Evolution of the carapace structure in Ostracoda (Crustacea:Arthropoda)

Thesis

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December 2005

Summary

In many ostracod species, the body plan and structure is specialized in each taxon and rich in morphological characters, and the carapace bears many characteristic features in spite of their small body sizes (most of them are less than one millimeter). The hard tissues of carapaces preserved as fossils are thought to be exceptional materials for understanding on evolution of exoskeleton in arthropods practically, because they have important features "various ornamentations," "hingement," "muscle scars," and "pore systems." Actually, many paleontologists have carried out taxonomy and analysis of phylogeny utilizing these features (Moore, ed., 1961; Pokorný, 1968; Benson, 1972; Tsukagoshi, 1990 and others). But the structural (histological) understandings of carapace are necessary to exert the high potential subsisting in the carapace features and to understand the evolution of Ostracoda profoundly. The hard tissues of carapaces preserved as fossils are, so to say, "dead tissues", and therefore information as organisms can not be found out from it unless the correlations between "dead tissues" and "living tissues" are duly understood. Namely, comprehensive elucidation between the hard and soft tissues is highly needed.

In this study, the carapace ultrastructures comprising the cuticular layers and cellular tissues in the modern ostracod taxa (eight superfamilies) are described. The diversification of cuticular structure is recognized conspicuously in the carapace of Podocopida, which has the highest species diversity. Especially, the epicuticles remarkably vary among the podocopid families or genera. The structure of procuticle is conservative at family level, but differs among superfamilies (e. g. organic frameworks, networks of pore canals). The development of membranous layer seems not to depend on the phylogenetic linage, thus this layer appears in separate taxa independently. The morphology of membranous layer shows homogenous, laminar, and fibre-like structures and these features are stable in their own families or genera. It is supposed that these structural diversities of cuticle reflect the difference of cuticle formation among the ostracods, and this fact suggests that the structure of cuticle varies among higher taxa. On the other hand, the structure of epidermis appears quite stable in Ostracoda, because dual epidermal layers fastened by the supporting fibres and amoeboid cells are observed in all examined podocopan carapaces. High electron-dense layer exists between the outer and inner epidermal cells in the platycopids and hemocoele of sinus network propped by the supporting fibres are confirmed in the myodocopids. In consequence, the basic structure of epidermis diversifies at order level in Ostracoda. This study clarifies that the cuticle structure of

ostracods diversifies as well as that of the other arthropods, and strongly suggests that these diversities may caused by their differences of habitats, cuticle secretion, and so on. Furthermore, the structural difference (compotion of organelles) between outer and inner epidermal layers in the calcified carapace taxa is not confirmed in the non-calcified taxa (e. g. Cladocera, Halocyprididae). The structural difference between dual epidermal layers may explain that the ostracod calcified-carapace accomplishes the both calcification by outer epidermal cells and efficiency of respiration by inner epidermal cells simultaneously.

Moreover, this study describes comprehensively the ultrastructure of marginal areas in ostracod carapace, especially hinges, and captures the morphogenesis of marginal areas utilizing the TEM and SEM. The hinge structures tend to be identified with the independent structure connecting the right and left valves, but this study demonstrates that the homology between the attached and free margins: the attached margin can be identified with the modification of free margin, because the ostracod ligament is a homologous structure to the marginal infold. Besides, this study establishes the new concept of hinge classification with the description of comprehensive ultrastructures. According to this, the hinge structures are classified into four types by the relative position of ligaments and hingements: *Basic type*-ligament connects to each calcified valves and hingement does not develop; *Exterior type*-overlap structure of one valve develops over ligament and ligament can not be observed from exterior view; *Transitive type*-overlap structure of one valve develops over ligament and hingement develops below ligament; *Interior type*-hingement develops below ligament without overlap structure and ligament can be observed from exterior dorsal view. The tripartite hingement (anterior, median, and posterior elements) consists of the combination three of above four types. New classification elucidates that the adont type hinges which emerge in some lineages parallely share the uniform simple structure in the "primitive" superfamilies (Platycopida, Darwinuloidea, and Bairdioidea) but the structures are diversified in the "derived" superfamilies (Cytheroidea and Cypridoidea). Additionally, it is explained that the high morphological diversification of cytheroid hingements has been accomplished by the structural plasticity of their hinge structures. Most cytheroid hinge structures can develop the complex hingements for only adjustment of calcification, because their hingements develop separately from the ligaments. In this study, that the evolutionary pathway of hinge structures is also clarifies on the basis of fossil records. This evolutionary pathway indicates that the developed hinge structures evolved from the simple ones when the derived taxa was evolved from the primitive taxa, but the evolutions of hinge structures within the derived taxa through

the adaptation for their habitats have been frequently reversible (i. e. the developed one to simple one). While, this study exhibits the morphogenesis of hinge structure based on the EM observation through the final molt. It was confirmed that the ligament has been already formed at the premolt stage. This observation signifies a possibility that the division of carapace into the two valves preceded the calcification of cuticle through the evolution of podocopan ostracods. The calcification of hingement of one valve precedes that of the other valve, therefore the preceded hingements (row of teeth) of one valve plays a role as the mold for opposite the other hingement.

Consequently, this study sublimated the recognition of ostracod carapace from "merely cover of animal body" to "evolved arthropod integuments " by the new understandings of structural and functional morphology. Furthermore, this comprehensive elucidation of carapace structure containing the hard and soft tissues reanimates the "dead tissues" of carapace as a part of active animal body. This result enables extinct species to be evaluated at cell level as same as extant species. These fruitions of this study would newly establish the biological views of the physiology and metabolism, which lack in the previous paleontological studies and they deepen the understandings on the evolution of Arthropoda conspicuously.

Key words: Ostracoda, Crustacea, Arthropoda, carapace, ultrastructure, hinge structure, evolutionary pathway, morphogenesis.

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Introduction

Arthropods emerged about five hundreds and seventy million years ago (the Early Cambrian) on the earth and they keep the highest taxonomic diversity in the Recent. Their taxonomic and morphological diversities have captivated many researchers from the past. But they had not been always the main materials to consider the evolution, since the preservable taxa as fossils are small in Arthropoda. However, since the rediscovery of the Burgess Shale fauna and the discussion of, what we call, "Cambrian Explosion" (Gould, 1991; Conway Morris, 1998 and others), Arthropoda has been the center of attentions in studies on evolution beyond the category of paleontology. Moreover, following the above, the understandings of the Homeobox genes (Bachiller, *et al.*, 1994; De Robertis, 1990 and others) progressed. As a result, using the arthropods as motif, the studies on correlations between morphogenesis and genes developed remarkably (Telford & Thomas, 1998; Damen *et al.*, 1998 and others). From these backgrounds, at present Arthropoda has been one of the most important taxa in both biology and paleontology as the materials of study on evolution.

In many ostracod species, the body plan and structure are specialized in each taxon and rich in morphological characters, and the carapace bears many characteristic features in spite of their small body sizes (most of them are less than one millimeter). The hard tissues of carapaces preserved as fossils are thought to be exceptional materials for understanding on evolution of exoskeleton in arthropods practically, because they have important features "various ornamentations," "hingement," "muscle scars," and "pore systems." Actually, many paleontologists have carried out taxonomy and analysis of phylogeny utilizing these features (Moore, ed., 1961; Pokorný, 1968; Benson, 1972; Tsukagoshi, 1990 and others). But the structural (histological) understandings of carapace are necessary to exert the high potential subsisting in the carapace features and to understand the evolution of Ostracoda profoundly. The hard tissues of carapaces preserved as fossils are, so to say, "dead tissues", and therefore information as organisms can not be found out from it unless the correlations between "dead tissues" and "living tissues" are duly understood. Namely, comprehensive elucidation between the hard and soft tissues is highly needed.

The previous studies on the structure of ostracod carapace have been carried out from the two points of views. One is the biological view to clarify the carapace structure anatomically. The other is the paleontological view to elucidate mainly the structure of hard tissues preserved as fossils. As the former cases, Claus (1865), G. W. Müller (1894, 1898), Fassbinder (1912), Cannon (1931,

1940), Rome (1947) and Kesling (1951) described the tissues and organs in the carapace as a part of animal body. Subsequently, these studies were taken over by Harding (1964) and Kornicker (1969). All the anatomical studies mentioned above were carried out by the optical observations. On the other hand, the latter studies for paleontology have been developed using mainly the SEM and the object of studies has been changed from the macro (e. g. external carapace morphology) to micro (e. g. crystal structure, organic framewaorks) features since 1970's (Sylvester-Bradley & Benson, 1971; Bate & East, 1972, 1975; Langer, 1973; Bate & Sheppard, 1982; Smith & Bate, 1983; Sohn & Kornicker, 1988; Yumoto, 1994, 1995MS). The number of species examined in these studies is more than one hundred. Though comprehensive studies on the carapace structure comprising "dead tissues" as fossils and "living tissues" should be carried out utilizing the both SEM and TEM, the number of species treated in these studies is less than twenty (Rosenfeld, 1979, 1982; Okada, 1981, 1982a, 1982b, 1983a, 1983b; Keyser, 1981, 1982, 1983, 1990, 1995, 2003, 2005; Yamada *et al.*, 2004, 2005) and thus, the taxonomic exhaustivity is lack entirely.

Delay of understanding on "living tissues" of Ostracoda would be caused by its small body and difficulty in application of histological methods. This delay causes the lack of not only taxonomic exhaustivity of cuticular structure and formation but also elucidation of biominelarization. The former and latter studies of Ostracoda get behind with those of Insecta and Decapoda, Bivalvia and Foraminifera, respectively. These facts suggest that the morphological information of hard tissues by superficial observations is not supported by the understandings of carapace structure including "living tissues." Especially the structural understandings of hingements, which is one of the most important characters, lag behind. They remain in morphological classifications in view of lateral observation, though many authors reported morphological diversity of hingements and regarded the hingements as taxonomic importance (Sylvester-Bradley, 1956; How & Laurencich, 1958; Hanai, 1961 and others).

In this situation, this study describes the ultrastructure of ostracod carapace comprising the cuticle and epidermis in eight extant superfamilies exhaustively: the structural diversity of ostracod carapace is pointed out; the generality as crustacean integuments and the particularity as ostracod carapace are discussed. Moreover, the ultrastructure of marginal area, especially the attached margin, is described exhaustively here. It is elucidated that the homology between attached and free margins by the successive sections in some species. The morphogenesis of hinge structure is also clarified by the EM observations through the final molt. In addition, the evolutionary pathway of hinge structures is

discussed with the fossil records.

Consequently, this study sublimated the recognition of ostracod carapace from "merely cover of animal body" to "evolved arthropod integuments " by the new understandings of structural and functional morphology. Furthermore, this comprehensive elucidation of carapace structure containing the hard and soft tissues reanimates the "dead tissues" of carapace as a part of active animal body. This result enables extinct species to be evaluated at cell level as same as extant species. These fruitions of this study would newly establish the biological views of the physiology and metabolism, which lack in the previous paleontological studies and they deepen the understandings on the evolution of Arthropoda conspicuously.

For the nomenclature of calcified parts of carapace, this study adopts the term of "hard tissues" which is used conventionally in the studies on invertebrates rather than "hard parts." The "carapace" consisting of the hard and soft tissues identified as the modification of arthropod integuments. The term of "valve" as two components of "carapace" is also adopted rather than "shell" which is used in studies on Bivalvia and Gastropoda. The anatomical terminology of "ultrastructure" here is used according to the convention of studies on crustacean cuticle.

Materials and methods

Living specimens used in this study are listed in Table 1. In preparation for observation by SEM (JEOL JSM-5600LV), specimens were fixed in either 5% formaldehyde or 70% ethanol solution. Carapace specimens were then air-dried and ion-coated with gold. For observations by TEM (HITACHI H-7500) living specimens were initially fixed in 2% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate sodium buffer (pH 7.4), with 5% sucrose, for 2 hours at 4°C ; post-fixed in 1% osmium tetroxide in the same buffer with 5% sucrose for 2 hours at 4°C ; decalcified in 10% EDTA in the same buffer with 7% sucrose for 3 days at 4°C ; dehydrated in an acetone series; and then embedded in Spurr's resin and polymerized. Sections were obtained using the ultramicrotome. Semi-thin sections were stained with 1% toludine-blue in 1% sodium tetraborate solution. Ultra-thin sections were stained with 1% potassium permanganate solution in distilled water for 2 minutes, and lead citrate (Reynolds, 1963) for 3 minutes.

Abbreviations: antel: anterior element, atm: attached margin, bc: body cavity, bcf: bundle of chitinous fibres, bl: basement lamina, cil: calcified inner lamella, cr: crenulation, dr: dorsal ridge, ef: ecdysial fluid, ep: epidermis, epc(i): epicuticle of inner lamella cuticle, epc(o): epicuticle of outer lamella cuticle, epc1: outermost epicuticle, epc2; epc3: middle or innermost epicuticle, es: ecdysial space, exc: exocuticle, endc: endocuticle, fl: flange, fn: filamentaous network, fp: feather-like projection, g: granule, ga: Golgi apparatus, hcy: hemocyte, hg: hingement, hg(lv): hingement of left valve, hg(rv): hingement of right valve, hl: hemolymph lacuna, hld: high electron-dense layer, ie: inner epidermal cell, if: intracuticular fibres, il: inner lamella, ilc: inner lamella cuticle, im: inner margin, la: lattice structure, ld: lipid droplet, lg: ligament, ls: list, m: mitochondorion, lv: left valve, mcy: melanocyte, mel: median element, mi: marginal infold, ml: membranous layer, mpc: marginal pore canal, ms: muscle, n: nucleus, nepc: new epicuticle, nepc1: new outermost epicuticle, nepc2: new middle epicuticle, nepc3: new innermost epicuticle, nilc: new inner lamella cuticle, nlg: new ligament, nolc: new outer lamella cuticle, novl: new overlap structure, nprc: new procuticle, ns: new sensillum, nsl: new selvage, oe: outer epidermal cell, oilc: old inner lamella cuticle, olc: outer lamella cuticle, olg: old ligament, om: outer margin, oolc: old outer lamella cuticle, osl: old selvage, ovl: overlap structure, poc: pore canal, postel: posterior element, prc(i): procuticle of inner lamella cuticle, prc(o): procuticle of outer lamella cuticle, r-ER: rough-surface endoplasmic reticulum, rv, right valve, sbd: subdermal cell, s-ER: smooth-surface endoplasmic reticulum, sf: supporting fibres, skt: socket, sl: selvage, sp: spine-like projection, svp: sieve plate, tth: tooth, v: vesicle.

Terminology of carapace structure

Initially, the ostracod carapace had been thought to consist of distinct right and left valves, with one valve being formed by concrescence of the outer and inner lamellae (Sylvester-Bradley, 1941). While, Harding (1964) suggested that the carapace is composed of one cuticular sheet and the same structure as any other joint in the exoskeleton of an arthropod (arthrodial membrane) develops in the hinge part. Kornicker (1969) emphasized that ligament is independent structure, but agreed with the opinion that the carapace consists of one cuticular sheet. Okada (1982a) confirmed the outer and inner lamella cuticles are comprised in one cuticular sheet and revised the terminology of carapace structure utilizing the TEM and SEM. Swanson (1989a, b) demonstrated this concept based on the observation through the ontogeny of *Manawa staceyi* practically. This concept was named "continuous sheet theory" by Hanai & Ikeya (1991).

Domiciliary region

The terminology of carapace structure is adopted from Okada (1982a) basically (Fig. 1A). The outermost layer of carapace is termed "epicuticle," and the inner layer of cuticle is termed "procuticle." The procuticle of outer lamella cuticle is strongly calcified and well preserved as fossils. In many arthropods, the procuticle is divided to distinct several layers based on the staining reaction or direction of crystallographic axis (recently based on function or the timing of formation). The name of these divided layers are various in each taxon or author (Neville, 1957; Price & Holdich, 1980a, b; Roar & Dillaman, 1984; Boxshall, 1992). According to Okada (1982a), this study adopt the terms "exocuticle" and "endocuticle" based on the difference of the structure of organic frameworks, but applies the term "procuticle" to the species which develops no boundary of the exocuticle and endocuticle. Innermost of the outer lamella cuticle, the organic layer called "membranous layer" often develops. The cellular tissue exists between the outer and inner lamella cuticle is called "epidermis," and this tissue comprises the "outer and inner epidermal cells." "Subdermal cell," which seems to be amoeboid, is often observed between the outer and inner epidermal cells.

Marginal areas

In previous studies, the term "attached margin" was identified with "hingement" and "ligament." In this paper, calcified teeth and crenulations are defined as "hingement" and the uncalcified connecting structure is given the name of "ligament" (Fig. 1B).

The ostracod "ligament" is not an elastic structure, like that of bivalves, but an uncalcified cuticular structure connecting both calcified valves with each other as part of a continuous cuticular sheet. Several researchers did not use the term "ligament" (see Table 2), and independently named this connecting flexible cuticular structure as "soft cuticle" (Harding, 1964), "connecting chitin (ligament of hinge)" (Bate & East, 1972), and the "intervalvar cuticle" (Jaanusson, 1985). This study uses the term "ligament" for the structural comparison of previous ostracod studies and for convenience of terminology.

The palaeontologist Sylvester-Bradley (1941) established influential terminology on the ostracod carapace as hard tissue. He divided the dual lamellar structure along the free margin into the outer and inner lamellae, by their relative location to the marginal pore canals. One continuous ridge running along the marginal zone of the outer lamella, and the other developing along the calcified inner lamella, were called "flange" and "selvage" respectively; and an additional continuous minor ridge inside of the selvage was called "list" (Fig. 1C). These terms were also used by Müller (1898) and by many other palaeontologists quoting Sylvester-Bradley's terminology (Triebel, 1950; Pokorný, 1957; van Morkhoven, 1962); but these structures do not always develop on all ostracod carapaces, and they show differences even between the right and left valves. As a result, there is often confusion in the use of these terms among different researchers, and thus it is not appropriate to apply these terms universally to the homology of the carapace structure. The conventional terminology, as mentioned above, probably comes from the idea that the ostracod carapace consists of distinct right and left valves, with one valve being formed by concrescence of the outer and inner lamellae.

In this study the marginal areas of the carapace were observed in detail, using the TEM and SEM. Consequently, the terminology of the marginal areas has been revised. The cuticular dual lamellae structure is called the "duplicature." The inner calcified one along the free margin was formerly called the "calcified inner lamella", but is now identified with the extension of the outer lamella cuticle due to its calcified structure and protective function (Okada, 1982; Yamada *et al.*, 2004). In this study the term of "marginal infold," after Kornicker (1969) and Hanai & Ikeya (1991) is adopted for this inner structure, and the external margin of this fold is the "outer margin." The boundary between the "marginal infold" and "inner lamella cuticle" (see Yamada *et al.*, 2004) is called the "inner margin." Uncalcified process along the free margin making a seal for the carapace can be observed, and have now adopted name of "selvage" (Fig. 1D). The selvage is often accompanied by a slight ridge at its root, and this ridge corresponds to the selvage defined by Sylvester-Bradley (1941).

Previous studies on carapace structure

In Ostracoda, the anatomical studies of large freshwater species were carried out by Claus (1865), G. W. Müller (1894, 1898), Rome (1947), Kesling (1951), and Hartmann (1966-1975). Canon (1931, 1940) illustrated the structure of each tissue and organ in Myodocopa (*Gigantocypris*).

Claus (1865) suggested that the epidermis consists of two layers and termed this tissue "Hypodermiszellen (Hypodermis, in English)." G. W. Müller (1894, 1898) described the basic structure of not only "soft tissue of carapace" but also "hard tissue of carapace." He found the calcareous matter to have an amorphous or fine-grained structure, and observed calcite prisms oriented perpendicular to the surface of carapace by using polarizing microscope. Furthermore, he determined the calcified layer of the carapace consisting of $CaCO_3$ and $MgCO_3$. His terminology was translated English from German by Sylvester-Bradley (1941). Rome (1947) redefined the carapace cellular tissue as "epidermis" consisting two layers of "epithelial cells" from the functional view of calcite secretion. Kesling (1951) renamed "epidermal cell" as the cells in "epidermis." Besides, he demonstrated the presence of calcite in the procuticle of outer lamella cuticle by using X-ray diffraction, and described the structure of carapace margin according to the terminology of Sylvester-Bradley (1941). These studies described the carapace structure as a part of description on animal body.

Fassbinder (1912) was the first study on the structure of ostracod carapace. He suggested the homology between the attached and free margins by observation of sections, and also mentioned the structure of inner lamella cuticle and reabsorb of Ca during the molt. Harding (1964) demonstrated that the ostracod carapace is composed of one cuticular sheet and concluded that cuticle in the hinge part has the same structure as arthrodial membrane. Kornicker (1969) developed this concept and emphasized that the ostracod carapace is one cuticular sheet which consists of four parts as "right and left shells," "ligament," and "vestment."

From the 1970's, most investigations have focused their attentions on the hard tissue of carapace utilizing the SEM. Sylvester-Bradley & Benson (1971) found the at least two distinct layers "foliated layer" and "laminar layer" in the hard tissue of fractured carapace by the SEM observations. Langer (1973) could distinguish different types of structural arrangements of the grains in some Ordovician to Recent ostracods. He classified the grains in the procuticle of outer lamella cuticle into three grates division. Jørgensen (1970) showed the hard tissue of carapace is built of small

calcite crystal enclosed by organic membrane and "pore canal (not sensory canal)" found in any other crustacean cuticle does not exist in the hard tissue of carapace. Bate & East (1975) observed the cuticle of carapace in *Cypridopsis vidua* and *Heterocypris incongruens* utilizing the TEM, and divided the outer lamella cuticle into three layers epicuticle, exocuticle and endocuticle from outer to inner side based on the difference of the structure of organic lattice. Moreover, they did not find the membranous layer in the carapace, though described the "secretary pore (pore canal in this study)" in the exocuticle. But, Okada (1982a, b) stated that the boundary between the exocuticle and endocuticle can be recognized not in the ornamented carapace but in the smooth carapace. Additionally, he found the membranous layer in the outer lamella cuticle of *Bicornucythere bisanensis*. Okada (1983a) reported the pore canal in the carapace of only *Neonesidea* and *Xestoleberis*. Bate & Sheppard (1982) reported the "secretary pore" and "circular disc of calcite " in the procuticle of outer lamella cuticle of *Halocypris inflata*. Smith & Bate (1983) suggested that the procuticle of outer lamella cuticle in *Halocypris inflata* can be divided into epicuticle, exocuticle and endocuticle, and emphasized that the organic lattice structure in the TEM photographs reflects the crystal structure captured by the SEM. Yumoto (1995MS) concluded that podocopan procuticle can not be divided into exocuticle and endocuticle by the SEM observations of fractured carapaces.

Histological studies on the ostracod carapace are few. Turpen & Angell (1971) carried out experiments on the ostracode carapace involving Ca^{45} as a tracer. Using a freshwater species of *Heterocypris* they showed that Ca is neither reabsorbed from the old carapace prior to molting nor stored for molting at the last molt stage. In addition, they reported that the amoeboid cells in the outer lamella move from the margin to the mid-region of the carapace during calcification, and then elaborate the calcite portion of the exoskeleton. Rosenfeld (1979, 1982) observed granules in the outer lamella cuticle and epidermal layer of the carapace using SEM and TEM, and subsequently analyzed the granules using X-rays in the three freshwater species *Cypris pubera*, *Eucypris virens*, and *E. lutaria*. He reported that the granules mostly consist of Ca and serve as the material for calcification of the carapace. The relationship between the cuticular layer and the epidermis was well described in the series of studies by Okada (1981, 1982a, 1982b). He described the carapace ultrastructure in *Bicornucythere bisanensis* (Okubo, 1975) by SEM and TEM, and classified the molt into three stages, i.e. C, D and A, following on from the studies on the crustacean cuticle by Drach (1939, 1944) and Passano (1960) (the molt stage B could not be distinguished in *B. bisanensis*). Stage D was subdivided into four sub-stages (D1, D2, D3, D4) with reference to studies of insect

cuticles by Locke (1966). The relationship between carapace ornamentation and the underlying epidermis was clarified by determining the degree of development of the cuticle at the various molt stages. Okada's works demonstrated that the polygonal fossae (reticulations) on the carapace surface represented the arrangement of outer epidermal cells, and the development of them, during ontogeny, and is controlled by the mitoses of the outer epidermal cells (according to their genotype). Okada (1983b) described the ultrastructure of muscle attachment area and discussed the correlation between the arrangement of addcutor mucle scars and epidermis. Keyser (1981) described the ultrastructure of sensory sensilla in nine podocopid species and considered their functions. Keyser (1982) carried out SEM and TEM observations of the ontogenetic development of the sieve-type pore system in *Hirschmannia viridis* and discussed its function and morphogenesis. Keyser (1990) observed the high density of mitochondria and the labyrinth structure of cell membrane at the area of the isthmus in *Cyprideis torosa* and discussed the osmoregulatory function of these structures. Furthermore, Keyser (1995) discussed the general forming of cuticle in *Cyprideis*, *Leptocythere*, and *Heterocypris*. Keyser & Walter (2003) explained that the formation process of the calcified layer of *Heterocypris salina* and the other freshwater species, and Keyser (2005) demonstrated the formation of node-like process in the carapace of *Cyprideis torosa* utilizing the histological methods. Abe & Vannier (1993) reported the sinus networks in the epidermis of *Vargula hilgendorfii* and discussed the circulatory systems of this species. Aladin (1993) observed many mitochondria in the caplike structure on the inner lamella of *Mytilocypris praenuncia* and thought this structure also has the osmoregulatory function. Yamada *et al.* (2004) described the prismatic layer inside the adult carapace of *Semicytherura* and discussed the respiratory systems with restricted epidemis of *Semicytehrura* species. Yamada *et al.* (in press) discussed the formation of the major ridges and prismatic layer in *Semicytherura kazahana*.

Systematic structural analysis on only the hard tissue are also few. Sohn & Kornicker (1988) has investigated the microstructure of myodocopid valve in seventeen species. They used component as the basic unit constructing the procuticle and identified five primary components (laminate, columnar, fine granular, coarsegranular, homogeneous) in the procuticle and reported the arrangements of these components were relative to taxonomy and ecology of moyodocopid ostracods. Yumoto (1994) divided the four components (foliated, granular, prismatic, and organic fibrous) in the fractured carapace of *Xestoleberis hanaii* using SEM and described their change through the ontogeny. Yumoto (1995MS) observed the fractured valves in eighty-one podocopan species and concluded

that the ontogenetic changes of crystal structure are conservative at the family level in Podocopa.

As above, even understandings of the base structure differ among the authors and most studies remained only the description of carapace structure. Structural studies on carapace, which applied the histological experiments and discussed the cuticle formation or metabolic activity of Ostracoda, were carried out in few species mentioned above. These are caused by the facts that each researcher treated the limited taxa and analysis methods differed in each study.

In this study, the ultrastructure of ostracod carapace in two superorders, seven superfamilies are described utilizing the TEM and SEM, and establish the basic ultrastructure of carapace in each higher taxon. Structural diversity and specificity of ostracod carapace are discussed based on structural comparison among higher taxa or other arthropod cuticle.

Ultrastructure of ostracod carapaces

In this chapter, the ultrastructure of carapace is described in each superfamilies and structural diversity at the superfamily level is recognized in ostracod carapace. As well, the descriptions are limited only for the adult carapace.

1. Podocopida

1-1. Cytheroidea

This study examined the carapace structure in the eighteen cytheroid families.

Epicuticle of outer lamella cuticle. The epicuticle is the outermost thin layer consisting of only the organic matters. This layer develops for protection of the inner calcified procuticle and forms the selvage to seal the free margin of each valve. Okada (1982a) described that the epicuticle of *Bicornucythere bisanensis* is composed of three layers and concluded that the outermost layer corresponds to the cuticulin layer of the insect *Calpodes ethlius* for its morphology and morphogenesis. Since this layer directly exposed to the circumstance, it is often wounded.

By the TEM observations, the epicuticle of many species in Cytheroidea can be divided into the two or three layers (Figs. 2, 3). In *Pontocythere japonica*, the three smooth layers can be recognized (Fig. 2A), and the outermost layer (epc1) is low electron-dense whereas the electron-dense of middle layer (epc2) is high. Thickness of the both layers are very thin as that of plasma membrane. The innermost layer (epc3) consists of most part of the epicuticle and its electron dense is high. Thickness of this layer increases towards the carapace margin and is up to 1 μm. *Loxoconcha pulchra* develops the three-layered epicuticle (Fig. 2B). The outermost layer (epc1) seems to be the row of spine and the middle layer (epc2) is the thickest in this epicuticle and high electron-dense, while the innermost layer (epc3) is thin and low electron-dense. In *Keijia* cf. *demissa*, *Cythere omotenipponica*, *Parakrithella pseudadonta*, *Semicytherura wakamurasaki*, *Callistocythere pumila*, *Perissocytheridea inabai*, *Microcythere* sp., and *Schizocythere kishinouyei*, the epicuticles are composed of the two layers and their morphological character can be recognized. In *Keijia* cf. *demissa*, the outer layer (epc1) develops the crenulate-like process and the electron-lucent thin layer (epc2) lines beneath the outer layer (Fig. 2C). In *Cythere omotenipponica* and *Parakrithella pseudadonta*, the low electron-dense smooth layer (epc1) exists as the outer layer. The inner layer (epc2) of *Cythere omotenipponica* is high electron-dense and thick (Fig. 2F), whereas the inner layer

(epc2) of *Parakrithella pseudadonta* is thin layer which formed by aggregation of organic fibres (Fig. 2G). The inner layer (epc2) of *Semicytherura wakamurasaki* is high electron-dense and thin (Fig. 2I). The outer layer (epc1) seems to be broken line and covers the inner layer. The characteristic layer is developed remarkably in the genus *Semicytherura*. In *Callistocythere pumila*, the outer layer (epc1) protrudes and takes various forms (Figs. 2D, 5E). A feather-like projection observed in the family Leptocytheridae (reported in Keyser, 1995) is composed of only this layer (Fig. 4C). The inner layer (epc2) is low electron-dense and smooth. The inner layer (epc2) of *Perissocytheridea inabai* continues as low electron-dense layer and often seems to be two-layered structure covered by high electron-dense thin layer. Thus, the inner layer may be divided into the two layers. The outer layer (epc1) appears to be broken line which consists of the granular-like components. The epicuticle of *Microcythere* sp. seems as node-like continuous layer (Fig. 2H). Electron-dense is high in the outer layer (epc1) but low in the inner layer (epc2). In *Schizocythere kishinouyei*, the epicuticle is composed of the high electron-dense outer layer (epc1) and low electron-dense inner layer (epc2) and appeared as wave-like layer (Fig. 2E). The epicuticle of *Linnocythere stationis* consists of the low electron-dense outer layer (epc1) and high electron-dense inner layer (epc2). The both layers are smooth and their thickness are almost equal each other (Fig. 3A). The epicuticle of this species often seems to be amorphous and the boundary between epicuticle and exocuticle are often obscure (Fig. 3A). In *Xestoleberis hanaii*, the epicuticle is high electron-dense thin layer and the exocuticle containing the dense lattice of organic fibres develops inside the epicuticle (Fig. 3C). The epicuticle of *Paradoxostoma triangulum* is electron-lucent wavy and often seems to be amorphous (Fig. 3B). The boundary between epicuticle and excuticle is often obscure. In *Paracobanocythere* sp., *Aurila hataii* and *Trachyleberis scabrocuneata*, the epicuticle is continuous high electron-dense layer (Fig. 3D, E, F). In five species mentioned above, the epicuticle seems to be mono-layered structure. The epicuticle of *Bythoceratina* sp. as the ancestor taxon of Cytheroidea is single layer and its electron-dense is low. This layer often appears to be amorphous (Fig. 3G). In *Schlerochilus* sp., which has the extremely thin carapace, the epicuticle often seems to be amorphous but consists of the low electron-dense outer layer and high electron-dense inner layer (Fig. 3H).

Procuticle of outer lamella cuticle. Most studies on the arthropod cuticle divided the procuticle into the exocuticle and endocuticle. As mentioned in the chapter of the previous studies, many structural studies on the ostracod carapace also divided the procuticle into the exocuticle and endocuticle. But the definition on the division of the procuticle differs among the authors and the

consensus on understanding of the carapace structure has not been established yet.

Okada (1982a, b) stated that the boundary between the exocuticle and endocuticle can be recognized not in the ornamented carapace but in the smooth carapace. On the contrary, Yumoto (1995MS) concluded that podocopan procuticle can not be divided into exocuticle and endocuticle by the SEM observations of fractured carapaces. In this study, the boundary of exocuticle and endocuticle can be recognized in the procuticle of only four species; *Xestoleberis hanaii*, *Paradoxostoma triangulum*, *Limnocythere stationis*, and *Cythere omotenipponica* utilizing the TEM (Figs. 2F, 3A, B, C). The exocuticles of these species line inside the epicuitcle and contain the dense lattice structure by aggregation of the organic fibres. In *Paradoxostoma triangulum*, *Limnocythere stationis*, and *Cythere omotenipponica* (Figs. 2F, 3A, B), this layer develops as a thin layer, but in *Xestoleberis hanaii*, the organic lattice of this layer seems to be sparse towards the inner of procuticle and continues to the endocuticle (Figs. 3C, 4A). The boundary between exocuticle and endocuticle can not be recognized using the SEM. In the other cytheroid species, the procuticle can not be divided into the exocuticle and endocuticle, though some taxa comprises abundant organic matters in the procuticle.

Okada (1983a) described the pore canal (for secretary of cuticle) in the procuticle of *Xestoleberis* sp. utilizing the TEM. This study also reconfirmed the same structure in the procuticle of *Xestoleberis hanaii* and *Xestoleberis setouchiensis* (Fig. 4A, B): 1) the pore canals are often filled up with electron-dense granular materials, 2) lack the membrane walls, 3) form networks by fusing each other and 4) develop just below the exocuticle. This study confirmed the characteristic features except above: 1) the number of pore canals decrease in the carapace margin, 2) the pore canals develop only the exterior part of outer lamella cuticle and 3) have not been formed in the procuticle at the premolt stage (Fig. 66). In Cytheroidea, the pore canal networks can be observed in the procuticle of *Xestoleberis hanaii* and *Xestoleberis setouchiensis* and the number of canals remarkably differs between the species.

Membranous layer. Many studies on cuticular structure of Decapoda and Isopoda reported the organic membranous layer (uncalcified endocuticle) as innermost part of endocuticle (Travis, 1963; Dalingwater & Mutvei, 1991; Wägele, 1992). Okada (1982b) also reported the homogenous membranous layer in the procuticle of *Bicornucythere bisanensis* (Cytheroidea).

In this study, three forms of the membranous layer can be recognized in the procuticle of cytheroid ostracods (Fig. 4D, E, F). The homogenous membranous layer is developed in the procuticle of

Xestolaberis hanaii, *Xestoleberis setouchiensis*, *Pontocythere japonica*, and *Pontocytehre miurensis* (Figs. 4D, 6C). The laminar thick membranous layer can be confirmed in the procuticle of *Cythere omotenipponica*, *Parakrithella pseudadonta*, *Loxoconcha pulchra*, *Loxoconcha japonica*, *Cytheromorpha acupunctata*, *Semicytherura kazahana*, *Hemicytherura kajiyamai*, *Hemicytherura tricarinata*, *Microcythere* sp., *Schizocythere kishinouyei*, *Paradoxostoma triangulum*, *Aurila hataii*, Caudites *asiaticus*, *Trachyleberis scabrocuneata*, and *Spinileberis quadriaculeata* (Figs. 4E, G, 6A, D). In *Paracobanocytehre* sp., the several (chitinous?) organic fibres form the membranous layer inside the procuticle (Fig. 4F). In *Bythoceratina* sp., *Sclerochilus* sp., *Keijia* cf. *denissa*, *Semicytherura* wakamurasaki, Callistocythere *pumila*, *Callistocythere setouchiensis*, *Callistocytehere rugosa*, *Ishizakiella miurensis*, *Tanella* sp., *Limnocythere stationis*, *Perissocytheridea japonica*, and *Perissocytheridea inabai*, the organic structure as the membranous layer do not develop (Figs. 5, 6B).

Epidermis. The morphological characters of epidermis, observed in this study, almost correspond to the description by Okada (1982a, b). In many cytheroid species, the epidermis consists of outer and inner epidermal cells and subdermal cell (Figs. 4E, 5, 6A, B, C). Body cavity often develops between the outer and inner epidermal cells (Fig. 6E). Outer epidermal cell contains various type granules, lipid droplets and abundant melanocytes especially in the colored species (Figs. 4E, 5, 6A, B, C). Inner epidermal cell contains mitochodria abundantly (Fig. 6C). These epidermal cells are fastened by the supporting fibres (Figs. 4E, 5A). Subdermal cell comprises abundant r-ER and is not fastened by the supporting fibres (Figs. 5D, 6A, B). This cell is often observed in the body cavity (hemolymph lacuna?) of carapace. In the case of *Microcythere* sp., the cell (subdermal cell?) contains abundant s-ER is observed and only this cell occupies the body cavity of carapace (Figs. 6D).

Inner lamella cuticle. According to the Okada (1982a), the inner lamella cuticle is the organic layer, consisting of the three-layered thin epicuticle and thick procuticle, and lacks the membranous layer. In many cytheroid species, the inner lamella cuticle can be divided into the high electron-dense thin layer and low electron-dense thick layer (Fig. 6G). The morphological difference among the taxa is not confirmed. In some taxa, the inner lamella cuticle near the marginal infold develops laminar structure as like the laminar membranous layer (Fig. 6F).

1-2. Bairdioidea

In this study, the ultrastructure of carapace in the family Bairdiidae (*Neonesidea oligodentata* and *Triebelina* sp.) is described.

Epicuticle of outer lamella cuticle. The epicuticle of *Neonesidea oligodentata* is composed of the

high electron-dense outer layer (epc1) and low electron-dense inner layer (epc2) (Fig. 7A). In *Triebelina* sp., the outer layer is low electron-dense and seems to be microvilli-like, while the inner layer consists of the granular materials and its electron-dense is high (Fig. 7B).

Procuticle of outer lamella cuticle. The procuticles of both species are divided into the exocuticle containing the organic fibres abundantly and endocuticle consisting almost the calcite (Fig. 7C, F, 8A). The pore canals develop beneath the exocuticle (Fig. 7E, F, 8A). They are often filled up with electron-dense substances and have their own membrane walls. The pore canals develop only the exterior part of outer lamella cuticle.

Membranous layer. In *Neonesidea oligodentata*, the laminar thick membranous layer develops as the innermost part of outer lamella cuticle (Fig. 7D). But in *Triebelina* sp., the membranous layer does not develop (Fig. 7F).

Epidermis. Outer epidermal cells comprising the various granules and inner epidermal cells containing the mitochodria arranges inside the cuticular layer, and they are fastened by the supporting fibres (Fig. 8A, B, D). Amoeboid cells like subdermal cell are often observed, but they contains not r-ER but abundant granules (Fig. 8B).

Inner lamella cuticle. As like Cytheroidea, the epictuticle and procuticle can be recognized but their electron-dense are low (Fig. 8C).

1-3. Cypridoidea

This study examined the three families belonging to the superfamily Cypridoidea.

Epicuticle of outer lamella cuticle. The epicuticles of *Cypridopsis vidua* and *Chrissia* sp. consist of the low electron-dense outer layer (epc1) and high elevtron-dense innerlayer (epc2) (Fig. 9A, F). The epicuticle of *Cypridopsis vidua* is smooth, but that of *Chrissia* sp. seems as a node-like layer. In the both species, the boundary between the epicuticle and exocuticle is obscure, for the innermost part of epicutcle fuses the outermost part of exocuticle. In the freshwater candonid species *Cypria reptans* and *Fabaeformiscandona* sp., the epicuticle shows the same structure in the cypridid species described above (Fig. 9D, E). But in marine candonid species Paracypridinae sp. A, the epicuticle is the high electron-dense single layer and often amorphous (Fig. 9G). In this species, the boundary between epicuticle and exocuticle can be observed distinctly. The epicuticle of *Ilyocypris japonica* is composed of the electron-lucent outer layer (epc1) and high electron-dense inner layer (epc2) (Fig. 9B) and the organic spine-like structure protrudes (Fig. 9C) and its root seems to extend like the hemi-circle.

Procuticle of outer lamella cuticle. In Cyprididae (*Cypridopsis vidua* and *Chrissia* sp.) and Candonidae (*Cypria reptans* and *Fabaeformiscandona* sp.), the procuticle can be divided into the exocuticle and endocuticle (Fig. 9A, B, E, F). The procuticles of *Cypridopsis vidua* and *Fabaeformiscandona* sp. contain the pore canals (Fig. 9A, E). In the former, the pore canals extend towards the exterior of carapace and interrupted beneath the innermost part of epicuticle. In the latter, the pore canals seems like those of Bardiidae and Xestoleridídae and develop below the exocuticle. In marine species Paracypridinae sp. A, the procuticle can be divided into the exocuticle and endocuticle by the density of organic fibres, but its boundary is sometimes obscure (Fig. 9G, 10A). In *Ilyocypris japonica*, sparse organic fibres run parallel to the epicuticle in the procuticle (Fig. 10B), then the boundary between exocuticle and endocuticle is not recognized.

Membranous layer. *Cypria reptans* develops the thick membranous layer only around the attached margin (Fig. 25D), but all other cypridoid taxa examined in this study lack this layer (Fig. 10A, B, E, F).

Epidermis. Thin epidermal cells arrange in the both lamellae and develop the basic structure like those of cytheroid species, but broader body cavity is often confirmed in most cypridoid species (Fig. 10A, E, F). In the body cavities of *Ilyocypris japonica* and *Fabaeformiscandona* sp., the sperms in their testes are observed (Fig. 10G, H, I). This study dose not confirm the "ameboid outer epidermal cell" reported by Turpen & Angell (1971).

Inner lamella cuticle. The inner lamella cuticle consists of the high electron-dense thin outer layer and low electron-dense thick inner layer (Fig. 10D). In *Cypria reptans*, the thick inner lamella cuticle develops and its procuticle contains the organic lattice structure like that of exocuticle of outer lamella cuticle (Fig. 10C).

1-4. Pontocypridoidea

This study described the ultrastructure of carapace in Pontocypridoidea at the first time. *Propontocypris* sp. is examined (Fig. 11).

Epicuticle of outer lamella cuticle. The epicuticle seems to be the single layer and often amorphous (Fig. 11A).

Procuticle of outer lamella cuticle. The exocuticle contains the organic fibres abundantly and the endocuticle is filled up with the calcite (Fig. 11B). The pore canals do not develop.

Membranous layer. The laminar thick membranous layer develops as the innermost part of outer lamella cuticle (Fig. 11B, C).

Epidermis. The epidermis consists of outer and inner epidermal cells and amoeboid cells comprising the granules are often observed between outer and inner epidermal cells (Fig. 11B).

Inner lamella cuticle. The inner lamella cuticle consists of the high electron-dense thin outer layer and low electron-dense thick inner layer (Fig. 11C).

1-5. Darwinuloidea

This study is the first description of the ultrastructure of carapace in Darwinuloidea. The carapace ultrastructures of *Vestalenula* sp., *Darwinula stevensoni*, and *Microdarwinula* sp. are examined (Fig. 12). The ultrastructures of these species show uniform.

Epicuticle of outer lamella cuticle. The epicuticle consists of the three layers; low elecron-dense thin layer (epc1), high electron-dense thin layer (epc2), and low electron-dense thick layer (epc3) (Fig. 12A). The innermost part of epicuticle continues the outermost past of exocuticle gradually. The selvage is quite small (Fig. 51D).

Procuticle of outer lamella cuticle. The exocuticle contains the dense organic lattice and the endocuticle comprises the sparse organic fibres (Fig. 12C). The pore canals, which contain electron-dense substances and have membrane walls, develop beneath the exocuticle (Fig. 12A). At the marginal areas in these species, the flexible inner lamella cuticle connects directly to the outer margin and the marginal infold does not develop fully (Fig. 51D).

Membranous layer. All taxa lack the membranous layer (Fig. 12C).

Epidermis. The epidermis consists of outer and inner epidermal cells, but they are extremely thin and contain sparse organelles (Fig. 12D). Many supporting fibres interrupt the body cavity and form as the channels like sinus networks (Fig. 12C). Subdermal cells contain r-ER abundantly and are often observed in the body cavity (Fig. 12B). Furthermore, the cells like adipocyte in Insecta are sometimes observed (Fig. 12E), but all specimens of these species are not well fixed and they may be deformed.

Inner lamella cuticle. The thickness of this cuticle is up to 500nm, and more thicker than those of the other superfamilies. The inner lamella cuticle consists of the high electron-dense thin outer layer and low electron-dense thick inner layer, and the procuticle develops the laminar structrue (Fig. 12B, D).

2. Platycopida (Cytherelloidea)

This study is the first description of the carapace ultrastructure in Platycopida. *Keijcyoidea*

infralittoralis is examined.

Epicuticle of outer lamella cuticle. The epicuticle consists of the two layers (Fig. 13A); low electron-dense amorphous layer (epc1) and low electron-dense rigid layer (epc2). The outer layer of epicuticle is often wounded and lost at the most part of carapace. The selvage is longer than that of the other superfamilies (Fig. 51C).

Procuticle of outer lamella cuticle. Sparse organic matters distribute in the procuticle and the boundary between exocuticle and endocuticle is not recognized (Fig. 13C). As Darwinuloidea, at the marginal areas, the flexible inner lamella cuticle connects directly to the outer margin, and the marginal infold does not develop fully (Fig. 51C).

Membranous layer. The high electron-dense single layer develops as the innermost of outer lamella cuticle and many organic fibres can be observed near the membranous layer (Fig. 13B).

Epidermis. The epidermis consists of outer and inner epidermal cells, and the high electron-dense layer develops between outer and inner epidermal cells (Fig. 13C, D). This layer is often interrupted by the supporting fibres and reduced towards the carapace margin. In this layer, amoeboid cells containing many granules are often observed and they are not fastened by the supporting fibres (Fig. 13F). In the carapace of eighth instar, similar cells are observed but the high electron-dense layer does not develop (Fig. 14A). The epidermis of juvenile (eighth instar) is thicker than that of adult and contains the organelle abundantly.

Inner lamella cuticle. The laminar structure develops in the cuticle (Fig, 13E). The high electron-dense thin layer covers over the low electron-dense thick layer. It is supposed that these layers correspond to the outer and inner epicuticle respectively. The electron-lucent laminar layer beneath the epicuticle is thought to be the procuticle. The fused zone of outer and inner lamella cuticles at the marginal area in juvenile (eighth instar) is narrower than that of adult (Fig. 14B, C).

3. Myodocopida (Cypridinoidea)

In this study, *Cypridina noctiluca*, *Melavargula japonica*, and *Vargula hilgendorfii* are examined.

Epicuticle of outer lamella cuticle. The epicuticles of these species are divided into the two layers and the both layers are low electron-dense (Fig, 15B). The boundary between these layers is sometimes obscure. The selvage develops at the outer margin (Fig. 15C).

Procuticle of outer lamella cuticle. The exocuticle contains the sparse organic fibres running parallel to the epicuticle (Figs. 15A, 16A). The organic lattice structure develops in the endocuticle

(Figs. 15A, 16A) and large calcite crystals are housed in this region (Fig. 15D).

Membranous layer. The laminar membranous layer develops as the innermost part of outer lamella cuticle (Figs. 15A, 16A, B).

Epidermis. The epidermis consists of outer and inner epidermal cells, and subdermal cells and the other amoeboid cells are not confirmed (Figs. 15A, 16). The sinus networks reported by Abe & Vannier (1993) develop in the carapace of these species. Free cells (hemocyte?) containing many granules are observed in the body cavity between the supporting fibres (Fig. 16B).

Inner lamella cuticle. The inner lamella cuticle is composed of the low electron-dense thin layer and high electron-dense thick layer, and they correspond to the epicuticle and procuticle respectively (Fig. 15D).

4. Halocyprida (Cladocopoidea)

This study examines *Polycope japonica* which has strongly calcified valves (Fig. 17).

Epicuticle of outer lamella cuticle. The epicuticle is a single layer and often amorphous (Fig. 17B). Bubble-like structure can be observed near the boundary between the epicuticle and procuticle. As the carapace in Myodocopida, the selvage develops at the outer margin (Fig. 17D).

Procuticle of outer lamella cuticle. The procuticle is filled up with the calcite and the prominent organic frameworks develop at the marginal area (Figs. 17A, 18A).

Membranous layer. This species lacks the membranous layer (Fig. 17A).

Epidermis. The epidermis consists of outer and inner epidermal cells, and the both cells contain mitochondria abundantly (Fig. 17A). The body cavities are often observed between outer and inner epidermal cells and subdermal cells are sometimes observed (Fig. 17C).

Inner lamella cuticle. The inner lamella cuticle is composed of the low electron-dense outer layer and high electron-dense inner layer, and they correspond to the epicuticle and procuticle respectively (Fig. 17A).

Previous studies on ostracod hinge structure

Previous studies on ostracod hinge structure have focused their attentions to apply for higher taxonomy of Ostracoda. Zalányi (1929) divided post-Paleozoic ostracod hingements into two types "dentate" and "non-dentate". He classified two hinge types into "dysodont", "kriptodont", "desmodont", "taxodont", "heterodont" and "schizodont" for three marginal elements (anterior, interangular or apical, posterior). These terms were adopted from studies on lamellibranch dentition. He insisted that the hingment must be considered the most important taxonomic character in fossil species, not only for species but also higher taxa. Afterwards, most studies on hinge structure were carried out for the classification of hingements based on their numbers of elements and hingement morphologies and have been emphasizing that the hingement is the most important criterion for higher taxonomy of Ostracoda (Bold, 1946; Malkin, 1953; Kingma, 1948; Berousek, 1952).

Triebel (1950) introduced term "merodont" for hingement in which only one valve carries hinge teeth, and term "amphidont" for hingement in which both valves are provided with one or more hinge teeth. He concluded that "merodont" preceded "amphidont" phylogenetically. Pokorný (1955) discussed phylomorphogenesis of the hingement of Hemicytherinae using the hinge character "protogenic hinge teeth".

Sylvester-Bradley (1956) classified the ostracod hinges into six types (lophodont, merodont, entomodont, lobodont, schizodont and amphidont) based on the components of hingement and redefined the terminology of hingement. He also concluded that the primitive hinge is (one or) tripartite and that more specialized hinge types originate by subdivision of the median element. This concept of hinge classification has been accepted to date, though each author use the different terms. Besides, he speculated that lophodont hinge is the most primitive for Devonian ostracods (Metacopina, Bairdiocopina) developed lophodont hinge. Hanai (1957) proposed "pentodont" which has tripartite median element and "pseudadont" which develops like "adont" from "desmodont" through the final molt. Howe & Laurencich (1958) illustrated the definitions of the most common types of Cretaceous hingement, and redefined the terminology of Sylvester-Bradley (1956). By these previous studies, the basic understanding of present hinge classification had established. Hanai (1961) summarized these classic studies up to that time and defined various types of hingement in Cenozoic cytheroid ostracods. He also evaluated the hingement as an evolutionary character and mentioned the limitation of hingement as a taxonomic character. This study not only

was directly reflected in the two checklists from Japan (Hanai *et al.*, 1977) and from Southeast Asia (Hanai *et al.*, 1980), but also greatly influenced subsequent investigations on ostracod systematics. Moore (ed., 1961) made a comprehensive systematic survey of cytheroid Ostracoda entirely on the basis of hingement. Gründel (1974) divided the hingements of post-Ordovician ostracods into five basic types (subdivided into twenty-four types). So far, most previous studies on the hinge classification depend on the numbers of elements in their own hingements. This hinge classification has been thought to be useful for higher taxonomy in post-Paleozoic cytheroid ostracods.

Previous studies stated the homologue between hinge structure and free margin are few. Fassbinder (1912) suggested that the attached margin is homologous structure for the free margin. Pokorný (1957) further advanced the concept of Fassbinder (1912) and Zalányi (1929) and stated the correlation between hingement and free margin. He recognized the two types of hingement, "hemisolenic" and "holosolenic." The former has a continuous contact groove around the entire periphery as in *Cytherella* (Platycopa), whereas in the latter the contact groove is interrupted by fusion of the selvage and list as in *Thlipsurella* (Podocopa). He conclued that the podocopa arose from a "holosolenic" platycopan ancester. Kornicker (1969) developed the concept of Harding (1964) that the ostracod carapace is one cuticular sheet. He emphasized that the carapace consists of four parts as "right and left shells," "ligament," and "vestment" and concluded that "ligament" is an independent structure from other parts of carapace. He also mentioned that it is important to recognize the hingement as "exterior part" or "interior part" of valve. The importance of this concept was also mentioned by Hanai (1988). But this concept is accepted by few reseacher, so taxonomic or paleontological utilities of this concept have not been found.

Few studies discussed the evolution of hinge structure, though many researchers recognized its taxonomic value. Sandberg (1964) regarded the ontogenetic change of hingement as representing evolutionary change. He assumed that the entomodont and holomerodont hinges evolved from the antimerodont hinge. The cytheroid hingements are generally thought to have an evolutionary trend from a simple one to a complicated one (Hartmann, 1963; Benson, 1966). Sylvester-Bradley (1948) found this trend in the lineage from the Middle Jurrasic *Oligocythereis* (entomodont) to the Tertiary and Recent *Trachyleberis* (amphidont). These studies suggested that the hinge structures (especially hingements) reflect the phylogeny of Ostracoda. On the other hand, Triebel (1954) assumed that the amphidont hinge has been achieved independently in the homeomorphic genera *Macrodentina* and *Amphicythere* in the Jurassic. Sylvester-Bradley (1956) also postulated parallel evolution from the

entomodont hinge to the amphidont hinge in the lineage from *Oligocythereis* in the Middle Jurassic to *Trachyleberis* in the Tertiary and Recent and in another ostracod lineage in the Middle Jurassic. Kamiya (1992) and Tsukagoshi (1994) explained the morphological differences of hingements, which appeared intra-specific or intra-genetic, as the heterochrony of the feature. Tsukagoshi & Kamiya (1996) indicated that all the basic hingement designs already appeared at least by the Paleogene and the designd became modified exclusively by paedomorphosis in the Neogene. Yamaguchi (2003) made the molecular phylogenetic trees using 18SrDNA of twenty-eight cytheroid ostracods represent sixteen families. He demonstrated that amphidont basic type hingements emerged at least four times independently in the lineage of cytheroid ostracods and concluded that the lophodont hinge is the plesiomorphic and various hinge types evolved from the lophodont hinge. These results indicated that hinge structures do not always reflect the ostracod phylogeny.

Some authors mentioned the correlation between the complication of hingement and increase of mineralization of the carapace. They stated that the complication of hingement must be associated with the complication of ornamentation or increase of calcification (Pokorný, 1957; Benson, 1966; Hinz, 1993; Hinz-Schallreuter & Schallreuter, 1999). Yamaguchi (2003) assumed that the complication of hingement was caused by the increase of calcification of carapace and the same hingement types emerged independently in the lineage of cytheroid ostracods.

As above, not all researchers agree with the phylogenetic availability of hinge structures. This study establishes the new classification of hinge structures based on homology using the ligament and hingement and traced the morphogenesis of hinge structure at the final molt utilizing the TEM and SEM. Consequently, the evolutionary pathways of hinge structure and the factor that carries their structural diversity are discussed.

Ultrastructure of marginal areas

1. Ultrastructure of ostracod hinge structures

1-1. Ultrastructure of ostracod ligament

In platycopid and podocopid species bearing strongly calcified carapaces a thick ligament is recognised without regard to the developmental intensity of the hingement (Fig. 18B, C, D). In the myodocopid *Polycope japonica*, a thin ligament is connected with each valve (Fig. 18A). The ultrastructure of these ligaments has certain common features, as listed below:

(1) In transverse section, thick bundles of chitinous fibres are found in the ligament. These fibres show a feather-like structure (Fig. 19A, B).

(2) In longitudinal section the feather-like fibres are parallel to each other. Curved parabolic fibres connect to adjacent feather-like fibres (Fig. 19C).

(3) A seamless epicuticular layer (at least for the outer epicuticle) covers the outermost ligament (Fig. 19B).

The bundles mentioned in (1) were found in *Cyprideis torosa*, as illustrated by Keyser (1995). The fibrous structures mentioned in (1) and (2) are similar to the layered structure in the exoskeleton of *Cypridopsis vidua*, shown by Bate & East (1972, 1975) (Table 2). Furthermore, they reported that this structure could be also observed in the chitinous body cuticle and selvage spine. Similar structures have also been reported widely in the arthropod cuticle (e.g., Insecta, Decapoda) (Neville, 1975).

1-2. New classification of podocopan ostracod hinge structures

In most previous studies, podocopan hinge structures have been classified into various types based on the hingement morphology. Thus, adont basic type, which develops the simple hingement, has been classified into few subtypes, and they are appeared in some lineages simultaneously. On the contrary, merodont and amphidont basic types have been classified into many subtypes.

