

Marsupial Development and Molt Cycle of *Archaeomysis kokuboi* (Crustacea: Mysidacea)

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Ovarian cycle, marsupial development and molt cycle of *Archaeomysis kokuboi* were studied to understand these processes as a whole event. Based on morphological characters the marsupial development is divided into 3 stages, Embryonic stage (duration time of 4 days), Nauplioid stage (5 days) and Postnauplioid stage (4 days). Morphological description was made for the 3 stages, and each stage was examined in relation to the corresponding stages of ovarian cycle and molt cycle.

Key words: *Archaeomysis kokuboi*, Marsupial development, Ovarian cycle, Molt cycle

Introduction

Crustacean reproduction is related to the molt cycle. Determination of the successive stages of the molt cycle is useful to understand the ovarian cycle and embryonic development in the marsupium of the adults. Most of studies on the molt cycle and the development in crustaceans have been known in various crustaceans.

Cuzin-Roudy and Tchernigovtzeff (1985) studied on a planktonic mysid, *Siriella armata* which is a marine species that lives in shoals or in swarms in the shallow water.

The molt cycles of crustaceans were formulated by Drach (1939), Passano (1960) and Aiken (1968). Successive stages of the molt cycle has been identified by the integumental observation and developmental changes in the setae (Drach and Tchernigovtzeff, 1967). The method has been adopted to a wide variety of crustaceans (Lyle and MacDonald, 1983). Electron microscopical studies have been useful for describing the details of the deposition of cuticular layers by epidermal cells (Green and Neff, 1972).

Marsupial development and the morphological characters of the mysid larvae have been described by the various authors. Matsudaira et al. (1952) and Brown and Talbot (1972) described embryonic development of *Gastrosaccus vulgaris* and *G. psammodytes*, respectively.

Cuzin-Roudy and Tchernigovtzeff (1985) attempted to relate the female molt cycle and the marsupial development of the larvae of *S. armata*. They observed antennal scale and telson only in the postmolt and the premolt stages of female.

In the present study, *Archaeomysis kokuboi* was examined to describe its marsupial development, ovarian cycle and molt cycle in relation to the cuticular cycle. The stages of the molt cycle of the female were determined by the state of development of the embryos in the marsupium. The results were compared to those of the previous studies on other mysid species.

Materials and Methods

A. kokuboi was collected from Songjeong sandy beach in March and May, 1992 by a modified R-H push net and a D-hand net during the low tide periods. The large females (total length: 20~24

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mm) were observed for ovarian cycle, marsupial development and molt cycle. Twenty females were numbered and individually reared without feeding in a glass bottle of 500 mL. They were daily taken out by a plastic spoon to examine their embryos under a dissecting microscope in live condition. Since the oostegites were transparent, development of ovaries and embryos can be easily observed in a short time without serious physical damages of the females.

For the morphological description of the embryos and the larvae, the large live females were sorted out from the field samples, and their embryos and larvae were taken out from the marsupium under a dissecting microscope. To determine the cuticle cycle, changes of the integument of the lateral spines and the setae of uropod were observed. Based on the photomicroscopic pictures, degree of retraction of soft tissues following apolysis were measured. It was estimated as the ratio of distance of epidermis from the cuticle to the thickness of the cuticle. The ovaries of live females were examined to estimate ovarian expansion which was defined as the ratio of thorax height occupied by the ovary in the lateral view.

For TEM microscopy telsons of females were measurably excised to 1 mm in length, and they were fixed in 2.5% glutaraldehyde at 4°C for 2~4 hrs, and then fixed in 1% osmium tetroxide (OsO_4) at 4°C for 2 hrs. Fixed specimens were rinsed in 0.1 M phosphate buffer (pH 7.3~7.4), and dehydrated in increasing ethyl alcohol concentrations for 10~20 min, respectively, and embedded in a mixture of Epon (A+B) and propylene oxide for 1~3 hrs. Embedded specimens were polymerized at 37°C for 12 hrs, and at 60°C for 48 hrs. Polymerized specimens were embedded in Epon 812. For TEM microscopy semi-thin sections (LKB ultramicrotome, Nova, Sweden) were cut, and contrast of the sectioned materials was enhanced with toluidine blue, and after observation they were trimmed. The trimmed parts were attached to copper grids with 200 mesh in size and cut (Ultra-thin section). The sections were double-strained with uranyl acetate and lead citrate, and viewed with a TEM (JEM 1200 Ex-II, JEOL).

