiomorphic for Dictyoptera, whereas that of the “non-reversion type” apparently is apomorphic; the “non-reversion type” is unique to Mantodea and Blaberoidea, not only in Dictyoptera, but also in non-holometabolan Pterygota. Thus, we present two parsimonious phylogenetic explanations of the blastokinesis types for Dictyoptera (Fig. 19). If the ancestor of Dictyoptera had the “reversion type” blastokinesis (R in black), then 1) blastokinesis of the “non-reversion type” (N) was convergently acquired in Mantodea (N) and Blaberoidea (N), and 2) Blattodea is shown in a trichotomy of “Blaberoidea (N) + Blattoidea (R) + Isoptera (R)” (Fig. 19A). Alternatively, provided that the “non-reversion type” blastokinesis (N) was acquired as an autapomorphy of Dictyoptera, then 1) it was inherited by the lineages of Mantodea (N) and Blattodea (N), and 2) in Blattodea, the blastokinesis of the “reversion type” (R in white) was reacquired by the stem of Blattoidea and Isoptera as the autapomorphy of the assemblage (Fig. 19B).

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Fig. 1. Eggs of *Eucorydia yasumatsui*. **A:** Ventral view, anterior to the top. **B:** Lateral view, anterior to the top, dorsal to the right. **C:** Differential interference contrast microscopy of micropyles (arrowheads), anterior to the top. **D:** SEM of micropyles (arrowheads), anterior to the top. Scale bars: **A**,**B** = 500 μm; **C** = 20 μm; **D** = 10 μm.

Fig. 2. Eggs of *Eucorydia yasumatsui* in Stage 1, anterior to the top. **A:** A sagittal section of a newly laid egg in anaphase of the first maturation division, showing the cytoplasmic island. **B:** A sagittal section of an egg about 1 day after oviposition, showing two pronuclei just before conjugation. **C:** A sagittal section of an egg, showing the nucleus at the first cleavage. **D:** A sagittal section of an egg with ca. 200 nuclei, showing the anterior mycetome. AMy, anterior mycetome; Chr, chromatin of egg nucleus; CI, cytoplasmic island; CN, cleavage nucleus; Pn, pronucleus; PYN, primary yolk nucleus. Scale bars: **A–C** = 20 μm; **D** = 50 μm.

Fig. 3. Eggs of *Eucorydia yasumatsui* in Stages 1 and 2, fluorescence microscopy (DAPI staining, UV-excitation). Ventral views, anterior to the top. **A:** Stage 1, blastoderm just completed, about 5 days after oviposition. **B:** Early Stage 2, paired areas with higher cellular density (asterisks) appeared. **C:** Middle Stage 2, with a V-shaped embryo newly formed by the fusion of paired areas with higher cellular density. AMy, anterior mycetome; Bd, blastoderm; Em, embryo; PMy, posterior mycetome; Se, serosa. Scale bar: 500 μm.

Fig. 4. Eggs of *Eucorydia yasumatsui* in late Stage 3, fluorescence microscopy (DAPI staining, UV-excitation). Ventral views, anterior to the top. The embryo starts to migrate anteriorly, accompanied by elongation of the protocorm (**A**), and the embryo takes its position in the middle of the ventral surface of the egg (**B**). AMy, anterior mycetome; AnS, antennal segment; HL, head lobe; Pco, protocorm; PMy, posterior mycetome; Sd, stomodaeum; Se, serosa. Scale bar: 500 μm.

Fig. 5. Sections of eggs of *Eucorydia yasumatsui* in Stage 1, focusing on mycetomes. Anterior to the top, ventral to the left. **A:** A sagittal section of egg in Stage 1. **B:** Enlargement of anterior mycetome shown in **A**. **C:** Enlargement of posterior mycetome shown in **A**. **D:** TEM of posterior mycetome. AMy, anterior mycetome; PMy, posterior mycetome. Scale bars: **A** = 200 μm; **B**,**C** = 50 μm; **D** = 5 μm.