In this study, new classification of podocopan hinge structures has been established by the relative position of ligaments and hingements. This classification is based on the concept of Kornicker (1969)'s hinge classification developed by the exhaustive TEM observations and redefined the nomenclature of hinge structures. The ligament is the flexible cuticular layer connecting the each valve and develops the conservative position in the ostracod cuticle. By this classification, the basic structures of podocopan hinges can be compared each other irrespective of the complexity of

hingements, and the morphological evolution of hinge structures can be discussed. New classfication of hinge structures is described below (Fig. 20).

Basic type: The ligament connects to each calcified valves and the hingement does not develop (Fig. 20A).

Exterior type: The overlap structure of one valve develops over the ligament and the ligament can not be observed from the exterior view. The hingement exists the exterior or lateral position of ligament (Fig. 20B).

Transitive type: The overlap structure of one valve develops over the ligament and the ligament can not be observed from the exterior view. In addition, the hingement develops below the ligament (Fig. 20C).

Interior type: The hingement develops below the ligament without the overlap structure and the ligament can be observed from the exterior dorsal view (Fig. 20D).

The results applied to podocopan hinge structures are showed in Table 3. The hinge structures, which have tripartite hingements, are showed in the each element (anterior-median-posterior). The details of each podocopan hinge structure are described below.

1. Platycopida (Cytherelloidea)

***Keijcyoidea infralittoralis* : Basic-Interior-Basic type**

This study examined *Keijcyoidea infralittoralis* belonging to the superfamily Cytherelloidea (Fig. 21A, B). The hinge structure of this species except the median element (a tooth) shows *Basic type* (Fig. 21C). In median element, the hinge structure shows *Interior type* (Fig. 21D). In the cytherelloid species excluding the genus *Keijcyoidea*, a tooth does not develop in their hingements. Thus, most cytherelloid hinge structures are thought to show the simple hinge structure *Basic type*.

2. Podocopida

2-1. Bairdioidea (Bairdiidae)

***Neonesidea oligodentata* : Exterior type**

***Triebelina* sp. : Exterior type**

In the previous studies, the bairdioid hinge structure has been identified with a simple "adont". But the overlap structure develops over the ligament and the bairdioid hinge structure shows *Exterior type* (Fig. 22).

2-2. Darwinuloidea (Darwinulidae)

***Darwinula stevensoni* : Basic type**

***Vestalenula* sp. : Basic type**

***Microdarwinula* sp. : Basic type**

Basic type hinge structures are recognized in these species (Fig. 23). The fibres of long ligament are partly sparse, especially in *Darwinula stevensoni* (Fig. 23C). The ligament of *Darwinula stevensoni* seems to be elongate perpendicularly, and in the innermost part of ligament, the fibres are dense and the ligament seems to be a general form. In *Vetstalenula* sp., the ligament is elongate and its fibres are low electron-dense (Fig. 23E). In *Microdarwinula* sp., the extreme thin ligament consists of a few fibres (Fig. 23D).

2-3. Cypridoidea

A. Cyprididae

***Chrissia* sp. : Basic type**

***Cypridopsis vidua* : Basic type**

Long ligaments develop in these species (Fig. 24). In *Cypridopsis vidua*, the inner part of left valve has a ridge. But this ridge does not work as the complementary structure; therefore this study does not identified the ridge with the hingement (Fig. 24D). As the same reason, this study classifies the hinge in *Heterocypris* shown by Harding (1964) into *Basic type*, though Kornicker (1969) classified this hinge into "Interior type". Harding (1964) also illustrated the sections of hinges in *Chlamydotheca*, *Heterocypris*, *Cypridopsis*, and *Cypris pubera*. Based on his illustrations, this study classifies the hinge of *Chlamydotheca*, *Heterocypris*, and *Cypridopsis* into *Basic type* and that of *Cypris pubera* into *Exterior type*.

B. Candonidae

***Fabaeformiscandona* sp. : Exterior type**

***Cypria reptans* : Exterior type**

Paracypridinae sp. A : Interior type

Paracypridinae sp. B : Interior type

The freshwater and marine species belong to the family Candonidae. In the freshwater species *Cypria reptans* and *Fabaeformiscandona* sp., their hinge structures show *Exterior type* (Fig. 25). On the contrary, the marine species Paracypridinae sp. A and B, their hinge structures exhibit *Interior type* (Fig. 26).

C. Ilyocypridae

***Ilyocypris japonica* : Basic type**

This species has the prominent ridges on the carapace surface, though it lives in the freshwater. The hinge structure is classified into *Basic type* (Fig. 27), but the external part of attached margin along the hinge line develops the crenulations modified from the surface ornamentation (Fig. 27C-F).

D. Notodoromatidae

***Notodoromas* : Exterior type**

This study adopts the illustration of this taxon from Harding (1964), since no specimen could be captured. The complementary structure develops over the ligament, so this hinge structure is classified into *Exterior type*.

2-4. Pontocypridoiodea (Pontocyprididae)

***Propontocypris* sp. : Interior type**

The hinge structure exhibits *Interior type* (Fig. 28). The calcified part of left valve extends over the ligament, but this hinge structure is not classified into *Transitive type* for the ligament is not wholly covered with the left valve.

2-5. Cytheroidea

A. Bythocytheridae

***Bythoceratina* sp. : Interior-Transitive-Interior type**

***Sclerochilus* sp. : Exterior type**

Bythoceratina sp., which develops the prominent ornamentations, develops the both terminal elements as *Interior type* and the median elemement as *Transitive type* (Fig. 29C, D). The both terminal elements consist of the smaller teeth than those of the other cytheroid species and the median element develops the crenulations (Fig. 29A, B).

In *Sclerochilus* sp. which has the extremely thin carapace the hinge structure is *Exterior type*, for the small overlap structure develops over the ligament (Fig. 30C). The two teeth like the terminal elements can be observed inside of right valve (Fig. 30A, B). But these features should not be evaluated as the hingement, for they develop in the free margin.

B. Eucytheridae

***Keijia* sp. cf. *K. demissa* : Interior type**

The terminal elements consist of the large teeth and exhibit *Interior type* (Fig. 31C, D). The crenulations develop in the median element. The hinge structure of median element shows *Interior*

type, though the crenulations become smaller towards the middle part (Fig. 31A, B).

C. Paradoxostomatidae

***Paradoxostoma triangulum* : Exterior type**

The hingement is composed of the bar-groove, though the terminal elements appear as vestiges (Fig. 32A, B). The hinge structure of all elements shows *Exterior type*, but the appearance differs between the terminal and median element. In the terminal elements, the cuticular layers of both valves extend over the ligament and form the overlap structure (Fig. 32C). In the median element, the membranous layer of right valve overlaps the ligament (Fig. 32D).

D. Cytheruridae

***Hemicytherura kajiyamai* : Transitive type**

***H. tricarinata* : Transitive type**

***Semicytherura kazahana* : Transitive type**

***S. wakamurasaki* : Transitive-Exterior-Transitive type**

In *Hemicytherura kajiyamai* and *Hemicytherura tricarinata*, the crenulations develop as the terminal elements and only the both ends of median elements. The middle part of median element appears as the bar-groove (Fig. 33A, B). The hinge structure of all elements develops as *Transitive type* (Fig. 33C, D). In *Semicytherura kazahana*, which has a thick carapace, the crenulations develop in all elements (Fig. 34C, D). But in *Semicytherura wakamurasaki*, which has a thin carapace, the teeth-sockets develop in the terminal elements and the bar-groove appears as the median element (Fig. 34A, B). The dimorphism of hinge structure is recognized in the genus *Semicytherura*. The terminal elements of these species show *Transitive type*, but the median elements in *Semicytherura kazahana* and *Semicytherura wakamurasaki* develop as *Transitive type* and *Exterior type* respectively (Fig. 34E, F).

E. Loxoconchidae

***Loxoconcha pulchra* : Interior-Transitive-Interior type**

***L. japonica* : Interior-Transitive-Interior type**

The terminal elements of genus *Loxoconcha* develop the biramous large teeth and the median element exhibits the crenulations (Fig. 35A, B). The hinge structures in the terminal elements and median element exhibit *Interior type* and *Transitive type* respectively (Fig. 35C, D).

F. Leptocytheridae

***Callistocythere pumila* : Interior-Exterior-Transitive type**

C. rugosa : **Interior-Exterior-Transitive type**

Ishizakiella miurensis : **Interior-Basic-Transitive type**

The dimorphism of hinge structure is recognized in the family Leptocytheridae (Fig. 36, 37). In the right valve of *Callistocythere* species, the large teeth of terminal elements connect via the median bar (Fig. 36A), but in *Ishizakiella miurensis*, the median element develops as the crenulations (Fig. 37A). In the left valve of *Callistocythere* species, the crenulations develop as median element and are reduced towards the middle part (Fig. 36B), but in *Ishizakiella miurensis*, these crenulations do not develop (Fig. 37B). The hinge structures of these species in the anterior and posterior element show *Interior type* and *Transitive type* respectively (Figs. 36D, 37C). The hinge structures of median elements in the genus *Callistocythere* and *Ishizakiella* are classified into *Exterior type* and *Basic type* respectively (Figs. 36C, 37D).

G. Cobanocytheridae

Paracobanocythere : **Basic type**

Paracobanocythere sp., which has an extremely thin carapace and lives in the interstitial pore water, develops the hingement containing the bar-groove, and its hinge structure exhibits *Basic type* (Fig. 38). The ligament arranges perpendicularly to the longitudinal axis of animal body.

H. Xestoleberididae

Xestoleberis hanaii : **Transitive type**

The hingement of this species consists of the terminal elements as the distinct crenulations and median element as the slight crenulations (Fig. 39A, B). Some xestoleberid species do not develop the median crenulations (Fig. 78E). The tripartite overlap structure develops over the hingement. The terminal elements have a thin ligament between the overlap structure and hingement (Fig. 39C). On the contrary, the median element has a thick ligament between the overlap structure and hingement (The outline of hingement is obscure, but the hingement locates below the ligament apparently.) (Fig. 39D). The hinge structures of all elements in this species are classified into *Transitive type.*

I. Limnocytheridae

Limnocythere stationis : **Interior type**

This species is the only non-marine cytheroid species examined in this study. Its hingement is much weaker than that of European species. In the European species *Limnocytherina sanctipatricii*, the prominent terminal elements develop (Fig. 40A, B), but those of *Limnocythere stationis* are poor

(Fig. 40C, D). Thus, the hingement of this species seems to consist of the bar-groove mainly. The hinge structures in all elements show *Interior type* (The outline of hingement is probably broken, but the hingement locates below the ligament apparently.) (Fig. 40E, F).

J. Entocytheridae

***Enotocythere* : Basic type**

This species is the non-marine parasitic taxon. This study adopts the illustration of this taxon from Harding (1964), since no specimen could be captured. The long ligament connects to each valve without the hingement. The hinge structure is classified into *Basic type.*

K. Cytheridae

***Cythere omotenipponica* : Transitive type**

The hingement consists of the crenulations (Fig. 41A, B) and the tripartite overlap structure develops over the ligament (Fig. 41C, D). The hinge structures in all elements are recognized as *Transitive type.*

L. Cytherideidae

***Perissocytheridea japonica* : Interior-Transitive-Interior type**

***P. inabai* : Interior-Transitive-Interior type**

The hingements of these species are composed of the large crenulations in the terminal elements and the small crenulations in the median elements (Fig. 42A, B). The hinge structures in the terminal elements and median element develop *Interior type* and *Transitive type* respectively (Fig. 42C, D).

M. Cushmanideidae

***Pontocythere miurensis* : Basic-Exterior-Interior type**

***P. japonica* : Basic-Exterior-Interior type**

The hingement of the genus *Pontocythere* consists of the bar-groove as the median element and the crenulations as the posterior element (Fig. 43A, B). The hinge structure of anterior element is classified into *Basic type*, since the anterior bar-groove element is not complementary structure and the elongate ligament connects to each valve (Fig. 43C). In the median element, the hinge structure exhibits *Exterior type* which a poor ligament develops below the overlap structure (Fig. 43D). In the posterior element, the small short crenulations develop below the ligament and the hinge structure shows *Interior type* (Fig. 43E).

N. Krithidae

Parakrithella pseudadonta : **Transitive type**

The hingement is composed of the bar-groove and short crenulations (Fig. 44A, B). This hingement has been classified into "pseudadont (adont basic type)" for its simple structure. But this hingement is tripartite and the hinge structures of all elements are classified into *Transitive type* which consisting of the small overlap structure and large hingement (Fig. 44C, D). In the terminal elements, the ligament is extremely elongate (Fig. 44C).

O. Schizocytheridae

Schizocythere kishinouyei : **Transitive type**

Spnileberis quadriaculeata : **Interior type**

The hingement consists of the biramous large tooth and crenulations in the anterior element and the other elements respectively (Fig. 45A, B). The hinge structures of all elements are classified into *Transitive type*, which have rather small overlap structure (Figs. 45C, D, 46C).

On the contrary, the hingement of *Spnileberis quadriaculeata* does not develop the biramous large tooth, though this species belongs to the family Schizocytheridae (Fig. 46A, B). The hinge structure of this species appears as *Interior type* (Fig. 46D).

P. Hemicytheridae

Caudites asiaticus : **Transitive type**

Aurila hataii : **Transitive type**

The hingement of Hemicytehridae is composed of the large teeth in the terminal elements and the median bar comprising the anteromedian large tooth with dorsal crenulations (Fig. 47A, B). The hinge structure exhibits *Transitive type*, which has the extremely small overlap structure and the large tooth below the ligament in all elements (Fig. 47C, D, E).

Q. Trachyleberididae

Trachyleberis scabrocuneata : **Interior type**

The hingement of Trachyleberididae consists of the large teeth in the terminal elements and the median bar comprising the anteromedian large tooth with lateral crenulations (Fig. 48A, B). The hinge structures in all elements are classified into *Interior type* (Fig. 48C, D, E).

2. Ultrastructure of free margins

2-1. Selvage

The selvage is composed only of epicuticle. Around its root lattice structures of feather-like fibres

develop, and this region seems to be slightly calcified (Fig. 49A, B, C).

In many species selvages seem to be homogenous with each other due to the presence of electron-dense materials (Fig. 49B, C). Though helicoidal structures exist in the selvage of *Neonesidea oligodentata* (Fig. 49A) these structures are different from the feather-like structure in the ligament (Fig. 19C).

2-2. Marginal infold

The broad marginal infold which develops along the free margin of adult podocopids (Fig. 5A) does so to a lesser extent in juveniles (Fig. 50B): the difference being due to the extent of the calcified area (Figs. 51A, B; 52A, B).

In Platycopida and podocopid Darwinuloidea, which have many primitive characteristics, adults are recognised for the poor development of the marginal infold, like other podocopid juveniles (Fig. 50C, D). But at the marginal areas in both taxa the flexible inner lamella cuticle connects directly to the outer margin (Figs. 51C, D; 52C), and the marginal infold is not fully developed. Therefore they lack a marginal infold rather than having one which is poorly developed.

Ultrastructure of carapace structure at the final molt

In this study, the various phases of cuticle and epidermis in the carapace margin of *Loxoconcha pulchra* are observed utilizing the TEM and SEM. The division of molt stages is adopted from Okada (1982a). Since the carapace structure in the adult has been described in the other chapter and the difference of carapace structure between the adult and juvenile are only the development of membranous layer, the carapace structure at the stage C (intermolt) is not described in this chapter.

1. Stage D (Premolt)

1-1. Stage D1 (Fig. 53)

Cell membranes are separated from the cuticle (apolysis) and the ecdysial fluid emerges between the cuticle and epidermis (Fig. 53A). The ecdysial fluid contains the electron-dense granular materials and its diameter is up to 1 μm. This granular material seems not to be calcareous substances, since the specimen is treated with EDTA.

At this stage, the new cuticle has not been formed yet in the marginal areas and the epidermis looks static (Fig. 53B, C).

1-2. Stage D2 (Figs. 54, 55)

The ecdysial space is filled up with low electron-dense substances, and the granular materials and amorphous substances containing lattice-like structures seem to increase in the ecdysial fluid (Fig. 54 A). The newly formed epicuticle appears as a dotted line consisting of the numerous grains in most parts of carapace (Fig. 54B). A continuous layer is sometimes recognized.

In the attached margin, the new continuous epicuticle develops but partly looks like still patches (Fig. 54 C, D, E). The epidermis commences the secretary activities and the granules are often confirmed in the cytoplasm. In the free margin, the new epicuticular fragments form a continuous layer and the formation of selvage has almost finished (Fig, 55A, B).

1-3. Stage D3 (Figs. 55, 56, 57)

In this stage, the new epicuticle has been formed as a continuous layer completely and is composed of the low electron-dense thin layer (Fig. 56A). The high electron-dense layer has been formed beneath the epicuticle at the ligament area and comb-like structure develops inside this layer (Fig. 56B). The epidermis secretes the various granules (e.g. high/low electron-dense, patch-like, cored) and the numerous granules are observed in the epidermis (Figs. 56D, 57A, B).

In advance of above phase, a spine-like layer begins to develop as the new epicuticle (Fig. 56C). The selvage, which has been formed almost in the stage D2, develops its root and the formation of selvage has completely finished by the stage D3 (Fig. 55C, D). At the ligament area, the fibres of comb-like structure elongate and joint each other to form the feather-like fibres of ligament beneath the new epicuticle (Fig. 56E). The cuticular projection as the new overlap structure can be confirmed adjacent to the new ligament.

The bundles of chitinous fibre emerge in the new ligament (Fig. 57E), when the spinous epicuticular layer has been formed completely in the whole areas of carapace (Fig. 57C, D).

1-4. Stage D4 (Figs. 58, 59, 60)

The formation of new epicuticle finished, when the high electron-dense layer (nepc2) has been formed between the spinous layer (nepc1) and low electron-dense thin layer (nepc3) completely (Fig. 58A). The new procuticle (up to 1 μ m thick) are formed beneath the epicuticle (Figs. 58A, 59A, C). The new procuticle is not still calcified and comprises only the organic matters. The numerous granules exist in the epidermis (Fig. 58B). Around the newly formed sieve pore, exocytosis of many vesicles towards the ecdysial space is observed (Fig. 58C, D). Since the supporing fibres are confirmed in all stages and penetrate the new cuticle to fix the cuticle and epidermis (Fig. 58E). The bundles of chitinous fibres in the new ligament develop as degree as the adult ligament in the stage C (Fig. 59). The hinge structure of *Loxoconcha pulchra* is classified into *Interior-Transitive-Interior type*. The new overlap structure does not develop in the terminal elements (Fig. 59A), whereas it develops in the median element (Fig. 59C). Furthermore, it is supposed that the series of epidermal cells lining below the ligament form the new ligament and overlap structure (Figs. 59A, C, 60A).

The formation of new ligament has finished, when the new epicuticle and procuticle (yet non-calcified) are formed completely (Fig. 60A). The new ligament comprises the bundles of chitinous fibres formed entirely (Fig. 60B).

2. Stage A (Postmolt) (Figs. 61, 62, 63, 64, 65)

The carapace just after ecdysis is flexible, but the calcification immediately is carried out. It is confirmed by the optical observations that many fine threads secreted from the spinneret seta fixed the animal body during the ecdysis, since the specimens are easily carried away by a weak flow of water. The cuticular layer just after ecdysis is one wrinkled sheet (Fig. 61). The cuticular layer, which commences the calcification, increases its thickness rapidly (Fig. 61, 62). The calcification of

carapace margin is earlier than that of central part (e.g. adductor muscle area) of carapace (Fig. 62). In the other crustacean studies, the stage B (e.g. the formation of endoxuticle) is recognized easily for the distinct boundary between the exocuticle and endocuticle (Passano, 1960). But in *Loxoconcha pulchra*, the stage B can not be recognized. The carapace becomes hard within a few hours. The end of formation of membranous layer after the calcification shows the beginning of the stage C. The membranous layer is not observed in the specimens past at least three days after ecdysis.

The formation of hingement is traced using the SEM, since the outline of hingement can not be seen by the TEM observations (probably due to the decalcified influence). The successive morphologies of hingement after ecdysis by five hours are shown in Figure 63, 64 and 65. In the right valve just after ecdysis, the hingement is not observed but the rudiments of terminal elements have been formed (Fig. 63A). All specimens of left valve just after ecdysis are broken, for they are broken easily by surface tension of water when mounted on the stage of SEM. In the specimen past five hours after ecdysis, the rudiments of terminal elements are formed in the both valves (Fig. 63B). The crenulations of median element in the right valve commence to develop, whereas the median element in the left valve appears as the smooth bar (Fig. 64A). In the specimen past ten hours after ecdysis, the crenulations of median element in right valve have been almost formed except the median part (Fig. 63C). On the contrary, the crenulations of median element in the left valve begin to be formed following the formation of hingement of right valve (Fig. 64B) and the formation of median part is the latest also in the left valve (Fig. 64B-E). All elements in the hingement of right valve have been formed by fifteen hours after ecdysis (Fig. 63C) and the formation of hingement has finished fully by thirty hours after ecdysis (Fig. 63G). On the other hand, the crenulations in the median element of left valve have been formed by twenty-five hours after ecdysis (Fig. 64E) and the formation of hingement has finished completely by fifty hours after ecdysis (Fig. 65D).

Discussion

1. Structural diversity of ostracod carapace

1-1. Cuticular structure

The studies on carapace structure of Ostracoda have been carried out much less than that of Insecta or Decapoda. Many authors found out the structural diversity of ostracod carapace only based on the descriptions of calcite crystal or organic lattice (Sylvester-Bradley & Benson, 1971; Bate & East, 1972, 1975; Yumoto, 1994 and others), and discussed the correlations between the carapace structure and phylogeny or ecology (Sohn & Kornicker, 1988; Yumoto, 1995MS). Okada (1982a) is the only study, which described the ultrastructure of ostracod carapace in detail. The significance of his work is that ostracod carapace is enabled to be compare with the cuticle of other arthropods or other cuticular integumental organisms (e.g. Nematoda). In this chapter, ostracod carapaces are compared with that of other arthropod integuments based on the descriptions of their ultrastructures, and their structural diversity is discussed.

Epicuticular structure

The epicuticle, organic thin layer, covers the outer surface of arthropod exoskeleton. This layer protects the procuticle and inhibits the evaporation of water from outer surface (Price & Holdich, 1980a; Powell & Holdich, 1985), and is often wounded for directly contact with the circumstance. The epicuticle is composed mostly of protein, lipids in most arthropods. In higher crustaceans (e. g. Decapoda), this layer contains the calcium salt (Martin, 1992). The epicuticle does not comprise chitin conventionally in Arthropoda (Neville, 1975). Kesling (1951) described the outermost thin layer of carapace in *Cypridopsis vidua* as "chitin coating of calcareous layer", but he had no evidence that this layer contains chitin.

In most arthropods, the epicuticle is divided into outer and inner epicuticle, and subdivided into several layers (Wigglesworth, 1947; Locke, 1964; Weis-Fogh, 1970 and others). The ostracod epicuticle is also divided into several layers as that of other arthropods (Table 4). The epicuticle has been divided for the three criterions; the difference of composition (e. g. lipid or protein), formation process (e. g. before or after ecdysis) and function (e. g. defense or resistance to desiccation).

Chemical analysis is needed to identify the composition of epicuticle accurately, but this can be speculated on the basis of the studies on insect and decapod cuticle. Wigglesworth (1947) divided the insect cuticle into four layers and termed "cement layer," "wax layer," "polyphenol layer," and

"cuticulin layer" from the outside. He reported that outer two layers are formed before ecdysis and inner two layers are formed after ecdysis. Locke (1964) termed the outermost low electron-dense layer "cuticulin layer" and described underlying the high electron-dense layer as "protein layer". In consequence, they both reported that low electron-dense outer layer contains tanned or amorphous lipids abundantly and inner layer comprises lipids, proteins, and polyphenols. According to the studies on decapod cuticle (Dennell, 1947; Green & Neff, 1972), outer and inner layer consists of mainly lipids and proteins respectively. Also in Ostracoda, it is supposed that the outer thin layer, which often seems to be amorphous, contains lipids abundantly and rigid inner layer is composed of proteins mainly (single layered epicuticle may be simple composition).

The recognition of homology among the arthropod epicuticles needs the detail elucidation of its formation in each taxon. Wigglesworth (1947) concluded that lipid layers are secreted from the pore canal and dermal galand duct, and formed after ecdysis. On the other hand, Locke (1964) reported that the outermost epicuticle (his "cuticulin layer"), which is saturated with tanned lipids, is formed initially as the new cuticle. Some authors reported that the crustacean epicuticles are formed from the outside to inside layer (Halcrow, 1976; Price & Holdich, 1980a; Powell & Halcrow, 1985). The homology between the ostracod and other arthropod epicuticles and formation process of ostracod epicuticle have not been discussed in few studies. Okada (1982a) stated that the outermost epicuticle of *Bicornucythere bisanensis* corresponds to the cuticulin layer in the insect epicuticle reported by Locke (1966) based on its structure and formation process. Yamada *et al.*, (2004) reported that the outer epicuticle emerges after the formation of inner epicuticle in *Semicytherura kazahana*. This study showed that the order of epicuticular formation in *Loxoconcha pulchra*; the innermost, outermost and middle epicuticle are formed in order (Fig. 53-58).

Futhermore, the microvilli structures at the apical membrane of epidermis and growing points of newly formed epicuticle of insect species reported by Locke (1966) have not been recognized in the above ostracod studies. These are the important structures for the insects to concern with the deposition of new cuticle. Powell & Holdich (1985) reported that the formation of outer epicuticle in the marine isopod *Idotea baltica* experiences the same process as a formation model proposed by Locke (1966), but some authors the new epicuticle is formed as a continuous layer by aggregation of many plaques without microvilli structure of epidermis in the freshwater branchiopod (*Daphnia magna*) and terrestrial isopod (*Oniscus asellus*) (Halcrow, 1976; Price & Holdich, 1980a).

These facts suggest that the mechanism of epicuticular formation in Ostracoda differs from that in

Insecta, and it is thought that the some patterns of epicuticular formation process exist in each ostracod taxon as the other crustaceans. The recognition of homology between the ostracod and other crustacean cuticle needs the detail elucidation of epicuticular composition and formation process in each higher taxon.

Procuticular structure

The procuticle in arthropods usually has been divided into the exocuticle and endocuticle (Neville, 1975; Dalingwater & Mutvei, 1991 and others) on the basis of staining reaction in optical sections (the difference of compositions) or direction of crystallographic axis (Travis, 1963; Taylor & Richard, 1965; Neville, 1975). Recently, some studies divided the procuticle based on the function or timing of formation (Price & Holdich, 1980b).

Some authors insisted the each understanding concerning with the division of procuticle. As the study utilizing the TEM, Bate & East (1972; 1975) observed the organic fibres in the carapace of freshwater ostracods and divided the procuticle into the exocuticle and endocuticle based on the structural difference of organic lattice. Keyser (1990; 1995) also showed that the latticed exocuticle and laminar endocuticle in the carapace of freshwater ostracod. As the study using the SEM, Sylvester-Bradley & Benson (1971) found the at least two distinct layers "foliated layer" and "laminar layer" in the hard tissue of fractured carapace, but did not mention that they correspond to the exocuticle and endocuticle. Smith & Bate (1983) and Sohn & Kornicker (1988) divided the myodocopan procuticle into the exocuticle and endocuticle on the basis of crystal structures. Kondo *et al.* (2005) divided the procuticle of *Xestoleberis hanaii* into the exocuticle and endocuticle by the morphological difference of organic matrix.

Consequently, Okada (1982a, b) concluded that the boundary between the exocuticle and endocuticle can be recognized not in the ornamented carapace but in the smooth carapace by the SEM and TEM observations. But Yumoto (1995MS) concluded that podocopan procuticle can not be divided into exocuticle and endocuticle by the SEM observations of fractured carapaces.

In this study, all examined non-marine ostracods, which have smooth carapace, develop the organic latticed exocuticle in the procuticle (Table 4). The boundary between the exocuticle and endocuticle is obscure in the procuticle of ornaneted non-marine species *Ilyocypris japonica*. In marine ostracods, the boundary between the exocuticle and endocuticle can be almost found in only the species which has the smooth or minor ornamented (e. g. slight ridge, pit) carapace (*Melavargula japonica*, *Cypridina noctiluca*, *Neonesidea oligodentata*, *Triebelina* sp., *Darwinula stevensoni*,

Vestalenula sp., *Microdarwinula* sp., *Propontocypris* sp., *Chrissia* sp., *Cypridopsis vidua*, *Fobaeformiscandona* sp., *Cypria reptans*, Paracypridinae sp. A, Paracypridinae sp. B, *Paradoxostoma triangulum*, *Xestoleberis hanaii*, *Xestoleberis setouchiensis*, *Limnocythere stationis*, and *Cythere omotenipponica*), but the smooth carapace species do not always develop the organic latticed exocuticle (*Sclerochilus* sp., *Pontocythere japonica*, *Pontocythere miurensis*, *Parakrithella pseudadonta*, *Praracobanocythere* sp., and *Microcythere* sp.). The development of exocuticle as dense organic lattice is thought to cause the aggregation of organic materials near the carapace surface. This causes to regulate the ratio of organic materials to calcite. Consequently, it is supposed to be difficult for ridges to develop by the extreme concentration of calcite. Exceptionally, the surface ornamentations develop in the cararpace of non-marine species *Limnocythere stationis*, which has the organic latticed exocuticle. It is supposed that much thinner exocuticle of this species (up to 100nm) than that of other freshwater species enables to develop the surface ornamentations. Conversely, these facts also suggest that the obscure boundary between the exocuticle and endocuticle is not directly associated with the development of surface ornamentations. Probably, the recognition of the boundary between the exocuticle and endocuticle is thought to relate to not only the development of surface ornamentations but also its habitat, phylogeny, and so on.

The pore canals develop the procuticle of outer lamella cuticle in some taxa (*Neonesidea oligodentata*, *Triebelina* sp., *Xestoleberis hanaii*, *Xestoleberis setouchiensis*, *Darwinula stevensoni*, *Vestalenula* sp., *Microdarwinula* sp., *Cypridopsis vidua*, and *Fabaeformiscandona* sp.). In many species, amorphous pore canals form the networks beneath the exocuticle and some of them are filled with electron-dense substances. The morphology of networks differs among the taxa. In *Xestoleberis hanaii* and *Xestoleberis setouchiensis*, the pore canals lack the wall membranes. In *Cypridopsis vidua*, the pore canals penetrate through the exocuticle and extend to just below the epicuticle.

Bate & East (1972) described the dot-like secretary pore canals in the exocuticle of *Cypridopsis vidua* and Okada (1983) reported the pore canals beneath the exocuticle in *Xestoleberis* sp. and *Neonesidea* sp.. It is sure that the pore canals are associated with cuticular secretion based on the morphology, developmental range, and comparison with the other crustacean pore canals, though the concrete function of them have never been clarified yet. It is confirmed that the pore canal networks have not seen in the new cuticle of *Xestoleberis hanaii* at the premolt stage, though the organic lattice of exocuticle has already developed (Figure 66). This fact suggests that the pore canal networks

develop after the ecdysis and indicates the possibility for transportation of the materials concerning with forming or tanning the endocuticle. Moreover, the ostracods, which develop the pore canals, has smooth or minor ornamented carapace. The mechanisms of cuticle formation in these taxa may be similar to each other.

Structure of membranous layer

The organic layer, which develops as the innermost layer of calcified cuticle (e. g. Decapoda, Isopoda), is termed membranous layer and this layer is also called uncalcified endocuticle (Dalingwater & Mutvei, 1991).

In Ostracoda, the membranous layer exists in the outer lamella of the following taxa; *Xestolaberis hanaii*, *Xestoleberis setouchiensis*, *Pontocythere japonica*, *Pontocytehre miurensis*, *Bicornucythere bisanensis*, *Cythere omotenipponica*, *Parakrithella pseudadonta*, *Loxoconcha pulchra*, *Loxoconcha japonica*, *Cytheromorpha acupunctata*, *Semicytherura kazahana*, *Hemicytherura kajiyamai*, *Hemicytherura tricarinata*, *Microcythere* sp., *Schizocythere kishinouyei*, *Paradoxostoma triangulum*, *Aurila hataii*, *Caudites asiaticus*, *Trachyleberis scabrocuneata*, *Spinileberis quadriaculeata*, *Paracobanocythere* sp., *Neonesidea oligodentata*, *Keijcyoidea infralittolaris*, *Melavargula japonica*, and *Cypridina noctiluca*. Thus, this layer develops among the several lineages simultaneously. The membranous layer shows homogenous, laminate, or fibre-like structures and the detail structures (e. g. electron-dense, the number of lamina) differ among the taxa. Further, in all non-marine taxa examined (three superfamilies; five families), the membranous layer does not develop. But, only around the attached margin of *Cypria reptans*, thick membranous layer develops and also around the attached margin of *Fabaeformiscandona* sp. belonging the same family, the weak-calcified layer similar to membranous layer is confirmed as the innermost part of endocuticle. The development and morphology of membranous layer do not well reflect the ostracod phylogeny but they are almost conservative in the each genus.

1-2. Epidermal structure

In many arthropods, the single layered epidermis arranges beneath the cuticle and contributes to the cuticular secretion (Neville, 1975). The epidermis in the free margin of ostracod carapace is composed of two epidermal layers, since the ostracod integument develops the dual structure covering the whole animal body as carapace (Okada, 1982a; Keyser, 1990 and others). The histological structure and morphology of epidermis are quite uniform comparing with those of cuticle (Figs. 4, 5, 6, 8, 10, 12, 13, 14, 16, 17).

The outer epidermal cells shape the rectangle like paving-stones and comprise the circular nucleus, granules and melanocytes. These cells have been thought to have the function of cuticle formation for the reason of containing abundant granules and vesicles at the premolt stage (Rosenfeld, 1979, 1982; Okada, 1982b). The TEM observations of this study support this opinion. The amoeboid outer epidermal cells, which can migrate in the body cavity of carapace, reported by Turpen & Angell (1971) are not found by this study. The inner epidermal cells are thicker than outer epidermal cells and contain mitochondria and lipid droplets abundantly (Fig. 6; Yamada *et al.*, 2004, text-fig. 7C). These cells have been supposed to have the role of respiratory metabolism and osmoregulation (Okada, 1982a; Keyser, 1990; Aladin, 1993). This study agrees with the above hypothesis and proposes the possibility for a function like adipocytes (e. g. reservoirs of nutrition) for abundant lipid droplets.

The outer and inner epidermal cells are fastened to the cuticle by the supporting fibres. The pillar cells, which contain the abundant microtubles and prop the hemicoele, develop in the decapod carapace and their structure is similar to that of supporting fibres (Taylor & Taylor, 1992). In the carapace of *Conchoecia*, the supporting fibres separate the body cavities and form the sinus network (Harding, 1964). This study also confirms the separation of body cavities by the supporting fibres in a portion of myodocopid and darwinuloid carapace (Figs. 12C, 16B). It is supposed that the supporting fibres have partly the same functions as pillar cells based on the structural similarity, though they are not homologue of pillar cells. But the supporting fibres have a role of fixation between the cuticle and epidermis rather than formation of the circulation, because the distinct circulation does not develop in Ostracoda excluding some taxa of Myodocopa.

In most ostracods, the subdermal cells, which comprise the abundant r-ER, exist in the body cavities of carapace without distinct circulation (Okada, 1982a, b). These cells are thought to be able to migrate in the body cavites of carapace, since they are free from the fixation by the supporting fibres (Okada, 1982b) and they have been observed in Cytheroidea and Cypridoidea generally (Kesling, 1951; Turpen & Angell, 1971; Okada, 1982a). Amoeboid cells, which are not fastened by the fibres without containing the abundant r-ER, are often observed in all podocopan carapaces (Figs. 5, 6, 8, 10, 12, 13, 14). This study agrees with Okada (1982b)'s opinion and suggests that the subdermal cells and other amoeboid cells migrate in the body cavities of carapace and transport the substances to the epidermal cells, because these cells always comprise the numerous granules and some of them contain the abundant r-ER. In *Keijicyoidea infralittlaris*, unique high electron-dense

layer develops in the adult carapace and the amoeboid cells containing the numerous granules exist in this layer (Fig. 13F). This layer is similar to hemolymph lacuna in the decapod carapace (Taylor & Taylor, 1992, Fig. 25A) for its position and electron-dense and has a possibility for hemocoele of this taxon, but this layer is not observed in the carapace of 8th instar (Fig. 14A).

2. Ostracod carapace as crustacean integument

The histological structure in arthropod integuments is quite conservative, in spite of their various external morphologies. Crustacean integuments basically consist of the epicuticle, exocuticle, endocuticle, membranous layer, epidermis, and basement membrane from the outside to inside. The ostracod carapace has the two characteristic features. One is that the pore canals forms a network structure in only the procuticle of outer lamella cuticle, though the pore canals develop as the extension of epidermis to the cuticle in the other arthropods. The other is that the epidermis in the free margin is always composed of dual epidermal layers.

2-1. Pore canal network

In many arthropods, the pore canals are identified with the extensions of epidermis and they seem to extend to their own cuticular layer perpendicularly (Compere & Goffinet, 1987a, b and others). But the ostracod pore canals appear as short amorphous canals in transverse sections, and they penetrate beneath or into the exocuticle. Similar structure to these pore canals exists as the sieve plate around the sensory sensillum in the carapace. This study captured the formation process of this plate in *Loxoconcha pulchra* utilizing the TEM (Figs. 58C, D, 61C). The outer epidermal cells around the nervous cells secrete the numerous vesicles beneath the newly formed cuticle at the premolt stage (Fig. 58C, D). These vesicles are trapped though the calcification after ecdysis and remain as a sieve plate in the procuticle (Fig. 61C). Keyser (1982) explained that a sieve plate is the rudiment of exocytosis during the molt, using *Hirschmannia, Cyprideia, Xestoleberis, Leptocythere* species. But, Keyser (1983) speculated that the sieve plate in *Aurila convexa* has some functions, since this structure is filled with electron-dense substances in the adult carapace and the envelop cell beneath the sieve plate contains abundant mitochondria.

For the same character as cavities trapped in the procuticle of outer lamella cuticle, it is speculated that the pore canal networks have a secretary function during the molt and are trapped in the procuticle through the calcification like the sieve plates. Furthermore, it is supposed that the formation of pore canals is carried out after the ecdysis and their function is associated mainly with the

formation of endocuticle, because the pore canals have not been formed in the new cuticle of *Xestoleberis hanaii* at the premolt stage (Fig, 66). Based on the analogy, the pore canals in the other taxa (*Neonesidea oligondentata*, *Triebelina* sp., *Darwinula stevensoni*, *Vestalenula* sp., *Microdarwinula* sp., *Fabaeformiscandona* sp.) have the similar function and are formed through the same process, but the concrete function of pore canals remains unclear.

The cuticle formation of ostracod carapace has more complex mechanism than that of the other arthropod integuments for the various degree of calcification. Therefore, the various formation processes are carried out in Ostracoda comparing with the other crustacean and various micro organs for cuticle secretion (e. g. pore canal, sieve plate, and so on) are found in the cuticle. The qualitative diversification and evolution of morphogenesis in the arthropod cuticles may be elucidated by the structural understanding of the ostracod hard tissues base on the its components, micro organs, the directions of crystal growth and so on, since the hard tissues of ostracod carapace are well preserved as fossils.

2-2. Dual epidermal layers

The ostracod epidermis consists of dual epidermal layers. In many arthropods, their integuments are composed of single epidermal layer, but dual epidermal layers exist in the carapace fold of Cladocara (Halcrow, 1976, Fig. 67C). A cypris larva (Cirripedia) as bivalved crustacean also has the narrow carapace fold, but single epidermal layer arranges beneath the cuticle (Fig. 68C). In Decapoda, the broad carapace fold develops as a respiratory chamber and single epidermal layer exists beneath the cuticle (Taylor & Taylor, 1992). Dual epidermal layers are found in the carapace fold of *Uca pugnax* (Decapoda), but this taxon has dual epidermal layers in the whole animal body.

As a result of this study and previous studies, the composition of organelles differs between the outer and inner epidermal cells in the calcified ostracod carapace. It is clear that this fact indicates the functional differentiation of epidermal cells. This characteristic feature is not recognized in the dual epidermal layers of Cladocera and non-calcified ostracods (*Conchoecia*: Harding, 1964). In calcified ostracoda carapaces, the functional differentiation of epidermal cells accomplishes the rigid protection and efficiency of respiration concurrently. Consequently, it is assumed that Ostracoda has developed the specialized carapace structure, as the cover of animal body, which demarcated from the other crustacean integuments, equipped not only the protection from the circumstance but also the efficiency of metabolic activities.

3. Understanding of ostracod ligament and selvage

In this chapter, the homology between the attached and the free margin, and the structural differences of the duplicature among the some taxa, are also discussed.

3-1. Correlation between "ligament" and "selvage"

The studies which established the homology between the attached and free margins are few (Table 2). Based on optical observations of sections of *Cypris pubera* (Cypridoidea) Fassbinder (1912) concluded that the selvage is continuous with the ligament, and that both are composed of epicuticle. Bate & East (1972) reported that the ligament and the selvage consisted of epicuticle and exocuticle, by TEM observation, but they did not mention their homology. On the other hand, the homology between the attached and free margins has been discussed by several palaeontologists. Pokorný (1957) and Hanai (1961) noted the correlation between the contact groove, "selvage" (used in previous palaeontological studies; see Fig. 1C), and the "list" in Podocopida (Cytheroidea). Kornicker (1969) divided the myodocopid carapace into four components: "right and left shells", "ligament", and "vestment", and concluded that the ostracod ligament is not continuous with the selvage and that the ligament is independent of other cuticular parts.

The TEM photographs of successive sections around attached and free margins of *Aurila hataii* (Podocopida) and *Keijcyoidea* sp. (Platycopida) are compared here (Fig. 69).

In *Aurila hataii*, the selvage of the right valve is incorporated into the epicuticle of the marginal infold of the left valve. Thus, the selvages of both valves join with the epicuticle of the ligament, and the procuticle of both marginal infolds connect to each other through the procuticle of the ligament, with a chitin-fibrous structure (Figs. 69A-C; 70A-C). Since the epicuticle and procuticle of the marginal infold corresponds to those of ligament, we recognise a general homology between the marginal infold and the ligament. These facts suggest that the ligament is an uncalcified cuticular structure developing along the marginal infold, and is not continuous with the selvage in Podocopa. This understanding is also supported by the other observations, namely that the ultrastructure of the ligament differs from that of the selvage (Figs. 19, 49), and the formation of the selvage precedes that of the ligament at the premoult stage (Figs. 54-56). In *Keijcyoidea* sp. without a marginal infold, the selvage of one valve connects to that of the other and both selvages form a continuous epicuticular layer of ligament (Figs. 69D-F; 70D-F). Consequently, the selvages of both valves are continuous with the epicuticle of the ligament, and the procuticle of the inner lamella cuticle meets the procuticle of the ligament. Also, in this case, the ligament is not completely identified with the selvage.

3-2. Elasticity of the ligament

Many previous studies assumed that the ligament of almost all ostracods is not elastic, and that it plays no role in the opening of valves (cf. Harding, 1964; Smith, 1965; Jaanusson, 1985). But Jaanusson (1985) reported that dead specimens of *Cypridopsis vidua* had their valves open, and stated the possibility of elasticity of the ligament to open them. According to observations reported here the feather-like fibres in many ostracod ligaments are thought to correspond to the parabolic pattern made from the helicoidal fibrous arrangement in the arthropod cuticle. This feather-like structure is supposed to contribute only to mechanical strengthening of the cuticle rather than elasticity (Locke, 1964). Besides, the ostracod ligament (and its fibrous structure) occasionally develops along the vertical plane to the animal body (Fig. 18D). In such a case this arrangement of the ligament is not thought to work effectively for opening the valves. This study also denies that the ligament has enough elasticity to open valves.

Kornicker (1969) and Bate & East (1975) referred to the possibility of the ligament contributing to the opening of the valves in myodocopids with thick ligament, but many ostracod researchers think that myodocopid ostracods open their valves utilising a hydrostatic system (Maddocks, 1992; Vannier *et al.*, 2001).

The elastic protein "resilin" was reported to be distributed in some particular regions of the arthropod cuticle (Neville, 1975). This rubber-like cuticle develops at the root of grasshopper's limb and mechanoreceptive hairs. For elucidation of the elasticity of the ligament, chemical analysis is needed.

3-3. Ultrastructure of the marginal infold

Many species of Podocopida develop a marginal infold, though the degree of development depends on the ontogenetic stage (Figs. 50A, B; 51A, B). In *Keijcyoidea* sp. (Platycopida) and *Vestalenula* sp. (Podocopida: Darwinuloidea), the "outer lamella cuticle" directly links with the "inner lamella cuticle" along the outer margin (Fig. 51C, D). In these taxa, the outer margin cannot be distinguished from the inner margin, i.e. both margins are identical, because the inner margin is defined as the boundary of calcified cuticle and uncalcified cuticle along the inside of the valves (Fig. 52C).

Fossils of platycopids and darwinuloids without marginal infolds have been reported from Palaeozoic formations, and as is often pointed out the two taxa retain some primitive characters. On the other hand, extinct Paleocopa and Leiocopa from the Palaeozoic are also lacking a marginal infold

(cf. Moore, ed., 1961; Swanson, 1989a, 1989b; Williams & Vannier, 1995). Therefore the feature of free margin is assumed to be almost equivalent to that of Platycopida and Darwinuloidea. On the contrary, in the fossil carapace of podocopid superfamilies excluding Darwinuloidea, from the Palaeozoic, a broad marginal infold is developed (cf. Moore, ed., 1961). These facts suggest that the marginal infold is a synapomorphic character of these higher taxa, when they derived from the common ancestor in the early Palaeozoic.

4. A new concept of podocopan hinge structures

The previous morphological studies on podocopan hinges have been carried out mainly the classification on the basis of the number of elements in the hingements (Sylvester-Bradley, 1956; Hanai, 1961 and others). This classification has been applied to the higher taxonomy on ostracod superfamilies and regarded as the important features in ostracod taxonomy. But all hinge structures, which develop the hingement consisting of bar and groove, have been classified into "adont" simply, though they are recognized in some ostracod lineages simultaneously. Therefore, the evolution of hinge structures among the higher ostracod taxa has never been discussed. This study evaluates the hinge structures including the ligaments and establishes the new classification of hinge structures based on their homology. Moreover, the evolutionary pathway of hinge structures is discussed utilizing the characteristics of hinge structures, which have never been recognized in the previous classification.

4-1. Structural diversity of hinge structures

In Platycopida, Bairdioidea, and Darwinuloidea, which emerged in the Early Paleozoic and retain the primitive features in their body, their hinge structures are classified into uniform in each taxon and simple types by the new classification (Table 3). The diversification of cytheroid hinge structures also is confirmed by the new classification, and this consequence corresponds to the result of previous classification.

On the other hand, the hinge structures of Cypridoidea, most of which have non-marine aquatic habitats mainly, have been identified with only the adont hinge, but they are reclassified into at least three types (*Basic type*, *Exterior type*, and *Interior type*) by the new classification, in spite of their simple hingements consisting of the bar and groove.

These facts suggest that the hinge structures of "primitive taxa" (Platycopida, Bairdioidea, Darwinuloidea) have not much diversified, but those of "derived taxa" (Cytheroidea, Cypridoidea)

have highly diversified irrespective of their hingement morphologies.

4-2. Plasticity of cytheroid hinge structures

In Cytheroidea, the hingement morphologies diversify and are classified into near the twenty types even only in the extant species (Hinz-Schallreuter & Schallreuter, 1999). The two characteristics of cytheroid hinge structures are considered to cause the various morphologies of hingements. One is that most cytheroid ostracods develop *Trasitive type* and *Interior type* hinge structures. The other is that most cytheroid hingements are composed of the three elements.

The former attains the high morphological plasticity for the hingement, due to the separation of ligament and hingement in *Transitive type* and *Interior type*. In *Basic* type, no hingement develops while in *Exterior type*, which develop the hingement at the ligament area, its hingement consists of only the bar and groove. It is ascertained that these hinge structures (*Basic* type and *Exterior type*) have no space for the development of hingement beneath the ligament. Besides, the hingement of *Exterior type* can not take complex morphologies, because numerous organic fibres aggregate in the ligament joint area and they bring the difficulty for quantitative change of calcification. Thus, it is supposed that the aggregation of organic fibres correlates not only the carapace ornamentation but also the hingement morphology. On the contrary, *Transitive type* and *Interior type* have enough space to develop the hingement beneath the ligament and they can develop the complex hingements for only adjustment of calcification.

The latter is recognized in Cytheroidea and a few other ostracods and their hingement morphologies have diversified remarkably by this tripartition (anterior, median, and posterior elements). Most cytheroid species develop the tripartite hingements, since it is assumed that they are benthos which crawl on or dig into the sediment surface and furnish the terminal elements for the resistance to the distortional force of heavy sediment particles. The tripartite hingements are observed in the marine taxa; *Keijcyoidea infralittlaris* (Fig. 21A, B), *Manawa staceyi* (see, Swanson, 1989a, b), *Neonesidea oligodentata* (Fig. 22A, B; almost invisible), and *Macrocypris* sp. (Fig. 71A, B), which live on or in the sediments (coarse sand). On the other hand, the tripartite hingements do not develop in the interstitial species *Paracobanocythere* sp. (Fig. 38A, B), the phytal species *Sclerochilus* sp. (Fig. 30A, B), *Padoxostoma trianglum* (Fig. 32A, B), and the parasitic species *Entocythere* (see Harding, 1964, Fig. 14). These facts support the hypothesis that the tripartition of hingements develops as the intensification of the resistance to the distortional force of heavy sediment particles.

It is concluded that the two characteristics above ensure the morphological plasticity of cytheroid hingement and promote the diversification.

4-3. What are hinge structures affected by?

Some previous studies discussed the correlation between the complexity of hingements and degree of calcification (Pokorný, 1957; Benson, 1966 and others). However, they have never mentioned the concrete affections on the complication of hingements.

Most cypridoid ostracods are found in non-marine aquatic habitats and their hinge structures are composed of the simple types; *Basic type* and *Exterior type* (Figs. 23, 24, 25). But the minor marine species (e. g. Paracypridinae) have the developed hinge structures; *Interior type* (Fig. 26). In Cypridoidea, the benthic (e.g. *Chrissia* sp., *Fabaeformiscandona* sp., and Paracypridinae sp. A) and neck to-benthic species (e.g. *Cypridopsis vidua*, *Cypria reptans*, and Paracypridinae sp. B) develop the same hinge structures in the each families.

On the contrary, most cytheroid ostracods live in marine and brackish environments and their hinge structures are highly complicated. The non-marine species *Limnocythere stationis* furnishes the developed hinge structure (*Interior type*) and tripartite hingement (Fig. 40), though the non-marine taxon *Entocythere* has the simple hinge structure (*Basic type*) and non-tripartite hingement (Harding, 1964, Fig. 14). The genus *Entocythere* is the peculiar taxon, which parasitizes the crayfish gills. Besides, the interstitial species *Paracobanocythere* sp. (Fig. 38), phytal species *Sclerochilus* sp. (Fig. 30), and *Padoxostoma trianglum* (Fig. 32) also have the simple hinge structure (*Basic type* and *Exterior type*) and non-tripartite hingement, though they are found in marine environments.

In consequence, the non-marine and marine cypridoid ostracods equip the simple (*Basic type* and *Exterior type*) and developed (*Interior type*) hinge structures respectively. In Cytheroidea, only the interstitial, phytal and parasitic cytheroid species have the simple hinge structures (*Basic type* and *Exterior type*), though most cytheroid species furnish the developed hinge structures (*Transitive type* and *Interior type*) irrespective of their saline environments. These facts conclude that the cypridoid hinge structures exhibit the difference of their habitats (freshwater or marine; mineral environment) distinctly and the cytheroid hinge structures are affected by the modification of the carapace features in connection with the difference of their microhabitats rather than aquatic environments.

4-4. Evolutionary pathway of podocopan hinge structures

Few studies discussed the evolution of ostracod hingements. In the previous studies, the hingements have been generally thought to reflect the ostracod phylogeny and have an evolutionary

trend from a simple one to a complicated one (Sylvester-Bradley, 1948; Hartmann, 1963; Sandberg, 1964; Benson, 1966). On the other hand, the studies on hingements, which reported the parallel evolution (Triebel, 1954; Sylvester-Bradley, 1956), discussed the pseudomorphosis (Kamiya, 1992; Tsukagoshi, 1994; Tsukagoshi & Kamiya, 1996), and assumed the parallel evolution based on the molecular phylogenetic relationships (Yamaguchi, 2003), ascertained that the hingements do not exactly have a regular evolutionary trends and concluded that the hingements do not always reflect the ostracod phylogeny. This study surveys the fossil records of major taxa in Podocopa from the literatures and shows the result in Figure 72. On the basis of this figure, the evolutionary pathway of ostracod hinge structures is presumed below.

In Ostracoda, it is supposed that the most primitive hinge is found in the Paleozoic ostracods Paleocopa, which can not close their own carapace completely (Hinz, 1993 and others). This study assumes the all ostracod hinge structures originate from the paleocopan one, since this can be thought to have a simple structure consisting of only the ligament. It is thought that the most simple hinge structure *Basic type* in Podocopa (Fig. 73A; Platycopida, Darwinuloidea) derived from the paleocopan hinge without structural modification by the Early Ordovician, though their carapaces modified as a covering structure.

By the Late Ordovician, the marginal infold was developed due to the increase of calcification in the podocopan free margin, while the hinge structure *Exterior type* comprising the overlap structure with simple hingement was emerged (Fig. 73B; Bairdioidea).

The hinge structures, mentioned above, had no complicated hingements, since their hingements did not develop or were formed in the connecting area. But the hinge structures *Transitive type* and *Interior type*, which equips the ligament and hingement independently, appeared by the Silurian (Fig. 73C; Bythocytheridae, Macrocypridoidea?). This order of emergence corresponds to the fact that the formation of overlap structure precedes that of hingement beneath the ligament. Besides, due to the development of tripartite hingement, the cytheroid lineage had achieved the high plasticity of hinge structures by the Early Paleozoic. In the Mesozoic, species diversity of Cytheroidea exploded at the family level and the various hingement morphologies emerged (Fig. 73D). To date, they have adapted the various aquatic habitats and some of them (Paradoxostomatidae, Conbanocytheridae, Entocytheridae, and *Sclreochilus*) reduced the developed hinge structures to the simple ones throughout their adaptation (Fig. 73E).

On the contrary, the superfamily Cypridoidea occurred by the Late Paleozoic and most cypridoid

species retained the simple hinge structures *Basic type* and *Exterior type* for the adaptation to non-marine aquatic habitats up to the present (Fig. 73F). But the marine cypridoid species (Paracypridinae) and Pontocypridoidea (derived from Cypridoidea in the middle Mesozoic?) adapted to the marine environment and equipped the developed hinge structure *Interior type* by the Cenozoic (Fig. 73G). But they have no tripartite hingements, in spite of the circumstance containing the minerals richly. Because it is supposed that they do not need the tripartite hingements like those of Cytheroidea for their nektonic or interstitial ecology. On the other hand, Macrocypridoidea is the marine benthic taxon like most cytheroid species and they develop the tripartite hingements (Fig. 71). It can be speculated that the macrocypridoid hinge structure was given rise to the cypridoid hinge structures for the adaptation to the marine habitat in the cypridoid lineage from the early Cenozoic based on their accurate fossil records (Fig. 73H). But it can be also assumed that the macrocypridoid hinge structure gave rise to the cypridoid hinge structures for the adaptation to the non-marine habitats by the Late Paleozoic (Fig. 73F), since the macrocypridoid species retain the primitive features and their unsure fossil records are reported from the Early Paleozoic. Consequently, it is concluded that the hinge structures of cypridoid, pontocypridoid, and macrocypridoid species correlate their own aquatic environments distinctly.

The evolutionary pathway of hinge structure inferred in this study suggests the facts below.

(1) The ostracod hinge structures have an evolutionary trend from the simple one to the developed one, when the derived taxa (Cytheroidea, Cypridoidea) were evolved from the primitive taxa (Platycopida, Darwinuloidea, and Bairdioidea) in the Paleozoic (Fig. 73A-C).

(2) The hinge structures of derived taxa diversified with the adaptation to various habitats in the Mesozoic and they had an evolutionary trend not only from a simple hinge structure to a developed one (Fig. 73D, G) but also from a developed hinge structure to a simple one (Fig. 73E, F).

(3) The hinge structures do not always reflect the ostracod phylogeny and are often influenced by the habitats or adaptive modification of carapace features. But the hinge structures are useful for the ostracod higher taxonomy, since they are conservative features at the family level.

5. Morphogenesis of podocopan hinge structures

Few studies discussed the cuticle formation of ostarcods (Okada, 1982b, Keyser, 1995, Keyser & Walter, 2003; Keyser, 2005; Yamada *et al.*, 2005), though many studies on the formation of arthropod cuticle were carried out (Locke, 1966; Price & Holdich, 1980a; Powell & Halcrow, 1984

and others). In this chapter, the morphogenesis of podocopan hinge structures, which has never been discussed in spite of the various hingement morphologies, is elucidated.

