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Embryonic development of *Reticulitermes speratus* (Kolbe, 1885) (Insecta: Blattodea, Rhinotermitidae): An outline

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ABSTRACT

The embryonic development of a termite, *Reticulitermes speratus* (Rhinotermitidae), was examined, and its outline was described. Observations were compared with those of previous studies to explore the groundplan of Isoptera, Blattodea, and Dictyoptera. In the species under study, micropyles are horizontally lined on the ventral side of the egg near its posterior pole. Micropyles on the ventral side of the egg are a groundplan of not only Isoptera but also Dictyoptera. The developing embryo, along with the amnion produced from its posterior, forms a small, ball-shaped embryo–amnion composite at the ventroposterior region of the egg. The ball-shaped embryo–amnion composite formed this way is an apomorphic groundplan feature of Isoptera. The embryo undergoes blastokinesis in which the reversion of the embryo's anteroposterior axis is involved, i.e., the blastokinesis of the reversion type, as in other termites. The blastokinesis of the reversion type, which is shared by the examined groups of Blattoidea, including Isoptera, may be regarded as being derived in Dictyoptera, of which the groundplan of blastokinesis may be the non-reversion type. In the late stage of development, the embryo rotates along the anteroposterior axis of the egg, as seen in other termites; this is a groundplan feature of Isoptera. The rotation of the embryo in Isoptera and Mantodea, in the latter of which the rotation occurs at an earlier developmental stage before katatrepsis, may have been independently acquired in each lineage.

KEYWORDS blastokinesis, comparative embryology, formation of embryo, Isoptera, micropyle, rotation of embryo, termites

1. INTRODUCTION

Isoptera (termites) had been regarded as one of the 11 orders of Polyneoptera, which was derived from the early explosive radiation of Neoptera, which comprises approximately 98% of Insecta in species. However, a closer affinity between Isoptera and the enigmatic wood-feeding cockroach Cryptocercidae was suggested by Cleveland et al. (1934) based on the feeding habit, behavior, sociality, and intestinal flagellate fauna. Various morphological, molecular, phylogenomic, and combined studies (e.g., Klass and Meier 2006; Inward et al. 2007; Djernæs et al. 2012; Evangelista et al. 2019) have strongly supported their sister group relationship. Thus, Isoptera is subordinate of Blattodea and is currently given an infraordinal rank within Blattodea, for which Beccaloni and Eggleton (2013) coined the new name "Epifamily Termitoidae". Blattodea, including Isoptera, and Mantodea constitute Dictyoptera, and its monophyly is strongly supported (Kristensen 1991; Beutel et al. 2014; Misof et al. 2014). Despite recent phylogenomic challenges (e.g., Misof et al. 2014; Wipfler et al. 2019; Evangelista et al. 2019), the position of Dictyoptera among Polyneoptera and the inter-relationships among major lineages in Blattodea remain unclear.

Comparative embryology is useful for reconstructing the groundplan of a group and resolving phylogenetic issues. We have been conducting comparative embryological studies on various blattodean groups (e.g., Fujita and Machida 2017; Fujita et al. 2020) to reconstruct the phylogeny and groundplan related to Blattodea. The present study focuses on termites

(Isoptera). To date, several embryological studies have been conducted on five termite families: Termitidae by Knower (1900), Strindberg (1913), Mukerji and Chowdhuri (1962), and Anderson (1972); Rhinotermitidae by Hu and Xu (2005); Kalotermitidae by Striebel (1960), Mukerji (1970), and Kawanishi (1975); Archotermopsidae by Striebel (1960); and Hodotermitidae by Roonwal and Rathore (1975). To further deepen our understanding of termite embryology, we started the embryological study using *Reticulitermes speratus* of Rhinotermitidae, which is widely distributed in Japan. As the first step of the embryological study of termites, the present study describes the outline of the embryonic development of the species and compares the results with those of previous studies to discuss the embryological issues, especially in terms of phylogenetic perspectives.

2. MATERIALS AND METHODS

Decayed trees inhabited by *Reticulitermes speratus* (Kolbe, 1885) were taken back from the woodlands in Tsukuba, Ibaraki Prefecture in May 2012 and March 2013; in Iyo, Ehime Prefecture in September 2013; and in Ueda and Nagano, Nagano Prefecture in June 2013 and August 2023. Two hundred workers, several soldiers, and a few reproductives (kings and queens) were collected from the decayed trees and reared at 25°C in a plastic case (90 mm × 60 mm × 20 mm) with a moistened paper (Comfort Service Towel; Nippon Paper Crecia Co., Ltd., Tokyo, Japan) bottom, in which some wood chips were placed. The deposited eggs were transferred to a small plastic case (35 mm × 35 mm × 15 mm) lined with moistened filter paper, and a few workers were introduced into the case to keep the eggs clean. The eggs were incubated at 28°C.

To observe the egg structure, the nano-suit method described by Takaku et al. (2013) was employed following the protocol of Fujita et al. (2016). Living eggs placed in distilled water (DW) were cleaned using an ultrasonic cleaner, transferred to a 1% polyoxyethylene sorbitan monolaurate (Tween 20) solution and immersed for 2 h. After blotting excess solution using a filter paper, the eggs were mounted on a stub and observed under a scanning electron microscope (SEM) (SM-300, TOPCON, Tokyo, Japan) under a high vacuum with an accelerating voltage of 5 kV.

For fixation, eggs were cleaned with a soft brush in Ephrussi–Beadle solution (0.75% NaCl, 0.035% KCl, 0.021% $CaCl_2$), transferred to Carl's fixative (ethyl alcohol : formalin : acetic acid : DW = 15 : 6 : 2 : 30) or alcoholic Bouin's fixative (saturated picric acid alcoholic solution : formalin : acetic acid = 15 : 5 : 1), punctuated with a fine tungsten needle, and left in the fixative for 24 h at room temperature. The eggs fixed with Carl's fixative or alcoholic Bouin's fixative were stored in 50%–70% or 90% ethyl alcohol, respectively.

