

TEM Observations of the Egg Membranes of a Webspinner, *Aposthonia japonica* (Okajima) (Insecta: Embioptera)

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Abstract

The ultrastructure of egg membranes of *Aposthonia japonica* was investigated in detail. The egg membranes are composed of: 1) an exochorion which is about 5 μm in thickness and becomes spongier towards the inside, to show a sedimented rods-like structure at the innermost, 2) an endochorion which is 0.6–0.8 μm in thickness and composed of 9–10 thin layers and columns, and 3) a single-layered vitelline membrane which is about 0.05 μm in thickness. The egg membranes are specialized at the operculum, micropyle and polar mound. At the operculum, the exochorion is denser in structure and thicker, and at the opercular collar, it swells and becomes highly spongy. The micropylar plate is the ellipsoidal area of about 150 μm in length and 100 μm in width around the micropyle, where the exochorion is denser in structure and the endochorion lacks columns. Around the polar mound, the exochorion is thickened and highly spongy, and the thin layers of the endochorion is dissociated except for its innermost layer and columns.

The ultrastructural features of the egg membranes in Embioptera closely resemble those in Phasmatodea with: 1) an endochorion composed of layers and columns, 2) a single-layered, thin vitelline membrane, and 3) specialization of the egg membranes around the operculum and micropylar plate. These similarities corroborate the close relationship between Embioptera and Phasmatodea previously proposed by us [Jintsu *et al.* (2007) *Proc. Arthropod. Embryol. Soc. Jpn.*, 42, 1–5].

Introduction

Embioptera are a small insect order distributed in tropical and temperate zones, members of which are characterized by the possession of silk glands in the first basitarsi. The interordinal relationships in Polyneoptera are highly controversial, and the placement of Embioptera in Polyneoptera is contentious. Whereas Embioptera are considered a sister group of Plecoptera with modifications of the male tenth tergum and paraprocts into copulatory claspers, suppression of phallogeres, and a reduction in the branching of veins Rs and M (Boudreaux, 1979), a close relationship between Embioptera and Zoraptera is suggested from the wing base structures (Yoshizawa, 2007), or the leg structures and biology (Engel and Grimaldi, 2000, 2006; Grimaldi and Engel, 2005). Also, the musculature of the paraglossae (Rähle, 1970) and recent molecular data (*e. g.*, Whiting *et al.*, 2003; Terry and Whiting, 2005; Kjer *et al.*, 2006) show a closer relationship between Embioptera and Phasmatodea.

There have been few studies on the egg structure of Embioptera, most of which were merely a part of studies on biology (Melander, 1903; Ross, 2000) and reproduction (Stefani, 1955, 1956; Niwa *et al.*, 1993). Jintsu *et al.* (2007) examined the eggs of Embioptera with light and

scanning electronic microscopes using a Japanese webspinner, *Aposthonia japonica*, as materials, and found a close resemblance with phasmatodean eggs including: 1) an operculum on the anterior pole of the egg, 2) a single micropyle on the ventral surface of the egg, and 3) a polar mound on the posterior pole of the egg. In the present study, the ultrastructure of the egg membranes of *Aposthonia japonica* was examined in detail by transmission electron microscopy and compared to that of Phasmatodea.

Materials and Methods

Females of *Aposthonia japonica* were collected in Kagoshima, Japan in June 2005. For light microscopy, eggs were fixed with Bouin's fixative, processed into 2- μm -thick methacrylate sections in accordance with Machida *et al.* (1994a, b), and stained with 1% Delafield's hematoxylin, 1.5% eosin, and 0.05% fast green FCF. For transmission electron microscopy, the eggs were prefixed with Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde) buffered with 0.1 M HCl-sodium cacodylate (pH 7.5) and post-fixed with 1% osmium tetroxide, dehydrated in a graded ethyl alcohol series, embedded in low viscosity Spurr's epoxy resin (Nissin EM), and processed with an ultramicrotome (RMC MT-

XL) into ultrathin sections. The sections were double-stained with uranyl acetate and lead citrate and observed under a transmission electron microscope (TOPCON LEM-2000) at 90 kV.

