Volume 270, Number 12, Month 2009

JOURNAL OF morphology

ISSN 0362-2525

WILEY-BLACKWELL

Editor: J. Matthias Starck

Journal of Morphology
Volume 270, Number 12, Month 2009
Pages 1413–0000
WILEY-BLACKWELL

JOURNAL OF MORPHOLOGY 270:1413–1430 (2009)

Comparative Anatomy of Internal Incubational Sacs in Cupuladriid Bryozoans and the Evolution of Brooding in Free-Living Cheilostomes

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ABSTRACT Numerous gross morphological attributes are shared among unrelated free-living bryozoans revealing convergent evolution associated with functional demands of living on soft sediments. Here, we show that the reproductive structures across free-living groups evolved convergently. The most prominent convergent traits are the collective reduction of external brood chambers (ovicells) and the acquisition of internal brooding. Anatomical studies of four species from the cheilostome genera *Cupuladria* and *Discoporella* (Cupuladriidae) show that these species incubate their embryos in internal brooding sacs located in the coelom of the maternal nonpolymorphic autozooids. This sac consists of a main chamber and a narrow neck communicating to the vestibulum. The distal wall of the vestibulum possesses a cuticular thickening, which may further isolate the brood cavity. The presence of this character in all four species strongly supports grouping *Cupuladria* and *Discoporella* in one taxon. Further evidence suggests that the Cupuladriidae may be nested within the Calloporidae. Based on the structure of brooding organs, two scenarios are proposed to explain the evolution of the internal brooding in cupuladriids. The evolution of brood chambers and their origin in other free-living cheilostomes is discussed. Unlike the vast majority of Neocheilostomina, almost all free-living cheilostomes possess nonprominent chambers for embryonic incubation, either endozooidal and immersed ovicells or internal brooding sacs, supporting the idea that internal embryonic incubation is derived. We speculate that prominent skeletal brood chambers are disadvantageous to a free-living mode of life that demands easy movement through sediment in instable sea-floor settings. J. Morphol. 270:1413–1430, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: Cupuladriidae; embryonic incubation; sexual reproduction

INTRODUCTION

Brooding of embryos in the bryozoan taxon Cheilostomata is considered a key evolutionary innovation, contributing to their massive radiation and dominance in benthic habitats that began shortly after the appearance of incubation in the Middle Cretaceous (Taylor, 1988, 2000; Jablonski et al., 1997). The vast majority of living cheilostomes brood embryos in externally prominent protective chambers with well-developed calcified walls (hyperstomial ovicells), in which all or at least half of the brooding cavity is above the colony surface. Some taxa, however, incubate internally in the brooding cavity below the colony surface. In this case, embryos develop in either 1) modified ovicells with a reduced ooecium (protective calcified fold of the ovicell)—endozooidal (brooding cavity is placed in the proximal part of the distal zooid in a series) or immersed (brooding in the distal part of the maternal zooid), or in 2) internal brooding sacs. In both ovicells and internal brooding sacs the cavity for embryonic incubation is topologically external, distinguishing it from viviparity found in the family Epistomiidae, whose species incubate progeny intracoelomically (reviewed in Hyman, 1959; Ström, 1977; Reed, 1991; Ostrovsky et al., 2006, 2007; Ostrovsky, 2008a,b).

Internal embryonic incubation is known in at least 34 families and has been suggested in 10 more (Table 1), comprising one fourth of all neocheilostome families (Gordon, 2008). At least 21 families comprise exclusively internal brooders,

Contract grant sponsor: SENACYT, Panama; FWF, Austria; Grant number: P19337-B17; Contract grant sponsor: RFBR, Russia; Grant number: 07-04-00928a; Contract grant sponsor: STRI, Panama. Contract grant sponsor: NSF, USA; Grant number: EAR03-45471.

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Received 6 December 2008; Revised 8 May 2009;
Accepted 17 May 2009

Published online 1 July 2009 in
Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/jmor.10767

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TABLE 1. Occurrence of internal brooding and prominent ovicells in cheilostome taxa

Taxon	Internal brooding sacs, immersed ovicells and endozooidal ovicells	Prominent ovicells	References
Flustrina			
Calloporidae	IBS, IMO, IBS/VO	+	
Cranosina	IBS	−	Harmer, 1926
Gontarella	IBS	−	Ostrovsky et al., 2006
Cauloramphus	IBS/VO	−	Ostrovsky et al., 2007, 2009a
Crassimarginatella	IMO	+	Cook, 1968a, 1985; Ostrovsky et al., 2009a
Aplousina	IMO	+	Cook, 1968a
Cymulopora	IMO	−	Winston and Håkansson, 1986
Septentriopora	*	+	Kuklinski and Taylor, 2006a
Vibracellina	IMO	−	Winston and Håkansson, 1986
Antroporidae	IMO, EZO	−	Hastings, 1930; Cook, 1968a; Gordon, 1986; Tilbrook, 1998; Tilbrook and Grischenko, 2004
Chaperiidae	IBS	+	
Chaperia	IBS	−	Gordon, 1970, 1982, 1984; Gordon and Mawatari, 1992
Quadricellariidae	*	+	
Quadricellaria	*	−	Harmer, 1926; Mawatari, 1974; Gordon, 1984
Bryopastoridae	*	−	
Bryopastor	*	−	Gordon, 1986
Pseudothyracella	*	−	d'Hondt and Gordon, 1999
Farciminariidae	IBS	+	
Farciminellum	IBS	−	Harmer, 1926
Heliodomidae	*	+	
Setosellina	*	+	Harmer, 1926; d'Hondt and Schopf, 1984; Lagaaij, 1963b
Cupuladriidae	IBS	−	
Cupuladria	IBS	−	Waters, 1921; Cook, 1965a, 1985; our data
Discoporella	IBS	−	Winston and Håkansson, 1986; our data
Reussirella	IBS	−	Waters, 1921; Winston and Håkansson, 1986; Winston, 1988
Flustridae	IBS, EZO	+	
majority of genera	EZO	+	Vigelius, 1884a, b; Calvet, 1900; Levinsen, 1909; Hayward, 1995
Carbasea	IBS	−	Grant, 1827; Hayward, 1995
Nematoflustra	IBS	−	Ostrovsky et al., 2006
Bugulidae	IBS, IMO	+	
Bugula	IMO	+	Ryland, 1962; Hastings, 1943; Prenant and Bobin, 1966
Caulibugula	*	+	Harmer, 1926; Liu, 1985
Himantozoum	IBS, IMO	−	Harmer, 1926; Hastings, 1943; Hayward, 1995
Cornucopina	IMO	+	Hayward, 1995
Camptoplites	IMO	+	Kluge, 1914; Hastings, 1943; Hayward, 1995
Beaniidae	IBS, IBS/VO, IMO(?)	+	
Beania	IBS, IBS/VO, IMO(?)	+	Waters, 1912, 1913; Harmer, 1926; Hastings, 1943; Marcus, 1955; Gautier, 1962; Prenant and Bobin, 1966; Gordon, 1970; Ryland and Hayward, 1977; Cook, 1968b, 1985
Candidae	IBS, IMO, EZO	+	
Menipea	IBS, IMO, EZO	+	Hastings, 1943; Gordon, 1986; Hayward, 1995
Bugulopsis	IMO	−	Hastings, 1943
Caberea	EZO	+	Hastings, 1943; Gordon, 1984, 1986
Microporidae	*	+	
Calpensia	*	−	Hayward and Ryland, 1998
Microporina	*	−	Canu and Bassler, 1929; Kluge, 1975
Ogivalia	*	−	Hayward, 1995
Lunulitidae	IMO, EZO	−	
Lunulites	IMO	−	Håkansson, 1975; Håkansson and Voigt, 1996
Pavolunulites	IMO, EZO		Håkansson and Voigt, 1996
Lunulariidae	IBS, IMO	−	
Lunularia	IBS, IMO	−	Cook and Chimonides, 1986
Otionellidae	IBS	−	
Otionella	*	−	Cook and Chimonides, 1985b; Bock and Cook, 1998
Otionellina	IBS	−	Cook and Chimonides, 1985b; Bock and Cook, 1998
Petatosella	IBS	−	Bock and Cook, 1998
Helixotionella	*	−	Cook and Chimonides, 1984b
Kausiaria	*	−	Bock and Cook, 1998
Selenariidae	EZO, IMO	−	
Selenaria	EZO, IMO	−	Chimonides and Cook, 1981; Bock and Cook, 1999
Onychocellidae	IMO, EZO	+	
Aechmella	EZO	−	Taylor and McKinney, 2006
Onychocella	IMO	−	Cook, 1985
Smittipora	IMO	−	Cook, 1968c, 1973, 1985
Floridina	IMO	−	Hastings, 1930

TABLE 1. (Continued)

