

# Early Stages in Morphogenesis of the Shell of *Crenella decussata* (Bivalvia: Mytilidae)

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## ABSTRACT

This study examines the morphological features of larval and postlarval shells of the paedomorphic bivalve *Crenella decussata* Montagu, 1808 from the Sea of Japan. During the early morphogenesis of the shell of *C. decussata* the following characteristics appear: prodissoconch I - the anterior and posterior provincial teeth, a broad primary ligament pit located on the chondrophore; nepioconch - the anterior and posterior juvenile teeth, primary lateral teeth, fine commarginal and radial sculpture. Larvae of *C. decussata* distinctly differ from other mytilid larvae by a D-shaped shell, absence of umbo, a straight hinge margin, and homogeneous fine-grained sculpture without co-marginal lines of growth. These shell characters indicate lecithotrophic development of this species. Some morphostructures are revealed which might be used in crenellin taxonomy.

**Key words:** prodissoconch I, II nepioconch provincial teeth, juvenile teeth, ligament pit, developmental type.

## Introduction

The family Mytilidae is one of the most widespread groups of bivalves in the intertidal and uppersubtidal zones of cold, boreal and subtropical waters of the World Ocean and often forms vast settlements that dominate in benthic communities (Seed, 1976; Scarlato, 1981; Lukanin et al., 1985; Zaika et al., 1990; Seed and Suchanek, 1992; Coan et al., 2000 and others.). On different sources, from 13 to 16 mytilid species inhabit Peter the Great Bay in the Sea of Japan (Kafanov, 1991; Scarlato, 1981), among them the investigated species *Crenella decussata*.

*C. decussata* is a boreal-arctic bivalve. In the Far Eastern seas, this species is found in the Sea of Japan - in Peter the Great Bay and off western Sakhalin Island, and in the Sea of Okhotsk - off eastern Sakhalin Island and the Kuril Islands. It is one of the smallest representatives of the family Mytilidae with a shell height not exceeding 4 mm. *C. decussata*

occurs in the subtidal and bathyal zones from 6 to 185 m depths at a temperature of 1.8 to 18.0°C on sand-pebble substrates. Individuals of this species usually live in groups (Scarlato, 1981; Evseev and Yakovlev, 2006).

Pelagic larvae of mytilids indeterminate are characterized by the morphological features peculiar to the Mytilidae larvae, namely shell outlines, shell dimensional ratios, hinge of the taxodont type, location of ligament, sculpture, and presence of an eye-spot (Rees, 1950; Chanley and Andrews 1971; Le Pennec, 1980; Ramorino and Campos, 1983; Kasyanov et al., 1998).

Three development types are found in mytilid mollusks: (1) direct development, in which the larva develops within an egg envelope in an egg mass on the bottom and the juvenile is released into the environment; (2) completely pelagic planktotrophic development, which occurs in the water column from gamete release to settlement, and the larva feeds on planktonic organisms; and (3) partly pelagic lecithotrophic development, which occurs within the egg until the larva hatches into the water column (Thorson, 1946, 1950; Mileikovskiy, 1971). Development of *Musculus laevigatus* (Gray, 1824),

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*Musculus niger* (Gray, 1824) is direct while in the life cycle of others there is a pelagic phase, which can be very short (*Seprifer keenae* Nomura, 1936 *Dacrydium vitreum* (Möller, 1842) or long, as is typical of most species of Mytilidae (Thorson, 1935; Jørgensen, 1946; Ockelmann, 1965 Kasyanov et al., 1998).

Data on the reproduction and development of mollusks of the Crenellinae and on morphology of their larvae are not numerous. The literature provides by limited data concerning general issues of the biology of reproduction and development of *C. decussata*. It is known that this species has separate sexes, usually not dimorphic in shell structure; fertilization occurs within the mantle cavity and young hatch as pelagic larvae (WoRMS, 2010). Ockelmann (1958, 1965) reported that the planktonic stage in this species is short or absent, probably lecithotrophic development. Jørgensen (1946) described the general structure and dimensions of the larvae of this species in the stage preceding settlement. Rusanova (1963) briefly described the embryonic development and indicated the size of larvae in the metamorphosis stage. Semenikhina with coauthors (2008) described only total morphology of larval shell size 190-200  $\mu\text{m}$ .