Results

Oogenesis and oviposition

In the mature female the ovary is located in the interspace between an alimentary canal and pericardial floor, and the developing eggs in the ovarian tube can be observed through the transparent carapace in live condition. The eggs are arranged in a row in the two parallel ovarian tubes.

To know the oviposition time the mysids were samples every 4 hrs for 24 hrs from 06:00 o'clock. Based on relative abundance of the females carrying the embryos of different developmental stages, the samples collected in the early morning have more females carrying the newly-laid eggs. This suggests that the oviposition occurred mainly during night time.

The newly-laid eggs are an unfertilized egg mass within the marsupium (Fig. 1, A). After fertilization the individual eggs are recognized (Fig. 1, B). Yolk material is evenly diffused within the egg, and a vitelline membrane is present. In the later stages the eggs are clustered by membranous material (Fig. 1, C) within the marsupium.

Marsupial development of embryos and larvae

According to the developmental stages proposed by Wittmann (1981), the marsupial development of *A. kokuboi* was divided into the following 3 stages: (1) Embryonic stage with development within the egg membrane; (2) Nauplioid stage after egg hatching; (3) Postnauplioid stage after the nauplioid molt. In addition to the 3 main stages, Embryonic stage can be further subdivided by morphological characters of the embryos.

Embryonic stage

Duration: 4 days

Embryonic stage begins with the egg is fertilized in the marsupium and lasts until the outer membrane bursts and sloughs off. Eggs have a very thin, inner membrane tightly applied to the uncolored yolk. They appear homogeneously in structure and are full of refringent yolk globules. Although the shape of the egg varies, most eggs are nearly spherical (Fig. 1, D). Size of the eggs is fairly constant, about 0.64 mm long and ca. 0.54 mm wide. In the posterior region of the germ a minute invagi-

