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The Internal-Brooding Apparatus in the Bryozoan Genus *Cauloramphus* (Cheilostomata: Calloporidae) and Its Inferred Homology to Ovicells

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We studied by SEM the external morphology of the oecium in eight bryozoans of the genus *Cauloramphus* Norman, 1903 (Cheilostomata, Calloporidae): *C. spinifer*, *C. variegatus*, *C. magnus*, *C. multiavicularia*, *C. tortilis*, *C. cryptoarmatus*, *C. niger*, and *C. multispinosus*, and by sectioning and light microscopy the anatomy of the brooding apparatus of *C. spinifer*, *C. cryptoarmatus*, and *C. niger*. These species all have a brood sac, formed by invagination of the non-calcified distal body wall of the maternal zooid, located in the distal half of the maternal (egg-producing) autozooid, and a vestigial, maternally budded kenozooidal oecium. The brood sac comprises a main chamber and a long passage (neck) opening externally independently of the introvert. The non-calcified portion of the maternal distal wall between the neck and tip of the zooidal operculum is involved in closing and opening the brood sac, and contains both musculature and a reduced sclerite that suggest homology with the oecial vesicle of a hyperstomial ovicell. We interpret the brooding apparatus in *Cauloramphus* as a highly modified form of cheilostome hyperstomial ovicell, as both types share 1) a brood chamber bounded by 2) the oecium and 3) a component of the distal wall of the maternal zooid. We discuss *Cauloramphus* as a hypothetical penultimate stage in ovicell reduction in calloporid bryozoans. We suggest that the internal-brooding genus *Gontarella*, of uncertain taxonomic affinities, is actually a calloporid and represents the ultimate stage in which no trace of the oecium remains. Internal brooding apparently evolved several times independently within the Calloporidae.

Key words: Bryozoa, *Cauloramphus*, ovicell, internal brooding, evolution, homology

INTRODUCTION

The majority of species of the bryozoan order Cheilostomata brood embryos in a specialized structure termed 'ovicell' by Busk (1852) and 'oecium' by Hincks (1873). These initially synonymous terms reflected an old view that the brood chamber contains an ovary (see Levinsen [1909] for historical references). However, anatomical descriptions (Nitsche, 1869; Vigelius, 1884a, b; Calvet, 1900) soon made it clear that a single term was insufficient to describe the complexity of the cheilostome brooding apparatus. The problem was that 'ovicell' and 'oecium' were used to refer to both the whole brooding apparatus and the externally visible covering of the brood chamber. While this loose usage did not affect taxonomists, it was unacceptable to anatomists.

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Terminological changes made by Woollacott and Zimmer (1972) and supported by Ryland (1976), Ryland and Hayward (1977), Hayward and Ryland (1979), and Reed (1991) reflected the dual necessities of 1) separating the entire brooding apparatus (ovicell) from its parts, which include the protective hood (oecium or oecial outfold) generated either by the maternal (egg-producing) zooid or the distal zooid, the brooding cavity, and the closing device (oecial vesicle or zooidal operculum), and 2) distinguishing between the contributions, in the majority of cases, of two zooids (maternal and daughter) to the structures of the brooding apparatus. Ryland and Hayward (1977), Hayward and Ryland (1979), Reed (1991), and Ostrovsky (1998) provided clear definitions. In this paper, we use the term 'ovicell' to refer to the entire brooding apparatus (including protective hood, brooding cavity, and closing device) and the term 'oecium' to refer to the protective hood alone; however, we note that in quotes from previous authors, the terms may have been considered synonymous.

Separation of the terms 'ovicell' and 'oecium' removes obvious contradictions that appear in some instances. An example is the commonly used phrase 'vestigial ovicell.'

Only the protective fold (oecium) can be vestigial, whereas the brooding chamber (brooding cavity) always remains well developed. If the term 'ovicell' is applied to the totality of the brooding apparatus, it cannot logically be referred to as vestigial in this case.

Usually cheilostome brooding structures are conspicuous, though they may be hidden to a greater or lesser extent by immersion in calcification associated with the frontal wall of the succeeding zooid. In some taxa, however, indications of the presence or absence of a brooding structure (indicated as either 'ovicell' or 'oecium') have been ambiguous in the literature, as is the case with the genus *Cauloramphus* Norman, 1903 of the cheilostome family Calloporidae.

Norman (1903) mentioned nothing about ovicells, oecia, embryos, or brooding in his original description of *Cauloramphus*. However, he designated *C. spinifer* (Johnston, 1832) as type species of the genus, and Hincks (1880, p. 149) had noted for this species (as *Membranipora spinifera*), "Ooecia shallow, smooth, with a rib across the front." This description may reflect an error in identification, since a rib on the oecium is characteristic of other calloporid genera such as *Callopora* and *Tegella*. Bassler (1953, p. G160) simply noted, "Ovicells inconspicuous" in his generic diagnosis of *Cauloramphus*. In their monographs on British bryozoans, Ryland and Hayward (1977, p. 98) and Hayward and Ryland (1998, p. 172) included a reduced ovicell in their diagnosis of *Cauloramphus*. Their generic diagnosis was likely based strongly on *C. spinifer*, the only *Cauloramphus* they treated, for which they noted, "ovicell partly immersed, very small and depressed, not readily apparent but seen as a shallow, crescentic cap at the distal end of the autozooid; closed by the autozooidal operculum." Prenant and Bobin (1966, Fig. 70-IV) also included a description and drawing of the reduced oecium in *C. spinifer* from France.