In the present work, we examine the early morphogenesis of the shell of *C. decussata* with the aim of identifying morphostructures of the larval-juvenile shell useful for taxonomy and showing the role of peculiarities of the prodissoconch structure for the development type of crenellid mollusks.

## Materials and Methods

Larval and early juvenile stages of *Crenella decussata* were taken in Vostok Bay of Peter the Great Bay in the Sea of Japan. Larvae were collected using a 96  $\mu\text{m}$  plankton net from the bottom to the water surface from July to August, 2003. Juveniles were sampled with a hand dredge.

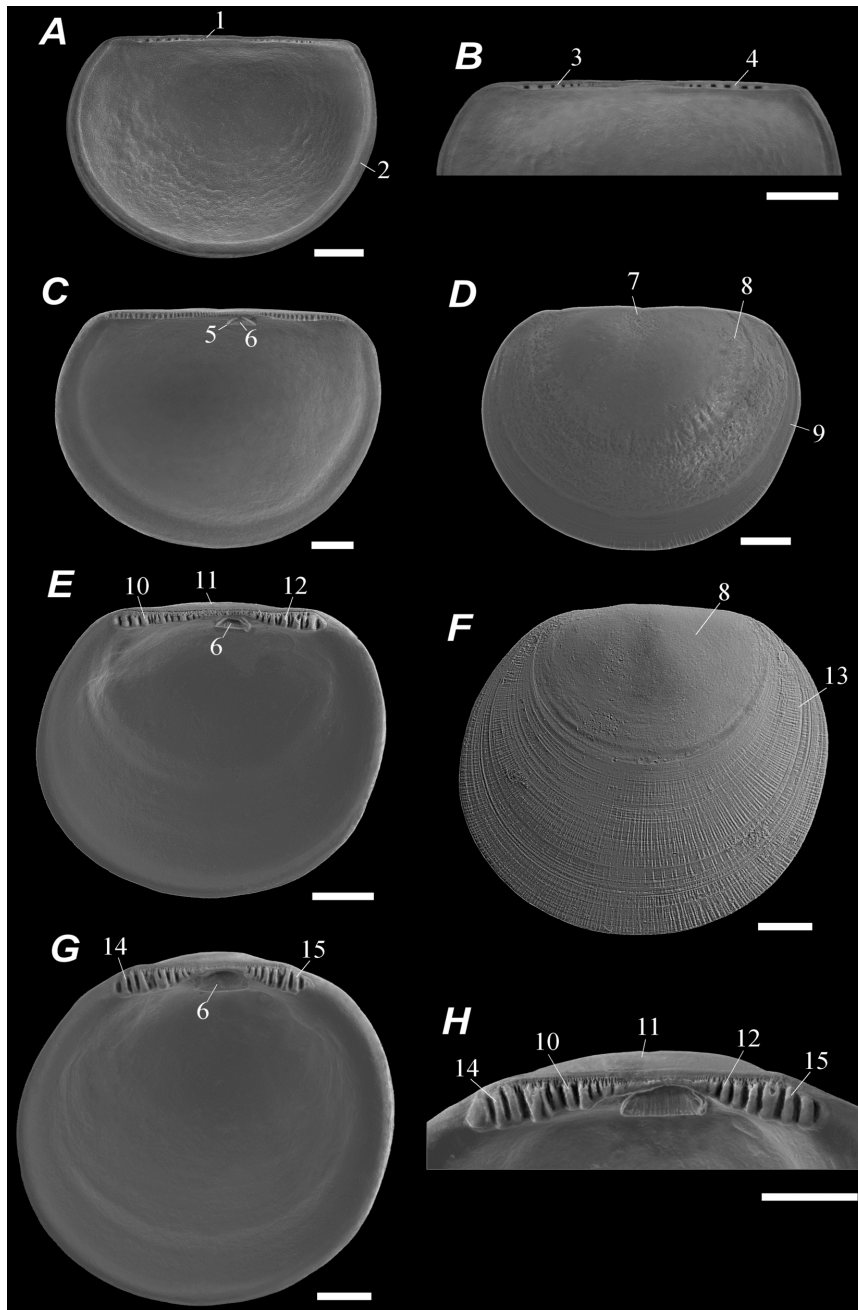
In the laboratory, samples of larvae were placed in 70% ethanol and sorted under a MBS-10 binocular microscope. Juvenile forms were extracted by washing the substrate samples through a series of sieves with a minimal mesh size of 250  $\mu\text{m}$ . For light and electron

microscopic observations, larvae and juveniles were submerged in a 5% solution of sodium hypochlorite for 1-3 min before their shells were disarticulated and cleared of the periostracum and soft tissues. The valves were washed in distilled water, and then placed in glycerol or dehydrated in 96% ethanol. The structure of the hinge system and the surface sculpture of larval and juvenile shells were examined under a Leica DM4500 B light microscope equipped with a Leica DFS300 FX camera and under a Leo430 scanning electron microscope.