5-1. Cuticle formation of hinge structures

The cuticle formation of the hinge structure can be analyzed as follows, based on the TEM and SEM observations of newly formed cuticle at the various phases in the attached margin of *Loxoconcha pulchra*. Initially, the apolysis occurs and ecdysial fluid is secreted (stage D1), prior to the formation new cuticle, as known in the other crustaceans (Travis, 1963). Ecdysial fluid contains the electron-dense granular materials. It is speculated that these are not calcium materials but the organic materials for the synthesis of new cuticle, since all specimens are experienced through EDTA treatment.

The cuticle formation of this species begins with the deposition of granular cuticular fragments in the fluid near the epidermal cell membranes (stage D2). The earlier development of cuticular layer in the marginal areas suggests that the cuticle formation commences from the marginal areas to central areas. In this stage, the new ligament in the attached margin has never been formed yet, though the new selvage in the free margin has been almost formed except its proximal part.

The deposition of granular cuticular fragments forms a continuous epicuticular layer (new innermost epicutcle) (stage D3) and the formation of fibrous structure begins beneath a continuous layer in the attached margin. When the new spinous epicuticle (new outermost epicuticle) emerges, the fibres beneath the new epicuticle in the attached margin commence to joint each other and form the ligament structure. In this phase, the new selvage in the free margin has been formed completely. Additionally, the newly formed overlap structure can be confirmed adjacent the new ligament. At the last phase of stage D3, the new epicuticle is composed of a continuous layer (new innermost epicuticle) and a spinous layer (new outermost epicuticle) and the formation of bundle of chitin fibres proceeds.

In the stage D4, the new epicuticle consists of three layers, and the newly formed thin procuticle comprising abundant organic substances appears beneath the new epicuticle. This newly formed procuticle is still non-calcified. The new ligament has been formed completely in this stage.

The new procuticle swells rapidly and begins its calcification and tanning after ecdysis (stage A). The calcification commences from the marginal area to central area (near the adductor muscle fields) as reported by Turpen & Angell (1971). The each hingement in the right and left valve has been formed completely about in one and two days after ecdysis respectively. The formation of

hingement in the both valves does not proceed simultaneously. The ligament and hingement are formed by the series of underlying epidermal cells along the attached margin. The epidermal cells secrete actively in the both premolt and postmolt stages, but the various granules in the each stage may contain the different materials (numerous granules at the postmolt stage seen in ultra-thin sections are often torn). Probably the granules at the postmolt stage comprise the minerals (e. g. Ca, Mg, and so on) abundantly.

This formation process of hinge structure demonstrates that the ligament has been formed before ecdysis and the hingement has been synthesized in one or two days after ecdysis, and suggests that the formation of them is carried out by the series of underlying epidermal cells along the attached margin. Moreover, this formation process also proposes the opinion on morphological evolutionary of ostracod carapace. "Continuous sheet theory (named by Hanai & Ikeya, 1991)" which was established by Harding (1964) and the hypothesis on morphological evolutionary of ostracod carapace proposed by Hinz (1993) are supported by the fact that a continuous epicuticular layer is formed initially in the whole areas of carapace and the formation of ligament follows this. The formation of ligament divides a newly formed carapace into new two valves and these are calcified after ecdysis. This order of formation process indicates that the calcification was acquired after the division of a non-calcified carapace in Ostracoda. This opinion conflicts with the presumption on morphological evolutionary of ostracod carapace represented by Swanson (1989a, b) based on the ontogenetic change of carapace in *Manawa staceyi*. Hinz (1993) stated the correlation between the increase of carapace mineralization and development of hinge structure, but did not mention whether the calcification followed the separation of carapace or not.

This study infers that the ostracod carapace was divided into two valves, prior to acquirement of calcification, because of the morphogenetic order of hinge structure, the wrinkled carapaces of early Archaeocopida (Hinz, 1993), and the hypothesis on secondary phosphatisation of early archaeocopid carapace (Kozur, 1974). This inference is supported by the facts that the fibrous structure of ligament is commonly seen in the arthropod cuticles (Bate & East, 1975; Neville, 1975) and the calcified cuticle should be identified with the specialized integuments rather than ligament, since the attached margin of myodocopid ostracods (Fig. 16C; Bate & East, 1972), which have weakly calcified or sclerotized bivalved carapace, and dorsal areas of the other bivalved crustaceans (Figs. 67D, E, 68D; Dahn, 1976) develop the usual integumental structure in Arthropoda comprising the similar fibrous structure as the podocopan ligament. More detail observation of ostracod embryos or

well-preserved fossils are needed for this discussion.

5-2. Formation of podocopan hingements

The detail formation of carapace morphologies was discussed in only Okada (1982b) exemplified by the reticulation of *Bicornucythere bisanensis*. This study proposes that the hypothesis on the morphogenesis of podocopan hingements on the basis of the TEM and SEM observations through the final molt.

Ridges and fossae expressed on the surface of arthropod exoskeletons are formed by the difference of secretary quantity among the parts of one epidermal cell (or epidermal cells), or external force to epidermis in the process of cuticle formation (Clarke, 1973). Locke (1966) explained the formation of cutiular reticulation (about 10 μ m in diameter) on the surface of *Calpodes ethlius* (Insecta) by the difference in the amount of cuticular deposition between cell margin and cell center. According to Locke (1966), the formation of carapace ornamentations situated above one epidermal cell in ostracods is also explained by the difference in the amount of cuticular deposition between cell margin and cell center, though these ornamentations of ostracods are much larger than those of insects. It can be supposed that the podocopan hingements are also formed by the difference in the amount of cuticular (containing the calcite) deposition among the parts of epidermal cells beneath the attached margin. This presumptive introduces the hypothesis on the morphogenesis of hingements as follow.

The formation of hingements in the both valves commences just after ecdysis (Fig. 74A). The terminal elements are formed initially and the formation of median elements follows in the both valves, though the formation of hingement in the both valves does not proceed simultaneously. In *Loxoconcha pulchra*, the hingement of the right valve is formed prior to that of the left valve (Figs. 63, 64, 75). This appears remarkably in the median elements (Figs. 63, 64, 75). The crenulations in the right median element develops by the aggregation of cuticular deposition, but the crenulations in the left median element has never been formed yet (Figs. 63, 64, 75). In this stage, the transverse sections of attached margin exhibit two different phases (Fig. 74B, C). One shows a developed tooth (of crenulations) of the right valve and a complement of the left valve. The other represents undeveloped hingememts in the both valves. With the advance of formation process, the epidermal cells beneath the undeveloped areas begin to form the hingements. In this time, if these epidermal cells bias the cuticular deposition towards the hingement of the left valve, the hingement of left valve is formed as crenulations (Fig. 74D). On the other hand, if these epidermal cells bias the cuticular deposition towards the hingement of the right valve, the each hingement of right and left valve is

formed as a major bar and groove respectively (Fig. 74E). Namely, the hingements in the both valves are not formed simultaneously and initially the crenulations in one valve has been formed by the bias of cuticular deposition of the underlying epidermal cells. The preceded crenulations have a function as a mold. Cytoplasm in the molds bias the cuticular deposition towards the either valve and forms a bar or crenularions as the hingement.

It is supposed that the various hingement morphologies (e. g. bar, groove, tooth, socket, and crenulations) of ostracods, especially Cytheroidea, can be formed by only adjustment of the bias of cuticular deposition. The complicated hingements tend to develop in many ornamented species irrespective of their lineages. This fact suggests that their epidermis may be input the bias mechanism of cuticular deposition.

5-3. Variability of podocopan hingements

The hingement is a conservative feature in most podocopan families or genera; therefore it has been thought to be an important character for higher taxonomy in Podocopa (Hanai, 1961; Hinz-Schallreuter & Schallreuter, 1999). In some taxa, however, the polymorphisms of hingement are found (Hanai, 1957) and few studies stated whether these morphological variations reflect their own phylogeny or the difference of habitats (Fukazawa, 2005MS in Japanese). Actually, the studies, which mentioned the range of variety in podoocpan hingements, are also few (Kamiya, 1992).

In this study, the morphological variability of podocopan hingements is discussed based on the result of a preliminary culture experiment. The juveniles (8th instar) of *Loxoconcha pulchra* were bred in the experimental artificial (brackish) seawaters and each individual was killed in three days after ecdysis. They were dissected by needles and their hingements were observed utilizing the SEM. In Ostracoda, the resorption of minerals from the old cuticle is denied (Turpen & Angell, 1971), thus, it is supposed that the new cuticle is synthesized by the minerals absorbed from the surrounding water. The parameters of experimental seawaters are written below.

(1) 25‰ (2) 12.5‰ (3) 25‰ (Ca-free) (4) 25‰ (Ca-half) (5) 25‰ (Ca-double)

Three males and two females are bred in each water condition. Consequently, all specimens bred in (3) and (4) were swelling and dead before ecdysis. Probably, the supporting fibres were torn due to extreme insufficiency of Ca. All specimens in the other seawaters shed their exoskeletons and their hingements were examined (Figs. 76, 77). The hingements of all specimens bred in (1) and (5) developed as almost usual (Figs. 76A, D, 77A, D). On the contrary, the hingements of all male specimens bred in (2) exhibited their poor development (Figs. 76B, 77B). The hingements formed

in (2) are similar to those formed in natural seawater (34‰) past ten or fifteen hours after ecdysis. Therefore, it is supposed that these hingement morphologies were caused by not redution of calcification but delay of calcification, due to decrease of salinity. This hypothesis is supported by the fact that the hingements of all female specimens, which are much smaller than males, in (2) developed as almost usual (Figs. 76C, 77C).

This hypothesis and morphogenesis of hingements indicate that the median elements, which are formed in the last phase, tend to express their own variations. Actually, the variations found in some podocopan families or genera often exhibit in the median elements (Fig. 78).

The hingement morphologies can not be always evaluated as a conservative character, since they may be affected even only by salinity. But the hingement morphologies are useful for analysis of adaptive evolution to other habitats in the family or genus, since they are usually quite conservative in most podocopan families or genera and their variations are always observed in particular elements.

Conclusions

1. New terminology for the ostracod carapace margin has been established, based on homology and ultrastructural observations.

The structure of cuticle diversifies in each family or genus, though the epidermal structure is more conservative and it diversifies at the order level. The organelle composition in ostracod epidermis should be evaluated as not only a mere cover of animal body, but an organism with the both functions of its own calcification and respiratory efficiency.

2. The ostracod ligament is a homologous structure not to the selvage but to the marginal infold. It is an uncalcified cuticular structure contacting the free margins of both valves. Furthermore, it has specialised fibrous structures to provide for strength, but probably it has no elasticity for opening the valves.

3. Since the Platycopida and podocopid Darwinuloidea do not develop the marginal infold at all, the structure of their free margin substantially differs from that of podocopid Cytheroidea. Marginal infolds may have appeared in the early Palaeozoic as a synapomorphic character of podocopid superfamilies excluding Darwinuloidea.

4. The ostracod hinge structures are classified into four types; *Basic type, Exterior type, Transitive type,* and *Interior type* based on the relative position of ligaments and hingements. This classification elucidates that the derived taxa (Cypridoidea, Cytheroidea) share all four types but the primitive taxa (Platycopida, Bairdioidea, Darwinulloidea) do only two types. This result the former has the structural diversity separately from its well development of hinge teeth. The various hingements in Cytheroidea, which has the highest species diversity, are caused by the plasticity of *Transitive type* and *Interior type* and three elements structure of hingements.

5. The four hinge types which are defined here must reflect the influences of their habitats, ecology and so on rather than their pathways of phylogeny. But the hingements are available for the ostracod higher taxonomy, since the hinge types and arrangements of teeth on hingements are conservative in each superfamily and family (or at least genus), respectively.

6. The morphogenesis of hinge structure is clarified in detail. The formation order of ligament and hingement suggest that the separation of a cuticular sheet precedes achievement of carapace calcification in the evolutionary pathway as "from one sheet to two valves". In Cytheroidea the hingements (teeth arrangements) in both valves are not formed concurrently. The formation of hingement in one valve precedes and it has almost finished before that of the other valve commences. Therefore, it is suggested that teeth arrangements of opposite valve develop between the teeth on the preceded valve. These phenomena must be caused by the polarization of cuticle secretion from epidermis.

7. The relationship between hingement morphology and salinity were demonstrated by the cultural experiment. The low salinity effects not the decrease of calcite deposition but the delay of calcification. Therefore, the diversity of median elements in some cytheroid families or genera may imply the evidence of adaptive evolution (e. g. adaptation for brackish water), because the median element with many interspecific variations is formed finally in morphogenesis of hingement.

8. The comprehensive studies on the carapace ultrastructures and morphogenesis of bivalved crustaceans including Recent ostracods enable to be taken in the structural and functional diversity of "bivalved integuments" in Crustacea. The consequences in this thesis provide clues to elucidate the reason for acquirement of "bivalved integuments" in each taxon, and they can be expected to reconstruct even the metabolic activities in extinct species. Furthermore, with unprecedented accuracy, these fruitions promote understandings on the evolutionary pathway of integuments in bivalved arthropods from the Paleozoic to Recent.

Acknowledgements

The author would like to express my deep gratitude to Dr. Akira Tsukagoshi (Shizuoka University) for critical reading of manuscript and valuable discussions. I greatly appreciate Prof. Emeritus Noriyuki Ikeya (Shizuoka University) for his helpful suggestions and continuous encouragements, and Prof. Shigeyasu Tanaka (Shizuoka University) for technical advice. Thanks are also due to Dr. Yutaro Suzuki (Shizuoka University), Dr. Gengo Tanaka (Shizuoka University), and Dr. Yuriko Nakao (Nihon University) for their valuable discussions and encouragements and to Dr. Rumi Sohrin (Shizuoka University) for adjusting the artificial marine water. I also greatly thank Satoshi Watanabe, Yasuhiko Sakumoto, Ryouichi Higashi, Tsukasa Fukazawa, Shihori Ito, Tomonari Kaji, Toru Takahashi, Hideaki Takahashi, Yasuhisa Kondo, Yumi Sugimoto, and Hayato Tanaka (Shizuoka University) for their valuable discussions and kindly helps for sampling. My deep thanks are extended to Dr. Takahiro Kamiya (Kanazawa University), Dr. Robin Smith (Lake Biwa Museum), and Mr. Tomoatsu Takenaka (Heiden-ji Temple) for kindly helps on collecting the samples. Gratitude is also expressed to my family and familiar friends for their precious supports.

The staff member of Shimoda Marine Research Center (University of Tsukuba), Misaki Marine Biological Station (University of Tokyo), and Sesoko Station Tropical Biosphere Research Center (University of the Ryokyus) provided various facilities to the author.

This study was partly funded by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

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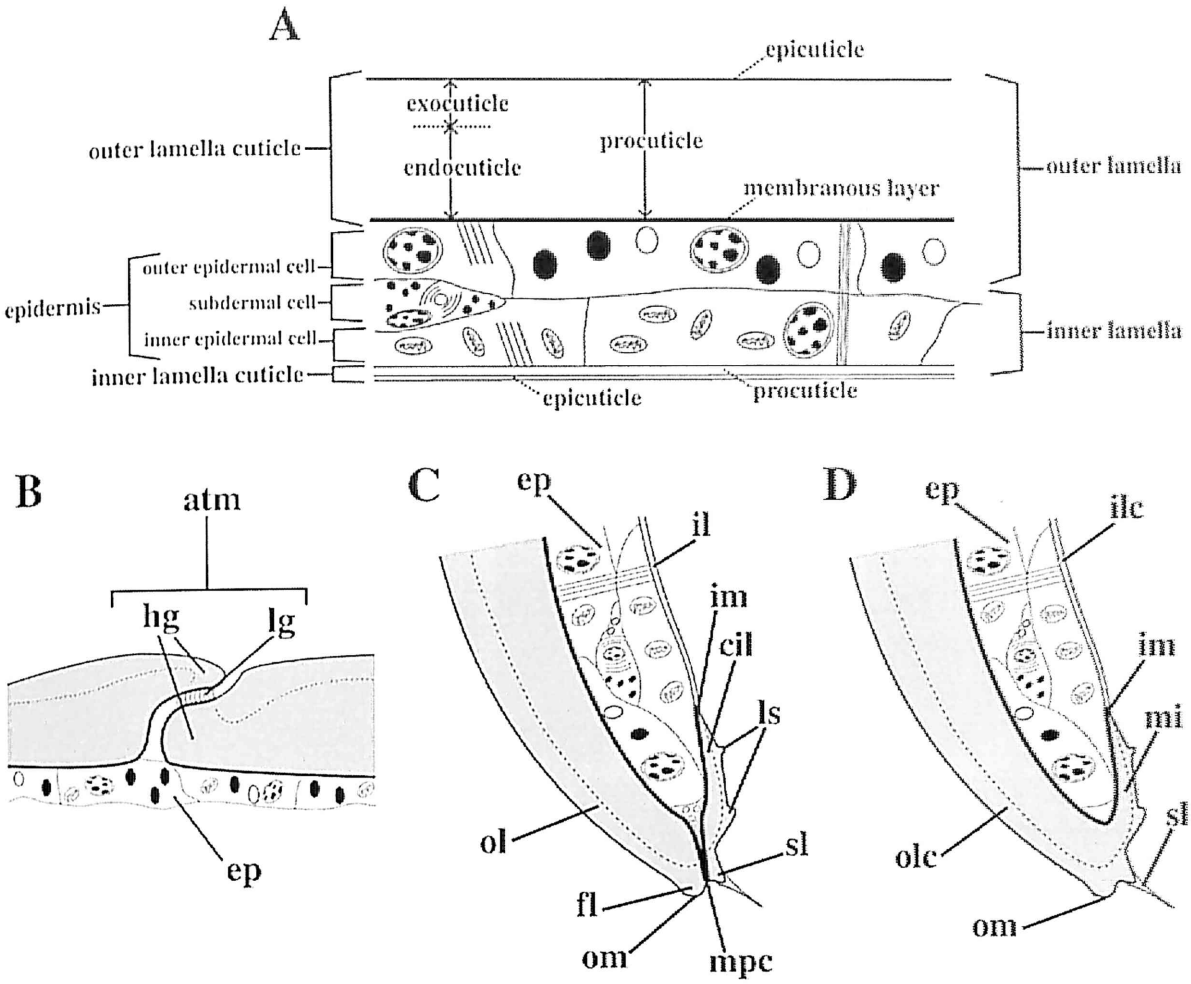

Figure 1 Terminology of the ostracod carapace.
A. Domicilar region. B. attached margin.
C. free margin (used by previous paleontological studies).
D. free margin (adopted by this study).
Grey areas in B, C and D indicate the calcified parts.

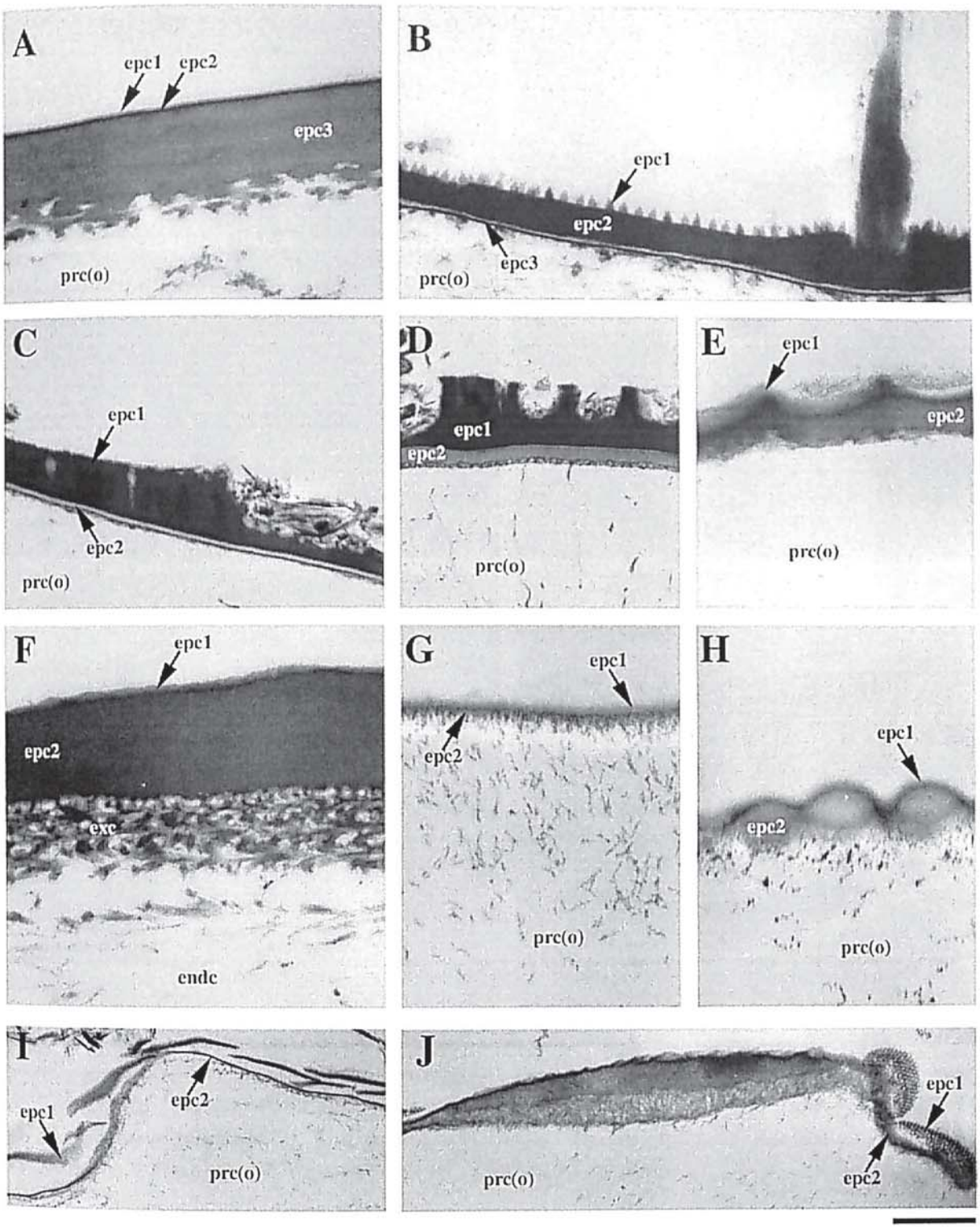

Figure 2 TEM photographs of cytheroid epicuticle.
A. *Pontocythere japonica.* B. *Loxoconcha pulchra.* C. *Keijia* cf. *demissa.*
D. *Callistocythere pumila.* E. *Schizocythere kishinouyei.* F. *Cythere omotenipponica.*
G. *Parakrithella pseudadonta.* H. *Microcythere* sp. I. *Semicytherura wakamurasaki.*
J. *Perissocytheridea inabai.*
Scale bar is 500nm (A, F, G, H,); 1.8 μm (B); 700nm (C, I, J); 1.5 μm (D); 300nm (E).

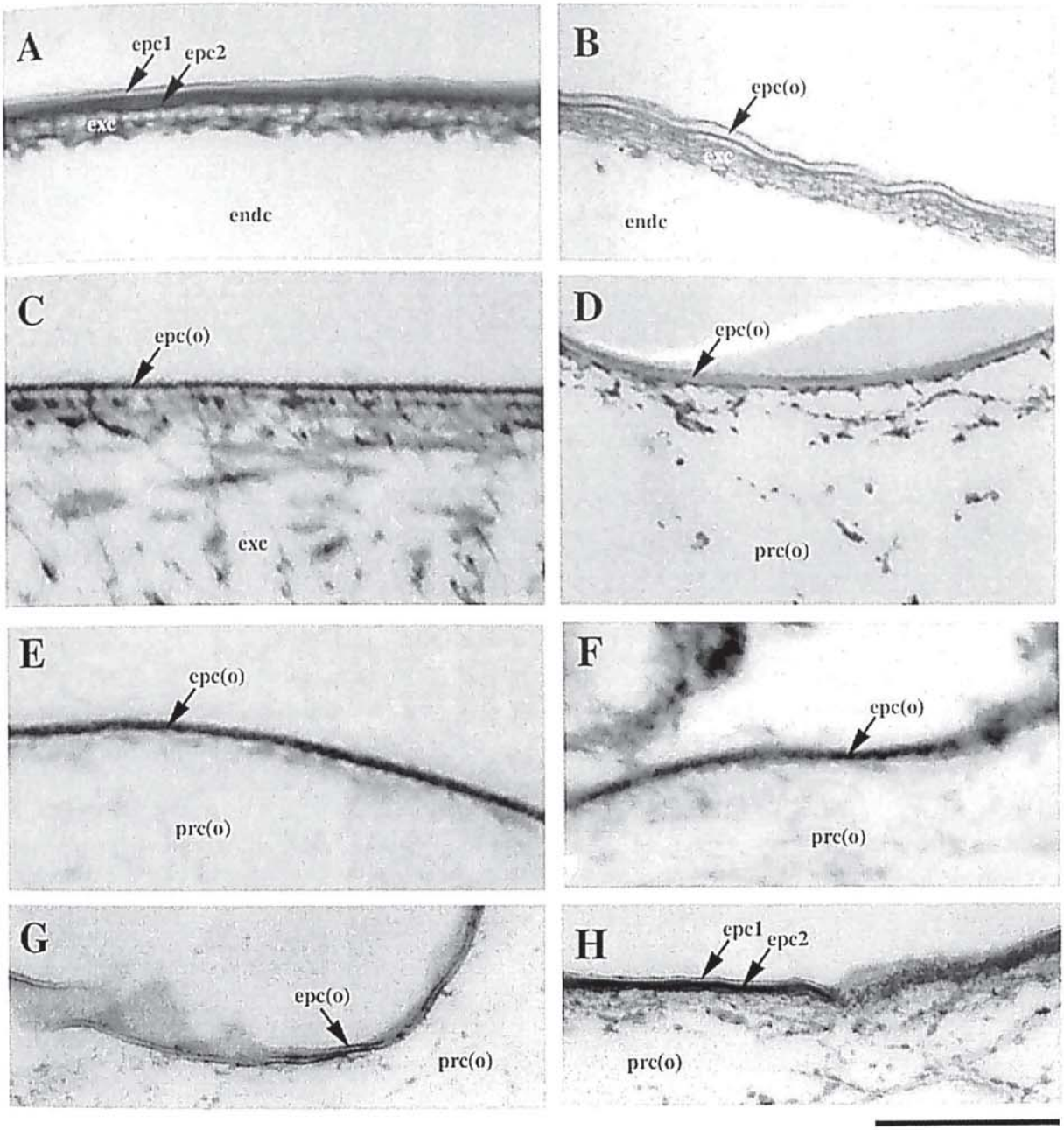

Figure 3 TEM photographs of cytheroid epicuticle.
A. *Limnocythere stationis.* B. *Paradoxostoma triangulum.* C. *Xestoleberis hanaii.*
D. *Paracobanocythere* sp. E. *Caudites asiaticus.* F. *Trachyleberis scabrocuneata.*
G. *Bythoceratina* sp. H. *Sclerochilus* sp. Scale bar is 500nm.

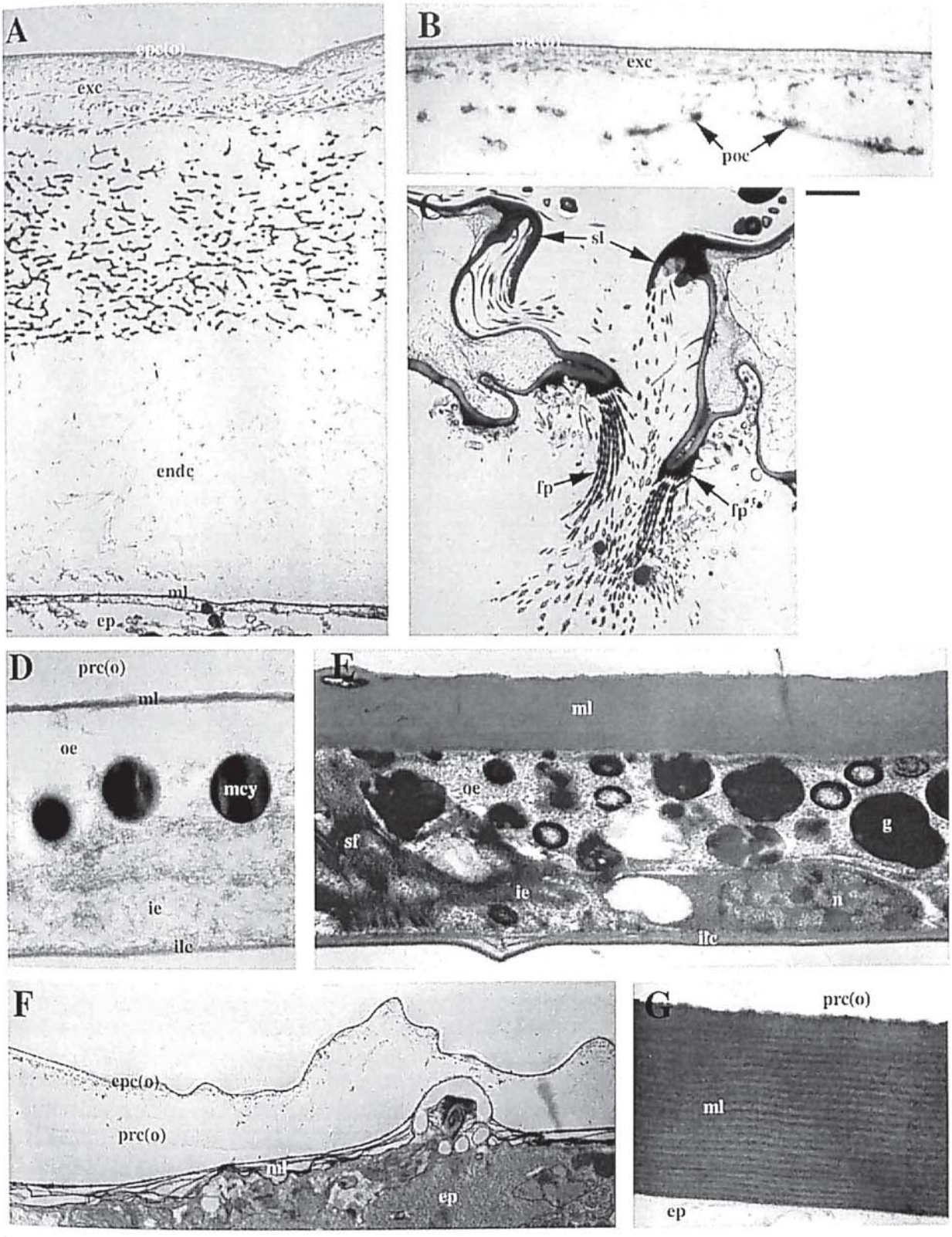

Figure 4 TEM photographs of cytheroid carapace.
A. pore canal of *Xestoleberis hanaii.* B. pore canal of *Xestoleberis setouchiensis.*
C. feather-like projections. D. membranous layer of *Xestoleberis hanaii.*
E. membrannous layer of *Cythere omotenipponica.*
F. membranous layer of *Paracobanocythere* sp. G. membranous layer of *Aurila hataii.*
Scale bar is 1.7 μm (A); 500nm (B, G); 3.3 μm(C); 400nm (D); 1 μm (E); 2 μm (F).

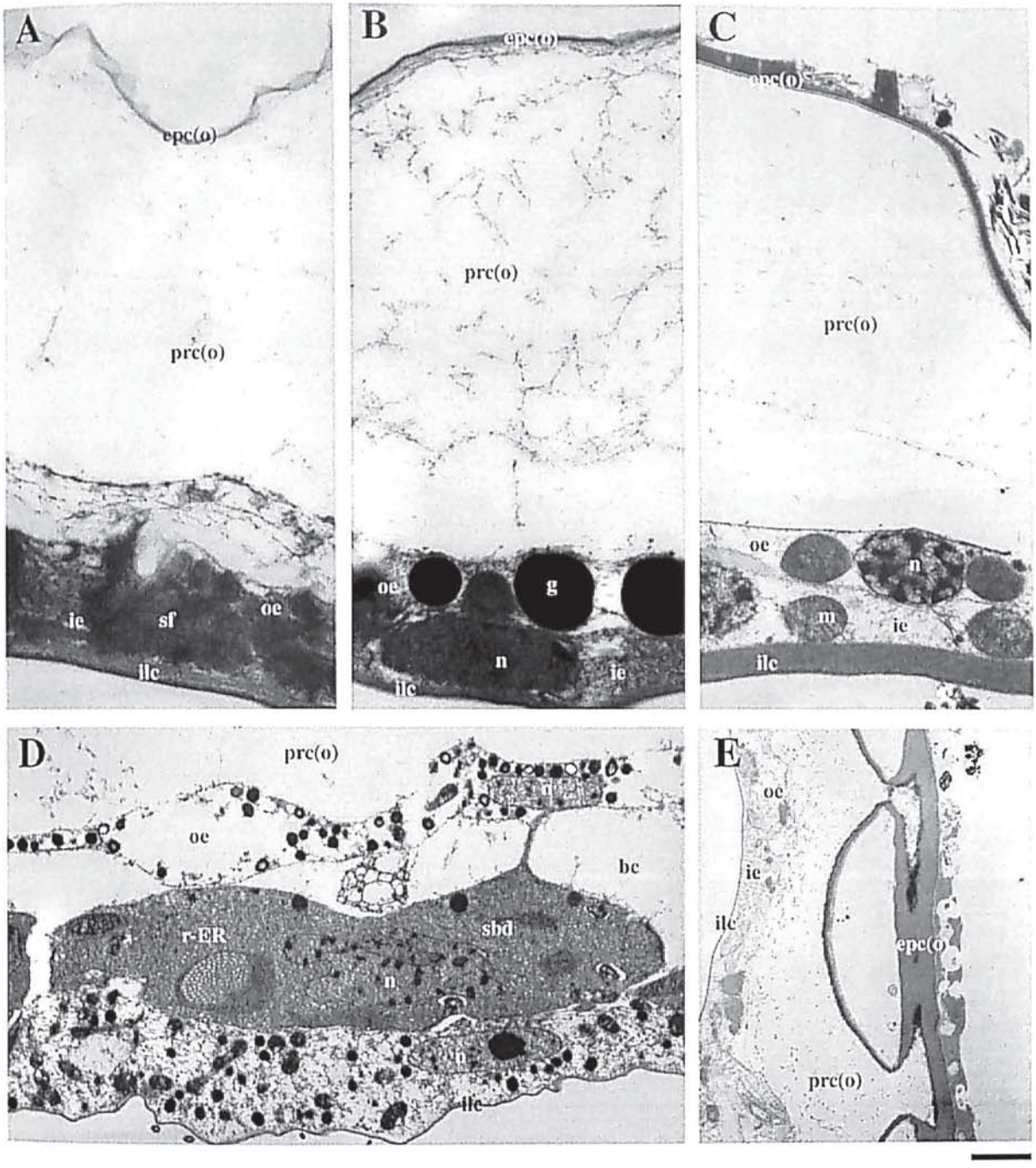

Figure 5 TEM photographs of cytheroid carapace.
A. *Bythoceratina* sp. B. *Sclerochilus* sp. C. *Keijia* cf. *demissa*.
D. *Perissocytheridea inabai*. E. *Callistocythere setouchiensis*.
Scale bar is 500nm (A, B); 1.3 μm(C); 2 μm (D); 5 μm (E).

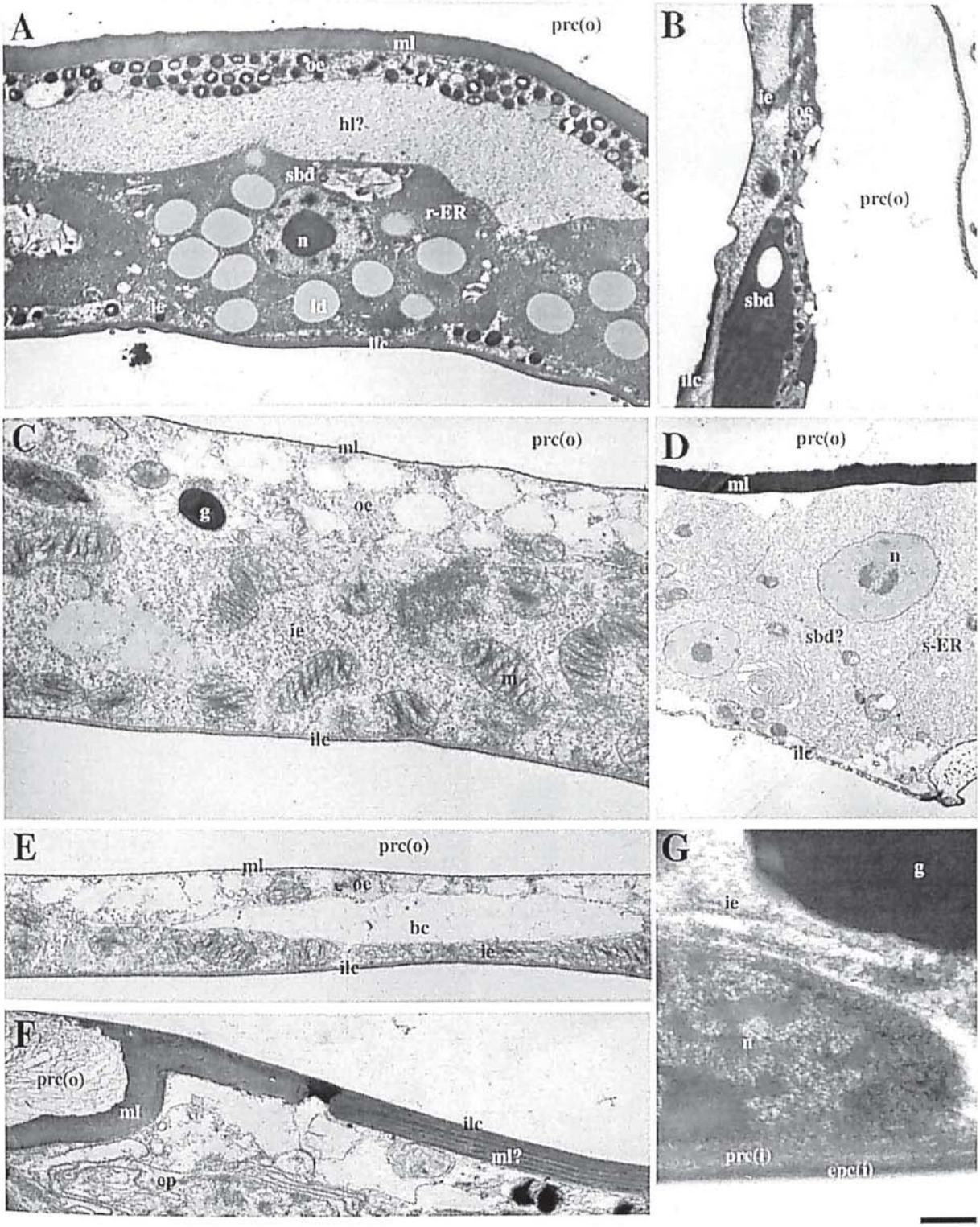

Figure 6 TEM photographs of cytheroid carapace.
A. epidermis of *Schizocythere kishinouyei.* B. carapace of *Limnocythere stationis.*
C. epidermis of *Pontocythere japonica.* D. epidermis of *Microcythere* sp.
E. body cavity of *Pontocythere japonica.* F. inner lamella cuticle of *Aurila hataii.*
G. inner lamella cuticle of *Cythere omotenipponica.*
Scale bar is 2.9 μm (A); 1.7 μm(B); 1 μm (C, F); 500nm (D); 2 μm (E); 350nm (G).

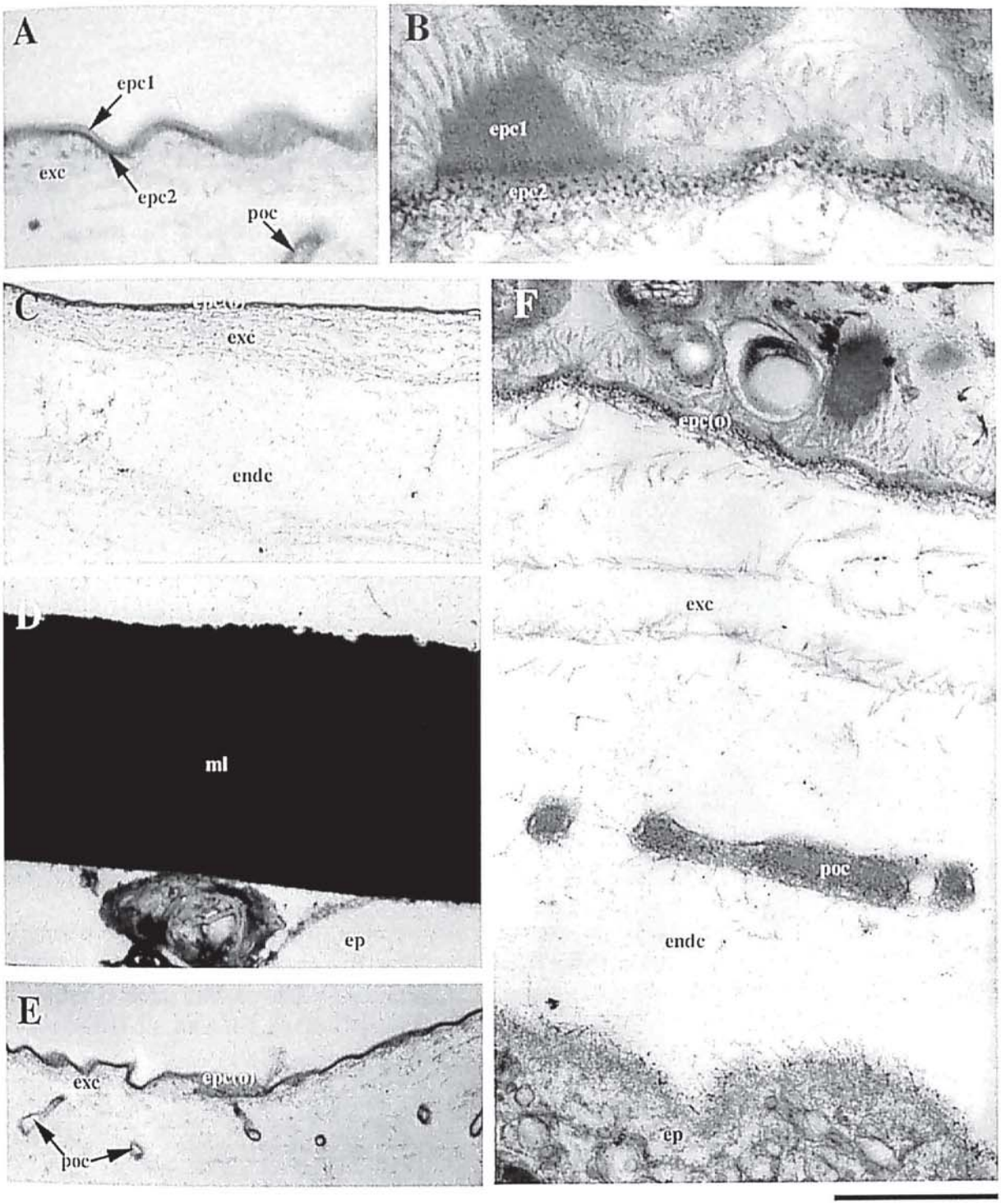

Figure 7 TEM photographs of baridioid carapace.
A. epicuticle of *Neonesidea oligodentata.* B. epicuticle of *Triebelina* sp.
C. exocuticle of *Neonesidea oligodentata.*
D. membranous layer of *Neonesidea oligodentata.*
E. pore canal of *Neonesidea oligodentata.* F. carapace of *Triebelina* sp.
Scale bar is 666nm (A); 500nm (B); 700nm (C); 1.4 μm(D); 2 μm (E); 1 μm (F).

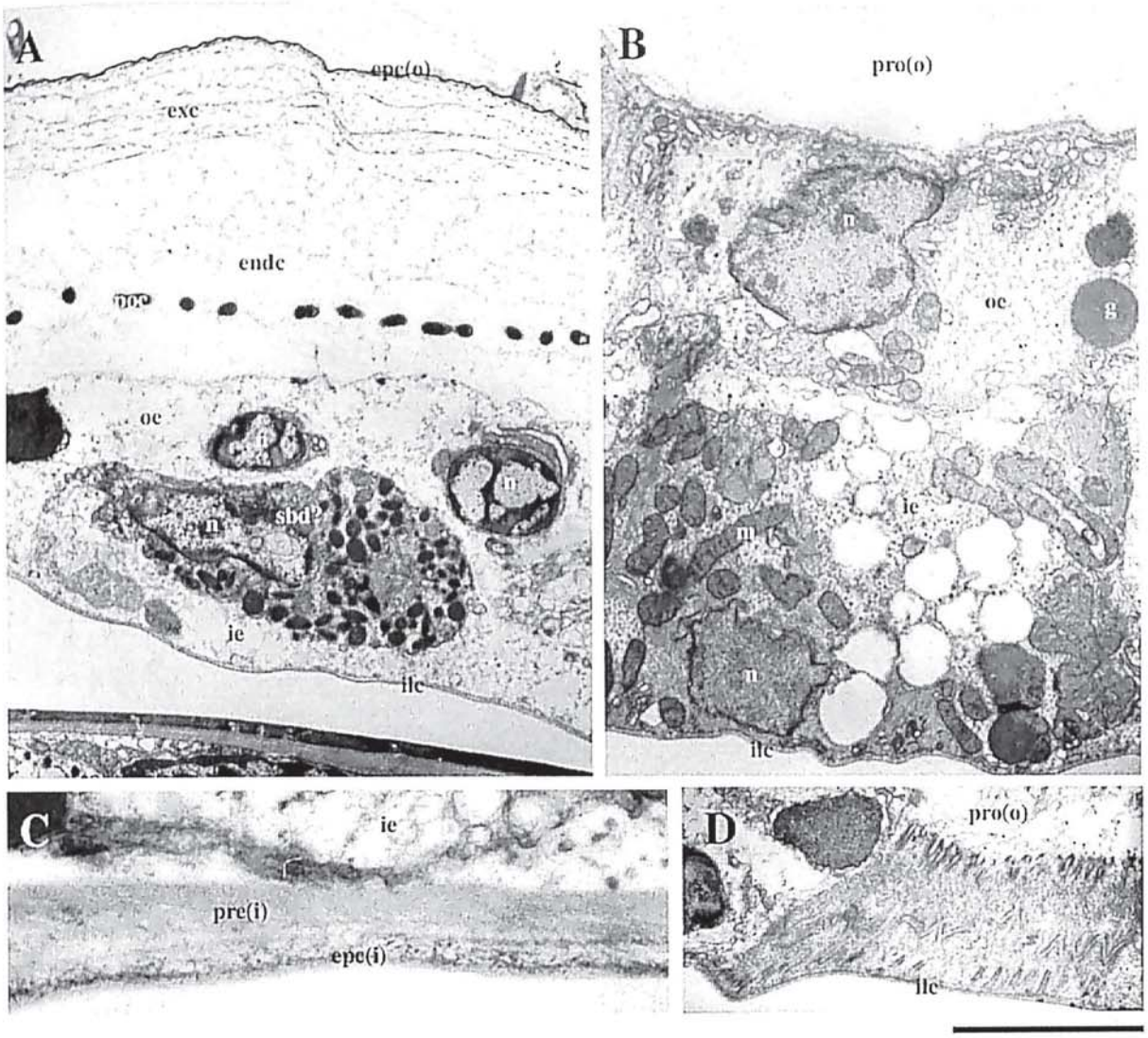

Figure 8 TEM photographs of baridioid carapace.
A. carapace of *Triebelina* sp. B. epidermis of *Triebelina* sp.
C. inner lamella cuticle of *Triebelina* sp. D. supporting fibres of *Triebelina* sp.
Scale bar is 5 μm(A); 1.2 μm (B); 1 μm (C); 350nm (D).

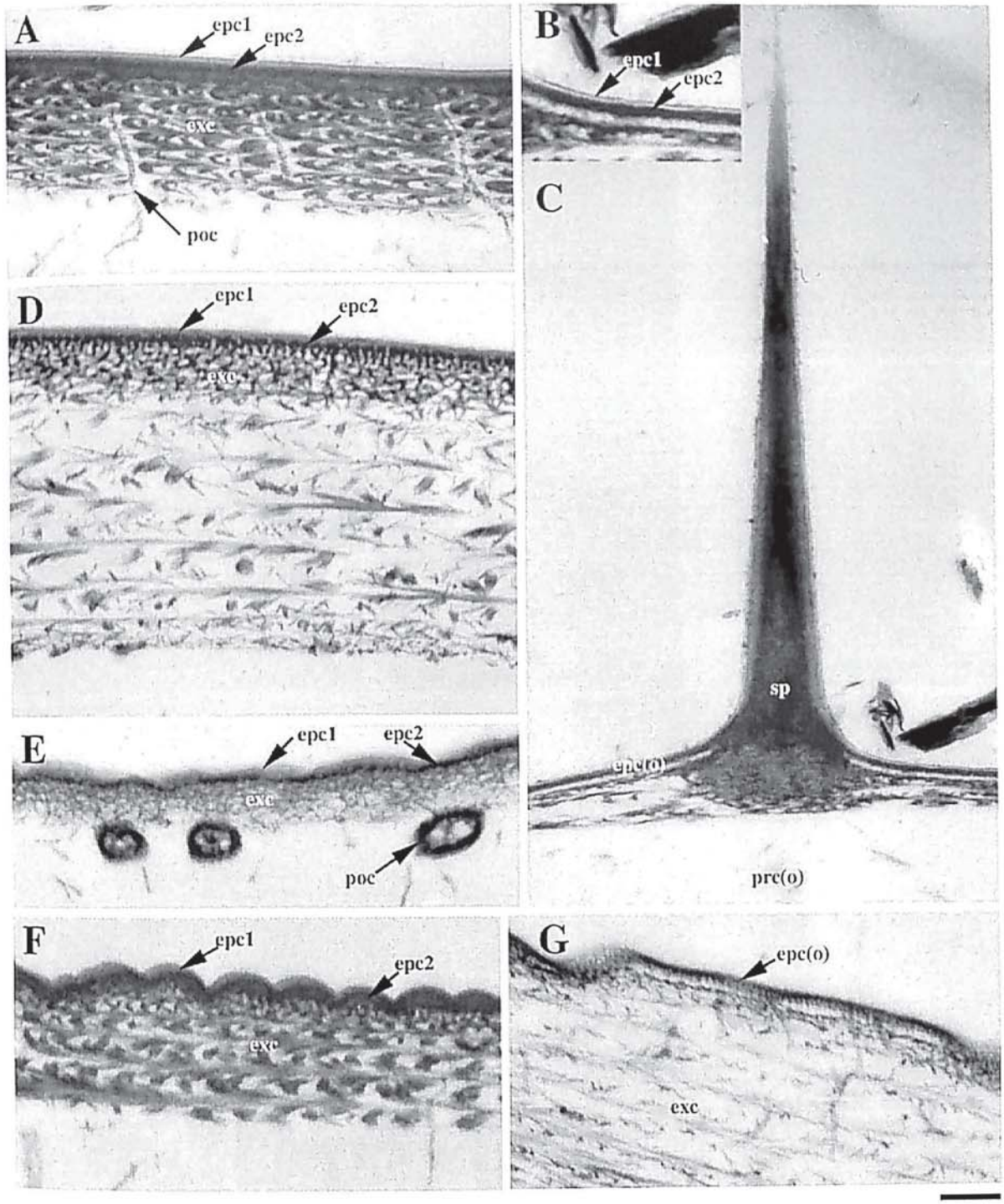

Figure 9 TEM photographs of cypridoid epicuticle.
A. *Cypridopsis vidua.* B, C. *Ilyocypris japonica.* D. *Cypria reptans.*
E. *Fabaeformiscandona* sp. F. *Chrissia* sp. G. Paracypridinae sp. A.
Scale bar is 333nm (A); 1.7 μm (B); 833nm (C); 170nm(D, G); 270nm (E); 500nm (F).

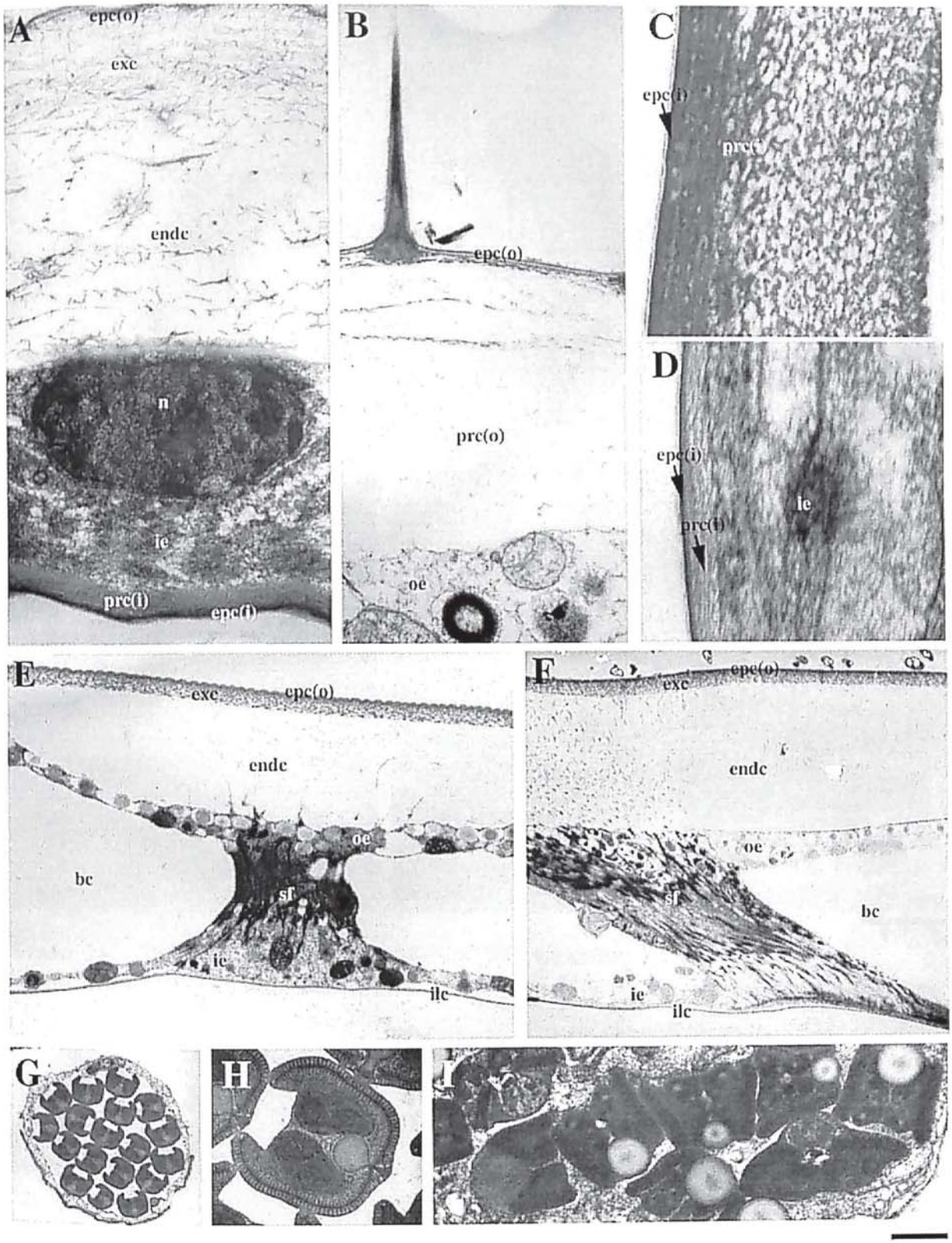

Figure 10 TEM photographs of cypridoid carapace.
A. carapace of Paracypridinae sp. A. B. carapace of *Ilyocypris japonica*.
C. inner lamella cuticle of *Cypris reptans*. D. inner lamella cuticle of *Ilyocypris japonica*.
E. carapace of *Chrissia* sp. F. carapace of *Cypridopsis vidua*.
G, H. tetes and sperm of *Ilyocypris japonica*. H. sperms of *Fabaeformiscandona* sp.
Scale bar is 500nm (A); 1 μm (B, G, I); 180nm (C); 300nm(D, H); 1.4 μm (E); 3 μm (F).