In fact, reports in *Cauloramphus* of structures related to brooding have been quite variable throughout the taxonomic literature. Hincks (1882, p. 250) indicated uncertainty in his original description of *C. echinus* by noting, "Ooecia (?)". Some species have been reported as having an inconspicuous, cap-like ovicell (e.g., Kluge, 1975; Mawatari and Mawatari, 1981; Dick and Ross, 1988). Despite previous reports of brood chambers in *C. spinifer*, Gostilovskaya (1978) noted for the White Sea population of this species that brood chambers were unknown. In other species, ovicells or oecia have never been reported, e.g., *C. cymbaeformis* (Hincks, 1877); *C. intermedius* Kluge, 1962; *C. pseudospinifer* Androsova, 1958; *C. disjunctus* Canu and Bassler, 1929; and *C. californiensis* Soule, Soule, and Chaney, 1995.

We suspect that failure to report brooding structures in *Cauloramphus* is due both to the difficulty of seeing the external parts of these structures without scanning electron microscopy (SEM) and to authors simply failing to understand the condition of the oecium, which is reduced. For example, Dick and Ross (1988) reported ovicells as "not observed" or "lacking" in three of the five *Cauloramphus* species they reported from Alaska; in *C. magnus*, they noted a vague connection between the internal brooding of embryos by zooids and a raised, crescentic lip distal to the orifice. As another example, Seo (2001, p. 225) noted in her

original description of *C. korensis*, "Ovicells not seen," yet she inadvertently described the oecium with "Distal to the orifice, transverse crescentic cap much raised above the level of mural rims," and her illustrations of this species (Seo, 2001, Fig. 1B, C, 2005, Figs. 32B, 33A) clearly show a fairly substantial oecium.

Dick *et al.* (2005) and Grischenko *et al.* (2007) reported, but did not describe in detail, both oecia and internal brooding in all seven species of *Cauloramphus* they treated between them, including five new species. This result suggests that an oecium, reduced to a variable extent, and internal brooding are features common to all species of *Cauloramphus*.

In this study, we examined and compared the gross development and external morphology of the oecium in eight species of *Cauloramphus* from the North Pacific. In addition, we examined by histological sectioning the anatomy of internal brooding in three of these species. On the basis of our results, we suggest hypotheses concerning the origin of reduced kenozooidal oecia and internal brooding sacs through the modification of conventional ovicells, and the evolution of internal brooding in the cheilostome families Calloporidae and Flustridae.

MATERIALS AND METHODS

Species examined

The eight species included in this study were collected intertidally from the following localities:

Akkeshi Bay, Hokkaido, Japan, early June–early July 2004:

- Cauloramphus spinifer* (Johnston, 1832);
- Cauloramphus magnus* Dick and Ross, 1988;
- Cauloramphus cryptoarmatus* Grischenko, Dick, and Mawatari, 2007;
- Cauloramphus niger* Grischenko, Dick, and Mawatari, 2007;
- Cauloramphus multispinosus* Grischenko, Dick, and Mawatari, 2007.

Narrow Strait, Kodiak, Alaska, USA, September–December 1982:

- Cauloramphus variegatus* (Hincks, 1881).

Ketchikan vicinity, Alaska, USA, 9–12 September 2003:

- Cauloramphus multiavicularia* Dick, Grischenko, and Mawatari, 2005;
- Cauloramphus tortilis* Dick, Grischenko, and Mawatari, 2005.

There has been much inconsistency in the literature regarding the suffixes used for species names in *Cauloramphus*. According to article 30.1.3 of the ICZN (1999), *Cauloramphus* must be treated as masculine in gender; hence, for species names other than those treated as nouns in apposition, we use the masculine form of the suffix.

Microscopy

Specimens of all species were collected alive and air-dried. Specimens were prepared for scanning electron microscopy (SEM) by immersion in a 7.5% sodium hypochlorite solution to remove the soft tissue, rinsed in distilled water, and air-dried. They were then coated with Pd-Pt in a Hitachi E-1030 sputter coater and observed with a Hitachi S-2380N scanning electron microscope at 15 kV accelerating voltage. All images were stored electronically as TIFF files at a resolution of 200 pixels/cm with ImageCatcher software (Denshi Kongaku Kenkyusyo Co.).

For light microscopy, selected specimens of *C. spinifer*, *C. cryptoarmatus*, and *C. niger* were fixed in Bouin's fluid without acetic acid. These colonies were decalcified in a 2 M HCl solution, dehydrated in an ethanol series, embedded in TAAB 812 epoxy resin, sectioned (3 μ m thick) with a glass knife, and stained with Richardson's stain using standard methods. Richardson's stain

(Richardson *et al.*, 1960) contains methylene blue, azure II, and borax. In our preparations, methylene blue non-selectively stained cells of all types in soft tissues, whereas azure II selectively stained decalcified skeletal tissue.

RESULTS

All eight species studied have a strongly reduced oecium evident externally (Figs. 1–3) and a sac in the coelomic cavity of the maternal zooid that functions for the brooding of embryos (Figs. 4, 5). The oecium, located on the distal mural rim of the maternal (egg-producing) autozooid inside the semicircle of mural spines distal to the orifice, is a kenozooidal polymorph budded from the maternal zooid. Distally is a non-calcified outer membrane (ec in Fig. 4C, D) that we interpret as homologous to the distal portion of ectooecium (external oecial wall) that is non-calcified in many conventional oecia. Beneath this membrane is a space that we interpret as homologous with the coelomic lumen between the ectooecium and entoecium of conventional oecia. We interpret the heavily calcified cap-like or crescentic structure (en in Fig. 4C, D) comprising the bulk of the oecium along the distal rim of the zooidal opesia as homologous with the calcified entoecium (lower oecial wall) of a conventional ovicell. In cleaned specimens, the border of the non-calcified ectooecium (removed in cleaning) is normally evident in both forming and completed oecia (arrowheads in Fig. 2C, D). The basal part of the entoecium is fused with the distal wall of the maternal zooid (Figs. 4A–D, 5). In cleaned specimens of seven of the eight species examined, the surface of the entoecium is finely tuberculate, similar in texture to the zooidal cryptocyst (Figs.