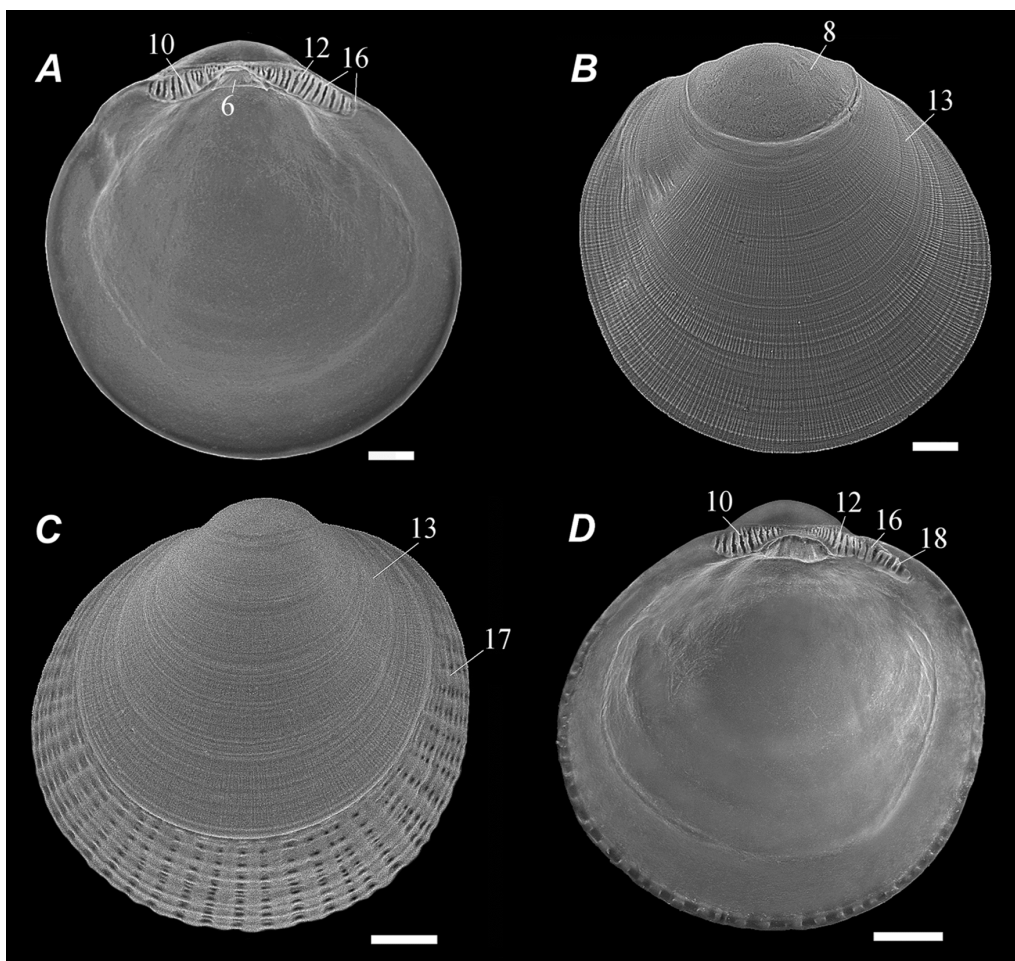
In the present study, morphogenetic series of two types were constructed (Evseev et al., 2001). Progressive series consisted of larval stages up to the juvenile and adult stages, and retrospective series consisted of adults to juveniles (including early post-larval) and larval stages. Larvae were also identified based on the descriptions of larval morphology of mytilid species available in the literature (Jørgensen, 1946; Rees, 1950; Chanley & Andrews, 1971; Le Pennec & Masson, 1976; Booth, 1977; Lutz & Hidu, 1979; Le Pennec, 1980; Redfearn et al., 1986; Fuller & Lutz, 1989; Kasyanov et al., 1998 Semenikhina & Kolotukhina, 2001; Malchus, 2006; Evseev & Kolotukhina, 2008; Semenikhina et al., 2008; Evseev et al., 2010). Larval shell terminology is the same as in the foregoing works.

