



FEEDING BEHAVIOUR AND THE OPERCULUM IN OLIVIDAE (GASTROPODA): THE CASE OF *CALLIANAX BIPLICATA* (G. B. SOWERBY I, 1825)

MOLLY KELLY¹, LINDA SCHROEDER², DANIEL L. MULLENDORE³, BENJAMIN F. DATTILO¹,
WINFRIED S. PETERS^{1,3,4*}

¹Purdue University Fort Wayne, Department of Biology, 2101 Coliseum Boulevard, Fort Wayne, IN 46805, USA (e-mails: dattilob@pfw.edu; petersw@pfw.edu);  BFD <https://orcid.org/0000-0002-2917-6777>,
 WSP <https://orcid.org/0000-0002-5061-1497>

²Pacific Northwest Shell Club (PNWSC.org), Seattle, WA, USA

³Washington State University, School of Biological Science, Abelson Hall, Pullman, WA 99164, USA
(e-mail: mullendore@wsu.edu)

⁴Goldring-Gund Marine Biology Station, Playa Grande, Santa Cruz, Guanacaste, Costa Rica

*corresponding author

ABSTRACT: Olividae are marine gastropods living as predators or scavengers on soft sediments. The complex prey handling behaviour of large predatory species includes the storage of food in a pouch formed temporarily by bending and contraction of the posterior foot. Such metapodial pouches had been observed only in Olividae that lack an operculum, prompting the hypothesis that the folding of the metapodium into a pouch biomechanically required the absence of the operculum. Here we report metapodial pouch formation in an operculate olivid, *Callianax biplicata* (formerly *Olivella biplicata*). Since the operculum is too small to close the shell aperture in mature *C. biplicata*, a protective function seems unlikely. The operculum may rather serve as an exoskeletal point for muscle attachment, but may also represent a ‘vestigial organ’ in the process of evolutionary reduction, or an ontogenetic remnant functional at early life stages but not at maturity. Consequently, our observations refute the notion that only inoperculate olivids can form pouches, but not necessarily the idea that the ability to form a metapodial pouch evolved in parallel with operculum reduction.

KEY WORDS: biomechanics; *Callianax*; Olividae; metapodial pouch; operculum; vestigial organ

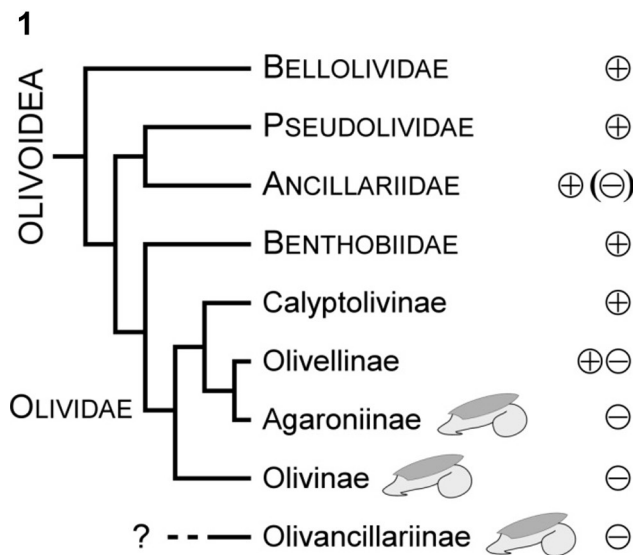
INTRODUCTION

Olive shells are marine gastropods which mostly live as scavengers and predators on soft sediments in shallow marine and intertidal habitats (ZEIGLER & PORRECA 1969, TURSCH & GREIFENEDER 2001). Because of their size and appealing appearance, the shells are sought after by collectors (STERBA 2004). The resulting tendency to describe even minor variations as new taxa has contributed to considerable taxonomic confusion, especially in the large genus *Oliva* (TURSCH & GREIFENEDER 2001). The most recent revision of olive shell systematics subdivided the superfamily Olivoidea (Caenogastropoda, Neogastropoda)

into five taxa of family rank (KANTOR et al. 2017). Of these families, the Olividae are the largest with about 270 species, more than half of all the species in the Olivoidea (MOLLUSCABASE 2020). Molecular data further suggested the division of the Olividae into four subfamilies: Olivinae, Calyptolivinae, Agaroniinae, and Olivellinae (KANTOR et al. 2017; Fig. 1). The inclusion of the Olivellinae was not necessarily expected, as this taxon, which consists of comparatively small species (OLSSON 1956), previously had been listed as a family of its own (Olivellidae; KANTOR 1991, BOUCHET & ROCROI 2005). Both the

maximum likelihood and Bayesian analysis of the available sequence data of four genes suggested the Olivinae as the sister group of a clade comprising the other three subfamilies, and within that clade the Olivellinae as the sister group of the Agaroniinae (Fig. 1; see also KANTOR et al. 2017: figs 3, 4).

