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PRELIMINARY OBSERVATIONS ON THE USEFULNESS OF HINGE STRUCTURES FOR IDENTIFICATION OF BIVALVE LARVAE

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ABSTRACT Difficulties associated with discrimination of bivalve larvae isolated from plankton samples have long hampered both applied and basic research efforts in estuarine and open coastal marine environments. The vast majority of practical barriers to identification of larval bivalves may be eliminated through routine optical microscopic examination of the hinge apparatus of disarticulated larval shells. Representative micrographs of various ontogenetic stages of larval hinge development are presented for 12 genera (*Mytilus*, *Geukensia*, *Crassostrea*, *Placopecten*, *Argopecten*, *Mya*, *Spisula*, *Mulinia*, *Ensis*, *Arca*, *Arctica*, and *Mercenaria*) from 9 bivalve superfamilies (Mytilacea, Ostreacea, Pectinacea, Myacea, Mactracea, Solenacea, Arcacea, Arcticacea, and Veneracea). The larval hinge apparatus (provinculum), by itself, is generally useful for superfamilial separation. When coupled with a consideration of gross shell shape, detailed examination of hinge line structures often permits generic, or even specific, identification. A format is suggested for organization of qualitative morphological life history data that will provide an adequate basis for comparison of the larval stages of various species of bivalves.

INTRODUCTION

An inability to identify bivalve larvae within the plankton has long hampered both applied and basic research efforts in estuarine and open coastal marine environments (Werner 1939; Jørgensen 1946; Sullivan 1948; Rees 1950; Loosanoff and Davis 1963; Loosanoff et al. 1966; Chanley and Andrews 1971; Lutz and Jablonski 1978a,b, 1979, 1981; Lutz and Hidu 1979; Jablonski and Lutz 1980; Le Pennec 1980). For example, as a result of existing practical barriers, detailed studies concerning spatfall predictions for aquacultural and fisheries management purposes have been extremely limited (for discussions, see Wisely et al. 1978, Lutz and Hidu 1979, Le Pennec 1980). Year-to-year fluctuations in larval abundance and juvenile recruitment often are not possible to define or predict because of the present inability of researchers to discriminate individual larval or early post-larval specimens with a high degree of certainty. Similarly, it has been virtually impossible in routine plankton identification studies to assess the impact of various environmental perturbations (natural "disasters," chemical pollutants, thermal discharges, oil spills, dredge spoil dumping, entrain-

ment through industrial cooling systems, etc.) on the larvae of individual species of bivalves. While a few keys for larval identification do exist (e.g., Chanley and Andrews 1971), their usefulness is limited and, at the present time, it is not possible to identify unambiguously the larvae of many bivalve species, particularly at the early (straight-hinge) developmental stages, because of the great morphological similarity of articulated shells. We offer in this paper an approach designed to eliminate many of the existing barriers to larval bivalve identification. Emphasis is placed on the usefulness of hinge (provinculum) structures in discriminating the early life-history stages of various species of bivalve molluscs.

In recent years, various workers have employed both optical and scanning electron microscopy to describe in detail the larval hinge structures of several bivalves and have suggested that such structures may be diagnostic at the generic, or even specific, level (Chanley 1965, 1969; Turner and Johnson 1969; Scheltema 1971; Pascual 1971, 1972; LaBarbera 1975; Boyle and Turner 1976; Culliney and Turner 1976; Dinamani 1976; Le Pennec and Masson 1976; Booth 1977, 1979a,b; Siddall 1977, 1978; Le Pennec

1978, 1980; Lutz and Jablonski 1978a,b, 1981; Carriker and Palmer 1979; Lutz and Hidu 1979; Chanley and Dinamani 1980; Jablonski and Lutz 1980). Despite these recent advances, much of the morphological data obtained over the past few years has not been presented in an adequate or sufficiently consistent format to permit unambiguous identification of early life-history stages. In this collaborative paper, we present representative micrographs of various ontogenetic stages of larval hinge development of nine bivalve superfamilies and suggest a format for organization of qualitative morphological life-history data that will provide an adequate basis for comparison of the planktonic stages of various species of bivalves.