Results and Discussion

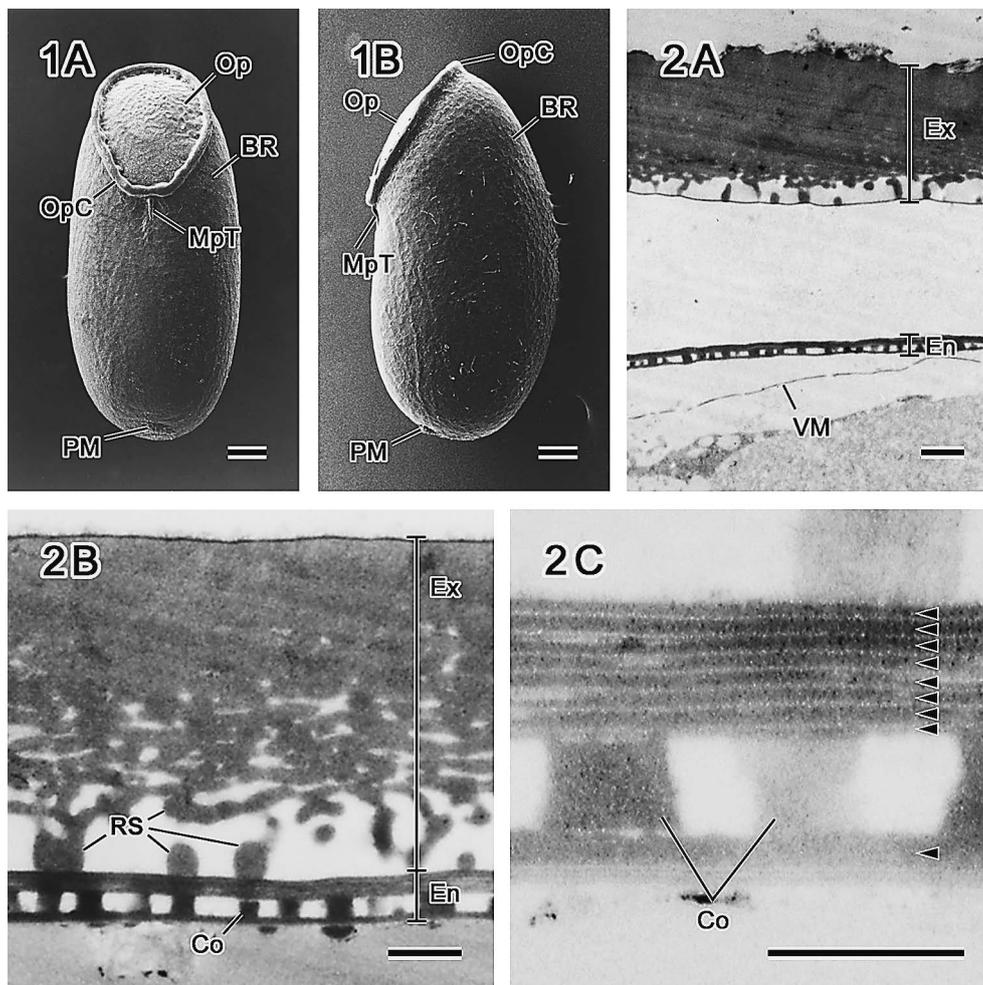
Egg structure

The general structure of *Aposhtonia japonica* eggs is as described by Jintsu *et al.* (2007). The eggs are ellipsoidal and about 1 mm in long and 0.5 mm in short diameter (Fig. 1A, B). They consist of the operculum in the anterior part and the remaining part or body region (= main body in Jintsu *et al.*, 2007). The operculum is ellipsoidal, 350–400 μm in length and 250–300 μm in width, and inclines ventrally (Figs. 1A, B, 3). It is surrounded by a thickened rim called the 'opercular collar' (Figs. 1A, B, 3). At hatching, the operculum is detached from the body region due to the splitting of the

opercular collar. On the ventral side of the egg about 5 μm from the opercular collar is a micropylar tube, which is a single tube 60–75 μm in length (Figs. 1A, B, 3, 5), and at its posterior tip a micropyle opens (Fig. 5). The micropylar passage or the micropylar canal starts at the micropyle, runs anteriorly in the micropylar tube up to just posterior to the opercular collar, then penetrating the chorion, then reverses its direction, and runs posteriorly just beneath the chorion. The inner opening of the micropylar canal is about 100 μm posterior to the opercular collar (Fig. 6). At the posterior pole of the egg slightly biased ventrally, there is a discoidal swelling 50–70 μm in diameter, named the 'polar mound' (Figs. 1A, B, 3, 9A).

Egg membranes

Transmission electron microscopy has revealed the specialization of egg membranes around the operculum,



Figs. 1, 2 General structural features of eggs and egg membranes of *Aposhtonia japonica*.