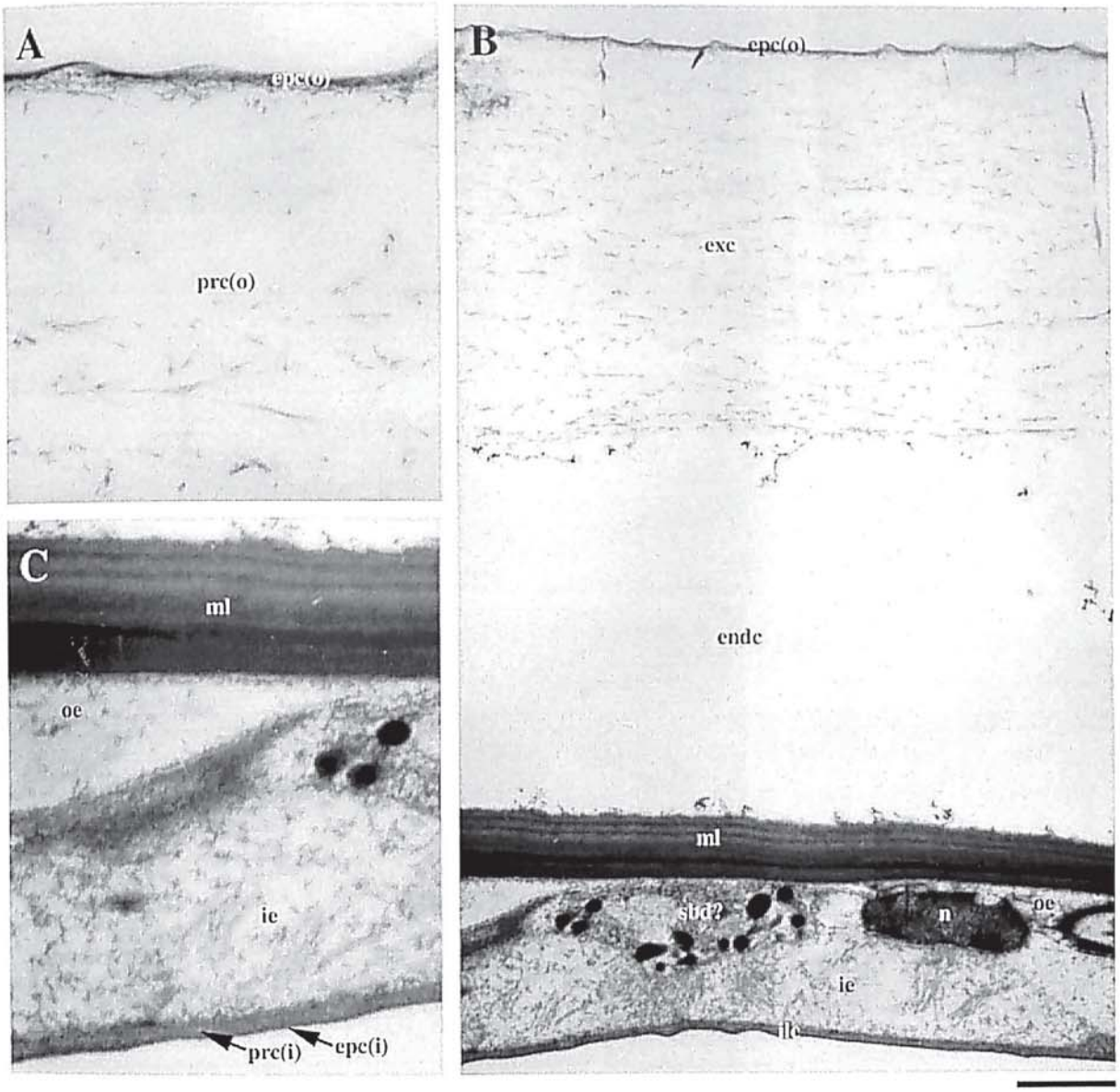

Figure 11 TEM photographs of pontocypridoid carapace.
A. epicuticle of *Propontocypris* sp. B. carapace of *Propontocypris* sp.
C. epidermis of *Propontocypris* sp.
Scale bar is 333nm (A); 1.4 μ m (B); 700nm (C).

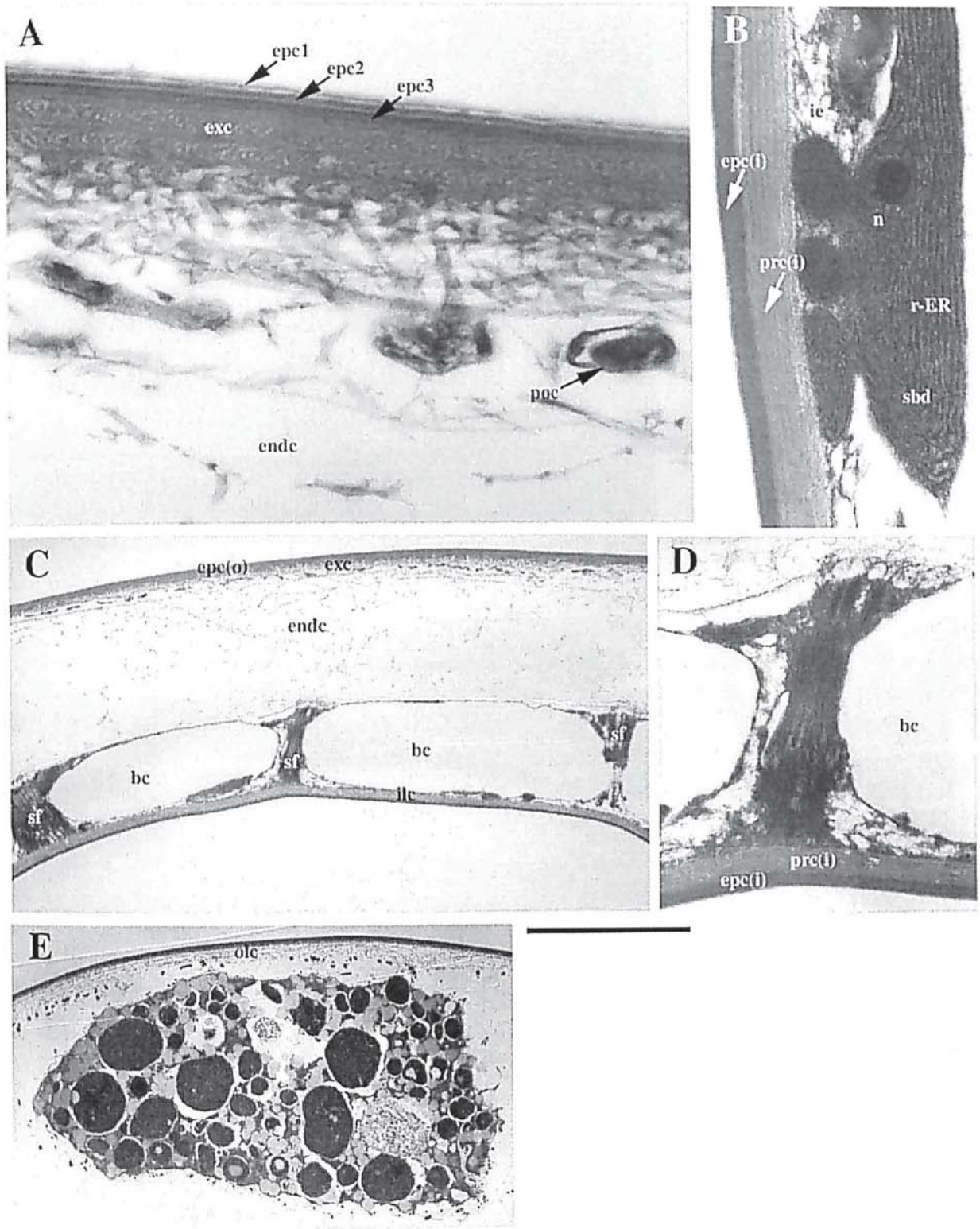

Figure 12 TEM photographs of darwinuloid carapace.
A. epicuticle of *Vestalenula* sp. B. epidermis of *Vestalenula* sp.
C. carapace of *Vestalenula* sp. D. supporting fibres of *Vestalenula* sp.
E. cell (adipocyte?) in the body cavity of *Vestalenula* sp.
Scale bar is 500nm (A); 1.3 μm (B); 6.3 μm (C); 2 μm (D); 15 μm (E).

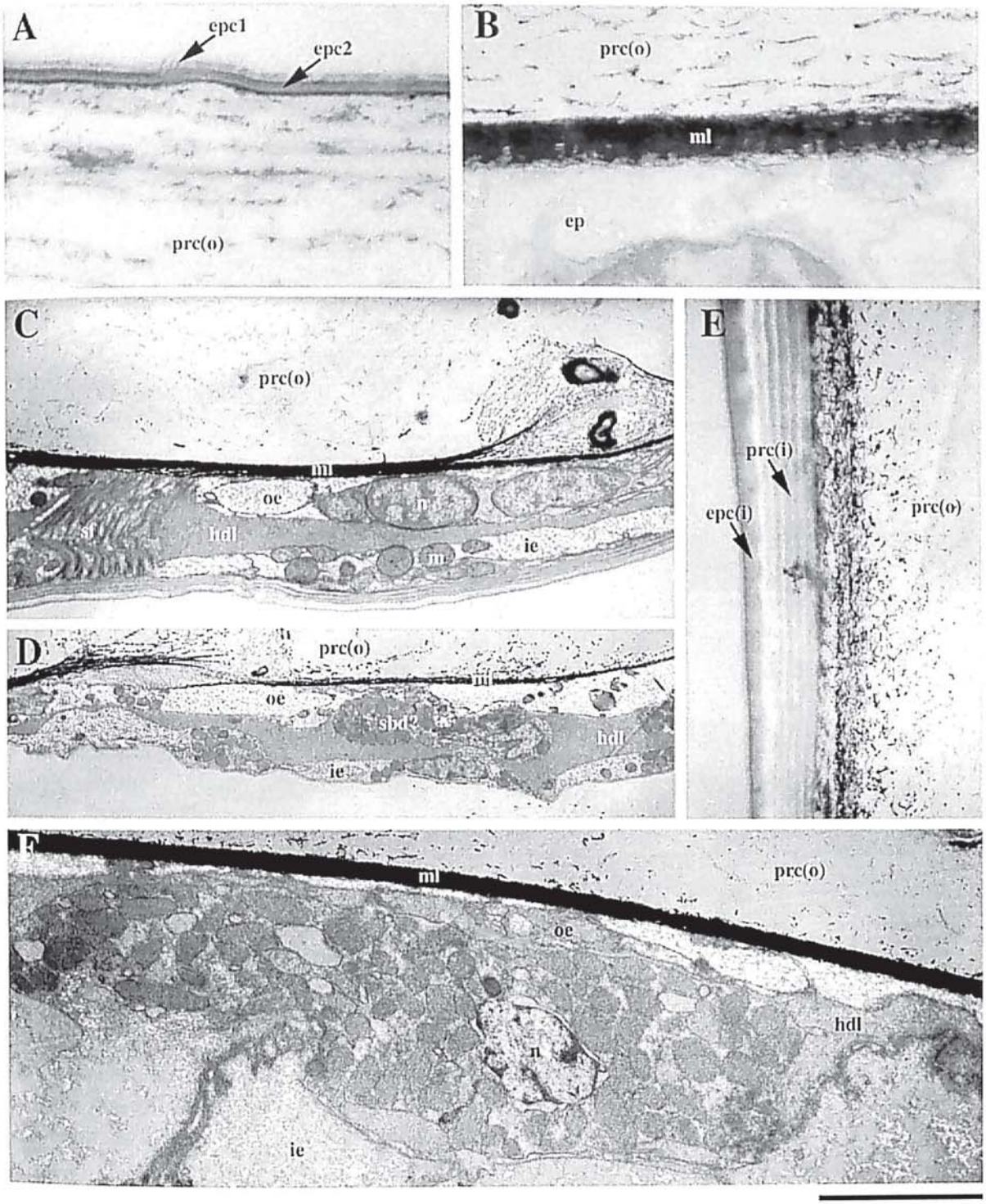

Figure 13 TEM photographs of carapace in *Keijcyoidea infralittolaris* (Cytherelloidea). A. epicuticle. B. memebranous layer. C, D. epidermis. E. inner lamella cuticle. F. cell in high-electron dense layer.
Scale bar is 500nm (A); 1.7 μm (B, D); 4.2 μm (C); 10 μm (E); 3.2 μm (F).

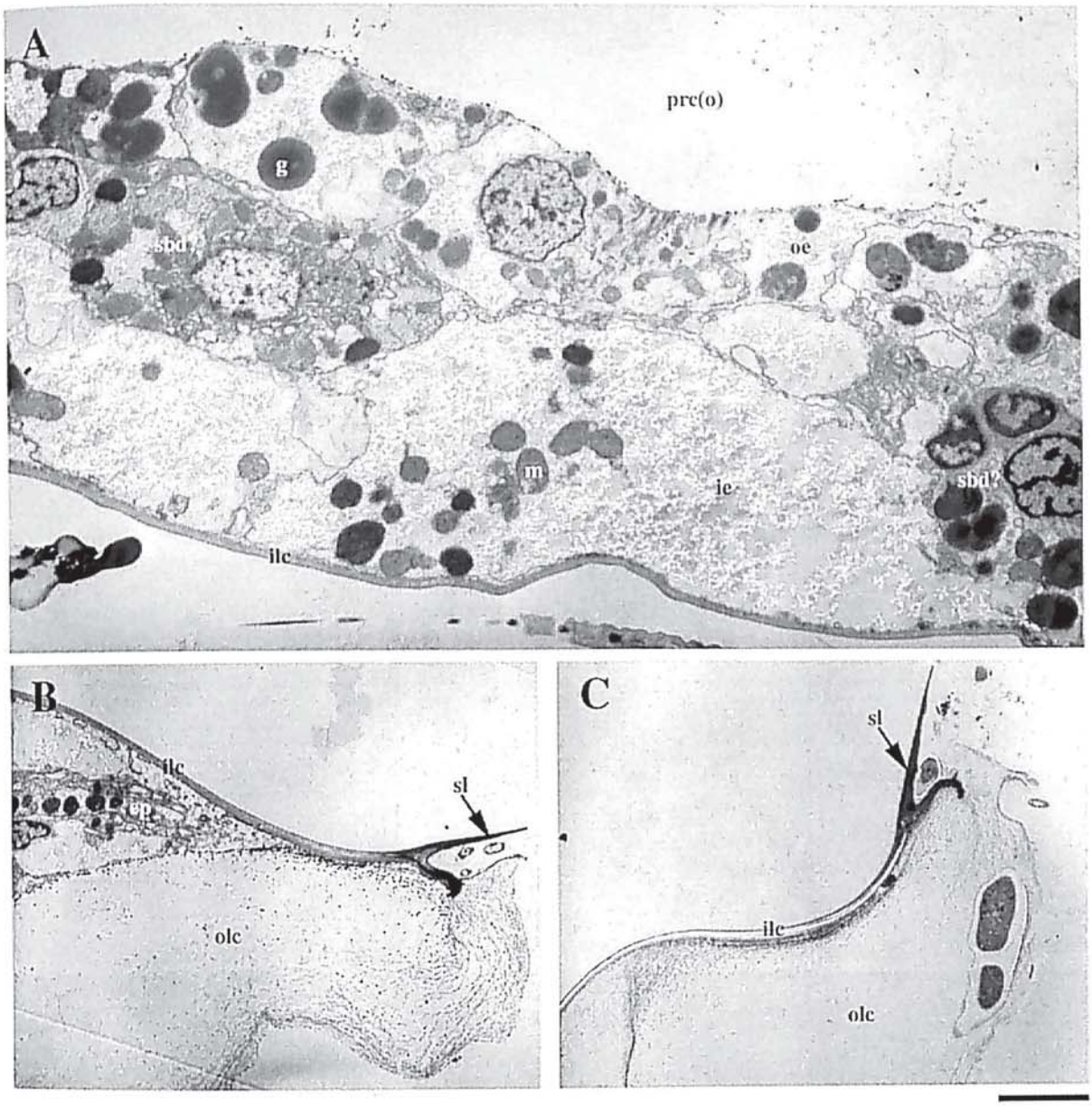

Figure 14 TEM photographs of carapace in *Keijcyoidea infralittolaris* (Cytherelloidea).
A. epidermis of *Keijcyoidea infrallittolaris* at the 8th instar.
B. free margin of 8th instar specimen. C. free margin of adult specimen.
Scale bar is 2.5 μ m (A); 2.7 μ m (B); 4.3 μ m (C).

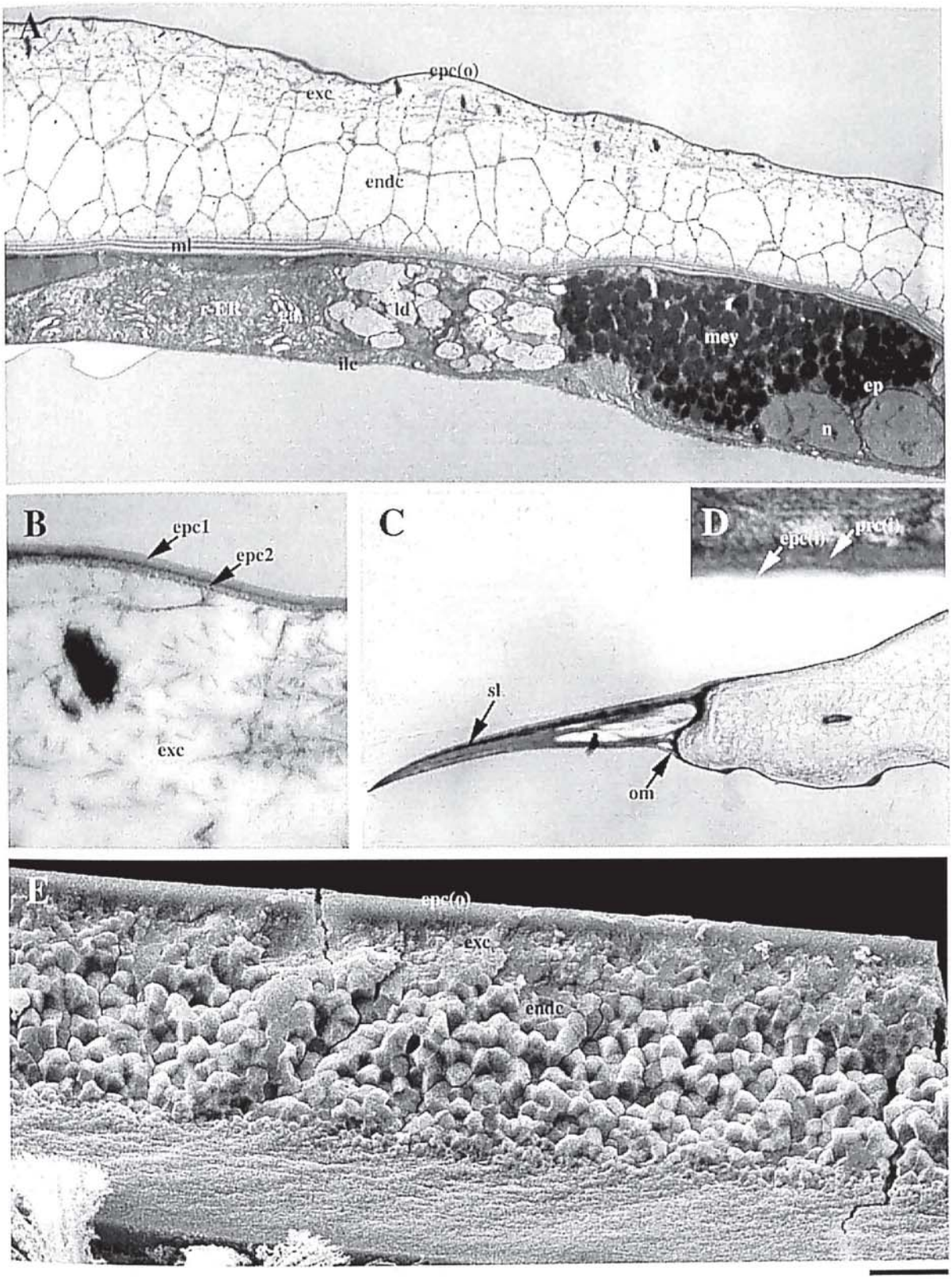

Figure 15 TEM and SEM photographs of myodocopid carapace.
A. carapace of *Melavargula japonica.* B. epicuticle of *Melavargula japonica.*
C. outer margin of *Cypridina noctiluca.* D. inner lamella cuticle of *Cypridina noctiluca.*
D. outer lamella cuticle of *Vargula hilgendorfii.*
Scale bar is 3.3 μ m (A, C); 500nm (B); 320nm (D); 10 μ m (E).

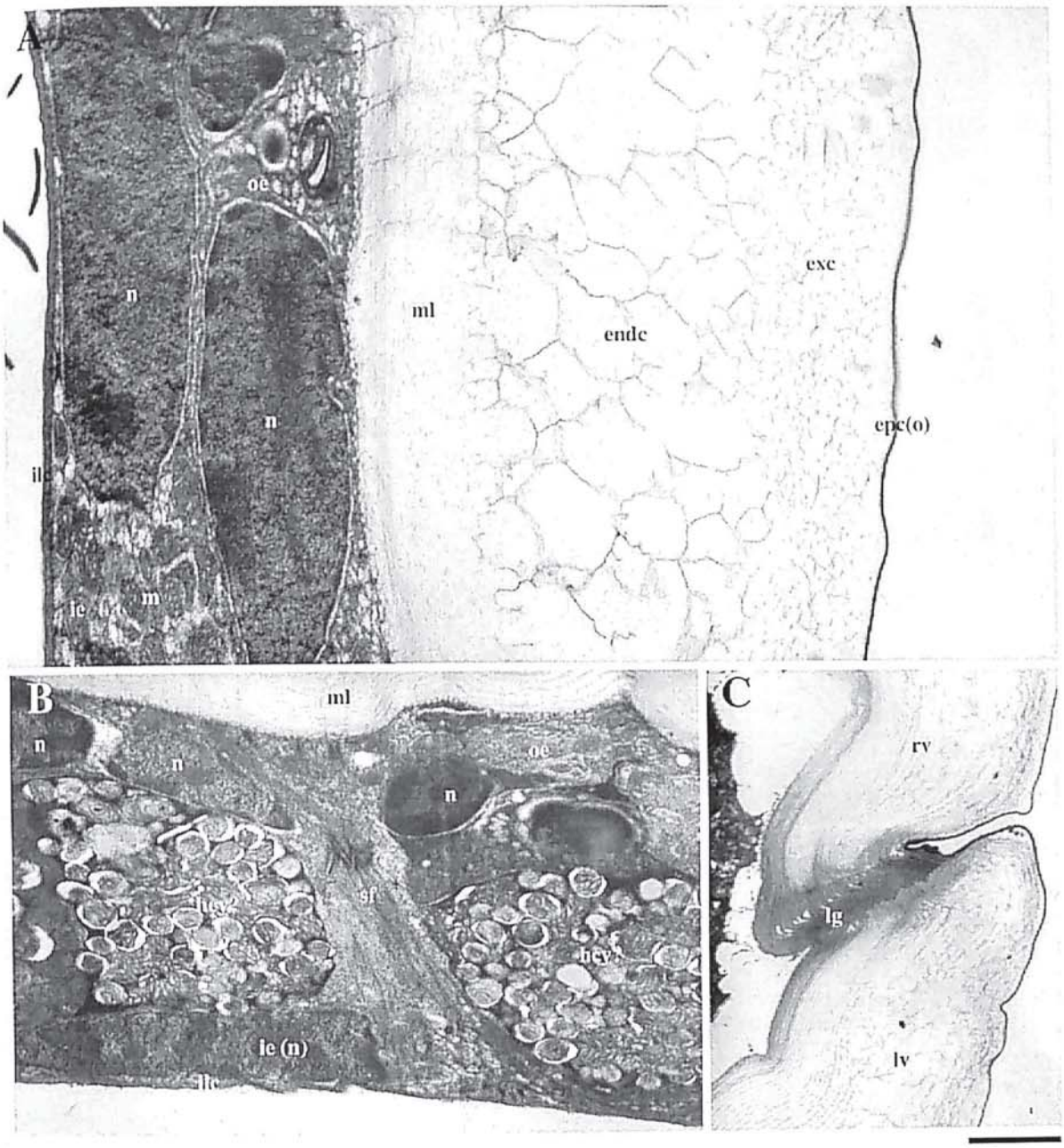

Figure 16 TEM photographs of myodocopid carapace.
A. carapace of *Cypridina noctiluca.* B. epidermis of *Cypridina noctiluca.*
C. ligament of *Cypridina noctiluca.* Scale bar is 1.3 μ m (A, C); 1 μ m (B).

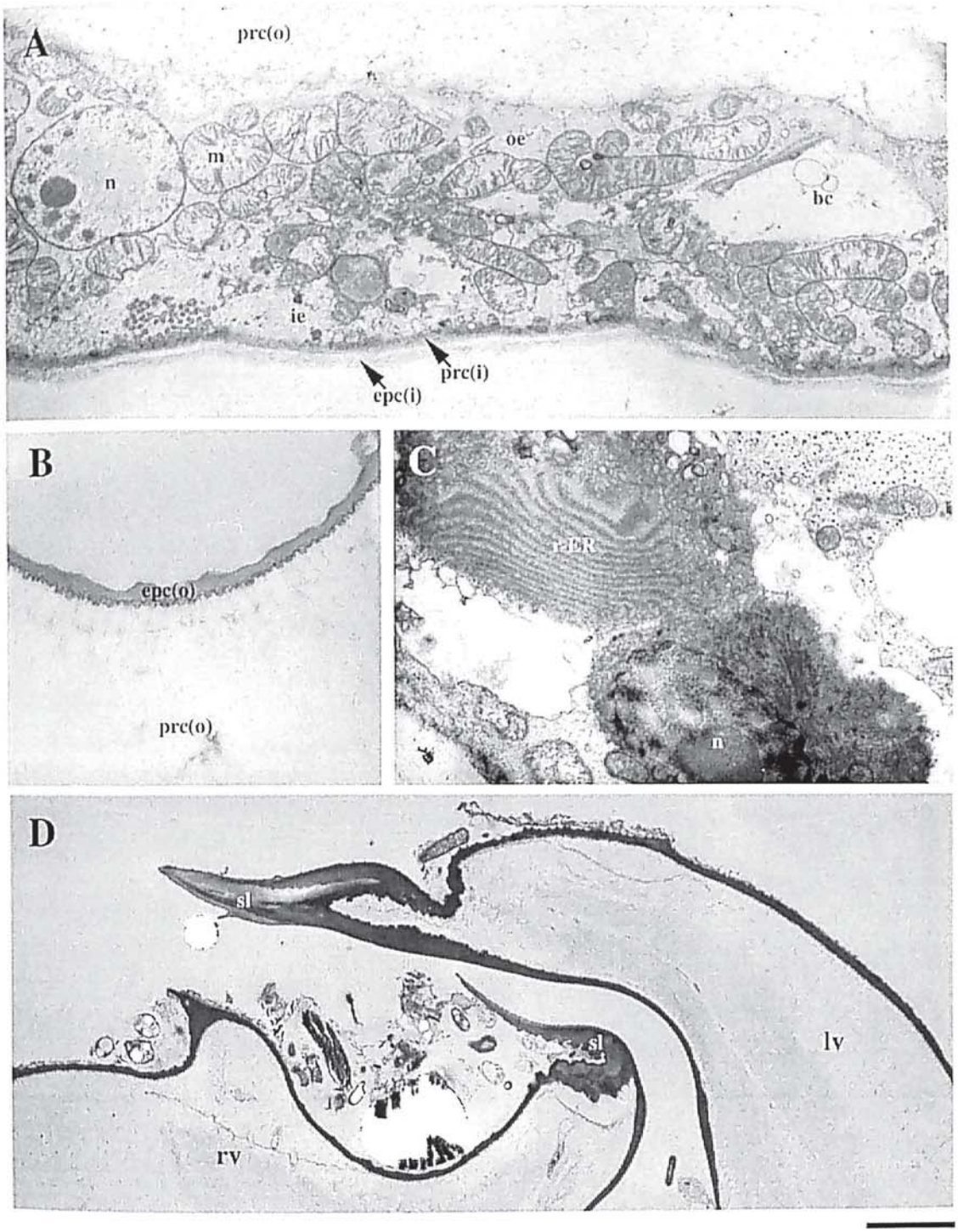

Figure 17 TEM photographs of halocyprid carapace.
A. epidermis of *Polycope japonica*. B. epicuticle of *Polycope japonica*.
C. subdermal cell? of *Polycope japonica*. D. free margin of *Polycope japonica*.
Scale bar is 2 μm (A); 500nm (B); 1.5 μm (C); 2.5 μm (D).

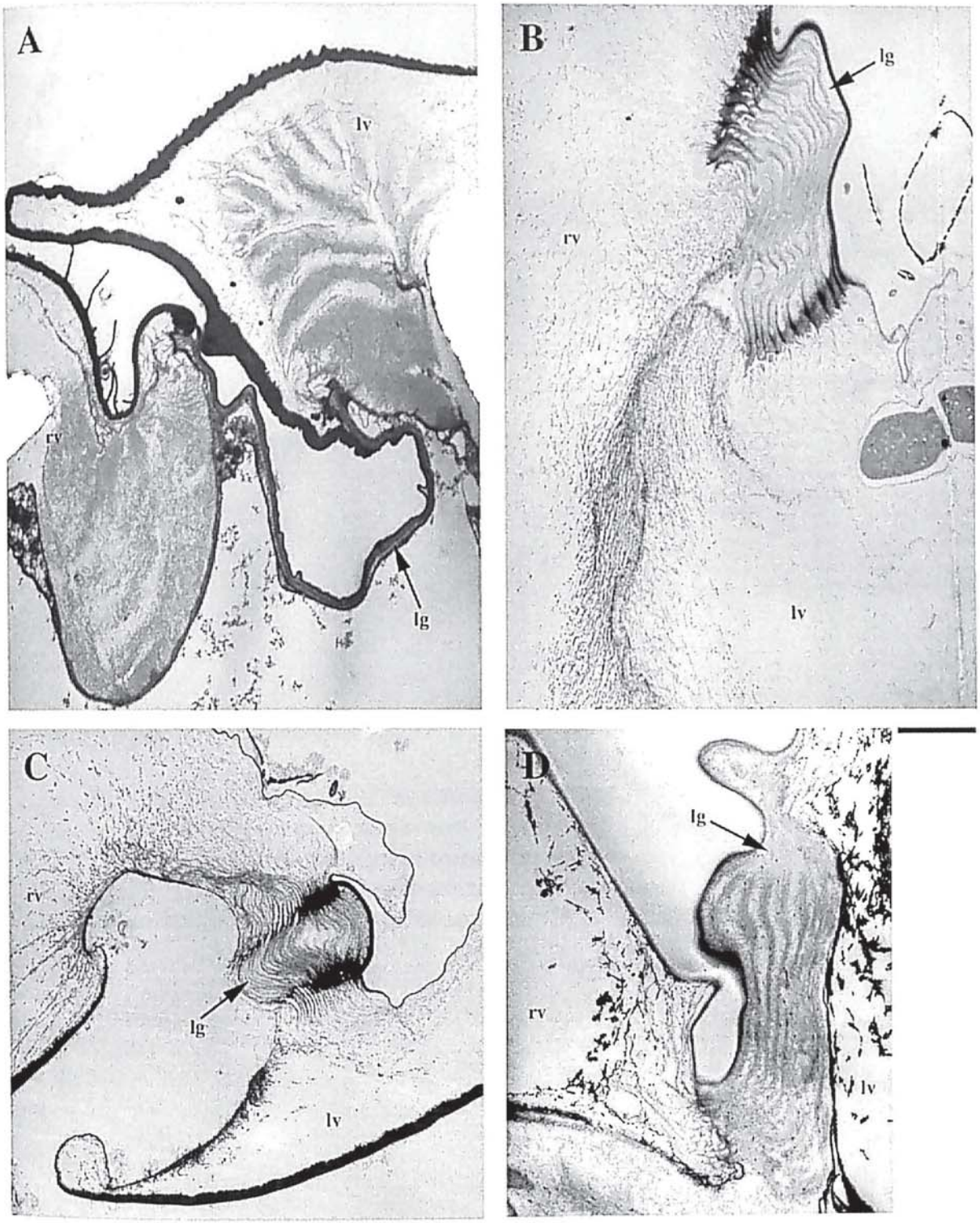

Figure 18 TEM photographs of the ostracod ligament.
A. *Polycope japonica.* B. *Keijcyoidea inflalittolaris.* C. *Aurila hataii.*
D. *Paracobanocythere* sp.
Scale bar is 2 μm (A); 3.3 μm (B); 2.5 μm (C); 800nm (D).

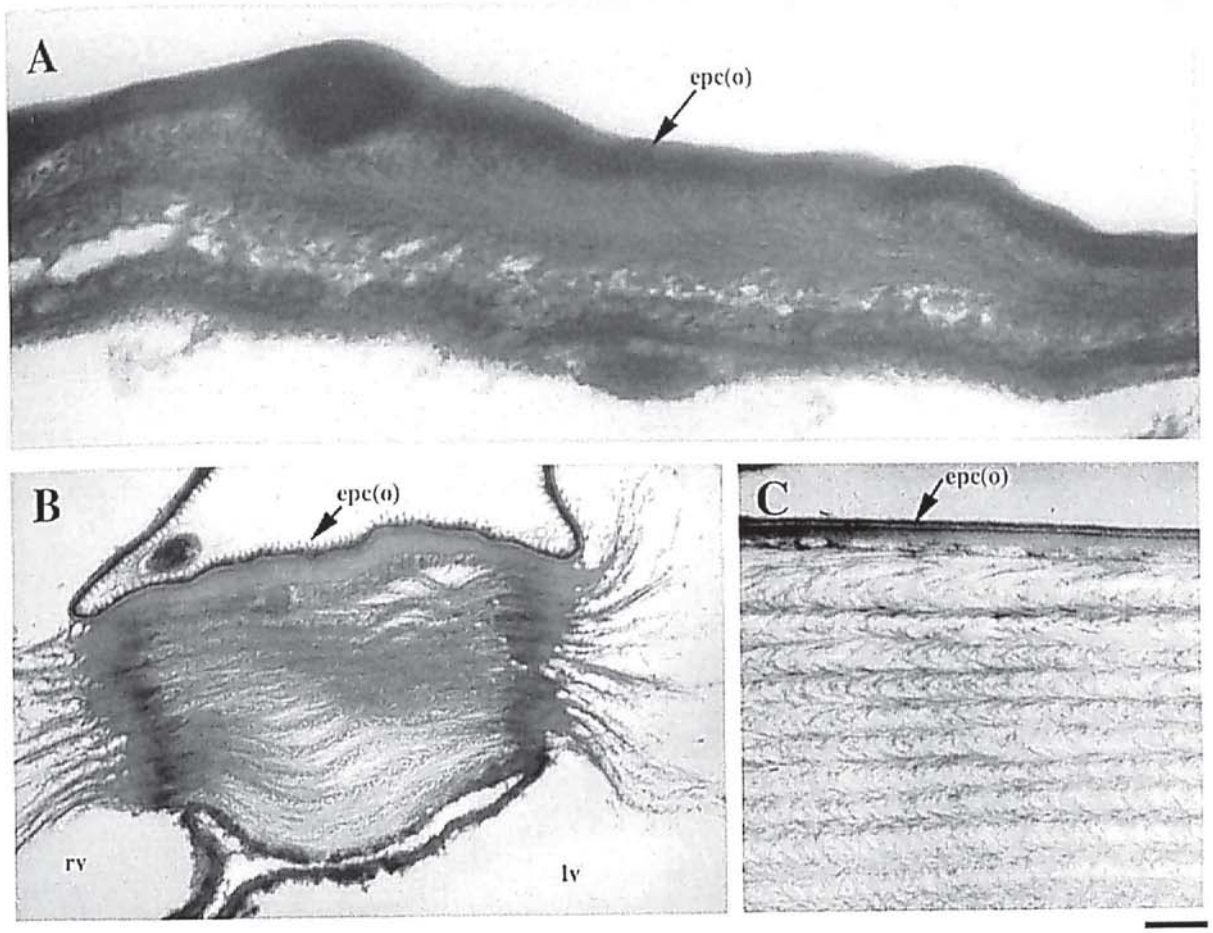

Figure 19 TEM photographs of the ostracod ligament.
A. ligament of *Polycope japonica* in transverse section.
B. ligament of *Loxoconcha pulchra* in transverse section.
C. ligament of *Loxoconcha pulchra* in longitudinal section.
Scale bar is 100nm (A); 400nm (B); 500nm (C).

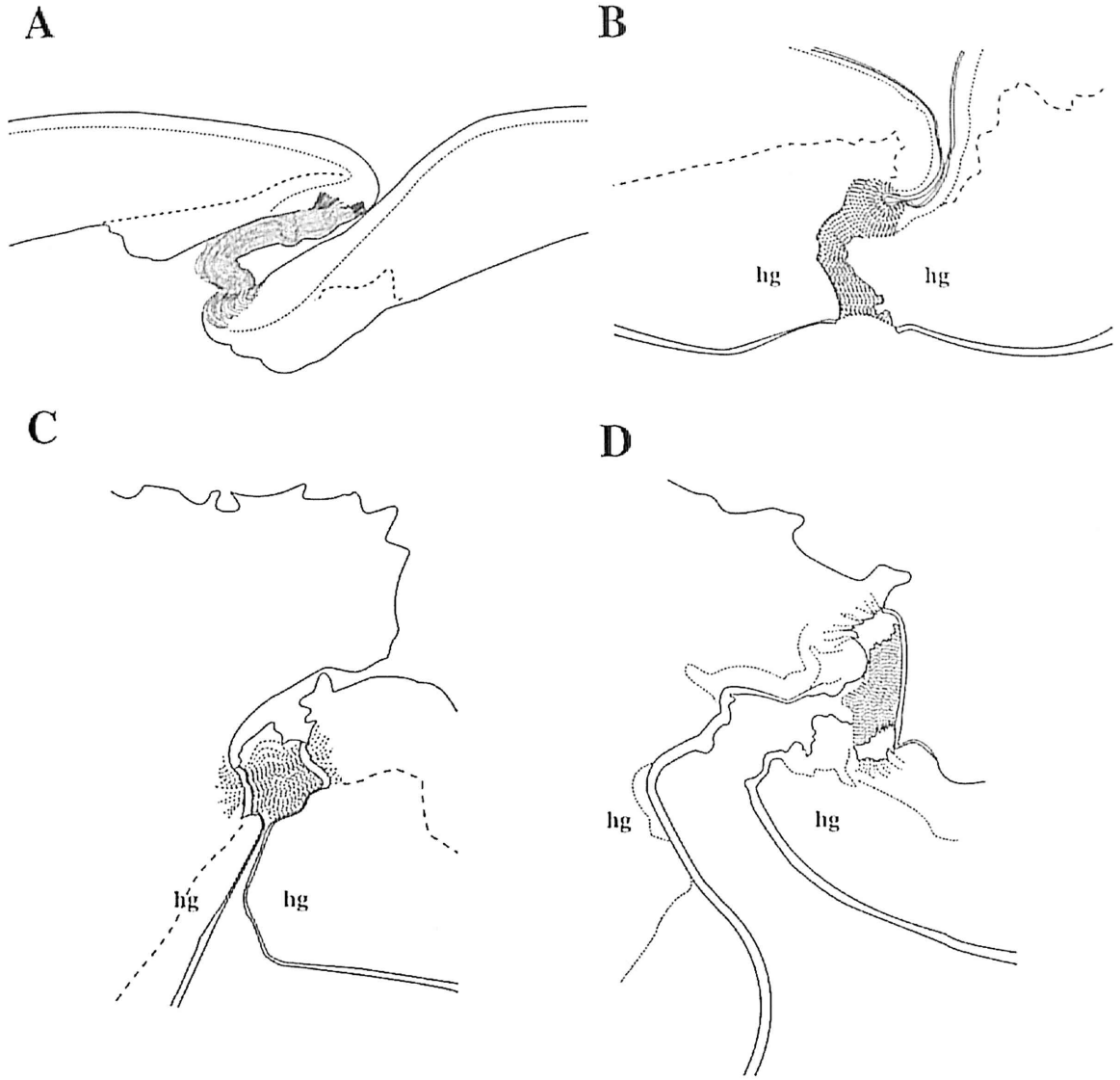

Figure 20 New classification of podocopan hinge sturctures.
A. transverse section of Basic type (*Chrissia* sp.).
B. transverse section of Exterior type (*Neonesidea oligodentata*).
C. transverse section of Transitive type (*Semicytherura kazahana*).
D. transverse section of Interior type (*Trachyleberis scabrocuneata*).

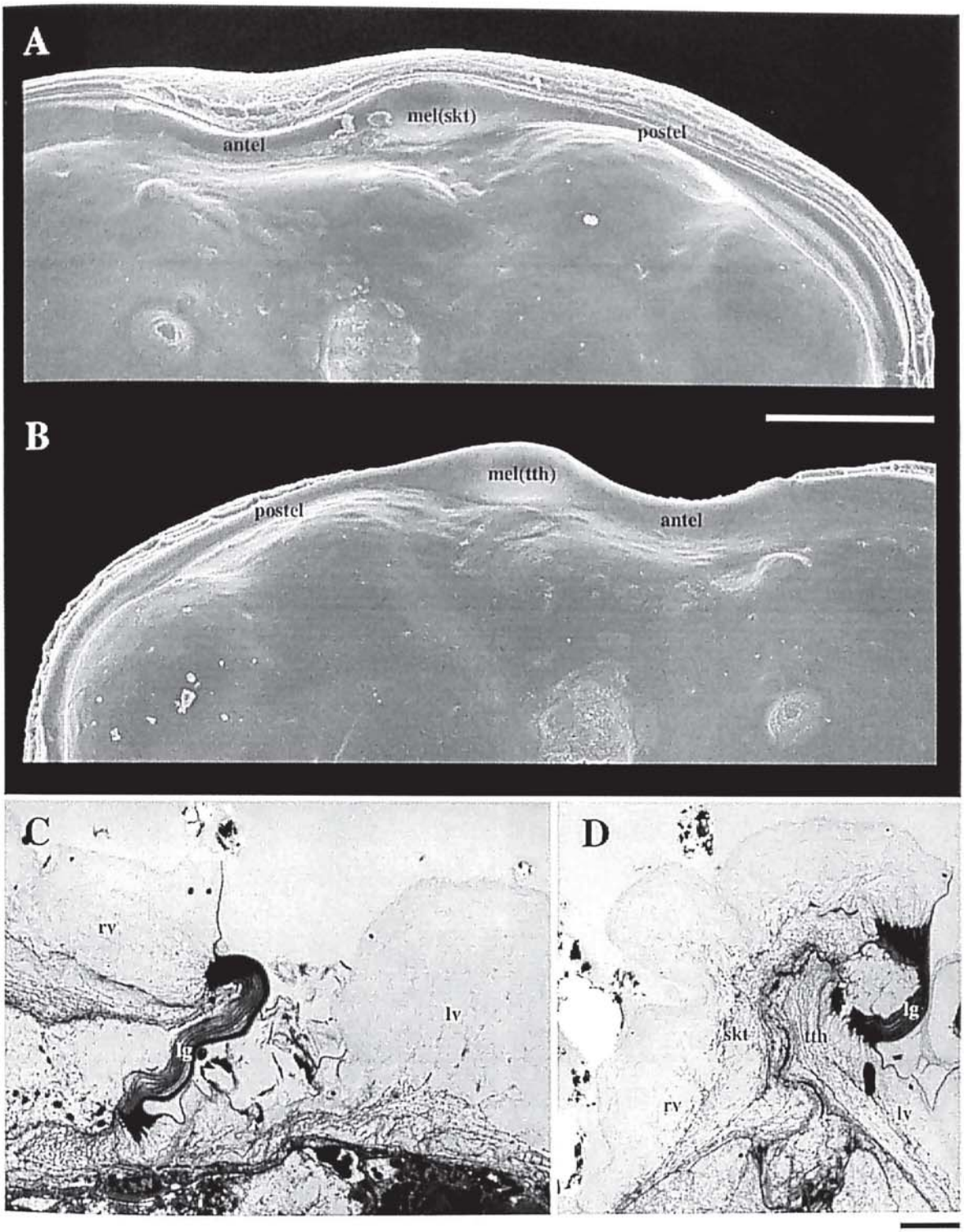

Figure 21 Hinge structure of *Keijcyoidea infralittolaris* (Cytherelloidea).
A. hingement of right valve. B. hingement of left valve.
C. transverse section of terminal element. D. transverse section of median element.
Scale bars are 100 μm (A, B); 2.5 μm (C); 2.9 μm (D).

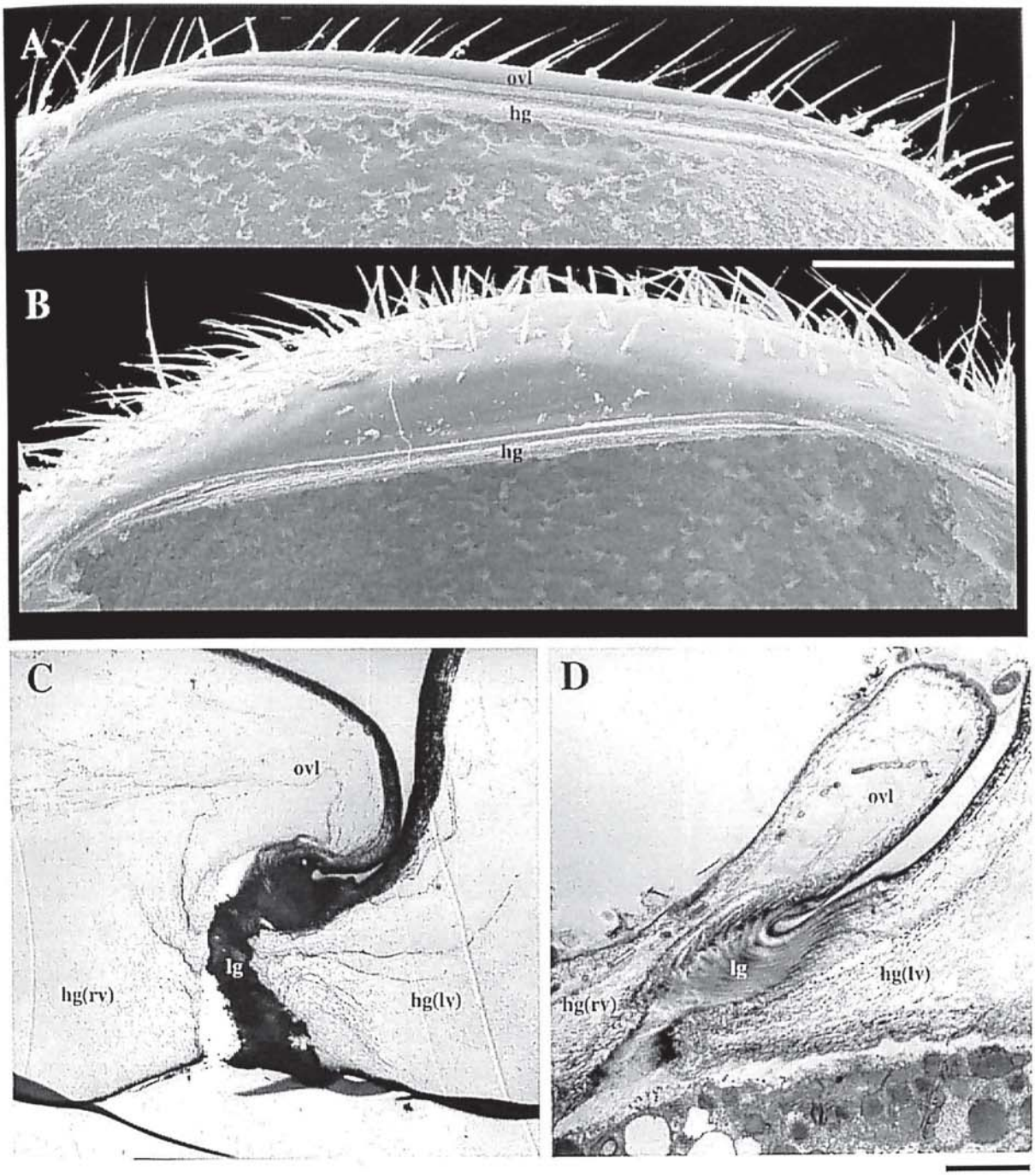

Figure 22 Hinge structure of bairdioid ostracods.
A. hingement of right valve (*Neonesidea oligodentata*).
B. hingement of left valve (*Neonesidea oligodentata*).
C. transverse section of hinge structure (*Neonesidea oligodentata*).
D. transverse section of hinge structure (*Triebelina* sp.).
Scale bars are 100 μm (A, B); 3.2 μm (C); 2.5 μm (D).

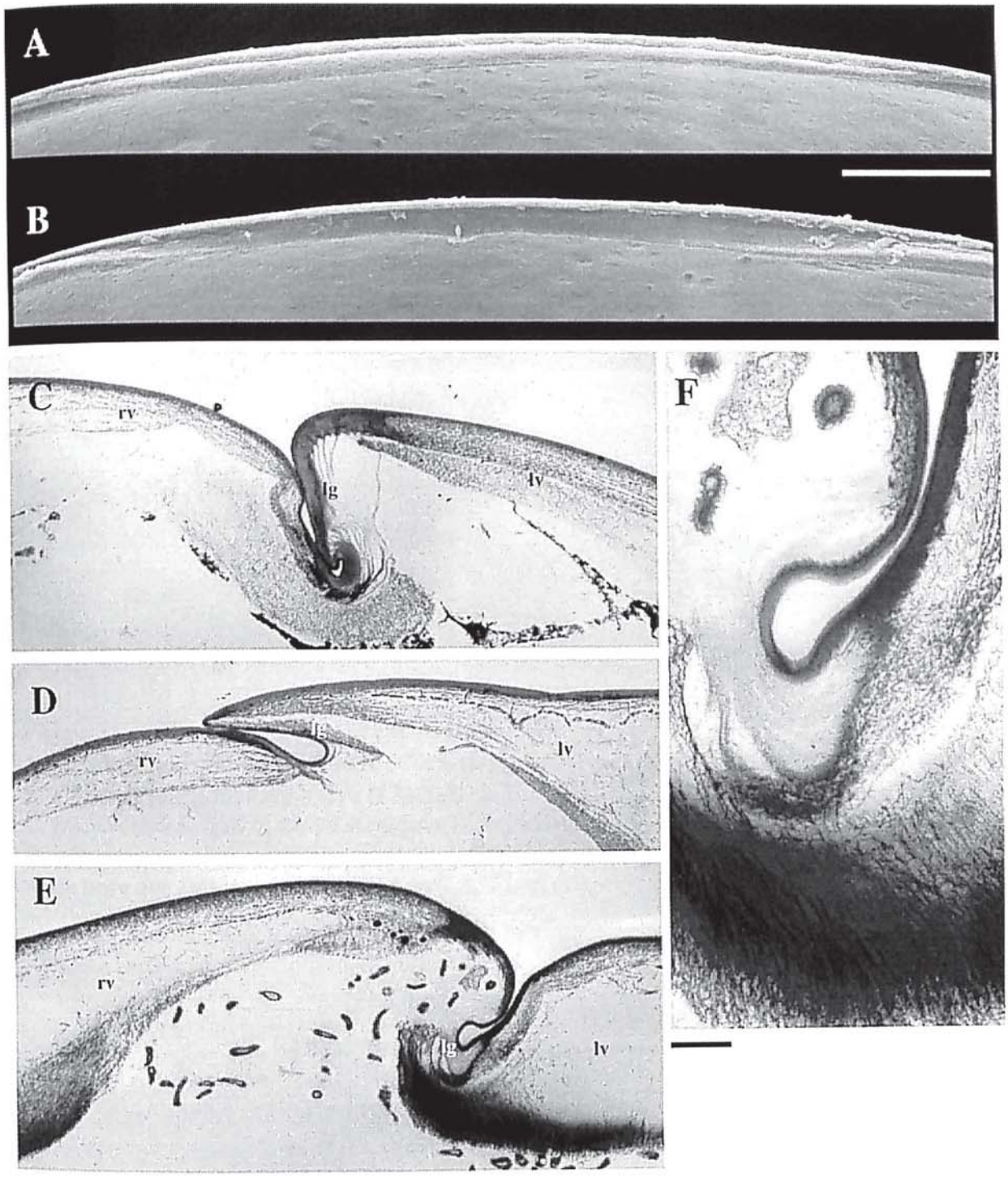

Figure 23 Hinge structure of darwinuloid ostracods.
A. attached margin of right valve (*Darwinula stevensoni*).
B. attached margin of left valve (*Darwinula stevensoni*).
C. transverse section of hinge structure (*Darwinula stevensoni*).
D. transverse section of hinge structure (*Microdarwinula* sp.).
E. transverse section of hinge structure (*Vestalenula* sp.)
F. ligament of *Vestalenula* sp.
Scale bars are 50 μ m (A, B); 2 μ m (C, E); 2.5 μ m (D); 500nm (F).

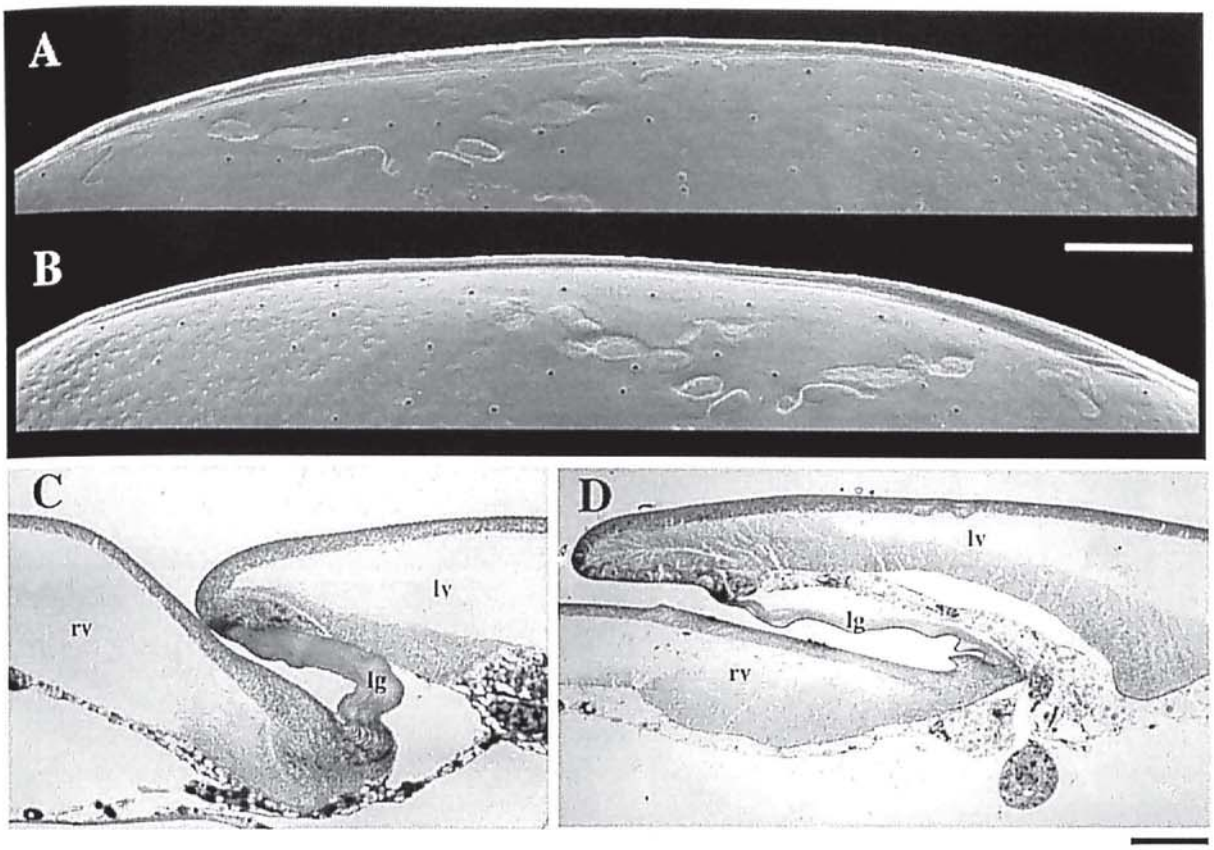

Figure 24 Hinge structure of cypridid ostracods.
A. attached margin of right valve (*Chrissia* sp.).
B. attached margin of left valve (*Chrissia* sp.).
C. transverse section of hinge structure (*Chrissia* sp.).
D. transverse section of hinge structure (*Cypridopsis vidua*).
Scale bars are 100 μm (A, B); 2.9 μm (C); 5 μm (D).