Predatory Olividae show complex hunting behaviour, as has been described for several *Oliva* spe-



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Figs 1–2. In olivid gastropods, the habit of storing food in a pouch formed by the posterior metapodium appears to correlate with the lack of an operculum: 1 – Simplified model of the phylogenetic hypothesis concerning the superfamily Olivoidea by KANTOR et al. (2017). Cladistic relationships between the five recognised families in the Olivoidea and the subfamily structure of the Olividae are shown. The Olivancillariinae were not included in the analysis, but may represent a fifth subfamily of the Olividae. The Agaroniinae, Olivinae, and Olivancillariinae are predators known to store captured prey in a metapodial pouch (compare Fig. 2), as highlighted by the cartoons. In these three taxa, opercula are generally missing. Opercula are absent also in some Olivellinae and a few Ancillariidae, but are present in all other taxa, as indicated by plus and minus signs on the right; 2 – *Agaronia propatula* with prey in a metapodial pouch (asterisk; from Supplemental Video 1)

cies (Olivinae; OLSSON & CROVO 1968, ZEIGLER & PORRECA 1969, TURSCH 1991, TAYLOR & GLOVER 2000, KANTOR & TURSCH 2001). The animals usually rest burrowed in the sediment, but emerge as soon as they sense the presence of food olfactorily. They then cruise rapidly on the substrate, apparently unguided by any long-distance sensory capabilities. Upon encountering a potential food item, they seize it with the anterior part of the foot, the propodium. Next, the propodium bends ventrally and backwards to transfer the item to the ventral side of the posterior foot, the metapodium, which simultaneously curves forward to form a more or less spherical pouch that encloses the item. Once the prey is securely stored in this metapodial pouch, *Oliva* usually burrows into the substrate where it feeds on its catch by inserting its long, protractile proboscis into the pouch. Members of the genus *Agaronia* (Agaroniinae) exhibit very similar behaviour. The trophic relationships and prey handling of one of them, tentatively identified as *A. propatula* (Conrad, 1849), have been studied in some detail (CYRUS et al. 2015, ROBINSON & PETERS 2018, VEELTENTURF & PETERS 2020), and this includes the usage of the metapodial pouch in storing prey (RUPERT & PETERS 2011, CYRUS et al. 2012). Fig. 2 and Supplemental Video 1 present an example of this behaviour.

As emphasised by KANTOR et al. (2017), all known Olivinae and Agaroniinae are devoid of opercula. Members of other taxa of the Olivoidea generally possess opercula, although some Olivellinae and a few Ancillariidae also appear to lack these structures. The phylogenetic hypothesis shown as Fig. 1 implies that an evolutionary loss of the operculum occurred at least three times independently in the Olividae: at the bases of the Olivinae and the Agaroniinae, and within the Olivellinae. Furthermore, a correlation seems to exist among the Olivoidea between the absence of the operculum and the habit of storing food items in a metapodial pouch (Fig. 1). Kantor and colleagues therefore suggested that “the disappearance of the operculum may be related with this ability to form a pouch by facilitating the bending of the metapodium” (KANTOR et al. 2017: 536). This is a plausible hypothesis; it receives additional support if one considers the Olivancillariinae. This taxon had not been included in the phylogenetic analysis by KANTOR et al. (2017) due to the lack of molecular data (Fig. 1), but it is listed as a fifth subfamily of the Olivoidea on MOLLUSCABASE (2020). All members of *Olivancillaria*, the only genus in the Olivancillariidae, lack opercula (TESO & PASTORINO 2011), and *O. auricularia* (Lamarck, 1811) stores subdued prey in a metapodial pouch (as *Lintrricula auricularia*; MARCUS & MARCUS 1959: 105–107 and table 1; for a confirmatory observation, see ROCHA-BARREIRA 2002).

Most Olividae have a tropical or subtropical distribution, with few species occurring in temperate waters. One of these exceptions is the Purple Olive, *Callianax biplicata* (G. B. Sowerby I, 1825), in the subfamily Olivellinae. *C. biplicata* can be found in large numbers on sandy substrates in the intertidal and shallow subtidal zone along the North American Pacific coast (ABBOTT 1954, OLSSON 1956, POWELL et al. 2020). The shells have been collected and used by indigenous peoples to produce jewellery and ornamental objects since prehistoric times (BENNYHOFF & HUGHES 1987, GROZA et al. 2011). Previous studies focused on the species' reproductive and developmental biology (EDWARDS 1968, STOHLER 1969),

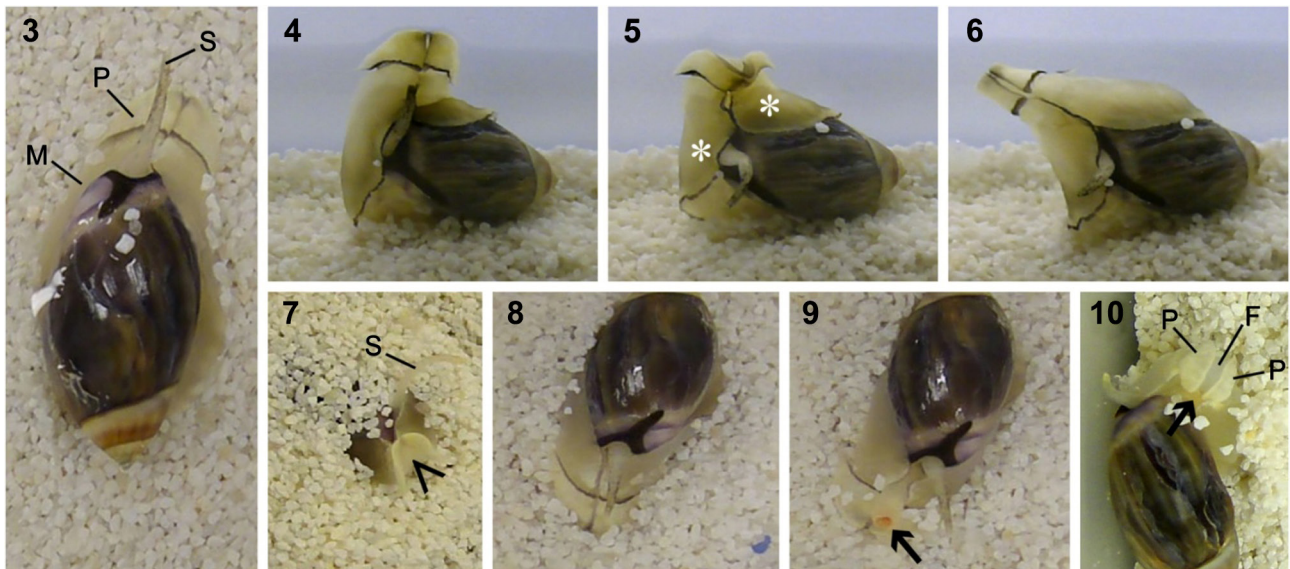
its ecological preferences (EDWARDS 1969a, PHILLIPS 1977a), and its diverse responses to a variety of predators (EDWARDS 1969b, PHILLIPS 1977b). Juvenile animals selectively ingest certain foraminiferans (HICKMAN & LIPPS 1983), but not much more is known about the feeding behaviour of the species. In particular, metapodial pouches have not been observed in *Callianax* or any other Olivellinae. Here we present observations of *C. biplicata* that throw additional light on the hypothesis of a possible link between the usage of the metapodial pouch in prey storage and the evolutionary loss of the operculum in the Olividae.

