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Egg Membranes of a Web-spinner, *Aposthonia japonica* (Okajima) (Insecta: Embioptera)

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Abstract

The egg structure of *Aposthonia japonica* was examined using light and scanning electron microscopies. An operculum with a thickened rim or the 'opercular collar' is in the anterior part of the egg. A single micropyle is on the ventral side of the egg just posterior to the opercular collar. A small chorionic swelling or the 'polar mound' is at the posterior pole of the egg. A set of the features in *Aposthonia japonica* eggs is shared by phasmatodean eggs. This may support Zompro [*Abh. Naturwiss. Ver. Hamburg (NF)*, **37**, 1–327 (2004)] who employed egg features as evidence of a closer affinity of Embioptera and Phasmatodea.

Introduction

Polyneoptera are a group composed of eleven orders, among which phylogenetical interrelationships remain highly controversial (cf. Kristensen, 1989). Within Polyneoptera, Embioptera are one of the orders whose phylogenetical position varies most according to the discipline employed (*e. g.,* Boudreaux, 1979; Kukalová-Peck, 1991; Whiting *et al*., 2003; Terry and Whiting, 2005; Kjer *et al*., 2006). Thus, Embioptera are a significant group for reconstructing the phylogeny and groundplan of Polyneoptera. For solving phylogenetical issues, the comparative embryological approach is one of the most promising. We have made several studies on the embryology of Embioptera (Melander, 1903; Kershaw, 1914; Stefani, 1955, 1956), but our knowledge remains insufficient and highly fragmented. Against such a background, we started an embryological study on Embioptera, using *Aposthonia japonica* as materials. In the present study, as a first step, we described the egg membranes of *Aposthonia japonica*.

Materials and Methods

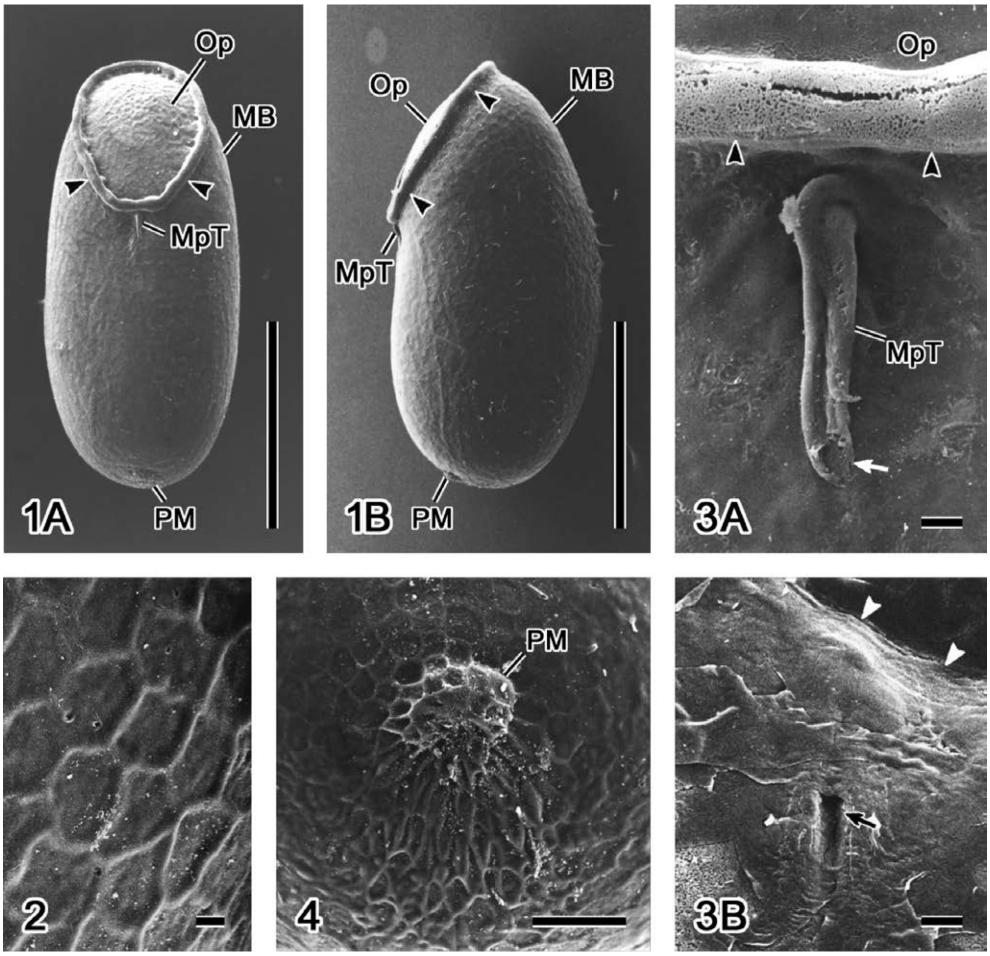
Females of *Aposthonia japonica* (Okajima) were collected from the bark mainly of palm trees in the campus of Kagoshima University (Kagoshima), in Apr. 2003, Aug. 2004, and May and Jun. 2005. Eggs, which were laid or dissected out of oviducts, were cleaned with fine forceps or a brush, and fixed with Bouin's fixative. Eggs were dried with a critical point dryer and coated with gold, then the surface structures of the eggs were observed under an SEM (TOPCON SM-300). Some of the eggs were processed into 2 μm-thick methacrylate sections in accordance with Machida *et al*. (1994a, b), which were stained with 1% Delafield's hematoxylin, 1.5% eosin, and 0.05% fast green FCF.