2B, D, F, H, 3B–D). In *C. spinifer* it is smooth (Fig. 3A). The most proximal surface of the entoecium, facing the maternal zooid, is smooth in all species examined (e.g., Fig. 1A). Among the species examined, *C. niger* has the least reduced, most prominent oecium, with a cap-like entoecium (Figs. 1D, 2H). In contrast, the entoecium is smooth and concave (cup-like) in *C. spinifer* (compare Figs. 1D and 2H with Figs. 1A and 3A).

In a completed oecium, the oecial coelomic cavity (Figs. 4A–D, 5) consists of a larger upper part (oc in Fig. 4C, D) leading through a simple pore (oecial pore; arrows in Figs. 2, 3) to a lower part comprising a narrow canal (Fig. 5). The canal connects to the visceral coelom of the maternal zooid by a single communication pore having a cuticular cincture and plugged by a specialized pore-cell complex (small arrows in Fig. 4C, D and Fig. 5). The oecial pore (arrows, Figs. 1–3) is visible externally in specimens after removal of the overlying non-calcified ectooecium and associated cellular layers by cleaning with sodium hypochlorite (compare Figs. 2 and 3 with Figs. 4C, D and 5). This pore is situated at or near the midline and in sections was sometimes seen to be filled with non-specialized epithelial cells. The lower part of the oecial coelom (canal) was often seen filled with a loose mass of peritoneal and outer-epithelial cells (Fig. 4D). These cells comprise the cellular layers between the coelom and outer wall in any bryozoan zooid; when part of the coelom becomes restricted in a narrow canal, these layers lining the canal become appressed as a more-or-less solid mass, with the coelomic cavity evident only as numerous intercellular spaces. The presence of a pore-cell complex clearly indicates that the oecium is a

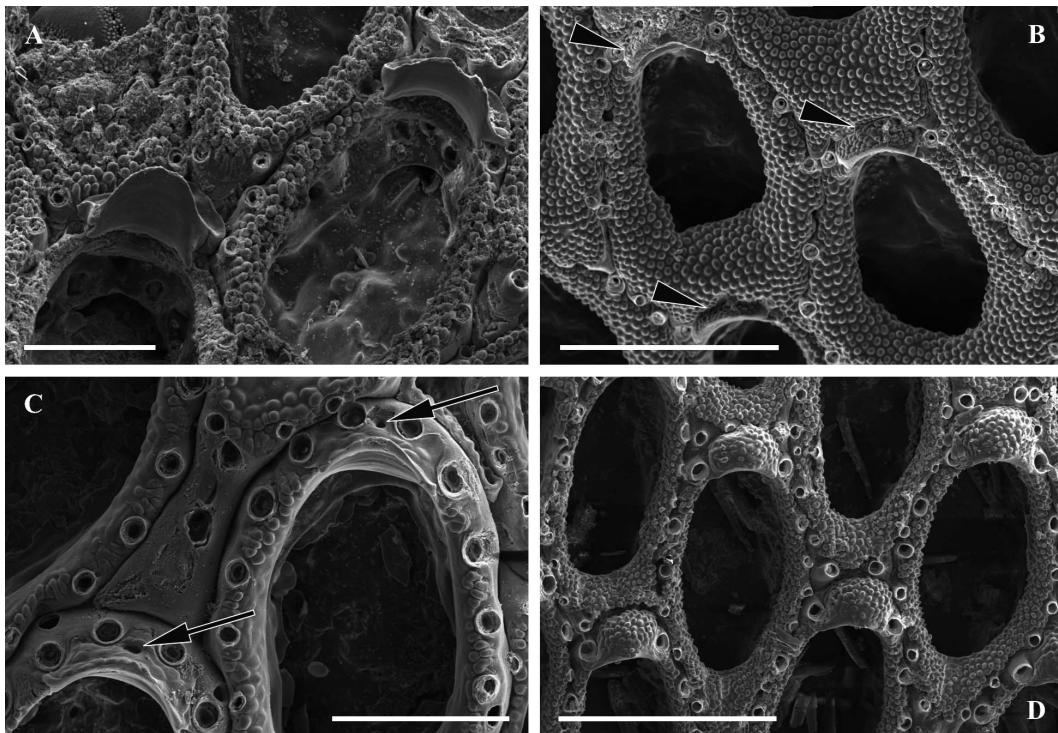


Fig. 1. Brooding autozooids with fully-formed vestigial oecia (hypochlorite-cleaned specimens; SEM). **(A)** *Cauloramphus spinifer*; **(B)** *C. cryptoarmatus*; **(C)** *C. multiavicularia*; **(D)** *C. niger*. Arrowheads, oecia; arrows, oecial pores. Scale bars: A, C, 200 µm; B, 400 µm; D, 500 µm.