The fixed eggs were stained with a DAPI (4',6-diamidino-2-phenylindole dihydrochloride, diluted to approximately 5 μg/ml with DW) for 1 week. Stained eggs were observed under a fluorescence stereomicroscope (MZ FL III + FLUO COMBI, Leica, Heerbrugg, Switzerland) with UV-excitation at 360 nm and photographed using a digital camera (Olympus E-620, Tokyo, Japan). Images with different depths of field were stacked using an image-processing software (Combine ZP, Alan Hadley; Helicon Soft Ltd., Kharkiv, Ukraine).

The fixed eggs were dehydrated through a graded series of ethyl alcohol, mediated with acetone, and embedded in methacrylate resin (Technovit 7100, Külzer, Wehrheim, Germany), following the methods of Machida et al. (1994a, b). The eggs were processed into 2-μm-thick serial sections using a semithin microtome (H-1500, Bio-Rad, Hercules, CA, USA) equipped with a tungsten carbide knife (Superhard Knife, Meiwafosis, Tokyo, Japan). The sections were stained with Mayer's acid hemalum for 2 h and 0.5% eosin G for 1 h. The stained sections were observed under a biological microscope (Optiphot-2, Nikon, Tokyo, Japan) and photographed using a CCD camera (DS-Fi2, Nikon).

3. RESULTS

3.1. Eggs

The orientation of insect eggs is defined relative to the orientation of the embryo just before hatching (Wheeler 1893), where the embryo forms on the ventral side of the egg. Unlike most insects, in Isoptera, including the present species *Reticulitermes speratus* (e.g., Striebel 1960; Kawanishi 1975), and Mantodea (e.g., Hagan 1917; Fukui et al. 2018), the embryos undergo 180° rotation around the anteroposterior axis of the egg during development (see "3.2. Embryonic development" and "4.4. Blastokinesis and rotation of embryo"). Therefore, we define the side on which the embryo develops as the ventral side of the egg, and the side opposite to it as the dorsal side of the egg.

Queens in the oviposition cycle lay several eggs each day, with egg-laying occurring over successive days. *R. speratus* eggs, of which surface is smooth, are ivory white in color, approximately 600 μm long and 300 μm wide, and have a slightly curved ellipsoid shape (Fig. 1A, B): the convex side, on which the embryo differentiates later in Stage 2, is the egg's ventral side (see Figs 2B, 3B), and the opposite concave side is the dorsal side. Five to 15 fan-shaped patterns are horizontally connected at the egg's ventral side near the posterior pole of the egg (Fig. 1A–C). Micropyles are positioned at the corners of each fan-shaped pattern (Fig. 1C).

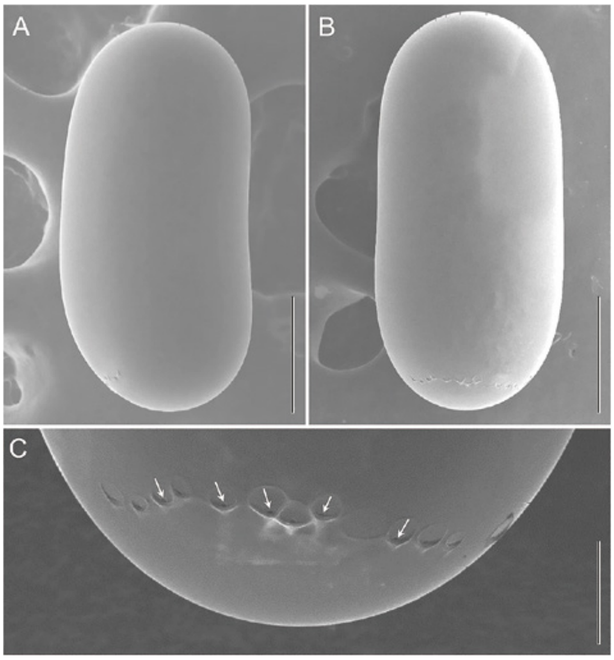

Fig. 1 SEMs of eggs of *Reticulitermes speratus*. A. Lateral view, anterior to the top, ventral to the left. B. Ventral view, anterior to the top. C. Enlarged view of the posterior ventral side of the egg, anterior to the top, showing horizontally connected fan-shaped patterns, each with a micropyle (white arrow) at its corner. Scales = A, B: 200 µm; C: 50 µm.

3.2. Embryonic development

The egg period of *Reticulitermes speratus* is approximately 25 days at 28°C. The outline of the embryonic development of the species was described, dividing it into 12 stages based on the changes in the external embryonic features.

Stage 1 (Figs 2A, 3A, 4A)

The cleavage is of the superficial type. At the fifth cleavage, some of the cleavage nuclei arrive at the egg periphery and proliferate to form a unicellular blastoderm (Figs 2A, 3A, 4A), and a small proportion of the cleavage nuclei remain in the yolk and develop into yolk nuclei (cf. Fig. 6A).

Stage 2 (Figs 2B, 3B, 4B, 5A–F, 6A, B)

Three to 5 days after oviposition (d.a.o), with the progressive proliferation of blastoderm cells on the posterior ventral side of the egg, regional differentiation occurs in the blastoderm, i.e., the embryonic and extraembryonic areas (Fig. 5A). A smaller area with higher cellular density, occupying the ventroposterior egg surface, represents the embryonic area, whereas the remaining large area of the egg's surface with lower cellular density represents the extraembryonic area. The embryonic area is more cellulated and once widened laterally (Fig. 5B, C) and condenses into a small discoidal embryo of approximately 100 µm in diameter (Fig. 5D). The extraembryonic area represents the serosa.

The posterior marginal region of the embryo curves ventrally and differentiates into the amnion as thick as the embryo proper. The produced amnion spreads over the embryo, forming the amnioserosal fold along with the serosa (Figs 5E, 6A). The amnioserosal fold finally reaches the anterior margin of the embryo, and the amniotic pore is closed. Consequently, a compressed ball-shaped "embryo–amnion composite" is formed, comprising the inner embryo, on the dorsal surface of which the mesoderm or inner layer is segregated, the outer amnion, and the amniotic cavity in between (Figs 2B, 3B, 5F, 6B). The anterior amnioserosal fold is not formed in *R. speratus*.

