POLYMORPHISM IN FREE-SWIMMING LARVAE OF *HALISARCA DUJARDINI* (DEMOSPONGIAE, HALISARCIDA)

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ABSTRACT

This paper discusses the embryonic development of *Halisarca dujardini* Johnston, 1842 (Demospongiae, Halisarcida). Different morphotypes of free-swimming larvae are formed: parenchymella-like, coeloblastula-like larvae and disphaerulae larvae. Larvae with different morphological characters may develop in the same parent and remain morphologically distinct until metamorphosis. We have identified a number of characters that emerge during embryogenesis, determined the developmental stage when they emerge as well as possible morphogenetical processes causing their variability. The complexity of morphogenesis in *H. dujardini* is indicative of the low level of determination that occurs during embryogenesis.

KEY WORDS

Larvae, larval polymorphism, sponges, embryology, *Halisarca dujardini.*

INTRODUCTION

The basic problem of developmental biology is description of pattern formation processes. Different morphogenetical processes compose pattern formation and their mechanisms are different for different kinds of Metazoa. Sponges occupy a basal position in all modern phyletic studies of metazoan relationships. For this reason the complexity of sponge's morphogenetical processes can be considered as one of the most archaic.

Polymorphism is a characteristic feature of Porifera (BERGQUIST, 1978; SIMPSON, 1984). The phenomenon of polymorphism characterizes earlier onthogenetic stages (embryonic development, larvae, metamorphosis) as well. The investigation of morphological variability in embryonic development and free-swimming larvae is important for understanding the mechanism of genetic regulation in comparison with unstable of cells morphogenesis.

Halisarca dujardini Johnston, 1842 is one of the most investigated species (LÉVI, 1956; CHEN, 1976; KOROTKOVA & ERMOLINA, 1982; KOROTKOVA & ERESKOVSKY, 1984; ERESKOVSKY & GONOBOBLEVA, 2000). Cleavage, embryonic development and formation of the disphaerula larva have been described previously (KOROTKOVA & ERESKOVSKY, 1984; ERESKOVSKY & GONOBOBLEVA, 2000). However, we paid no attention to polymorphism phenomenon.

The aim of the present study is to describe the morphogenetic processes which causing variability of larval morphology.

MATERIAL AND METHODS

Reproducing specimens of *Halisarca dujardini* Johnston, 1842 were collected in the Chupa Inlet near the Srednij Island 33°05' E 66°15' N (Kandalaksha Bay, White Sea) from the depth of 1,5 - 5 m in June - July 2000 - 2001. Larvae and tissue fragments were prefixed in 1 % OsO_4 for 10 min and fixed in 2.5 % glutaraldehyde in phosphate buffer (pH 7.4) at room temperature for 1 h. After fixation, larvae were washed in the phosphate buffer (pH 7.4) and postfixed in 1 % OsO_4 in phosphate buffer for 1 h. Samples were dehydrated through a graded ethanol series and embedded in Epon-Araldit. For each stage serial semi-thin sections 1 μm thick were cut, mounted on glass slides, and stained with methylene blue-borax. For Scanning Electron Microscopy (SEM) the specimens were fractured in liquid nitrogen, critical-point-dried, sputter-coated with gold-palladium, and observed under a Hitachi S 570 SEM.