Results

The orientation of the egg or the anteroposterior and dorsoventral axes of the egg are designated according to those of the embryo after blastokinesis (cf. Fig. 6A). The egg of *Aposthonia japonica* is ellipsoidal, being about 1 mm in long and 0.5 mm in short diameter (Fig. 1A, B), and whitish yellow in color, the inside yolk being visible through a whitish translucent egg membranes (chorion and vitelline membrane). The chorion is elastic and shows a reticular pattern on its surface (Figs. 1A, B, 2, 4), which is more distinct at the dorsal side and around the posterior pole of the egg. A large operculum is on the anteroventral side of the egg (Fig. 1A, B). On the ventral side of the egg, just posterior to the operculum, there is a short tube, the 'micropylar tube' (Figs. 1A, B, 3A), and at the posterior pole there is a small swelling, here named the 'polar mound' (Figs. 1A, B, 4).

Operculum

The operculum, which locates in the anterior part of the egg, being inclined ventrally, is ellipsoidal and 350–400 μm in length and 250–300 μm in width (Figs. 1A, B, 5A). The operculum slightly swells, with a



Figs. 1–4 SEMs of eggs of *Aposthonia japonica* (Okajima).
Fig. 1 An egg. Anterior to the top. A. Ventral view. B. Lateral view. Ventral to the left. Arrowheads show the opercular collar. Scales = 500 μm.
Fig. 2 Enlargement of the dorsal surface of the egg. Scale = 10 μm.
Fig. 3 Micropylar structures. A. Micropylar tube, at the posterior tip of which a micropyle (arrow) opens. Arrowheads show the opercular collar. B. Inner opening (arrow) of the micropylar canal. Arrowheads show the boundary between the operculum and the main body of the egg. Scales = 10 μm.
Fig. 4 Polar mound. Ventral to the top. Scale = 50 μm.
MB: main body of the egg, MpT: micropylar tube, Op: operculum, PM: polar mound.

thickened rim or the 'opercular collar' (Fig. 1A, B). The opercular collar is structurally porous in its outer part (asterisks in Figs. 5B, 6B), and in its inner part is found a demarcation between the operculum proper and main body of the egg (arrowheads in Fig. 5B). Due to the split of the opercular collar along the demarcation (Fig. 6B), the operculum opens and the first instar larva hatches out (Fig. 6A).

Micropyle

At the posterior tip of the micropylar tube, which is a single tube of 60–75 μm in length located just posterior to the operculum on the ventral side of the egg, a micropyle of *ca*. 7 μm in diameter opens (Figs. 1A, 3A, 5A). Figure 7A is a sagittal section of a micropylar tube. The micropylar passage or the micropylar canal starts at the micropyle, runs anteriorwards up to just posterior to the opercular collar, there penetrating the chorion, reverses its direction, and runs posteriorwards just beneath the inner surface of the chorion. The inner opening of the micropylar canal is situated about 30 μm posterior to the position of the opercular collar (Fig. 3B): an arrow in Figure 7A shows the approximate position of the inner opening of the micropylar canal. Figure 7B–D is a set of transverse sections of the micropylar canal at different levels shown with B to D in Figure 7A: these sections clearly show the micropylar tube to be a structure fused with the chorion as well as the micropylar passage aforementioned.

