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LARVAL SKELETOGENESIS IN THE CRINOID *ANTEDON MEDITERRANEA* AND THE OPHIUROID *AMPHIURA FILIFORMIS*: A MORPHOLOGICAL, MOLECULAR AND EVOLUTIONARY APPROACH

The endoskeleton is a distinctive feature of echinoderms and consists of a true skeletal tissue with a complex microstructure (stereome). Skeletal elements can be present also in larval stages, although they differ markedly among classes. Therefore, the comparative study of larval skeletogenesis in different echinoderm classes can provide insights into the origin and evolution of this character.
In this study we analysed the skeletogenic process in the crinoid *Antedon mediterranea,* which represents the basal echinoderm class, and the ophiuroid *Amphiura filiformis*. In *A. mediterranea* the doliolaria and the post-metamorphic pentacrinoid stages were mainly investigated, whereas in *A. filiformis* the blastula, gastrula, prism and pluteus stages were analysed. Detailed morphological investigations included both light and electron (SEM, TEM) microscopy techniques. Furthermore, a preliminary molecular analysis was carried out in *A. mediterranea* by cloning for the first time twelve skeletogenic genes already studied in *A. filiformis*, and studying their expression and presence by whole mount *in situ* hybridization (WMISH) and Quantitative-PCR (Q-PCR).
Our results confirm that in *A. mediterranea*, primordial pentacrinoid skeletal elements are already present in the doliolaria stage. Spicule and plate formation starts in a planar multi-branched arrangement and continues throughout the metamorphosis process, until their complete three-dimensional stereome-like architecture in the pentacrinoid stage. Sclerocytes differentiate, produce short cellular expansions and develop calcitic elements in a highly cellularized and collagen rich mesenchyme. It is still unclear, however, if these elements originate within a syncytium. Differently, in *A. filiformis* thin tri-radiate and three-dimensional spicules are formed by primary mesenchyme cells (PMCs), following a process very similar to that described in sea urchins. PMCs differentiate in an extremely loose mesenchyme, produce long and branched fillopodia and eventually form a syncytium where calcium carbonate is deposited without furtherly developing in a stereom microstructure.
Preliminary molecular analysis indicates that skeletogenesis gene orthologues of *A. filiformis* are present and expressed in areas of active skeleton formation also in the crinoid, suggesting that the overall genetic program for skeleton development was already present in the ancestor groups of echinoderms and has been conserved in all the five extant classes for several millions of years.
Interestingly, in terms of skeletogenesis processes, the data obtained so far in different classes, comparing larval stages characterized by different life-style and cycle, indicate indeed that the cellular mechanisms can significantly diverge among groups adapting to specific functional requirements. In this view the skeleton of the lecitotrophic doliolaria, which represents the primordium of a persisting adult stereome structure, could be the primitive adaptive solution for these short-range dispersion larvae; in contrast, the specialized temporary skeleton of planktotrophic ophiuroid larvae could be an advanced strategy designed to maximize long-range dispersion capabilities.

Further comparative studies on skeleton formation in different echinoderm classes, and particularly in crinoids, are necessary to complete the knowledge about its evolution.