MATERIAL AND METHODS

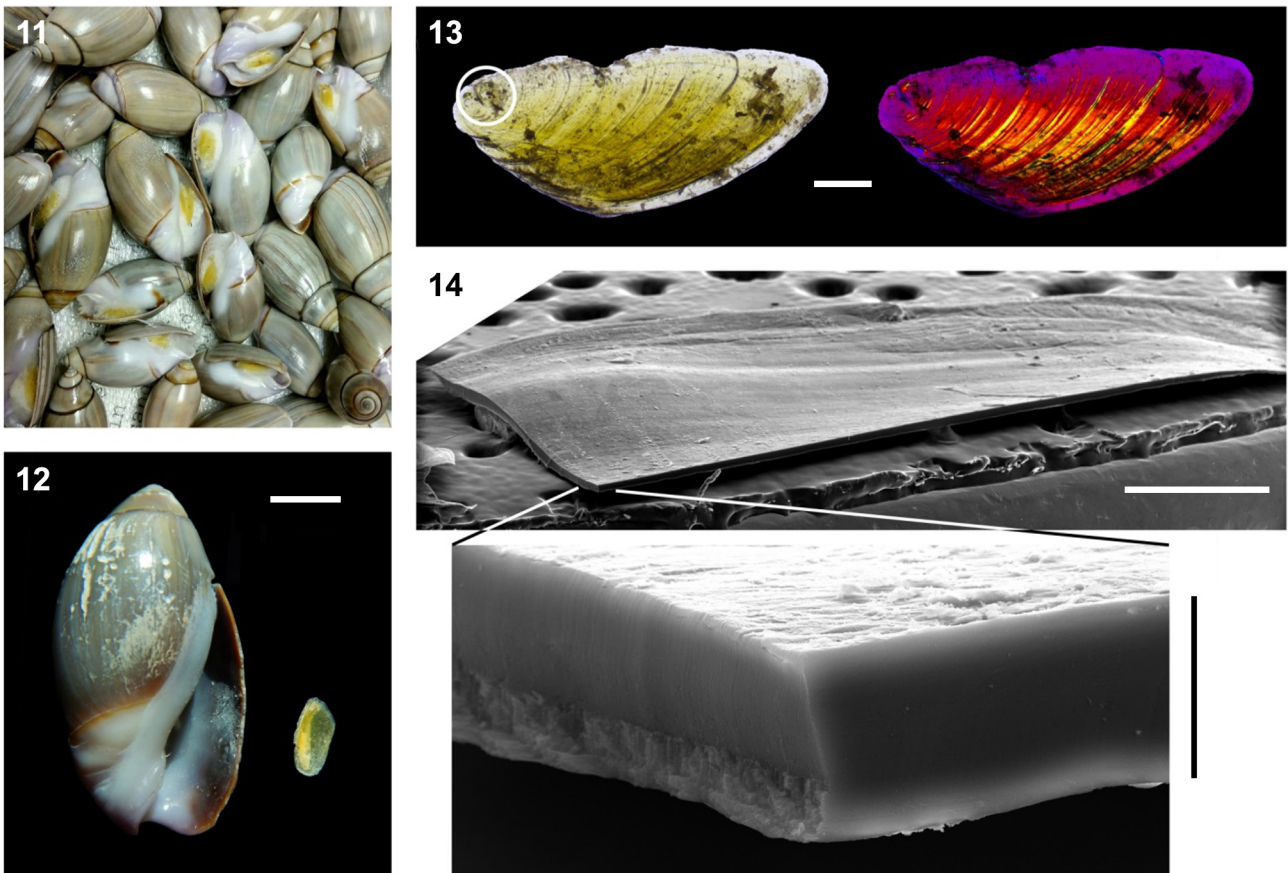
This paper compiles observations that are mostly by-products of unrelated studies, but which contribute to our understanding of the feeding behaviour of *C. biplicata* in its morphological context. The attack of an *Agaronia propatula* on *Olivella semistriata* was filmed in the natural habitat at Playa Grande, Guanacaste, Costa Rica, in 2014 (Fig. 2 and Supplemental Video 1). The behaviour of *C. biplicata* in aquaria with natural seawater was filmed at the Bodega Marine Laboratory (Bodega Bay, California, USA) in 2012, and at the Bamfield Marine Sciences Centre (Bamfield,

Vancouver Island, British Columbia, Canada) in 2013 (Figs 3–10, 18–26 and Supplemental Videos 2, 3). Videos were taken with various standard digital cameras and were processed using QuickTime v.7.7.9 (www.apple.com/quicktime) and ImageJ v.1.50i (imagej.nih.gov/ij).

