

SUPPLEMENTARY MATERIAL

Detailed description of the operculum of *Hexaplex trunculus*

Comprehensive knowledge on the gastropod operculum is essential to elucidate the underlying principle of using this structure for ageing purposes. In this context, the following paragraphs present a detailed description of the operculum of the banded murex (*Hexaplex trunculus*), including information on the type, function, morphology, structure and composition.

The operculum of *H. trunculus* is brownish (light-brown in smaller specimens and dark-brown in older individuals), roughly oval-shaped and moderately slim (Figures 1A,B). The operculum is corneous or horny, i.e. the bulk of the opercular material is composed mainly by a proteinaceous substance known as conchiolin, more or less impregnated with salts of calcium (Fretter & Graham 1994; Checa & Jiménez-Jiménez 1998).

As typically found in siphonostomatous shells, *H. trunculus* has a rigiclaudent operculum (aperture fitting) (Figure 1A) that is shaped to conform perfectly to the shell aperture and almost does not flex when the organism retracts and seals the opening (Checa & Jiménez-Jiménez 1998). Nevertheless, because conchiolin is not rigid, the edges of the operculum are somewhat flexible (the edges flex and bend outwards as the animal withdraws into the shell, except for the columellar edge which is buried in the opercular groove) (Fretter & Graham 1994).

Like most neogastropods, *H. trunculus* has a concentric operculum (Checa & Jiménez-Jiménez 1998) with a nucleus in the left side of the siphonal edge of the operculum (siphonal nucleus, representing the beginning of its formation) (Figure 2B). In species with a relatively long and wide siphonal canal (such as *H. trunculus*), concentric opercula with siphonal nucleus allow for a more precise fitting of the opercular edge to the shell aperture (Fretter & Graham 1994; Checa & Jiménez-Jiménez 1998). Indeed, concentric opercula have a band in the internal surface (close to the labrum), which wedges outward and becomes concave towards the edge of the operculum (Figure 2A), providing an exact fit to the interior of the shell aperture (Checa & Jiménez-Jiménez 1998).

The underside of the operculum is attached to the dorsal surface of the foot by the operculigerous disc (metapodium) (Fretter & Graham 1994), which is a conspicuous disc-like epithelium quite distinct from the surrounding foot (Checa & Jiménez-Jiménez 1998). The operculigerous disc is never fully adhered to the internal surface of the operculum, being restricted mainly to the labial half of the operculum (Checa & Jiménez-Jiménez 1998). Most part of the operculum underside is roughened and with a fingerprint-shape (coinciding with the area of attachment of the columellar muscle) (Figure 2A) (Fretter & Graham 1994). In horny opercula, a shiny material (known as varnish or gloss) secreted by the foot epithelium is applied underneath the conchiolin (Fretter & Graham 1994; Checa & Jiménez-Jiménez 1998). This varnish layer is limited to a strip around the labial and siphonal edges of the operculum, where the concentric marks are covered and sealed underneath by the gloss (Figure 2A) (Fretter & Graham 1994).

Concentric opercula grow by addition of marginal increments of new material to the edges of the operculum (Fretter & Graham 1994; Checa & Jiménez-Jiménez 1998). For this reason, the operculum of *H. trunculus* displays consecutive concentric lines on the external surface (surface striae), lying around the terminal nucleus (Figure 2B), which represent growth marks (Fretter & Graham 1994). The internal surface of the operculum also shows a series of concentric marks related to an eccentric nucleus (Figure 2A), but without relationship with the growth marks on the opposite side (Fretter & Graham 1994). These concentric marks are due to plates of conchiolin added at the underside of the operculum and lying below the main layers,

known as adventitious layers (Fretter & Graham 1994) or accessory layers (Checa & Jiménez-Jiménez 1998) (Figures 3C,D). Adventitious or accessory layers have only been reported in concentric opercula, are secreted by the operculigerous disc, and presumably strengthen the operculum in the area of attachment of the columellar muscle (Fretter & Graham 1994; Checa & Jiménez-Jiménez 1998).

References

- Checa AG, Jiménez-Jiménez AP. 1998. Constructional morphology, origin, and evolution of the gastropod operculum. *Paleobiology* 24:109–32.
- Fretter V, Graham A. 1994. *British Prosobranch Molluscs: Their Functional Anatomy and Ecology*. London: The Ray Society. 820 pages.