## Results

The post-trochophore development of *C. decussata* begins with the formation of a D-shaped prodissoconch I (PD-I), usually at a length of 170-190  $\mu\text{m}$  (Fig. 1A). The shell (PD-1) is equilateral, in the form of an almost regular semicircle. The anterior and posterior ends are rounded; the anterior end is more obtuse. The ventral margin is rounded. Umbo is absent. The shell is relatively high a ratio of height to length is from 0.78 to 0.79. The inner broad marginal ridge is clearly visible from the outside of the shell. The larval valves are thin; larvae are dark-grey. The eye-spot is imperceptible. The narrow provinculum bears 8-10 anterior and posterior teeth (Fig. 1B); in the centre of the hinge line denticles are not distinct. Before long, the number of these teeth is more than



**Fig. 1.** Larval and earliest postlarval development of *Crenella decussata*. **A.** Right valve of the prodissoconch I (PD-I) internally. **B.** Provincular morphostructures. **C.** Primary ligament formation. **D.** PD-I externally and the earliest stage of nepioconch (N). **E.** Original umbo, formation of the postprovinculum and juvenile teeth. **F.** Sculpture of the N. **G.** General outlines of shell and widened pit of the primary ligament. **H.** Details of the postprovincular teeth and ligamental pit. 1, provinculum; 2, marginal rim; 3, anterior provincular teeth; 4, posterior provincular teeth; 5, chondrophore; 6, pit of the primary ligament; 7, middle saddle; 8, vesicular sculpture of the PD-I; 9, the initial phase of N-formation; 10, anterior juvenile teeth; 11, umbo; 12, posterior juvenile teeth; 13, fine comarginal-radial ridges of the N; 14, anterior new teeth of the postprovinculum; 15, posterior new teeth of the postprovinculum. Scale bars: A - D = 30  $\mu$ m; E - H = 50  $\mu$ m.



**Fig. 2.** Juvenile development of *Crenella decussata*. **A.** The initial phase of formation of the primary lateral teeth (L1). **B.** General outlines of the nepioconch N. **C.** Early dissoconch(D). **D.** The secondary lateral teeth L2. 16, primary lateral teeth L1. 17, commarginal-radial ridges of the D. 18, secondary teeth L2. For others see Fig. 1. Scale bars: A, B = 50  $\mu$ m; C-D = 100  $\mu$ m

double. Under the provinculum, a pit of the primary (inner) ligament appears, being placed on a triangular chondrophore (Fig. 1C). Externally, the surface of PD-I (Fig. 1D) is covered with small knobs and pits; in the middle the surface is somewhat smooth and saddled the growth lines are absent.

At a shell length of 200  $\mu$ m, the pelagic larva settles to the sea bottom. The shell becomes less transparent and acquires a dark grey color. This stage begins with the development of a new morphostructure, the nepioconch (N), which grows until it reaches the margins of the PD-I. Initially, the shell shape is close to oval-truncated (Fig. 1E). The postprovinculum bears irregular anterior and posterior juvenile teeth.

Among them, some are formed by the union and elongation of two or seldom three provincular teeth, others are newly formed following the anterior and posterior growth of the postprovinculum. At the same time, the number of teeth decreases to 9-10 anteriorly and 6-7 posteriorly. The juvenile teeth of the central part of the postprovinculum are indistinct. The primary ligament pit is broadened. The surface of the nepioconch (Fig. 1F) is covered with an irregular sculpture of very fine radial and commarginal ridges.

At the middle stage, when the shell length is about 350-400  $\mu$ m (Fig. 1G), its form becomes nearly round, and a low umbo begins to rise above the postprovinculum. The length of juvenile teeth

increases markedly. The number of teeth, however, has not changed (Fig. 1H). The pit of the primary ligament is more broadened, with thick edges anteriorly and posteriorly.