Fig. 1 SEMs of an egg (Jintsu *et al.*, 2007). A. Ventral view. B. Lateral view.

Fig. 2 TEMs of egg membranes. A. Exochorion, endochorion and vitelline membrane, each artificially separated. B. Enlargement of the exochorion and endochorion. The exochorion becomes spongier towards the inside, showing a sedimented rods-like structure. C.

Enlargement of the endochorion, which is composed of 9–10 thin layers (arrowheads) and columns.

BR: body region, Co: column, En: endochorion, Ex: exochorion, MpT: micropylar tube, Op: operculum, OpC: opercular collar, PM: polar mound, RS: rod-like structure, VM: vitelline membrane. Scales = 1: 100 μm ; 2A, B: 1 μm ; 2C: 0.5 μm .

micropyle and polar mound. First the egg membranes of the body region, and then their regional specialization, are described.

1. Egg membranes of the body region

The chorion is composed of the exochorion and endochorion, the latter more electron-dense than the former (Fig. 2A, B). The exochorion is about $5\ \mu\text{m}$ thick. Its outer half is uniform in structure, but its inner half becomes spongier towards the inside, exhibiting a sedimented rods-like structure (Fig. 2B). The endochorion is $0.6\text{--}0.8\ \mu\text{m}$ thick, and composed of 9–10 thin layers. The innermost layer (about $0.15\ \mu\text{m}$ thick) and the second innermost layer (about $0.05\ \mu\text{m}$ thick) are connected to each other by columns about $0.25\ \mu\text{m}$ in height (Fig. 2C). The vitelline membrane is a single thin layer about $0.05\ \mu\text{m}$ thick (Fig. 2A).

In a study on the oogenesis of *Aposthonia japonica*, Niwa *et al.* (1993) reported the vitelline membrane as a layer $0.5\text{--}0.8\ \mu\text{m}$ thick. This measurement is ten times as thick as ours, and corresponds to the thickness of the endochorion. It is possible that they mistook the endochorion for the vitelline membrane.

The egg membranes of Phasmatodea, similar to those of *Aposthonia japonica*, consist of an exochorion, an endochorion and a vitelline membrane (*Carausius morosus*: Hinton, 1981; Mazzini *et al.*, 1993; *Bacillus libanicus*: Moscona, 1950). The endochorions of both orders have columns. However, there are some differences as follows: 1) the exochorion shows a successive change in structure in *Aposthonia japonica*, while that in Phasmatodea is composed of clearly defined

layers [*e. g.*, three layers in *Carausius morosus* (Hinton, 1981; Mazzini *et al.*, 1993) and eight in *Bacillus libanicus* (Moscona, 1950)], and 2) the vitelline membrane of *Aposthonia japonica* is very thin, but that of Phasmatodea is rather thick (*ca.* $2\ \mu\text{m}$ in *Carausius morosus*).

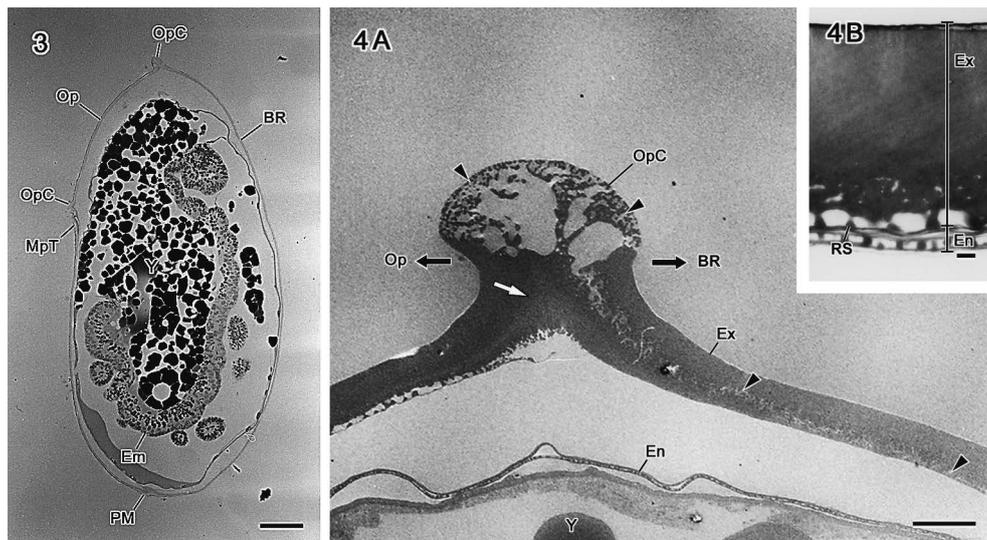
2. Specialization of the egg membranes around the operculum