Stage 3 (Figs 2C, 3C, 4C)

Approximately 6 d.a.o., the embryo begins to extend along the egg surface, with its posterior end reaching and passing the posterior pole toward the dorsal side of the egg (Figs 2C, 3C, 4C). In this stage, the anterior protocephalon and posterior protocorm differentiate (Figs 2C, 3C, 4C).

Stage 4 (Figs 2D, 3D, 4D)

Approximately 7 d.a.o., the embryo further elongates, and its anterior and posterior ends reach approximately 35% of the egg length from the posterior pole (Figs 2D, 3D, 4D). The protocephalon enlarges to form the head lobe, with the newly differentiated antennal segment appearing in its posterolateral regions (Fig. 3D).

Stage 5 (Figs 2E, 3E, 4E)

Approximately 8 d.a.o., the embryo elongates along the dorsal side of the egg with its posterior end ahead, which reaches approximately >60% of the egg length from the egg's posterior pole, although its anterior end remains in the same position as in the previous stage (Figs 2E, 3E, 4E). The intercalary, gnathal, and thoracic segments differentiate (Figs 2E, 3E, 4E). The appendages develop in the mandibular, maxillary, labial, and thoracic segments but not in the intercalary segment. The stomodaeum appears at the center of the head lobe, and just anterior to it, the clypeolabrum swells (Fig. 3E).

Stage 6 (Figs 2F, 3F, 4F)

Approximately 9 d.a.o., the embryo elongates, and its posterior end reaches the anterior pole of the egg (Figs 2F, 4F). The segmentation proceeds to the sixth abdominal segment. The ventral flexure begins posterior to the sixth abdominal segment (Figs 2F, 4F). The gnathal and thoracic appendages elongate posterolaterally. The proctodaeum appears at the caudal end (Fig. 4F).

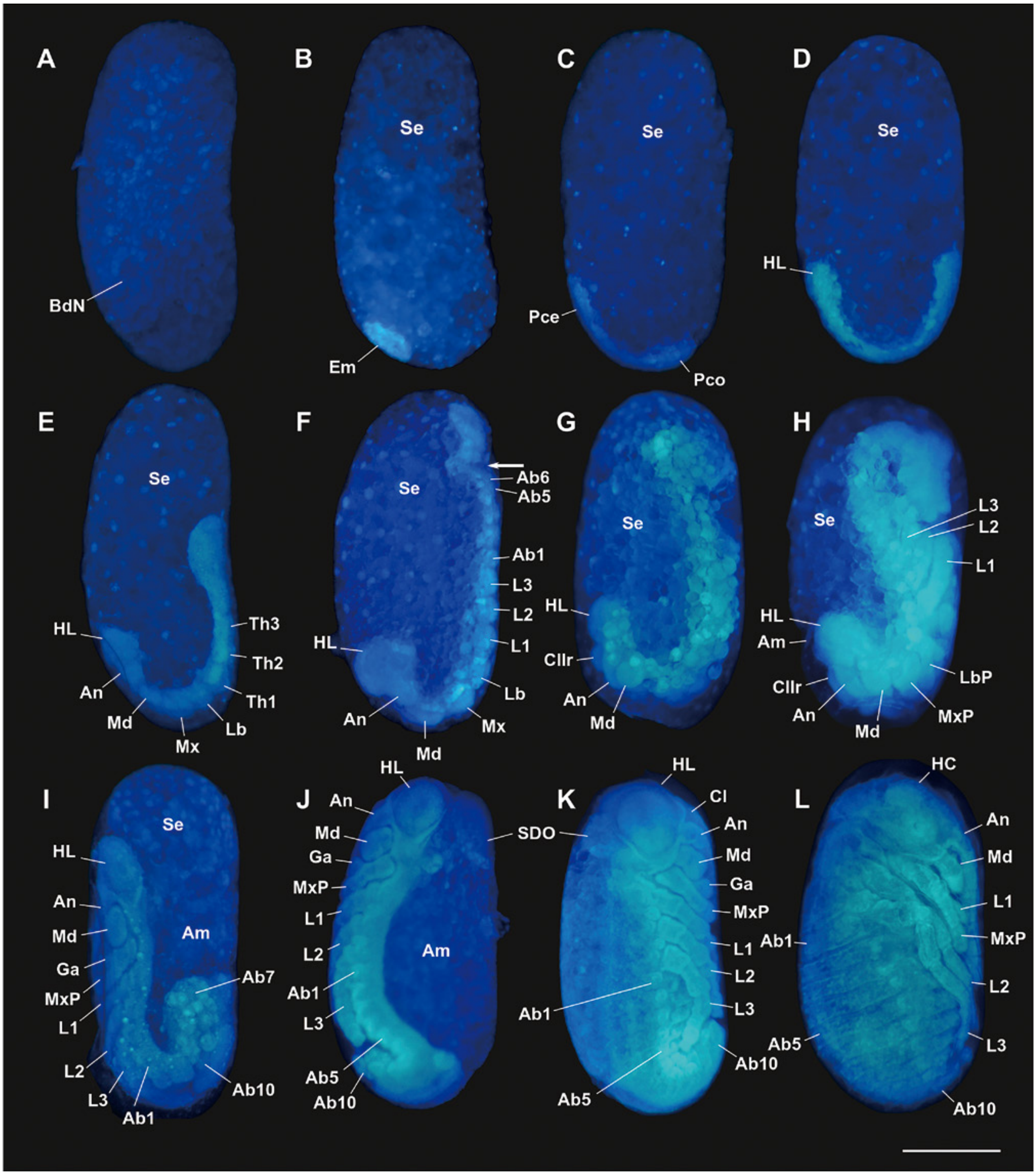

Fig. 2 Embryonic development of *Reticulitermes speratus*, fluorescence microscopy (DAPI staining, UV-excitation). Lateral views. A. Stage 1. B. Stage 2. C. Stage 3. D. Stage 4. E. Stage 5. F. Stage 6. G. Stage 7. H. Stage 8. I. Stage 9. J. Stage 10. K. Stage 11. L. Stage 12. Ab1, Ab5, Ab6, Ab7, Ab10: first, fifth, sixth, seventh and 10th abdominal segments, Am: amnion, An: antenna, BdN: blastoderm nucleus, Cl: clypeus, Cllr: clypeolabrum, Em: embryo, Ga: galea, HC: head capsule, HL: head lobe, Lb: labium, LbP: labial palp, L1–3: pro-, meso-, and metathoracic legs, Md: mandible, Mx: maxilla, MxP: maxillary palp, Pce: protocephalon, Pco: protocorm, SDO: secondary dorsal organ, Se: serosa, Th1–3: pro-, meso- and methathorax. Arrow shows the invagination of the posterior region of the abdomen into the yolk. Scale = 200 μm.