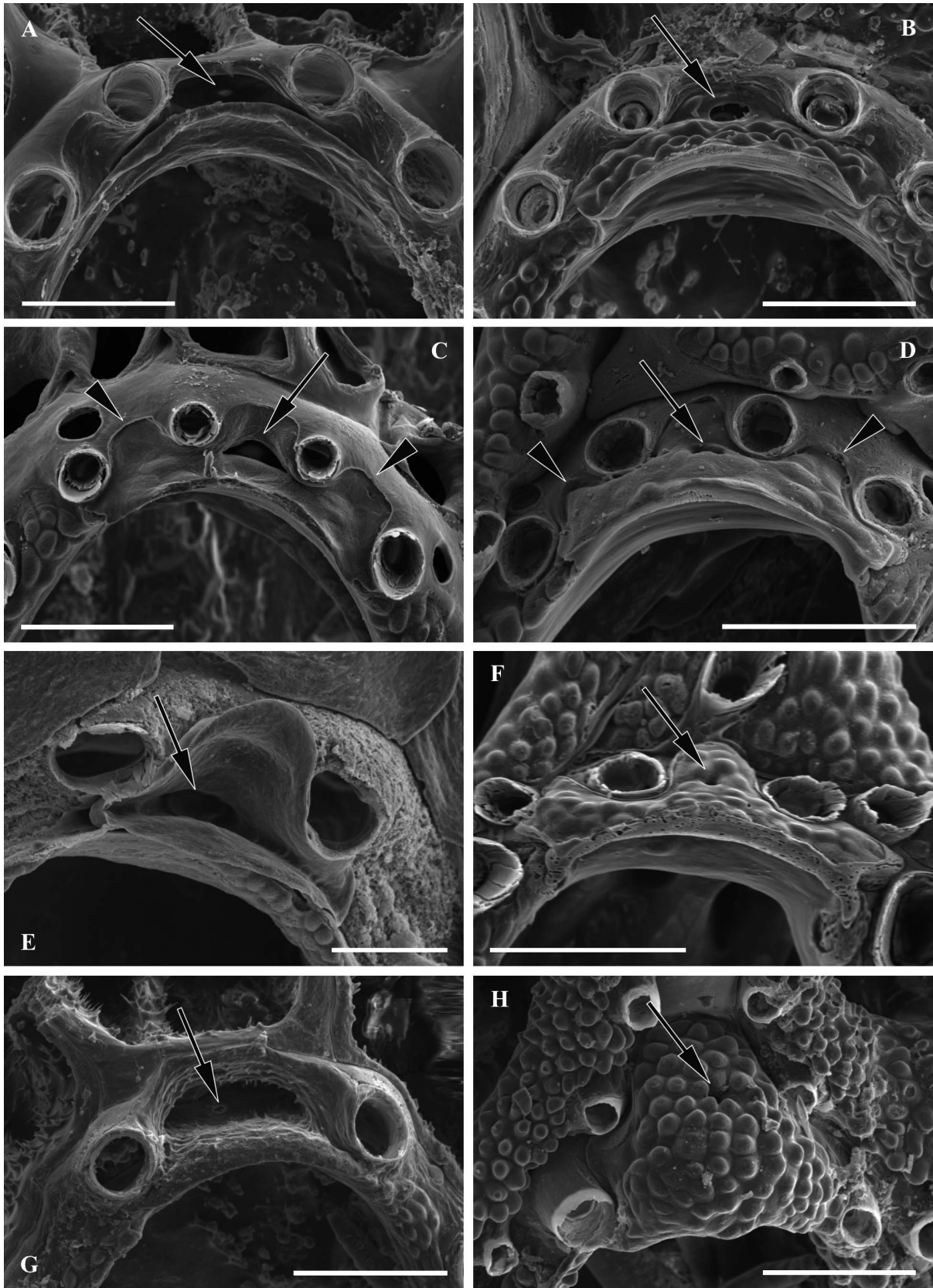


Fig. 2. Developing (left panels) and fully-formed (right panels) vestigial oecia (hypochlorite-cleaned specimens; SEM). Arrows indicate oecial pores; arrowheads in C and D point to the edge of the non-calcified ectooecium, which has been removed by cleaning. (A, B) *Cauloramphus multispinosus*; (C, D) *C. multiavicularia*; (E–F) *C. tortilis*; (G, H) *C. niger*. Scale bars: A–D, F–H, 100 µm; E, 50 µm.

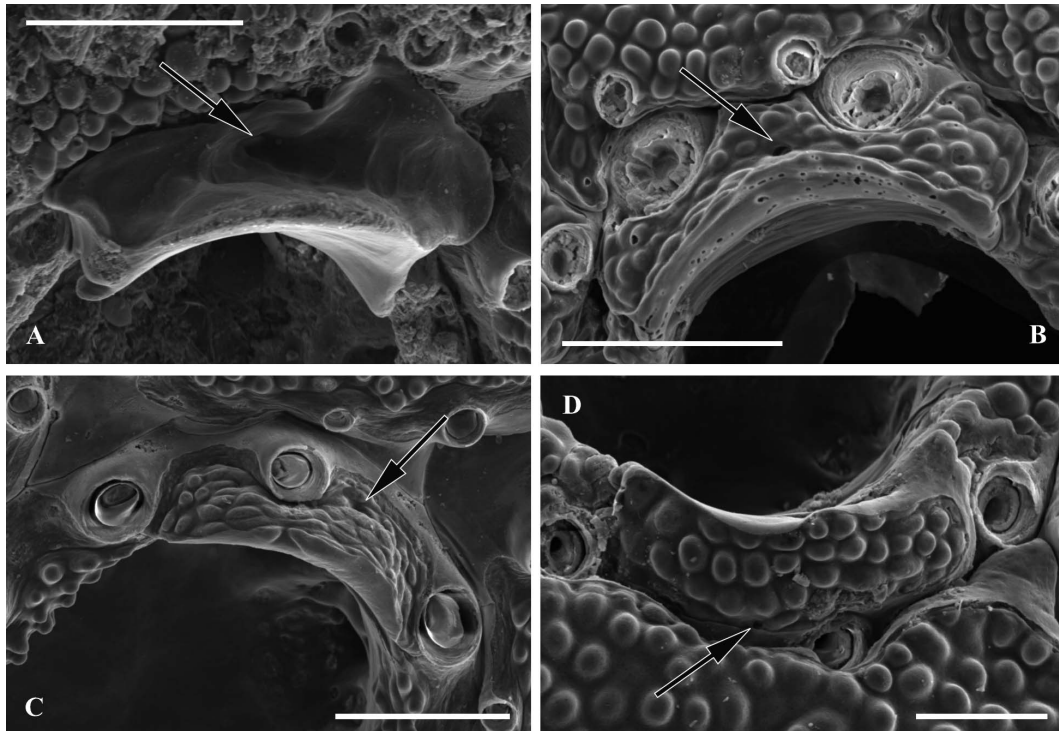


Fig. 3. Fully-formed vestigial oecia. Arrows show oocial pores. (A) *Cauloramphus spinifer*; (B) *C. variegatus*; (C) *C. magnus*; (D) *C. cryptoarmatus*. Scale bars: A–C, 100 µm; D, 50 µm.

daughter zooid budded from the maternal zooid; furthermore, since the oecium is a highly modified zooid lacking a polypide and without either orifice or muscles, it is by definition a kenozooid (Hayward and Ryland, 1998).