MATERIALS AND METHODS

Culture Techniques

Sexually mature adults of the bivalves were obtained from the following locations: *Mytilus californianus* Conrad—Puget Sound, Washington; *Geukensia demissa* (Dillwyn)—Wachapreague, Virginia; *Crassostrea virginica* (Gmelin)—Cape May, New Jersey; *Placopecten magellanicus* (Gmelin)—Damariscotta River, Maine; *Argopecten irradians* (Lamarck)—Cape Cod Bay, Massachusetts; *Mya arenaria* L.—Damariscotta River, Maine; *Spisula solidissima* (Dillwyn)—Rhode Island (open coast); *Mulinia lateralis* (Say)—Cape May, New Jersey; *Ensis directus* Conrad—Damariscotta River, Maine; *Arca noae* L.—northern Adriatic Sea (Istrian Peninsula, Yugoslavian coast); *Arctica islandica* (L.)—New Jersey (open coast) and Rhode Island (open coast); and *Mercenaria mercenaria* (L.)—Damariscotta River, Maine, and Wachapreague, Virginia; and *Diplothyra smithii* Tryon—Mississippi Sound, Mississippi.

Spawning was induced using standard techniques developed by various workers (see Loosanoff and Davis 1963, Bayne 1965, Morse et al. 1977) or, in the case of *Arctica islandica*, using the ammonium hydroxide treatment described by Loosanoff and Davis (1963) and Landers (1976) (i.e., 15 to 30-minute exposure to a solution of 3 ml of 0.1N NH₄OH for every 100 ml of egg culture, followed by addition of stripped sperm).

Scanning Electron Microscopy

Larval specimens were sampled at frequent intervals (frequency dependent upon the growth of organisms since the previous sampling period) from the various cultures of each species and placed in distilled water for 30 minutes (see Calloway and Turner 1978). Immediately following this treatment, specimens were preserved in 95% ethanol. After various lengths of time (up to 2 months), specimens were removed from the ethanol, rinsed in distilled water, and immersed in a 5% solution of sodium hypochlorite (Rees 1950) for approximately 10 minutes to facilitate separation of shell valves. After rinsing in distilled water, disarticulated valves were mounted on copper tape, coated

(under vacuum) with approximately 400 Å of gold-palladium or a combination of gold and carbon, and examined under an ETEC Autoscan scanning electron microscope. Care was taken to achieve consistent orientations of shell valves prior to photographing: each specimen was carefully manipulated under the microscope so that four points, each 90° apart, along the edge of the shell margin were in the exact same plane of focus at a magnification of approximately 9,000; when this is done, it can be calculated that the tilt of a specimen in any direction is less than 2°. This technique provides a means of obtaining a consistent, repeatable orientation, which, in turn, provides a basis for accurately comparing the gross shell morphometry of various species.

RESULTS

Representative scanning electron micrographs of disarticulated larval shell valves at various stages of development are depicted in Figure 1. Higher magnification micrographs of the hinge region of all but one (i.e., Figure 1C') of these specimens are presented in Figure 2. These micrographs illustrate the striking differences in provinculum morphology among 12 genera (*Mytilus*, *Geukensia*, *Crassostrea*, *Placopecten*, *Argopecten*, *Mya*, *Spisula*, *Mulinia*, *Ensis*, *Arca*, *Arctica*, and *Mercenaria*) from 9 bivalve superfamilies (Mytilacea, Ostreacea, Pectinacea, Myacea, Mactracea, Solenacea, Arcacea, Arcticea, and Veneracea). The morphology of the hinge ranges from distinctly taxodont dentition in the case of the Mytilacea, Arcacea, and Pectinacea to a lack of prominent denticular structures in the Mactracea, Veneracea, and Arcticea. The provincular structures seen in the specimens depicted in Figures 1 and 2 are also present (although often reduced) in the early (straight-hinge) developmental stages (Figure 3).