Figure 11 shows shells of *C. biplicata* in the mollusc collection of the Burke Museum, University of Washington (Seattle, Washington, USA). Shell structure was studied in material available in the teaching collection of the Biology Department, Purdue



Figs 3–10. General morphology and behaviour of *Callianax biplicata*: 3 – Crawling animal showing the long siphon (S) and the propodium (P) that is separated from the metapodium (M) by a crescent-shaped groove marked by a darkly pigmented line; 4–6 – Animal demonstrating the flexibility of the foot; note the median longitudinal groove in the propodium and anterior part of the metapodium. The outer edges of the anterior metapodium form parapodia that usually are folded upwards, covering part of the shell (asterisks in 5); 7 – Snail burrowed in the sediment. The siphon (S) protrudes into the water and the propodium is curved upwards (arrowhead pointing to ventral face of the anterior foot); 8, 9 – Crawling snail without (8) and with (9) extended proboscis (arrow); the mouth orifice (9) is visible at the tip of the proboscis; 10 – Animal eating from a food particle (F) held between the left and right halves of the propodium (P; arrow indicates the proboscis). Shell lengths of the animals shown: 16.8 mm (3, 8, 9), 17.2 mm (4, 5, 6, 10), and 14.8 mm (7). Figs 3–10 correspond to Supplemental Video 2



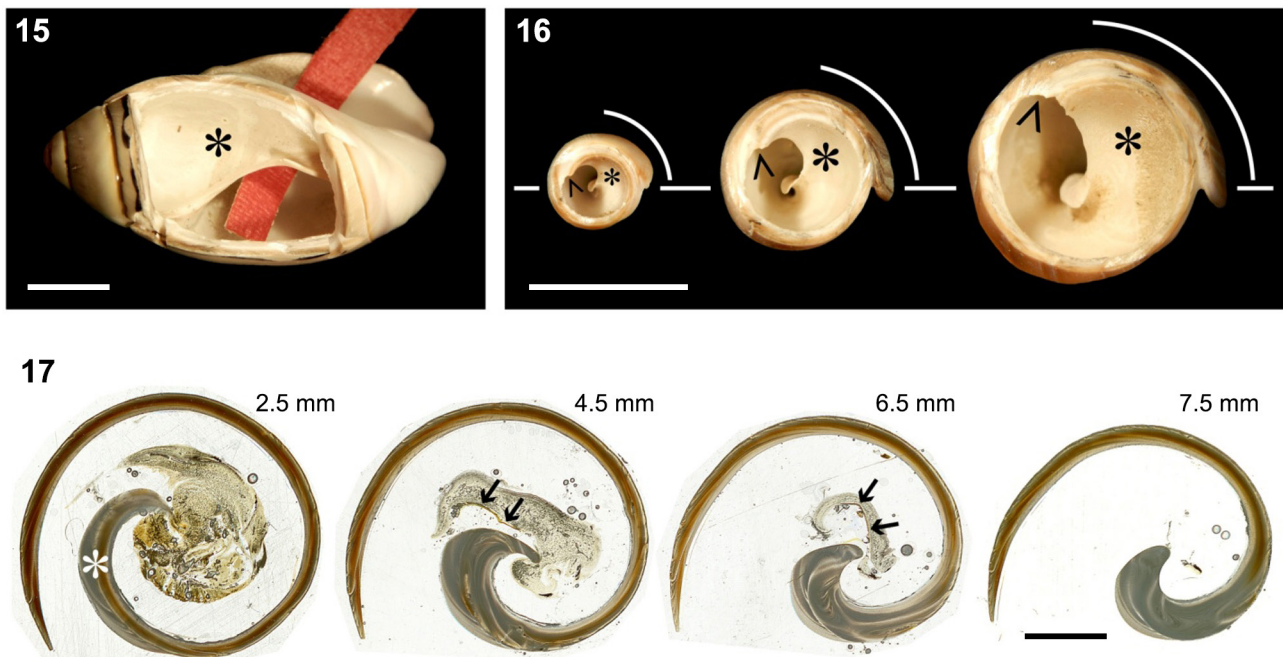
Figs 11–14. The operculum in *Callianax biplicata*: 11 – Sample of shells with their yellowish opercula attached; 12 – Example showing an operculum that is much smaller than the shell aperture; 13 – Opercula are concentric with a marginal nucleus (white circle on the left). They are translucent in plane polarized light (left) and exhibit birefringence in cross-polarized light (right; photographed at the orientation of maximum brightness with 1st order red accessory plate); 14 – Scanning electron micrographs of one quarter of an operculum divided by two perpendicular cuts (top), and magnified view of the corner produced by the cuts (bottom). The dehydrated operculum reaches a maximal thickness of less than 30 μm and thins towards its margins. Scale bars: 5 mm (12), 1 mm (13), 0.5 mm (top Fig. 14) and 25 μm (bottom Fig. 14)

University Fort Wayne, which originated from Charleston (Oregon, USA; Figs 12, 14), Manzanita (Oregon, USA; Figs 13, 17), and Brady Beach (British Columbia, Canada; Figs 15, 16). The shells were cut open with a hand-held rotary tool (7300 MiniMite; Dremel, Racine, Wisconsin, USA).

For thin-sectioning (Fig. 17), whole animals were dehydrated in an ethanol series and then vacuum-embedded (three cycles of 1 to 0.1 atm for 4 min each) in Epo-Tek-301 epoxy resin (Epoxy Technology, Billerica, Massachusetts, USA), followed by curing at room temperature for 24 h. Slices 2 mm thick were cut perpendicular to the shell axis with a Buehler Isomet 1000 (Buehler, Lake Bluff, Illinois, USA), polished on one side with 600 grit sand paper, glued to a microscope slide, and thinned to 30 μm thickness using a Hillquist Thin Section Machine (Hillquist, Arvada, Colorado, USA). Finally, the open surfaces of the sections were polished through a series of grits (600/1000/1200) and finished with aluminium oxide 1 μm MicroPolish Suspension (Buehler).