Taxon	Internal brooding sacs, immersed ovicells and endozooidal ovicells	Prominent ovicells	References
Steginoporellidae	IBS	−	
Steginoporella	IBS	−	Waters, 1913; Marcus, 1922; Harmer, 1926; Cook, 1964, 1968c, 1985; Winston, 1984
Labioporella	IBS	−	Cook, 1985
Chlidoniidae	IBS	−	
Chlidonia	IBS	−	Waters, 1913; Harmer, 1926
Crepis	*	−	Harmer, 1926
Poricellariidae	IBS/VO	−	
Poricellaria	IBS/VO	−	Waters, 1913
Ascophora			
Cribrilinidae	IMO, EZO	+	
Jullienula	*	−	Osburn, 1950; Hayami, 1975
Anaskopora	*	−	Arnold and Cook, 1997; Bock and Cook, 2001a
Cribrilina	IMO, EZO	+	Hayward and Ryland, 1998; Ostrovsky, 1998
Puelleina	EZO	+	Hayward and Ryland, 1998; Ostrovsky, 2002
Eurystomellidae	IMO, EZO	−	
Eurystomella	IMO	−	Gordon et al., 2002
Integripelta	IMO	−	Gordon et al., 2002
Zygoplane	IMO	−	Gordon et al., 2002
Selenariopsis	EZO	−	Bock and Cook, 1996
Pasytheidae	*	−	
Pasythea	*	−	Cook, 1985
Gemellipora	*	−	Cook, 1985
Exechonellidae	IBS	−	
Exechonella	*	−	Gordon, 1984; Cook, 1985
Triporula	IBS	−	Cook, 1985
Anexechona	*	−	Osburn, 1950
Adeonidae	IBS	−	
Adeona	IBS	−	Waters, 1912
Adeonellopsis	IBS	−	Waters, 1913
Reptadeonella	IBS	−	Winston, 1984
Adeonellidae	IBS	−	
Adeonella	IBS	−	Waters, 1912, 1913
Laminopora	IBS	−	Waters, 1912
Inversiulidae	*	−	
Inversiula	*	−	Harmer, 1926; Powell, 1967; Gordon, 1984; Hayward, 1995
Romancheinidae	IBS	+	
Arctonula	IBS	−	Gordon and Grischenko, 1994
Umbonulidae	IBS, IMO	+	
Oshurkovia	IBS	−	Hastings, 1944, 1964; Eggleston, 1972
Desmacystis	IMO	−	Gordon and Grischenko, 1994
Sclerodomidae	EZO	+	
Cellarinella	EZO	−	Hayward, 1995
Cellarinelloides	EZO	−	Hayward, 1995
Watersiporidae	IBS	−	
Watersipora	IBS	−	Waters, 1909, 1913; Mawatari, 1952; Cook 1985 Zimmer, in Reed, 1991
Uscia	*	−	Banta, 1969
Veleroa	*	−	Osburn, 1952
Stomachetosellidae	*	+	
Fatkullina	*	−	Grischenko et al., 1998
Tetraplariidae	*	+	
Tetraplaria	*	+	Harmer, 1957
Porinidae	EZO	+	
Porina	EZO	−	Ostrovsky, unpubl. data
Myriaporidae	EZO	−	
Myriapora	EZO	−	Ostrovsky, unpubl. data
Cheiloporinidae	IMO	+	
Cheiloporina	IMO	−	Ostrovsky, unpubl. data
Cryptosulidae	IBS	−	
Cryptosula	IBS	−	Smitt, 1863; Calvet, 1900; Gordon, 1977; Zimmer, in Reed, 1991; Gordon and Mawatari, 1992
Harmeria	IBS	−	Kuklinski and Taylor, 2006b
Urceoliporidae	IBS/VO	+	
Reciprocus	IBS/VO	−	Ostrovsky, unpubl. data

TABLE 1. (Continued)

Taxon	Internal brooding sacs, immersed ovicells and endozooidal ovicells	Prominent ovicells	References
Euthyrisellidae	IBS	–	
Pleurotoichus	IBS	–	Cook and Chimonides, 1981
Euthyrisella	IBS	–	Cook, 1979
Tropidozoum	IBS	–	Cook and Chimonides, 1981
Siphonicytaridae	*	–	
Siphonicytara	*	–	Bock and Cook, 2001b
Hippoporidridae	IBS	+	
Odontoporella	IBS	–	Gordon, 1970, 1989; Carter and Gordon, 2007

Data taken either from the literature or personal observations. The type of brooding is either observed anatomically or inferred from a presence of embryos in reproducing colonies. Bryozoans with immersed (IMO) and endozooidal (EZO) ovicells are classified as internal brooders because their embryos are incubated inside an internal brooding cavity below the colony surface. *Cauloramphus*, *Poricellaria, Reciprocus* and some species of *Beania* represent a special case in having both the internal brooding sac (IBS) and a vestigial kenozooidal ooecium (VO). Asterisks indicate cases in which brooding in the internal sac is suggested by the absence of ooecia or the presence of polymorphic zooids. The viviparous family Epistomiidae as well as Cellariidae with endotoichal ovicells were not included in the list. Note that the genera *Gontarella* and *Vibracellina* are provisionally placed in the family Calloporidae.

whereas others include both, species with internal brooding and species with prominent ovicells. In some taxa, zooids that brood internally are skeletally different from nonbrooding zooids. Such polymorphs can be larger than nonbrooding autozooids, and often have a special zooidal and orifice shape.

The fossil record suggests that ovicells were the earliest (skeletal) brooding structures in the cheilostomes (Taylor, 1988; Taylor and McKinney, 2002; Ostrovsky and Taylor, 2004, 2005; Cheetham et al., 2006), although incubation in the external membranous sacs in the first brooders can not be excluded. In any case, it is presumed that internal brooding is derived. Endozooidal and immersed ovicells are considered intermediate between prominent (hyperstomial) ovicells and internal brooding sacs (Ostrovsky et al., 2006, 2009a). The presence of both prominent ovicells and internal brooding within families (and, less frequently, genera; Table 1) suggests that internal embryonic incubation evolved independently several times within the Neocheilostomina: in both taxa, Flustrina and Ascophora (Ostrovsky et al., 2006, 2007, 2009a). The cause(s) underlying the shift from brooding in prominent ovicell to internal brooding remain unknown. Unfortunately, only a few anatomical studies of the internal sacs of cheilostomes have been published (Calvet, 1900; Waters, 1909, 1912, 1913, 1921; Mawatari, 1952; Ostrovsky et al., 2006, 2007, 2009a).

With this in mind we explore the evolution of internal brooding in free-living Bryozoa by 1) reviewing anatomical, ecological and fossil data and 2) conducting a comparative anatomical study of four cheilostome bryozoans from the free-living family Cupuladriidae in which internal brooding is the only method of embryonic incubation.

Mode of Life in Free-Living Cheilostome Bryozoa

Free-living cheilostomes are an ecological group of bryozoans adapted to life on soft, unstable sediments (Marcus and Marcus, 1962; Lagaaij, 1963a; Winston, 1988; Cook and Chimonides, 1983, 1994a). Larvae metamorphose on a sand grain, a foraminiferan, a small shell fragment, or less frequently without a substratum. They are transformed into the founding zooid(s) (ancestrula or ancestral complex) and begin budding zooidal generations to form discoidal or conical, cap-shaped colonies. These colonies rest freely on the sediment surface (O'Dea et al., in press).

Many taxa possess avicularian polymorphs with long tapered mandibles, which appear crucial to a successful life in shallow marine unstable sea-floor habitats. Motions of the setiform mandibles clean the colony surface of depositing sediment and epibiotic growth. They can lift the colony above the sea-floor, and by coordinated movements enable it to "walk." The mandibules uncover the colony when buried within sediment, and in some species overturn themselves if flipped over. This remarkable morphology and mode of life is termed "lunulitifom," and occurs among several unrelated bryozoan taxa, indicating convergent evolution (McKinney and Jackson, 1989).

The lunulitiform bryozoans are mainly represented by anascans of the Cupuladriidae, Heliodomidae (Calloporoidea), and Lunulitidae, Lunulariidae, Otionellidae and Selenariidae (Microporoidea) (see Gordon, 2008). A number of species of the Ascophora (Mamilloporidae, Eurystomellidae and Petraliellidae) show similar colony morphologies but anchor the colonies in the sediment by chitinous rootlets (Cook and Chimonides, 1981, 1983, 1994b). The free-living discoidal colonies are

TABLE 2. Data on stations and material collected

Station data				*Discoporella cookae*			*Discoporella marcusorum*			*Discoporella* sp. nov. P1			*Cupuladria exfragminis*		
Sample	Longitude	Latitude	Depth (m)	Living	Dead	% fertile	Living	Dead	% fertile	Living	Dead	% fertile	Living	Dead	% fertile
1	08° 16.86′ N	78° 53.04′ W	16.8	82	–	7.3									
2	08° 16.68′ N	78° 52.92′ W	17.1	22	2	9									
10	08° 17.09′ N	78° 52.73′ W	15.9	204	11	18.6									
11	08° 17.02′ N	78° 52.67′ W	16.1	81	3	17.3									
17	08° 18.21′ N	79° 04.64′ W	26.0				417	104	42.4	2	—	100	50	24	8

sometimes found living on soft substrata in species of Lanceoporidae, Bitectiporidae, Schizoporellidae and Lepraliellidae, which normally encrust hard substrata (Cook, 1965b).