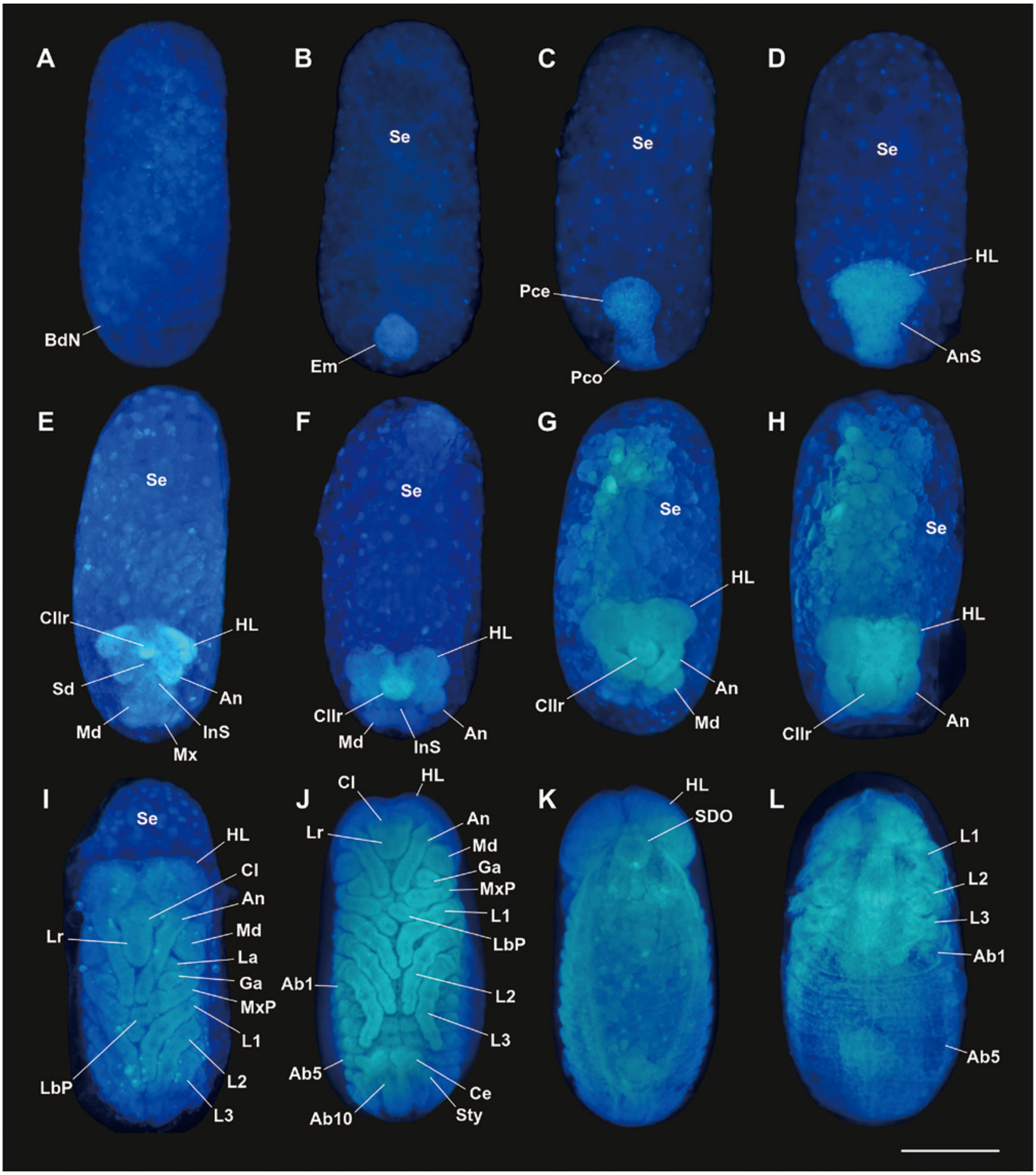

Fig. 3 Embryonic development of *Reticulitermes speratus*, fluorescence microscopy (DAPI staining, UV-excitation). Ventral views. A. Stage 1. B. Stage 2. C. Stage 3. D. Stage 4. E. Stage 5. F. Stage 6. G. Stage 7. H. Stage 8. I. Stage 9. J. Stage 10. K. Stage 11. L. Stage 12. Ab1, Ab5, Ab10: first, fifth and 10th abdominal segments, An: antenna, AnS: antennal segment, BdN: blastoderm nucleus, Ce: cercus, Cl: clypeus, Cllr: clypeolabrum, Em: embryo, Ga: galea, HL: head lobe, InS: intercalary segment, La: lacinia, LbP: labial palp, Lr: labrum, L1–3: pro-, meso-, and metathoracic legs, Md: mandible, Mx: maxilla, MxP: maxillary palp, Pce: protocephalon, Pco: protocorm, Sd: stomodaeum, SDO: secondary dorsal organ, Se: serosa, Sty: stylus. Scale = 200 µm.