DISCUSSION

An extensive literature exists on the identification of bivalve larvae. For over one half of a century, workers have attempted to define larval morphological characters diagnostic at various systematic levels (for discussions, see Stafford 1912; Odhner 1914; Lebour 1938; Werner 1939; Jørgensen 1946; Sullivan 1948; Rees 1950; Miyazaki 1962; Loosanoff and Davis 1963; Newell and Newell 1963; Loosanoff et al. 1966; Chanley and Andrews 1971; Le Pennec 1978, 1980; Lutz and Jablonski 1978a,b, 1979, 1981; Lutz and Hidu 1979; Chanley and Chanley 1980). The larval characteristics generally used in routine plankton identifications are shell length, height, and depth, as well as length of the "straight-hinge line" (Loosanoff et al. 1966, Chanley and Andrews 1971, Chanley and Chanley 1980). Differences in larval shell shape, color, and texture have also been of assistance, as have the presence or absence of a byssal notch, eyespot, or apical cilia ('apical flagellum') (Chanley and Andrews 1971, Culliney et al. 1975, Turner and Boyle 1975). In the present study we have presented a number of representative micrographs depicting striking

differences in the morphologies of the larval hinge apparatus of certain bivalve species, as well as subtle differences in the shell shape of these organisms. We have attempted to present the micrographs in a manner (i.e., consistent orientation) that will provide an adequate basis for comparing the morphologies of different species. While differences among various taxa are often subtle, we believe that they can be defined, permitting unambiguous identification at the specific level. For example, the hinge structures of larval stages of *Arctica islandica* closely resemble those of corresponding stages of *Mercenaria mercenaria* (see Figures 1 and 2), as well as various other species within the family Veneridae (see Le Pennec 1978, 1980). (Interestingly, such striking similarities in early ontogenetic development suggest a closer relationship between the arcticids and venerids than has heretofore been proposed.) Despite such similarities, careful examination of the fine structures of the hinge of *A. islandica* illustrated in Figure 2G reveals subtle differences that permit discrimination of early life-history stages of this species and those of *M. mercenaria* (Figure 2H), as well as those of other venerids. It should also be emphasized here that, while we have presented scanning electron micrographs of the hinge apparatus of selected organisms, a scanning electron microscope is not necessary to observe even fine hinge structures. Such structures are readily visible under a normal, optical compound microscope equipped with a high-intensity reflected light source. Scanning electron microscopy, however, is necessary to depict photographically the three-dimensional structure of the hinge region. In routine optical microscopic studies, the disarticulated shells must be viewed in several planes of focus to discern the subtle morphological details seen in Figures 1 through 3.

We suggest that in future descriptive studies morphological data should be organized into a format that includes

not only the "minimal information" recommended by Chanley and Andrews (1971, pp. 107–109) for "detailed descriptions of laboratory-reared bivalve larvae," but also detailed scanning electron micrograph sequences of the hinge structure and gross shell morphology of the various larval stages. It is imperative that such descriptions include micrographs of all the ontogenetic stages of larval development from the Prodissoconch I through settlement and metamorphosis rather than merely representative micrographs such as those that have been included in this introductory presentation (see also, Lutz et al. 1982). The use of such a comprehensive format for presentation of life-history data should help eliminate most of the practical barriers to the identification of early stages of bivalve molluscs.