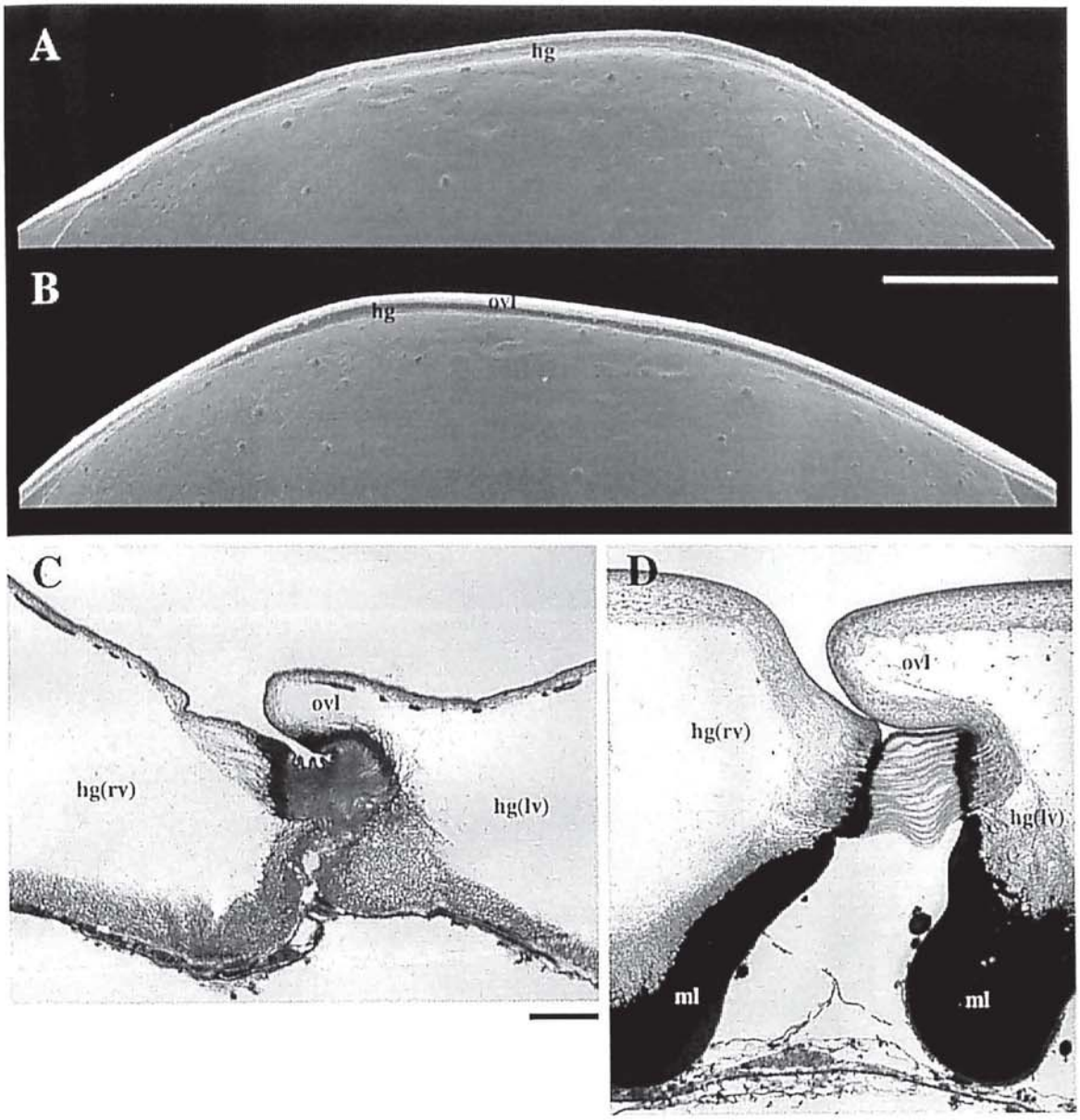

Figure 25 Hinge structure of freshwater candonid ostracods.
A. hingement of right valve (*Fabaeformiscandona* sp.).
B. hingement of left valve (*Fabaeformiscandona* sp.).
C. transverse section of hinge structure (*Fabaeformiscandona* sp.).
D. transverse section of hinge structure (*Cypria reptans*).
Scale bars are 100 μm (A, B); 1.3 μm (C); 2 μm (D).

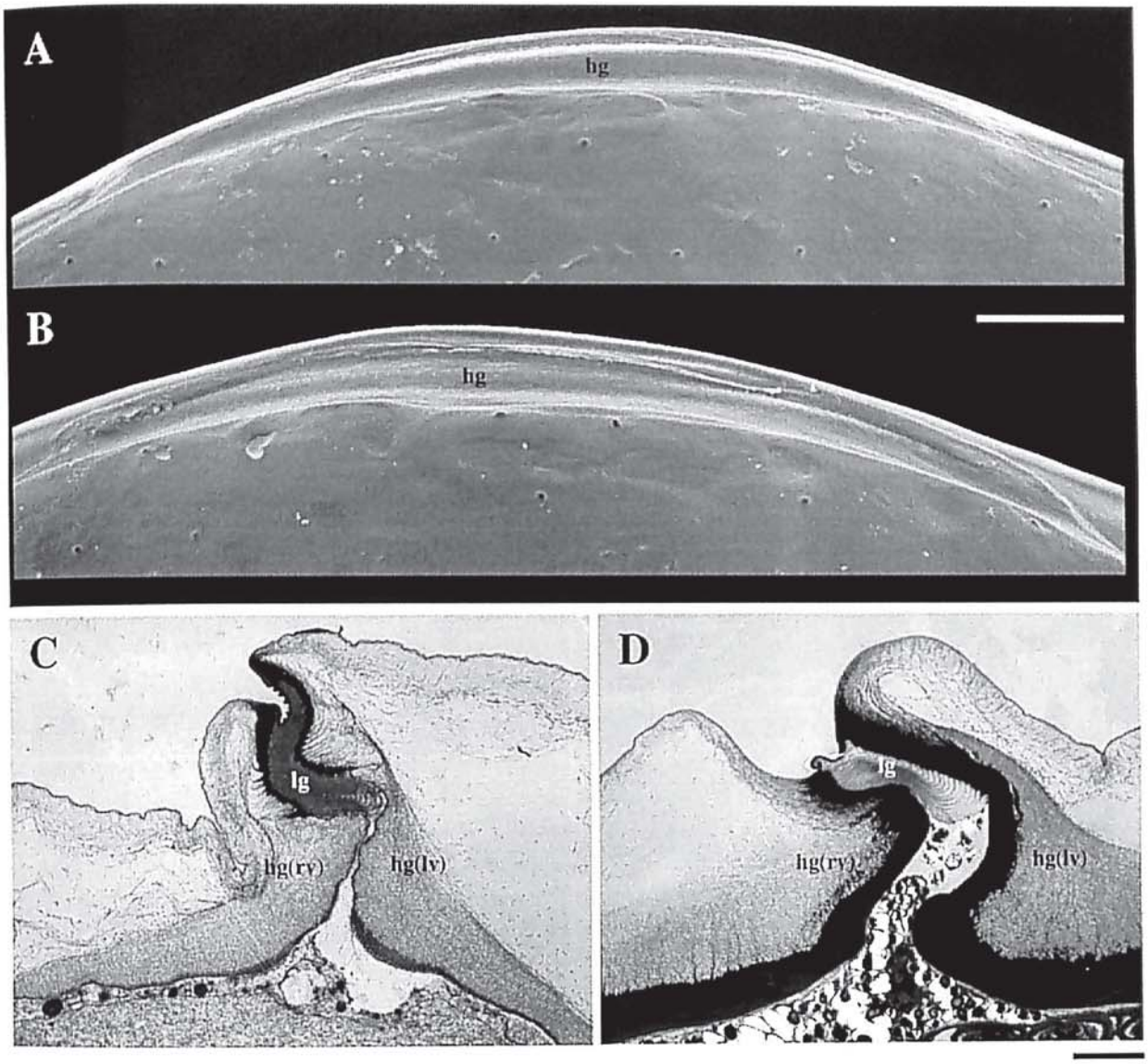

Figure 26 Hinge structure of marine candonid ostracods.
A. hingement of right valve (Paracypridinae sp. A).
B. hingement of left valve (Paracypridinae sp. A).
C. transverse section of hinge structure (Paracypridinae sp. A).
D. transverse section of hinge structure (Paracypridinae sp. B).
Scale bars are 50 μm (A, B); 2.5 μm (C, D).

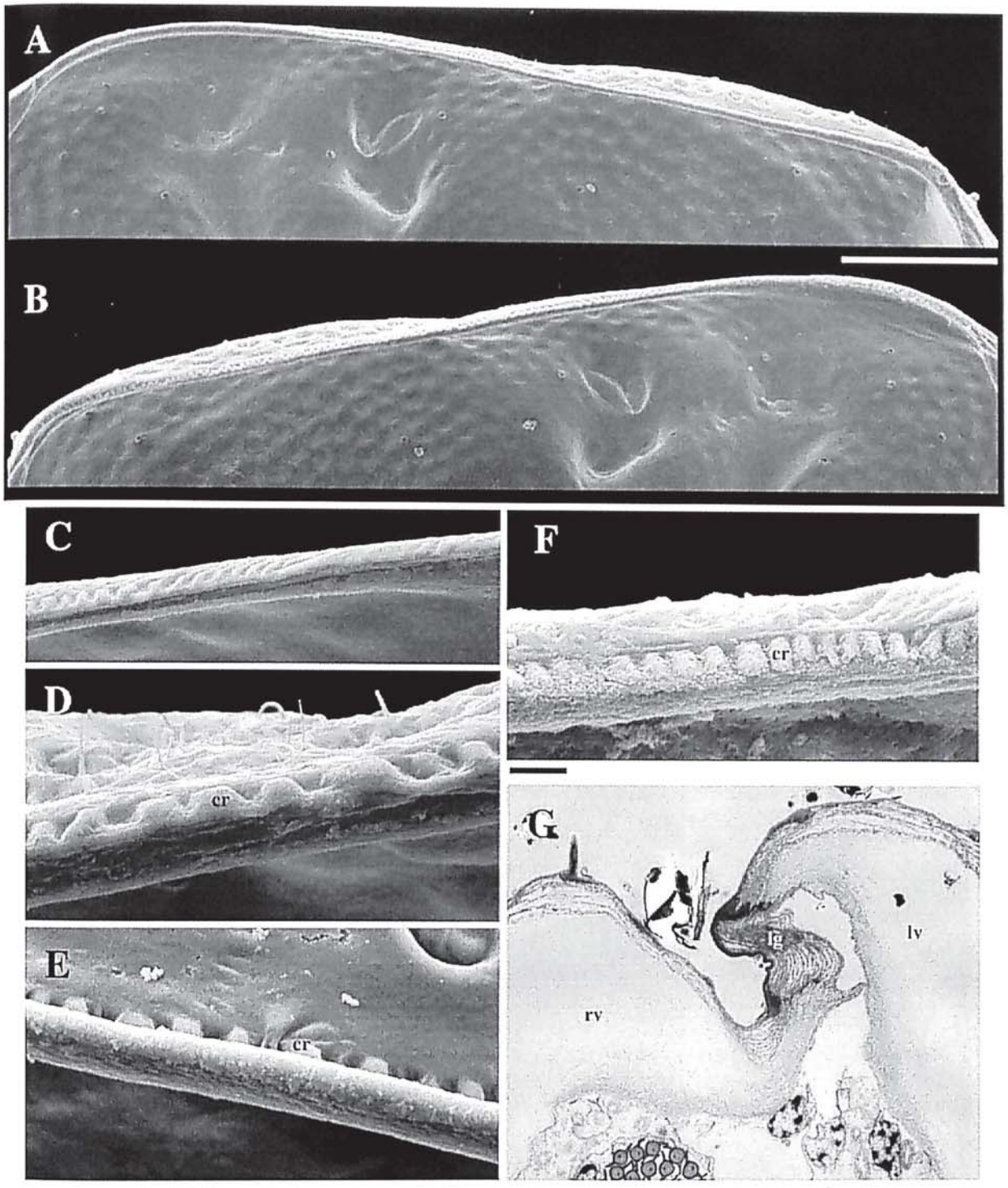

Figure 27 Hinge structure of ilyocyprid ostracods.
A. attached margin of right valve (*Ilyocypris japonica*).
B. attached margin of left valve (*Ilyocypris japonica*).
C. dorsal ornamentations of left valve (*Ilyocypris japonica*).
D. crenulate ornamentations of left valve (*Ilyocypris japonica*).
E. crenulate ornamentatons of right valve (*Ilyocypris japonica*).
F. crenulate ornamentations of left valve (*Ilyocypris* sp.).
G. transverse section of hinge structure (*Ilyocypris japonica*)
Scale bars are 100 μm (A, B); 10 μm (C); 5 μm (D, E, F); 1.9 μm (G).

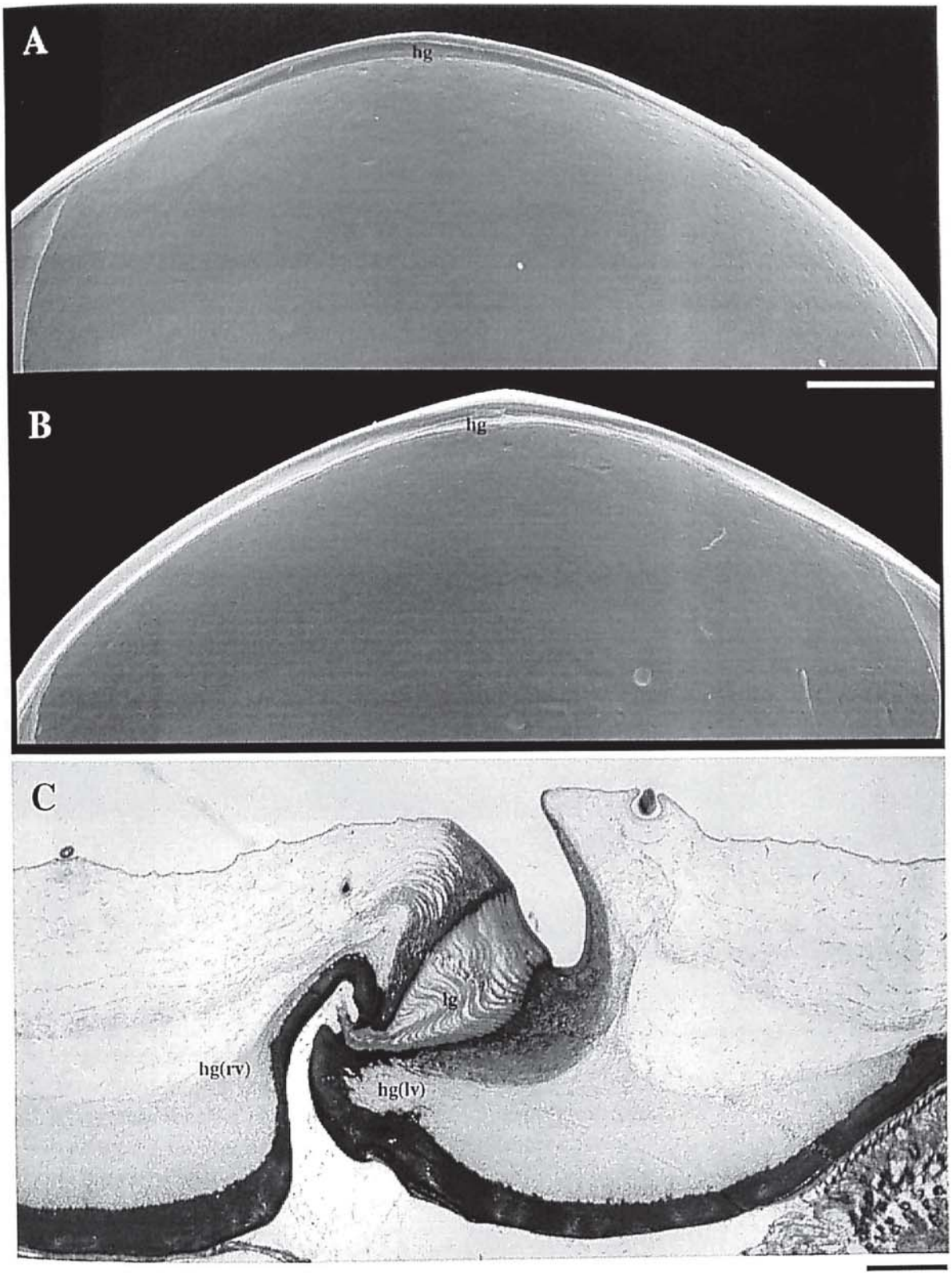

Figure 28 Hinge structure of pontocypridoid ostracods.
A. hingement of right valve (*Propontocypris* sp.).
B. hingement of left valve (*Propontocypris* sp.).
C. transverse section of hinge structure (*Propontocypris* sp.).
Scale bars are 100 μm (A, B); 3.3 μm (C).

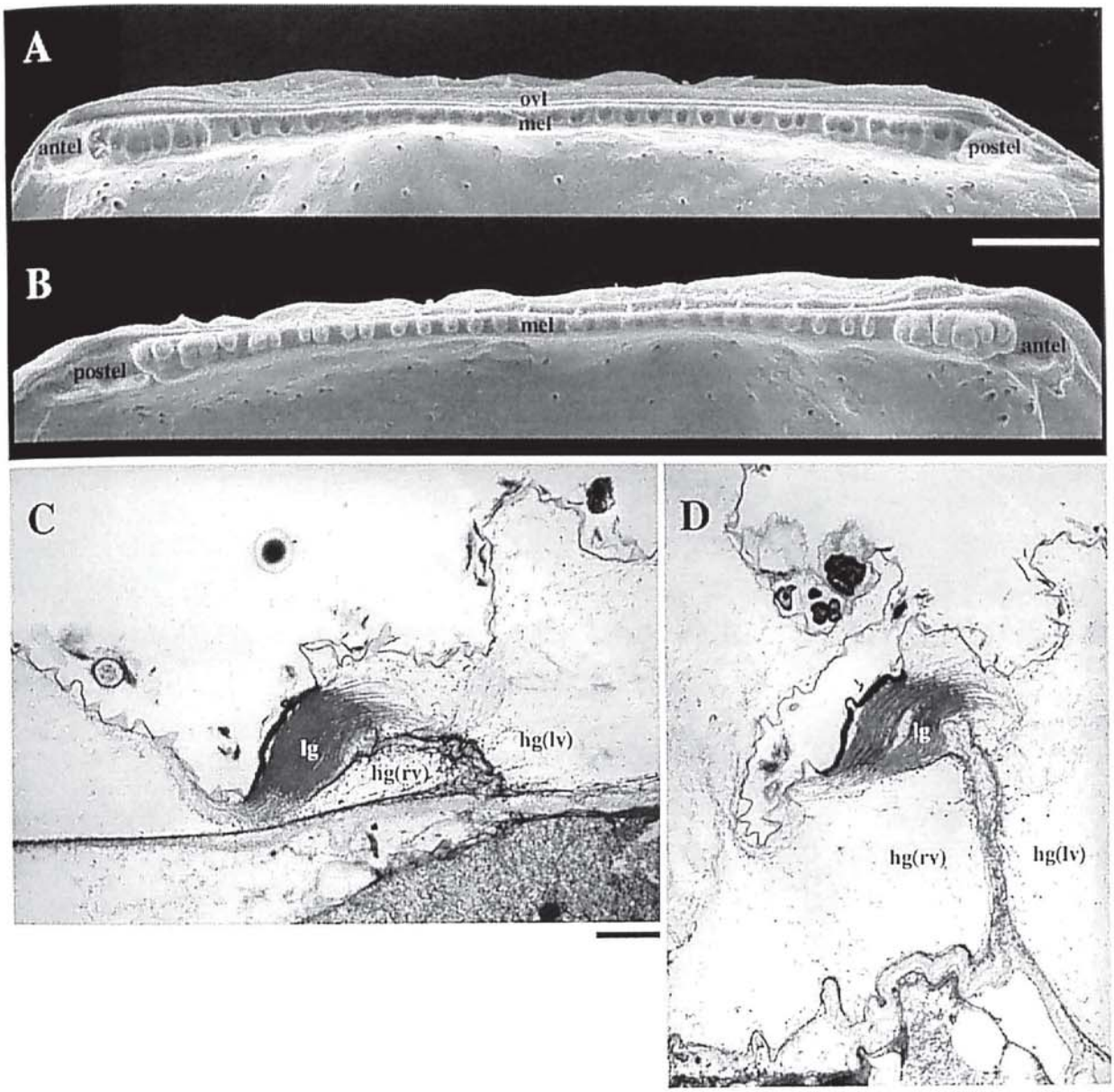

Figure 29 Hinge structure of bythocytherid ostracods.
A. hingement of right valve (*Bythoceratina* sp.).
B. hingement of left valve (*Bythoceratina* sp.).
C. transverse section of terminal element (*Bythoceratina* sp.).
D. transverse section of median element (*Bythoceratina* sp.).
Scale bars are 50 μm (A, B); 1.4 μm (C, D).

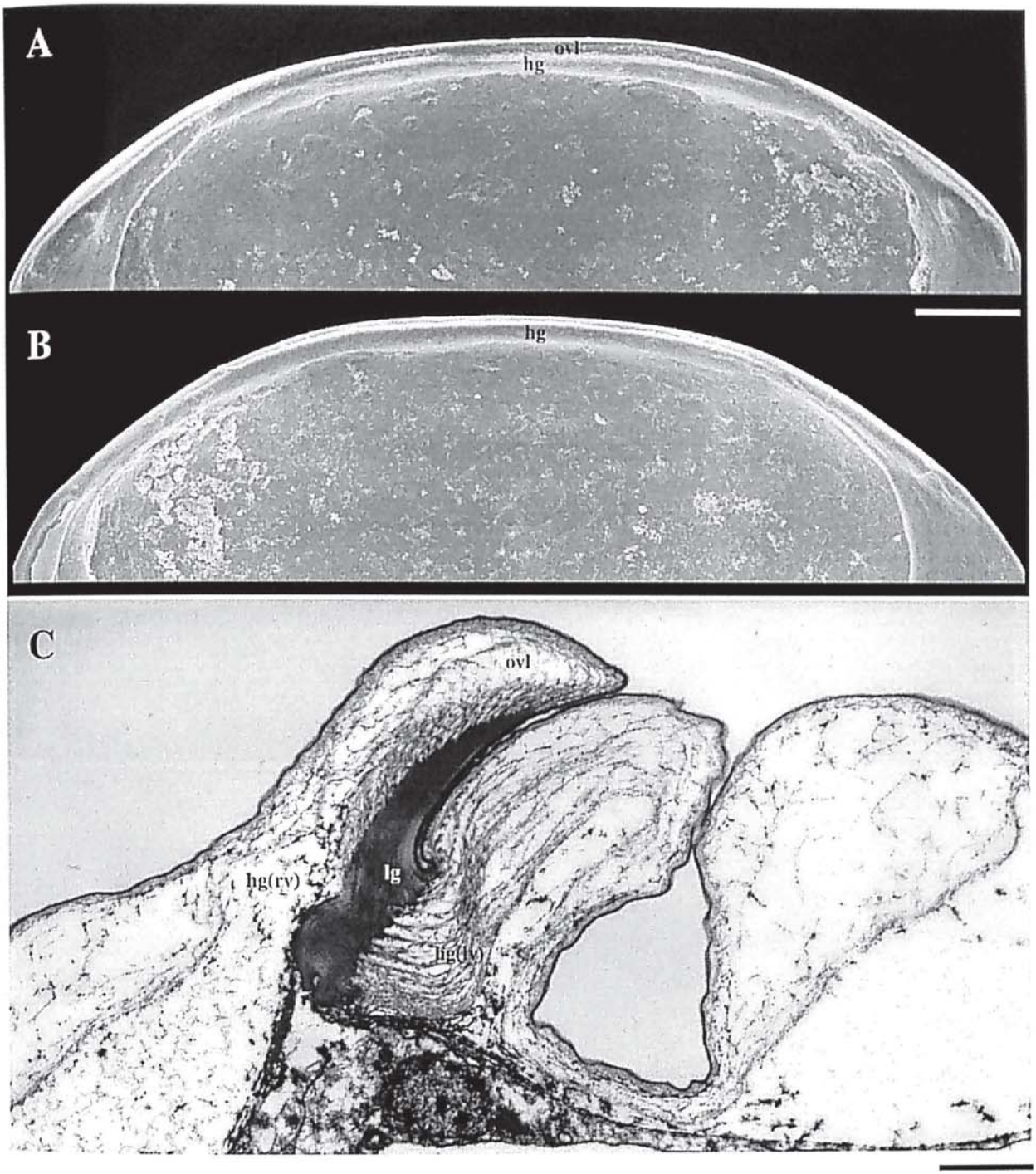

Figure 30 Hinge structure of bythocytherid ostracods.
A. hingement of right valve (*Sclerochilus* sp.).
B. hingement of left valve (*Sclerochilus* sp.).
C. transverse section of hinge structure (*Sclerochilus* sp.).
Scale bars are 50 μ m (A, B); 1.4 μ m (C).

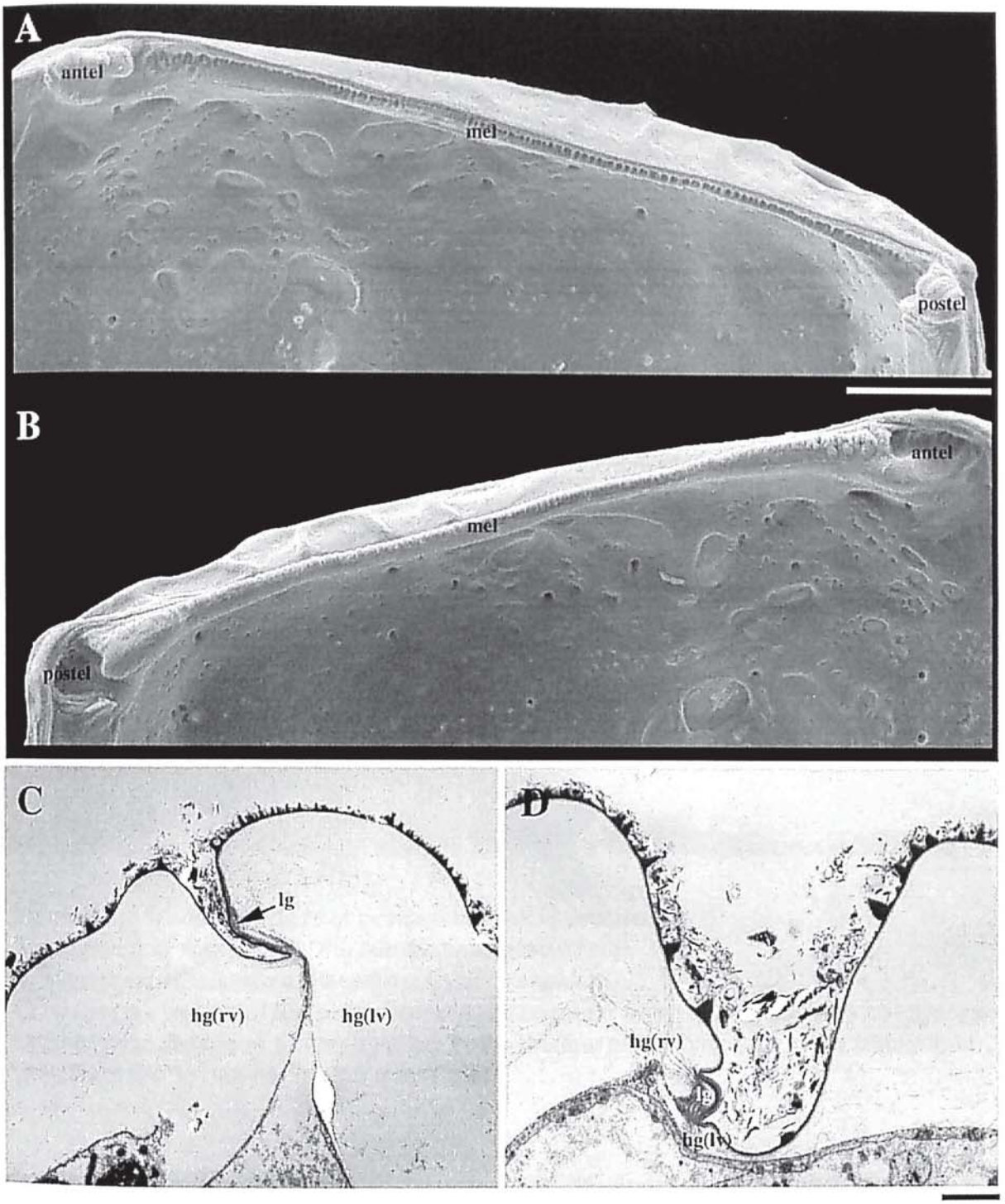

Figure 31 Hinge structure of eucytherid ostracods.
A. hingement of right valve (*Keijia* cf. *demissa*).
B. hingement of left valve (*Keijia* cf. *demissa*).
C. transverse section of terminal element (*Keijia* cf. *demissa*).
D. transverse section of median element (*Keijia* cf. *demissa*).
Scale bars are 50 μm (A, B); 4 μm (C); 2.5 μm (D).

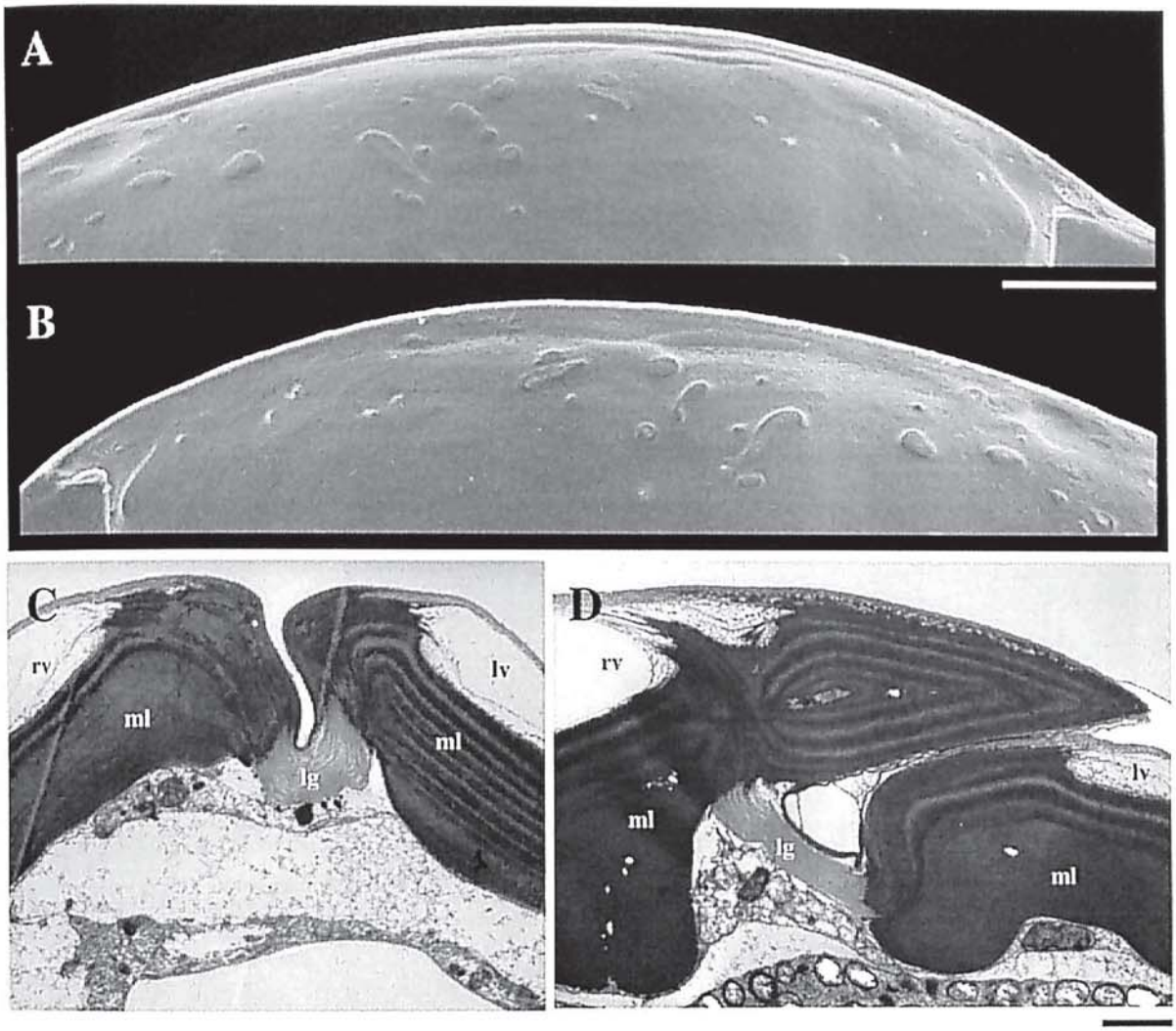

Figure 32 Hinge structure of paradoxostomatid ostracods.
A. hingment of right valve (*Paradoxostoma triangulum*).
B. hingement of left valve (*Paradoxostoma triangulum*).
C. transverse section of hinge structure in the terminal area (*Paradoxostoma triangulum*).
D. transverse section of hinge structure in the median area (*Paradoxostoma triangulum*).
Scale bars are 50 μm (A, B); 1.3 μm (C, D).

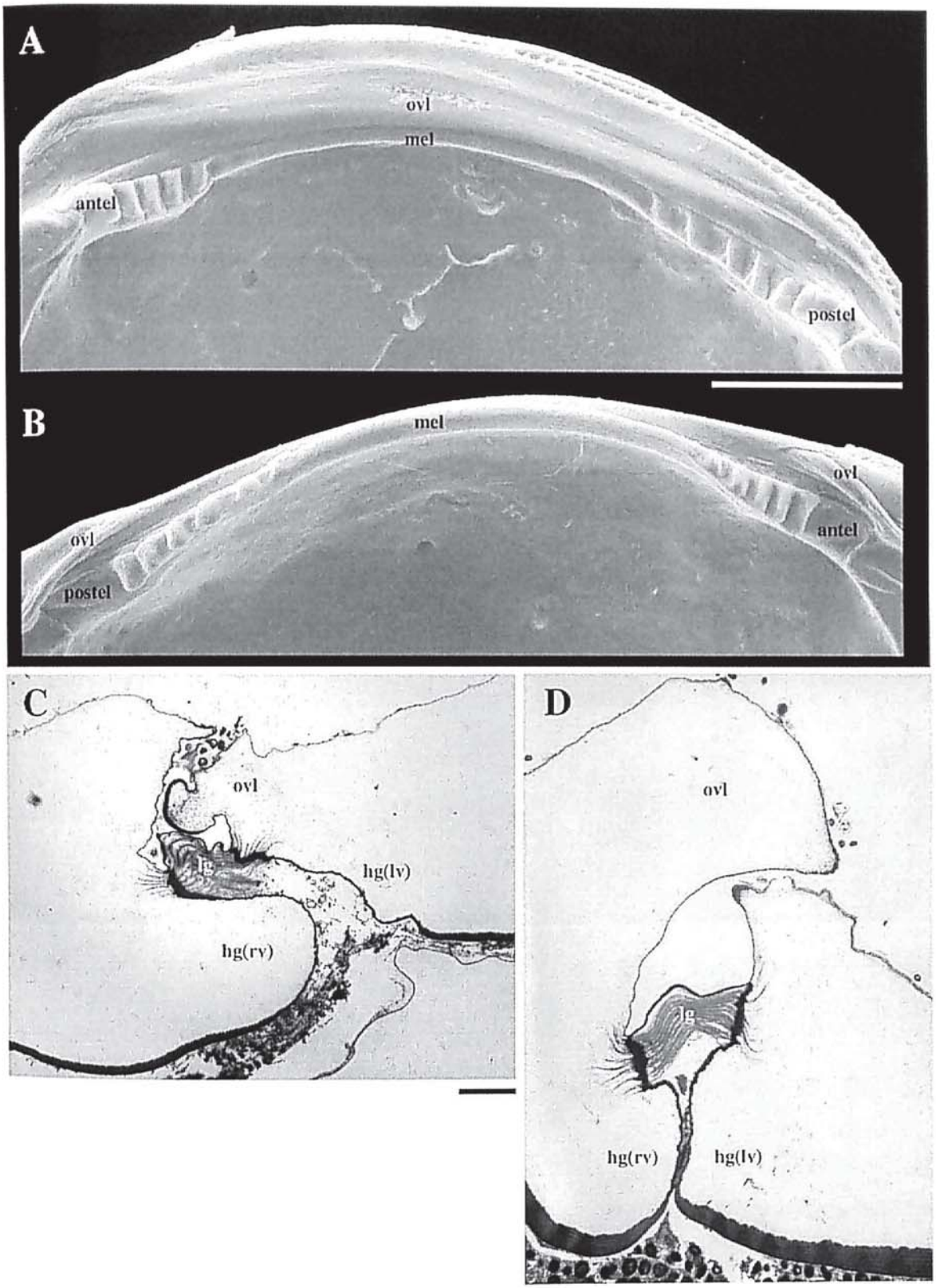

Figure 33 Hinge structure of cytherurid ostracods.
A. hingement of right valve (*Hemicytherura kajiyamai*).
B. hingement of left valve (*Hemicytherura kajiyamai*).
C. transverse section of terminal element (*Hemicytherura kajiyamai*).
D. transverse section of median element (*Hemicytherura tricarinata*).
Scale bars are 50 μm (A, B); 4 μm (C); 2 μm (D).

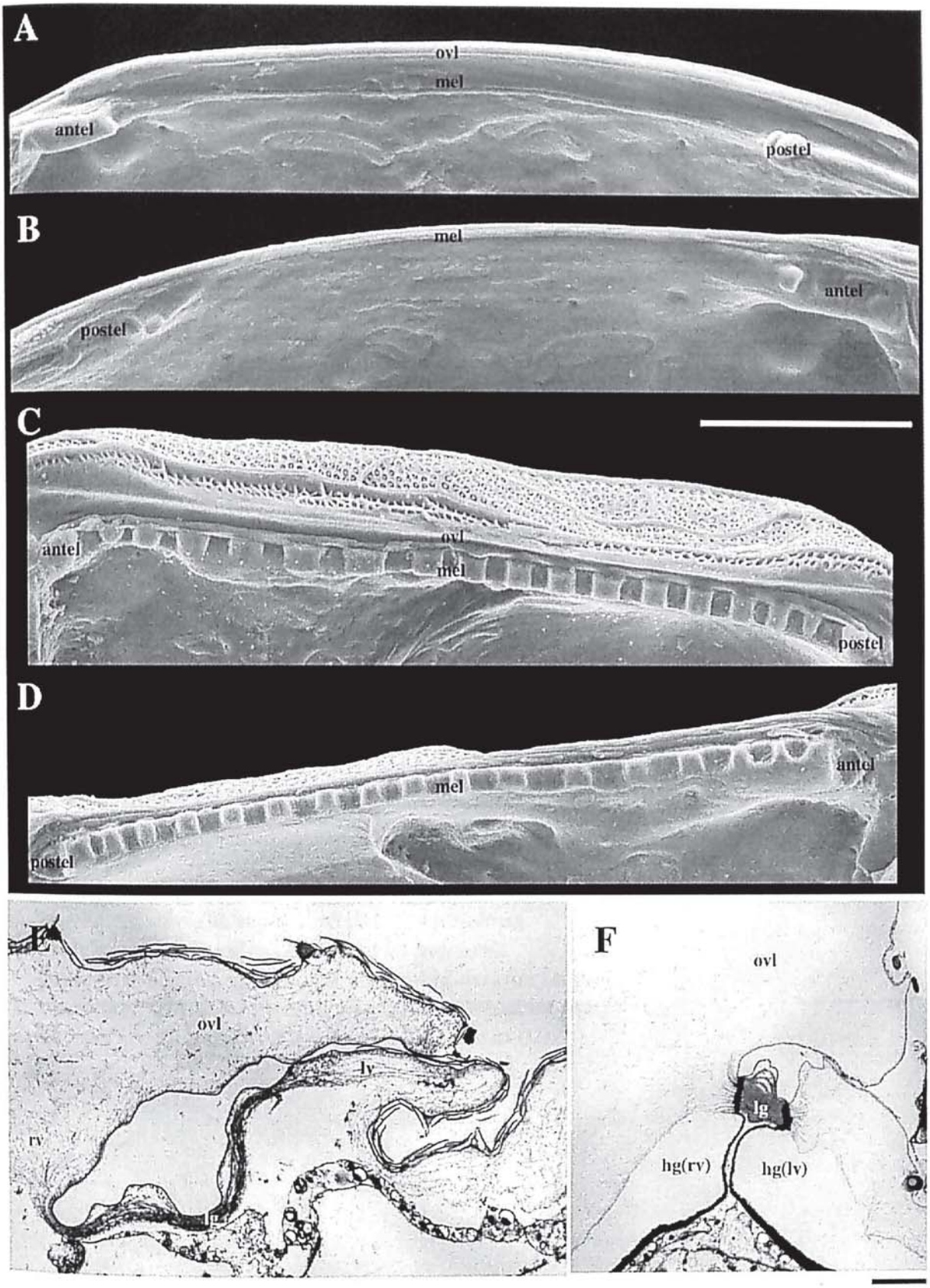

Figure 34 Hinge structure of cytherurid ostracods.
A, B, E. *Semicytherura wakamurasaki.* C, D, F. *Semicytherura kazahana.*
A, C. hingement of right valve. B, D. hingement of left valve.
E, F. transeverse sections of median element.
Scale bars are 50 μm (A, B); 1.4 μm (C); 2.7 μm (D).

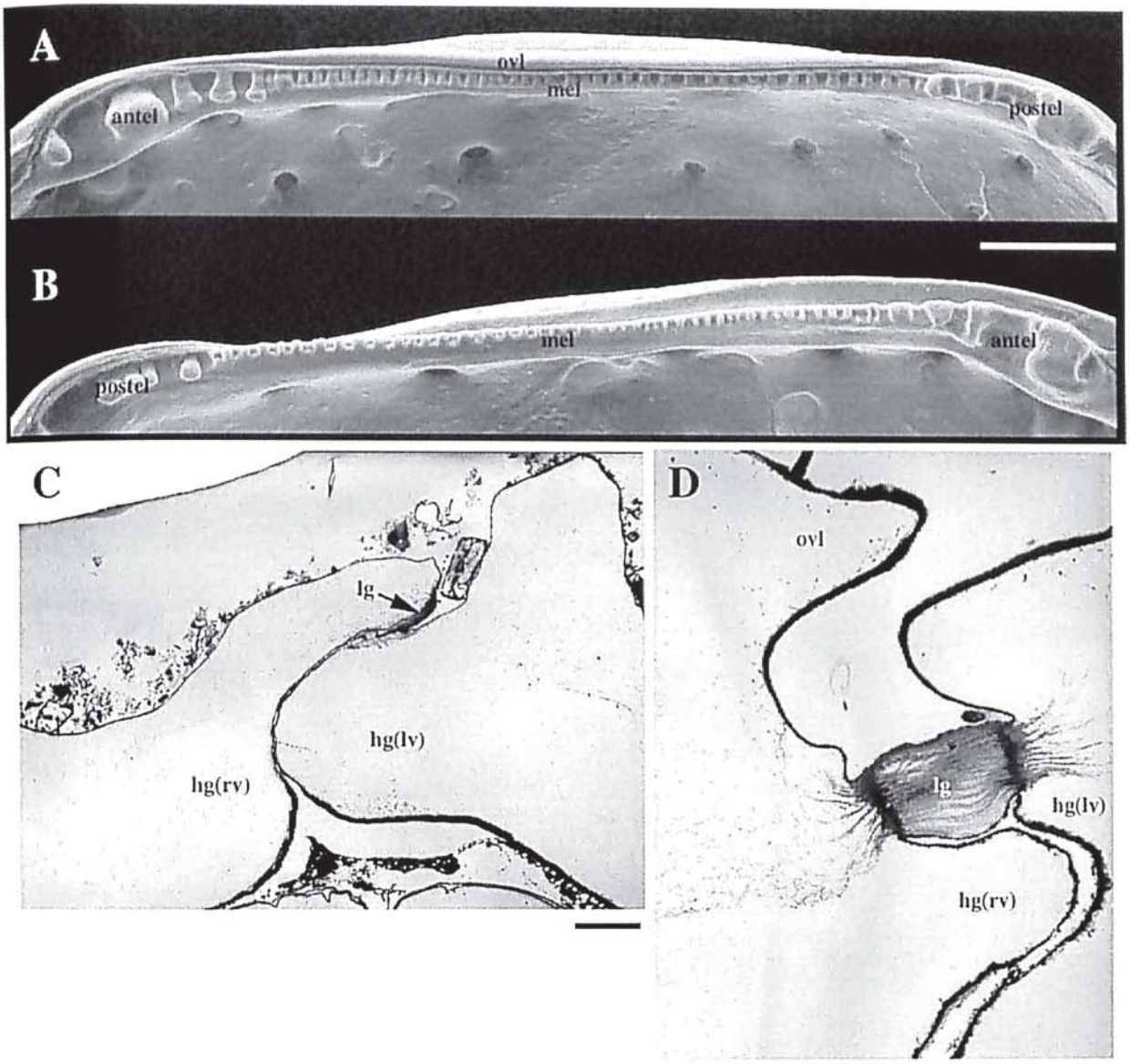

Figure 35 Hinge structure of loxoconchid ostracods.
A. hingement of right valve (*Loxoconcha pulchra*).
B. hingement of left valve (*Loxoconcha pulchra*).
C. transverse section of terminal element (*Loxoconcha pulchra*).
D. transverse section of median element (*Loxoconcha pulchra*).
Scale bars are 50 μ m (A, B); 6.7 μ m (C); 1.4 μ m (D).

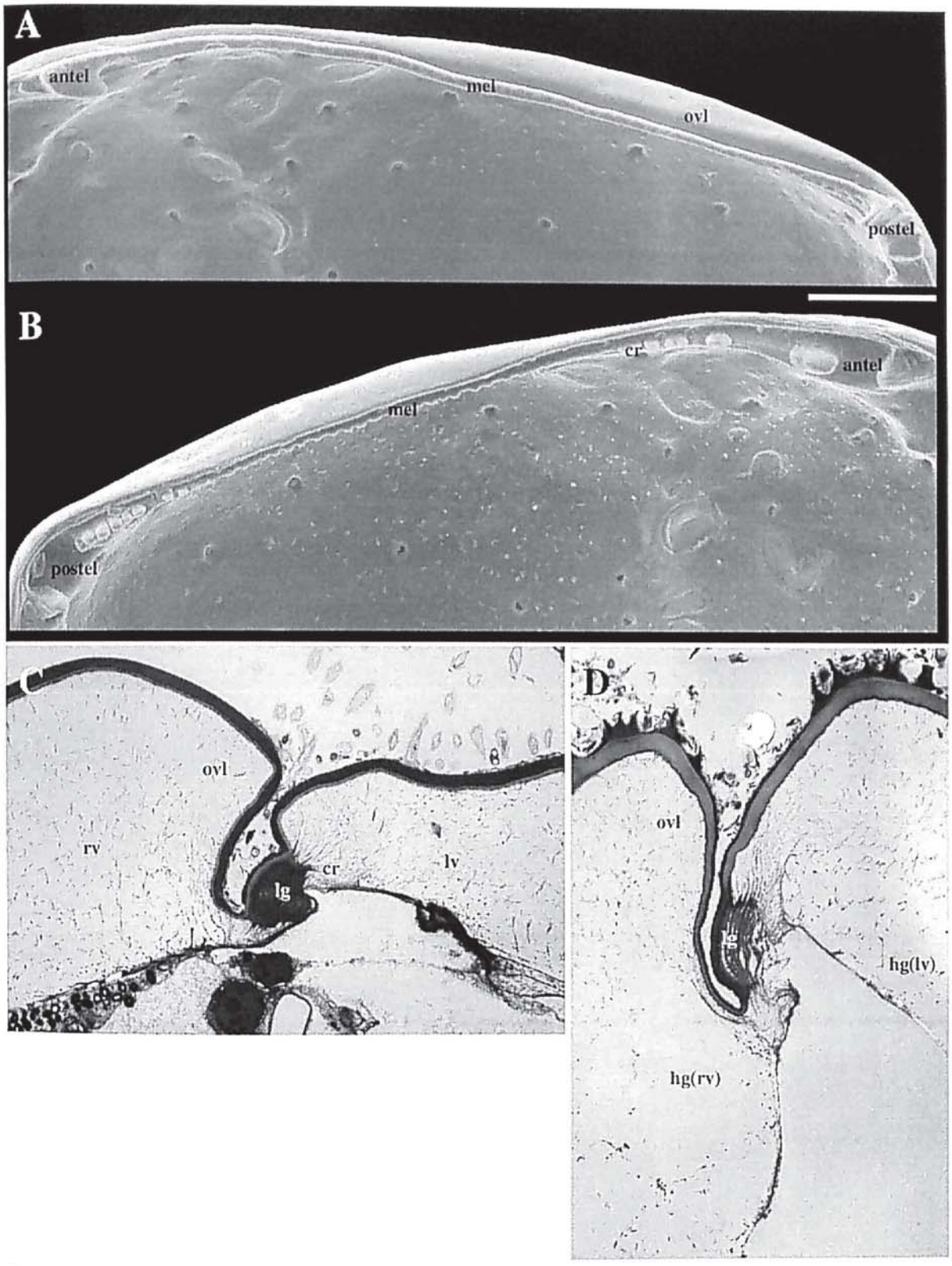

Figure 36 Hinge structure of leptocytherid ostracods.
A. hingement of right valve (*Callistocythere pumila*).
B. hingement of left valve (*Callistocythere pumila*).
C. transverse section of median element (*Callistocythere pumila*).
D. transverse section of posterior element (*Callistocythere pumila*).
Scale bars are 50 μm (A, B); 2.5 μm (C); 2 μm (D).

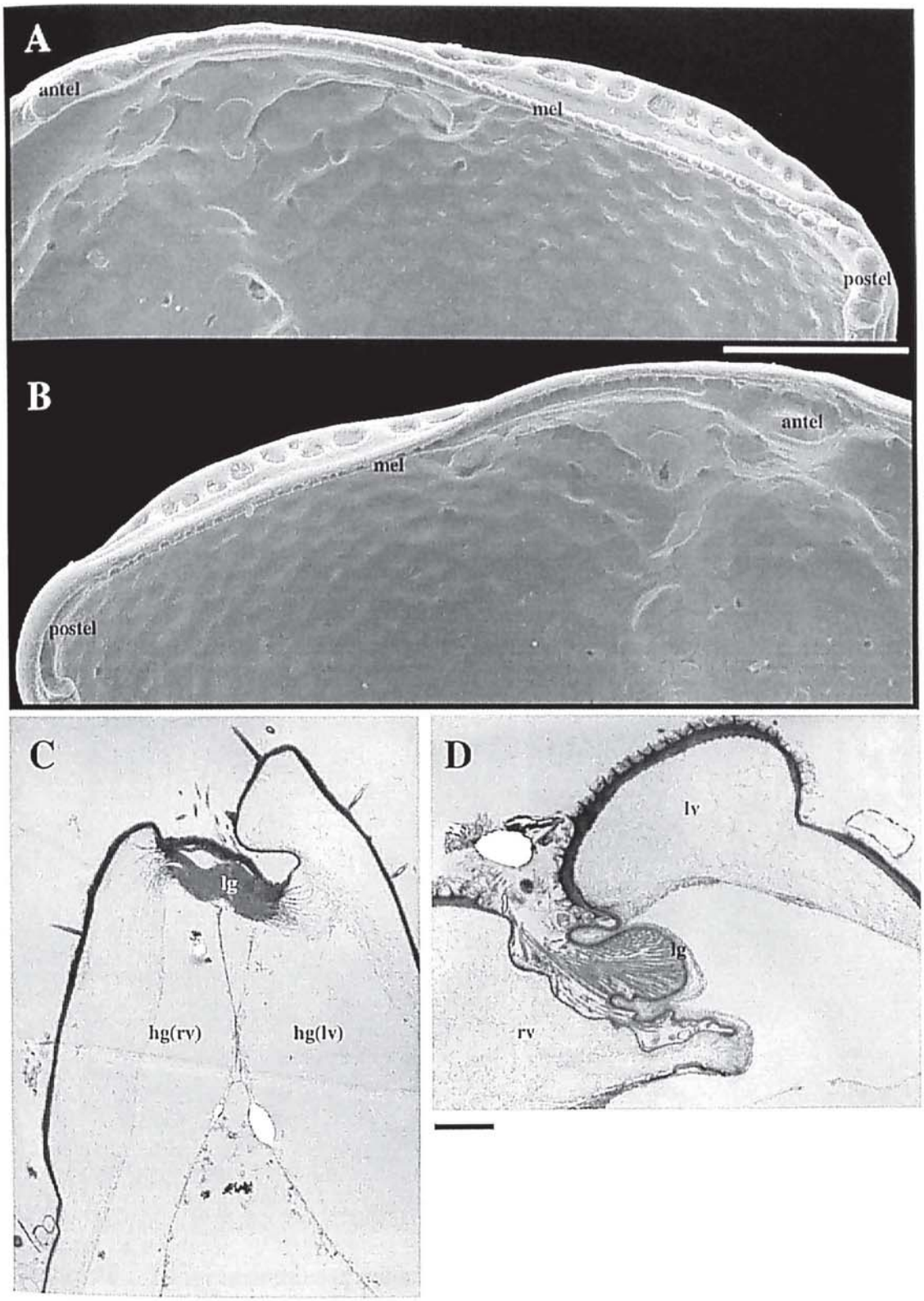

Figure 37 Hinge structure of leptocytherid ostracods.
A. hingement of right valve (*Ishizakiella miurensis*).
B. hingement of left valve (*Ishizakiella miurensis*).
C. transverse section of anterior element (*Ishizakiella miurensis*).
D. transverse section of anteromedian tooth (*Ishizakiella miurensis*).
Scale bars are 50 μm (A, B); 6.7 μm (C); 3.3 μm (D).

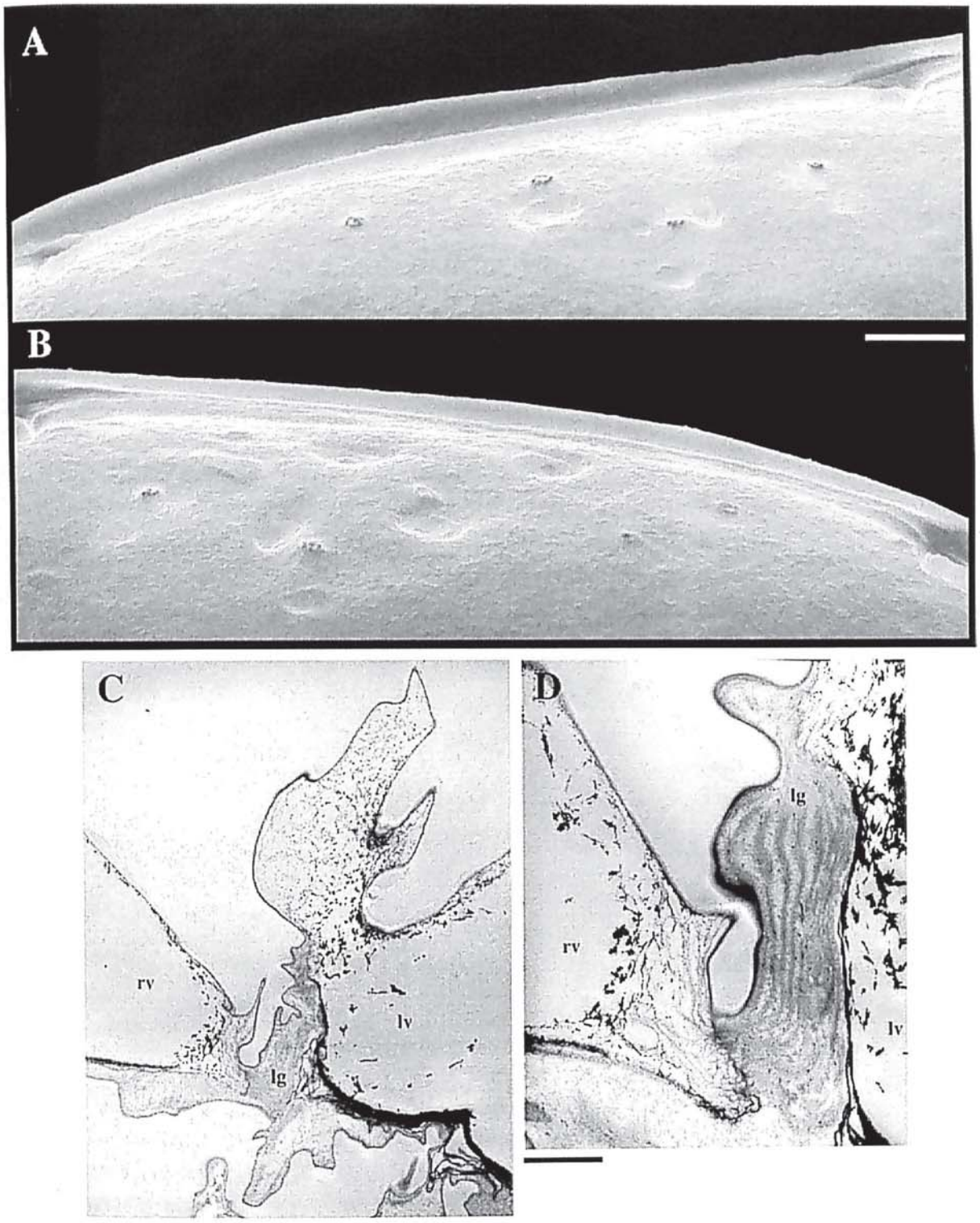

Figure 38 Hinge structure of cobanocytherid ostracods.
A. attached margin of right valve (*Paracobanocythere* sp.).
B. attached margin of left valve (*Paracobanocythere* sp.).
C. transverse section of hinge structure (*Paracobanocythere* sp.).
D. ligament of *Paracobanocythere* sp.).
Scale bars are 50 μ m (A, B); 2.5 μ m (C); 800nm (D).