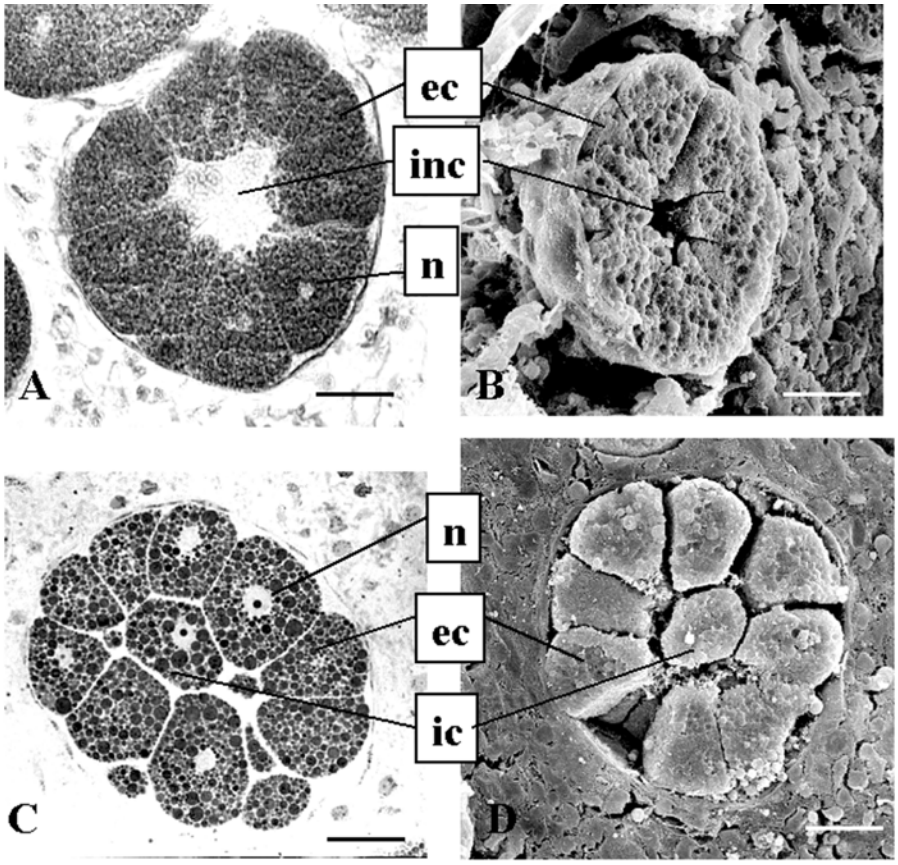

Fig. 1. *Halisarca dujardini.* Two types of blastulae are formed during compaction. **A, B,** hollow blastula and **C, D,** blastula with internal cells in cavity. **A, C,** light microscopy. **B, D,** scanning electron microscopy. ec - external cells; ic - internal cells; inc - internal cavity of blastula; n - nucleus. Scale bars - 30 μm.

RESULTS

Embryogenesis and larval development of *Halisarca dujardini* proceed synchronously within the population. The volume of reproductive elements reaches to about 70 % of sponge volume. In the White Sea population the larvae of *H. dujardini* exit synchronously from different specimens in the middle of July.

Several stages compose the embryonic development of *H dujardini*. Equal, asynchronous, complete cleavage results in the formation of a blastula. The blastula becomes compacted: the contact area between blastomeres is increased, and blastomeres acquire apical-basal polarity. Within the same parent sponge, compaction of the embryos can occur at the different stage - about 16 - 64 blastomeres.

Two morphological types of earlier blastulae are formed during compaction. One type consists of external and internal layer of cells (Figs 1C, D), while the other type only has external cells (Figs 1A, B). Both types of embryos may be found in the same parent. From this stage on to the final stage of embryogenesis, multipolar ingression takes place: single external cells move into the internal cavity. All mitotic spindles in the external cells of the blastula are oriented parallel to the embryonic envelope. The formation of the flagellum can first be seen at the 32 - 64 cell stage (which is partly due to the embryo compaction). External cells of the blastula will differentiate into flagellated cells, while internal cells will differentiate to become the nucleolar amoebocytes.

We can observe these two types of embryos at the middle blastula stage (~ 200 cells) (Figs 2A-D). From the embryonic stage with about 800 cells up to the final stage of embryogenesis one can observe two important events: first, the maternal granular eosinophilic cells penetrate into the embryos and second, the blastulae become wrinkled or convoluted as they increase in size. The arrangement and depth of wrinkles are different in each embryo in the same parent sponge. One of the deep invaginations of external flagellated cells becomes an internal flagellated chamber in some embryos (Figs 3A, B).