Recruitment in Free-Living Cheilostome Bryozoa

Many free-living cheilostomes reproduce sexually and asexually by fragmentation of colonies (Lagaaij, 1963a; Cook and Chimonides, 1984a,b; Winston, 1988; O'Dea et al., 2008). Several recent studies have explored processes of asexual reproduction and its role in the life-cycle of some of the free-living cheilostome bryozoans, in particular the family Cupuladriidae (O'Dea, 2006; O'Dea et al., 2004, 2008). However, little is known about their sexual reproduction. The presence of the ovaria, oocytes and large embryos "in the zooecia near the periphery" was first recorded by Waters, (1921, p 404) in cupuladriids. From sections, he illustrated a large macrolecithal oocyte inside the cystid of *Cupuladria canariensis* (Busk, 1821) (described as *Cupularia*) (Waters, 1921, pl. 30, Fig. 25), concluding that embryos "emerge directly without passing into any external ovicell" (Waters, 1921, p 411). In *Reussirella doma* (d'Orbigny, 1853) (described as *C. johnsoni*) Waters found that "embryos (about 0.5-mm long) nearly fill the zooecial chambers" (Waters, 1921, p 414). Oocytes and, possibly, clusters of sperm were also observed inside zooids by Hastings (1930) in *Discoporella depressa* (Conrad, 1841) (described as *D. umbellata*).

Cook (1965a) summarized the few data on egg diameters and breeding seasons in Cupuladriidae from the literature and collections available (see also Cook and Chimonides, 1983), citing absence of external calcified brood chambers (ovicells) as a character of the family Cupuladriidae. In a later monograph, Cook (1985) recorded large pale yellow embryos in *C. canariensis* and orange-yellow embryos in *C. biporosa* (Canu and Bassler, 1923).

Winston and Håkansson (1986) noted internal embryonic brooding in *Reussirella doma* and *Discoporella depressa* (as *D. umbellata* subspecies *depressa*) without giving any further details apart from noting the pinkish-orange color of the larvae in the former. Later, Winston (1988) recorded 1–10 orange embryos in colonies of *R. doma*, stressing that sexual reproduction can start even in small colonies consisting of just 30 zooids. Finally, Cook and Chimonides (1994a) updated the diagnosis of the family Cupuladriidae by including brooding in internal ovisacs that sit within zooids of the same size and shape as normal autozooids. Besides these limited studies and observations, little is known about embryonic brooding in cupulidriids. For example, it remains speculation that cupuladriids possess internal sacs for incubation (Ostrovsky et al., 2006).

In other free-living cheilostomes, (Cook and Chimonides, 1985b) described in sections large embryos within nonovicellate peripheral autozooids with wide opesia in the genus *Otionellina* (Otionellidae). Later, Bock and Cook (1998, p 201) wrote that "brooding [is] known to be in an interior ovisac in recent species" for the genus *Petasosella* (Otionellidae). The same could be suggested for all genera in this family since they possess nonovicellate brooding autozooids that may or may not be dimorphic and zoned across the colony (Cook and Chimonides, 1984a,b, 1985b; Bock and Cook 1998). Within the family Selenariidae anatomy of male and female autozooids (the latter with ooecia) has been studied in *Selenaria maculata* Busk, 1852 in sections (Chimonides and Cook, 1981). In Lunulitidae the small arched ooecia of the immersed and, obviously, endozooidal ovicells have been illustrated (Håkansson, 1975; Håkansson and Voigt, 1996). "Enlarged brooding zooids" with or without ooecia have been recorded in different species of Lunulariidae. Embryos are described as "enormous. . ., and . . . deeply pigmented" (Cook and Chimonides, 1986, p 696).

MATERIALS AND METHODS

Colonies of four species from Cupuladriidae: *Cupuladria exfragminis, Discoporella cookae, D. marcusorum* (see Herrera-Cubilla et al., 2008) and *Discoporella* sp. nov. P1, were collected from the *R/V Urraca* by dredge and bottom grab sampling in the Las Perlas Archipelago, tropical Eastern Pacific, Gulf of Panama. Table 2 lists location information, the number of colonies and fragments collected (living, dead and fertile) for each

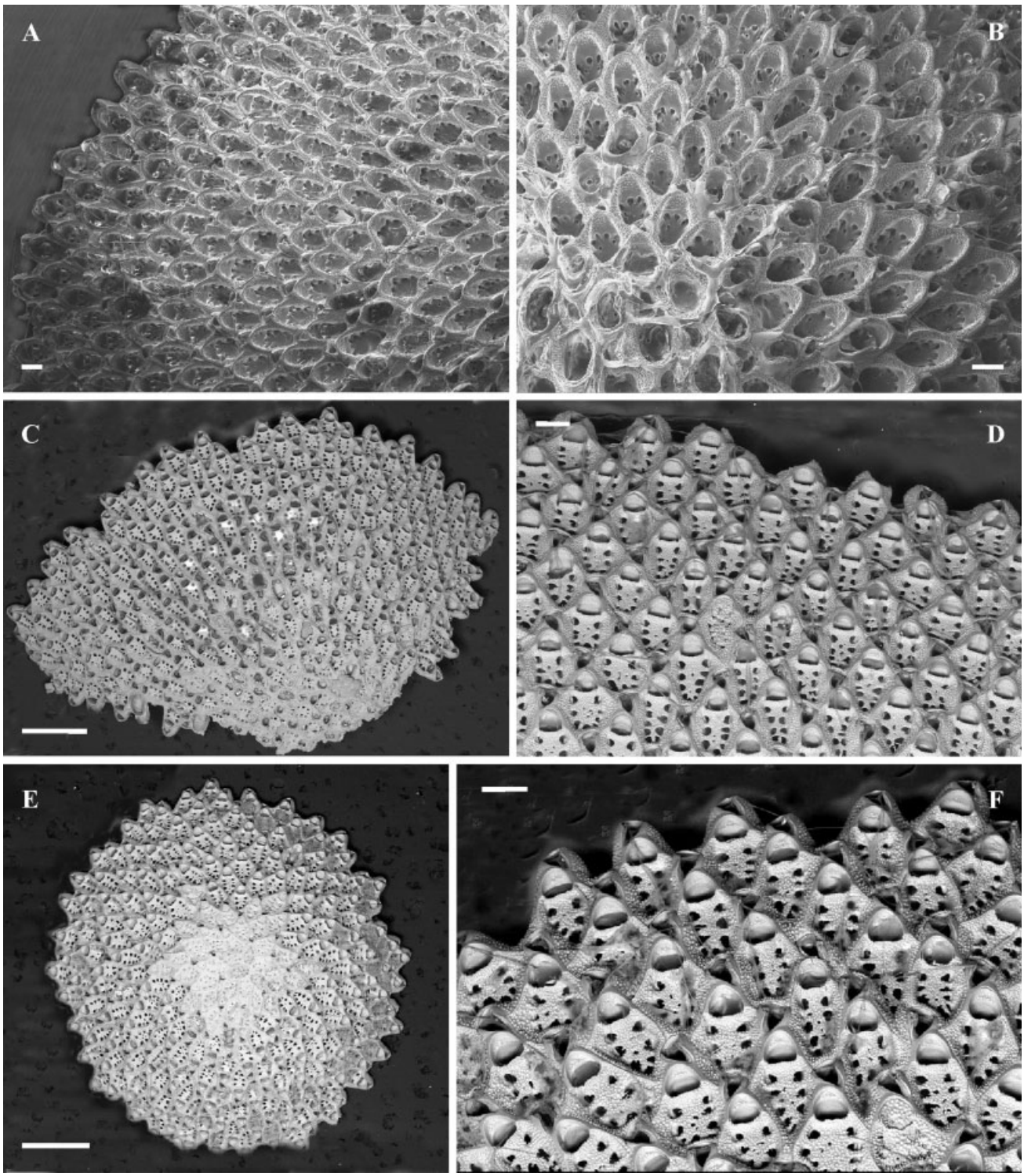

Fig. 1. Colonies and fragments of: **A**,**B**, *Cupuladria exfragminis*; **C**,**D**, *Discoporella cookae*; **E**,**F**, *Discoporella marcusorum* (SEM). Scale bars: A, B, D, F, 200.0 μm; C, E, 1.0 mm.

species. Samples 1, 2, 10, and 11 were taken on December 2, 2007, sample 17 on December 3, 2007.