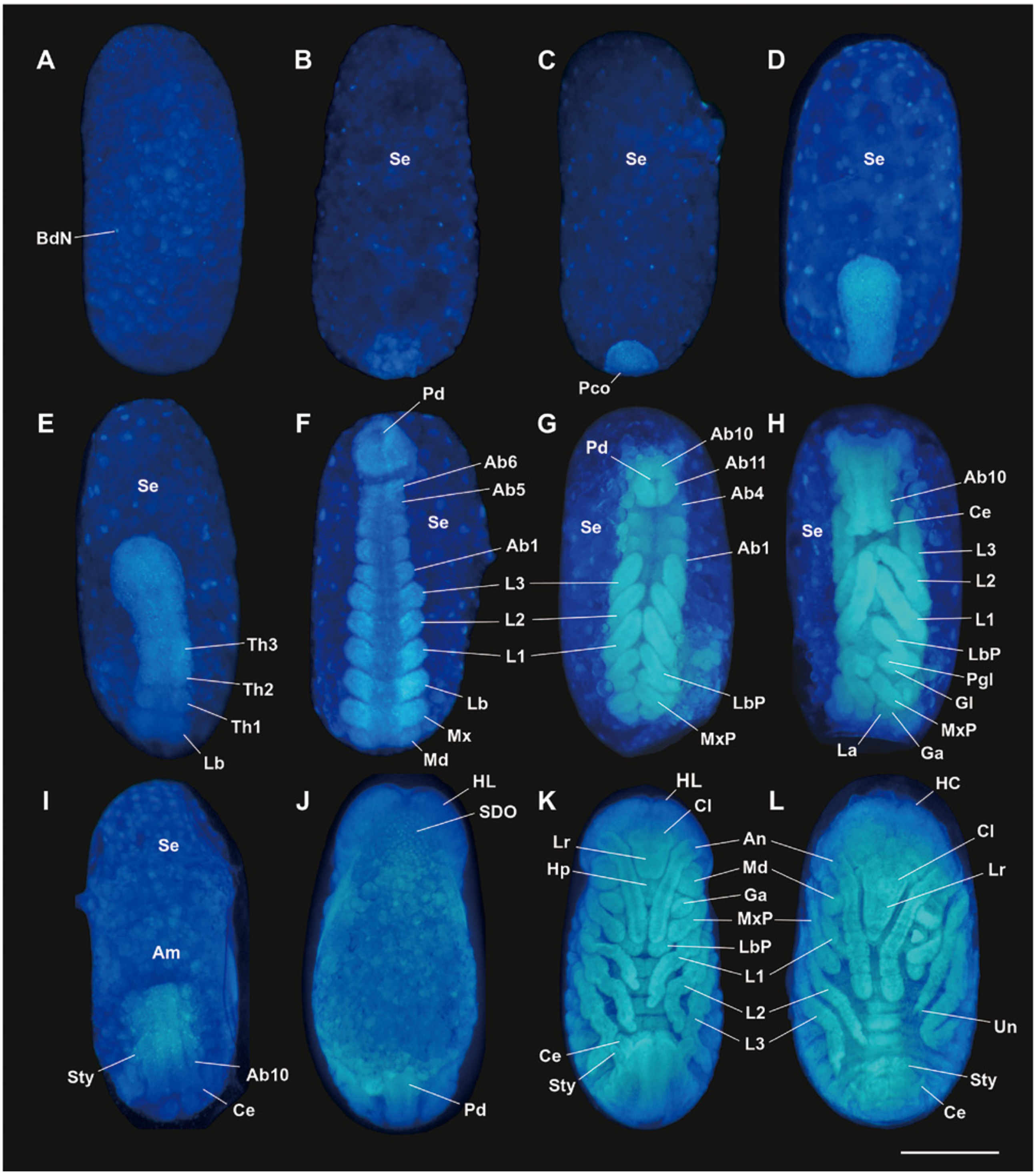

Fig. 4 Embryonic development of *Reticulitermes speratus*, fluorescence microscopy (DAPI staining, UV-excitation). Dorsal views. A. Stage 1. B. Stage 2. C. Stage 3. D. Stage 4. E. Stage 5. F. Stage 6. G. Stage 7. H. Stage 8. I. Stage 9. J. Stage 10. K. Stage 11. L. Stage 12. Ab1, Ab4, Ab5, Ab6, Ab10, Ab11: first, fourth, fifth, sixth, 10th and 11th abdominal segments, Am: amnion, An: antenna, BdN: blastoderm nucleus, Ce: cercus, Cl: clypeus, Ga: galea, Gl: glossa, HC: head capsule, HL: head lobe, Hp: hypopharynx, La: lacinia, Lb: labium, LbP: labial palp, Lr: labrum, L1–3: pro-, meso-, and metathoracic legs, Md: mandible, Mx: maxilla, MxP: maxillary palp, Pco: protocorm, Pd: proctodaeum, Pgl: paraglossa, SDO: secondary dorsal organ, Se: serosa, Sty: stylus, Th1–3: pro-, meso- and methathorax, Un: unguis. Scale = 200 μm.