The exochorion of the operculum is about $7\ \mu\text{m}$ thick, and the outer part with a uniform structure, which is about $5\ \mu\text{m}$ thick, is more thickened than the body region (Fig. 4B). At the opercular collar, the exochorion swells up to $40\text{--}50\ \mu\text{m}$ in thickness and its outer part is highly spongy (Fig. 4A), while in the inner part of the exochorion, there is an electron lucent area (white arrow in Fig. 4A), which corresponds to the demarcation between the operculum and the body region as shown in our previous histological study (Jintsu *et al.*, 2007). The spongy structure in the outer part at the opercular collar shifts and continues to the spongy structure in the body region (Fig. 4A).

In Phasmatodea, the egg membranes are also specialized in the operculum (Hinton, 1981). Phasmatodean eggs often have a projection of the exochorion or a capitulum at the center of the operculum, and in the operculum of *Carausius morosus*, the layers of the endochorion are fused together and lack columns.

3. Specialization of the egg membranes around the micropyle

The micropylar tube is a tubular elongation of the exochorion (Fig. 6). In the ellipsoidal region around the



Figs. 3, 4 Sections of eggs of *Aposthonia japonica*.

Fig. 3 Sagittal section of an egg. Ventral is to the left.

Fig. 4 Ultrastructure of egg membranes around the operculum. A. Sagittal section around the opercular collar. The operculum is to the left. Arrowheads show the spongy structure in the outer area of the opercular collar and in the body region. A white arrow shows the electron lucent area in the exochorion. B. Enlargement of egg membranes of the operculum.

BR: body region, Em: embryo, En: endochorion, Ex: exochorion, MpT: micropylar tube, Op: operculum, OpC: opercular collar, PM: polar mound, RS: rod-like structure, Y: yolk. Scales = 3: $100\ \mu\text{m}$; 4A: $10\ \mu\text{m}$; 4B: $1\ \mu\text{m}$.

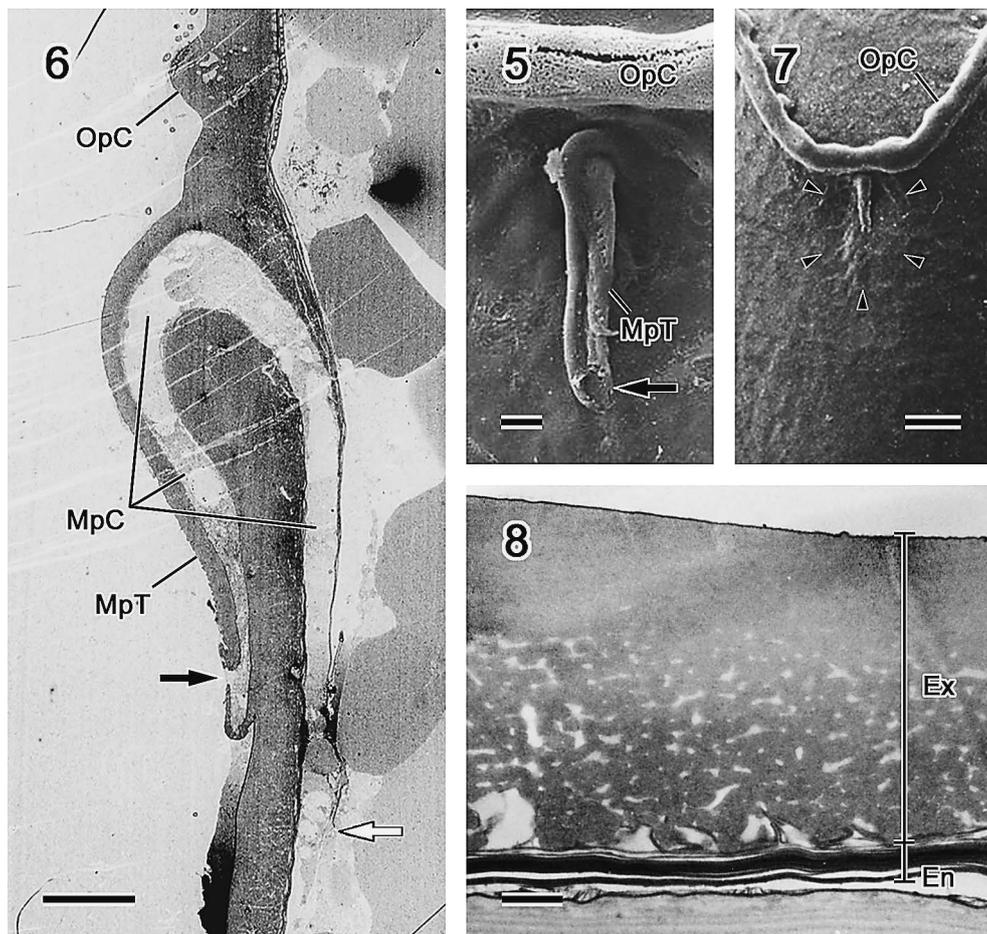
micropyle, *ca.* 150 μm long and *ca.* 100 μm wide (Fig. 7), the spongy structure of the exochorion is denser in structure than in the other areas of the body region, without a sedimented rods-like structure. In the region, the endochorion is also specialized and lacks columns (Fig. 8).