The sections were examined with a DM 2700 P microscope equipped with a DFC 450 camera (Leica Microsystems, Wetzlar, Germany). Isolated opercula preserved in 70% ethanol were examined in water with the same microscope under standard and cross-polarized illumination (Fig. 13).

For scanning electron microscopy (SEM; Fig. 14), material stored in 70% ethanol was cut with a razor blade and dehydrated in 80%, 90%, and 100% (3 times) ethanol for 10 minutes each, before drying in a Samdri-PVT-3B Critical Point Dryer (Rockville, Maryland, USA). Specimens mounted on an aluminium SEM stub with double-sided sticky carbon tape were sputter-coated with 4 nm platinum-palladium using a Cressington High-Resolution Sputter Coater (Watford, UK). Images were taken using an Everhart-Thornley detector in a Thermo-Fisher Scientific Apreo scanning electron microscope (Thermo-Fisher, Hillsboro, Oregon, USA) using 25 kV and 0.10 nA current.



Figs 15–17. Internal shell remodelling in *Callianax biplicata*: 15 – Shell of 24.7 mm length with ventral portion of the body whorl removed. Internal shell structures are missing except for a part of the most recent previous whorl which now forms a roof (asterisk) covering the apertural entrance. Red paper strip added to clarify three-dimensional structure; 16 – Anterior views into shells cut open 1 mm behind the aperture (original shell lengths 13.3, 21.6, and 29.0 mm, from left to right). The advancing resorption of the older whorl forms steps on the internal shell surfaces (arrowheads). Resorption is most advanced in the middle of the roofs over the apertures (asterisks), with angular distances to the posterior edge of the aperture of 85–90° (indicated by circle segments). This geometry is found in shells of all sizes; 17 – Cross-sections of a fixed specimen (shell length 20 mm) at 2.5 mm, 4.5 mm, 6.5 mm, and 7.5 mm from the aperture's posterior end (as indicated); the length of the aperture from its posterior end to the shell's base was 10.5 mm. In these posterior views, the outer lip is on the left. Callus is distinguished from other parts of the shell by its characteristic grey colour. Internal shell structures are absent except for the roof covering the aperture (white asterisk). The thin operculum (arrows) shows at 6.5 mm and 4.5 mm, but not at 2.5 mm and 7.5 mm, and appears to have deformed together with the soft tissues during preparative dehydration. Scale bars: 5 mm (15), 10 mm (16), 3 mm (17)

RESULTS

GENERAL MORPHOLOGY AND BEHAVIOUR

C. biplicata shows the typical morphology of olivid gastropods (Fig. 3; footage from which Figs 3–10 were extracted is compiled in Supplemental Video 2). The bullet-shaped shell, which reaches 30 mm length from base to apex, is smooth, glossy, and of variable colour ranging from light grey to dark brown. The body lacks a prominent head and cephalic tentacles. The foot consists of a narrow, crescent-shaped propodium, separated by a shallow groove from the large metapodium which has flat lateral parapodia capable of covering most of the shell (Figs 4–6). The propodium is divided lengthwise into halves by a median cleft, and terminates in a free pointed tip on each side (Figs 4, 5). In many specimens, the edges of both the groove between pro- and metapodium and the longitudinal cleft in the propodium are darkly pigmented, and therefore clearly visible (Figs

3–6). The animal has a long siphon (Fig. 3). Snails kept in tanks with sediment will burrow to assume a resting position. The siphon often protrudes from the sediment, sometimes together with the propodium which is folded lengthwise and bent upwards (Fig. 7); both organs apparently are used to monitor the environment. If in this situation a small piece of shrimp, clam, or fish meat is added to the tank, the snails soon will emerge and begin to search for the food. Like in other olivids (MARCUS & MARCUS 1959, TURSCH 1991, CYRUS et al. 2012), the propodium seems to be an important sensory organ, as it probes the surroundings continuously (Figs 4–7), especially in crawling animals (Figs 3, 8, 9). The mouth is located at the end of a trunk-like proboscis that can be extended from the most anterior dorsal part of the metapodium between the bases of the parapodia (Fig. 9). Animals feeding on large food items rest next to the object so that their proboscis can access it.



Smaller food items are often grasped with the propodium, which folds lengthwise so that its left and right halves act against each other, clamping the object between their ventral surfaces. At the same time, the propodium also bends upwards and backwards so that the proboscis can reach the food (Fig. 10).