The size of the larvae varies from 120 - 152 μm in diameter and 112 - 136 μm in length. Anterior-posterior polarity is evident in the structure of the external layer of flagellated cells. The number of cells in the layer at the posterior pole and the anterior hemisphere of the larva vary, and so does their size (Figs 4, 5). Thus, the curvature of the poles and, consequently, the shape of the larvae are slightly different.The larval cells belong to three types: flagellated, nucleolar amoebocytes and granular mother cells. The external layer of flagellated cells borders the internal cavity. The number of amoeboid cells in the cavity varies significantly. Larval morphotypes of *H. dujardini* are delimited on the basis of the differences in the internal cavity structure. If it is filled by a conglomerate of amoeboid cells, the larvae are called parenchymulae-like (Figs 4B, C). The larvae with only single amoeboid cells in the cavity we called coeloblastulae-like (Fig. 4A). In some larvae, an inner chamber (rarely two) is present, formed by flagellated cells, whose ultrastructure is identical to that of the external flagellated cells of the anterior larval hemisphere. The number of the cells forming the chamber, their shape, and, correspondingly, the size of the chamber, varies (Figs 4D-F). We called these larvae disphaerulae.

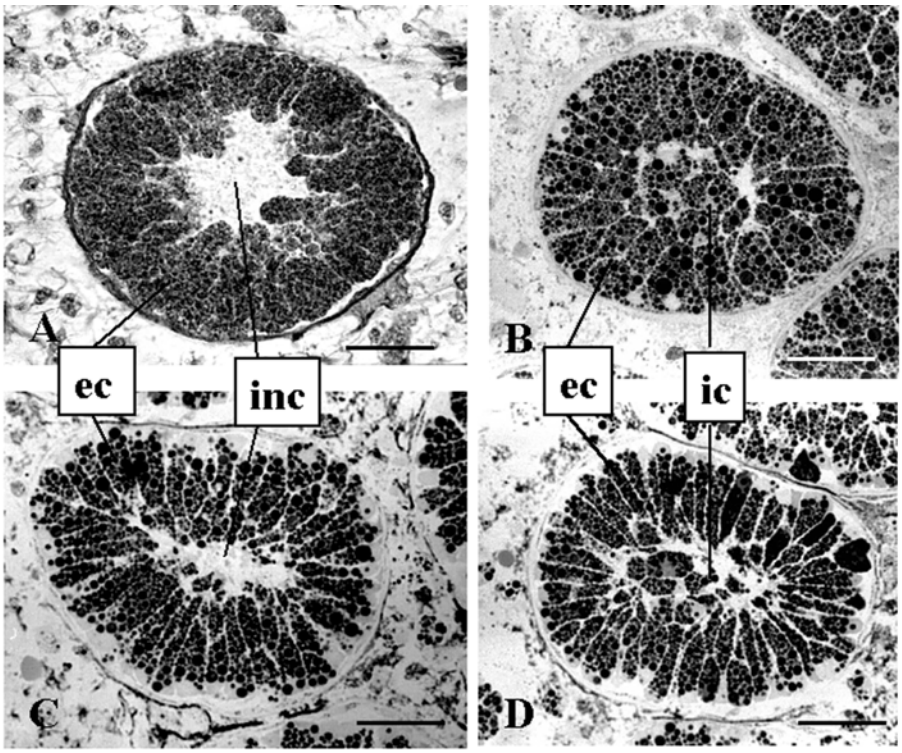

Fig. 2. *Halisarca dujardini*. Two types of blastulae at the middle stages of embryogenesis. **A, B,** ~ 200 cells. **C, D,** ~ 600 cells. **A, C,** hollow blastula. **B, D,** blastula with internal cells in cavity. ec - external cells; ic - internal cells; inc - internal cavity. Scale bars - 30 μm.