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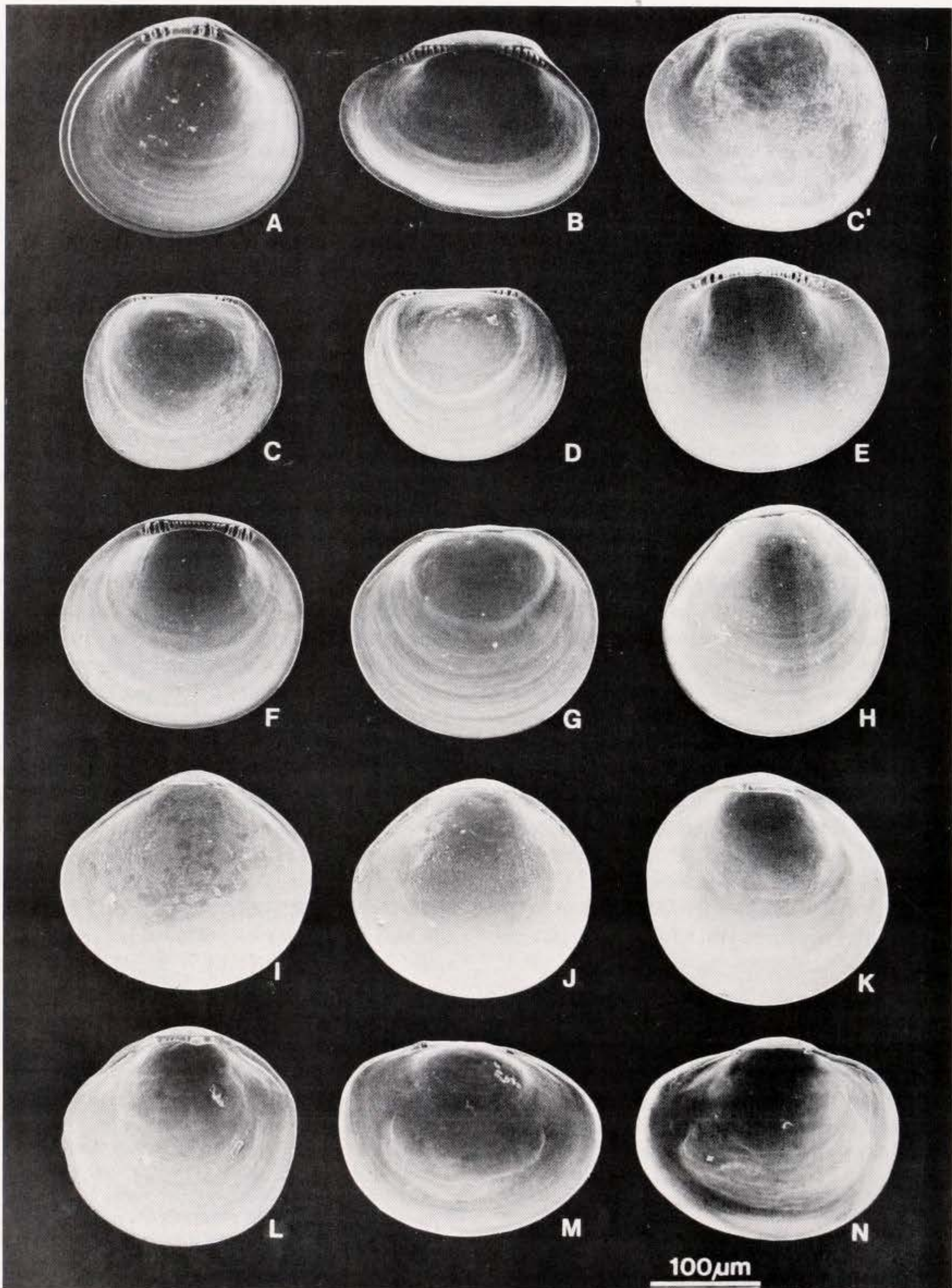


Figure 1. Scanning electron micrographs of disarticulated shell valves of planktonic larvae of various species of bivalve molluscs. A. *Crassostrea virginica* (right valve; mature larva). B. *Arca noae* (right valve; mature larva). C'. *Argopecten irradians* (right valve; mature larva). C. *Argopecten irradians* (left valve; straight-hinge larva). D. *Placopecten magellanicus* (left valve; straight-hinge larva). E. *Mytilus californianus* (left valve; mature larva). F. *Geukensia demissa* (right valve; mature larva). G. *Arctica islandica* (right valve; mature larva). H. *Mercenaria mercenaria* (right valve; mature larva). I. *Mya arenaria* (right valve; mature larva). J. *Mulinia lateralis* (right valve; mature larva). K. *Spisula solidissima* (left valve; mature larva). L. *Spisula solidissima* (right valve; mature larva). M. *Ensis directus* (left valve; mature larva). N. *Ensis directus* (right valve; mature larva).