The oecium is budded by the maternal zooid; it lies above the distal mural rim of the maternal zooid and is functionally and morphologically separate from the distal autozooid (Fig. 5) that is also budded by the maternal zooid. Developing oecia seen in young, peripheral zooids appear as a narrow groove or concavity that is actually the rudimentary oocial coelom (Fig. 2A, C, E, G). During calcification, the entoecium thickens, the oocial coelom is reduced, and the diameter of the communication canal decreases (compare Fig. 2A, C, E, G with Fig. 2B, D, F, H). *Cauloramphus spinifer* is atypical in that the completely formed oecium as seen in cleaned specimens is characteristically a broad, rather deep cavity (Fig. 3A) distal to a raised, crescentic lip (Figs. 1A, 3A).

From the exterior, brooding zooids are distinguishable from non-brooding zooids by the presence of reduced oecia and either oocytes or embryos visible through the semi-transparent frontal membrane (Fig. 4E). We observed embryos to be pink in *C. spinifer* (Fig. 4E) and up to 200×120 µm in size, and orange in *C. cryptoarmatus* and about 160×90 µm in size. Some were seen in sections to be surrounded by a fertilization envelope. In the species of *Cauloramphus* we examined, mature oocytes were positioned laterally either in the middle or proximal part of the zooid (asterisks, Fig. 4E); embryos were brooded inside the internal brood sac in the distal half of the maternal autozooid (Fig. 4E).

We observed the internal anatomy of brooding zooids

for three species; although only *C. spinifer* and *C. niger* are illustrated in Fig. 4, *C. cryptoarmatus* showed similar structure, which is reflected in Fig. 5. The brood sac consists of a main chamber connecting with the exterior via a long, narrow neck. Although the neck appears cylindrical in longitudinal sections, it is actually a wide, dorsoventrally compressed passage. The neck opens to the exterior at the frontoproximal edge of the entoecium. The opening is located beneath the distal edge of the zooidal operculum when the latter is closed; it is also located near the vestibular opening, but is independent of the introvert (Figs. 4A–D, 5). The neck is closed distally by a fold of the distal-most part of the non-calcified frontal wall of the maternal zooid that is appressed against the adjoining entoecium, comparable with an oocial vesicle in conventional ovicells (Fig. 4A, D; Fig. 5). In the lower base of the fold, there is a cuticular thickening that is presumably a reduced sclerite (in hyperstomial ovicells, the sclerite is a zone of thickened cuticle of the oocial vesicle where the latter touches the oocial fold). The sclerite is developed to different degrees in different brooding zooids within the same colony; sometimes it is slight (Fig. 4B, C). Two muscle bundles attach to the distal wall of the closing fold, one above and one below the sclerite; we were unable to trace the origins of these bundles. Numerous thin muscle bundles also attach to the basal wall of the neck of the brood sac (Figs. 4A–C, 5). Their origins are anchored onto the transverse, basal, and lateral cystid walls, and they presumably function to expand the neck during oviposition and larval release. We were not able to find muscles inserted on the sclerite.

The thin-walled brood sac and neck are an invagination of the non-calcified portion of the distal wall of the maternal