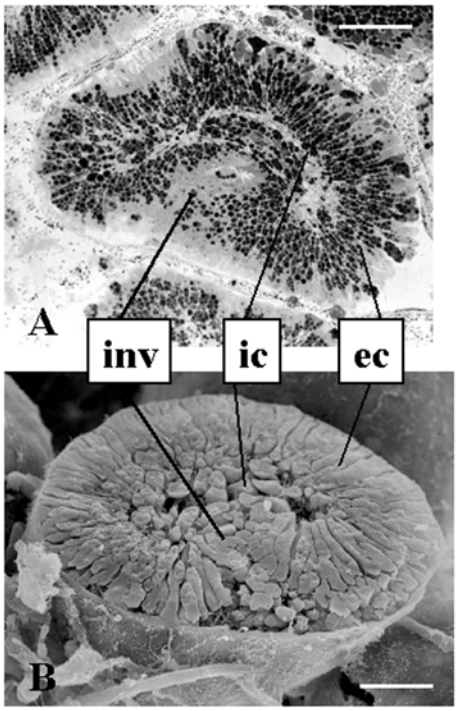

Fig. 3. *Halisarca dujardini.* Formation of disphaerulae larvae by invagination of the external flagellated cells. **A,** light microscopy. **B,** scanning electron microscopy. inv - invagination of external cells layer; ic - internal cells of embryo; ec - external cells of embryo. Scale bars - 30 μm.