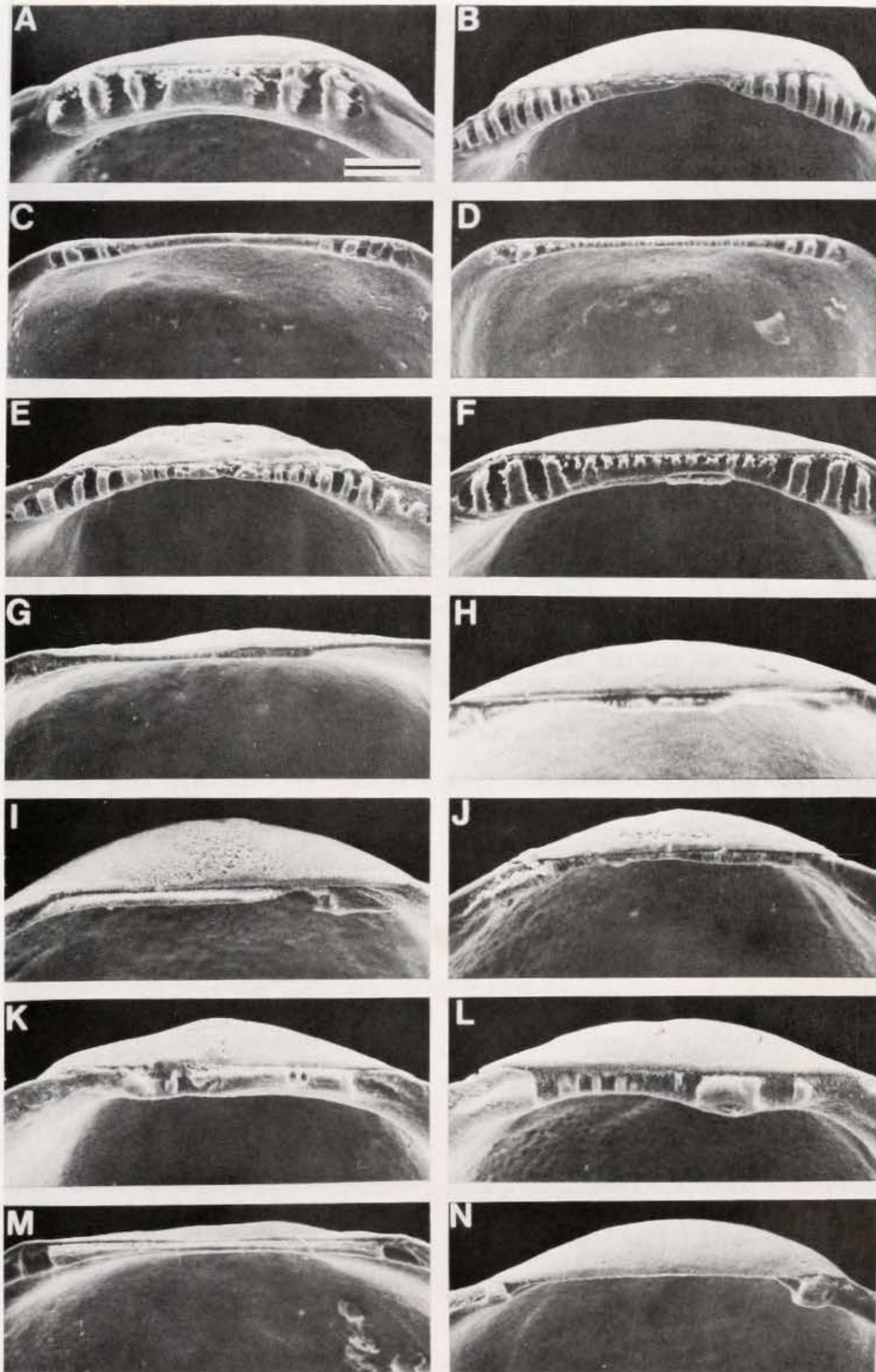


Figure 2. Scanning electron micrographs of the hinge region of the disarticulated shell valves of the specimens depicted in Figure 1. A. *Crassostrea virginica* (right valve), B. *Arca noae* (right valve), C. *Argopecten irradians* (left valve; straight hinge), D. *Placopecten magellanicus* (left valve; straight hinge), E. *Mytilus californianus* (left valve), F. *Geukensia demissa* (right valve), G. *Arctica islandica* (right valve), H. *Mercenaria mercenaria* (right valve), I. *Mya arenaria* (right valve), J. *Mulinia