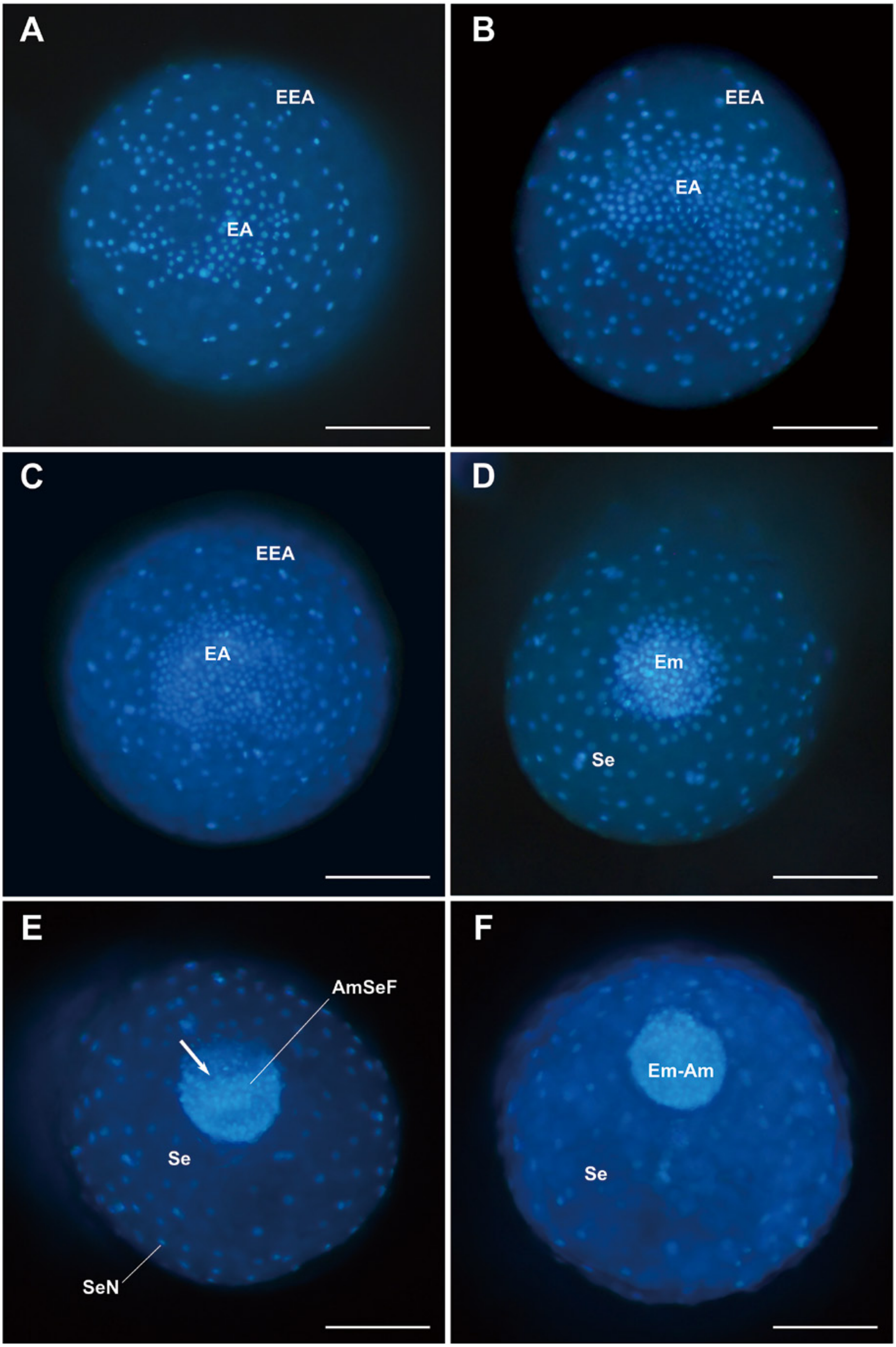

Fig. 5 Eggs of *Reticulitermes speratus* in Stage 2, fluorescence microscopy (DAPI staining, UV-excitation), successive stages A to F. Posterior views, ventral sides of the eggs to the top. A–D. Formation of embryo. E, F. Formation of amnioserosal fold and ball-shaped embryo. See the text. AmSeF: amnioserosal fold, EA: embryonic area, EEA: extraembryonic area, Em: embryo, Em-Am: embryo-amnion composite, Se: serosa, SeN: serosal nucleus. Arrow shows the frontier of spreading amnioserosal fold. Scales = 100 μm.

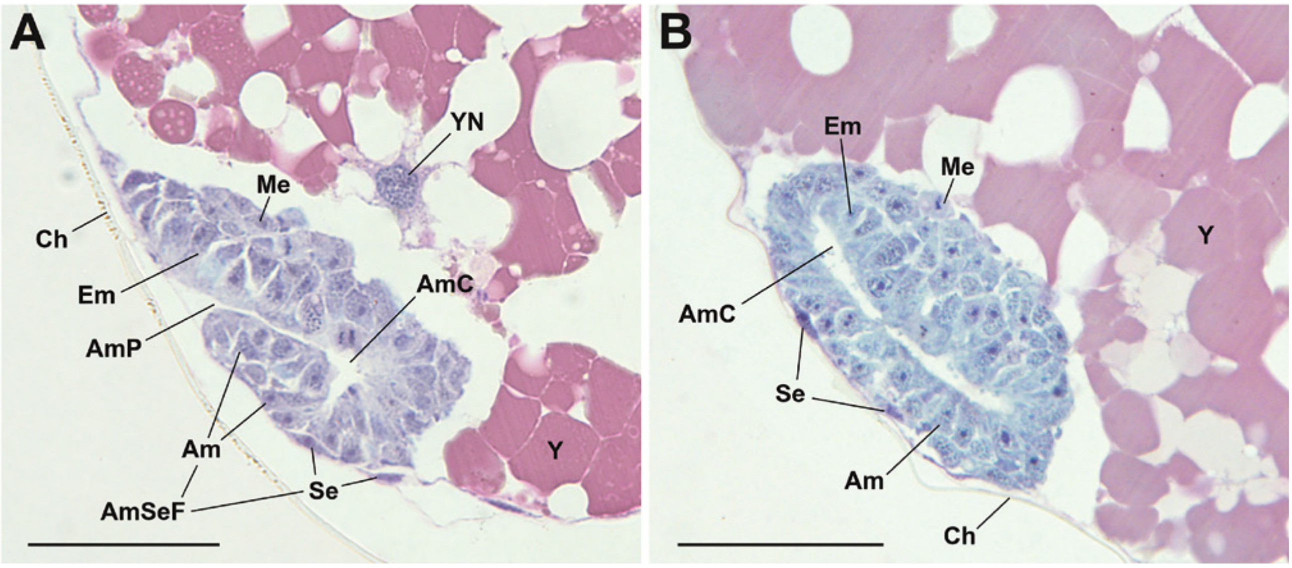

Fig. 6 Sections of eggs of *Reticulitermes speratus* in Stage 2. Anterior to the top, ventral to the left. A. Sagittal section of the egg in the same phase as shown in Fig. 5E, showing the developing amnioserosal fold. B. Sagittal section of egg in the same phase as shown in Fig. 5F, showing the ball-shaped embryo (embryo–amnion composite) just completed. Am: amnion, AmC: amniotic cavity, AmP: amniotic pore, AmSeF: amnioserosal fold, Ch: chorion, Em: embryo, Me: mesoderm, Se: serosa, Y: yolk, YN: yolk nucleus. Scales = 50 μm.

Stage 7 (Figs 2G, 3G, 4G)

Approximately 10 d.a.o., the embryo further elongates, with its abdomen anteriorly bent (ventral flexure), and 11 segments are complete in the abdomen (Figs 2G, 4G). The ventral flexure deepens, and the abdomen bends, with its posterior end directing anteriorly (Figs 2G, 4G). The appendages, which were directed posterolaterally in the previous stage, change their direction posteromedially (Fig. 4G). In the maxillary and labial appendages, the telopodites differentiate into their respective palps (Fig. 4G).