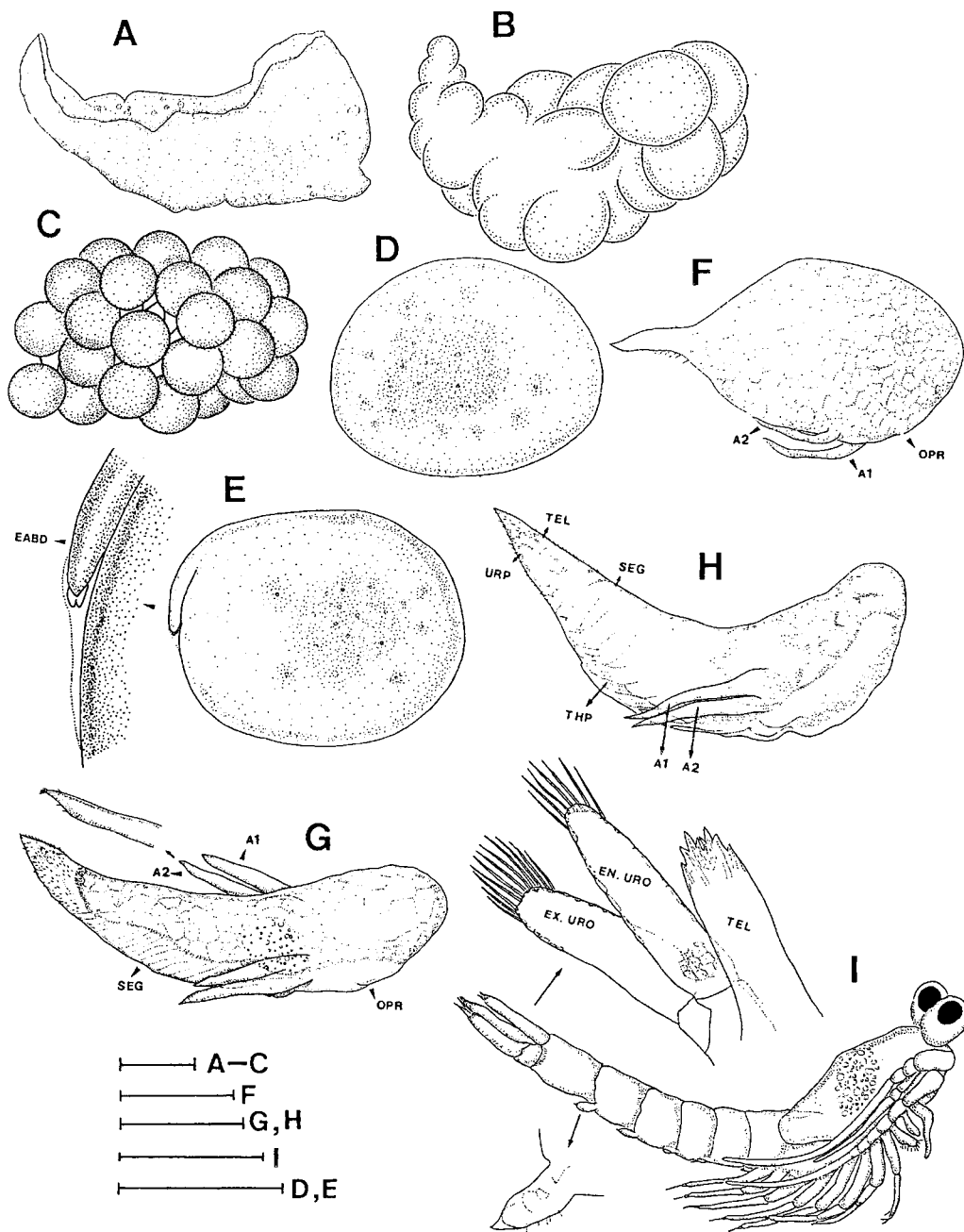


fig. 1. *A. kokuboi*. A~C: The newly-laid egg mass within the marsupium of the ovi-gerous female. A, unfertilized egg mass within the marsupium. B, fertilized egg mass within the marsupium after 24 hours of copulation. C, egg mass within the marsupium. D, E: the Embryonic stage. D, the early Embryonic stage; the egg is already surrounded by a continuous blastoderm. E, The late Embryonic stage; stretching of the embryonic abdomen starts, in this way hatching is initiated (EABD: embryonic abdomen). F~H: the Nauplioid stage. F, the early Nauplioid stage; a short period after hatching, the larval abdomen is already strongly bent dorsally. G, Nauplioid stage; the body segmentation is already clearly visible ventrally but not dorsally. H, the late Nauplioid stage; the larval abdomen is well-segmented over all of its part; the thoracopods and uropods appear; the old cuticle starts to separate from the body at the terminal tip of the abdomen (A1: antennule, A2: antenna, OPR: optical rudiment, SEG: segmentation of body, THP: thoracopod, URP: uropod, TEL: telson). I: Postnauplioid stage; al. appendages of the adult stage are present and already free but less specialized; there are still the large dorsal yolk mass (TEL: telson, EN. URO: endopod of uropod, EX. URO: exonod of uropod). Scale bars=0.3 mm.

nation appears to separate the abdominal rudiment. The invagination becomes progressively deeper into the embryos. The embryonic abdomen continues to grow in the anterior direction and in this way comes to lie folded back over the remainder of the body. At the end of the embryonic abdomen there is an anal appendix (Fig. 1, E). With development the thin egg membrane is apparent around the egg. The embryonic abdomen is now a long cod-like structure which folds back over the embryos end already reaches the region of the nauplius appendages. The buds of antennule and antenna continue to grow, and in some individuals the bud of antenna is biramous.

Nauplioid stage

Duration: 5 days

This stage is from hatching prior to the first naupliar molting within the marsupium. Just after hatching the nauplioid larvae (Fig. 1, F) is a comma form with the buds of antennule and antenna. The main body mass is represented by yolk. The abdomen represents a cod-like process, and in most case it is still slightly bent ventrally. With development the segmentation of thorax and abdomen (Fig. 1, G) becomes clearly visible. The dorsal part in the larva is still occupied by yolk. The larva develops the buds of appendages of the adult within the cuticle. A lateral dorsal organ appears as a circular form. The nauplius cuticle begins to separate more from the larval tissue. This phenomenon is at first visible at the distal part of the abdomen. In the late Nauplioid stage (Fig. 1, H) 8 pairs of the thoracopods become visible. Under the transparent cuticle the telson and the endopods and exopods of uropods can be distinguished. The larva no longer resembles the classic nauplius stage, but is still enclosed within the nauplius cuticle which is lifted up from the larval tissue in wide parts. The yolk is reduced to the antero-dorsal region, and in the posterior parts it is reduced within the gut. The lateral dorsal organ is reduced in size and eventually disappears completely. Body segmentation is already present on dorsal surface except for the region occupied by the yolk. Pleopods, maxillules and maxillae well develop. The rudiment of carapace appears.