OPERCULUM STRUCTURE

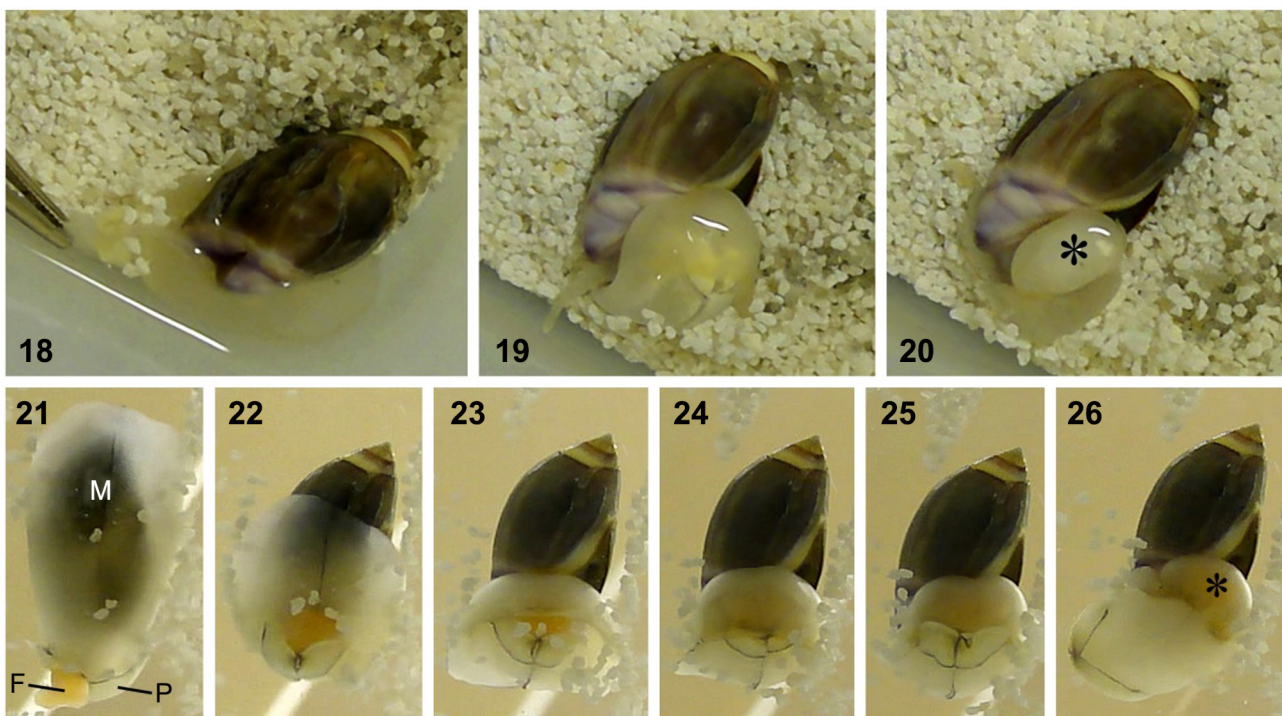
C. biplicata is one of the operculate Olivellinae. However, the operculum in this species appears to be smaller than the corresponding shell aperture (Figs 11, 12). The corneous opercula are of the concentric type with pronounced growth lines and a marginal nucleus. They appear translucent in the light microscope (Fig. 13, left) and show birefringence under polarized light illumination (Fig. 13, right). Optical extinction occurs when the direction of growth is parallel to one of the polarizing filters, and maximum brightness (as seen in Fig. 13, right) when the filters are at 45° to the growth direction. This suggests optical axes aligned along the direction of growth, perpendicular to the growth lines. Most opercula are about twice as long as they are wide, but their shapes vary widely. They appear to fray along the edges and are flexible and fragile when isolated. Scanning electron micrographs

of cut opercula suggest that they are not more than 30 µm thick (Fig. 14). However, fixation for the SEM includes dehydration, and it is possible that fully hydrated opercula are somewhat thicker.

SHELL REMODELLING

Older whorls do not persist as internal shell structures but are mostly resorbed. The only remaining internal component is a part of the wall of the most recently overgrown whorl which forms a cover or roof over the apertural entrance (Fig. 15). When viewed along the axis of a shell cut open just above the posterior end of the aperture, this older wall is seen extending in the counter-clockwise direction from the aperture (Fig. 16). The inward-facing edge of this wall is curved. In its centre, it reaches to about 85° measured from the posterior (upper) edge of the aperture, while it merges with the inner surface of the body whorl at around 160°. This geometry was observed in shells of all available sizes (13 to 29 mm shell length; Fig. 16).

An operculum that is smaller than the aperture itself may still close the aperture if the apertural channel constricts in the inward direction and the animal withdraws far enough into its shell. To see whether



Figs 18–26. *Callianax biplicata* stores food in a pouch formed by the posterior metapodium: 18–20 – A snail reaches with its propodium for a food item offered with forceps (18). Then the anterior foot holding the item bends toward the ventral side of the posterior metapodium, which forms a pouch around propodium and food item (19). Finally the propodium is withdrawn and the snail starts burrowing while the food remains secured in the metapodial pouch (asterisk; 20, 26); 21–26 – Stages of pouch formation recorded from below in a glass bottom aquarium (F, food item; M, metapodium; P, propodium). Shell lengths of the animals shown: 16.8 mm (18–20), 17.6 mm (21–26). Figs 18–26 correspond to [Supplemental Video 3](#)



this might be the case in *C. biplicata*, we produced serial shell sections. The sections confirmed not only that the aperture opened into a single, coherent cavity, but also demonstrated that the apertural channel widened rather than narrowed from the aperture inwards (Fig. 17).

FORMING A METAPODIAL POUCH

In animals kept in tanks, we repeatedly observed storage of food items in metapodial pouches (Figs 18–26 were extracted from the footage compiled in Supplemental Video 3). In the example shown in Figs 18–20, the snail reaches with its propodium for a small piece of shrimp meat offered with forceps, grabs the food, and bends the anterior part of its foot ventrally and posteriorly. At the same time, the posterior part of the foot bends ventrally while its margin contracts so that a sack-like cavity is produced. The anterior foot with the food item is inserted into this cavity, where it remains visible due to the partial translucence of the metapodium (Fig. 19). Next, the

propodium releases the item and the anterior foot is withdrawn. Simultaneously, the margin of the posterior foot continues to contract, reminiscent of a drawstring bag, and firmly encloses the food item. In this way, a metapodial pouch (asterisk in Figs 20, 26) is formed.

In tanks containing sand, snails forming metapodial pouches had to be turned over in order to witness the entire procedure (Figs 18–20, and the corresponding first part of Supplemental Video 3). To observe the behaviour in undisturbed animals, we filmed the process from below an aquarium with glass bottom and without sand. The snails performed the same sequence of movements as described above to deposit food in their metapodial pouches (Figs 21–26).