In Phasmatodea, the egg membranes around the micropyle are specialized, forming the micropylar plate (Hinton, 1981). At the micropylar plate, some of the layers comprising the exochorion become thicker and spongy. Near the micropyle, a pillar called the micropylar stalk develops, by which the exochorion and endochorion are connected. In *Carausius morosus*, the endochorion near the micropylar stalk lacks columns. As the egg membranes are specialized around the micropyle in *Aposthonia japonica* like the micropylar plate in Phasmatodea, we designate this region in *Aposthonia japonica* the micropylar plate.

4. Specialization of the egg membranes at the polar mound

At the polar mound, the exochorion is extremely thick, *ca.* 5 μm , and the inner part *ca.* 10 μm thick is spongy (Fig. 9A). The spongy structure shows numerous vertical arrangements (arrows in Fig. 9B). The layered construction of the endochorion except the innermost layer and columns is disrupted at the polar mound, and the endochorion which is represented by the innermost layer and columns is in direct contact with the spongy structure of the exochorion (Figs. 9B, C, 10B).

The serosal cuticle, a cuticular egg envelope secreted by serosa during the intertrepis stage, is specialized in structure at the polar mound. That is, it is *ca.* 2 μm thick with a clear multi-layered structure (Fig. 10A). However, that secreted beneath the polar mound is about half as thick and its layered construction is obliterated (Fig. 10B). The specialization of the serosal cuticle resembles the serosal cuticle hypople in



Figs. 5–8 Ultrastructure of egg membranes of *Aposthonia japonica* around the micropyle.

Fig. 5 SEM of a micropylar tube (Jintsu *et al.*, 2007). Anterior is toward the top. The arrow shows a micropyle.

Fig. 6 Sagittal section of a micropylar tube. Black and white arrows show a micropyle and an inner opening of the micropylar canal, respectively.

Fig. 7 Area of the micropylar plate, shown by arrowheads.

Fig. 8 Section of the egg membranes at the micropylar plate. The spongy structure of the exochorion is dense and thick, but lacks the rods-like structure. The endochorion lacks columns.

En: endochorion, Ex: exochorion, MpC: micropylar canal, MpP: micropylar plate, MpT: micropylar tube, OpC: opercular collar. Scales = 5, 6: 10 μm ; 7: 50 μm ; 8: 1 μm .

caeliferans, *Melanoplus differentialis* (Slifer, 1938; Slifer and Sekhon, 1963) and *Locusta pardalina* (Matthee, 1951). The serosal cuticle hydropyle is secreted by large serosal cells or the hydropyle cells (Hinton, 1981), but such specialized serosal cells have not been observed in *Aposthonia japonica* eggs.

The findings in the polar mound lead us to suspect that the structure is a chorionic hydropylar apparatus. Phasmatodean eggs usually develop a projection at the posterior pole, which varies greatly in shape among species (Sellick, 1997). There have been no ultrastructural studies on these projections, but they are likely to be homologous to the polar mound of *Aposthonia japonica*.

Comparison of embiopteran and phasmatodean egg membranes

The egg membranes of *Aposthonia japonica* are characterized by: 1) an exochorion which becomes spongier towards the inside, 2) an endochorion

composed of 9–10 thin layers and columns, and 3) a single-layered, thin vitelline membrane, and are regionally specialized as follows: 4) the exochorion of the operculum is denser and thicker, and at the opercular collar is swollen and highly spongy, 5) the egg membranes around the micropyle are specialized as a micropylar plate, where the exochorion is denser in structure and the endochorion lacks columns, and 6) the exochorion around the polar mound is thickened and highly spongy, and the thin layers of the endochorion around the polar mound is dissociated except for its innermost layer and columns.