Postnauplioid stage

Duration: 4 days

In the newly molted Postnauplioid (Fig 1, I), there is still a large yolk mass present in the anterodorsal position, and during this stage the yolk mass is absorbed and integrated into the thorax. This allows the eye to rotate upwards from the antero-ventral into the anterior position. All thoracic appendages are well-developed and elongated. Telson and uropods are separated. The pleopods are simple buds. As the yolk mass becomes absorbed and integrated, the carapace develops and its posterior part separates from the thorax giving place to the respiratory chamber between the carapace and the cephalothorax. During this stages the stomach (foregut) becomes increasingly visible. Within the old cuticle the juvenile stage becomes apparent; the antennules, antennae and thoracopods become articulated with setae and spines. After 4 days the larvae molted to juveniles and emerged from the marsupium.

Ovarian cycle

Development of the ovary is a cyclic change and a continuous process. After oviposition the ovary continues to develop. According to successive stages of the marsupial development we can match the ovarian cycle. In the females bearing embryos within the marsupium, the ovary of the females is very flat and thin. In lateral view melanophores on the surface of the ovary are not clearly visible, but the level of the ovary indicates its position (Fig. 2, A, A'). At the end of Embryonic stage and the beginning of the Nauplioid stage, a row of small oocytes is distinguishable inside each ovary. Ovarian expansion was estimated by the proportion of thorax height occupied by the ovary in lateral view. In the females bearing nauplioid larvae within the marsupium (Fig. 2, B, B') the ovary occupied about 15% of thorax height. At the end of Nauplioid stage (Fig. 2, C, C') the oocytes grow continuously and form a segmented arrangement. In this stage ovary occupied about 25% of the thorax height. In the females (Fig. 2, D, D', E, E') bearing postnauplioid larvae the ovary is in a ripe stage, and it occupies about 40% of thorax height. Upon releasing the postnauplioid larvae the unfertilized egg mass is laid into the marsupium again for the next brooding.