At a shell length of 450-500  $\mu\text{m}$  (Fig. 2A), the umbo takes an almost symmetric form. The postprovinculum bears 9-10 irregular anterior and 6-7 posterior teeth, as previously observed. Nevertheless, at the very end of the posterior tooth row, 3-5 primary lateral teeth appear. These teeth do not differ markedly from the juvenile teeth, excluding their inclination. At the end of the nepioconch stage, when shell length is more than 500  $\mu\text{m}$ , they are reduced suddenly. The nepioconch surface (Fig. 2B) bears almost regular and fine radial riblets and low commarginal ridges.

Thus, during the early morphogenesis of the shell of *C. decussata* the following characteristics appear: prodissoconch I - the anterior and posterior provincial teeth, a broad primary ligament pit located on the chondrophore; nepioconch - the anterior and posterior juvenile teeth, primary lateral teeth, fine commarginal and radial sculpture (Fig. 2 C, D).

### Discussion

Pelagic larvae of the family Mytilidae can be distinguished by their shell outlines; dimensional parameters including length of the anterior shoulders; shell height to length ratio; outlines and size of umbones; sculpture; color, and shape and size of an eye-spot. The hinge of the taxodont type consists of large regular rectangular denticles located on each end of the hinge line and, in most cases, small denticles in the central part of the provinculum that is invariably a common internal feature of larvae of all mytilid species. Also in all mytilid larvae studied the ligament is placed in the central part of the provinculum (Rees, 1950; Chanley and Andrews 1971; Le Pennec, 1980; Ramorino and Campos, 1983; Kasyanov et al., 1998).

Larvae of *C. decussata* distinctly differ from other mytilid larvae by a D-shaped shell, absence of umbo, the straight hinge margin, and homogeneous

fine-grained sculpture without co-marginal lines of growth (Semenikhina et al., 2008). Morphostructures of *C. decussata*, which form in the pre-dissoconch (PD-I, N) and early dissoconch (D-I) stages, can be regarded as specific or generic features. In determining the taxonomic status of these characters, it is necessary to compare them with the early morphostructures of other taxa of Crenellinae.

In the ontogeny of other small forms of this group the morphostructures of some taxa, including *Exosiperna rex* Iredale, 1929 and *Rhomboidella kuroharai* Habe, 1961 show a strong resemblance to those of *C. decussata*: nepioconch with a fine commarginal-radial sculpture and teeth L1. In ontogenetic patterns of large forms, the morphostructures homologous to those of *C. decussata*, for instance teeth L1, and L2, also occur. However, in this case, none of them are fully identical to the morphostructures of *C. decussata* and other small-sized taxa. Thus, like *C. decussata*, *E. rex* and *R. kuroharai*, the shells of larger-sized *Megacrenella columbiana* (Dall, 1897) and *Solamen spectabilis* (A. Adams, 1862) are rounded, but their nepioconchs lack radial ribs (Evseev et al., 2007).

The foregoing shell characters of the larvae of *C. decussata* indicate lecithotrophic development of this species. Development with lecithotrophic larva is common to small Autobranchia forms (often less than one centimeter long). This development type is found also in all Protobranchia, irrespective of the habitat. A brief pelagic stage can be present or lacking. Like most species characterized by small size, *C. decussata* possesses a short pelagic stage, which can continue for several hours to several days, depending on the temperature conditions. According to our data, in Peter the Great Bay, Sea of Japan, larvae were recorded in the plankton in July-August at a water temperature of 18-22°C and the pelagic stage lasts no longer than 1 day. In Kandalaksha Bay, White Sea, mature oocytes of this species are found from November to June and have a size of 120-200  $\mu\text{m}$ . In June, at a temperature of 4-7°C, light green egg masses shaped like a globe are found from 4 to 10 m depth attached on one side to the bottom. There are

about 500 eggs per egg mass. Larvae in the trochophore stage are released. The shell begins to form in the trochophore at a length of 175  $\mu\text{m}$ . Development from fertilized egg to settlement continues for 10 days at a temperature of 7-8°C (Rusanova, 1963).

Thus, we showed in the example of *C. decussata* that the specific features of the morphostructures of the larval shell (the presence of only prodissoconch I and nepioconch, the lack of prodissoconch II and the lack of growth lines), as well as the relatively large size of ripe eggs and larvae indicate the lecithotrophic type of development and a short pelagic stage. The morphostructures of the larval and early postlarval shells identified in the example of *C. decussata* can be useful in diagnostics and taxonomy, as in the issues relating to the reproductive strategy of Bivalvia.

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