All material collected was washed on board through a 2-mm-mesh sieve. Living colonies were kept in open seawater tanks with running water sourced from the Gulf of Panama. Selected specimens with embryos were fixed in either Bouin's fluid or 70% ethanol. For light microscopy, colonies were decalcified for 6–12 h using a few drops of a 2 N solution of hydrochloric acid, gradually dehydrated, cut in smaller pieces, embedded in plastic (epoxy resin type TAAB 812), sectioned (1.0-μm thick) with a

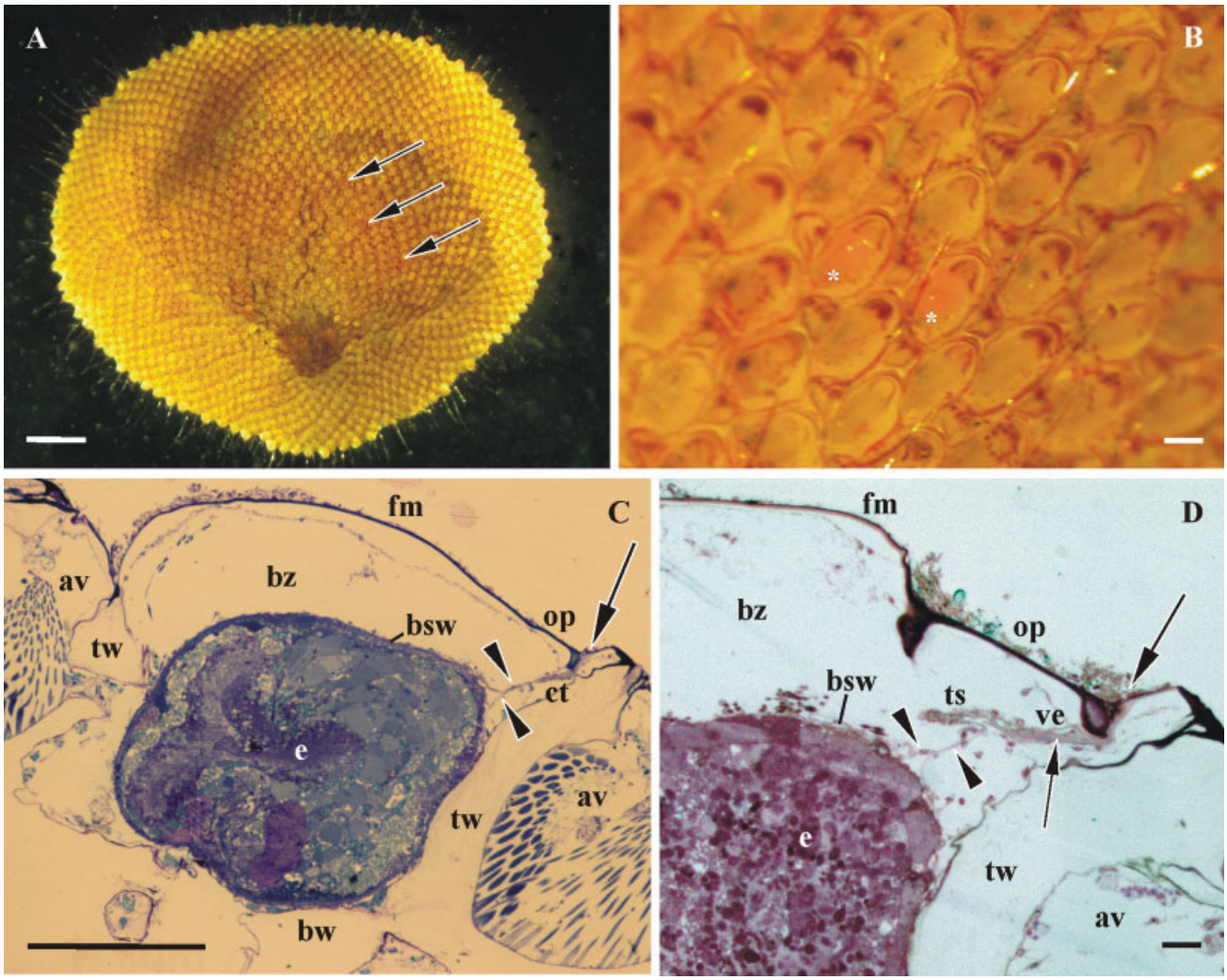

Fig. 2. *Cupuladria exfragminis*. **A:** Living colony regenerated from a fragment (arrows show brooding zooids with oocytes/embryos); **B:** close up of same colony, showing two zooids containing oocytes/embryos in the center (shown with stars); **C**,**D:** longitudinal sections through brooding zooids with the late (**C**) and early (**D**) embryos in the internal sac (walls of the neck of the brood sac shown with arrowheads, opening of the vestibulum shown with an arrow; cuticular thickening is seen in the vestibulum right below the operculum, also shown with arrow) (light microscopy). Abbreviations: av, avicularium; bsw, wall of the brood sac; bz, brooding zooid; bw, basal wall; ct, cuticular thickening; e, embryo; fm, frontal membrane; op, operculum; ts, tentacle sheath; tw, transversal wall; ve, vestibulum. Scale bars: A, 2 mm, B,C, 100 μm, D, 10 μm.

glass knife, and stained with Richardson's stain (1% water solutions of methylene blue, Na-tetraborate and azur II mixed in proportions 1:1:2) using standard methods (Richardson et al., 1960). In our preparations, methylene blue nonselectively stained cells of all types in soft tissues, whereas azur II selectively stained decalcified skeletal tissue. For scanning electron microscopy (SEM), colonies fixed in alcohol were cleaned in a 7.5% solution of sodium hypochlorite, rinsed, air-dried, and coated with gold. Specimens were observed with Zeiss EVO 40XVP scanning electron microscope at 15, 25.1, and 26.1 kV accelerating voltage.

RESULTS

Embryo Color and Position

Brooding zooids were recognized by the presence of embryos visible through the semi-transparent frontal or basal walls. No evidence was found for sexual dimorphism, and the external morphology of all brooding zooids appeared identical to non-brooding zooids (Figs. 1–4).

Embryos were pinkish in *Cupuladria exfragminis* (Fig. 2A,B), orange in *Discoporella cookae* (Fig. 3A–E) and scarlet in *Discoporella* sp. nov. P1. In *D. marcusorum*, embryos were scarlet or crimson in most colonies, but sometimes orange and rarely yellow (Fig. 4A–B). In one case, this full range of color was seen in several embryos of a single colony (Fig. 4B). In all other cases however, ripe eggs and embryos showed little intra-colony variation.

In both *C. exfragminis* and *D. cookae* colonies are mostly asexually recruited, and embryos appeared to be located predominantly within the central part of the colony. In contrast to this

