

**Title:**

Periostracum formation in *Sepia officinalis* (Sepiidae, Cephalopoda)

**Authors (first name, family name) and affiliations:**

Ernesto, Ruiz Villaespesa<sup>1</sup> (first author)

Carmen, Salas <sup>2</sup> (second author)

Antonio G., Checa<sup>1</sup> (third author)

<sup>1</sup> Departamento de Estratigrafía y Paleontología, Universidad de Granada, 18071 Granada, Spain.

<sup>2</sup> Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain

**Keywords:**

*Sepia officinalis*, embryo, periostracum, shell sac epithelium, vesicles, chitin

**Abstract:**

The morphology, composition and mechanical properties of the *Sepia* cuttlebone have been intensively studied. Since the first detailed descriptions by Appellöf (1893), much work has been conducted on the adult shell structure and microstructure. Evolution of the shell from the embryo to the adult was thoroughly detailed by Bandel and Boletzky (1979) and more recent contributions were added by Le Pabic et al. (2016, 2019) and Dauphin et al. (2020). Nevertheless, the shell sac epithelium and his involvement in shell formation have been little studied. At present, there is no clear evidence of how the cells of shell sac epithelium participate in biomineralization. Our goal is to characterize the shell-forming tissues and structures in *Sepia officinalis*, elucidate their role in biomineralization and search possible homologies with other molluscs. Embryos of *S. officinalis* collected from the coast of Málaga were anesthetized and fixed in glutaraldehyde. Histochemical and TEM analyses of the shell sac were conducted in paraffin- and resin-embedded specimens, respectively. Four types of epithelia have been differentiated: simple squamous (dorsal), transitional (lateral bending zones), columnar (lateral fold tips) and simple cuboidal (ventral). The dorsal shield originates at the lateral fold tips where columnar cells secrete numerous vesicles, possibly containing chitin, as indicated by calcofluor dyes. These folds seem deeper than previously depicted and resemble a periostracal groove, from which a periostracum emerges. This periostracum coarsens due to the addition of laminae secreted by the upper and lower epithelia. Thus, the dorsal shield is homologous to the periostracum, which constitutes a support for further mineralization.