Fig. 6. Sections of eggs of *Eucorydia yasumatsui* in Stages 4 to 10, focusing on mycetomes. Anterior to the top, ventral to the left. **A:** A sagittal section of egg in late Stage 4. **B:** A sagittal section of egg in Stage 5. **C:** A sagittal section of egg in Stage 6. **D:** A sagittal section of egg in Stage 9. **E:** A sagittal section of egg in Stage 10. **F:** Enlargement of bacteria nesting in the mesodermal cellular mass in **E**. AMy, anterior mycetome; B, bacteria; Em, embryo; FB, fat body; My, mycetome; PMy, posterior mycetome. Scale bars: **A–E** = 200 μm; **F** = 100 μm.

Fig. 7. Embryonic development of *Eucorydia yasumatsui*, fluorescence microscopy (DAPI staining, UV-excitation). Lateral views, ventral to the left **A:** Stage 1. **B:** Stage 2. **C:** Stage 3. **D:** Stage 4. **E:** Stage 5. **F:** Stage 6. **G:** Stage 7. **H:** Stage 8. **I:** Stage 9. **J:** Stage 10. **K**: Stage 11. **L:** Stage 12. Am, amnion; AMy, anterior mycetome; An, antenna; Bd, blastoderm; CE, compound eye; Ce, cercus; CN, cleavage nucleus; Em, embryo; HC, head capsule; HL, head lobe; Lb, labium; L1-3, pro-, meso-, and metathoracic legs; Md, mandible; Mx, maxilla; My, mycetome; Pce, protocephalon; Pco, protocorm; PMy, posterior mycetome; SDO, secondary dorsal organ; Se, serosa; Sty, stylus. Scale bar: 1 mm.

Fig. 8. Embryonic development of *Eucorydia yasumatsui*, fluorescence microscopy (DAPI staining, UV-excitation). Ventral views. **A:** Stage 1. **B:** Stage 2. **C:** Stage 3. **D:** Stage 4. **E:** Stage 5. **F:** Stage 6. **G:** Stage 7. **H:** Stage 8. **I:** Stage 9. **J:** Stage 10. **K**: Stage 11. **L:** Stage 12. Am, amnion; AMy, anterior mycetome; An, antenna; AP, amniotic pore; Bd, blastoderm; CE, compound eye; Ce, cercus; CN, cleavage nucleus; Em, embryo; HC, head capsule; HL, head lobe; Lb, labium; L1-3, pro-, meso-, and metathoracic legs; Md, mandible; Mx, maxilla; Pce, protocephalon; Pco, protocorm; PMy, posterior mycetome; SDO, secondary dorsal organ; Se, serosa; Sty, stylus. Scale bar: 1 mm.

Fig. 9. SEMs of *Eucorydia yasumatsui* embryos in Stages 4 to 6. Ventral views. Each image was produced by merging separate photos using Adobe Photoshop CS2 software. **A:** Stage 4. **B:** Early Stage 5. **C:** Late Stage 5. **D:** Stage 6. An, antenna; AnS, antennal segment; Cllr, clypeolabrum; Ga, galea; Gl, glossa; HL, head lobe; IcS, intercalary segment; La, lacinia; Lb, labium; LbS, labial segment; L1-3, pro-, meso-, and metathoracic legs; Md, mandible; MdS, mandibular segment, Mx, maxilla; MxS, maxillary segment; NG, neural groove; Pd, proctodaeum; Pgl, paraglossa; Pp, pleuropodium; Sba, subanal lobe; Sd, stomodaeum; Spa, supraanal lobe; Th1-3, pro-, meso-, and metathoracic segments; I-V, IX-XI, first to fifth, and ninth to 11th abdominal segments. Scale bars: 100 μm.

Fig. 10. SEMs of *Eucorydia yasumatsui* embryos in Stages 7 to 9. Ventral views. Each image was produced by merging separate photos using Adobe Photoshop CS2 software. **A:** Stage 7. **B:** Stage 8. **C:** Stage 9. Black and white arrowheads and asterisks show spiracles, ectodermal invaginations, and developing cerebral ganglia, respectively. Ce, cercus; Cl, clypeus; Cx, coxa; Cxp, coxopodite; Fe, femur; Fl, flagellum; Ga, galea; Gl, glossa; HC, head capsule; HL, head lobe; La, lacinia; Lb, labium; Lr, labrum; L1-3, pro-, meso-, and metathoracic legs; Md, mandible; Mx, maxilla; MxP, maxillary palp; Pe, pedicellus; Pgl, paraglossa; Pta, pretarsus; Pp, pleuropodium; Sba, subanal lobe; Sc, scapus; Scx, subcoxa; Spa, supraanal lobe; Sty, stylus; Ta, tarsus; ThT1-3, pro-, meso-, and metathoracic terga; Ti, tibia; Tr, trochanter; V, X, XI, fifth, 10th, and 11th abdominal segments. Scale bars: 100 μm.