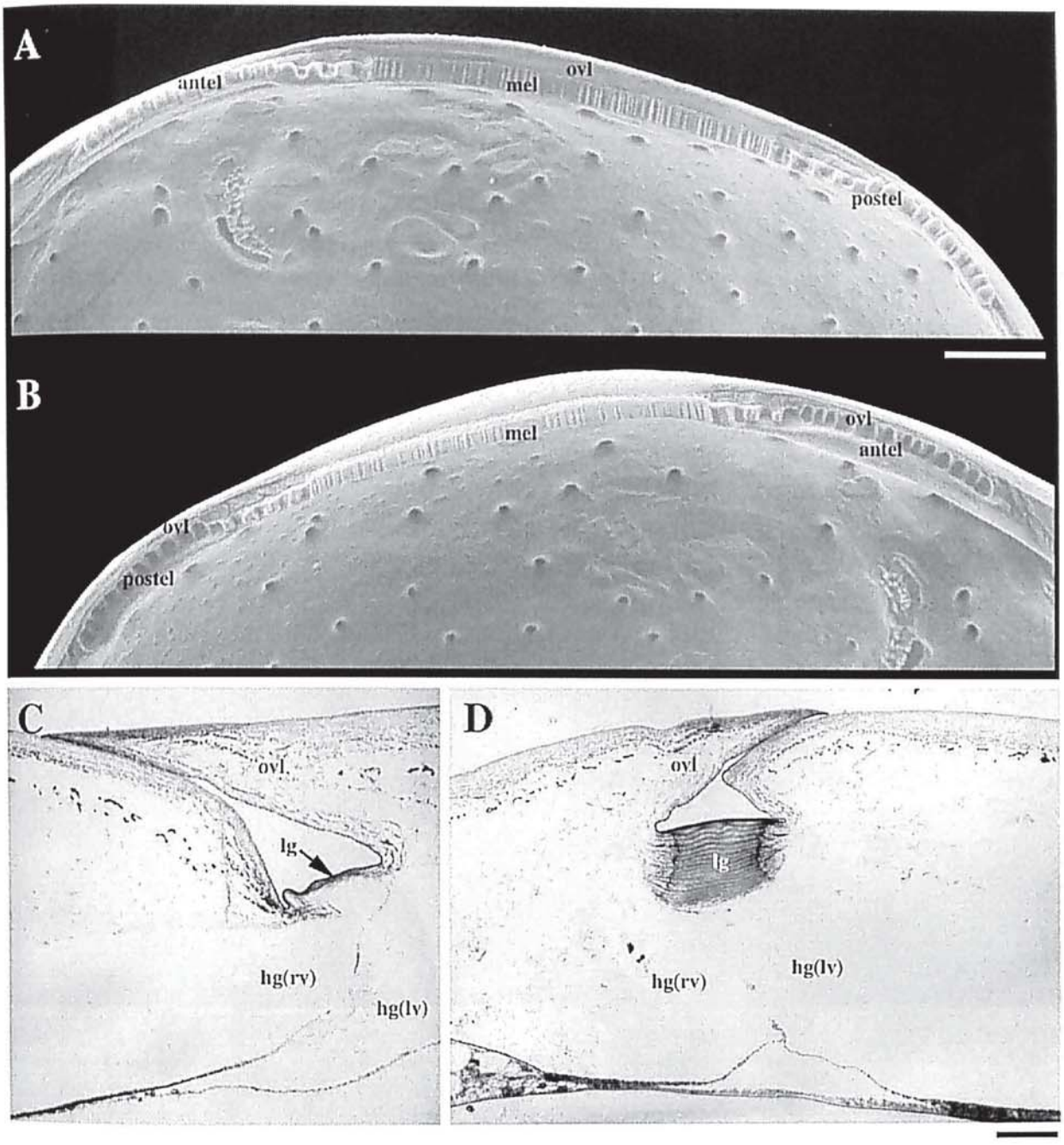

Figure 39 Hinge structure of xestoleberidid ostracods.
A. hingement of right valve (*Xestoleberis hanaii*).
B. hingment of left valve (*Xestoleberis hanaii*).
C. transverse section of terminal element (*Xestoleberis hanaii*).
D. transverse section of median element (*Xestoleberis hanaii*).
Scale bars are 50 μ m (A, B); 2.9 μ m (C); 3.3 μ m (D).

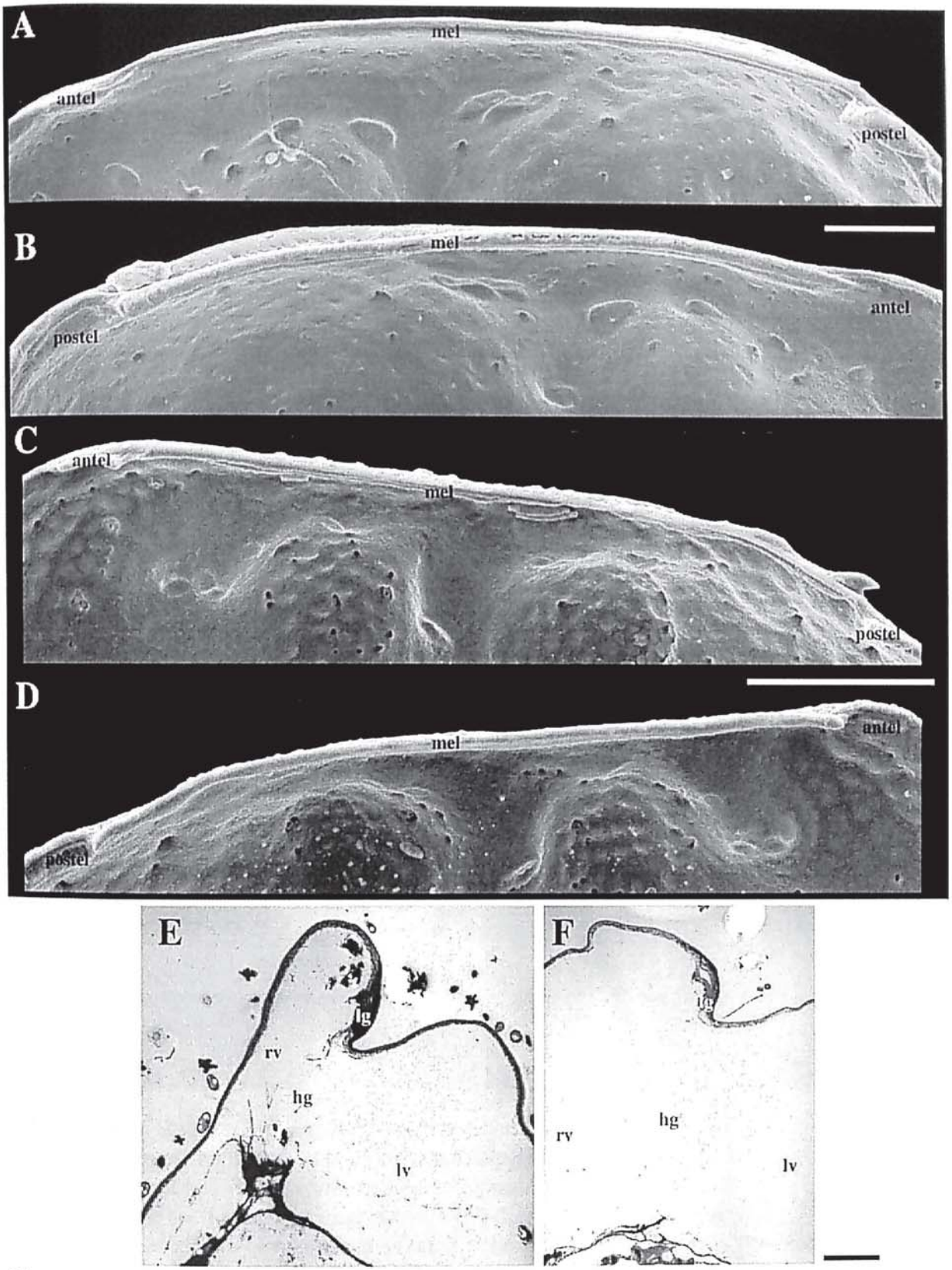

Figure 40 Hinge structure of limnocytherid ostracods.
A, B. hingment of *Limnocytherina sanctipatricii* (A; rv B; lv).
B. hingment of *Limnocythere stationis* (A; rv B; lv).
C. transverse section of terminal element (*Limnocythere stationis*).
D. transverse section of median element (*Limnocythere stationis*).
Scale bars are 50 μ m (A-D); 2.5 μ m (E); 1.4 μ m (F).

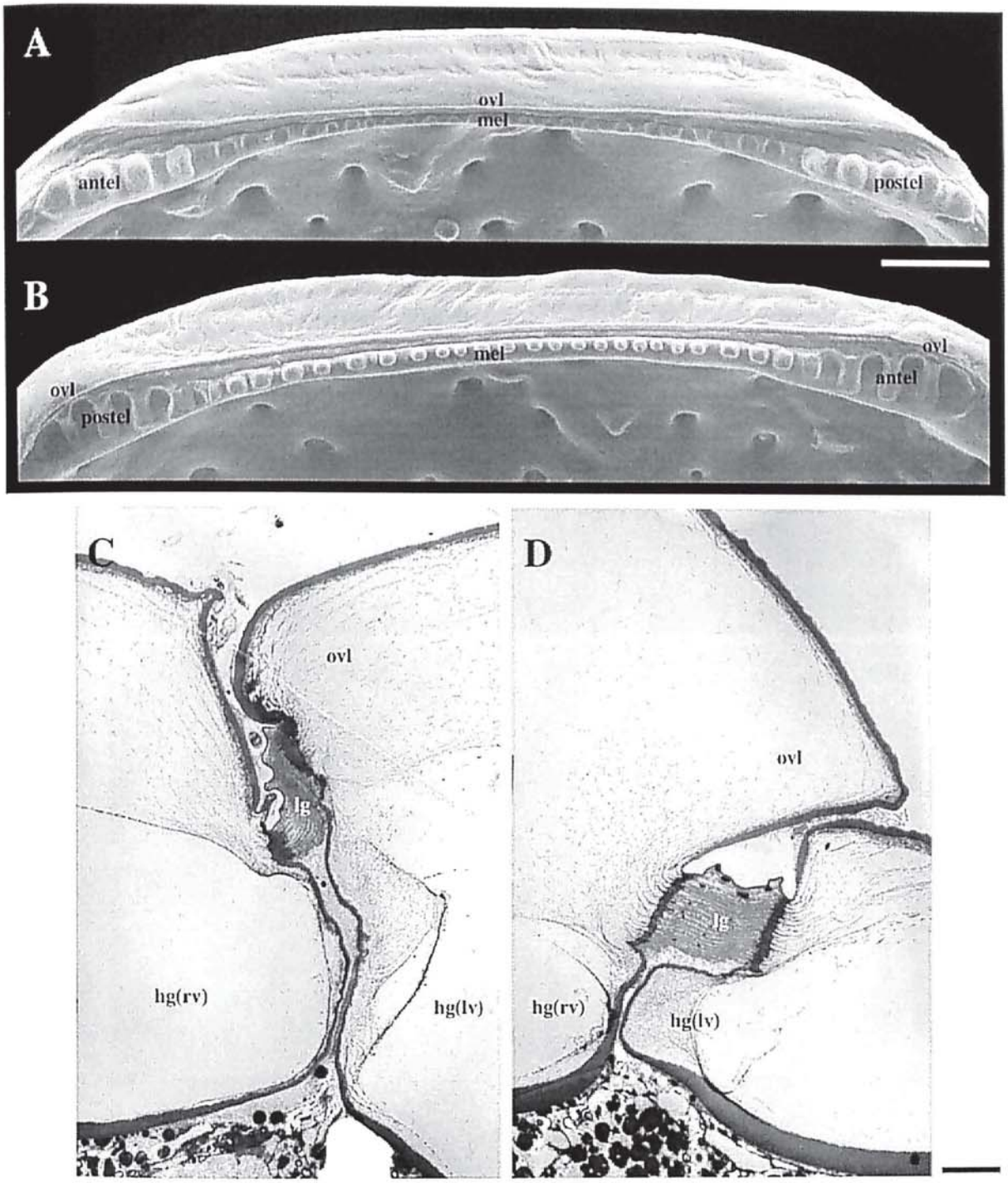

Figure 41 Hinge structure of cytherid ostracods.
A. hingement of right valve (*Cythere omotenipponica*).
B. hingment of left valve (*Cythere omotenipponica*).
C. transverse section of terminal element (*Cythere omotenipponica*).
D. transverse section of median element (*Cythere omotenipponica*).
Scale bars are 50 μm (A, B); 4 μm (C); 2.9 μm (D).

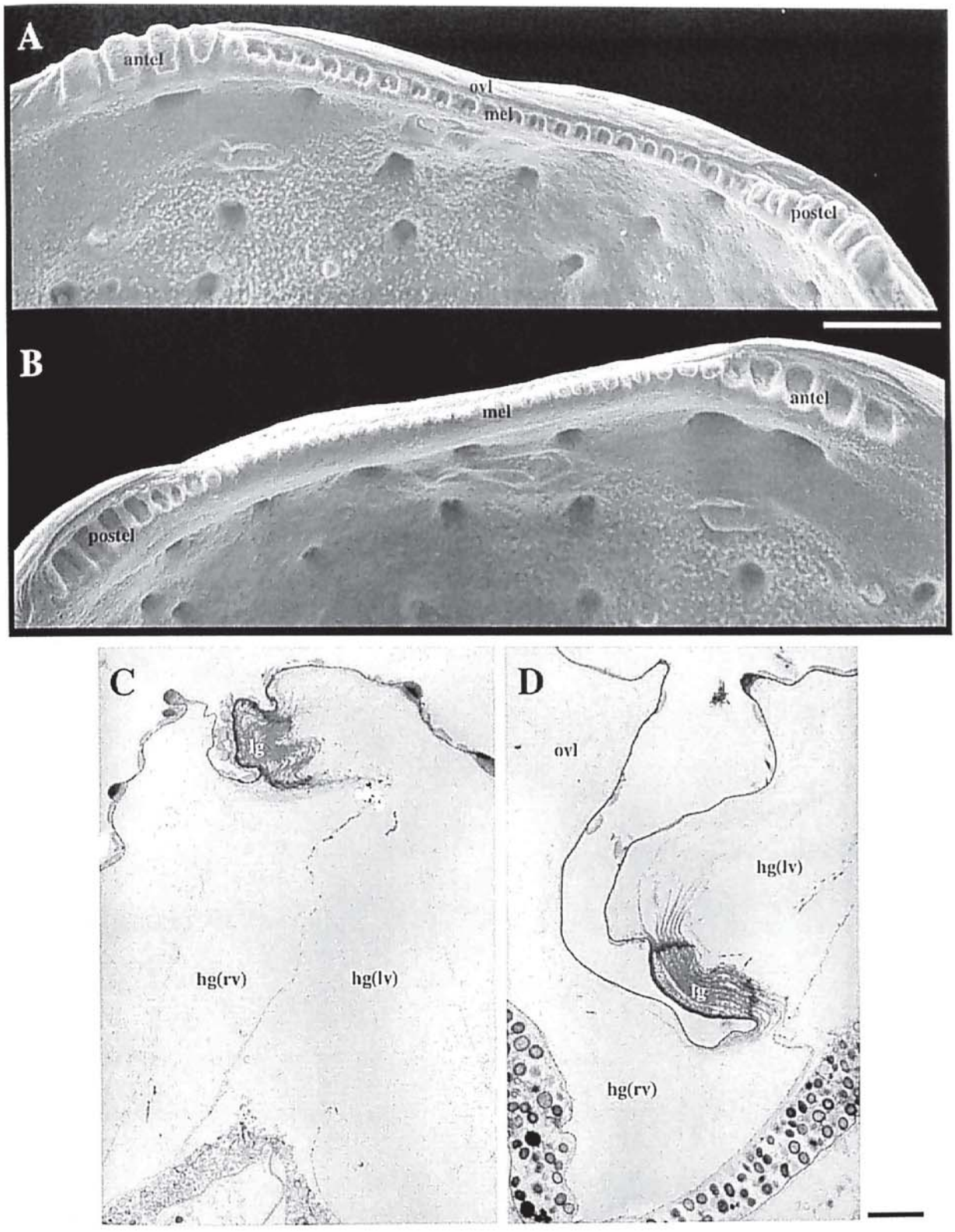

Figure 42 Hinge structure of cytherideid ostracods.
A. hingement of right valve (*Perissocytheridea inabai*).
B. hingment of left valve (*Perissocytheridea inabai*).
C. transverse section of terminal element (*Perissocytheridea japonica*).
D. transverse section of median element (*Perissocytheridea japonica*).
Scale bars are 50 μm (A, B); 2.9 μm (C); 1.3 μm (D).

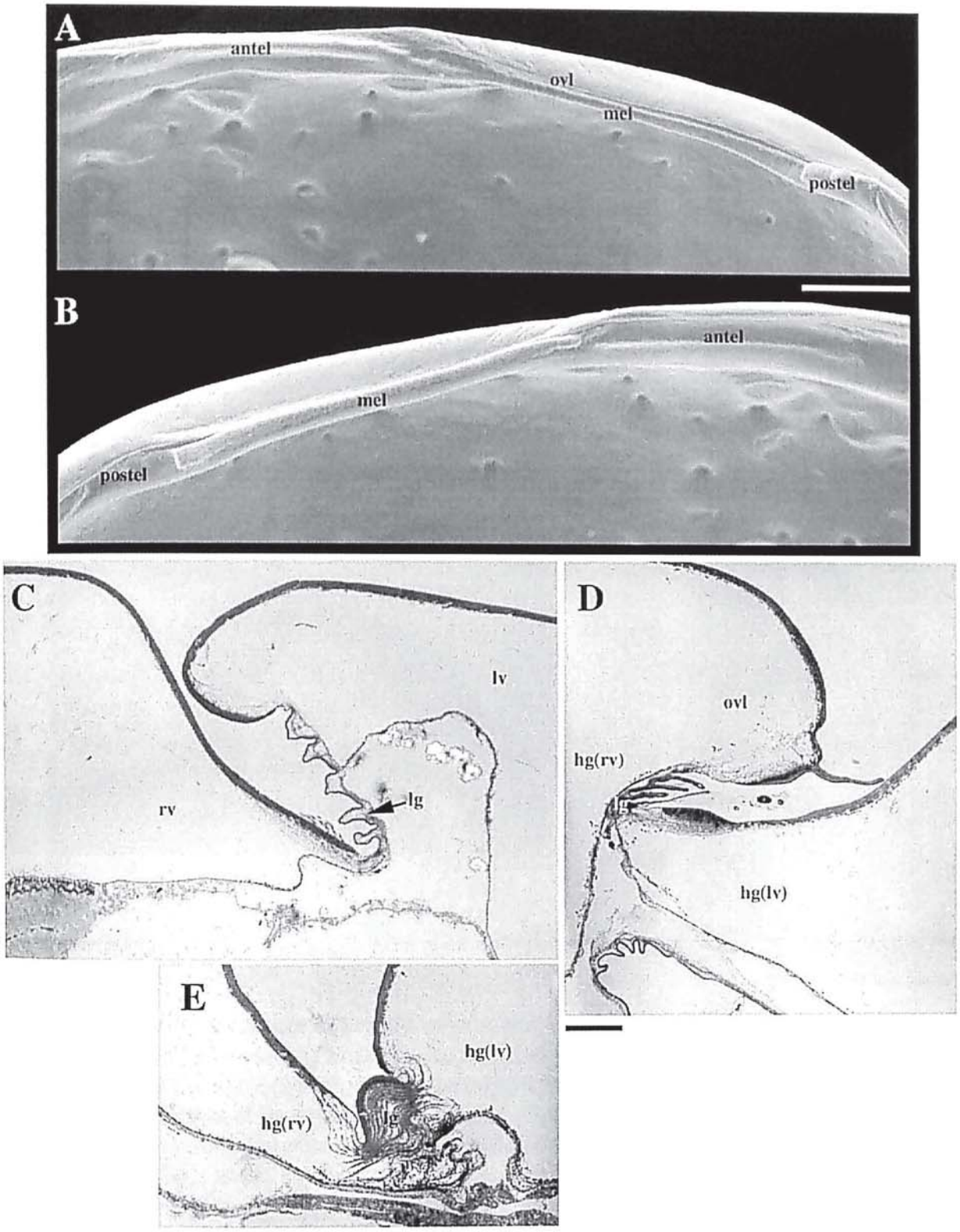

Figure 43 Hinge structure of cushmanideid ostracods.
A. hingement of right valve (*Pontocythere miurensis*).
B. hingment of left valve (*Pontocythere miurensis*).
C. transverse section of anterior element (*Pontocythere japonica*).
D. transverse section of median element (*Pontocythere japonica*).
E. transverse section of posterior element (*Pontocythere japonica*)
Scale bars are 50 μm (A, B); 3.3 μm (C); 2.9 μm (D); 2 μm (E).

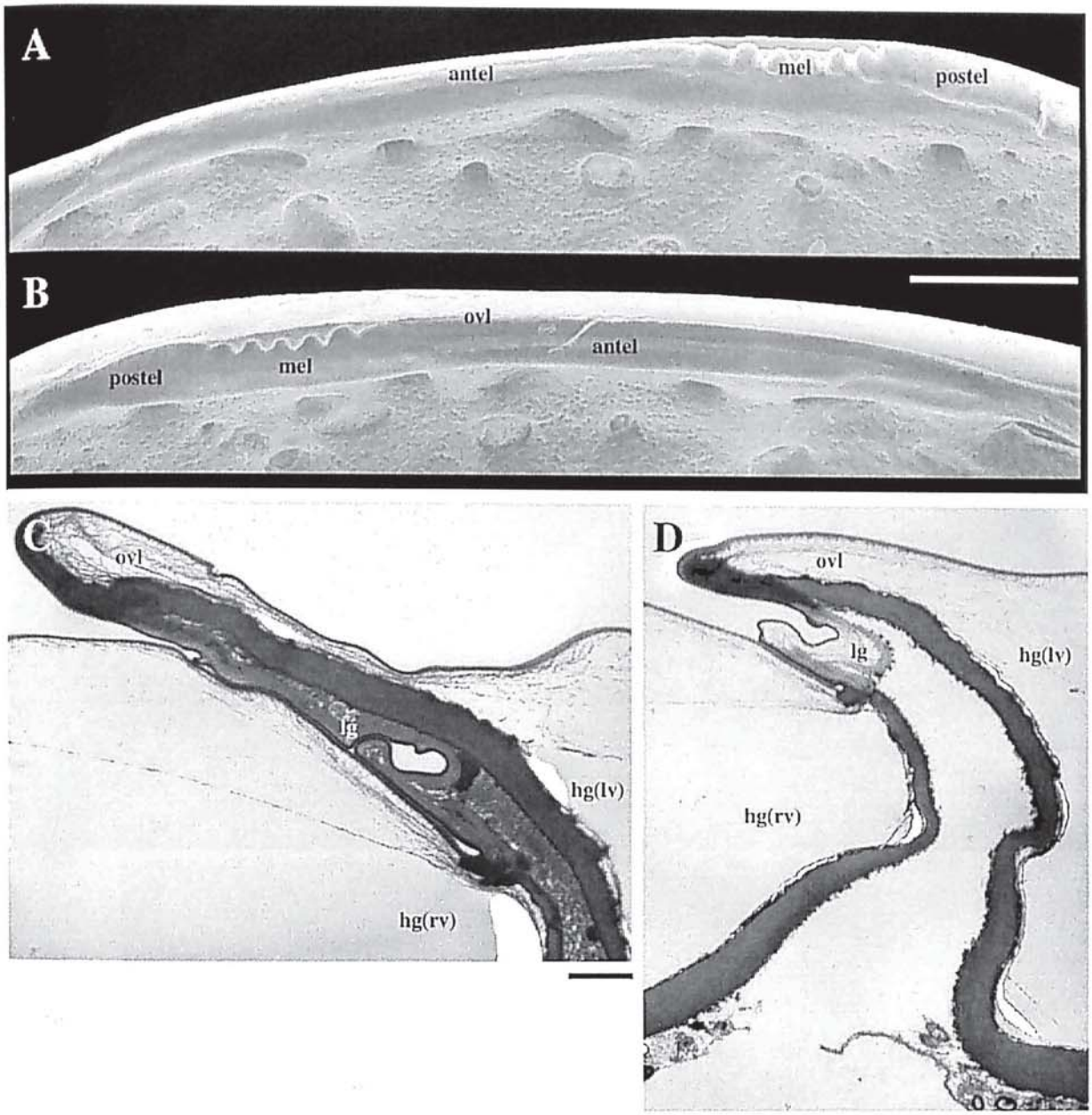

Figure 44 Hinge structure of krithid ostracods.
A. hingement of right valve (*Parakrithella pseudadonta*).
B. hingment of left valve (*Parakrithella pseudadonta*).
C. transverse section of anterior element (*Parakrithella pseudadonta*).
D. transverse section of median element (*Parakrithella pseudadonta*).
Scale bars are 50 μm (A, B); 1.4 μm (C); 1.7 μm (D).

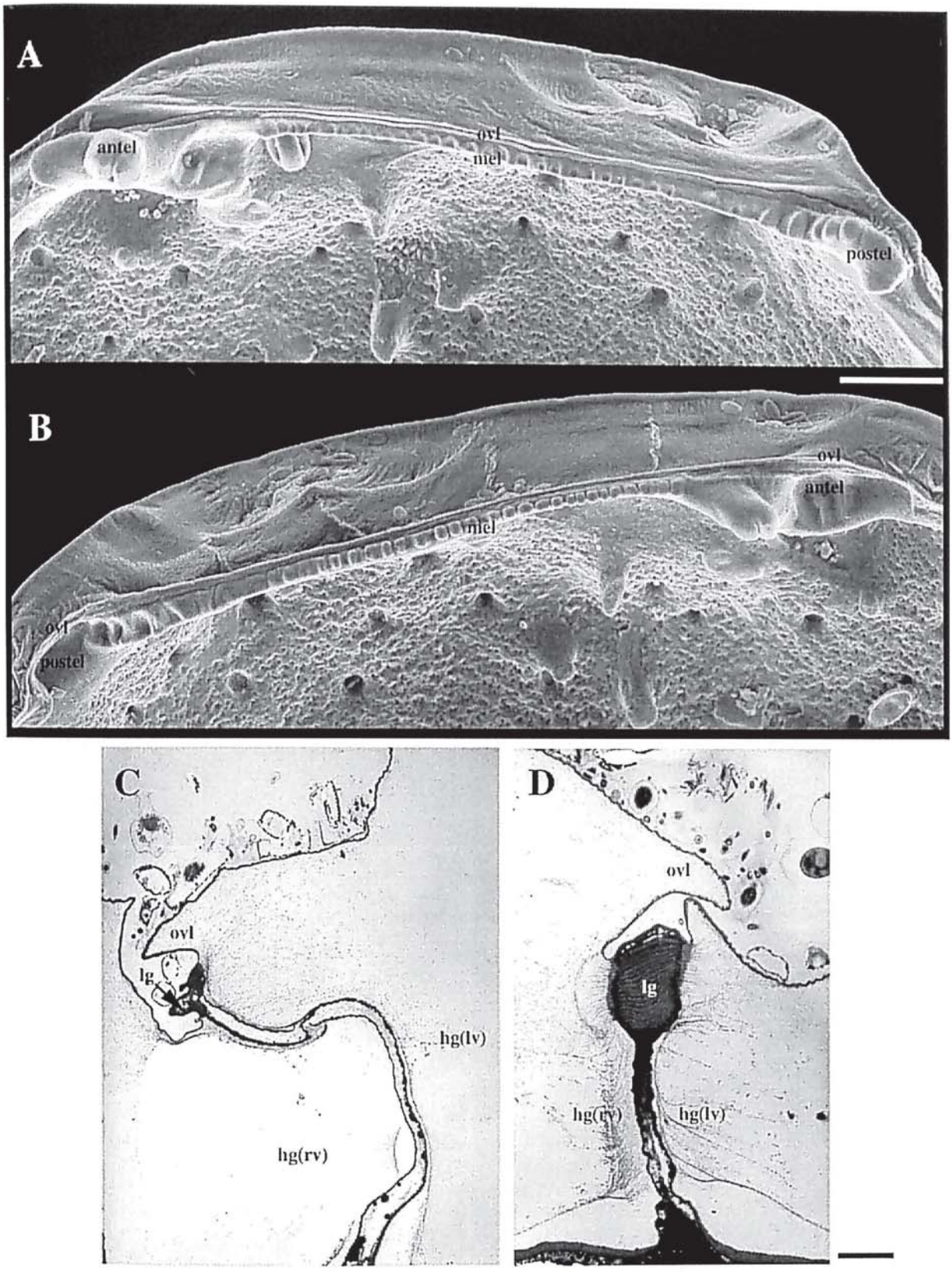

Figure 45 Hinge structure of schizocytherid ostracods.
A. hingement of right valve (*Schizocythere kishinouyei*).
B. hingment of left valve (*Schizocythere kishinouyei*).
C. transverse section of anterior element (*Schizocythere kishinouyei*).
D. transverse section of median element (*Schizocythere kishinouyei*).
Scale bars are 50 μm (A, B); 5.6 μm (C); 2.7 μm (D).

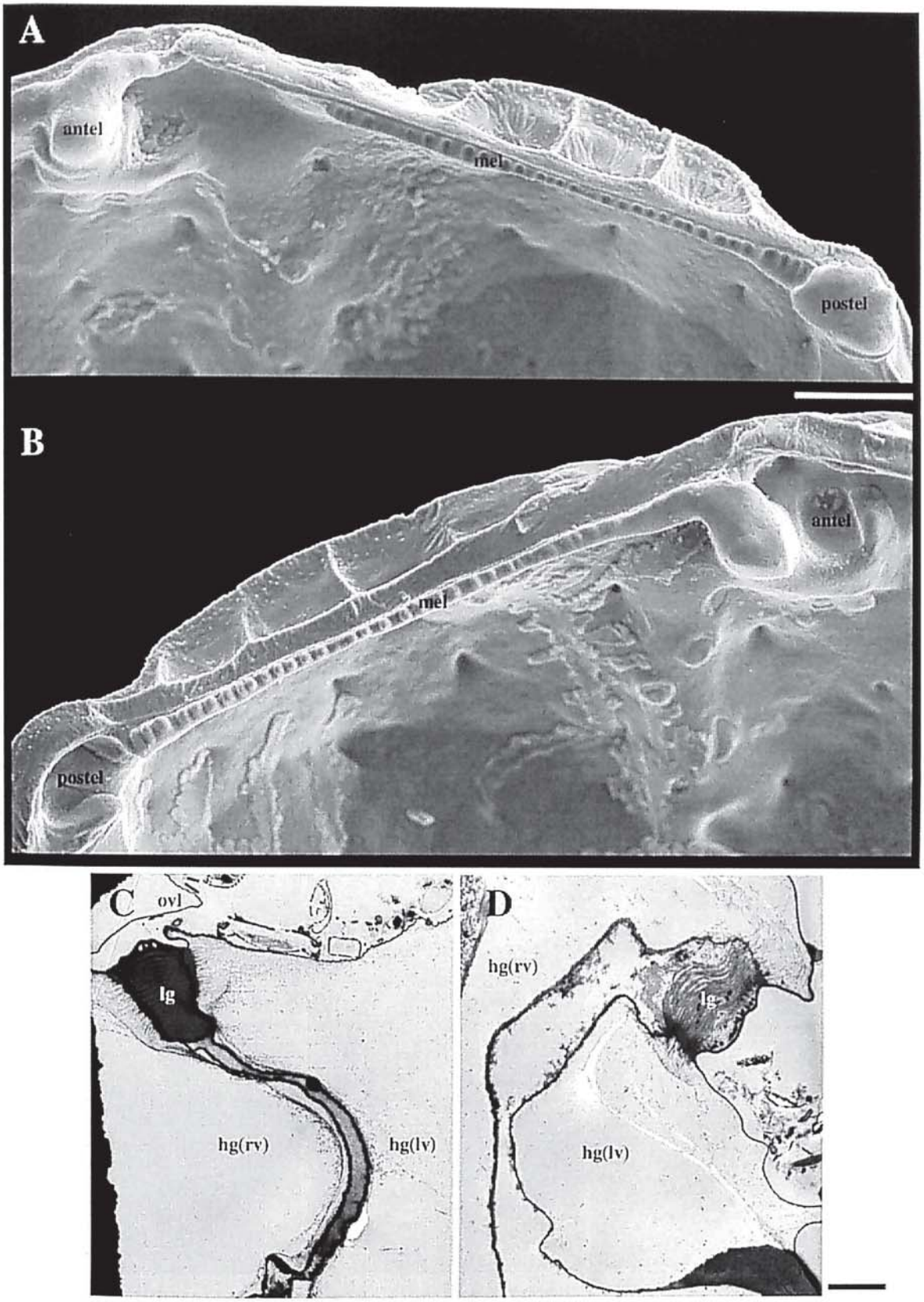

Figure 46 Hinge structure of schizocytherid ostracods.
A. hingement of right valve (*Spinileberis quadriaculeata*).
B. hingment of left valve (*Spinileberis quadriaculeata*).
C. transverse section of posterior element (*Schizocythere kishinouyei*).
D. transverse section of anteromedian tooth (*Spinileberis quadriaculeata*).
Scale bars are 50 μm (A, B); 4.5 μm (C); 1.8 μm (D).

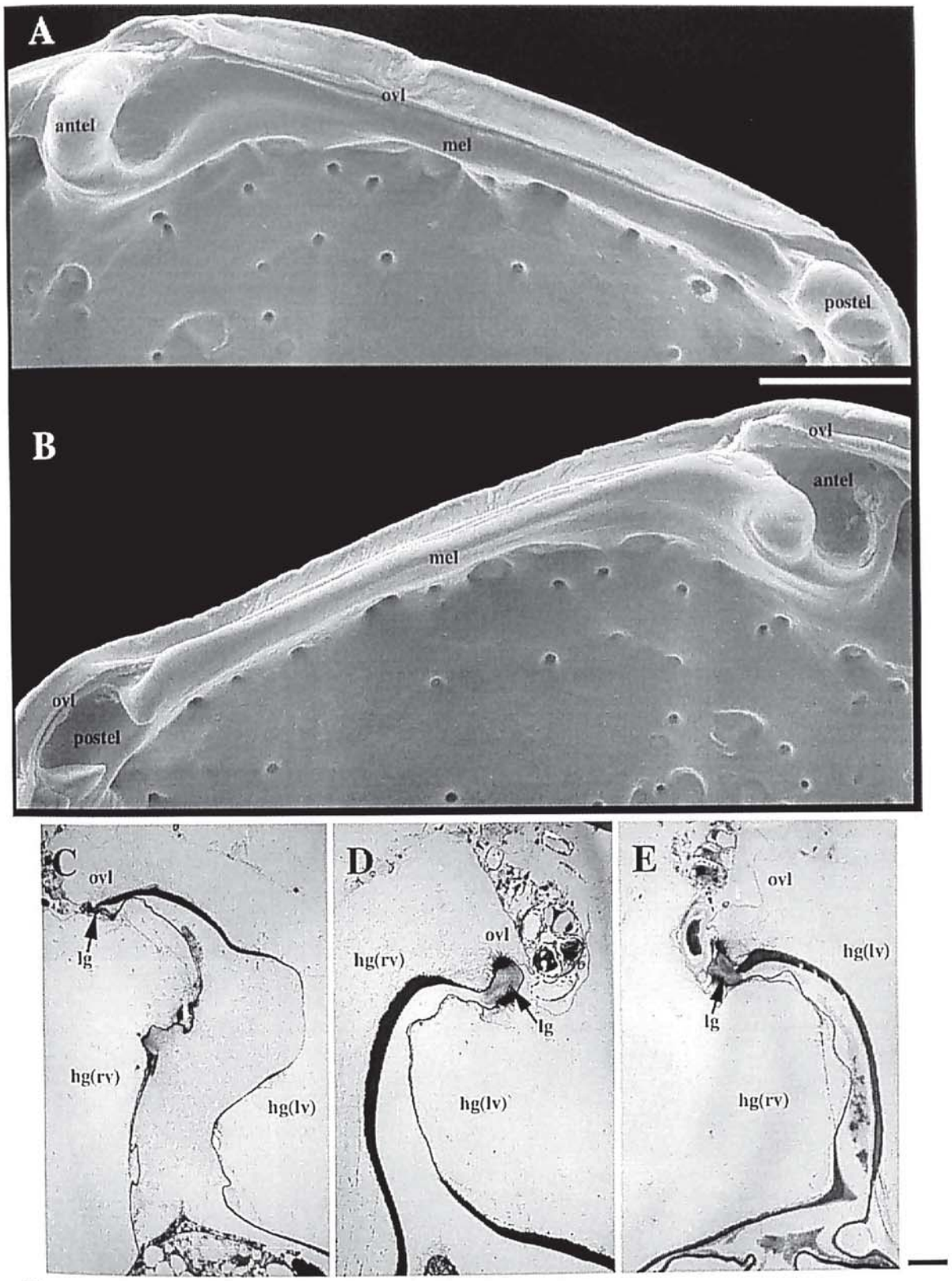

Figure 47 Hinge structure of hemicytherid ostracods.
A. hingement of right valve (*Caudites asiaticus*).
B. hingment of left valve (*Caudites asiaticus*).
C. transverse section of postterior element (*Caudites asiaticus*).
D. transverse section of anteromedian tooth (*Caudites asiaticus*).
E. transverse section of posterior element (*Caudites asiaticus*).
Scale bars are 50 μm (A, B); 5.6 μm (C); 3.3 μm (D); 4 μm (E).

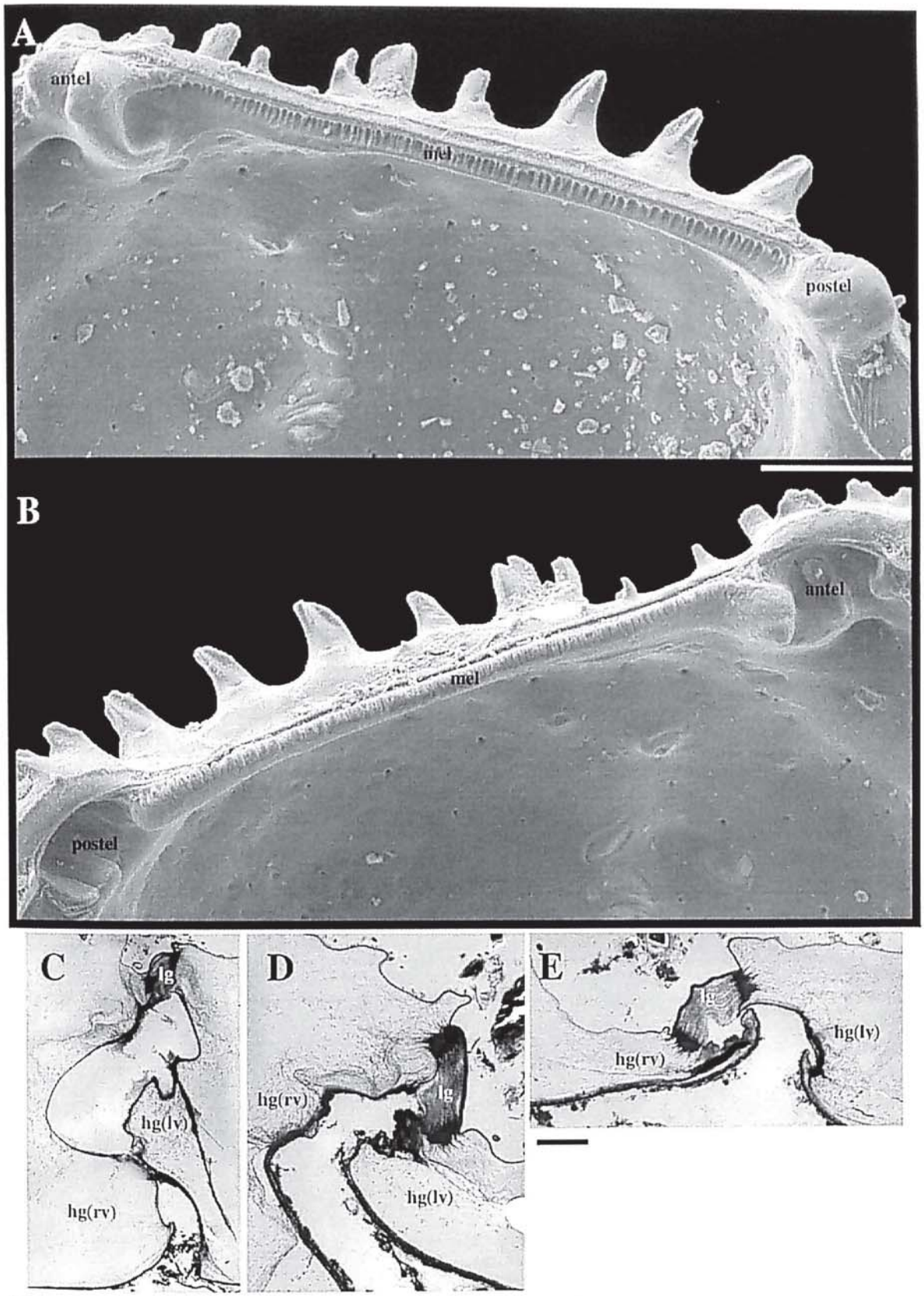

Figure 48 Hinge structure of trachyleberidid ostracods.
A, B. hingement of *Trachyleberis scabrocuneata* (A; rv B; lv).
C. transverse section of anterior element (*Trachyleberis scabrocuneata*).
D. transverse section of median element (*Trachyleberis scabrocuneata*).
E. transverse section of posterior element (*Trachyleberis scabrocuneata*)
Scale bars are 100 μm (A, B); 4 μm (C); 1.6 μm (D); 2 μm (E).

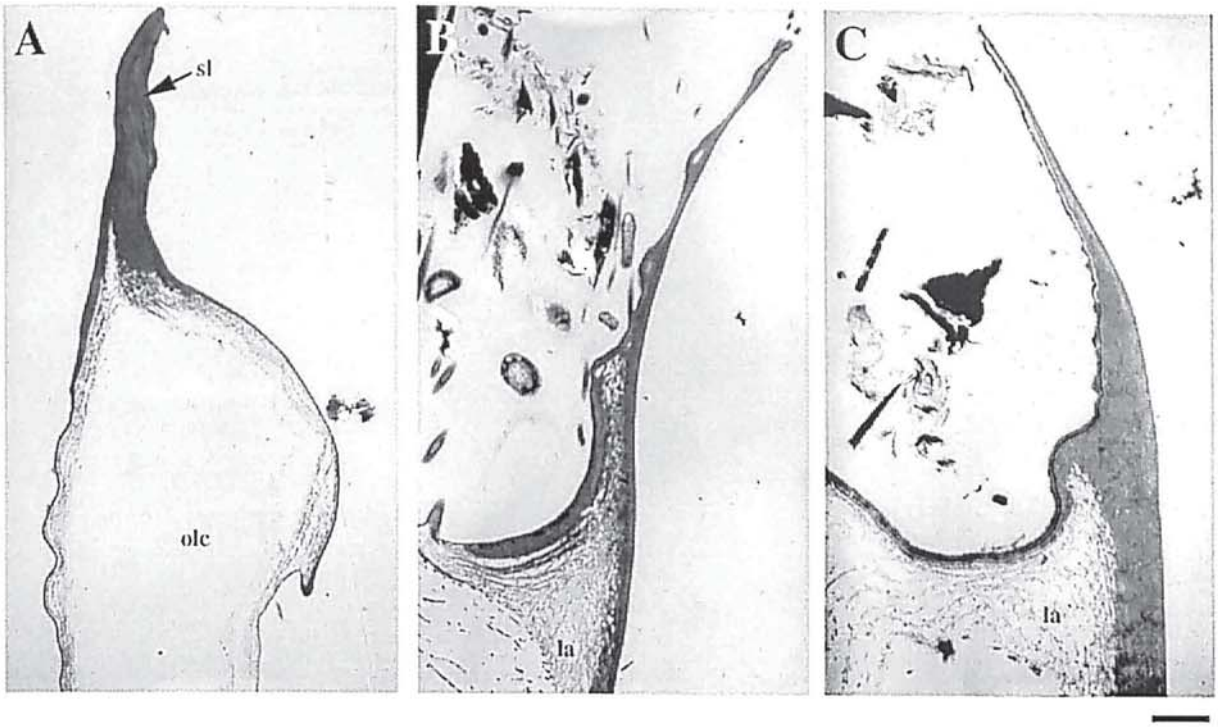

Figure 49 TEM photographs of the ostracod selvage.
A. *Neonesidea oligodentata.* B. *Aurila hataii.* C. *Callistocythere setouchiensis.*
Scale bar is 5 μm (A); 1.3 μm (B); 700nm (C).

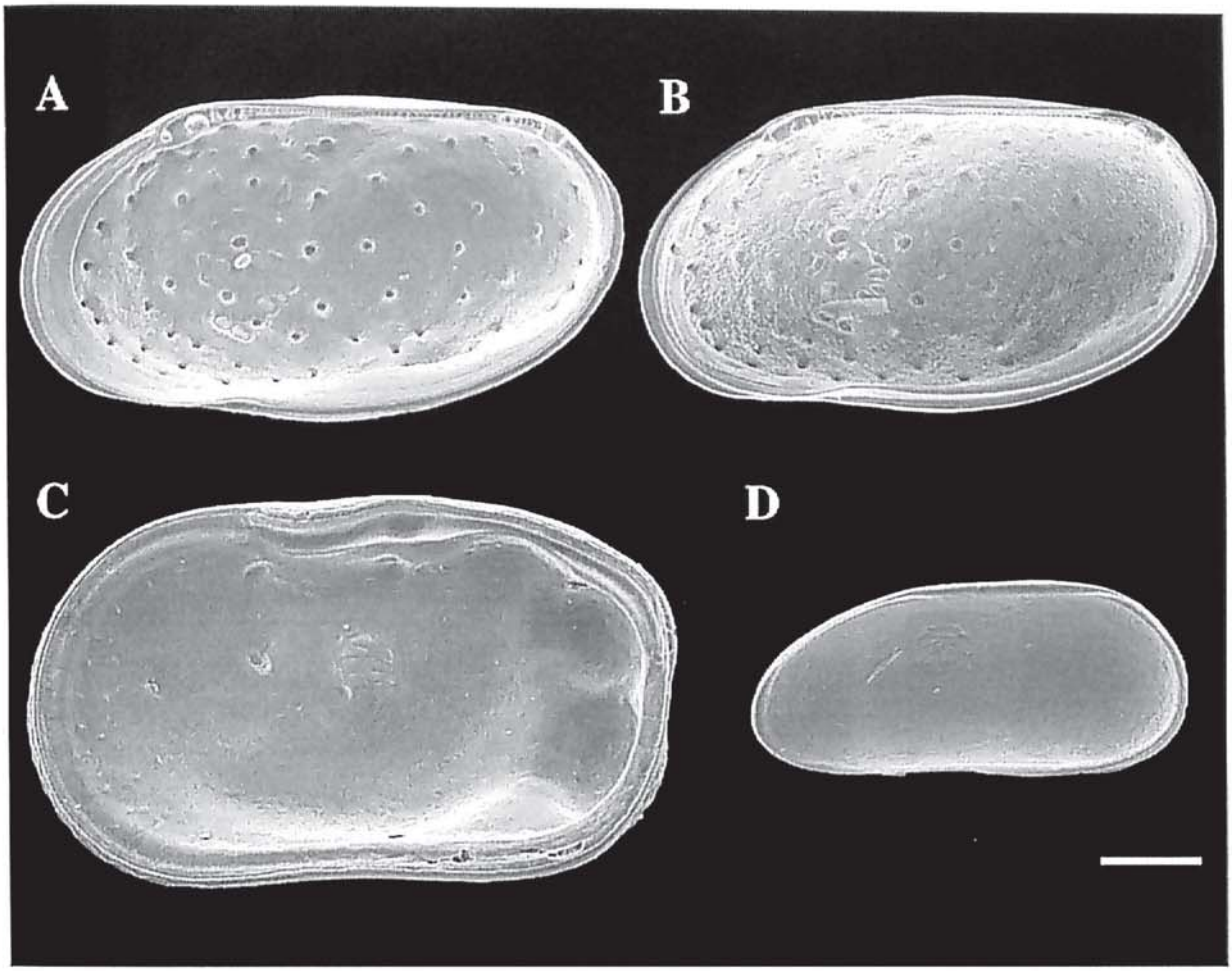

Figure 50 SEM photographs of the carapace in internal lateral view.
A. *Loxoconcha pulchra.* B. 8th instar of *Loxoconcha pulchra.*
C. *Keijcyoidea inflalittolaris.* D. *Vestalenula* sp.
Scale bar is 100 μ m.

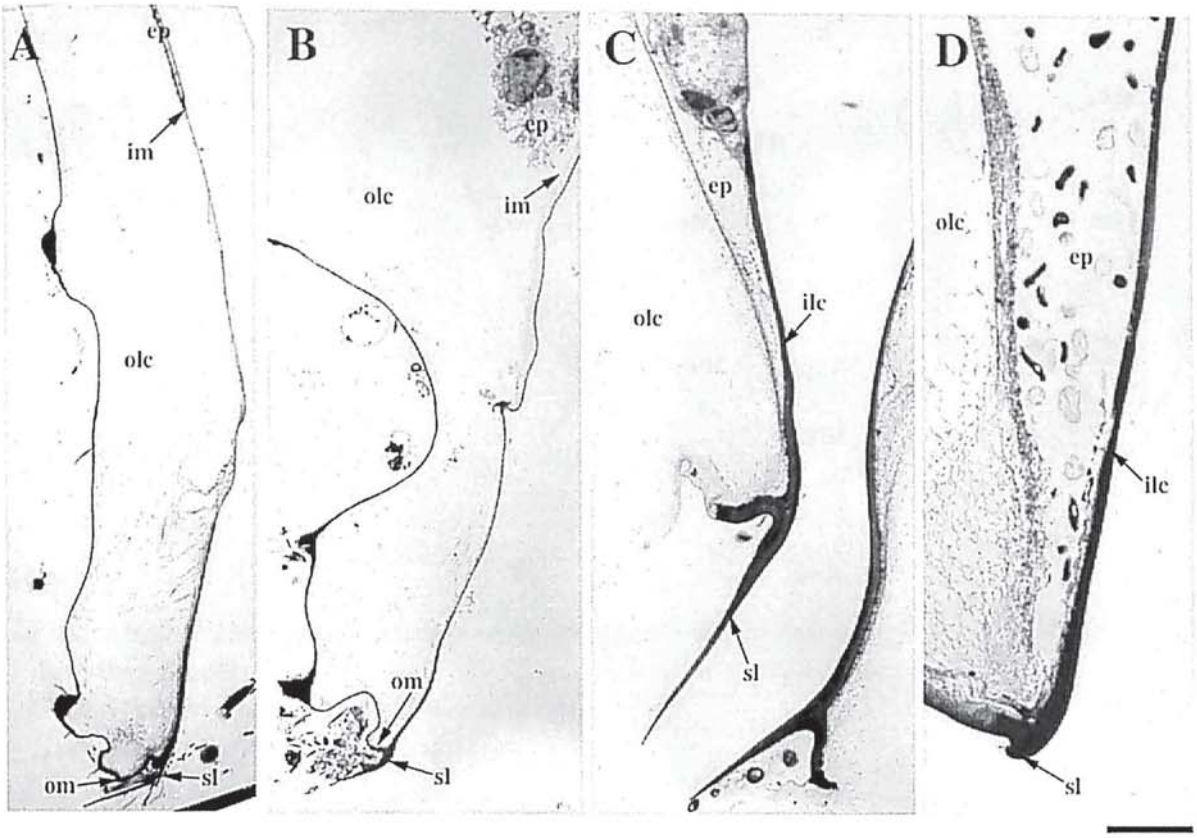

Figure 51 TEM photographs of the duplicature.
A. *Loxoconcha pulchra.* B. 8th instar of *Loxoconcha pulchra.*
C. *Keijcyoidea inflalittolaris.* D. *Vestalenula* sp.
Scale bar is 5 μm (A); 3.3 μm (B, C); 2 μm (D).

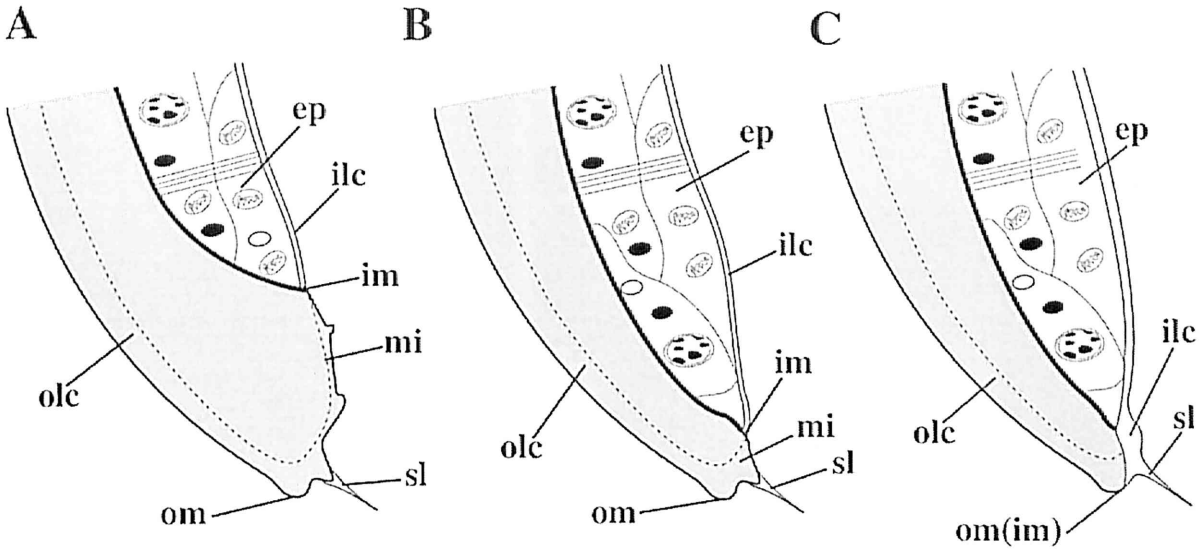

Figure 52 Schematic drawings of the structure of the free margin.
A. cytheroid ostracod (adult). B. cytheroid ostracod (8th instar).
C. platycopid and darwinuloid ostracods.
Grey areas represent the calcified parts.

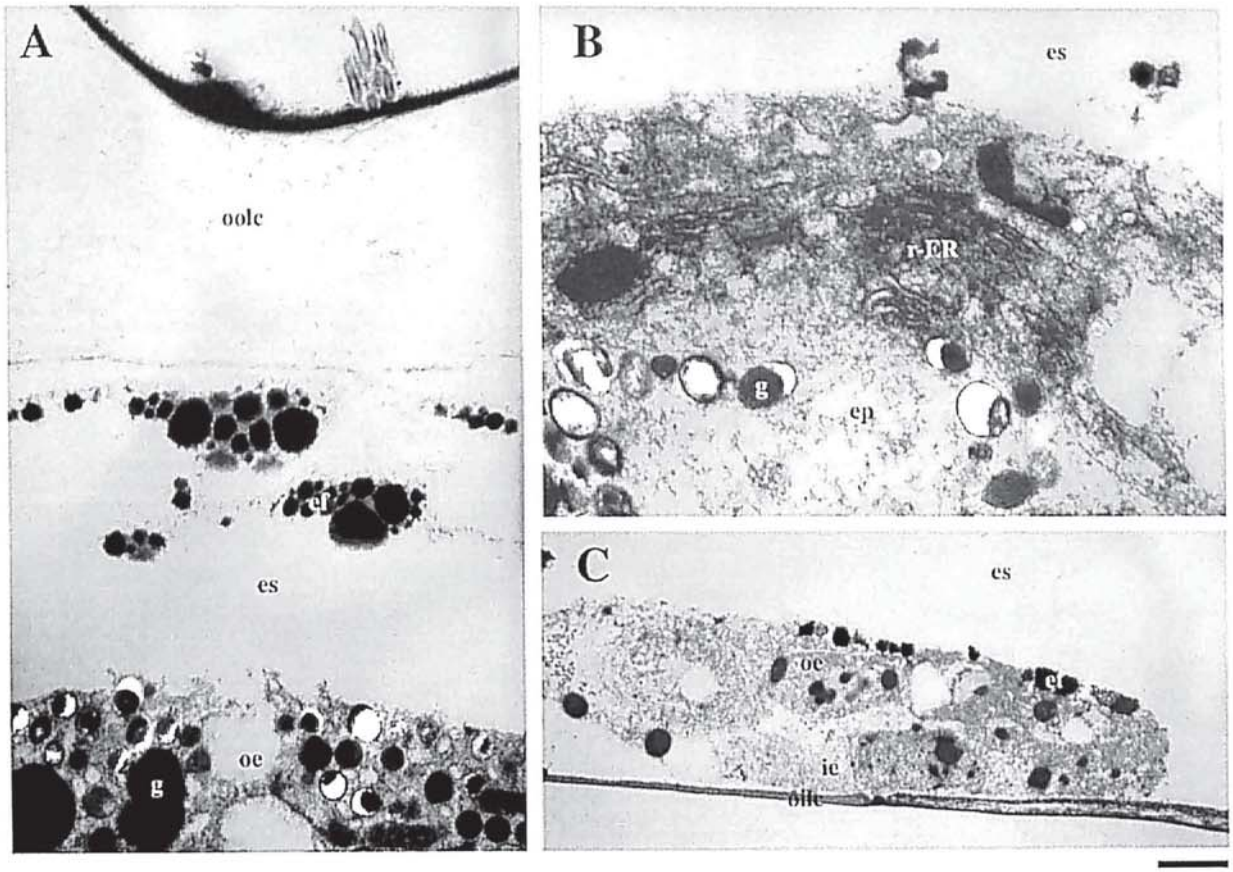

Figure 53 Carapace of *Loxoconcha pulchra* (8th instar) at the premolt stage D1. A. ecdysial fluid (apolysis occurs). B. epidermis beneath the attached margin. C. free margin. Scale bar is 1 μm (A); 500nm (B); 1.7 μm (C).