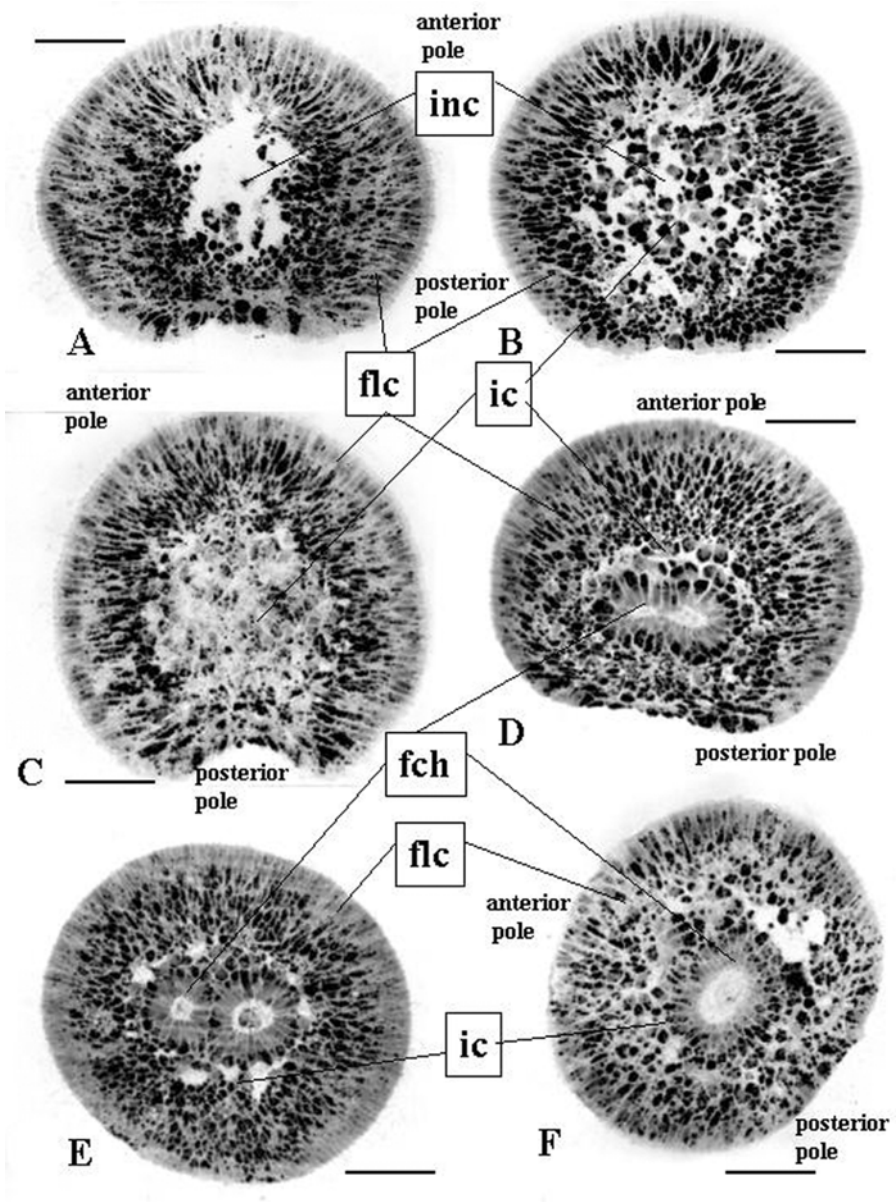

Fig. 4. *Halisarca dujardini*. Semi-thin sections of different types of free-swimming larvae *H. dujardini*. **A,** coeloblastula-like larva; **B, C,** parenchymella-like larvae. **D-F**, disphaerulae larvae. flc - external flagellated cells of larva; fch - internal flagellated chamber; ic - internal cells; inc - internal cavity. Scale bars - 30 μm.

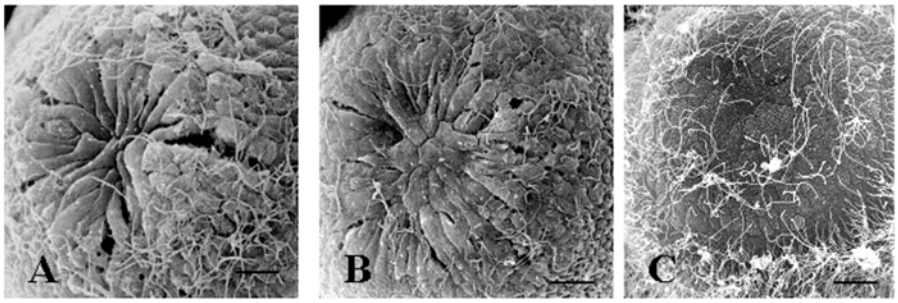

Fig. 5. *Halisarca dujardini*. Scanning electron micrographs of external cells at the posterior pole of different larvae. Scale bars - 10 μm.

DISCUSSION AND CONCLUSIONS

Although the phenomenon of polymorphism of larval morphology has been discussed by other authors (WOOLLACOTT, 1993; IVANOVA, 1997; ERESKOVSKY & GONOBOBLEVA, 2000), no studies have addressed the genesis of these structures.

Taking as basis results of our investigation we can conclude that all structures of the free-swimming larvae of *H. dujardini* are formed during embryonic development. Variability of embryonic morphogenesis results in morphological variability. There are a few key morphogenetic processes in embryonic development of *H. dujardini*: cleavage, embryo compaction, multipolar ingression, formation of a wrinkled embryo and invagination and pinching off of the external flagellated epithelium. These set of types of morphogenesis are characteristic of development in all Metazoa. At the same time, the high degree of variability of these morphogenetic processes in *H. dujardini* testify to the low level of determination that must occur during embryogenesis.

Recent data has confirmed that many of the fundamental components of the highly conserved regulatory program used in bilaterian development were present in the ancestors of present day sponges (DEGNAN *et al.,* 2002). Though the cellular pattern of embryonic morphogenetic processes of sponges is also similar to that of other Metazoa, it is likely that sponges have significant differences in morphogenesis regulation mechanisms.

Another important problem is the function of the larval structures. On the basis of our observations, different morphotypes of *H. dujardini* larvae can settle and form new sponges. But the differences in metamorphosis of these larvae remain unclear.

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