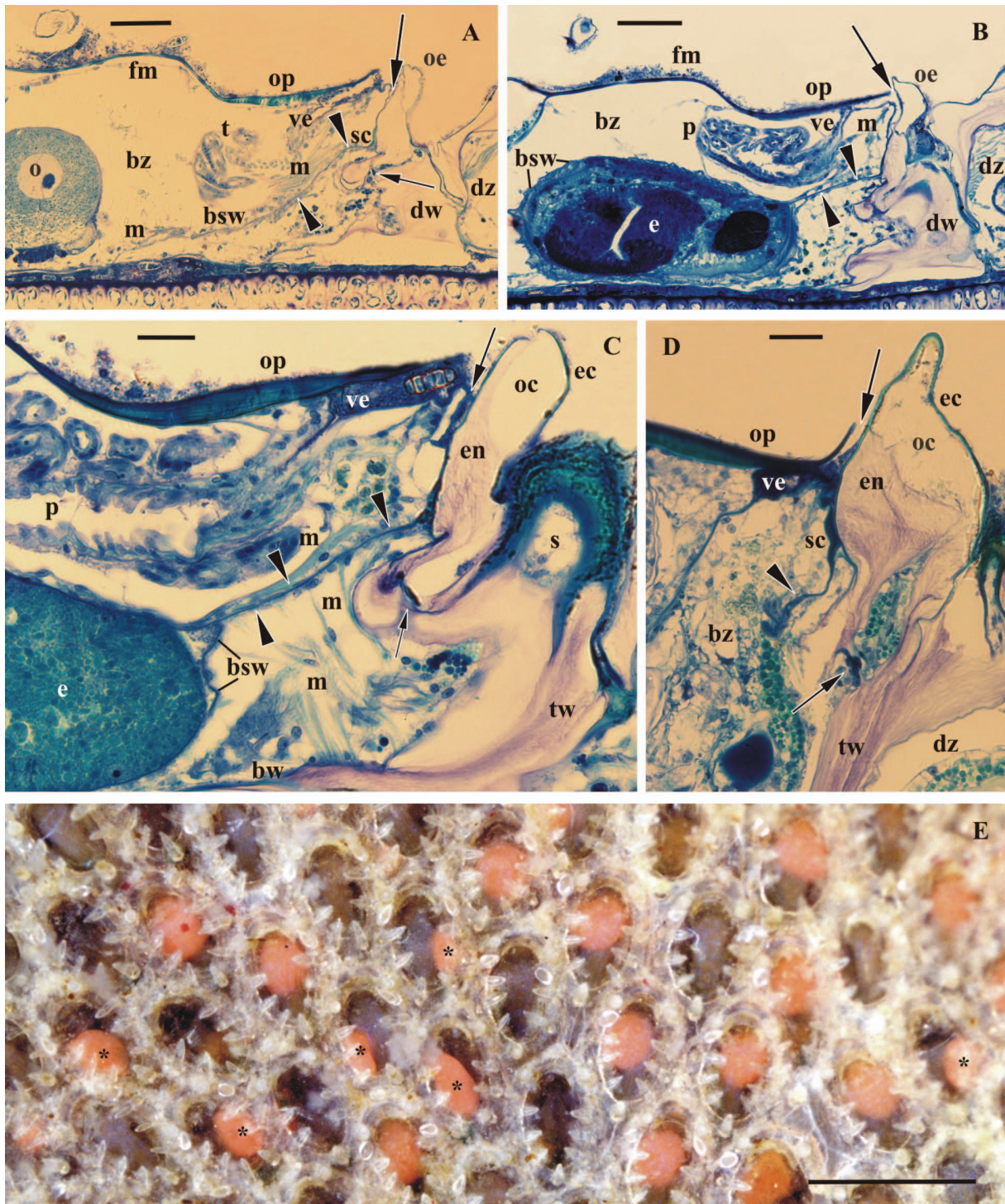


Fig. 4. Brooding zooids of *Cauloramphus spinifer* (A–C, E) and *C. niger* (D); light microscopy. **(A)** Longitudinal section of a zooid with an empty brood sac and a macrolecithal oocyte in the ovary. **(B)** Longitudinal section of a brooding zooid with an early embryo in the brood sac. **(C)** Enlargement of the distal end of a brooding zooid, showing part of the internal brood sac containing an embryo, the neck of the sac, and the vestigial kenozooidal oecium. **(D)** Enlargement of the distal end of a brooding zooid, showing the neck, internal brood sac, and vestigial kenozooidal oecium. In A–D: arrowheads, walls of the neck of the brood sac; larger arrows, entrance to the brood sac; smaller arrows, oocelial communication pore. **(E)** General view of living colony of *C. spinifer*. Asterisks indicate laterally positioned oocytes in different stages of development; embryos are located more centrally and toward the distal end of a zooid. Abbreviations: bsw, wall of the brood sac; bz, brooding zooid; bw, basal wall; dw, distal wall of brooding zooid; dz, distal zooid; e, embryo; ec, ectooecium; en, entoecium; fm, frontal membrane; m, muscular bundles of the brooding sac and closing fold of the non-calcified frontal wall of the maternal zooid; o, oocyte; oc, oocelial coelom; oe, oecium; op, operculum; p, polypide; s, spine; sc, sclerite; t, tentacles; tw, transversal wall; ve, vestibulum. Scale bars: A–B, 50 μ m; C–D, 20 μ m; E, 500 μ m.

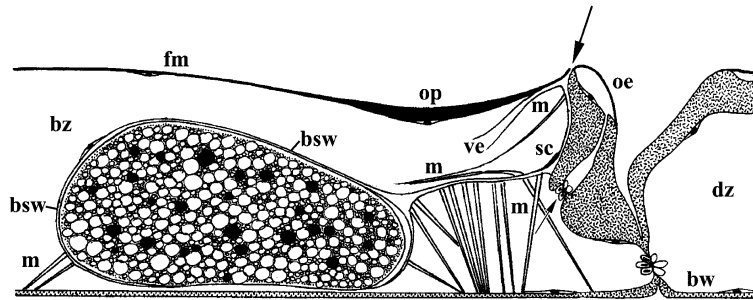


Fig. 5. Schematic longitudinal section through a brooding zooid of *Cauloramphus spinifer* with an early embryo in the internal brood sac. Larger arrow, entrance to the brood sac; smaller arrow, oocial communication pore. Abbreviations: bsw, wall of the brood sac; bz, brooding zooid; bw, basal wall; dz, distal zooid; fm, frontal membrane; m, muscular bundles of the brood sac and closing fold of the non-calcified frontal wall of the maternal zooid; oe, oocium; op, operculum; sc, sclerite; ve, vestibulum.

zooid. The brood sac is easily deformed; when not containing an embryo, it is completely flattened, positioned just above the basal wall, and extends for up to half the length of the cystid (Fig. 4A). Its wall consists of a thin cuticle and underlying epidermal and peritoneal epithelia. Several groups of muscles insert on the basal and lateral walls of both the distal and proximal parts of the main chamber of the sac. Their origins are on the basal wall and basal part of the lateral cystid walls (Fig. 4A; Fig. 5). These muscles possibly serve to extend the brood sac during larval release, and perhaps during oviposition as well.

Polypide recycling can occur during brooding. The polypide degenerates some time after oviposition, and a new one begins to grow.

DISCUSSION

Kenozooidal oecia among cheilostomes

In most cheilostomes that have been studied, the oecium is an outfold of the distal autozooid (discussed in Ostrovsky, 1998); oecia with direct pore connections to the maternal zooid (that is, maternally budded) appear to be rare. A maternally derived oecium has been reported in the cribrimorph cheilostome *Cribrilina annulata* (Fabricius, 1780) (Levinsen, 1909; Ostrovsky, 1998). In this species, the oecium is much larger than in *Cauloramphus*, covering the whole distal part of the maternal zooid. In addition, Winston and Håkansson (1986) illustrated a knob-like and obviously kenozooidal oecium for *Cymulopora uniserialis*. At present, these two examples and *Cauloramphus* should be considered as exceptions among cheilostomes, and are the only cases that support Silén's (1945, 1977) general model of derivation of the oecium from the maternal zooid.