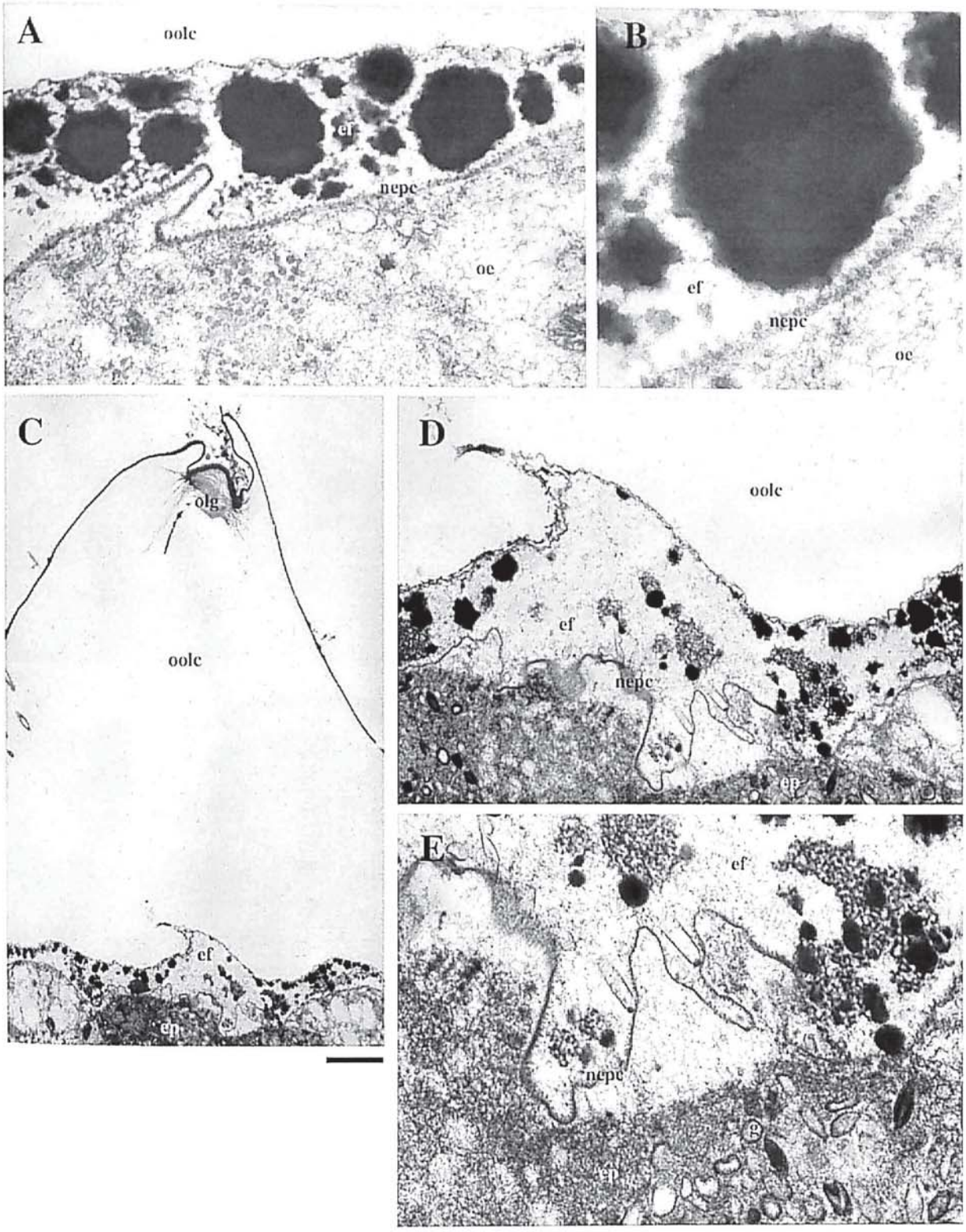

Figure 54 Carapace of *Loxoconcha pulchra* (8th instar) at the premolt stage D2. A. ecdysial fluid and epidermis. B. new epicuticle and materials in ecdysial fluid. C. attached margin. D. ligament area. E. new epicuticle at the ligamnet area. Scale bar is 500nm (A); 200nm (B); 3.3 μm (C); 1 μm (D); 500nm (E).

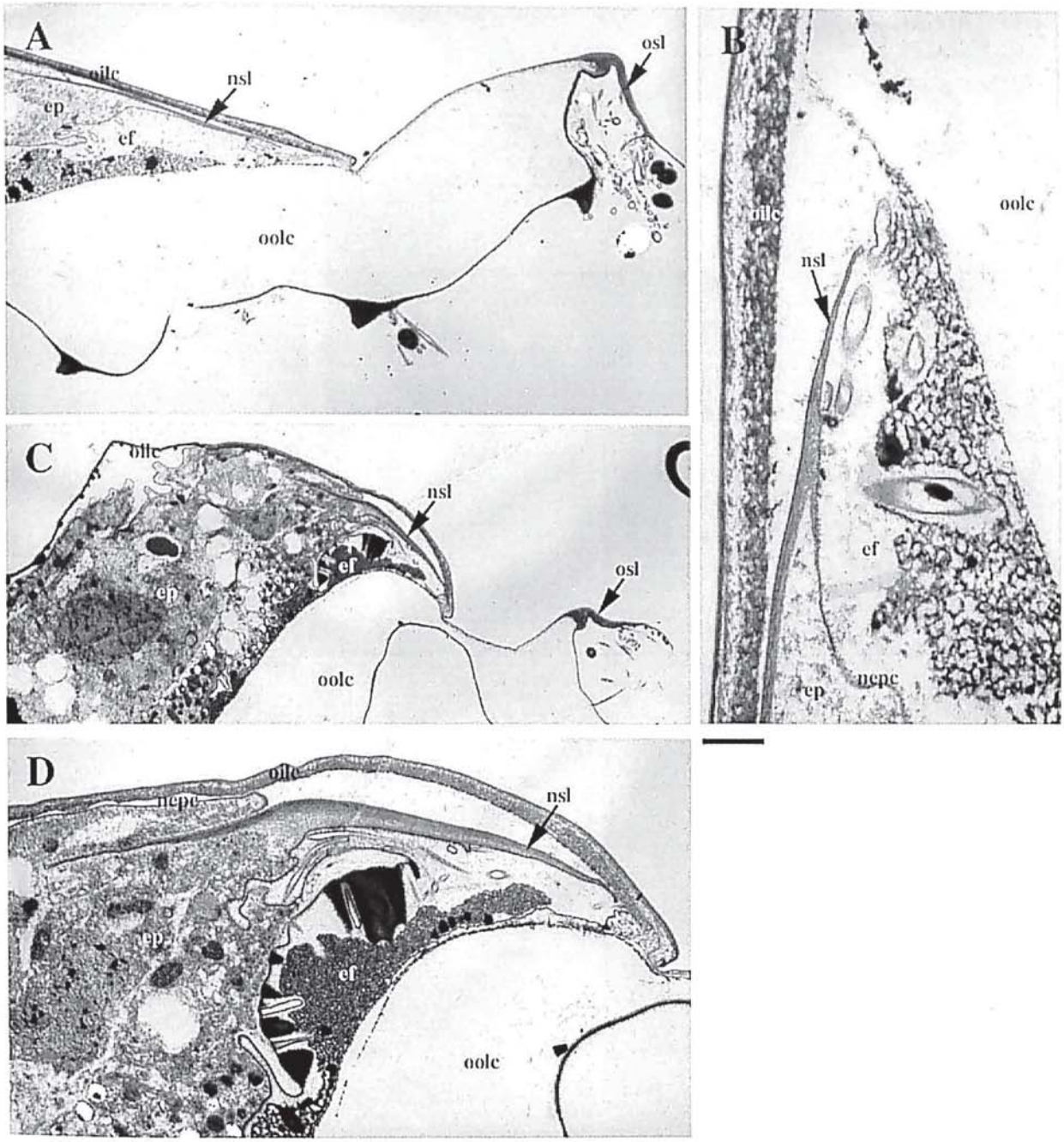

Figure 55 Selvage of *Loxoconcha pulchra* (8th instar) at the premolt stage D2 and D3.
A. free margin at the premolt stage D2. B. new selvage at the premolt stage D2.
C. free margin at the premolt stage D3. D. new selvage at the premolt stage D3.
Scale bar is 2.5 μ m (A); 500nm (B); 4 μ m (C); 1.3 μ m (D).

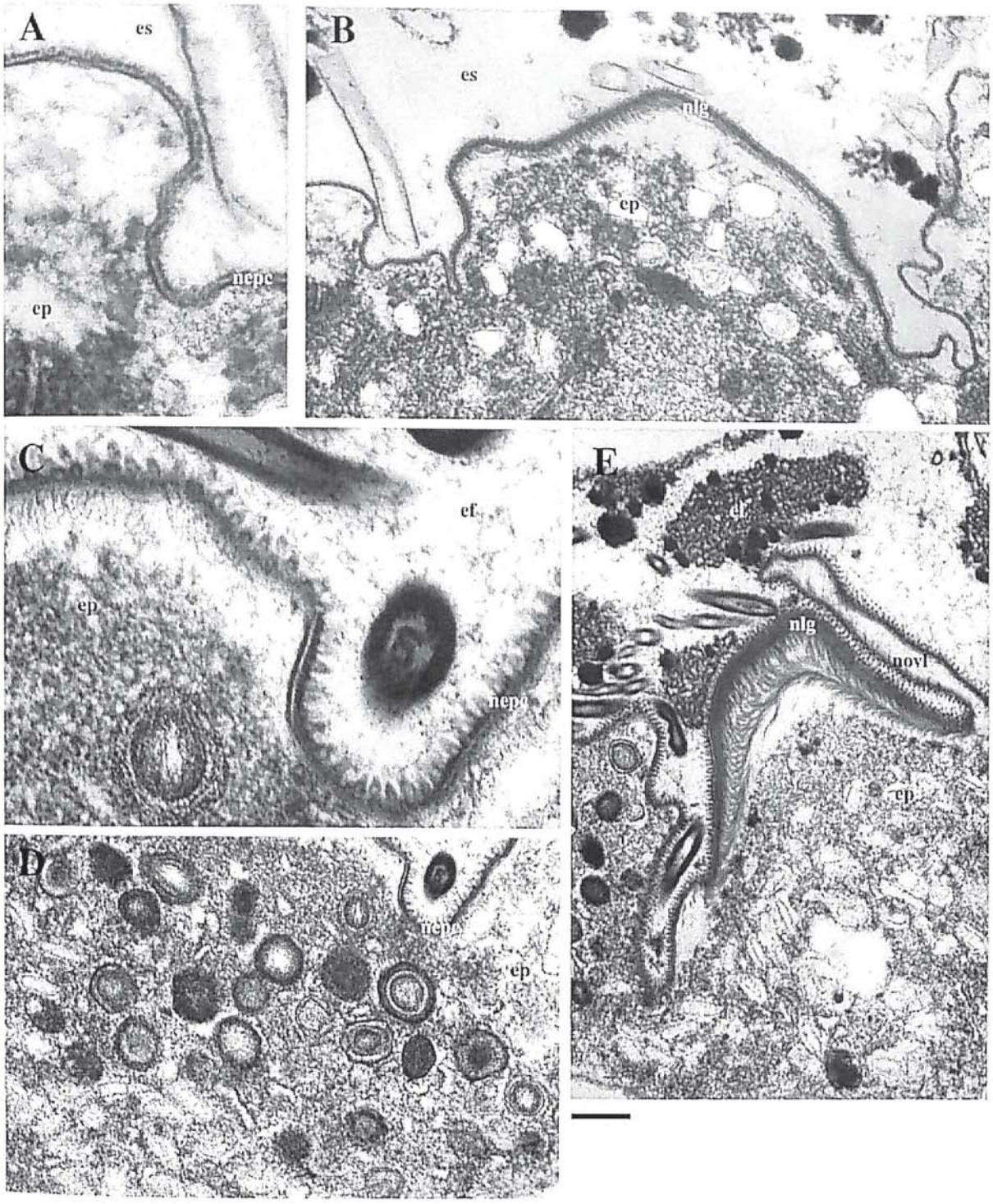

Figure 56 Carapace of *Loxoconcha pulchra* (8th instar) at the premolt stage D3.
A. new epicuticle at the early phase of stage D3.
B. new ligament at the early phase of stage D3.
C. new epicuticle at the middle phase of stage D3.
D. various granules in the epidermis.
E. new ligament at the middle phase of stage D3.
Scale bar is 10nm (A, C); 333nm (B); 400nm (D); 500nm (E).

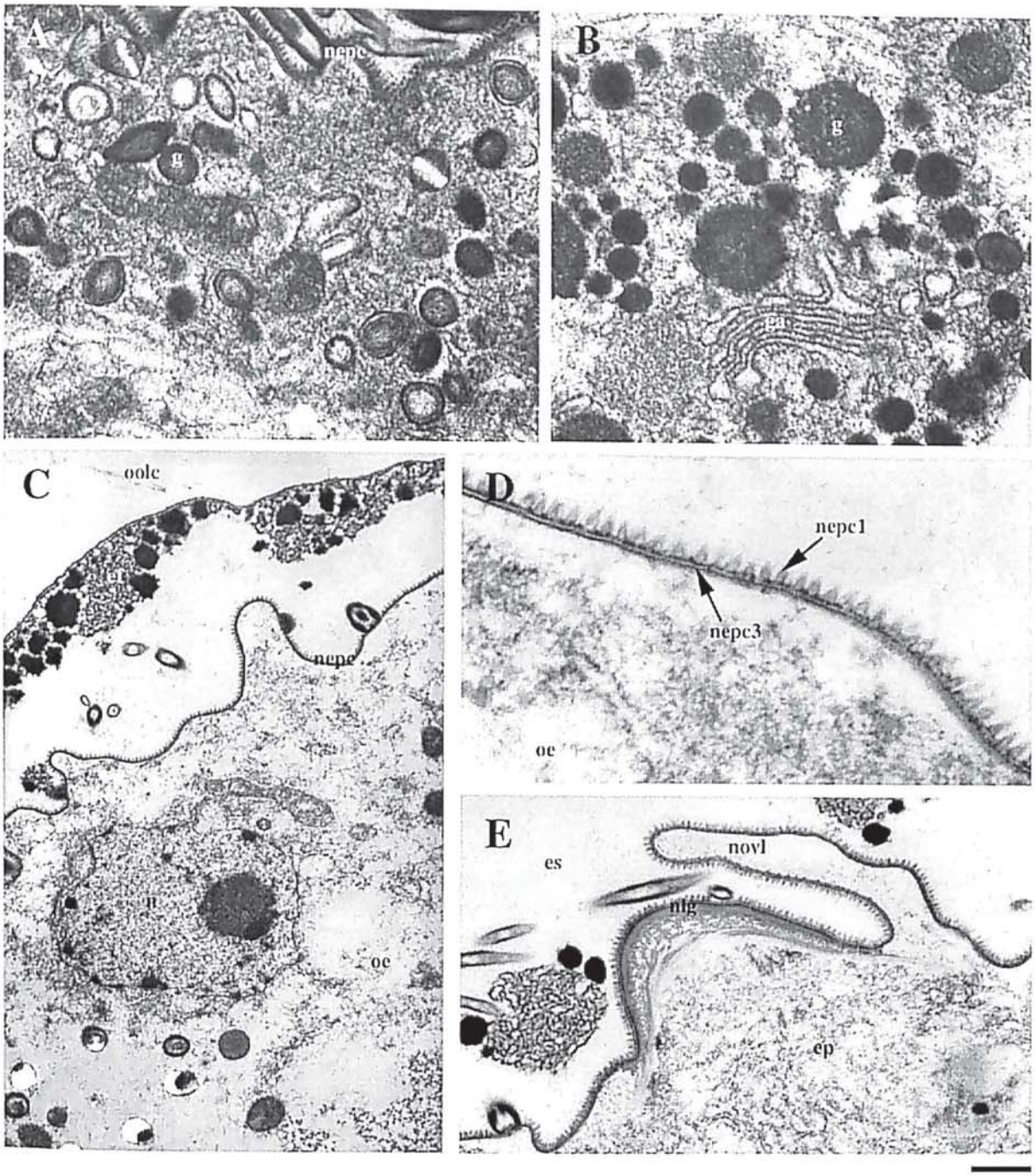

Figure 57 Carapace of *Loxoconcha pulchra* (8th instar) at the premolt stage D3.
A. various granules in epidermis at the middle phase of stage D3.
B. various granules in epidermis at the middle phase of stage D3.
C. epidermis at the last phase of stage D3.
D. new epicuticle at the last phase of stage D3.
E. new ligament at the last phase of stage D3.
Scale bar is 400nm (A, B); 833nm (C); 20nm (D); 500nm (E).

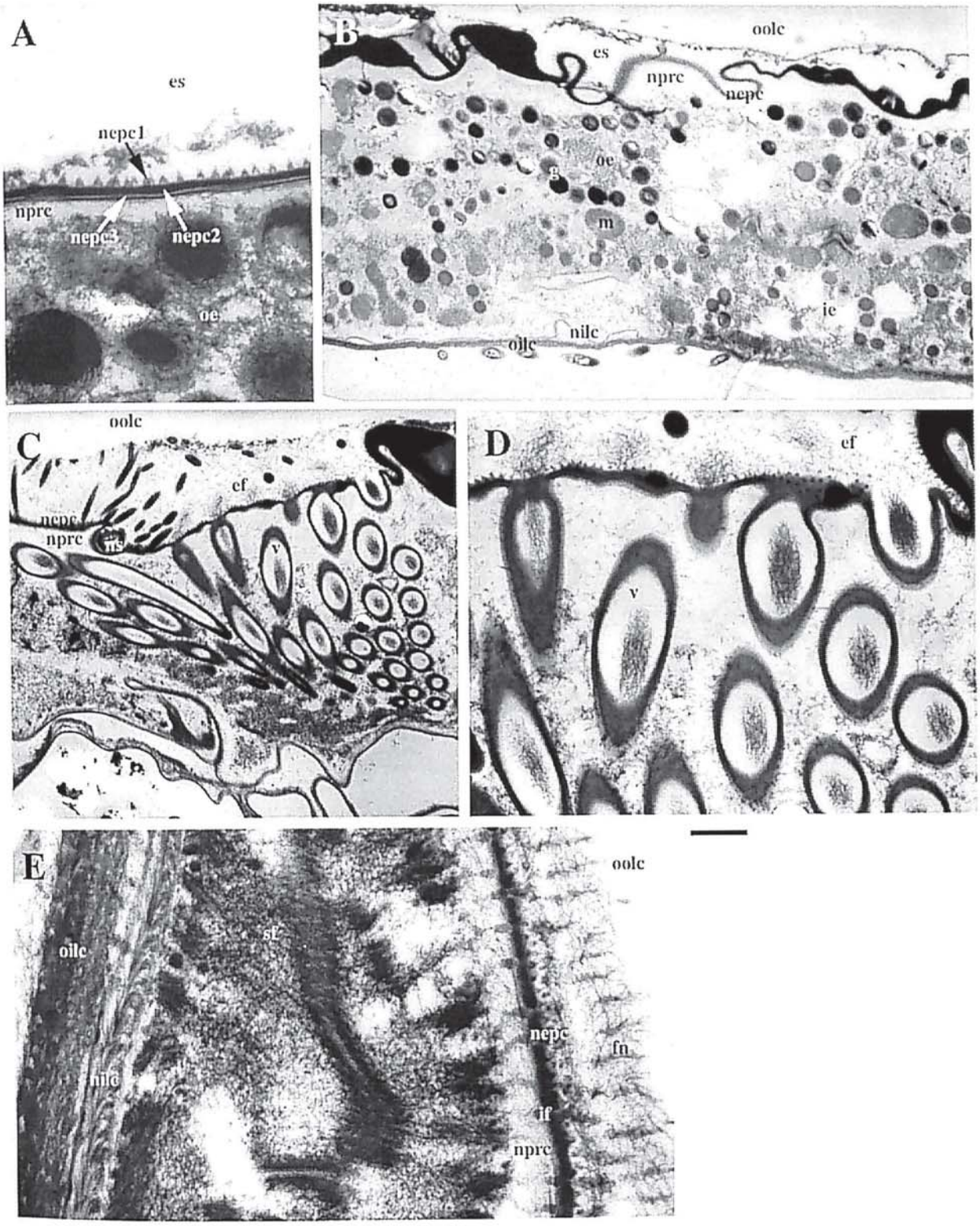

Figure 58 Carapace of *Loxoconcha pulchra* (8th instar) at the premolt stage D4.
A. new epicuticle. B. various granules in epidermis.
C. epidermis around the new sensillum. D. exocytosis.
E. supporting fibres at the stage D4.
Scale bar is 250nm (A); 1.3 μm (B, C); 500nm (D); 333nm (E).

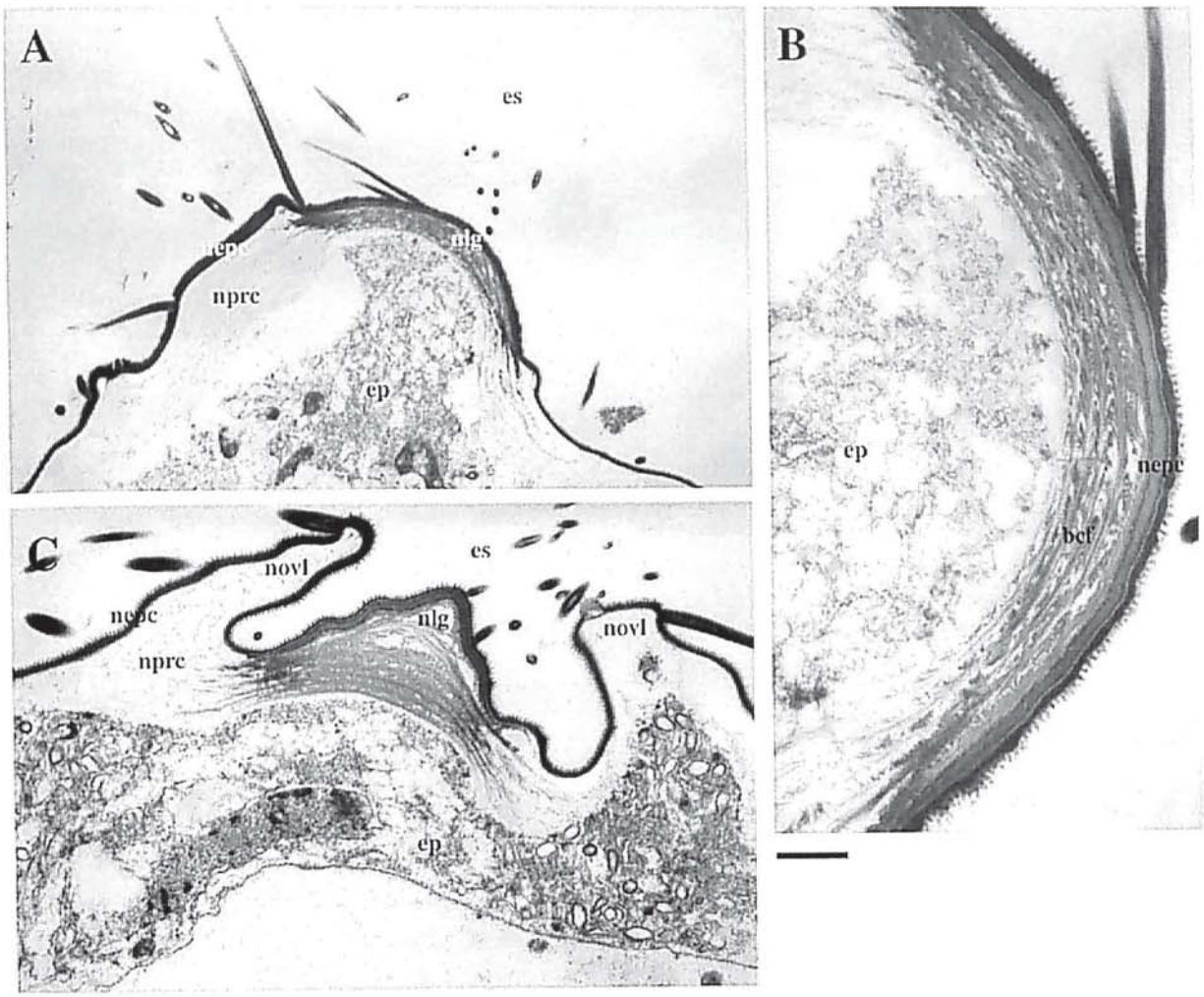

Figure 59 Carapace of *Loxoconcha pulchra* (8th instar) at the premolt stage D4.
A. new ligament of terminal element. B. bundle of chitinous fibres.
C. new ligament of median element. Scale bar is 1.7 μm (A); 500nm (B); 1 μm (C).

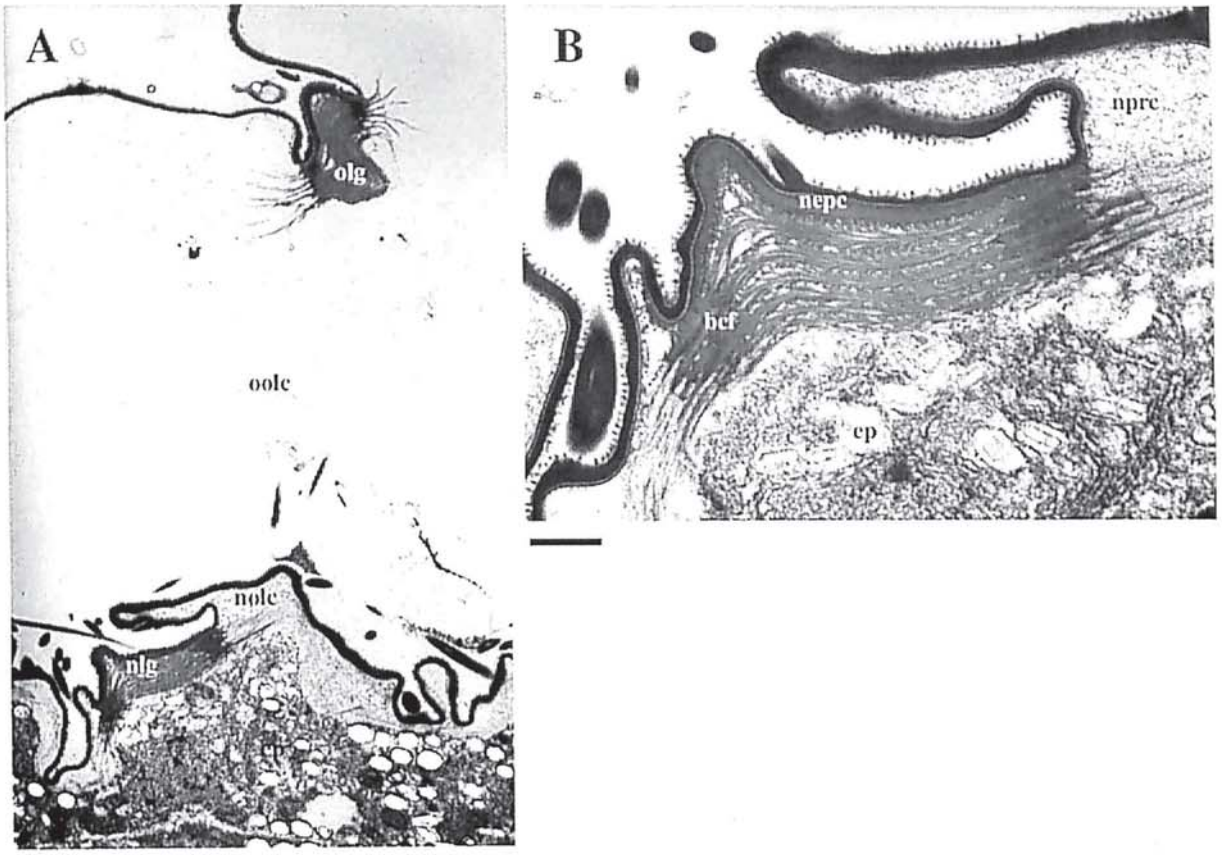

Figure 60 Carapace of *Loxoconcha pulchra* (8th instar) at the premolt stage D4. A. attached margin. B. new ligament. Scale bar is 1.7 μm (A); 500nm (B).

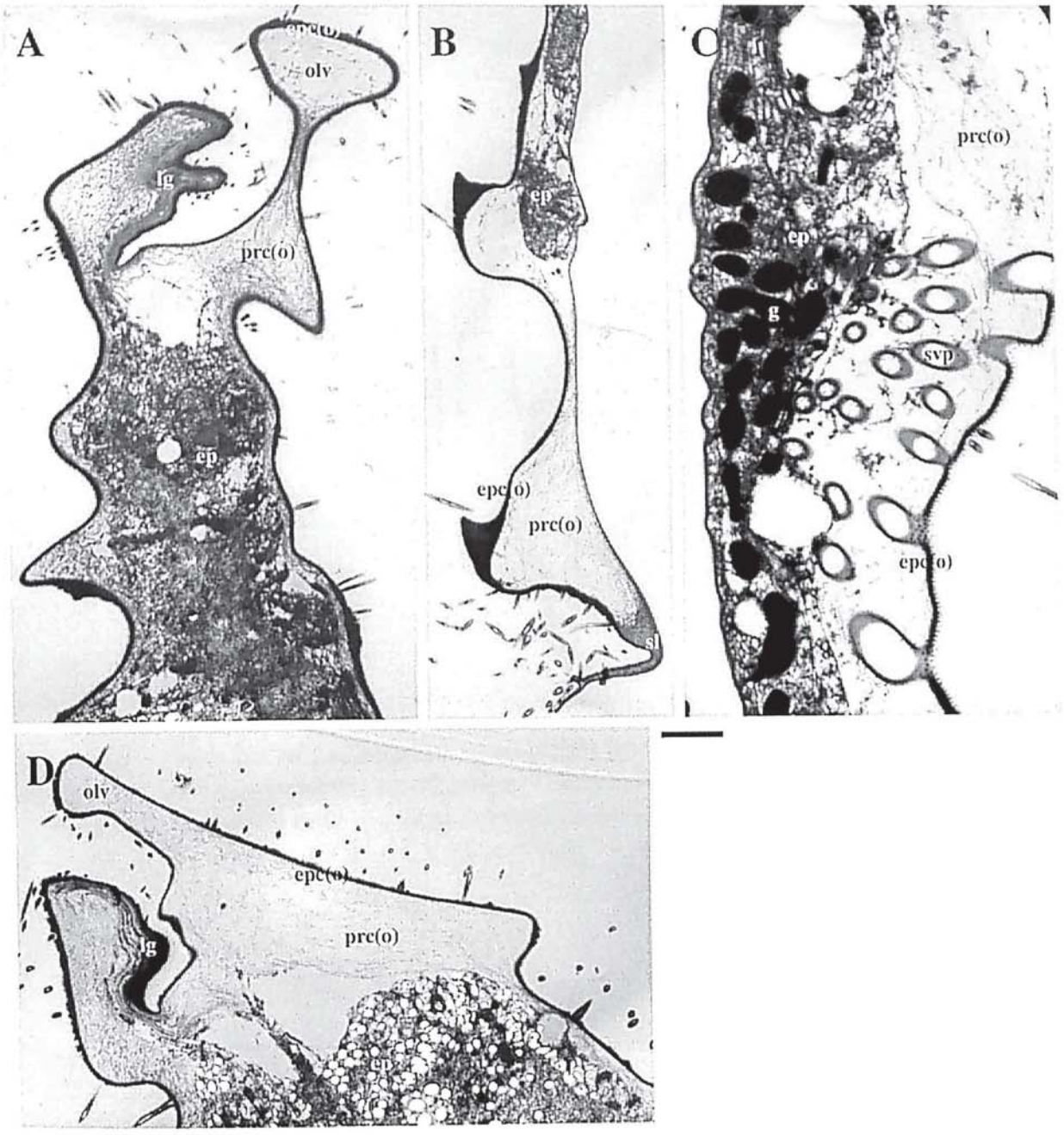

Figure 61 Carapace of *Loxoconcha pulchra* (8th instar) at the postmolt stage A.
A. attached margin at the early phase of stage A.
B. free margin at the early phase of stage A.
C. sieve pore system at the early phase of stage A.
D. attached margin when the calcification begins.
Scale bar is 2 μ m (A, B); 833nm (C); 2.5 μ m (D).

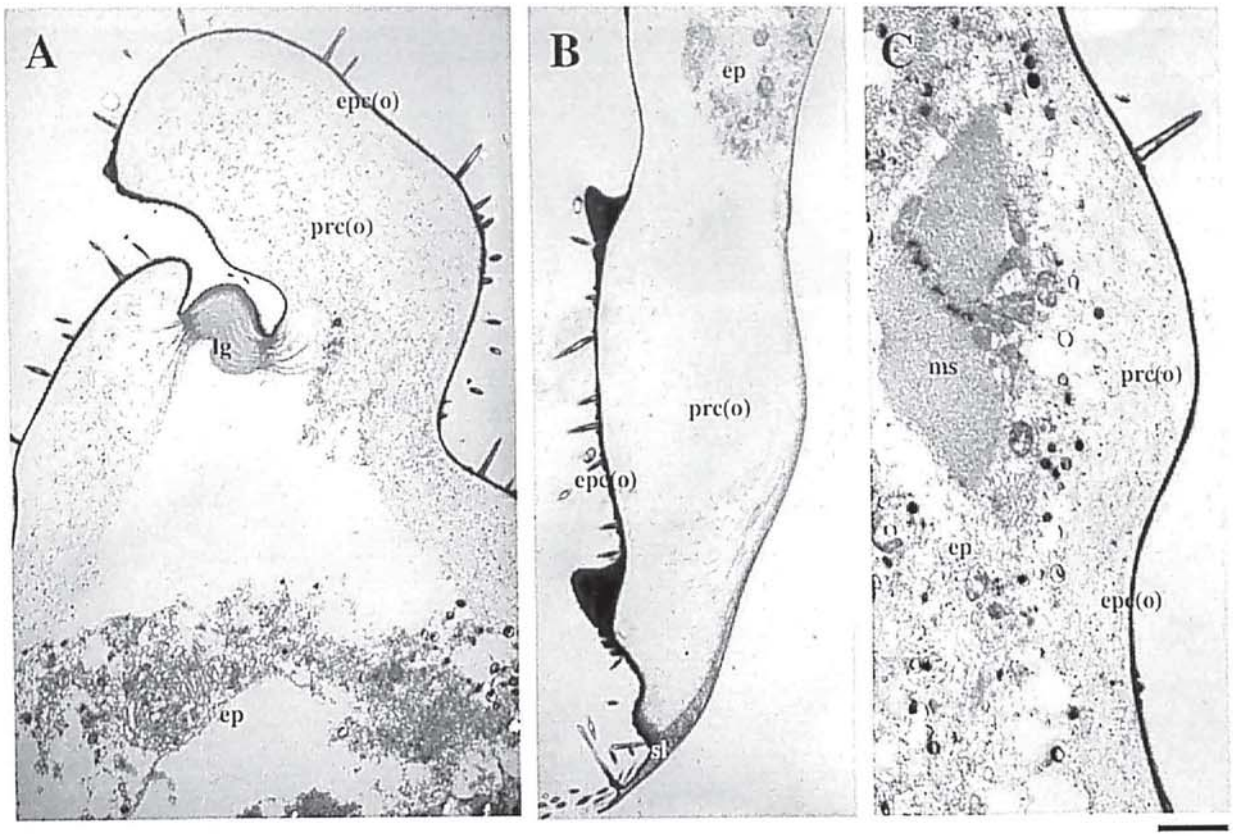

Figure 62 Carapace of *Loxoconcha pulchra* (8th instar) at the postmolt stage A.
A. attached margin during the calcification. B. free margin during the calcification.
C. outer lamella cuticle near the adductor muscle area.
Scale bar is 2.9 μm (A); 2.5 μm (B); 1.7 μm (C).

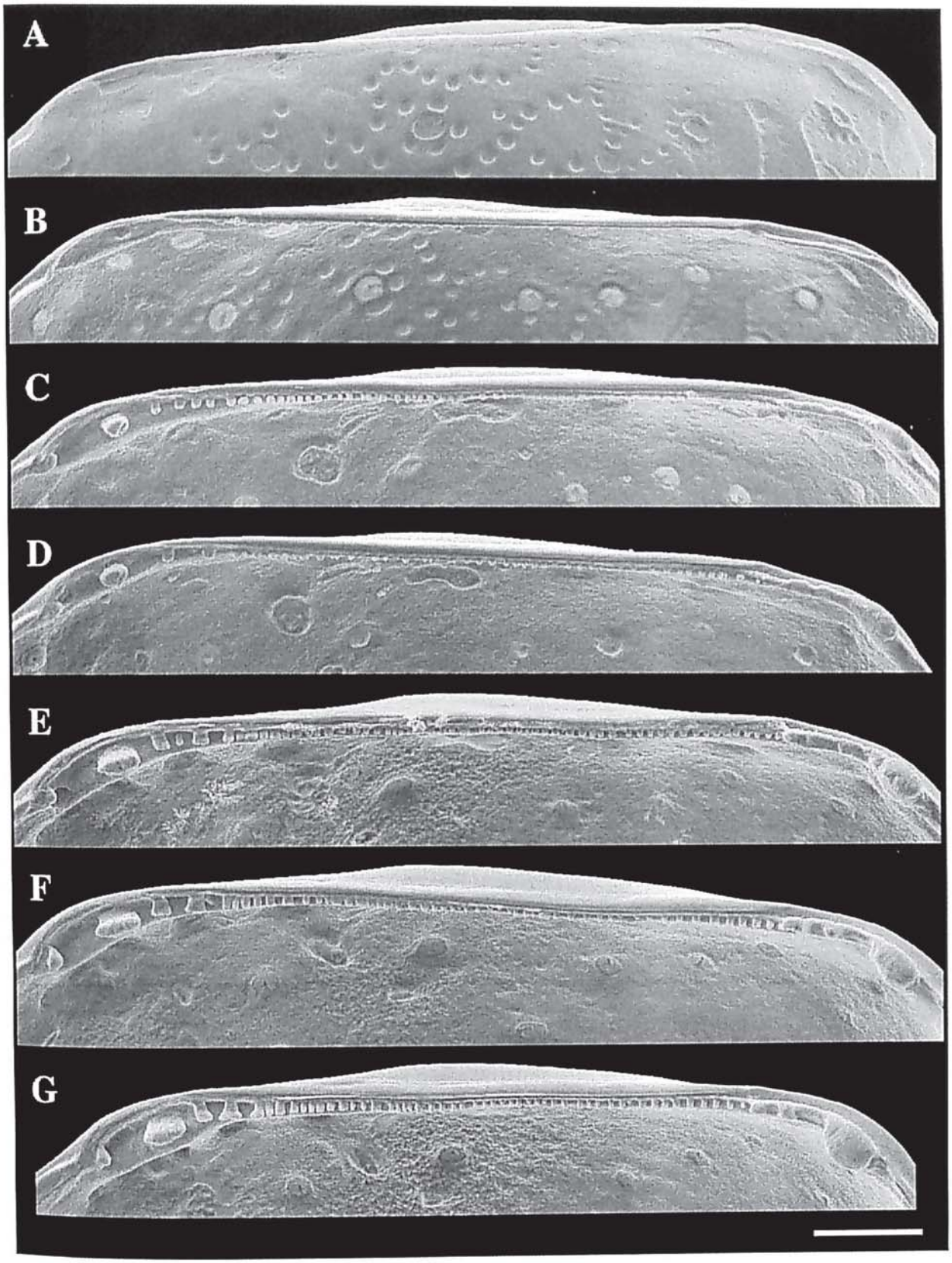

Figure 63 Hingement formation of *Loxoconcha pulchra* at the postmolt stage A (rv).
A. just after ecdysis. B. 5 hours after ecdysis. C. 10 hours after ecdysis.
D. 15 hours after ecdysis E. 20 hours after ecdysis. F. 25 hours after ecdysis.
G. 30 hours after ecdysis. Scale is 50 μ m.

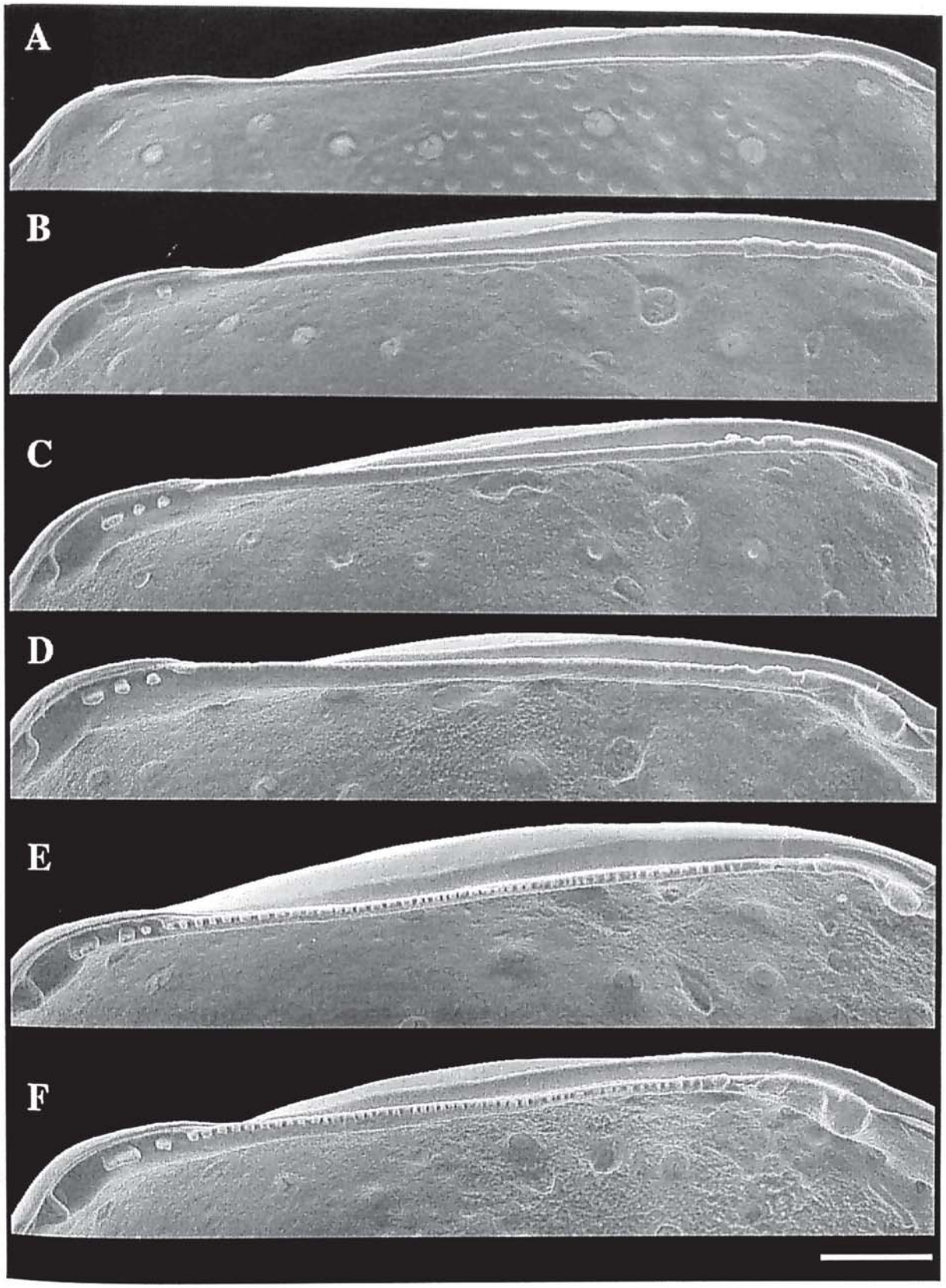

Figure 64 Hingement formation of *Loxoconcha pulchra* at the postmolt stage A (lv).
A. 5 hours after ecdysis. B. 10 hours after ecdysis. C. 15 hours after ecdysis
D. 20 hours after ecdysis. E. 25 hours after ecdysis. F. 30 hours after ecdysis.
Scale is 50 μ m.

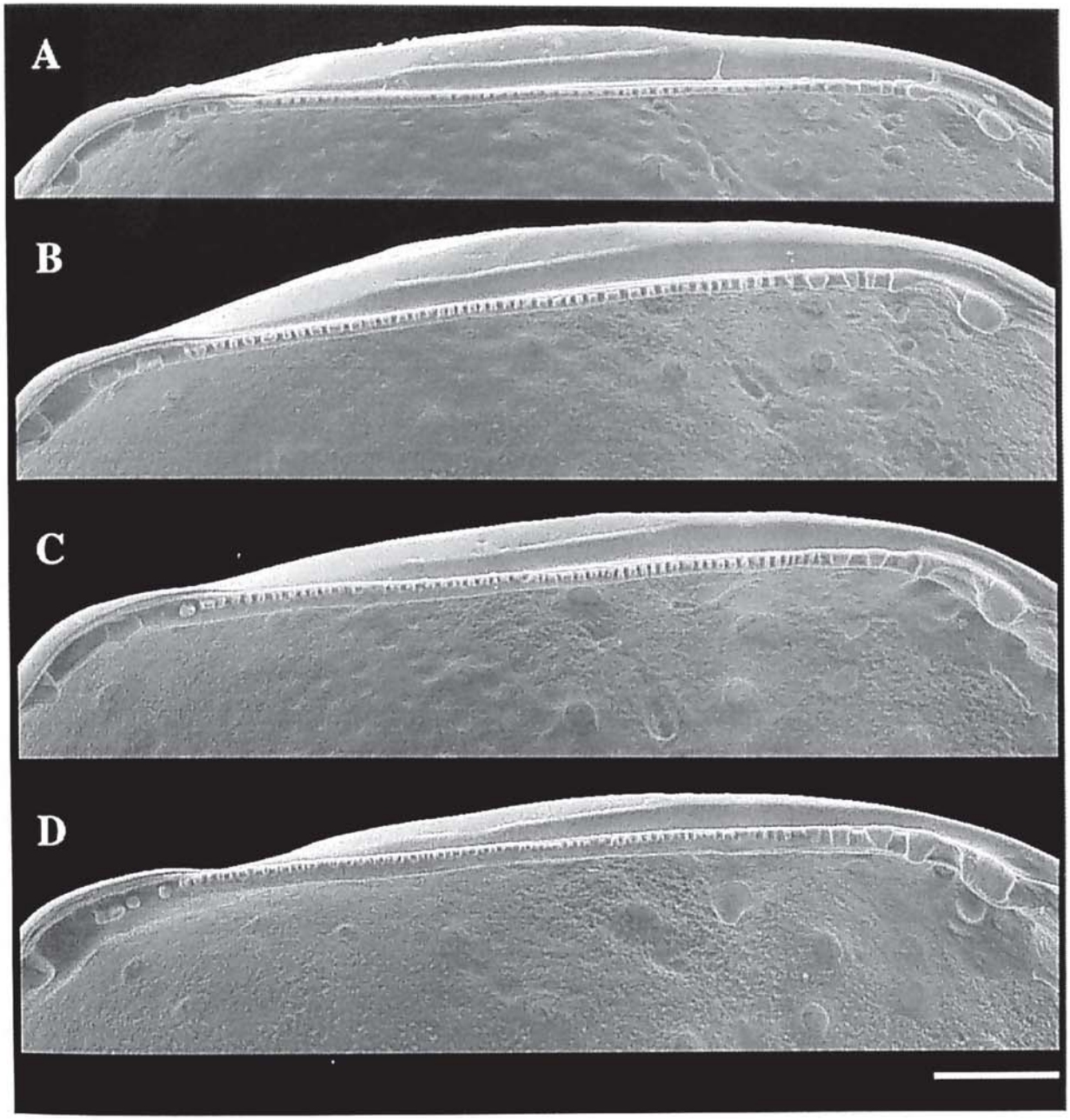

Figure 65 Hingement formation of *Loxoconcha pulchra* at the postmolt stage A (lv).
A. 35 hours after ecdysis. B. 40 hours after ecdysis. C. 45 hours after ecdysis
D. 50 hours after ecdysis. Scale is 50 μ m.

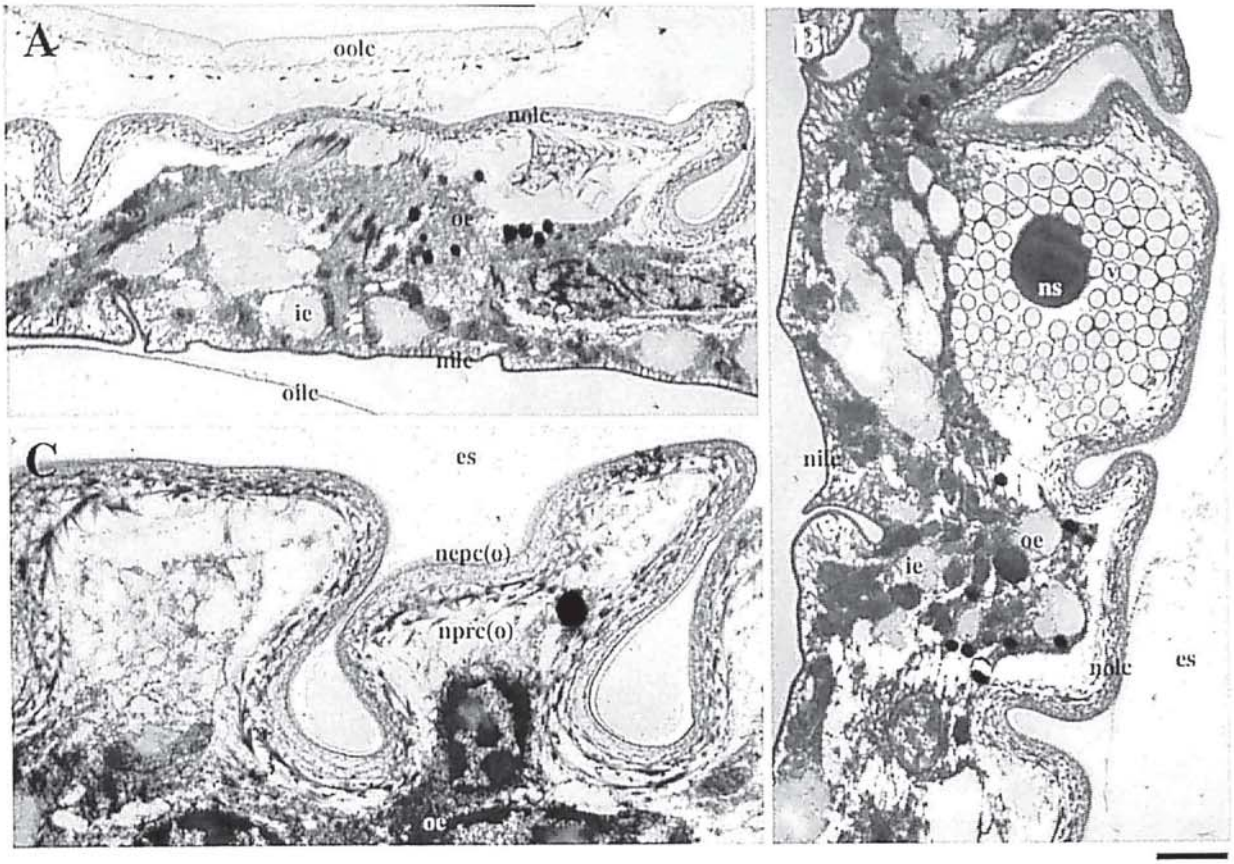

Figure 66 Carapace of *Xestoleberis hanaii* (8th instar) at the premolt stage D4.
A. outer and inner lamella cuticle. B. epidermis around the sieve pore.
C. new epicuticle and procuticle of outer lamella cuticle.
Scale bar is 2 μ m (A); 700nm (B); 1.7 μ m (C).

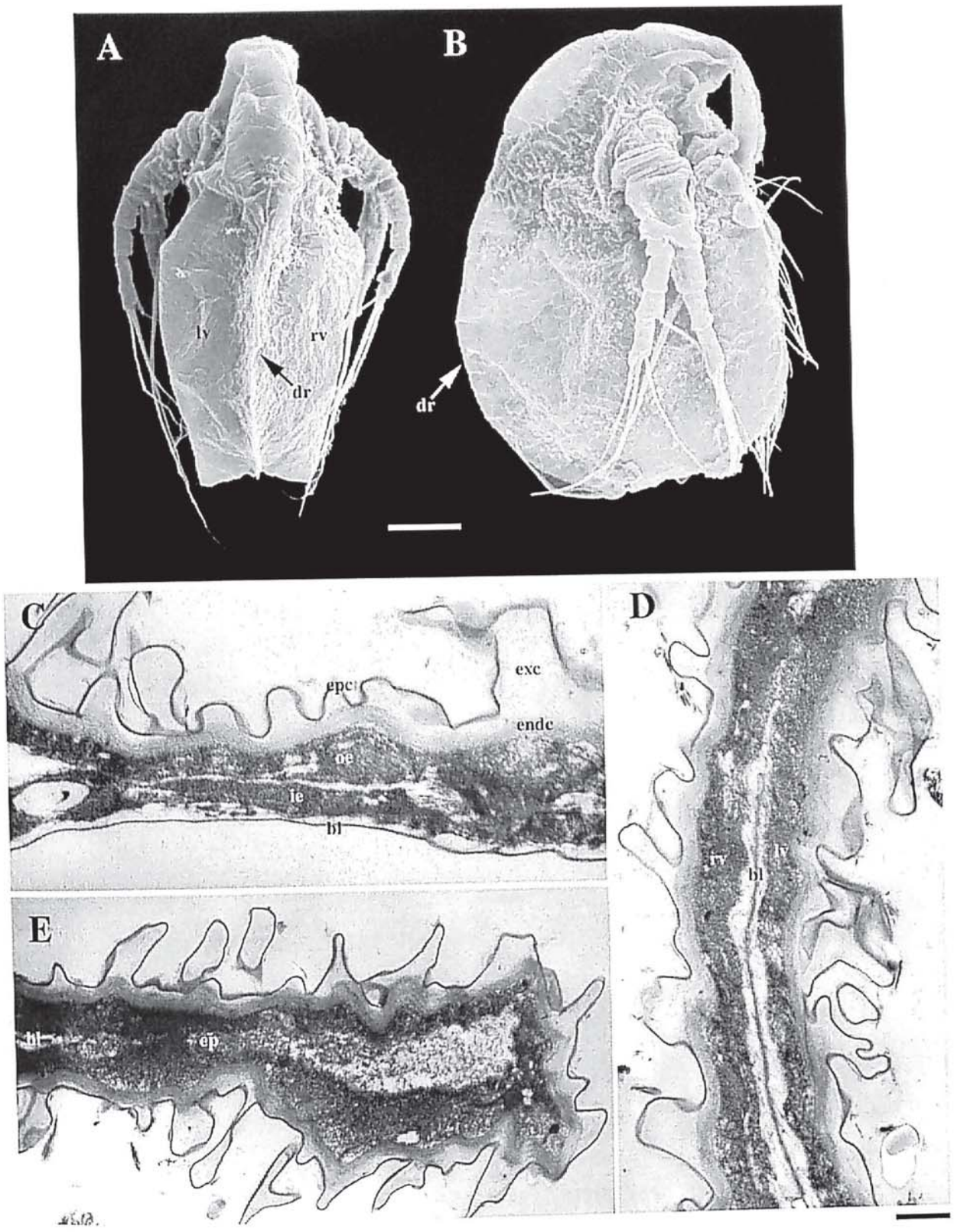

Figure 67 Carapace structure of Cladocera (Branchiopoda).
A. SEM photgraph in dorsal view. B. SEM photograph in lateral view.
C. carapace. D, E. transverse section of dorsal ridge.
Scale bar is 50 μ m (A, B); 1 μ m (C); 833nm (D); 500nm (E).