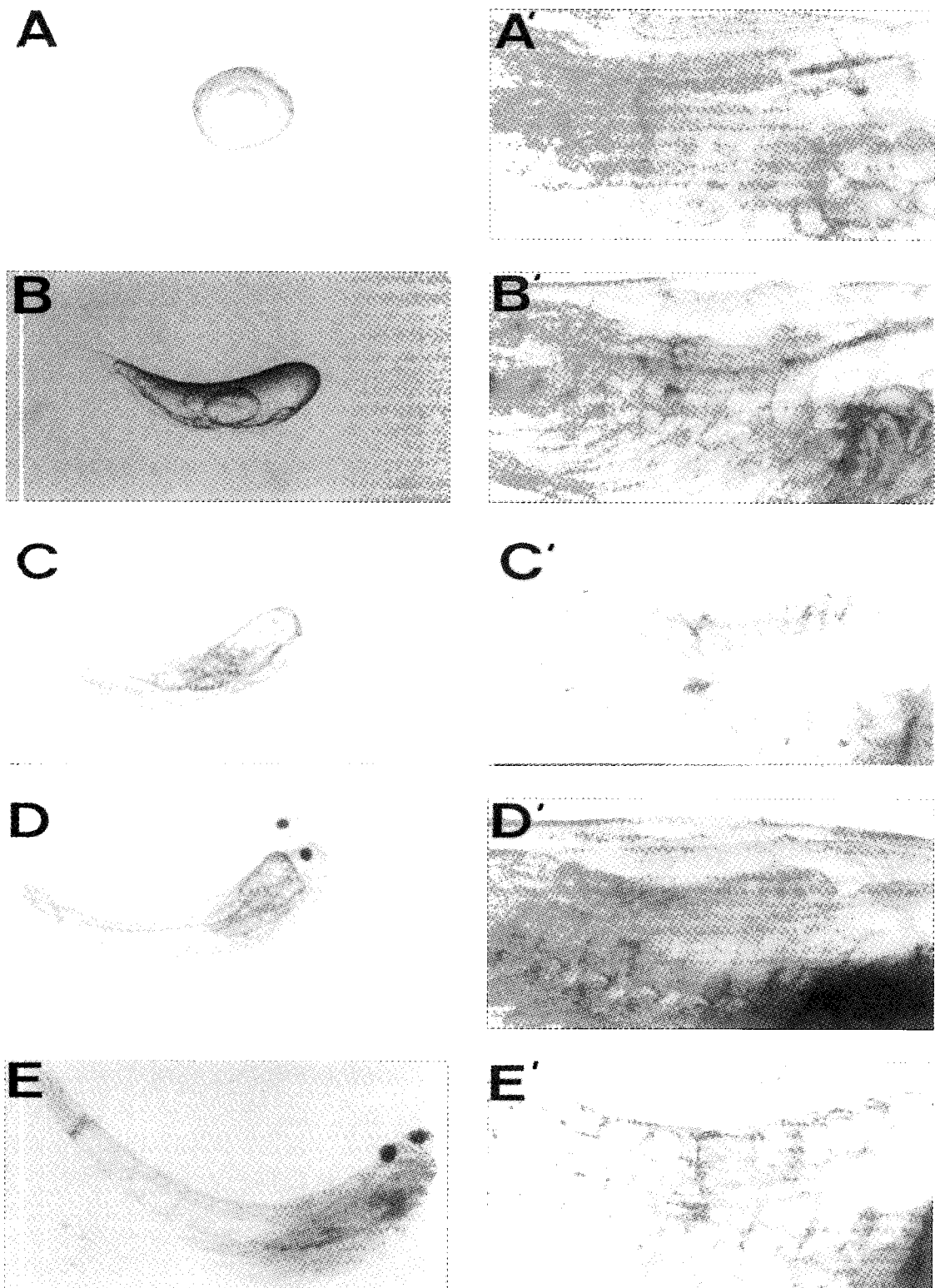


Fig. 2. *A. kokuboi*. Marsupial development and corresponding ovarian cycle in breeding females. A, the Embryonic stage. B, the early Nauplioid stage with cephalic appendages. C, the late Nauplioid stage with eye pigmentation. D, early Postnauplioid stage with free thoracic appendages and uropods. E, late Postnauplioid stage with complete carapace. A'~E', lateral view of the ovigerous female showing correlative states of development of the ovary.

Molt cycle of females

When the larvae are released from the marsupium, the females molt, and then the unfertilized egg mass is laid in the marsupium. Therefore, in mysids the ovarian cycle and egg laying are closely related to molting cycle. In view of molt cycle the development of ovaries and the larvae can be described. According to the molting stages defined by Drach (1939), Passano (1960) and Aiken (1968), *A. kokuboi* has four distinctive molting stages: Postmolt (Stage A, B), Intermolt (Stage C), Premolt (Stage D) and Ecdysis (Stage E). Molt cycle was observed based on the molt of the uropods and telson of the females.

Formation of setae and spines

Postmolt stage (Stage A, B) (Fig. 3, A, B). Just after the molt the cuticle of the uropods appears thin and flexible. Lacunae and haemocytes invade setae and spines, and epidermal cells are vacuolized. Later the lacunae condense to form a main blood lacuna which occupies a central position in the appendage with conspicuous border at the bases of setae and spines. This molting stage of the female corresponds the Embryonic stage of the marsupial development.

Intermolt stage (Stage C) (Fig. 3, C). This stage is a long phase in the molt cycle. The cuticle of the uropod is thickened. This stage corresponds to the stages from the end of the Embryonic stage to the Nauplioid stage.

Premolt stage (Stage D) (Fig. 3, D, E). At the beginning of Postnauplioid stage apolysis occurs, and separation of the epidermis from the cuticle can be first seen in regions such as the large indentation of the uropods or telson spines. This stage corresponds to the Postnauplioid stage of the marsupial development.

This stage can be divided into 4 Substages (D_0 , D_1 , D_2 , D_3) on the basis of the schemes proposed by Drach (1939), Passano (1960) and Aiken (1968). During Substage D_0 (Fig. 3, D) the underlying epidermis was separated from the exoskeleton. In Substage D_1 (Fig. 3, E) retraction of the epidermis is at maximum, and invaginations appear at each side of the bases of the setae matrices. This marks the beginning of Substage D_1 . New setae appear to form inside the uropod and telson and slowly begin

to evert. Shafts of the new setae apparently grow deep inside the uropod. Setal articulation also corresponds to the structure described by Tchernigovtzeff (1976) for the matrices of setae which involves a splitting of the matrices rather than invagination. For each seta and spine, splitting of the matrix proceeds in a centripetal way, from the base of the seta toward the inner part of the appendage. When the splits have attained their full development, Substage D_1 is terminated, and Substage D_2 starts with the secretion of the new cuticle on the surface of the matrices. In Substage D_2 , cuticle secretion occurs. This can not be pictured because it was not visible under the microscope, however, this stage can be clearly seen under the TEM (Fig. 4).