Stage 8 (Figs 2H, 3H, 4H)

Approximately 13 d.a.o., the embryo further grows. The appendages elongate, and segmentation proceeds (Figs 2H, 4H). Two endites develop in the maxillary and labial appendages, i.e., the medial lacinia and lateral galea and the medial glossa and lateral paraglossa, respectively. The cerci develop as the appendages of the 11th abdominal segment (Fig. 4H).

Stage 9 (Figs 2I, 3I, 4I)

At 15–16 d.a.o., katatrepsis occurs. The embryo moves along the ventral surface of the egg, with its head ahead, toward the anterior pole of the egg (Figs 2I, 3I, 4I). Consequently, the anteroposterior axis of the embryo is reversed, corresponding to that of the egg (cf. Figs 2J, 3J, 4J). The antennae elongate to reach the ends of the maxillary palps (Fig. 3I). The clypeolabrum is divided into the proximal clypeus and distal labrum (Fig. 3I). The appendages of the ninth abdominal segment develop into styli (Fig. 4I). The labial appendages on both sides move medially and are hidden under the maxillae and prothoracic legs (Fig. 3I).

Stage 10 (Figs 2J, 3J, 4J)

By 17 d.a.o., the serosa condenses toward the anterodorsal region of the egg to form the secondary dorsal organ (Figs 2J, 4J). The area from which the serosa regressed is occupied by the amnion, which functions as the provisional dorsal closure (Figs 2J, 4J).

Stage 11 (Figs 2K, 3K, 4K)

Approximately 20 d.a.o., the lateral walls of the embryo extend dorsally to form the definitive dorsal closure, replacing the provisional dorsal closure or amnion. The dorsum of the embryo is covered by definitive dorsal closure (Figs 2K, 3K). At this stage, the embryo undergoes 180° rotation around the anteroposterior axis of the egg, a process that takes approximately half a day. Because of rotation, the ventral and dorsal sides of the embryo reverse, facing the dorsal and ventral sides of the egg, respectively (Figs 2K, 3K, 4K). The hypopharynx develops behind the clypeolabrum (Fig. 4K).

Stage 12 (Figs 2L, 3L. 4L)

Approximately 25 d.a.o., the definitive dorsal closure completes (Figs 2L, 3L). The antennae further elongate, and the flagella are divided into 11 annuli (Fig. 4L). Paired ungues are distinct at the tips of the thoracic legs (Fig. 4L). The embryo acquires its definitive configuration.

4. DISCUSSION

4.1. Eggs

In the species *Reticulitermes speratus* of Rhinotermitidae, five to 15 micropyles are arranged in a straight line on the ventral side of the egg near its posterior pole, as reported by Yashiro and Matsuura (2014). In other termites, micropyles are also found on the ventral side of the egg, although they differ in number, position, or arrangement pattern among species. In Termitidae, in *Nasutitermes* (= *Eutermes*) *rippertii*, 12–18 micropyles are arranged in a semicircle or a single line on the ventral side of the egg near the posterior pole (Knower 1900), in *Rotunditermes* (= *Eutermes*) *rotundiceps*, on average nine, up to 12 micropyles are on the ventral side of the egg, arranged in the same manner as in *N. rippertii* (Strindberg 1913), and in *Odontotermes redemanni*, approximately 10 micropyles are arranged in a semicircular line on the ventral side of the egg near its posterior pole (Mukerji and Chowdhuri 1962). In *Kalotermes flavicolis* of Kalotermitidae, 14–26 micropyles are arranged in a straight or slightly curved line on the posterior ventral side of the eggs, and in *Zootermopsis nevadensis* of Archotermopsidae, eight to 15 micropyles are dispersed in the middle of the ventral side of the egg (Striebel 1960) [Striebel (1960) described that micropyles were on the dorsal side of the egg because he designated the convex side of the egg, which is our ventral side of the egg (see "3.1. Eggs"), as the dorsal side]. Thus, the presence of micropyles on the ventral side of the egg is a common feature in Isoptera. The eggs of Blattodea other than Isoptera (see Fujita and Machida 2017) and Mantodea (see Fukui et al. 2018) are on the ventral side of the egg. Therefore, micropyles on the ventral side of the egg can be considered as an apomorphic groundplan of Dictyoptera because this type of micropylar distribution is rarely found in Polyneoptera other than dictyopteran members.

Compared with other termites, micropyles vary widely in number in *R. speratus*. Based on evolutionary ecological studies of reproductive strategies in *R. speratus*, Yashiro and Matsuura (2014) reported that the number of micropyles decreases with aging of the queen. Once the micropyles are completely lost, the eggs develop parthenogenetically, producing only females, consequently increasing the number of successors of the queen.

4.2. Formation of embryo

Mashimo et al. (2014) proposed embryological autapomorphies for Polyneoptera, one of which concerns the formation of the embryo. In Polyneoptera, the embryo forms through the fusion of paired regions in the blastoderm that exhibit higher cellular density (e.g., Dermaptera: Shimizu and Machida 2024; Blattodea: Fujita and Machida 2017; Zoraptera: Mashimo et al. 2014; Embioptera: Jintsu 2010; Orthoptera: Miyawaki et al. 2004; Nakamura et al. 2010; Grylloblattodea: Uchifune and Machida 2005; Phasmatodea: Bedford 1970). In contrast, in other hemimetabolans, i.e., Palaeoptera (e.g., Ephemeroptera: Tojo and Machida 1997; Odonata: Ando 1962) and Acercaria (e.g., Thysanoptera: Heming 1979; Psocodea: Goss 1952; Hemiptera: Muir and Kershaw 1912; Shinji 1919), the fusion of paired regions with higher cellular density is not conspicuous (except for some hemipterans: Seidel 1924; Butt 1949; Sander 1956), and the embryo primarily forms through the simple concentration and proliferation of blastoderm cells around the posterior pole of the egg as known for the apterygote Ectognatha (e.g., Archaeognatha: Machida et al. 1990; Zygentoma: Masumoto and Machida 2006), and this mode of embryo formation is considered to be plesiomorphic in Pterygota. Consequently, the formation of the embryo by the fusion of paired blastoderm areas with higher cellular density, may be regarded as an apomorphic groundplan of Polyneoptera.