The ultrastructural features of egg membranes revealed in Embioptera resemble those reported in Phasmatodea, including 1) an endochorion composed of layers and columns, 2) a single-layered vitelline membrane, and 3) specialization of the egg membranes around the operculum and micropyle. These similarities are consistent with the close relationship between these two orders which we (Jintsu *et al.*, 2007) previously

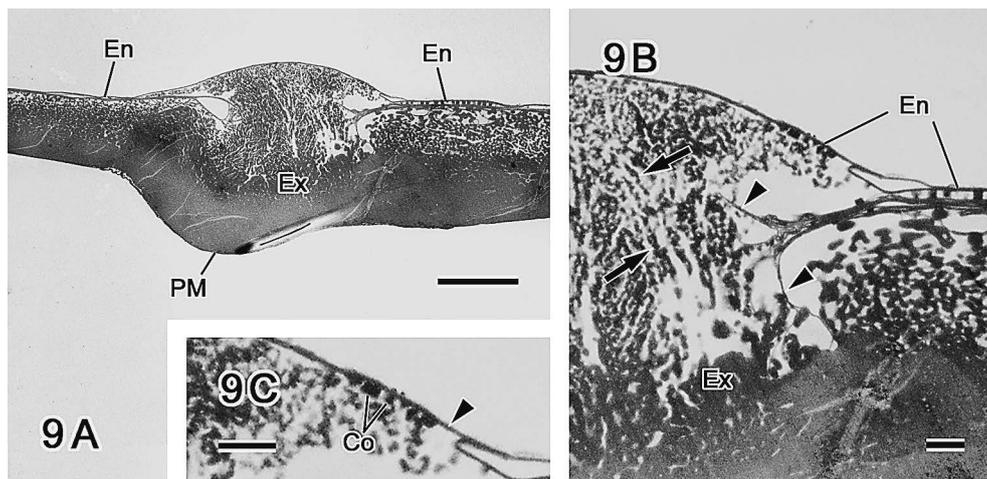


Fig. 9 Ultrastructure of egg membranes of *Aposthonia japonica* around the polar mound. A. Sagittal section of a polar mound. Anterior is toward the top and ventral is to the left. B. Enlargement of the dorsal part of the polar mound. Thin layers (arrowheads) of the endochorion except the innermost layer and columns are dissociated, and the spongy structure of the exochorion fills between these layers. There are numerous vertical arrangements (arrows) in the spongy structure of the exochorion. C. Enlargement of the innermost layer and columns of the endochorion. Co: column, En: endochorion, Ex: exochorion, PM: polar mound. Scales = 9A: 10 μ m; 9B, C: 1 μ m.

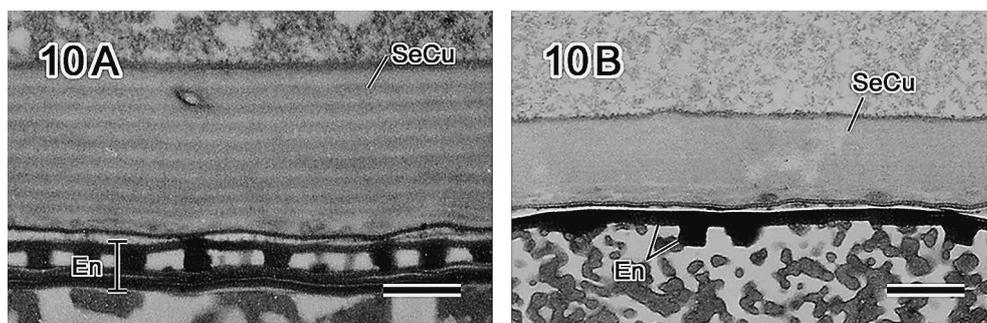


Fig. 10 Sections of the serosal cuticle of *Aposthonia japonica*. The inside of the egg is toward the top. A. General structure of the serosal cuticle. B. Serosal cuticle secreted beneath the polar mound. This section also clearly shows that the endochorion is dissociated except for the innermost layer and columns in the polar mound. En: endochorion, SeCu: serosal cuticle. Scales = 1 μ m.

proposed based on general features of the eggs.

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