Formation of ultrastructure of cuticles

In this molting stage of the females the marsupial development is in Embryonic stage. Observation by the TEM revealed formation of ultrastructure of the cuticles. The epicuticular layer (Fig. 4, A) consists of three layers: the outer epicuticle, the central zone, and the inner epicuticle. The epicuticular layer is as thick as the exocuticle. The endocuticular is thinner than each of the epicuticular and exocuticular layers. These layers appear at the end of Postmolt stages (Stage A, B). A newly secreted material is visible at the surface of the epidermal cells (Fig. 4, B, C). The cuticle structure of the telson of the females bearing Nauplioid stage larvae is shown in Fig. 4, D, E. Fully organized endocuticular layer (Fig. 4, D) is thicker than each of the epicuticular and exocuticular layers. These layers appear at the end of Intermolt stage (Stage C). Epidermis is close to the cuticle, and it has microvilli and pore canals (Fig. 4, E). The cuticle structure of the telson of the females bearing the early Postnauplioid stage is shown Fig. 4, F, G. Development of cuticular layers is similar to that of Intermolt stage (Fig. 4, F), however, this stage begins to soften the cuticular layer and to depress the epicuticular and exocuticular layers (Fig. 4, G). These layer and marked by the Premolt stage (Stage D_0 , D_1). The cuticle structure of the telson of the females bearing the late Postnauplioid stage is shown Fig. 4, H, I. At this time the microvilli have disappeared and cuticle secretion is terminated. New cuticle is completely organi-