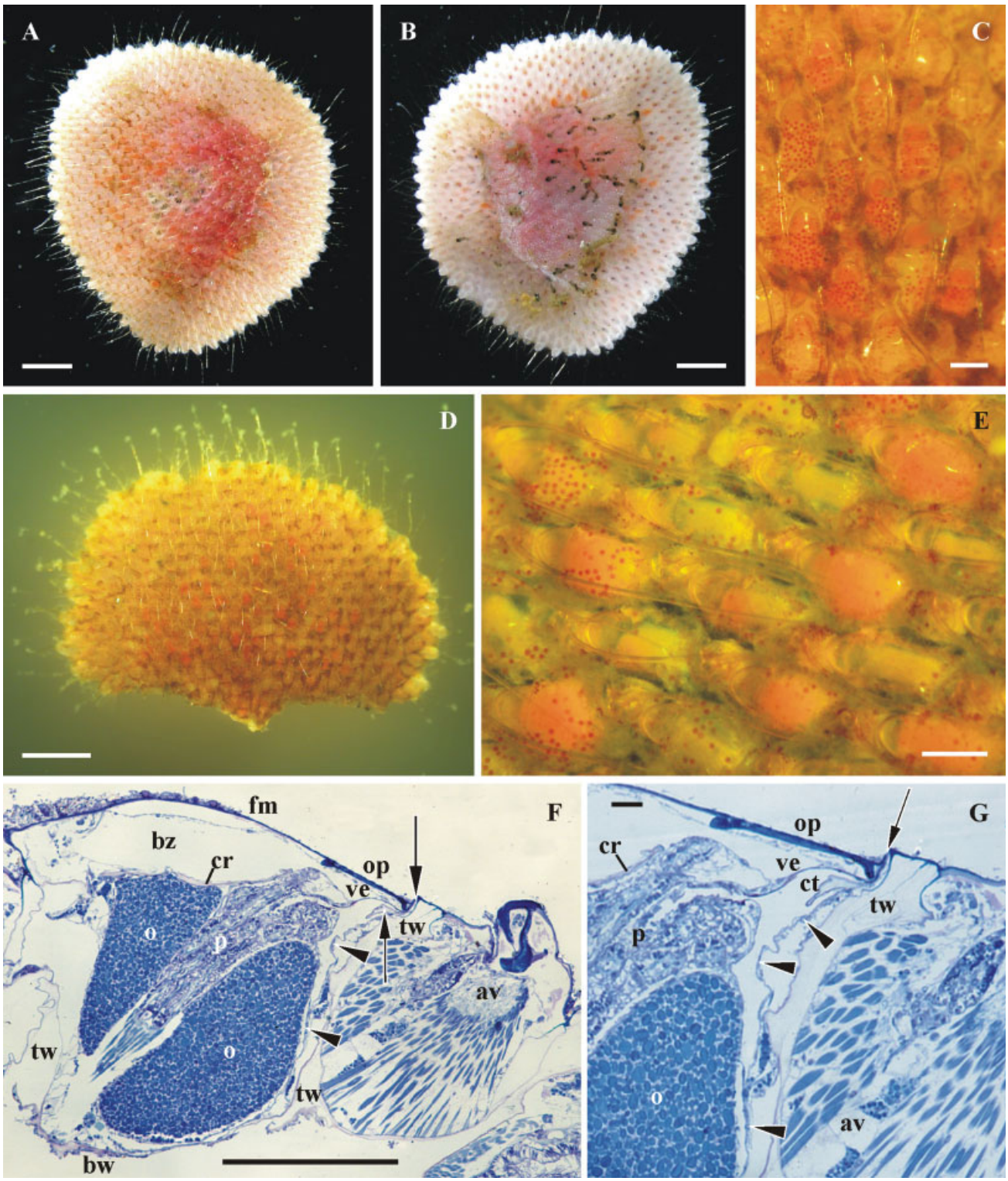

Fig. 3. *Discoporella cookae*. **A**,**B**: Frontal (**A**) and basal (**B**) view of living colony regenerated from a fragment; **D:** living fragment with oocytes/embryos (orange oocytes/embryos are mainly seen in the central part of the colony or fragment); **C**,**E:** part of the colony with several zooids containing oocytes/embryos (**E**, decalcified colony); **F**,**G:** longitudinal section through fertile zooid with the ovulated oocyte around the polypide [**G**, enlarged part of the same section showing the cuticular thickening in the vestibulum right below operculum; distal walls of empty brood sac shown with arrowheads, opening of the vestibulum and cuticular thickening (in **F**) shown with arrows] (light microscopy). Abbreviations: av, avicularium; bz, brooding zooid; bw, basal wall; cr, cryptocyst; ct, cuticular thickening; fm, frontal membrane; o, oocyte; op, operculum; p, polypide; tw, transversal wall; ve, vestibulum. Scale bars: A–B, D, 1 mm, C, E, F, 100 μm; G, 10 μm.

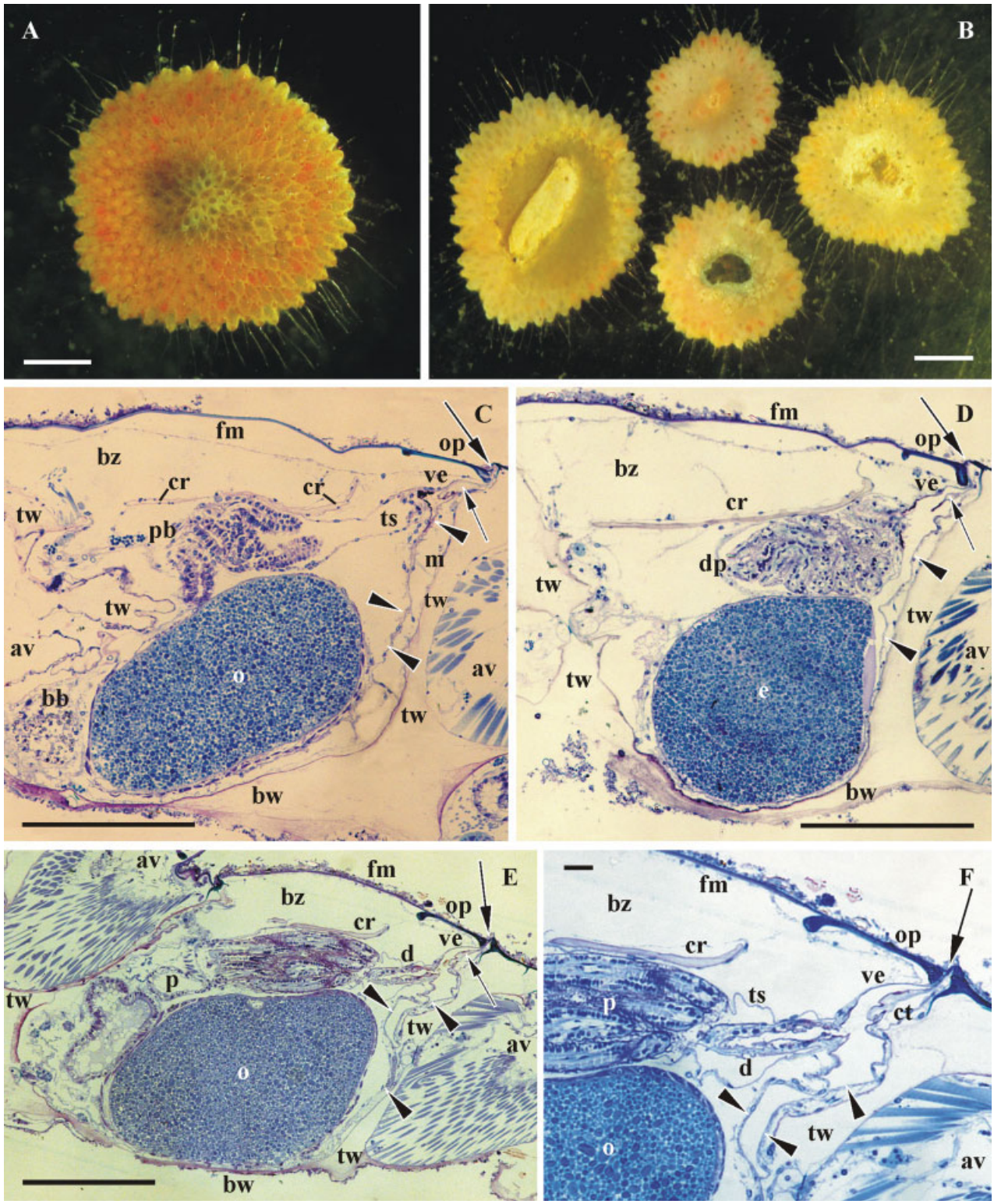

Fig. 4. *Discoporella marcusorum* (**A–D**, **F**) and *Discoporella* sp. nov. P1 (**E**). **A,B:** Living colonies (frontal and basal views) formed from larvae with substrate for the larval settlement observable in several colonies in **B**. Note that oocytes/embryos have different colors in different colonies, and that they are mainly located in the colony periphery. **C–F:** longitudinal sections through fertile zooids with the mature ovarial oocyte and empty brood sac (**C**,**E**,**F**), and early embryo inside the sac (**D**) (walls of brood sac shown with arrowheads, opening of the vestibulum and cuticular thickening shown with arrows) (light microscopy). Polypide is forming (**C**), degenerating (**D**) and functional (**E**,**F**). Abbreviations: av, avicularium; bb, brown body; bz, brooding zooid; bw, basal wall; cr, cryptocyst; ct, cuticular thickening; d, diaphragm; dp, degenerating polypide; e, embryo; fm, frontal membrane; m, muscular bundles of the brooding sac; o, oocyte; op, operculum; p, polypide; pb, polypide bud; ts, tentacle sheath; tw, transversal wall; ve, vestibulum. Scale bars: A, B, 1 mm; C, E, 100 μm, F, 10 μm.

pattern, in *D. marcusorum* and *Discoporella* sp. nov. P1, the colonies develop from larvae, and appear to brood embryos predominantly in the peripheral parts of the colony (see also O'Dea et al., in press, for details). Cuticles were more heavily pigmented in *D. marcusorum* and *Discoporella* sp. nov. P1 than in *D. cookae*.

Observations made here on reproductive morphology corroborate taxonomic division of species derived from skeletal morphology (Herrera-Cubilla et al., 2006, 2008). We suggest that the color of embryos and cuticle and the location of embryos within colonies can help identify living material preliminarily in the field when it is often difficult to study the skeletal morphology.

Comparative Brooding Anatomy

In all four cupuladriid species, oocytes were found to be large and macrolecithal. Oocytes appear to be produced and brooded sequentially, corresponding to the "second reproductive pattern" in Bryozoa (Reed, 1991; Ostrovsky et al., 2009b). Superficially, mature oocytes cannot be distinguished from embryos because they are of the same size and color and occupy the same position inside the maternal zooid. However, embryos are enclosed in internal brooding sacs while oocytes are not.

In one case of *D. cookae*, the contracted polypide was found to be ensheated by the ripe oocyte (Fig. 3F). Dyrynda and King (1983) observed mature ovulated oocytes wrapping round the tentacle sheath before oviposition in the flustrid cheilostome *Chartella papyracea* (Ellis and Solander, 1786), and Ostrovsky (1998) described oocytes wrapped around the vestibule in this manner in the cribrimorph *Cribrilina annulata* (Fabricius, 1789).

