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[SHORT COMMUNICATION]

Usefulness of low-vacuum scanning electron microscopy in descriptive insect embryology

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Scanning electron microscopy enabling observation at high resolution and increased depth is very useful in the field of descriptive insect embryology. However, it does present a problem in observation of later stage embryos. In the later stage embryos in which an embryonic cuticle has been secreted but not yet digested, the embryonic cuticular layer is often swollen and separated from the embryo proper. When such specimens are coated by ion-sputtering and observed by an ordinary high-vacuum scanning electron microscope, we only see the surface structure of the embryonic cuticle which covers the embryo either tightly as a wrinkled sheath or sometimes loosely like a bladder, and the true surface structures of the embryo cannot be observed (*e. g.*, Fig. 2A, B).

In order to solve this problem, I tried to observe non-coated, later stage embryos of a jumping bristletail *Pedetontus unimaculatus* [embryos with almost completed dorsal closure which have finished embryonic cuticular secretion: *i. e.*, stages 12 to 13 of Machida (1981)] under a low-vacuum scanning electron microscope. The resolution of images was not very high, but it was possible to satisfactorily observe the surface structures of the embryos themselves through the embryonic cuticular layer. I succeeded in obtaining images possibly suggestive of mandibular basal articulation which have not previously been well documented (Fig. 1A, B; cf. Machida, 2000).

I achieved a high performance level of low-vacuum scanning electron microscopy of non-coated specimens in descriptive insect embryology, which is reported here. The procedure used is as follows.

1. *Pedetontus unimaculatus* later stage embryos dissected out of eggs in physiological saline, were anesthetized with adding a few drops of saline solution mixed with chloroform to the solution containing themselves.
2. Embryos dissected out were rinsed several times with fresh saline.
3. Rinsed embryos were fixed with 4% paraformaldehyde solution for 1 h, rinsed with buffer solution, and postfixed with 1% OsO_4 solution for 3 h. In low-vacuum scanning electron microscopy, OsO_4 fixation is indispensable for the improvement of the resolution of images and the structural stability of specimens.
4. Fixed embryos were rinsed with water, dehydrated through a graded acetone series, and dried using a critical point dryer.
5. Processed embryos were observed under a scanning electron microscope TOPCON SM–300 Wet–4, as reflected electron images with a robinson detector, at an accelerating voltage of 20 kV under the pressure of 13 Pa. With a higher degree of vacuum, the resolution of images increases, but the specimens are apt to be electrically charged.

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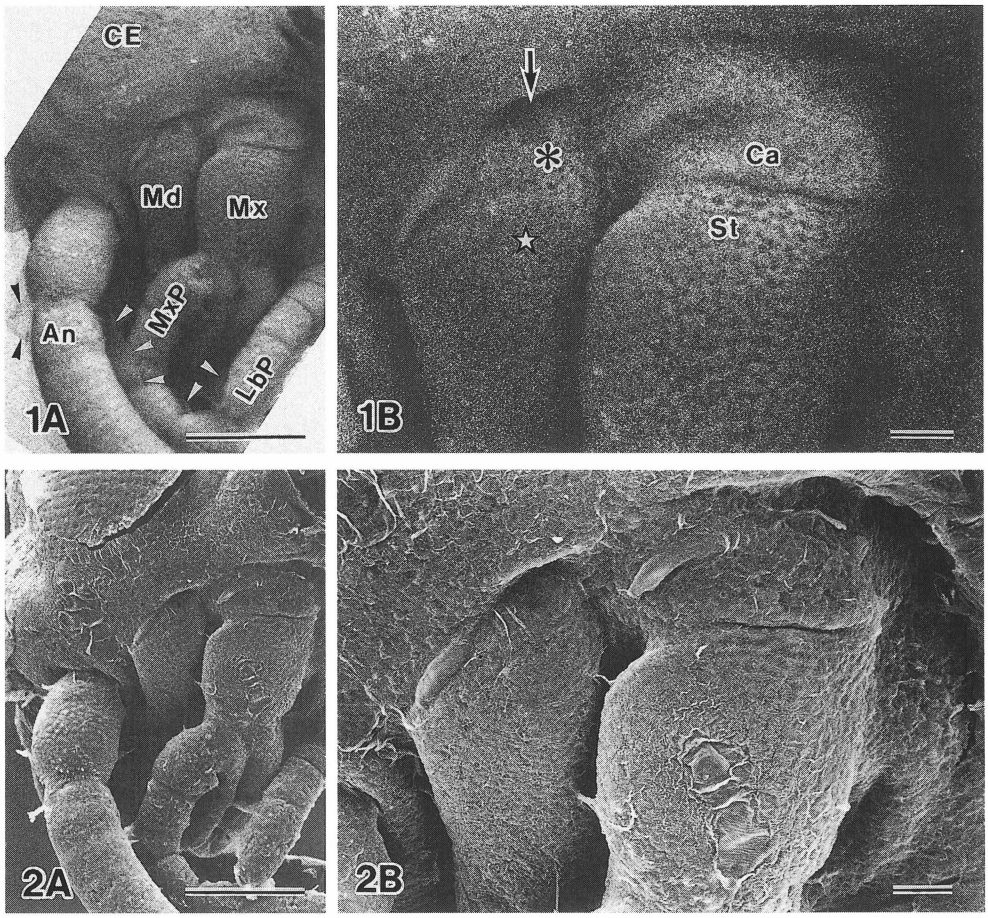

Fig. 1 A. A low-vacuum scanning electron micrograph of *Pedetontus unimaculatus* grown embryo, non-coated. Arrowheads show the embryonic cuticular layer, through which the surface structure of the embryo itself can be observed. B. Enlargement of the basal regions of the mandible and maxilla. The maxilla is proximally divided into the cardo and stipes. Likewise, it is well documented that the mandible is proximally divided into two parts shown by an asterisk and a star and they are homologized to the maxillary cardo and stipes, respectively. The arrowhead in the mandible is the precursor of the condyle. For details, see Machida (2000).

Fig. 2 A. An ordinary high-vacuum scanning electron micrograph of the same specimen as shown in Fig. 1, gold-coated. The embryonic cuticular layer conceals and adheres to the embryonic surface as a wrinkled sheath. B. Enlargement of the basal regions of the mandible and maxilla. The basal partition in the maxilla is recognizable, but that in the mandible is not detected.

An: antenna, Ca: cardo, CE: compound eye, Md: mandible, Mx: maxilla, MxP: maxillary palp, LbP: labial palp, St: stipes. Bars = Figs. 1A, 2A, 100 μm; Figs. 1B, 2B, 20 μm.

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