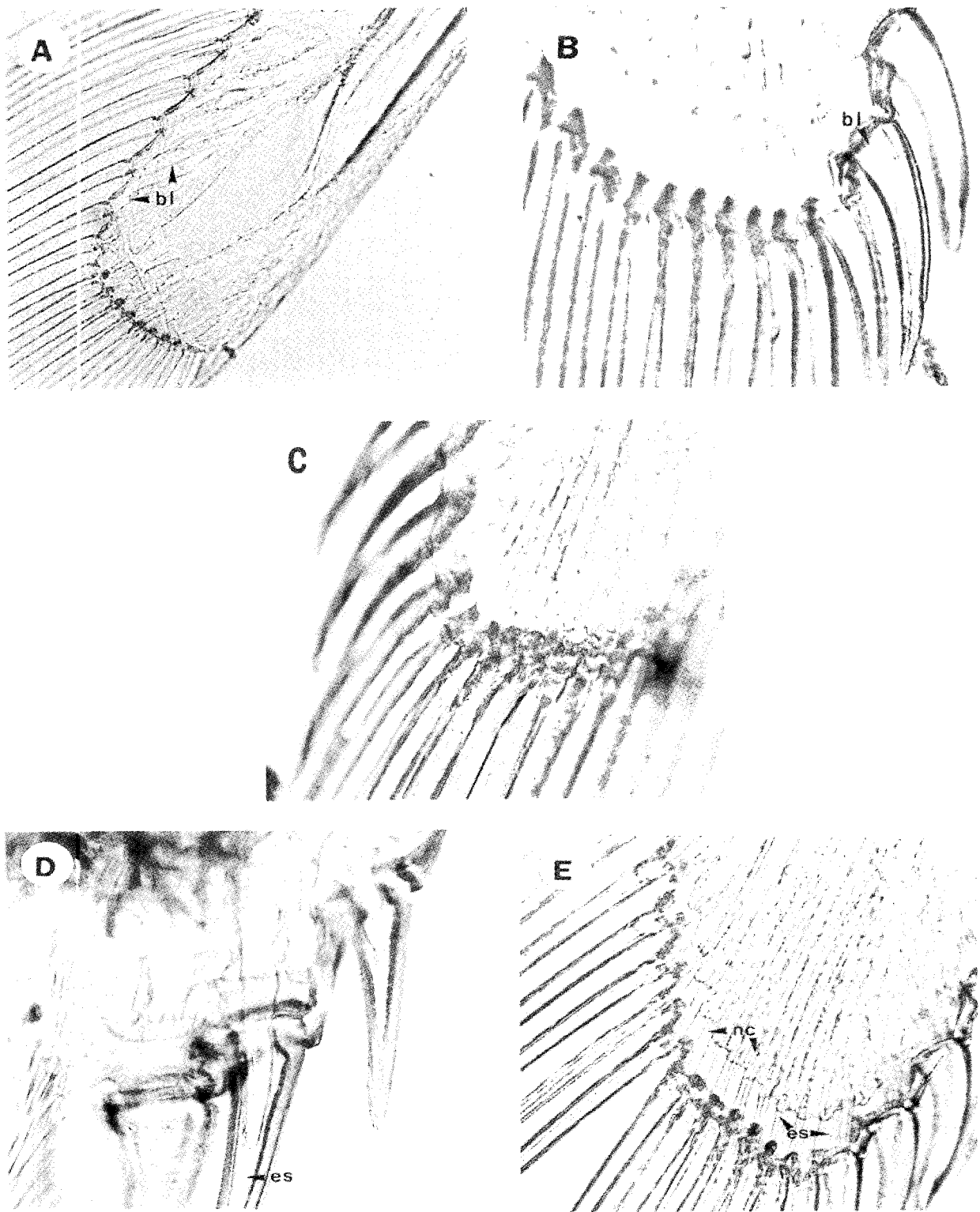


Fig. 3. *A. kokuboi*. Molt cycle of setae and spines during the marsupial development. A, external ramus of antennal scale during the Embryonic stage (10×20). B, exopod of uropod at Embryonic stage (Stage A, B) (10×40). C, exopod of uropod at Nauplioid stage (Stage C) (10×40). D, telson at Postnauplioid stage (Stage D₂). E, exopod of uropod at Postnauplioid stage (Stage D₂) (10×40) (bl: blood lacuna, nc: new cuticle, es: exuvial space).