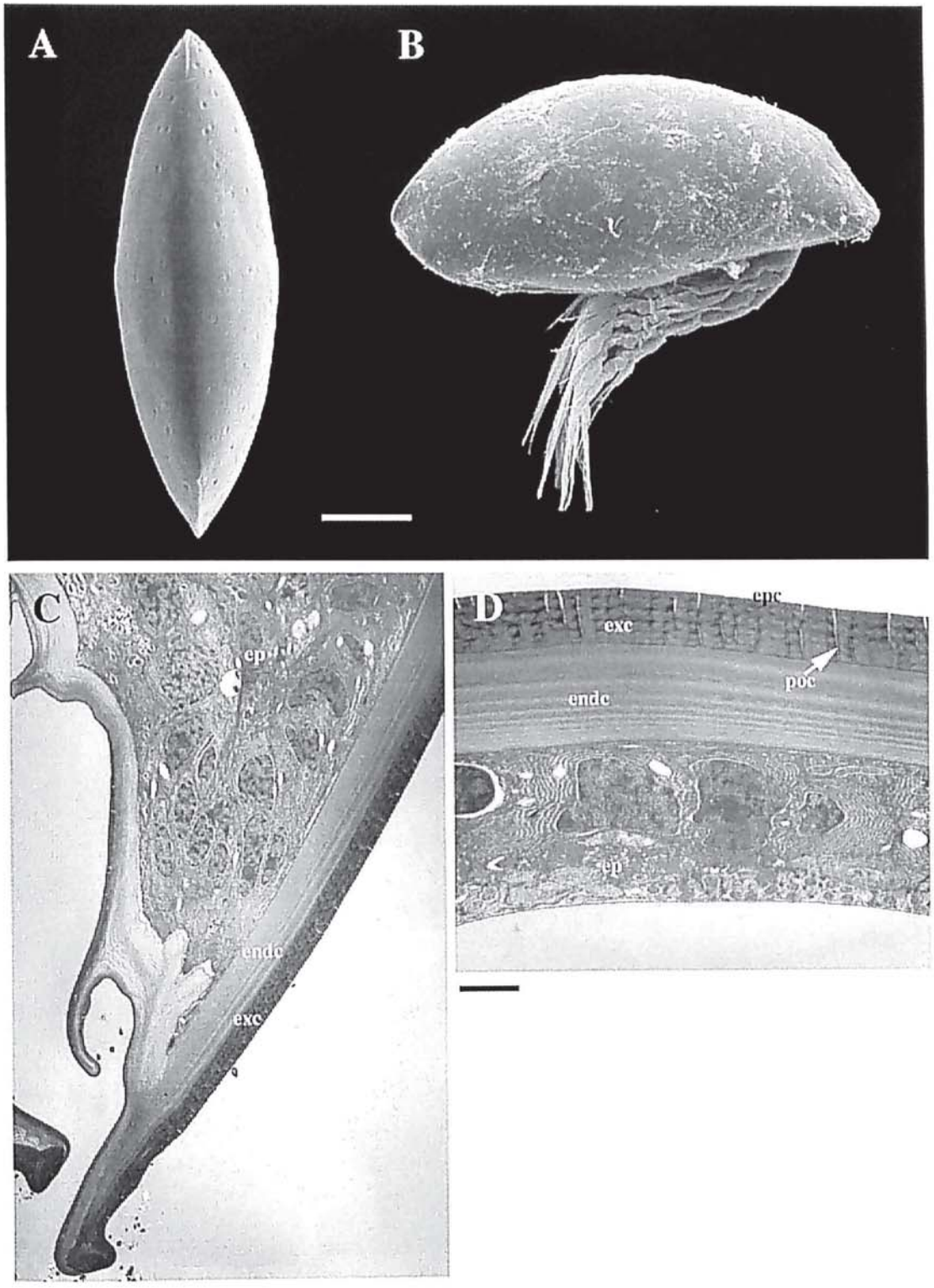

Figure 68 Carapace structure of Cypris larva (Cirripedia).
A. SEM photgraph in dorsal view. B. SEM photograph in lateral view.
C. transverse section of carapace fold. D. transverse section of dorsal region.
Scale bar is 100 μm (A, B); 5 μm (C); 2 μm (D).

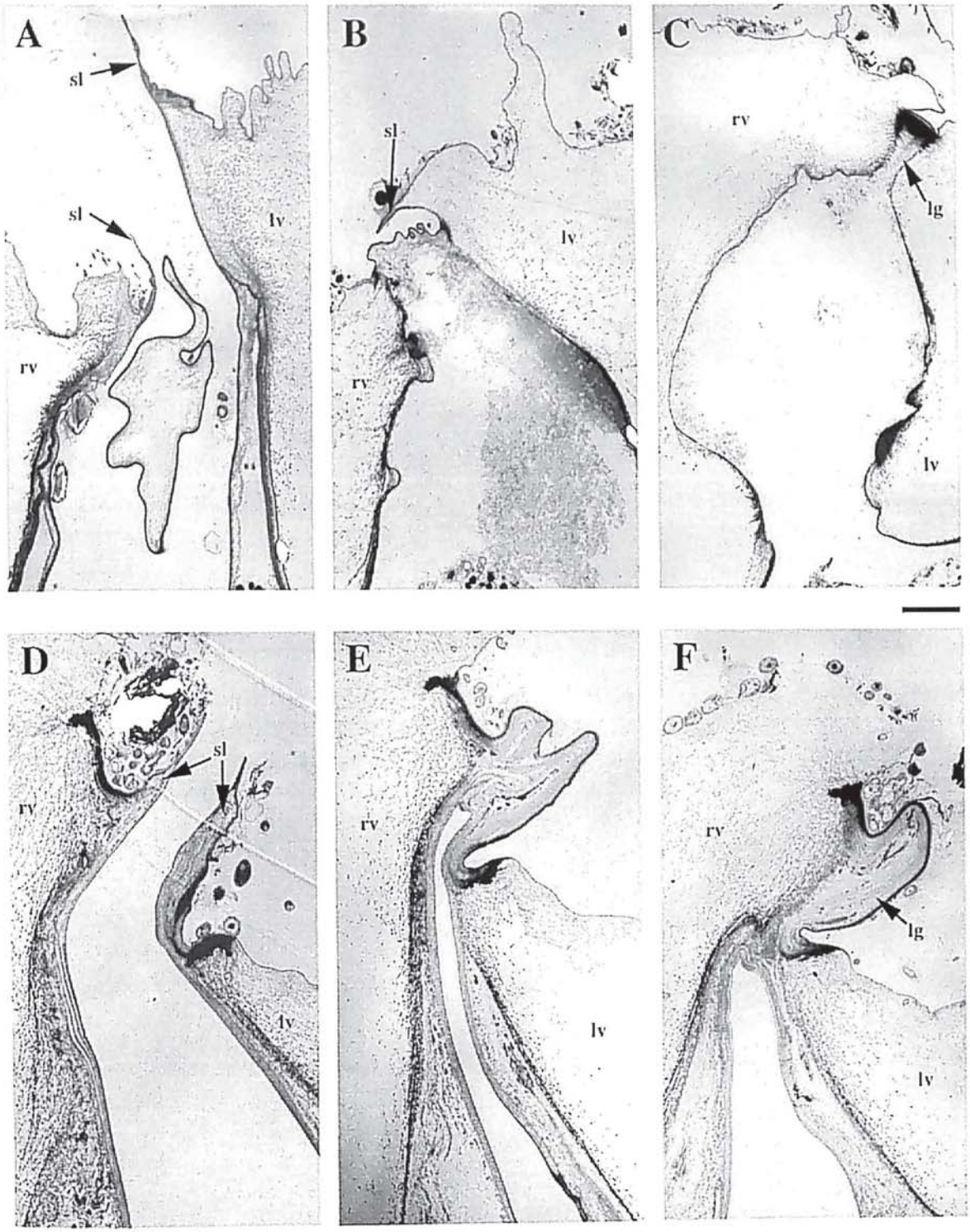

Figure 69 Successive sections near the boundary between free and attached margin of *Aurila hataii* (A-C) and *Keijcyoidea infralittolaris* (D-F).
A, D. free margins. B, E. transitional areas between free and attached margin.
C, F. attached margins.
Scale bar is 4 μ m (A-C); 2.9 μ m (D-F).

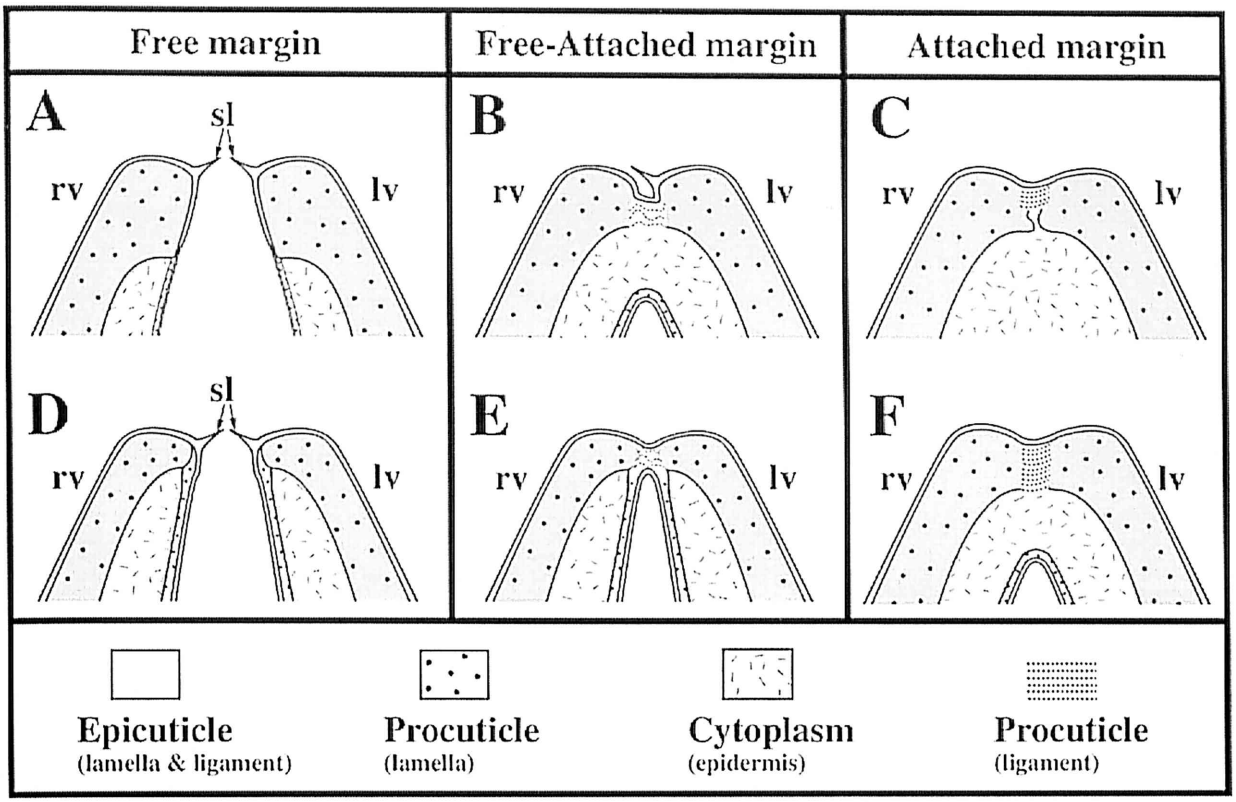

Figure 70 Schematic drawing of successive sections near the boundary between free and attached margin of *Aurila hataii* (A-C) and *Keijcyoidea infralittolaris* (D-F).
A, D. free margins. B, E. transitional areas between free and attached margin.
C, F. attached margins. Grey areas represent the calcified parts.

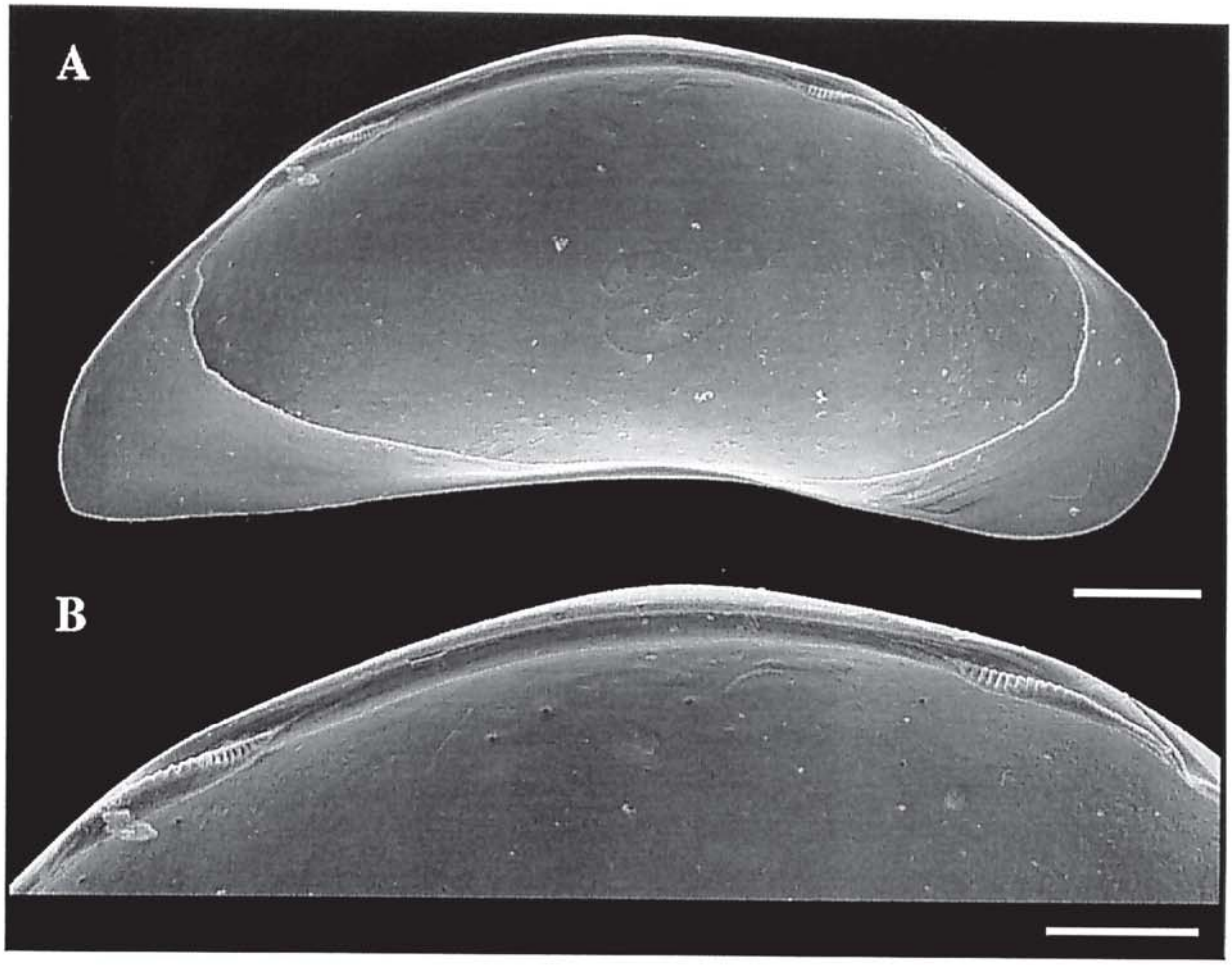

Figure 71 Hingement of *Macrocypris* sp. (Macrocypridoidea).
A. internal view of left valve. B. hingement of left valve.
Scale is 100 μ m (A); 50 μ m (B).

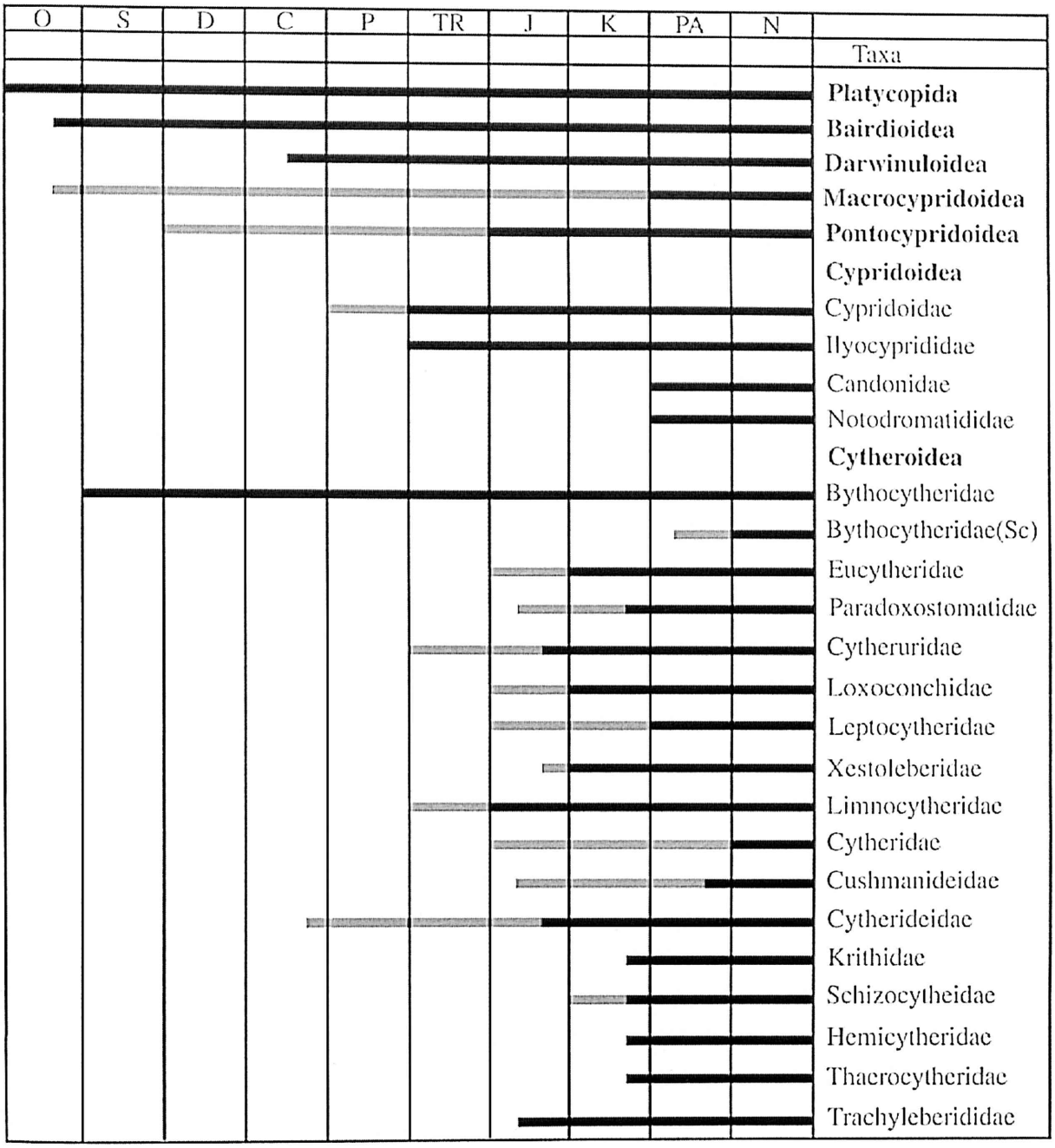

Figure 72 Fossil records of podocopan ostracod superfamilies.
Grey bars indicate unsure fossil records.

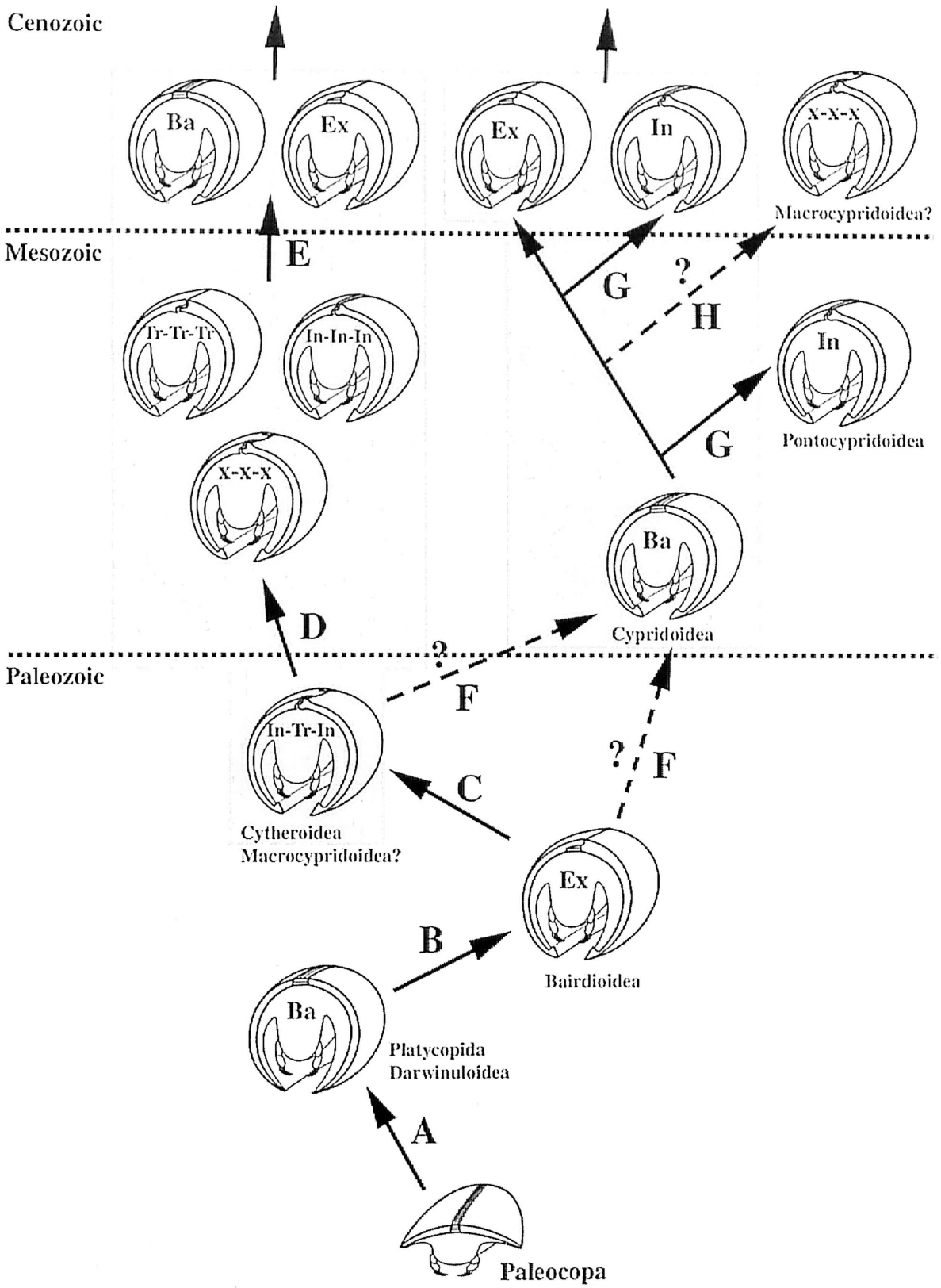

Figure 73 Evolutionary pathway of podocopan hinge structures.
Ba: Basic type. Ex: Exterior type. Tr: Transitive type. In: Interior type.
x-x-x: various combination of 3 elemented hinge structure.

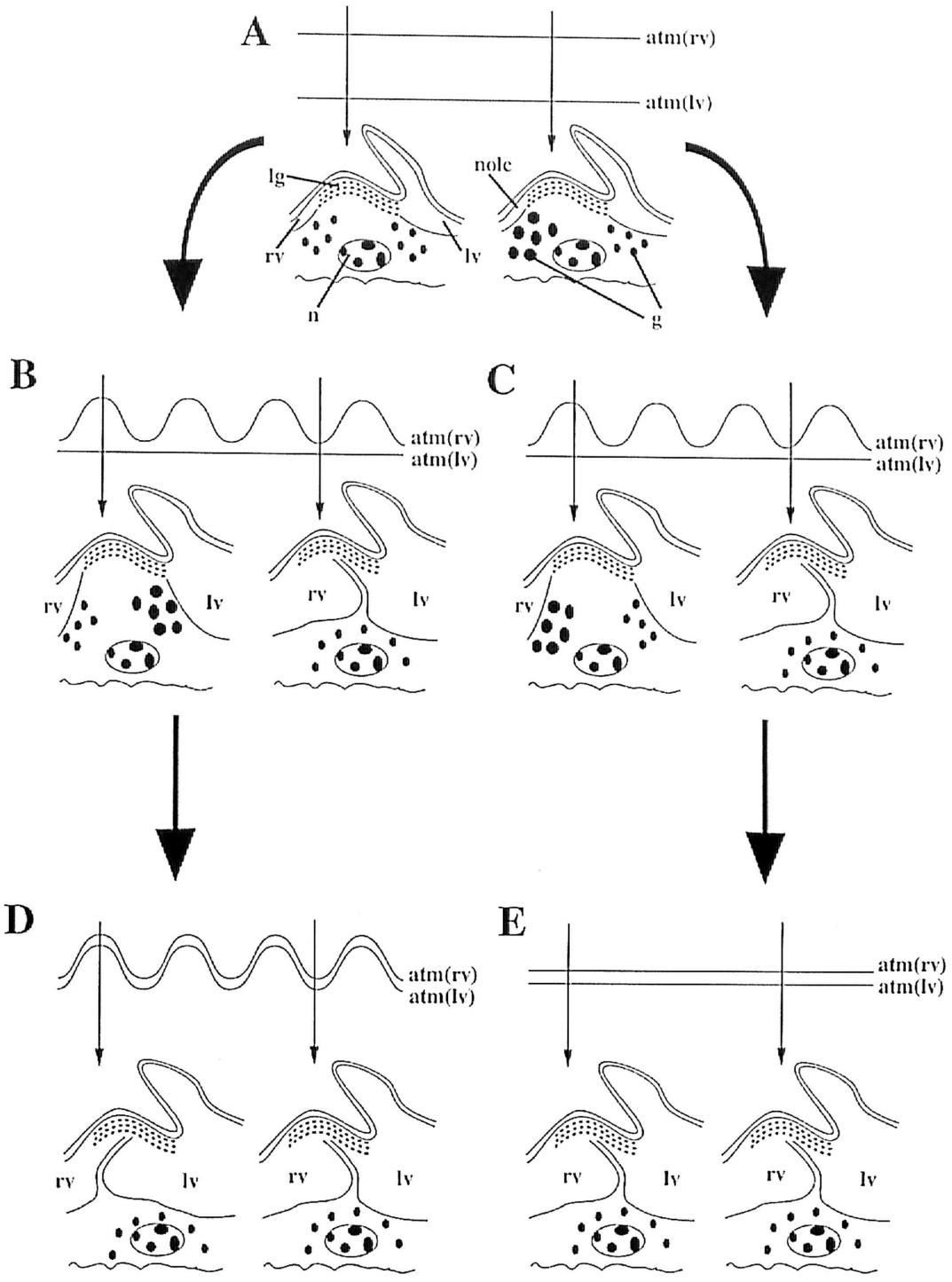

Figure 74 Morphogenesis of podocopan hingements.
Longitudinal section (upper) and transeverse sections (lower) of hingement are shown.
A-B-D: formation process of crenulate hingement.
A-C-E: formation process of simple hingement.
The size of granules represents the difference in the amount of cuticular deposition between right and left valve.

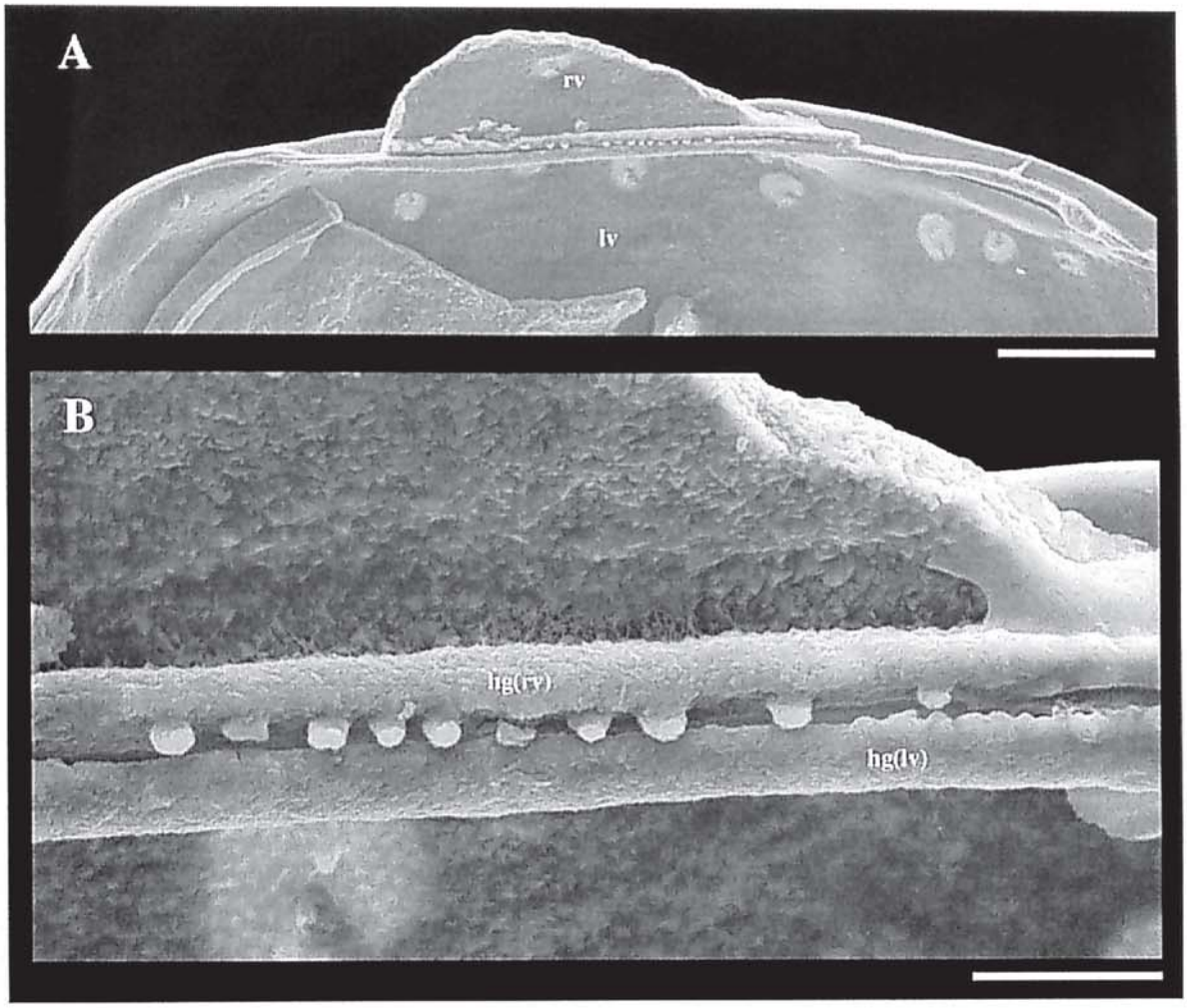

Figure 75 Hingement formation of *Loxoconcha pulchra* at the postmolt stage A. A. carapace past 10 hours after ecdysis. B. hingement past 10 hours after ecdysis. Scales are 50 μm (A); 10 μm (B).

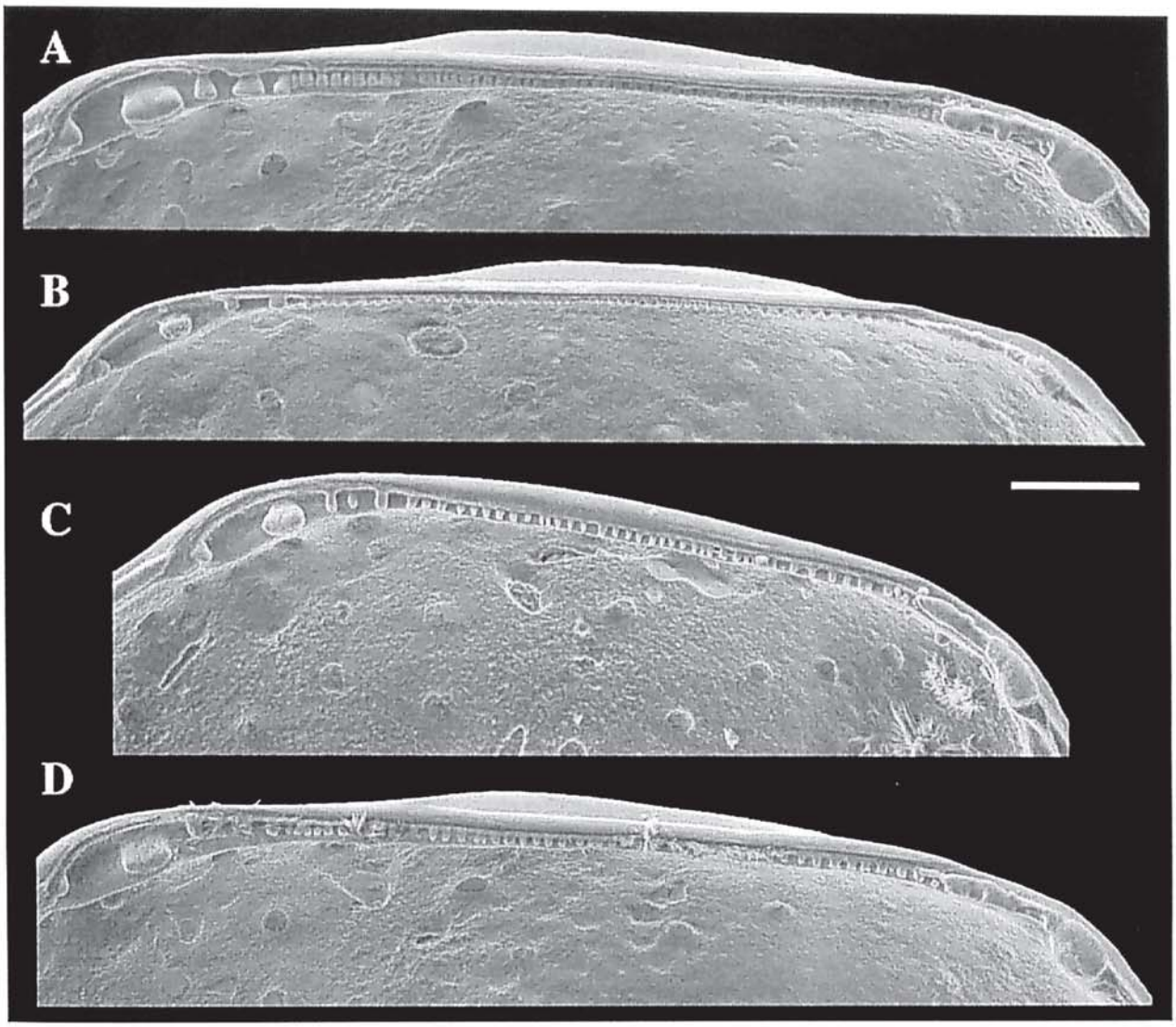

Figure 76 Hingements of *Loxoconcha pulchra* formed in the experimental brackish waters. A. male right valve under 25 ‰ condition. B. male right valve under 12.5 ‰ condition. C. female right valve under 12.5 ‰ condition. D. male right valve under 25 ‰ (Ca-double). Scales is 50 μ m.

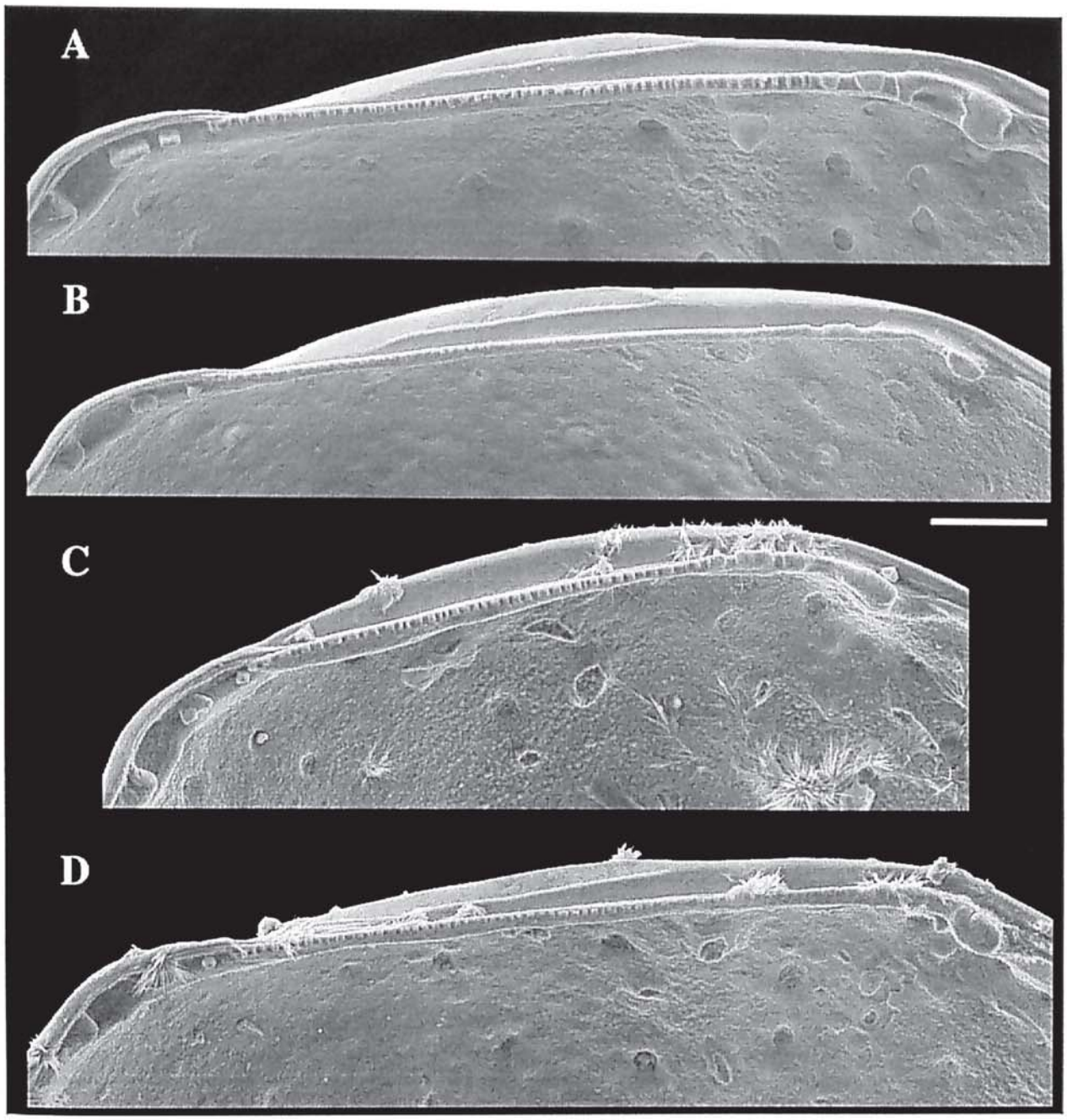

Figure 77 Hingements of *Loxoconcha pulchra* formed in the experimental brackish waters. A. male left valve under 25 ‰ condition. B. male left valve under 12.5 ‰ condition. C. female left valve under 12.5 ‰ condition. D. male left valve under 25 ‰ (Ca-double). Scales is 50 μ m.

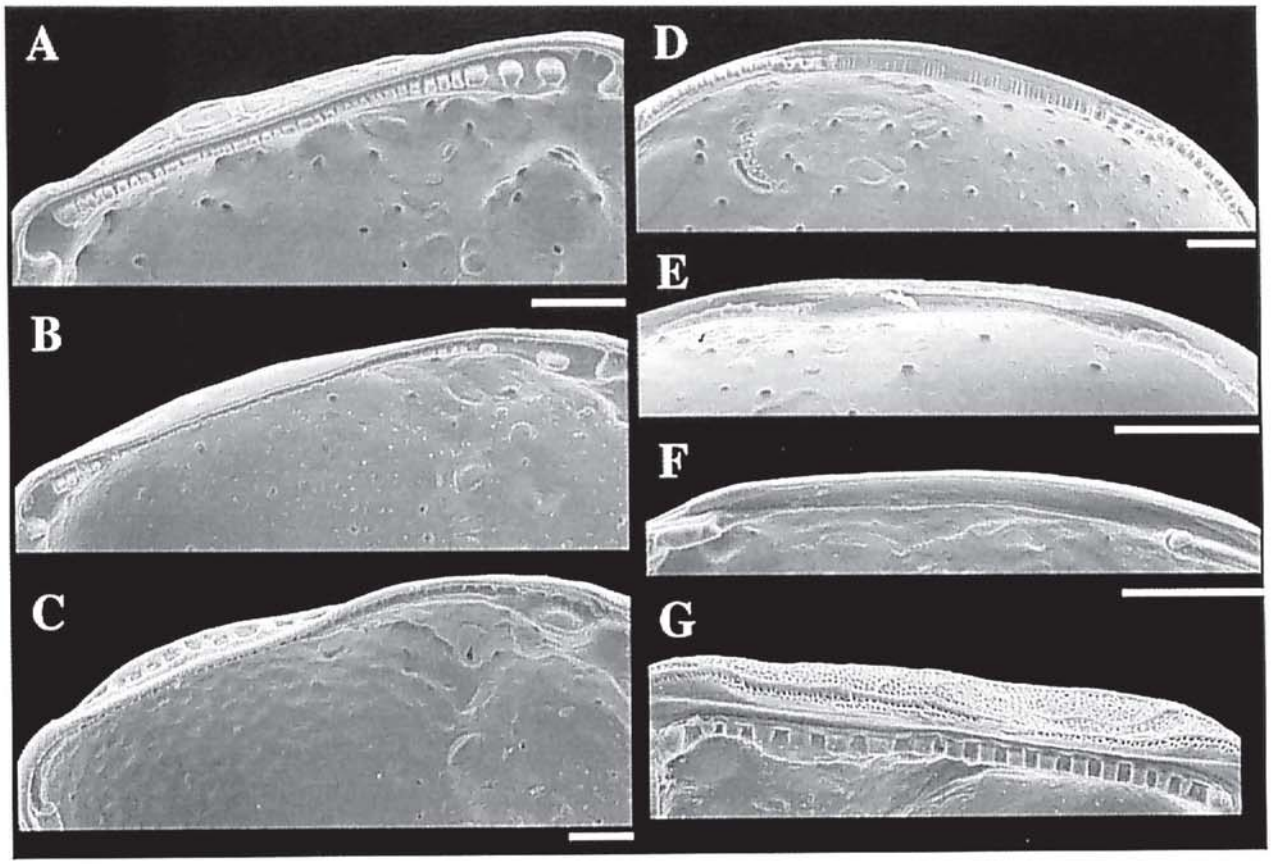

Figure 78 Dimorphisms of hingement in some cytheroid families and genera.
A. *Callistocythere rugosa.* B. *Callistocythere pumila.* C. *Ishizakiella miurensis.*
D. *Xestoleberis hanaii* E. *Xestoleberis* sp. F. *Semicytherura wakamurasaki.*
G. *Semicytherura kazahana.* Scales are 50 μ m.

Table 1 Specimens list

Species name	Locality	Algae / Sediments
Myodocopa		
Myodocopida		
Cypridinoidea		
Cypridinidae		
Vargula hilgendorfii	Shimoda beach (Shizuoka Pref. Japan)	algae
Melavargula japonica	Shimoda beach (Shizuoka Pref. Japan)	algae
Cypridina noctiluca	Shimoda beach (Shizuoka Pref. Japan)	algae
Halocyprida		
Cladocopoidea		
Polycopidae		
Polycope japonica	Shimoda beach (Shizuoka Pref. Japan)	sediments
Podocopa		
Platycopida		
Cytherelloidea		
Cytherellidae		
Keijcyoidea inflalittoralis	Shimoda beach (Shizuoka Pref. Japan)	algae
Podocopida		
Bairdioidea		
Bairdiidae		
Neonesidea oligodentata	Shimoda beach (Shizuoka Pref. Japan)	algae
Triebelina sp.	Shimoda beach (Shizuoka Pref. Japan)	algae
Darwinulloidea		
Darwinulidae		
Darwinula stevensoni	Lake Yamanaka (Shizuoka Pref. Japan)	sediments
Vestalenula sp.	Yaku Island (Kagoshima Pref. Japan)	sediments
Microdarwinula sp.	Ofudoson (Shizuoka Pref. Japan)	sediments
Macrocypridoidea		
Macrocyprididae		
Macrocypris sp.	Shimoda Ebisujima (Shizuoka Pref. Japan)	sediments
Pontocypridoidea		
Pontocyprididae		
Propontocypris sp.	Shimoda Ebisujima (Shizuoka Pref. Japan)	sediments

Table 1 Specimens list (continue)

Species name	Locality	Algae / Sediments
Podocopa		
Podocopida		
Cypridoidea		
Cyprididae		
Chrissia sp.	Ofudoson (Shizuoka Pref. Japan)	sediments
Cypridopsis vidua	Oya rice field (Shizuoka Pref. Japan)	sediments
Candonidae		
Fobaeformiscandona sp.	Lake Yamanaka (Shizuoka Pref. Japan)	sediments
Cypria reptans	Yaku Island (Kagoshima Pref. Japan)	sediments
Paracypridinae sp. A	Shimoda Ebisujima (Shizuoka Pref. Japan)	sediments
Paracypridinae sp. B	Kanna beach (Okinawa Pref. Japan)	sediments
Ilyocyprídae		
Ilyocypris japonica	Oya rice field (Shizuoka Pref. Japan)	sediments
Ilyocypris sp.	Myojin pond (Shizuoka Pref. Japan)	sediments
Cytheroidea		
Bythocytheridae		
Bythoceratina sp.	Shimoda beach (Shizuoka Pref. Japan)	algae
Sclerochilus sp.	Shimoda beach (Shizuoka Pref. Japan)	algae
Eucytheridae		
Keijia cf. *demissa*	Kanna beach (Okinawa Pref. Japan)	sediments
Paradoxostomatidae		
Paradoxostoma triangulum	Shimoda beach (Shizuoka Pref. Japan)	algae
Cytheruridae		
Semicytherura kazahana	Shimoda beach (Shizuoka Pref. Japan)	algae
Semicytherura wakamurasaki	Lake Hamana (Shizuoka Pref. Japan)	sediments
Hemicytherura kajiyamai	Shimoda beach (Shizuoka Pref. Japan)	algae
Hemicytherura tricarinata	Lake Hamana (Shizuoka Pref. Japan)	sediments
Loxoconchidae		
Loxoconcha japonica	Shimoda beach (Shizuoka Pref. Japan)	algae
Loxoconcha pulchra	Obitsu river (Chiba Pref. Japan)	sediments
Cytheromorpha acupunctata	Lake Hamana (Shizuoka Pref. Japan)	sediments
Leptocytheridae		
Callistocythere setouchiensis	Shimoda beach (Shizuoka Pref. Japan)	algae
Callistocythere pumila	Kurose river (Hiroshima Pref. Japan)	sediments
Callistocythere rugosa	Hayama beach (Kanagawa Pref. Japan)	algae
Ishizakiella miurensis	Obitsu river (Chiba Pref. Japan)	sediments
Tanella sp.	Kanna beach (Okinawa Pref. Japan)	sediments
Cobanocytheridae		
Paracobanocythere sp.	Shimoda beach (Shizuoka Pref. Japan)	sediments

Table 1 Specimens list (continue 2)

Species name	Locality	Algae / Sediments
Cytheroidea		
Xestoleberidae		
Xestoleberis hanaii	Shimoda beach (Shizuoka Pref. Japan)	algae
Xestoleberis setouchiensis	Shimoda beach (Shizuoka Pref. Japan)	algae
Limnocytheridae		
Limnocythere stationis	Lake Yamanaka (Shizuoka Pref. Japan)	sediments
Limnocytherina sanctipatricii	Spree river (Berlin Germany)	sediments
Microcytheridae		
Microcythere? sp.	Mochimune beach (Shizuoka Pref. Japan)	sediments
Cytheridae		
Cythere omotenipponica	Hayama beach (Kanagawa Pref. Japan)	algae
Cytherideidae		
Perissocytheridea inabai	Obitsu river (Chiba Pref. Japan)	sediments
Perissocytheridea japonica	Lake Hamana (Shizuoka Pref. Japan)	sediments
Cushmanideidae		
Pontocythere miurensis	Obitsu river (Chiba Pref. Japan)	sediments
Pontocythere japonica	Lake Hamana (Shizuoka Pref. Japan)	sediments
Krithidae		
Parakrithella pseudadonta	Shimoda beach (Shizuoka Pref. Japan)	algae
Schizocytheridae		
Schizocythere kishinouyei	Hayama beach (Kanagawa Pref. Japan)	algae
	Ise Bay (Off Aichi Pref. Japan)	sediments
Schizocytheridae?		
Spinileberis quadriaculeata	Lake Hamana (Shizuoka Pref. Japan)	sediments
Hemicytheridae		
Aurila hataii	Shimoda beach (Shizuoka Pref. Japan)	algae
Caudites asiaticus	Kanna beach (Okinawa Pref. Japan)	sediments
Trachyleberididae		
Trachyleberia scabroqueata	Aburatsubo Bay (Kanagawa Pref. Japan)	sediments
Bicornucythere bisanensis	Aburatsubo Bay (Kanagawa Pref. Japan)	sediments

Table 2. Studies on the ostracod ligament and selvage

Author name	Homologue of ligament	Nomenclature of ligament	Ultrastructure of ligament	Ultrastructure of selvage
Fassbinder (1912)	selvage	ligament	no figured	no figured
Harding (1969)	no mention	soft cuticle	no figured	no figured
Kornicker (1969)	independent	ligament	no figured	no figured
Bate & East (1972, 1975)	no mention	connecting chitin	layered chitin structure (parabolic pattern)	layered chitin structure (parabolic pattern)
Jaanusson (1985)	no mention	intervalvar cuticle	no figured	no figured
Keyser (1995)	no mention	no mention	chitinous bundles	no figured
This study	specialized cuticular structure	ligament	chitin bundle of feather-like fibers	homogeneous with lattice structure

Table 3 Podocopan hinge structures

Species name	Hinge structure	Hingement
Keijcyoidea inflalittoralis	Basic - Interior - Basic	a tooth
Neonesidea oligodentata	Exterior	adont
Triebelina sp.	Exterior	adont
Darwinula stevensoni	Basic	adont
Vestalenula sp.	Basic	adont
Microdarwinula sp.	Basic	adont
Propontocypris sp.	Interior	adont
Chrissia sp.	Basic	adont
Cypridopsis vidua	Basic	adont
Fobaeformiscandona sp.	Exterior	adont
Cypria reptans	Exterior	adont
Paracypridinae sp. A	Interior	adont
Paracypridinae sp. B	Interior	adont
Ilyocypris japonica	Basic	adont
Bythoceratina sp.	Interior - Transitive - Interior	lohodont
Sclerochilus sp.	Exterior	lohodont
Keijia cf. *demissa*	Interior - Interior - Interior	pentodont
Paradoxostoma triangulum	Exterior	lohodont
Semicytherura kazahana	Transitive - Transitive - Transitive	merodont
Semicytherura wakamurasaki	Transitive - Transitive - Transitive	merodont
Hemicytherura kajiyamai	Transitive - Transitive - Transitive	merodont
Hemicytherura tricarinata	Transitive - Transitive - Transitive	merodont
Loxoconcha japonica	Interior - Transitive - Interior	gongylodont
Loxoconcha pulchra	Interior - Transitive - Interior	gongylodont
Callistocythere setouchiensis	Interior - Exterior - Transitive	entomodont
Callistocythere pumila	Interior - Exterior - Transitive	entomodont
Callistocythere rugosa	Interior - Exterior - Transitive	entomodont
Ishizakiella miurensis	Interior - Basic - Transitive	entomodont
Paracobanocythere sp.	Basic	adont
Xestoleberis hanaii	Transitive - Transitive - Transitive	merodont
Limnocythere stationis	Interior - Interior - Interior	lohodont
Cythere omotenipponica	Transitive - Transitive - Transitive	merodont
Pontocythere miurensis	Basic - Exterior - Interior	desmodont
Pontocythere japonica	Basic - Exterior - Interior	desmodont
Perissocytheridea inabai	Interior - Transitive - Interior	merodont
Perissocytheridea japonica	Interior - Transitive - Interior	merodont
Parakrithella pseudadonta	Transitive - Transitive - Transitive	pseudadont
Schizocythere kishinouyei	Transitive - Transitive - Transitive	schizodont
Spinileberis quadriaculeata	Interior - Interior - Interior	schizodont
Aurila hataii	Transitive - Transitive - Transitive	amphidont
Caudites asiaticus	Transitive - Transitive - Transitive	amphidont
Trachyleberia scabroquneata	Interior - Interior - Interior	amphidont

Table 4 Outer lamella cuticular structures

Species name	Epicuticular layers	Division of procuticle	Ornamentation	habitat
Melavargula japonica	2	exocuticle / endocuticle	smooth	marine
Cypridina noctiluca	2	exocuticle / endocuticle	smooth	marine
Polycope japonica	1	procuticle	ornamented	marine
Keijcyoidea inflalittoralis	2	procuticle	ornamented	marine
Neonesidea oligodentata	2	exocuticle / endocuticle	minor ornament	marine
Triebelina sp.	2	exocuticle / endocuticle	minor ornament	marine
Darwinula stevensoni	3	exocuticle / endocuticle	smooth	freshwater
Vestalenula sp.	3	exocuticle / endocuticle	smooth	freshwater
Microdarwinula sp.	3	exocuticle / endocuticle	smooth	freshwater
Propontocypris sp.	1	exocuticle / endocuticle	smooth	marine
Chrissia sp.	2	exocuticle / endocuticle	smooth	freshwater
Cypridopsis vidua	2	exocuticle / endocuticle	smooth	freshwater
Fobaeformiscandona sp.	2	exocuticle / endocuticle	smooth	freshwater
Cypria reptans	2	exocuticle / endocuticle	smooth	freshwater
Paracypridinae sp. A	3	exocuticle / endocuticle	smooth	marine
Paracypridinae sp. B	3	exocuticle / endocuticle	smooth	marine
Ilyocypris japonica	2	procuticle	ornamented	freshwater
Bythoceratina sp.	1	procuticle	ornamented	marine
Sclerochilus sp.	1	procuticle	smooth	marine
Keijia cf. *demissa*	2	procuticle	ornamented	marine
Paradoxostoma triangulum	1	exocuticle / endocuticle	smooth	marine
Semicytherura kazahana	2	procuticle	ornamented	marine
Semicytherura wakamurasaki	2	procuticle	ornamented	marine
Hemicytherura kajiyamai	2	procuticle	ornamented	marine
Hemicytherura tricarinata	2	procuticle	ornamented	marine
Loxoconcha japonica	3	procuticle	ornamented	marine
Loxoconcha pulchra	3	procuticle	ornamented	marine
Callistocythere setouchiensis	2	procuticle	ornamented	marine
Callistocythere pumila	2	procuticle	ornamented	marine
Callistocythere rugosa	2	procuticle	ornamented	marine
Ishizakiella miurensis	2	procuticle	ornamented	marine
Paracobanocythere sp.	1	procuticle	smooth	marine
Xestoleberis hanaii	1	exocuticle / endocuticle	smooth	marine
Xestoleberis setouchiensis	1	exocuticle / endocuticle	smooth	marine
Limnocythere stationis	2	exocuticle / endocuticle	ornamented	freshwater
Microcythere sp.	2	procuticle	smooth	marine
Cythere omotenipponica	2	exocuticle / endocuticle	minor ornament	marine
Pontocythere miurensis	3	procuticle	smooth	marine
Pontocythere japonica	3	procuticle	smooth	marine
Perissocytheridea inabai	2	procuticle	ornamented	marine
Perissocytheridea japonica	2	procuticle	ornamented	marine
Parakrithella pseudadonta	2	procuticle	smooth	marine
Schizocythere kishinouyei	2	procuticle	ornamented	marine
Spinileberis quadriaculeata	1	procuticle	ornamented	marine
Aurila hataii	1	procuticle	ornamented	marine
Caudites asiaticus	1	procuticle	ornamented	marine
Trachyleberis scabroquneata	1	procuticle	ornamented	marine