Cupuladria exfragminis

Brooding occurs in a special internal sac for embryonic incubation placed in the maternal (egg-producing) zooid (Figs. 2C–D, 5A). The sac is a spacious invagination of the vestibular wall, and therefore the brooding cavity is extracoelomic. The sac wall consists of a thin cuticle and underlying epidermal and peritoneal epithelia and is easily deformed. When containing an embryo, the sac occupies more than a half of the zooidal volume (Figs. 2C, 5A).

The brood sac consists of a central chamber and a narrow short "neck" that communicates with the vestibulum close to the diaphragm of the tentacle sheath (Figs. 2C–D, 5A). The distal wall of the vestibulum bears a cuticular thickening (flap) above the place where the "neck" opens into the vestibular cavity. The position and shape of this flap may act like a cover, plugging the internal space of the main chamber and providing additional isolation from the vestibulum and the tentacle sheath (Figs. 2D, 5A).

Several thin muscular bundles attach to the "neck" of the brood sac (Fig. 5A). Their opposite ends are anchored onto the transverse and possibly, lateral cystid walls, presumably serving to expand the neck during oviposition and larval release. No fibers were found attached to the main chamber wall except for a few funicular strands.

Embryos reached up to 206 × 162 μm. Some embryos were seen to be surrounded by a thin fertilization envelope.

Discoporella cookae, *D. marcusorum* and *Discoporella* sp. nov. P1

In the three *Discoporella* species brooding occurs in a special internal sac for embryonic incubation placed underneath the cryptocyst of the maternal (egg-producing) zooid (Figs. 4D–F, 5B). The thin-walled brooding sac is an oval invagination of the vestibular wall. Thus, although the brood sac lies internally to the maternal zooid, its cavity is topologically external. Such sac wall consists of a thin cuticle and underlying epidermal and peritoneal epithelia, and is easily deformed. When containing an embryo, the sac occupies more than one third of the zooidal cavity, whereas it is flat or slightly folded when empty. Such sac is positioned just above the basal wall and extends up to half the length of the cystid (Figs. 3F, 4C–E).

The brood sac consists of a main chamber and a narrow "neck." It is confluent with the vestibulum, close to the diaphragm of the tentacle sheath (Figs. 3F–G, 4C–F, 5B). Right above where the "neck" opens to the vestibular cavity, the distal wall of the vestibulum bears a cuticular thickening similar to *Cupuladria exfragminis* (Figs. 2D, 3F–G, 4C–F, 5).

Several thin muscular bundles attach to the distal wall of the brood sac and its "neck" (Figs. 4C, 5B). Their opposite ends are anchored to the transverse and, possibly also to the lateral cystid walls, presumably serving to expand the "neck" during oviposition and larval release.

Embryos and ripe oocytes reached 163 × 160 μm (*D. cookae*), 173 × 131 μm (*D. marcusorum*), and 197 × 119 μm (*D.* sp. nov. P1) in size. In section, some embryos were seen to be surrounded by a thin fertilization envelope.

Polypide Recycling and Brooding

Polypide recycling is clearly connected with embryonic incubation (Fig. 4D), but, without seasonal observations our data on polypide degeneration-regeneration and reproductive cycle are provisional. Observation of the mature oocyte wrapped round the tentacle sheath of the contracted

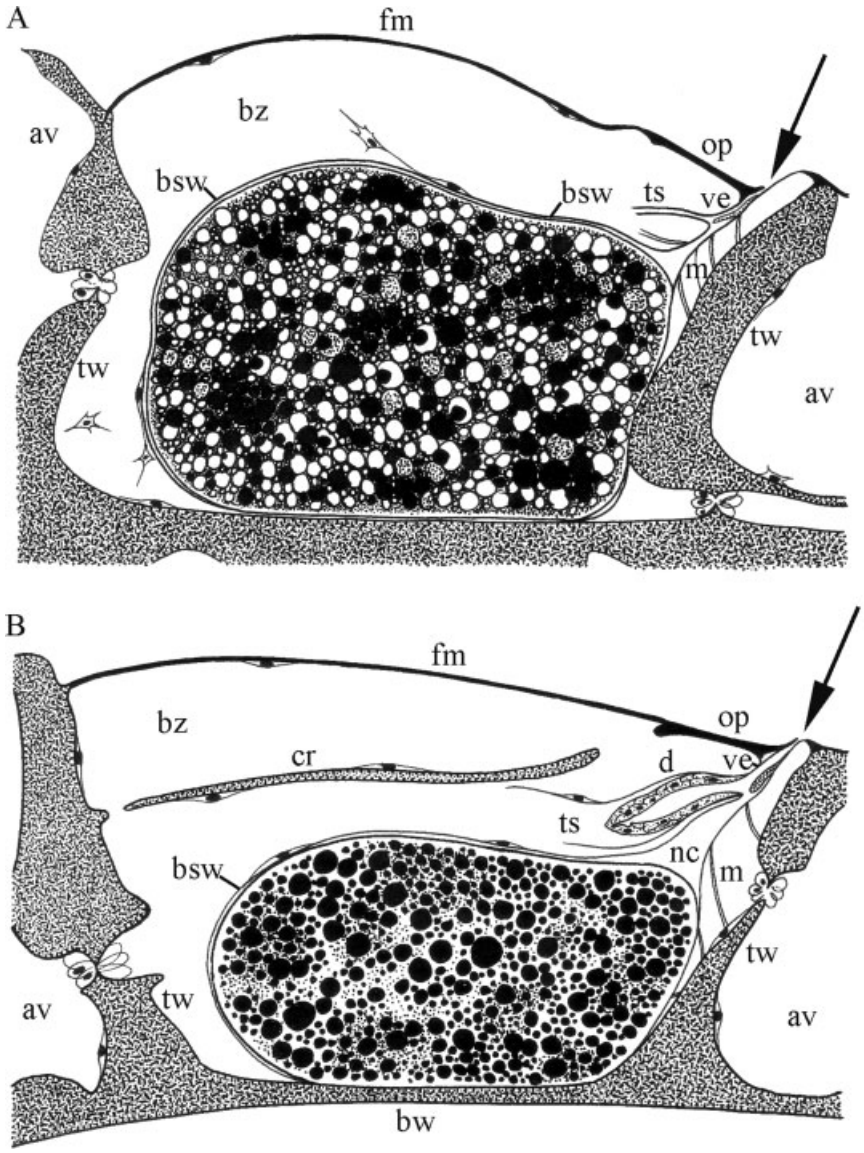

Fig. 5. Schematic longitudinal sections through brooding zooids with early embryos in the internal sac (opening of the vestibulum shown with arrows). **A,** *Cupuladria exfragminis*; **B,** *Discoporella marcusorum*. av, avicularium; bsw, wall of the brood sac; bw, basal wall; bz, brooding zooid; cr, cryptocyst; d, diaphragm; fm, frontal membrane; m, muscular bundles of the brood sac; nc, cavity of the "neck"; op, operculum; ts, tentacle sheath; tw, transverse wall; ve, vestibulum.

polypide in *D. cookae* suggests that oviposition occurs by the activity of the polypide through a supraneural coelomopore, similar to what has been described in other brooding cheilostomes (Gerwerzhagen, 1913; Silén, 1945; Nielsen, 1981; Dyrynda and King, 1983). There might be behavioral differences, because a zygote must be placed into the internal brooding sac and not in an ovicell as in other brooders.

Polypides degenerate some time after oviposition and brown bodies are placed near to the basal cystid wall in the proximal part of the zooid (Fig. 4C). A fresh polypide forms after the larva is released and the next oocyte begins to grow (Fig. 4C, E). The precise sequence of events remains unknown but the new polypide must be functional at the time of oocyte maturation in order to perform oviposition.

DISCUSSION

Polypide Degeneration in Internal Brooders

In species with internal brooding, the polypide of the zooid either obligatorily degenerates or continues to feed during incubation of the embryo. Post-oviposition, obligatory polypide degeneration probably occurs because of the need for space for the large embryo that may fill most of the zooidal coelom (Ostrovsky et al., 2006). Similarly to cupuladriids studied, this has been observed in several

encrusting species with internal brooding including *Steginoporella buskii* (Harmer, 1900), *S. magnilabris* (Busk, 1854), *Crassimarginatella falcata*, Cook, 1968, *Nematoflustra flagellata* (Waters, 1904), *Watersipora subtorquata* and *Gontarella* sp. (Mawatari, 1952; Cook, 1964, 1973, 1985; Ostrovsky et al., 2006). It is probable that polypide degeneration and regeneration are triggered hormonally by corresponding shifts in oogenesis.

In contrast, in such internally brooding species as *Smittipora levinseni* (Canu and Bassler, 1917), *Onychocella alula* Hastings, 1930, *Cryptosula pallasiana* (Moll, 1803) and, obviously "*Biflustra*"*perfragilis* MacGillivray, 1881 (Calvet, 1900; Cook, 1973, 1985; Ostrovsky et al., 2006), "polypides may either continue to feed, regress, or regenerate" during embryonic development (Gordon, 1977, p 341). In those species, we suggest that polypide recycling is associated with ontogenetic aging (Gordon, 1977).