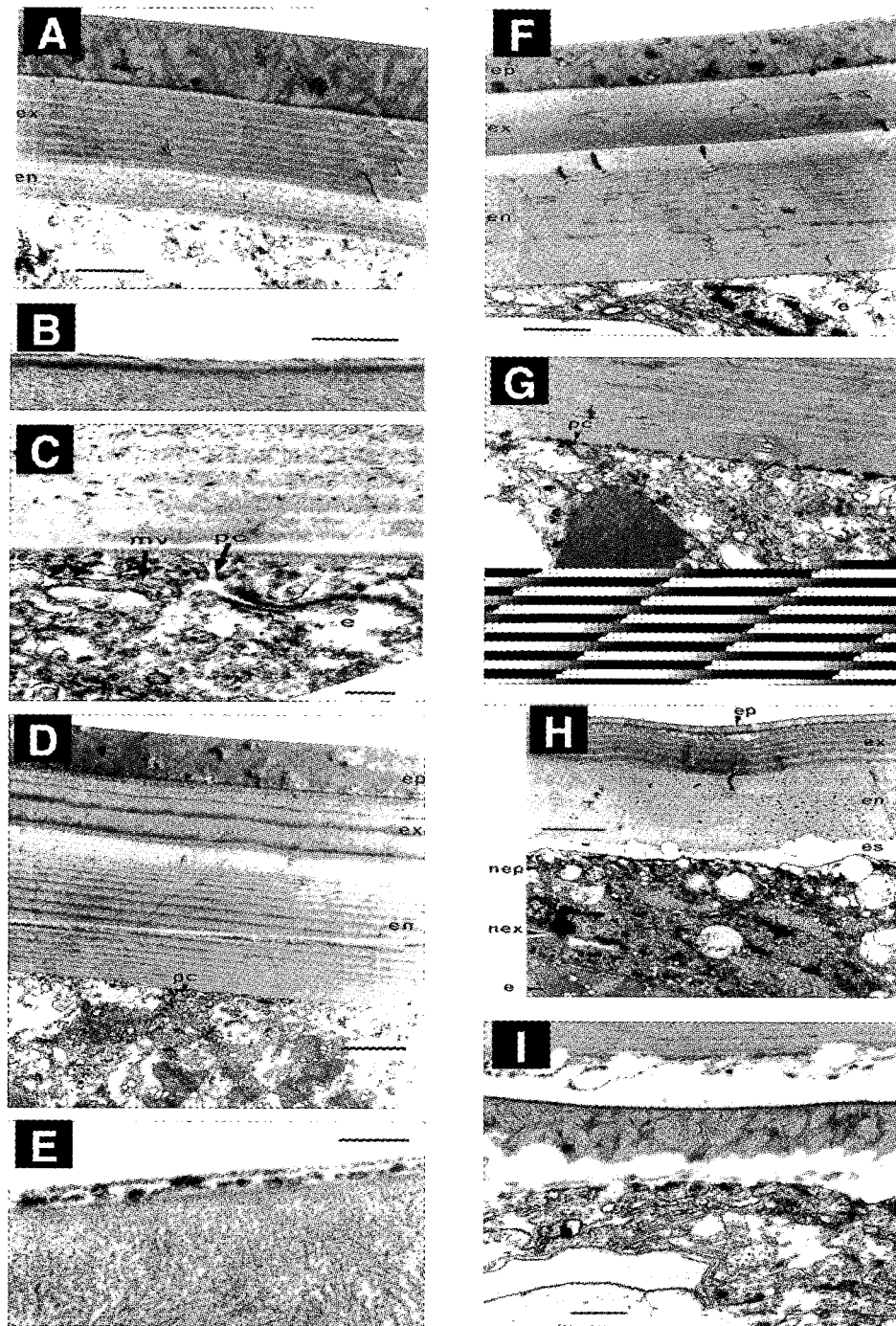


Fig. 4. *A. kokuboi*. Ultrastructure of telson cuticle. A~C: the Embryonic stage. A, transverse section of integument. Scale bar=2.0 μm . B, transverse section of the epicuticle. Scale bar=0.1 μm . C, transverse section of the endocuticle and epidermis. Scale bar=0.2 μm . D, E: the early Nauplioid stage. D, transverse section of integument. Scale bar=2.0 μm . E, transverse section of the epicuticle. Scale bar=1.0 μm . F, G: the late Nauplioid stage. F, transverse section of integument. Scale bar=2.0 μm . G, transverse section of the endocuticle and epidermis. Scale bar=1.0 μm . H, I: the Postnauplioid stage. H, transverse section of integument. Scale bar=2.0 μm . I, transverse section of the endocuticle and epidermis (ep: epicuticle, ex: exocuticle, en: endocuticle, e: epidermis, pc: pore canal, mv: microvilli). Scale bar=0.5 μm .

zed (Fig. 4, H, I). The epidermis has retracted to much greater extent, so that the cuticle is completely separated from the epidermis. This stage is followed by ecdysis.

Discussion

The developmental stages within the marsupium of mysids have various terminology. Generally the marsupial development of mysids is fairly uniform and occurs in three stages: the egg, the eyeless larva and the eyed larva, as reported by Mauchline (1980). However, some authors described the three stages as "embryos" (Nair, 1939; Jepsen, 1965; Davis, 1966; Berrill, 1969; Amaratunga and Corey, 1975), while others used the term "embryos" only for the developmental stages occurring inside the egg membrane, and "larvae" after hatching within the marsupium (Nusbaum, 1887; Matsudaira et al., 1952; Green, 1970; Wittmann, 1981; Cuzin-Roudy and Tchernigovtzeff, 1985). The developmental stage of *A. kokuboi* were described based on Wittmann (1981).

In *A. kokuboi* the Embryonic stage hatch by shedding egg membrane, and the Nauplioid stage molts to the Postnauplioid stage within the marsupium. This fact supports that the marsupial development of mysids passes the Nauplioid stages like other stages. The present study showed that hatching of eggs and two successive molting of Nauplioid and Postnauplioid stages within the marsupium. This type of development are common in mysids as proved by Jepsen (1965) for *Boreomysis arctica*, by Davis (1966) for *Mysidium columbae*, by Green (1970) for *Acanthomysis sculpta* and by Wittmann (1981) for *Leptomysis lingvura*.

There are controversies on the time of the second molt of the Nauplioid stage to be the juvenile. Jepsen (1965) reported that the Nauplioid stage of *B. arctica* molts before emergence from the marsupium. According to Amaratunga and Corey (1975), the larvae of *Mysis stenolepis* in the marsupium may undergo molting before but also after emergence. Nair (1939) and Wittmann (1981) described the larvae performed the second molt after emergence. In the larval development of *L. lingvura*, Wittmann (1981) reported that the second larval molt was coupled with release from the brood

pouch and may occur shortly before or immediately after liberation. Whereas Jepsen (1965) reports that the larvae of *B. arctica* molts before emergence. It is difficult to decide the molting time of the Nauplioid stage. In the present study neither the molting casts of the Postnauplioid nor the juveniles were found in the marsupium. This fact postulates that the Postnauplioid larvae molt after releasing from the marsupium.

In order to follow the endocuticular secretion from Postmolt and Intermolt stages, and to know when the Intermolt stage starts, a histological study of the integument by a TEM was necessary. In addition to this an alternative staging method is proposed by Cuzin-Roudy and Tchernigovtzeff (1985). They described that the method is valid only for female mysids and is based on the synchrony between marsupial development of the larvae and the molt cycle of the female. In *A. kokuboi* the molt cycle is synchronized with the development cycle of the eggs and larvae. Conclusively, in *A. kokuboi* the method can be used for rapid staging of the ovigerous female by direct observation of the eggs and larvae within the marsupium. Direct observation is not only efficient, but also it keeps the females intact in live condition.

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