Polar mound

The polar mound is a discoidal swelling about 60 μm in diameter, which is situated at the posterior pole of the egg, slightly biased ventrally (Figs. 1A, B, 4, 5A). The reticular pattern shown over the chorion is denser around the polar mound (Fig. 4). A section through the polar mound (Fig. 8) reveals that this structure is a thickened chorion with vertical striations running inside and that it tight contacts with the vitelline membrane.

Discussion

The general features of *Aposthonia japonica* eggs revealed in the present study are consistent with the brief descriptions previously made for other embiopteran eggs (*Embia texana*: Melander, 1903; *Embia ramburi*, *Embia mauritanica*: Krauss, 1911; *Haploembia solieri*: Stefani, 1956). Thus, we may accept *Aposthonia japonica* eggs as representing embiopteran eggs in general features.

The present study first described in detail the structure of the opercular collar. The opercular collar is composed of two parts: the outer porous part and the inner solid part with a demarcation in aid of the opening of the operculum at hatching. It is well known that the embiopteran larvae hatch out, pushing up the operculum (*e. g.*, Melander, 1903; Yokoyama, 1952).

A single micropyle is located on the ventral side of embiopteran eggs (Melander, 1903; Stefani, 1956; Hinton, 1981). As for the micropylar passage, Melander (1903) referred to it for *Embia texana* and speculated that the micropylar canal leads to the porous lumen in the opercular collar. His speculation proved to be misleading. The present study revealed that the micropylar canal anteriorly running from the micropyle does not attain the opercular collar but just posterior to it penetrates the chorion.

The reference to a swollen chorionic structure in the posterior pole of the egg or the 'polar mound' is the first for embiopteran eggs. The polar mound is a thickened chorion with vertical striations running inside, and internally it keeps a tight contact with the vitelline membrane. These features of the polar mound remind us of the hydropylar structures shown in various insects (cf. Hinton, 1981). Around the polar mound, the reticular pattern of the chorion is denser. This suggests that the concentration of follicular cells should be intensive there.

Thus, the embiopteran eggs can be characterized by: 1) an operculum at the anterior pole of the egg in aid of hatching, 2) a single micropyle on the ventral side, independent of the operculum, and 3) a polar mound at the posterior pole of the egg. In Polyneoptera, it is only the eggs of Phasmatodea that share a set of the features characterizing embiopteran eggs. The phasmatodean operculum is a circular or ellipsoidal plate in the anterior pole of the egg. At hatching, the operculum opens along the demarcation between the operculum and egg's main body (Leuzinger *et al*., 1926; Hinton, 1981; Sellick, 1997, 1998; Zompro, 2004), as in the case of embiopteran eggs. The micropyle of phasmatodean eggs has been reported to be single in the dorsal side of the egg (cf. Sellick, 1997, 1998). The embiopteran and phasmatodean micropyles seem to be in conflict with each other in position. However, this is not so. The determination of the micropylar positions in Phasmatodea was based on the eggs after embryo's blastokinesis. The phasmatodean embryos suffer from a 180 degree-rotation around the egg axis during early embryogenesis (Bedford, 1978), and so the position of micropyles in Phasmatodea should be designated originally as ventral, the same as in Embioptera, of which embryos suffer from no rotation around the egg axis during embryogenesis: note that in *Aposthonia japonica* both the embryos before (Fig. 5A) and after (Fig. 6A) blastokinesis stand with their venter to the side of the egg with micropyle or the ventral side of the egg. In the eggs of some phasmatodean forms, chorionic swellings at the posterior pole of the eggs are reported (Mazzini *et al*., 1993; Sellick, 1997, 1998; Zompro, 2004). They may be comparable to the embiopteran polar mound.

The present study revealed a close resemblance in egg structure between Embioptera and Phasmatodea, to propose an affinity of these orders. Recent molecular evolutionary (Whiting *et al*., 2003; Terry and Whiting, 2005; Kjer *et al*., 2006) and comparative morphological (Zompro, 2004) studies have suggested a close affinity of Embioptera and Phasmatodea. Zompro (2004) incorporated a comparison of egg structures in his discussion, referring to Hinton's (1981) review [based on Stefani's (1956) classical brief description on the embiopteran egg structure]: therein Zompro (2004) misleadingly described that the micropyle is situated on the dorsal side of the egg both in Embioptera and Phasmatodea.

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References

Bedford, G.O. (1978) Biology and ecology of the Phasmatodea. *Annu. Rev. Entomol*., **23**, 125–149.

Boudreaux, H.B. (1979) *Arthropod Phylogeny with Special Reference to the Insects*. John Wiley, New York.