Feeding by introduction of the proboscis into the metapodial pouch was not observed. The animals rather released food from the metapodial pouch, seized it with the propodium, and fed as described above (Fig. 10).

DISCUSSION

A LITTLE OLIVA

Etymologically, *olivella* is a Latin diminutive of *oliva*, and indeed, *C.* (formerly *Olivella*) *biplicata* behaves much like a little *Oliva*. Not only does it grab and manipulate food items with an ‘opposable propodium’ (Fig. 10), it also stores the food in a pouch formed by the posterior metapodium, just as *Oliva* and *Agaronia* do. The body movements which the operculate *C. biplicata* performs to form the pouch and transfer food into it (Figs 18–26, Supplemental Video 3) are essentially the same as those previously documented for the inoperculate Olivinae (OLSSON & CROVO 1968, ZEIGLER & PORRECA 1969, KANTOR & TURSCH 2001), Agaroniinae (RUPERT & PETERS 2011, CYRUS et al. 2012; Fig. 2, Supplemental Video 1), and probably also the Olivancillariinae (MARCUS & MARCUS 1959).

Filled metapodial pouches look like swellings of the snail’s body (Supplemental Video 1), which has led to misinterpretations. OLSSON (1956: 164) observed *Oliva undatella* (Lamarck, 1811) attacking the smaller *Olivella semistriata* (Gray, 1839; probably *Olivella columellaris*, G. B. Sowerby I, 1825; compare TROOST et al. 2012) on a beach in Ecuador. He reported that “the smaller shell would be swallowed whole, the body of the *Oliva* swelling into a large, rounded, ball-like mass”. This appears implausible, particularly since the live victims could be “freed shortly after having been swallowed”. OLSSON (1956) did not specify what exactly he did to “free” the *Olivella*, but

we note that olivid predators readily release the contents of their metapodial pouches if disturbed following a catch. Considering this example, it seems possible that pouch formation especially in small olivids, like the operculate *C. biplicata*, may have been observed but misinterpreted in the past.

In any case, our observations indicate that the correlation between the inoperculate state and the ability to form metapodial pouches for food storage in the Olividae is not as strict as our graphic representation (Fig. 1) of the hypothesis by KANTOR et al. (2017) had suggested. What do we know about function and structure of the operculum in *C. biplicata*?

PROTECTIVE FUNCTIONS OF THE OPERCULUM

It is sometimes claimed to be “axiomatic that shells provide protection for gastropods by enclosing the soft tissues. [...] To complete the protection offered by the shell, the operculum should be strong and fit tightly into the aperture” (PAUL 1991: 127–128). Concerning this protective function of the operculum that “fits like a door across the shell aperture after the snail has withdrawn” (HUGHES 1986: 10), CHECA & JIMÉNEZ-JIMÉNEZ (1998) distinguished two morphofunctional types. Rigidclaudent opercula are comparatively solid, as the name suggests. They have the size and cross-sectional shape of the path they are supposed to block, and may in fact fit like a rigid door. In contrast, flexiclaudent opercula are elastic and often larger than the shape of the path

they block; they do not necessarily have the exact shape of that path. Flexiclaudent opercula may bend as they are pressed against the apertural walls when the animal retracts, and the overlapping edges curve outward, sealing the aperture. Only rigidlaudent opercula can be calcified, while flexiclaudent ones generally are corneous, for obvious reasons.

The assumed protective function of both types of opercula is threefold. Firstly, opercula are thought to prevent predatory attacks through the aperture (HUGHES 1986, CHECA & JIMÉNEZ-JIMÉNEZ 1998). However, unequivocal quantitative evidence demonstrating an operculum's efficiency in the prevention of predation in natural habitats has been produced in only a few cases (e.g., SNYDER & KALE 1983). Even in the probably most convincing example for an efficient anti-predator function of an operculum in the wild (GIBSON 1970), the dog-whelk *Nucella lapillus* (Linnaeus, 1758; formerly included in the genera *Purpura* and *Thais*), significant proportions of natural populations possess opercula that are too small to block the aperture, or lack opercula altogether (COLGAN 1910, COOKE 1917, KEPPENS et al. 2008). Secondly, tightly fitting opercula can prevent the entrance of harmful fluids such as digestive juices into the shell, and have been interpreted as essential for the ability of some operculate gastropods to survive passage through the digestive tract of birds and fishes (NORTON 1988, BROWN 2007, CADÉE 2011). However, some small eupulmonates accomplish the same (WADA et al. 2012), although mature Eupulmonata generally lack opercula (GITTEBERGER 1996). Thirdly, opercula have been hypothesised to reduce water loss in animals exposed to dehydrating conditions (MCQUAID 1982, PAUL 1991). While this physical effect certainly can be observed in experimental settings, its physiological relevance under natural conditions has been questioned (GIBSON 1970, GITTEBERGER 1996). In any case, opercula might serve any of the three protective functions if they are flexiclaudent or rigidlaudent, but not if they are too small to occlude the aperture, in other words, if they are 'aclaudent'.