The interpretation by Woollacott and Zimmer (1972) of the oecium of *Bugula neritina* (Bugulidae) as a kenozooidal polymorph is not correct, since there is no pore-cell complex between the oocial cavity and the visceral coelom of the distal autozooid; for details and discussion, see Ostrovsky and Schäfer (2003). It is possible that the unusual, complex oecia of Scrupariidae and Alysidiidae also consist of two (*Scruparia*, *Brettiopsis*, *Alysidium*) to eight (*Catenicula*) kenozooids budded from the maternal zooid (Levinsen, 1909; Hyman, 1959; Mawatari, 1973; Hayward, 1995), but this needs checking. Furthermore, the brood chambers in these two families could have evolved independently from those of other cheilostomes (Osburn, 1950; Taylor, 1988).

The internal-brooding apparatus as a highly modified ovicell

Hyperstomial ovicells are the most common type of brood chamber in cheilostome bryozoans and consist of the following elements: 1) the oocium, a double-walled, often hemispherical outgrowth, produced either by the maternal (egg-producing) zooid or the distal auto- or kenozooid, containing an enclosed coelomic lumen that connects with the zooid of origin through one or more pores; 2) a component of non-calcified distal wall from the maternal zooid, often in the form of an oocial vesicle (=oocial plug), and 3) a brood chamber (brooding cavity) bounded by the oocial and maternal-wall components (Ryland, 1976; Ryland and Hayward, 1977; Reed, 1991; Ostrovsky, 1998; Ostrovsky *et al.*, 2003, 2006). The brooding apparatus in *Cauloramphus* contains all these elements (all maternally derived) and thus shows homology with cheilostome hyperstomial ovicells, though highly modified in form. The oecium in *Cauloramphus* is kenozooidal and reduced, ranging in extent from a moderately reduced hemispherical cap in *C. niger* to a highly reduced, vestigial oecium lying on the distal opesial rim, as in *C. spinifer*. In *Cauloramphus*, the component of non-calcified distal wall from the maternal zooid has shifted from the more elevated position it would occupy at the proximal side of a hyperstomial oocial brood chamber to a position beneath the distal end of the zooidal operculum. The sclerite correspondingly is shifted basally and reduced, and has lost its musculature. Nonetheless, even in this position, the distal maternal-wall component and associated muscles are still involved in the closing and opening of the internal brood sac, performing the same function they do, for example, in the oocial vesicle of a hyperstomial ovicell.

The internal brood sac is a deep invagination of the distal wall of the maternal zooid. Though the brood sac lies internal to the maternal zooid, its cavity is topologically external, just as is the brood chamber of a hyperstomial ovicell. That is, the inner surface of the invaginated brood sac is continuous with and corresponds to the outer surface of the non-calcified distal wall of the maternal zooid. The external opening of the 'neck' abuts the proximal surface of the kenozooidal oecium. The brood sac is thus bounded by the reduced oecium (oocial component) and invaginated brood pouch (maternal-wall component), similarly to a hyperstomial ovicell. Nonetheless, although internal brooding in *Cauloramphus* involves structures homologous to those

comprising the typical cheilostome hyperstomial ovicell, internal brooding in this and some other cheilostome genera must be regarded as an evolutionary novelty. That is, the deep invagination in the non-calcified portion of the maternal distal wall to produce the brood sac is a novel structure not present in hyperstomial ovicells.

Evolution of internal brooding in Calloporidae

Ostrovsky *et al.* (2006) argued and provided supporting evidence that internal brooding has evolved independently in several different neocheilostome lineages. They also postulated hypothetical steps that might have been involved in the evolutionary transition from a hyperstomial ovicell to an internal brood sac in cheilostomes. These steps are 1) reduction of the oecium and proximal displacement of the brooding cavity, followed by 2) reduction of the sclerite of the oecial vesicle and the sclerite musculature, further proximal displacement of the main chamber, and consequent lengthening of the neck of the brood sac. Though most calloporids have hyperstomial ovicells, it may be that internal brooding evolved independently multiple times even within this neocheilostome group.

The genus *Cauloramphus* exemplifies a late stage in the evolution of internal brooding in the Calloporidae, with the vestigial kenozooidal oecium representing the nearly complete reduction of the oecium-forming (distal) zooid and its oecial outfold (for details and discussion, see Bishop and Househam, 1987; Ostrovsky, 1998). In most cheilostomes, the oecium is formed from the zooid distal to the maternal (egg-producing) zooid (Ostrovsky, 1998). This distal zooid can be either an autozooid, with a functional feeding polypide (the majority of cases), or a kenozooid that lacks a feeding polypide. In a transition series in which a distal, oecium-forming kenozooid becomes increasingly reduced, a point is reached where this distal zooid no longer has interzooidal connections with any adjacent zooids other than the proximal (maternal) zooid, which is the case in *Cauloramphus*. In evolutionary derivation, what we are calling the kenozooidal oecium is equivalent to both the formerly distal zooid (lower part) and its oecial outfold (upper part). In *Cauloramphus*, the maternal zooid is also connected to next autozooid in series; an ability to contribute to budding the next autozooid in a column is necessary unless growth of the colony is to stop with every zooid that produces a kenozooidal oecium. This ability to contribute to the next distal autozooid in addition to budding the oecial kenozooid requires nothing more than establishment of a new pore chamber, which should be an evolutionarily easily effected change.