Variation of Embryo Color

Embryo color has been considered diagnostic in some cheilostome species (Ryland, 1958), although more research is required to support the confident use of embryo color to discriminate species. Jebram (1975) showed, using controlled culture experiments, that diet can strongly influence embryo color in the ctenostome *Bowerbankia gracilis* Leidy, 1855. From the other hand, the same author observed that the coloration of the embryos varies "from very light to reddish pink" in natural populations across different habitats in this species (Jebram, 1976, p 75).

Intercolonial embryo color variation observed in *Discoporella marcusorum* may therefore be due to environmental differences experienced by geographically separated populations rather than signaling the presence of a cryptic speciation. Indeed, several tones of embryo color were observed once within a single colony of *D. marcusorum*. Nonetheless, it remains unclear if this was due to asynchronous oocyte/embryo ontogeny or, perhaps intraoocytic parasitism that has been observed in some cheilostomes (Ostrovsky, 1998).

Positioning of Brooding Zooids and Asexual Propagation

Patterns of the distribution of brooding zooids in the cupuladriids studied may be linked to the rate of asexual reproduction by fragmentation (O'Dea et al., in press), leading us to ascertain if other free-living bryozoans show similar patterns. Some data from the literature support the idea that positioning of embryos in the central part of the colony correlates with common colony fragmentation, while it appears that colonies that are less likely to fragment preferentially brood in the colony periphery. For example, reproductively active zooids are positioned on the periphery in *Selenaria fenestrata* Haswell, 1880 and *S. maculata* (Selenariidae) (Chimonides and Cook, 1981; Cook and Chimonides, 1983) and these species do not reproduce asexually (Cook and Chimonides, 1985a, 1987; Bock and Cook, 1999). Similarly, *Helixotionella* (Otionellidae) has peripheral brooding autozooids and fragmentation is not known in this genus (Cook and Chimonides, 1984b; Bock and Cook, 1998). This is similar to the situation in *Discoporella marcusorum* in which brooding mainly occurs in the peripheral zooids and the preferred mode of reproduction is sexual. In contrast, *Otionella tuberosa* Canu and Bassler, 1920, and *Petasosella moderna* Bock and Cook, 1998 (Otionellidae) distribute reproductive zooids across the colony and often reproduce by fragmentation (Cook and Chimonides, 1985b; Bock and Cook, 1998). Also brooding zooids are scattered across the colony in *Lunularia capulus* (Busk, 1852) (Lunulariidae), which often propagates asexually by fragmentation (Cook and Chimonides, 1986), and this is reminiscent of the situation in *Discoporella cookae*.

This pattern is not universal across all free-living bryozoa, however. *Otionellina* (Otionellidae) broods peripherally and fragmentation is known in at least three species of this genus (Cook and Chimonides, 1984a). In the genus *Lunulites* (Lunulitidae), ovicellate brooding zooids are positioned peripherally or sub-peripherally, and while some species do not fragment it is sometimes the main method of propagation in others (Håkansson, 1975; Thomsen and Håkansson, 1995; Håkansson and Voigt, 1996). Ovicells occur in peripheral regions in the ascophoran genera *Anoteropora* and *Mamillopora* (Mamilloporidae) whose conical colonies are not free-living but anchored in soft sediment by kenozooidal rhizoids, but they do commonly fragment (Cook and Chimonides, 1994b; Cheetham and Jackson, 2000).

Comparative Anatomy of Internal Brooding in Cheilostomata

Although internal brooding was already described by Grant (1827) and Smitt (1863) in cheilostomes, the first anatomical study was not conducted until Calvet (1900). He described and illustrated a longitudinal section of the internal brood chamber (pouch or diverticulum of the vestibulum), showing the muscles attached to the wall of the brooding sac in *Cryptosula pallasiana*. Later, Waters (1909, 1912, 1913) described that embryos were brooded in an internal sac in a number of anascan and ascophoran cheilostome genera (*Beania, Steginoporella* [described as *Steganoporella*], *Watersipora* [as *Lepralia*], *Adeona*, *Adeonella*, *Adeonellopsis*, *Laminopora*), but the author

did not give details of brood sac structure or its connection with the exterior.

Marcus (1922), Harmer (1926), and Cook (1964) made similar findings while studying the genus *Steginoporella* (as *Steganoporella*), describing the embryo as occurring in "a spacious, thin-walled ovisac", and mentioning that it extends nearly to the zooidal basal wall, being attached "to the lateral walls ... by a number of muscle-fibers" (Harmer, 1926, p 271). These authors, however, were unable to ascertain whether the sac was connected to the vestibulum. Waters (1913) and Harmer (1926) also mentioned an embryo or egg inside a female brooding zooid in *Chlidonia pyriformis* (Bertoloni, 1810).

Hastings depicted (1930, p 709, pl. 5, Fig. 17) the embryo located in the brooding sac of the immersed ovicell in *Antropora tincta* (Hastings, 1930) (Antroporidae). The sac is situated beneath the vestibulum, proximal to the vestigial ooecium, and several muscle bundles are attached to the sac walls laterally on both sides. A similar position of embryo in the immersed ovicell was depicted by Cook, (1968a, Text-Fig. 8) in the calloporid *Aplousina major* (Calvet, 1907).

Hastings (1944, pp 273–274) observed "zooecia ...[with] embryos in the body-cavity" in *Oshurkovia littoralis* (Hastings, 1944) (as *Umbonula verrucosa*), that possesses neither ovicells nor zooidal sexual dimorphism. Later she mentioned "internal ovisacs" (Hastings, 1964), and this was confirmed by Eggleston (1972) who noted internal brooding of several embryos in this species.

Mawatari (1952, Figs. 34–35, 44), who studied in section some aspects of sexual reproduction in *Watersipora subtorquata* (d'Orbigny, 1852) (as *W. cucullata*), mentioned "the embryo sac," which was seen to envelop the developing embryo and connect to the vestibulum (see also Zimmer, pers. comm. in Reed, 1991); Cook, (1979, p 200) mentioned that "brood chambers may be membranous diverticula housed within zooid body walls" in dimorphic female zooids of *Tropidozoum cellariiforme* Harmer, 1957.

More recently, the anatomical structure of internal sacs for embryonic incubation was studied in the anascans *Nematoflustra flagellata* (Waters, 1904), *Gontarella* sp. and *"Biflustra" perfragilis* and a number of species from the genus *Cauloramphus* (Ostrovsky et al., 2006, 2007, 2009a). The latter is characterized by a vestigial ooecium (the ovicell's protective fold), otherwise the structure of its brood sac is very similar to the other three species mentioned. In all species, the neck of the brood sac opens to the outside independently of the vestibulum, and the brood sac is consequently closed by the (often modified) ooecial vesicle, sometimes in conjunction with the zooidal operculum.

The opening of the brood sac in the four cupuladriids species studied here communicates with the vestibulum. This is unlike all anascans previously studied but similar to the ascophorans *Cryptosula* and *Watersipora*. Interestingly, closure of the brood sac in cupuladriids is enhanced by a special cuticular thickening (flap) that has not been described before in cheilostomes. Because it occurs in all four species and is absent in other cheilostomes, *Cupuladria* and *Discoporella* should be maintained within the same family.

Evolution of Internal Brooding in Free-Living Bryozoans

The brood sac in cupuladriids consists of a main chamber and a narrow "neck" with a muscular system, which probably expands during oviposition and larval release. Considerable morphological variation occurs among cheilostome internal brooders, particularly with regard to the length of neck (which is absent in species with immersed ovicells and very long in some species with brooding sacs), the mode of external communication, and the method of closure and arrangement of the muscular system. Such variation may exist because internal brooding evolved independently many times in different lineages of Cheilostomata (Ostrovsky et al., 2006, 2009a). This suggestion is also strongly supported by a presence of both ovicells and internal brood sacs within the same taxa (Table 1).

Most free-living anascans have an internal brooding cavity, and the protective part of the ovicell (ooecium) is either completely or partially reduced. Some taxa (notably members of the Lunulitidae, Selenariidae and one species of Lunulariidae) possess either immersed or endozooidal ovicells with a different degree of ooecial reduction, whereas others (the rest of the Lunulariidae and Otionellidae) have no ooecia, brooding their embryos internally, obviously within membranous sacs in either modified or nonmodified zooids (Håkansson, 1975; Chimonides and Cook, 1981; Cook and Chimonides, 1983, 1984a,b, 1985a,c, 1987; Håkansson and Voigt, 1996; Bock and Cook, 1998, 1999). Cupuladriidae also have no ooecia and brood embryos in internal membranous sacs.

Internal brooding is almost entirely universal across the free-living Bryozoa. Exceptions include *Heliodoma implicata* Calvet, 1906, *Setosellina capriensis* (Waters, 1926) and *S. roulei* Calvet, 1903 (Heliodomidae), which possess terminal ovicells with well-developed ooecia (Jullien and Calvet, 1903; Harmelin, 1977), but ovicells have not been recorded in all other species of this family, both free-living and encrusting (Harmer, 1926; Lagaaij, 1963b; d'Hondt and Schopf, 1984).