The operculum of *C. biplicata* is non-calcified and flexible, but it appears to be aclaudent rather than flexiclaudent due to its comparatively small size (Figs 11, 12, 17); see also images of the species in HOLM (2007, as *Olivella biplicata*) and POWELL et al. (2020: figs 22A and 23A). Therefore a protective function of the operculum that depends on the tight occlusion of the aperture is unlikely, based on the specimens we have examined. One may argue that if the diameter of the whorl decreases in the inward direction, even a relatively small operculum could block the entrance path if the animal only retracts far enough. This argument may apply to gastropods which retain older whorls as internal shell structures (EDGELL

& MIYASHITA 2009), but not to those that partially or wholly resorb older whorls that have become overgrown and thus internalised by younger whorls (KOHN et al. 1979, SOLEM 1983). Interior shell remodelling occurs in *Oliva* (ZEIGLER & PORRECA 1969: 78), and the near complete resorption of the inner walls of older whorls had been recognised as a character of the Olivellinae (formerly family Olivellidae) by KANTOR (1991). In fact, *C. biplicata* resorbs older, internal shell components almost entirely, turning the shell interior into a single coherent space (Figs 15–17). Shells of all sizes examined exhibited the same internal geometry (Fig. 16), indicating that the breakdown of older whorls proceeds in step with shell growth. The inward passage from the outer edge of the aperture into the shell's interior therefore widens rather than shrinks in shells of all sizes. Consequently, the operculum in *C. biplicata* cannot function like a door that seals the shell interior at a distance from the aperture. The conclusion that the operculum has no protective function in the species is supported also by the small thickness of the operculum (Fig. 14).

OPERCULUM ULTRASTRUCTURE

In developmental studies, larval opercula routinely are identified by their birefringence (CLEMENT 1986, SWEET 1998). We are not aware, though, of systematic investigations into the causes of birefringence in corneous opercula of mature gastropods, as detected in *C. biplicata* (Fig. 13). Corneous opercula consist of operculins, scleroproteins which resemble collagen (HUNT 1970, 1976). Collagens assemble into fibrillar arrays of high molecular order which show both intrinsic and form birefringence (for a brief technical introduction, see WOLMAN & KASTEN 1986). Operculins may develop similar molecular arrangements. Our scanning electron micrographs could not be expected to resolve any crystal-like molecular order causing birefringence in opercula of *C. biplicata* (Fig. 14). Generally, further insights into the molecular structure of corneous opercula are desirable. As a simple and inexpensive method for acquiring information on sub-microscopic structures without any damaging preparation of valuable specimens, polarized light microscopy could facilitate comparative studies on the structure, mechanics, and evolution of corneous opercula in the Olividae and other taxa.

METAPODIUM BIOMECHANICS AND THE OPERCULUM

The formation of the metapodial pouch in the Olividae including *C. biplicata* requires ventral bending as well as contraction along the margin of the posterior metapodium (Figs 18–26, Supplemental



Videos 1, 3). To evaluate the biomechanical effects an operculum might have on the process, we have to remember that opercula connect to the foot at the opercular disc, a specialised epithelium which is histologically distinct from the regular dorsal foot integument (CHECA & JIMÉNEZ-JIMÉNEZ 1998). Because the opercular disc usually lies on or close to the mid-sagittal line on the foot surface, it appears unlikely that an operculum could interfere mechanically with contractions of the foot's margins. On the other hand, an inflexible operculum certainly could prevent any bending of the opercular disc. It must be noted, though, that the opercular disc is always smaller than the area of the operculum (CHECA & JIMÉNEZ-JIMÉNEZ 1998). Therefore the presence of even a very large operculum does not necessarily imply a limited ability for ventral bending of the posterior foot; direct observations of live animals will be necessary to evaluate the operculum's biomechanical effects. Our observations of live *C. biplicata* demonstrated that the operculum does not affect the ability to form a metapodial pouch in this species.

An operculum also may have indirect mechanical effects. When the metapodium bends ventrally as in forming a pouch, the part of the operculum that is unconnected to the integument might protrude from the pouch's curved surface and become entangled with sediment, inhibiting locomotion and burrowing. Again, this does not seem to apply to *C. biplicata*, which we observed moving and burrowing into the sediment without apparent difficulties while carrying food in its pouch.

As larvae, all gastropods are operculate (PONDER & LINDBERG 1997), and various structural and developmental aspects of larval opercula have attracted scrutiny (BONAR 1978, KANO 2006, HASHIMOTO et al. 2012). The operculum in adult *C. biplicata* may represent a vestigial organ or, more precisely, an ontogenetic remnant that had functional significance at

early life stages but no discernible function at maturity. For example, major muscles involved in the retraction of the foot into the protoconch attach to the operculum in larvae of various gastropod taxa (BONAR & HADFIELD 1974, EVANS et al. 2009, PAGE & FERGUSON 2013). It is unclear whether the operculum in mature *C. biplicata* and other operculate Olividae also functions as an exoskeletal attachment point for muscles. If it does, the evolutionary loss of the operculum in the Olividae would appear to have depended on modifications of the muscular system that rendered the opercular attachment dispensable. In this context it is of interest that the body movements which the inoperculate *Agaronia propatula* performs when it withdraws into its shell seem to differ from those observed in several operculate *Olivella* species (RUPERT & PETERS 2011). We will need sufficiently detailed anatomical data concerning the muscular systems of various Olividae, together with comparative kinematic analyses of their movement patterns, to develop a biomechanically sound reconstruction of the multiple independent losses of the operculum that seem to have occurred in this family.

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SUPPLEMENTAL VIDEOS

Feeding behaviour and the operculum in *Callianax biplicata* (G. B. Sowerby 1, 1825) ([Supplemental Video 1](#), corresponds to [Fig. 2](#)).

Feeding behaviour and the operculum in *Callianax biplicata* (G. B. Sowerby 1, 1825) ([Supplemental Video 2](#), corresponds to [Figs 3–10](#)). The video runs at double speed.

Feeding behaviour and the operculum in *Callianax biplicata* (G. B. Sowerby 1, 1825) ([Supplemental Video 3](#), corresponds to [Figs 18–26](#)). The video runs at double speed.