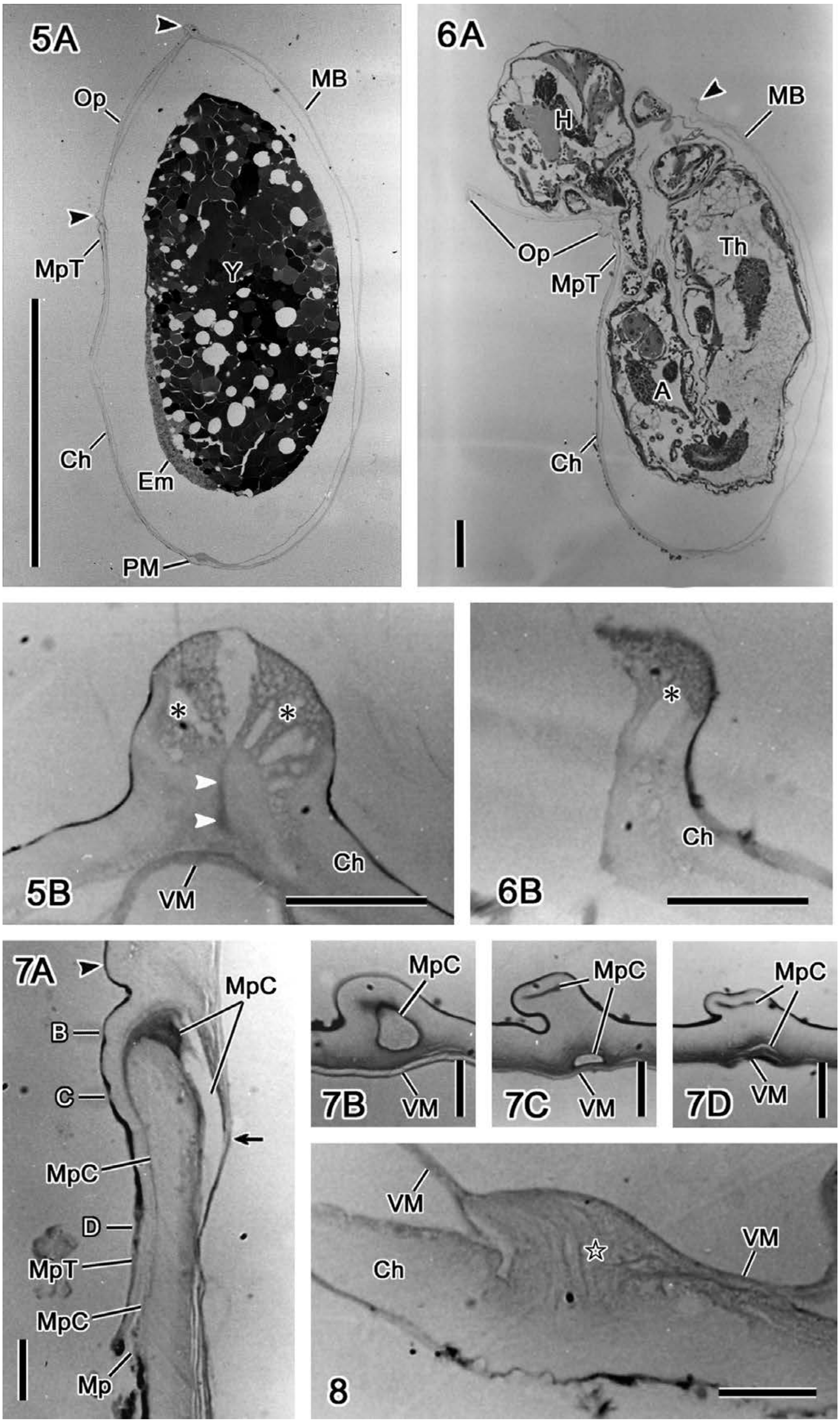
Hinton, H.E. (1981) *Biology of Insect Eggs, Vol. 2*. Pergamon Press, Oxford.

Kershaw, J.C. (1914) Development of an embiid. *J. R. Microsc. Soc*., **34**, 24–27.

Kjer, K.M., F.L. Carlie, J. Litman and J. Ware (2006) A molecular phylogeny of hexapoda. *Arthropod Syst. Phyl*., **64**, 35–44.

Krauss, H.A. (1911) Monographie der Embien. *Zoologica*, **23**, 1–78.