Cauloramphus may not represent the ultimate stage in the evolution of internal brooding in calloporids. The internal brood sac in the genus *Gontarella* (Grischenko *et al.*, 2002; Ostrovsky *et al.*, 2006) is quite similar to that in *Cauloramphus*, in terms of the structure of the sac, the positions of its attached muscle bands, and the long neck. These similarities, along with the simple zooidal morphology and encrusting growth form of *Gontarella*, suggest that *Gontarella* might belong in the Calloporidae rather than in the Flustridae as Ostrovsky *et al.* (2006) previously suggested. This is supported by the observation (Ostrovsky *et al.*, 2006) that the internal brood sacs of *Cauloramphus* and

Gontarella differ from those in representative species of the flustrid genera *Nematoflustra* and *Biflustra*, which have a different muscle arrangement and a shorter neck. In *Gontarella*, there is no trace of a kenozooidal oecium, nor of a vestigial sclerite of the oecial vesicle. Thus, if *Gontarella* is indeed a calloporid, it represents a stage of ovicell reduction and modification beyond that of *Cauloramphus*, and perhaps the ultimate stage in this evolutionary series.

The question arises as to whether earlier stages are evident in the Calloporidae with regard to the reduction of the distal ovicell-forming autozooid and ovicell leading to the morphology seen in *Cauloramphus* and *Cymulopora*. A well-developed kenozooidal oecium directly budded from the maternal zooid, such as that seen in *Cribrilinea annulata* discussed above, possibly exemplifies an intermediate morphology; however, this ovicell type has not been reported in any calloporid, whereas terminal oecia formed by a reduced distal kenozooid are widespread among calloporids.

Another theoretical possibility for the evolution of an internal sac in calloporids is by modification of an immersed ovicell, with the vestigial oecium (outfold) formed by the distal zooid. Such immersed ovicells with vestigial oecia are well known among Calloporidae, for instance in the genera *Crassimarginatella* (Hastings, 1945, 1964; Cook, 1968; Winston, 1984; Tilbrook *et al.*, 2001) and *Cranosina* (Osburn, 1950; Chimonides and Cook, 1994). Immersed ovicells in calloporids differ from the endozooidal ovicells of flustrids, since their brooding cavity is immersed in the distal part of the maternal zooid, rather than in the proximal part of the daughter zooid as in flustrids (Ostrovsky, unpublished data). Of particular interest are *Aplousina filum* Jullien and Calvet, 1903 and *A. gigantea* Canu and Bassler, 1927 (see Cook, 1968), which are quite similar to *Gontarella* in zooid shape and size but possess immersed ovicells with small, cap-like oecia. We suggest that an ovicell of this type could transform to an internal brood sac by complete reduction of the oecium (and, possibly, the sclerite of the oecial vesicle). Further proximal displacement could lead to formation of an internal sac with a prominent neck, as seen in *Gontarella*.

Relevant to this discussion is the species *Septentriopora karasi* Kuklinski and Taylor, 2006. All other known species of the calloporid genus *Septentriopora* have subimmersed ovicells with small (but not vestigial) oecia formed by the distal autozooid. In contrast, supposedly brooding zooids of *S. karasi* have in place of the conventional oecium a small kenozooid. Kuklinski and Taylor (2006) referred to this kenozooid as a "vestigial ovicell," noting that it originates from the two proximolateral corners of the distal zooid, with which it communicates by multiporous septula. However, this interpretation is questionable, since no other cheilostomes with this type of oecium are presently known (see above). The vast majority of Cheilostomata possess oecia that are outfolds of the distal zooid. These oecia have no septula or pore-cell complexes; rather, their coelomic cavity is connected with the visceral coelom of the distal zooid via slits or simple pores. We speculate that *S. karasi* is an internal brooder that lost its oecia in a similar manner to *Gontarella*. However, it has acquired a kenozooid that positionally substitutes the oecium.

Although in the course of this study we directly observed

embryos in only two of the species examined (*C. spinifer* and *C. cryptoarmatus*), internally brooded embryos have been reported for all the species examined (Dick and Ross, 1988; Dick *et al.*, 2005; Grischenko *et al.*, 2007). These data coupled with our observation of oocidia in the eight species studied strongly suggest that a reduced, maternally produced kenozooidal oocidium and internal brood sac are characters common to all species of *Cauloramphus* and should thus be appended as diagnostic characters for this genus. As for the *Cauloramphus* species for which neither oocidia nor embryos have been reported (see Introduction), we suggest that these reproductive features have simply not yet been found due to lack of detailed observation.

A number of interesting questions remain. It is not yet known, for example, whether internal brooding is a synapomorphy for *Cauloramphus*, as opposed to a plesiomorphic character in the common ancestor of *Cauloramphus* and its sister group (also unknown), or in an even more distant ancestor. In a similar context, verification that internal brooding arose independently multiple times in Calloporidae will require a reliable (i.e., molecular) phylogeny of this family.

Another problem is exactly how or in response to what stimuli the fertilized egg travels from the coelom of the maternal zooid to the external brood sac. Presumably, oviposition is carried out in a manner similar to that in other brooding cheilostomes, in which a highly plastic oocyte squeezes through the supraneural coelomopore and enters the opening of the brood cavity (Gerwerzhagen, 1913; Silén, 1945; Corrêa, 1948; Nielsen, 1981; Dyrinda and King, 1983).

Finally, why internal brooding evolved from hyperstomial brooding repeatedly both among and within neocheilostome lineages is an open question. Ostrovsky *et al.* (2006) proposed several hypotheses (not mutually exclusive) to explain this transition: (1) reduction of oocidia could conserve energy for somatic growth and reproduction; (2) internal brooding cavities may be more spacious than ovicells, allowing the brooding of larger larvae; and (3) internal brooding may be an evolutionary response to the evolution of predators that feed on embryos held in ovicells. In any case, the numerous internal brooders known among different cheilostome families shows that the internalization of brooding is a novelty that allows them to successfully compete with relatives possessing oocidia in the form of prominent protective hoods. Ultimately, the selective forces driving this transition will need to be empirically investigated.

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