The previous studies (e.g., Knower 1900; Striebel 1960) described that the embryos of termites are formed by a simple concentration and proliferation of blastoderm cells around the posterior pole of the egg, and the embryo formation with the fusion of paired areas with higher cellular density has not been reported for termites. As a possible explanation, Shimizu and Machida (2024) suggested that this may be related to their unique early embryogenesis, in which the embryo is formed as a small, compressed ball-shaped embryo–amnion composite. However, detailed fluorescence microscopical observation for *Reticulitermes speratus* revealed that the embryonic area is once widened laterally (Fig. 5B, C) and then condenses into a small discoidal embryo (Fig. 5D). This laterally widening of embryonic area may represent the embryonic formation through the fusion of paired regions with higher cellular density, a characteristic process in Polyneoptera.

4.3. Ball-shaped embryo–amnion composite

In *Reticulitermes speratus*, the posterior margin of the developing embryo curves ventrally and differentiates into the amnion as thick as the embryo proper. The amnion spreads over the embryo, forming the amnioserosal fold together with the serosa. The anterior amnioserosal fold is not formed in *R. speratus*. The amnioserosal fold reaches the anterior margin of the embryo, the amniotic pore is closed, and a compressed ball-shaped (= sac-shaped) "embryo–amnion composite" is formed. In other termites, e.g., *Nasutitermes rippertii* (Knower 1900) and *Odontotermes redemanni* (Mukerji and Chowdhuri 1962) of Termitidae and *Kalotermes flavicolis* (Striebel 1960) and *Cryptotermes brevis* (Kawanishi 1975) of Kalotermitidae, the ball-shaped embryo–amnion composite is formed similar to that in *R. speratus* of Rhinotermitidae, and the ball-shaped embryo–amnion composite formed in this way can be a groundplan feature of Isoptera.

The ball-shaped embryo–amnion composite similar to that of Isoptera is observed in Plecoptera (Miller 1939; Kishimoto and Ando 1985; Mtow and Machida 2018a, b). However, the ball-shaped embryo–amnion composites of Isoptera and Plecoptera differ in terms of amnion formation: in Isoptera, the amnion is formed only from the posterior of the embryo (see the papers cited above), whereas in Plecoptera, it is produced from all margins of the embryo (Kishimoto and Ando 1985; Mtow and Machida 2018a). Additionally, considering that, although ball-shaped embryo–amnion composites are the structures only found in these two groups in Polyneoptera, Dictyoptera (including Isoptera) and Plecoptera are not closely related lineages according to current understanding (e.g., Misof et al. 2014; Wipfler et al. 2019), these structures may have been independently acquired by Isoptera and Plecoptera as an apomorphic groundplan for each group. The structures resembling ball-shaped embryo–amnion composites in Isoptera and Plecoptera are sporadically found in Holometabola, i.e., fireflies of Coleoptera (e.g., Ando and Kobayashi 1975; Kobayashi et al. 2001), some families of Trichoptera (e.g., Miyakawa 1973; Akaike et al. 1982), primitive groups of Lepidoptera (e.g., Kobayashi and Ando 1981; Kobayashi et al. 1981) (for Amphiesmenoptera, see Kobayashi and Ando 1988), and Panorpodidae of Mecoptera (Suzuki 1990).

4.4. Blastokinesis and rotation of embryo

In the examined termites (e.g., Termitidae: Knower 1900, Anderson 1972; Kalotermitidae: Striebel 1960; Archotermopsidae: Striebel 1960; Rhinotermitidae: Hu and Xu 2005), blastokinesis involves the reversion of the embryo's anteroposterior axis, and the present study revealed that the embryo of *Reticulitermes speratus* also undergoes blastokinesis involving the reversion of the embryo's axis, which is called blastokinesis of the "reversion type" (see Fujita and Machida 2017). Thus, blastokinesis of the reversion type can be regarded as an embryological groundplan feature of Isoptera. Fujita et al. (2020), reviewing blastokinesis in Dictyoptera, noted that the examined Blattoidea, including Isoptera of Blattodea, undergo blastokinesis of the reversion type, whereas Blaberoidea, Corydioidea, and Mantodea undergo blastokinesis without the reversion of the embryo's axis, which is called the "non-reversion type". They argued that the ancestral blastokinesis type in Dictyoptera would have been the non-reversion type in light of parsimony and that the non-reversion type of blastokinesis could be regarded as a derived feature acquired in Dictyoptera, considering that the reversion type is predominant not only in Polyneoptera but also in Hemimetabola (see also Fujita and Machida 2017). Blastokinesis of the reversion type could be a feature acquired in the lineage Blattoidea including Isoptera within Dictyoptera, of which the groundplan of blastokinesis is the non-reversion type.

In the late stage (Stage 11) of the post-katatrepsis period, the embryo of *R. speratus* undergoes 180° rotation around the anteroposterior axis of the egg, similar to other termites (e.g., Termitidae: Anderson 1972; Rhinotermitidae: Hu and Xu 2005; Kalotermitidae: Striebel 1960, Kawanishi 1975; Archotermopsidae: Striebel 1960), and the rotation of the embryo in the late stage of development may be considered a groundplan feature of Isoptera. Knower (1900) did not mention the rotation in his embryological study on *Nasutitermes rippertii* of Termitidae, but Anderson (1972, fig. 21) clearly showed that *Nasutitermes exitiosus*, which belongs to the same genus as the material Knower (1900) used, undergoes a similar rotation as other termites [Fukui et al. (2018) mistakenly cited Knower (1900) as an example referring to the rotation in Isoptera].

The rotation of the embryo occurs in Mantodea, another member of Dictyoptera (Hagan 1917; Görg 1959; Fukui et al. 2018). However, the rotation of embryo in Mantodea differs from that in Isoptera: in the former the embryo's rotation occurs, before katatrepsis, at much earlier stages than in the latter. As Fukui et al. (2018) suggested, the rotation may have been independently acquired in the lineages of Isoptera and Mantodea.

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