Kristensen, N.P. (1989) Insect phylogeny based on morphological evidence.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Figs. 5–8

In B. Fernholm *et al*. (eds.), *The Hierarcy of Life*, pp. 295–306. Elsevier, Amsterdam.

Kukalová-Peck, J. (1991) Fossil history and the evolution of hexapod structures. *In* CSIRO (ed.), *The Insects of Australia, Vol. 1, 2nd ed*., pp. 141–179. Melbourne University Press, Carlton.

Leuzinger, H., R. Wiesmann and F.E. Lehmann (1926) *Zur Kenntnis der Anatomie und Entwicklungsgeschichte der Stabheuschrecke* Carausius morosus *Br.* Gustav Fischer, Jena.

Machida, R., T. Nagashima and H. Ando (1994a) The early embryonic development of the jumping bristletail *Pedetontus unimaculatus* Machida (Hexapoda, Microcoryphia, Machilidae). *J. Morphol*., **206**, 181–195.

Machida, R., T. Nagashima and T. Yokoyama (1994b) Mesoderm segregation of a jumping bristletail, *Pedetontus unimaculatus* Machida (Hexapoda, Microcoryphia), with a note on an automatic vacuum infiltrator. *Proc. Arthropod. Embryol. Soc. Jpn*., **29**, 23–24. (in Japanese).

Mazzini, M., M. Carcupino and A.M. Fausto (1993) Egg chorion architecture in stick insects (Phasmatodea). *Int. J. Insect Morphol. Embryol*., **22**, 391–415.

Melander, A.L. (1903) Notes on the structure and development of *Embia texana*. *Biol. Bull*., **4**, 99–118.

Sellick, J.T.C. (1997) Descriptive terminology of the phasmid egg capsule, with an extended key to the phasmid genera based on egg structure. *Syst. Entomol*., **22**, 97–122.

Sellick, J.T.C. (1998) The micropylar plate of the eggs of Phasmida, with a survey of the range of plate form within the order. *Syst. Entomol*., **23**, 203–228.

Stefani, R. (1955) Divisioni amitotiche e modificazioni durante l'oogenesi nell' ovario degli Embiotteri. *Boll. Zool*., **22**, 79–91.

Stefani, R. (1956) Il problema della partenogenesi in *Haploembia solieri* Ramb. *Atti Accad. Nazionale, Lincei, Mem. Sci, Ser. 8*, **5**, 127–201.

Terry, M.D. and M.F. Whiting (2005) Mantophasmatodea and phylogeny of the lower neopterous insects. *Cladistics*, **21**, 240–257.

Whiting, M.F., S. Bradler and T. Maxwell (2003) Loss and recovery of wings in stick insects. *Nature*, **421**, 264–267.

Yokoyama, A. (1952) Ecological study on *Oligotoma japonica* O. *Bull. Fac. Educ. Kagoshima Univ*., **4**, 88–97. (in Japanese).

Zompro, O. (2004) Revision of the genera of the Areolatae, including the status of *Timema* and *Agathemera* (Insecta: Phasmatodea). *Abh. Naturwiss. Ver. Hamburg (NF)*, **37**, 1–327.

Figs. 5–8 Sections of eggs of *Aposthonia japonica* (Okajima).

Fig. 5 A. Sagittal section of an egg. Arrowheads show the opercular collar. B. Enlargement of the opercular collar shown by the upper arrowhead in A. Asterisks and arrowheads respectively show the porous structure in its outer part and a demarcation between the operculum and the main body of the egg. Scales = A: 500 μm; B: 10 μm.

Fig. 6 A. Sagittal section of an egg at hatching. Anterior to the top, ventral to the left. B. Enlargement of the opercular collar shown by an arrowhead in A. Asterisk shows the porous structure in its outer part. Scales = A: 100 μm; B: 10 μm.

Fig. 7 Sections of micropylar structures. A. Sagittal section. Anterior to the top, ventral to the left. Arrow and arrowheads respectively show the approximate position of the inner opening of the micropyle and the opercular collar. B–D. Cross sections at the levels shown by 'B,' 'C,' and 'D' in A. Ventral to the top. Scales = 10 μm.

Fig. 8 Sagittal section of the polar mound. Ventral to the left. Star shows the thickened chorion with numerous vertical striations running inside. Scale = 10 μm.

A: abdomen, Ch: chorion, Em: embryo, H: head, MB: main body of the egg, Mp: micropyle, MpC: micropylar canal, MpT: micropylar tube, Op: operculum, PM: polar mound, Th: thorax, VM: vitelline membrane, Y: yolk.