The Lunulitidae are suggested to have evolved from the Onychocellidae (Cook and Chimonides, 1983, 1986), whose species possess prominent, endozooidal and immersed ovicells (Voigt, 1989;

Harmer, 1926; Taylor and McKinney, 2006; Tilbrook, 2006, see Table 1). Based upon zooidal and ovicellar morphology, a similar origin might also be inferred for the Selenariidae. Anatomical sections show that ovicells in *Selenaria maculata* are endozooidal with the vestigial ooecium and brooding cavity placed in the proximal part of the distal (ooecium-producing) zooid (Chimonides and Cook, 1981), and judging from illustrations of Bock and Cook (1999) immersed ovicells occur in a few species of *Selenaria*. The phylogenetic origins of the Cupuladriidae remain unclear (Cook and Chimonides, 1983, 1994a), but we assume that affinities with Calloporidae and Antroporidae exist, given that some or all members of these clades possess reduced or no ooecia and incubate embryos inside an internal brooding cavity of either internal sac or immersed ovicell (Ostrovsky et al., 2006, 2007, 2009a).

We present two hypotheses about the evolution of internal brooding in the Cupuladriidae. The first presumes that the ancestor had either immersed ovicells with strongly reduced ooecium, such as in some Calloporidae and Antroporidae, or internal brooding sacs with a vestigial kenozooidal ooecium, as in the calloporid *Cauloramphus*. A total reduction of the ooecium in descendants would be then a derived condition (Ostrovsky et al., 2007, 2009a). In this case, cheilostomes similar to the species from the genus *Vibracellina* might be considered as possible intermediates. They form not free-living but encrusting colonies growing on the sand grains and small pieces of the shells, and have immersed but not endozooidal ovicells with vestigial ooecia (Canu and Basler, 1929; Winston and Håkansson, 1986).

The second hypothesis suggests that the Cupuladriidae evolved directly from a nonovicellate ancestor similar to the recent calloporids *Cranosina*. *C. coronata* (Hincks, 1881) is an internal brooder in which Harmer (1926, p 266) found "an embryo, lying in an ovisac, in a cavity of a zooecium". Noteworthy, *C. coronata* forms encrusting colonies, whereas colonies of *C. spiralis* Chimonides and Cook, 1994, are virtually free-living. There are also similarities in the construction of colonies of *Cranosina* and cupuladriids (Chimonides and Cook, 1994). It should be noted, however, that the earliest cupuladriids are known from the Paleocene (Gorodiski and Balavoine, 1961), thus predating both, *Vibracellina* that appeared in the Middle Eocene (Canu and Bassler, 1917), and *Cranosina* that is known since the Late Eocene (Canu and Bassler, 1920). Although further paleontological and neontological work is required to clarify the origins of internal brooding in the cupuladriids, we deduce from the nature of internal brooding and simple zooidal morphology that the cupuladriids are nested within the calloporids or their close relatives.

The Heliodomidae evolved from an ancestor with prominent ovicells that also might be a calloporid. Since there are species both with and without ovicells in this family, independent loss of the ooecia and evolution of the internal brooding is quite likely.

Both Selenariidae and Lunulitidae could potentially have inherited either endozooidal or immersed ovicells from the variety of onychocellid ancestors (see earlier). The Lunulariidae are considered closely related to Lunulitidae, and include species both with and without ooecia (Cook and Chimonides, 1986). The Otionellidae on the other hand presumably never had ovicells, probably having evolving from a nonovicellate free-living ancestor, possibly lunulitid.

Comparison of the brood sac structure found in Cupuladriidae with *Cauloramphus* and the supposed calloporid *Gontarella* shows that the two latter cheilostomes possess much longer "necks", opening to the outside independently from the vestibulum. In contrast, "necks" in *Cupuladria* and *Discoporella* are much shorter, but they do open into the vestibulum. We consider the "fusion" of the "neck" with a vestibulum as another trend in the evolution of the internal brood chambers. Such a trend is also inferred in two lepraliomorph ascophorans (*Cryptosula*, *Watersipora*). Additionally a new closure device, the cuticular thickening, had evolved in cupuladriids.

Ostrovsky et al., (2006, 2007, 2009a) suggested that internal brooding sacs are the ultimate stage in the evolution of the internal embryonic incubation. They suggest that an immersion of the brooding cavity may have been triggered by several events including energy balance within colonies (formation of the ooecium requires an energy that can be re-directed to a somatic growth), the increase in larval size (internal brooding cavity may host larger larva with more chances to survive), and increased predation (evolution of a predator specialized on feeding on embryos in the ovicells). Repeatedly, in many cheilostome clades there has been a trend towards the loss of a protective hood (ooecium) and the immersion of the brooding cavity. Apart from free-living cheilostomes, a presence of both, ovicells and internal brooding sacs within the same families shows that a transition from the embryonic incubation in the ovicells to the incubation in the internal brooding sacs has occurred at least 11 times within Neocheilostomina. We suggest the same has happened in 6 more families (Table 1), but the lack of anatomical data prevent us from more certain statement. Recent studies on the brooding structure in Calloporidae showed that even within this family the process may have occurred twice (Ostrovsky et al., 2007, 2009a).

Further changes in the internal brood sac structure during this process led to either a lengthening

of the neck region or its fusion with the vestibulum accompanied by a loss of the ooecial vesicle. It is not known what could trigger these changes, although they may be effective against predators that feed on individual zooids. The evolution of a longer neck and the reduction in number of openings from two to one when the neck connects to the vestibulum may be effective in reducing the possibility of a parasite or predator from entering into the brood cavity since a common opening is closed by zooidal operculum. The functional significance of the cuticular thickening found in the Cupuladriidae may be also connected to the additional isolation of the brooding cavity.

Adaptive Significance of Internal Brooding

Vast majority of anascan free-living bryozoans brood internally, being characterized by either a complete or partial reduction of the ooecium. The loss of ooecia and acquisition of internal brooding sacs appears to have occurred independently in the cheilostome families Heliodomidae and Lunulariidae. Similarly, the independent loss of ooecia and evolution of internal embryonic incubation occurred in the lineages leading to Cupuladriidae and, possibly, Otionellidae. Both Selenariidae and Lunulitidae possess strongly reduced ooecia and internal brooding cavities of the endozooidal or immersed ovicells. This strongly suggests that there is considerable selection pressure upon bryozoans that assume a free-living mode of life to move away from external towards internal brooding. The three aforementioned species of heliodomids with terminal ovicells do not strongly contradict our hypothesis. Judging from the illustrations of Calvet (Jullien and Calvet, 1903) and Harmelin (1977) their ooecia sit only slightly above the colony surface (see also Ostrovsky, 2008b).

We speculate that the absence of prominent skeletal brood chambers is a necessary precursor to the transition to a "lunulitiform" mode of life. Life on unstable sediments results in frequent burial and colonies with reduced dynamic resistance are better able to survive burial events by moving through the sediment (O'Dea, in press). The near-universal reduction of prominent ovicells, and hence evolution of internal brooding, in free-living bryozoans could therefore facilitate the colony movement through the sediment. Reduction of ooecia would also allow greater movement of vibracular mandibles whose role in digging colonies out of sediment and maintaining colony surfaces clear of sediment and epibionts is crucial to life on unstable sediments (O'Dea, in press). The explosive radiation of free-living cheilostomes from the Late Cretaceous/Early Paleogene (Cook and Chimonides, 1983; Jackson and McKinney, 1990, Håkansson and Thomsen, 2001) may therefore have only been possible because of the common reduction and/or removal of prominent ovicells. These inferences on the potential adaptive causes for the shift from external to internal brooding are supported by those cheilostomes with a similar colony form that are not free-living, but rooted in soft sediments; many such species have prominent hyperstomial ovicells (Cook and Lagaaij, 1976; Hayward and Cook, 1979; Cook and Chimonides, 1981; Cook and Chimonides 1994b), but they lack vibracula setae, and their rooted mode of life suggests that hydrodynamic stability is less of a problem.

ACKNOWLEDGMENTS

The authors thank Drs. R. Collin and M. Seid of the Smithsonian Tropical Research Institute for access to their laboratories, the crew of the research vessel Urraca and students of the University of Panama for their help collecting and sorting material, Dr. A. Anker, STRI, for photoimages, and Drs. D.P. Gordon, New Zealand Institute of Water and Atmospheric Research, Wellington, and P.D. Taylor the Natural History Museum, London, for their help with references. Dr. D.P. Gordon and Professor C. Nielsen, Zoological Museum, Copenhagen, provided very useful comments and corrections. The Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT) and National Geographic Society Exploration Committee supported the project financially. A. O'Dea was also supported by the Tupper fellowship program at the Smithsonian Tropical Research Institute and the National Science Foundation grant EAR03-45471. A. Ostrovsky thanks FWF (grant P19337-B17), Austria, and RFBR (grant 07-04-00928a), Russia, for financial support. They also thank E. Bermingham and H. Lessios of STRI for additional financial support.

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