Fig. 11. SEMs of *Eucorydia yasumatsui* embryos in Stages 10 to 12. Ventral views. Each image was produced by merging separate photos using Adobe Photoshop CS2 software. **A:** Stage 10. **B:** Stage 11. **C:** Stage 12, processed with the nano-suit method. Ce, cercus; Cl, clypeus; Cx, coxa; Fe, femur; Fl, flagellum; Ga, galea; HC, head capsule; Hp, hypopharynx; Lr, labrum; L1-3, pro-, meso-, and metathoracic legs; Md, mandible; MxP, maxillary palp; Pe, pedicellus; Pta, pretarsus; Sba, subanal lobe; Sc, scapus; Scx, subcoxa; SDO, secondary dorsal organ; Spa, supraanal lobe; Sty, stylus; Ta, tarsus; ThT1-3, pro-, meso-, and metathoracic terga; Ti, tibia; Tr, trochanter; Vx, vertex; II, V, IX-XI, second, fifth, and ninth to 11th abdominal segments. Scale bars: 100 μm.

Fig. 12. SEMs of *Eucorydia yasumatsui* embryos in Stages 5 to 8. Lateral views. Each image was produced by merging separate photos using Adobe Photoshop CS2 software. **A:** Late Stage 5 (the same embryo as shown in Fig. 9C). **B:** Stage 6 (the same embryo as shown in Fig. 9D). **C:** Stage 7 (the same embryo as shown in Fig. 10A). **D:** Stage 8 (the same embryo as shown in Fig. 10B). Arrowheads and asterisk show spiracles and developing cerebral ganglia, respectively. An, antenna; Ce, cercus; Cl, clypeus; Cllr, clypeolabrum; Cx, coxa; Cxp, coxopodite; Fl, flagellum; Ga, galea; HL, head lobe; Lb, labium; LbP, labial palp; Lr, labrum; L1-3, pro-, meso-, and metathoracic legs; Md, mandible; Mx, maxilla; MxP, maxillary palp; Pd, proctodaeum; Pe, pedicellus; Pp, pleuropodium; Sba, subanal lobe; Sc, scapus; Scx, subcoxa; Sd, stomodaeum; Spa, supraanal lobe; Sty, stylus; ThT1-3, pro-, meso-, and metathoracic terga; Tr, trochanter; Y, yolk; I-V, IX-XI, first to fifth, and ninth to 11th abdominal segments; I, VT, first, and fifth abdominal terga. Scale bars: 100 μm.

Fig. 13. SEMs of *Eucorydia yasumatsui* embryos in Stages 9 to 11. Lateral views. Each image was produced by merging separate photos using Adobe Photoshop CS2 software. **A:** Stage 9 (the same embryo as shown in Fig. 10C). **B:** Stage 10 (the same embryo as shown in Fig. 11A). **C:** Stage 11 (the same embryo as shown in Fig. 11B). An, antenna; CE, compound eye; Ce, cercus; Cl, clypeus; Cx, coxa; Fe, femur; Ga, galea; HC, head capsule; Lr, labrum; Md, mandible; MxP, maxillary palp; Pf, palpifer; Pp, pleuropodium; Scx, subcoxa; SDO, secondary dorsal organ; Sty, stylus; Ta, tarsus; ThT1-3, pro-, meso-, and metathoracic terga; Ti, tibia; Tr, trochanter; Y, yolk; X, XI, 10th and 11th abdominal segments; I, V, XT, first, fifth, and 10th abdominal terga. Scale bars: 100 μm.