lateralis* (right valve), K. *Spisula solidissima* (left valve), L. *Spisula solidissima* (right valve), M. *Ensis directus* (left valve), N. *Ensis directus* (right valve). Scale bar (= 20 μ m) in A is applicable to all micrographs in this figure.

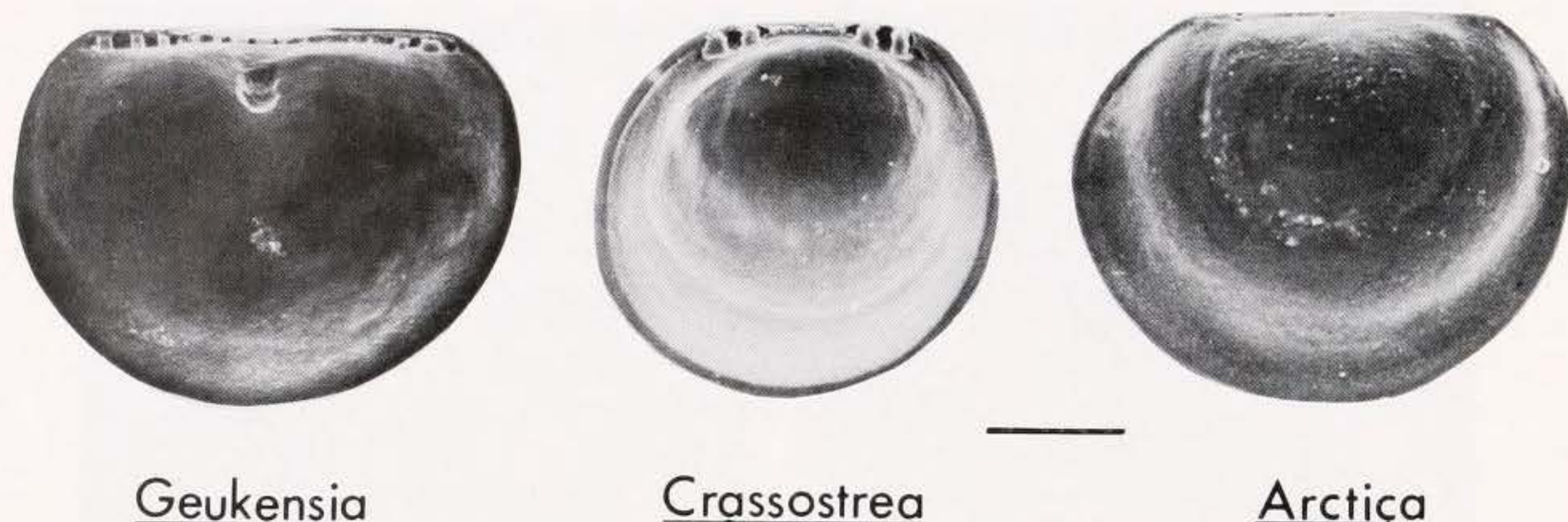


Figure 3. Scanning electron micrographs of disarticulated shell valves of straight-hinge larvae of three species of bivalve molluscs (*Geukensia demissa*, *Crassostrea virginica*, and *Arctica islandica*). Scale bar: 30 μm).

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