Fig. 14. Sections of embryos of *Eucorydia yasumatsui*. Anterior to the left, dorsal to the top. **A:** A sagittal section in late Stage 2, showing the segregation of the inner layer, or mesoderm, beneath the newly formed embryo. **B:** A sagittal section in early Stage 3, showing the developing amnioserosal fold. Inset: anterior region of posterior amnioserosal fold. Arrowheads show the apex of amnioserosal fold. **C:** A sagittal section in late Stage 4. **D:** A sagittal section in late Stage 5. **E:** A sagittal section in Stage 6. **F:** A sagittal section of postabdomen in Stage 12. Am, amnion; AmSeF, amnioserosal fold; An, antenna; AnS, antennal segment; Cllr, clypeolabrum; H, head; Lb, labium; LbS, labial segment; L1, prothoracic leg; Md, mandible; MdS, mandibular segment; Me, mesoderm; MpT, malpighian tubule; Mx, maxilla; MxS, maxillary segment; My, mycetome; Pce, protocephalon; Pco, protocorm; Pd, proctodaeum; PMy, posterior mycetome; Sba, subanal lobe; Sd, stomodaeum; Se, serosa; Spa, supraanal lobe; SYN, secondary yolk nucleus; Th1-3, pro-, meso-, and metathoracic segments; Th2,3Coe, coelomic sacs of meso- and metathoracic segments; I-III, V-X, first to third and fifth to 10th abdominal segments; I-VCoe, coelmic sacs of the first to fifth abdominal segments; VIII-XT, eighth to 10th abdominal terga. Scale bars: **A–D** = 50 μm; inset of **B** = 20 μm; **E**, **F** = 100 μm.

Fig. 15. SEMs of *Eucorydia yasumatsui* embryos in Stage 10, flagella removed. Asterisks show incisors. An, antenna; Cl, clypeus; Ga, galea; Hp, hypopharynx; LbP, labial palp; Lr, labrum; Md, mandible; MxP, maxillary palp; Pgl, paraglossa. Scale bar: 100 μm.

Fig. 16. SEMs of *Eucorydia yasumatsui* embryos in Stage 12. **A:** Median region of vertex, processed with the nano-suit method. **B,C:** Thoracic region, low-vacuum SEMs of a non-coated specimen, embryonic cuticle removed, lateral view (**B**), ventral view (**C**). Arrowheads show spiracles. **D:** Postabdomen, low-vacuum SEM of a non-coated specimen, ventral view. An, antenna; Bs, basisternum; CE, compound eye; Ce, cercus; Cx, coxa; Epm, epimeron; Eps, episternum; ET, egg tooth; Hp, hypopharynx; LbP, labial palp; Lr, labrum; Md, mandible; MxP, maxillary palp; PlS, pleural suture; SA, sternal apophysis; Sba, subanal lobe; Sty, stylus; ThT1-3, pro-, meso-, and metathoracic terga; Th2,3, meso- and metathoracic segments; Tn, trochantin; V, IX, fifth and ninth abdominal segments. Scale bars: **A** = 20 μm; **B**, **C** = 100 μm; **D** = 200 μm.

Fig. 17. SEMs of *Eucorydia yasumatsui* embryos in first instar larvae. **A:** Head, posterior view. **B:** Same specimen as **A**, with labium removed. **C:** Thoracic legs, ventral view. **D:** Distal region of mesothoracic leg. **E:** Mesothoracic sternum, ventral view. An, antenna; Bs, basisternum; Ca, cardo; Cx, coxa; Epm, epimeron; Eps, episternum; Fe, femur; Ga, galea; HC, head capsule; Hp, hypopharynx; La, lacinia; LbP, labial palp; MxP, maxillary palp; Pf, palpifer; PlS, pleural suture; Pm, postmentum; Prm, prementum; Pta, pretarsus; Spn, spina; St, stipes; Ta, tarsus; Ti, tibia; Tn, trochantin; Tr, trochanter. Scale bars: 100 μm.

Fig. 18. Two types of blastokinesis distinguished in Dictyoptera. **A:** Reversion type. **B:** Non-reversion type. See the text. Am, amnion; AmSeF, amnioserosal fold; Em, embryo; SDO, secondary dorsal organ; Se, serosa; Y, yolk.

Fig. 19. Mapping of blastokinesis types in Dictyoptera on phylogenetic trees. See the text.