Comparative Anatomical Study of Internal Brooding in Three Anascan Bryozoans (Cheilostomata) and Its Taxonomic and Evolutionary Implications

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ABSTRACT The anatomical structure of internal sacs for embryonic incubation was studied using SEM and light microscopy in three cheilostome bryozoans—*Nematoflustra flagellata* (Waters, 1904), *Gontarella* sp., and *Biflustra perfragilis* MacGillivray, 1881. In all these species the brood sac is located in the distal half of the maternal (egg-producing) autozooid, being a conspicuous invagination of the body wall. It consists of the main chamber and a passage (neck) to the outside that opens independently of the introvert. There are several groups of muscles attached to the thin walls of the brood sac and possibly expanding it during oviposition and larval release. Polypide recycling begins after oviposition in *Gontarella* sp., and the new polypide bud is formed by the beginning of incubation. Similarly, polypides in brooding zooids degenerate in *N. flagellata* and, sometimes, in *B. perfragilis*. In the evolution of brood chambers in the Cheilostomata, such internal sacs for embryonic incubation are considered a final step, being the result of immersion of the brooding cavity into the maternal zooid and reduction of the protecting fold (ooecium). Possible reasons for this transformation are discussed, and the hypothesis of Santagata and Banta (1996) that internal brooding evolved prior to incubation in ovicells is rejected. J. Morphol. 267:739–749, 2006.   © 2006 Wiley-Liss, Inc.

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The origin of parental care in the bryozoan order Cheilostomata is considered one of the key evolutionary innovations contributing to their success in benthic marine communities from the mid-Cretaceous to the present day (Taylor, 1988). Two facts that favor this supposition are: 1) despite antedating brooders, nonbrooders are represented by very few fossil and Recent species, and most cheilostomes instead hold their embryos in special embryonic chambers called ovicells; and 2) the earliest known fossil ovicells coincide in time with the onset of an explosive radiation of cheilostomes in the Late Albian.

Incubation in the coelom (more precisely, in the ovary) is restricted to one cheilostome family (Epistomiidae). Only a few species, belonging to the primitive genera *Aetea* (Aeteidae), *Eucratea* (Eucrateidae) and *Leiosalpinx* (Leiosalpingidae), brood their embryos externally in a membranous sac. The overwhelming majority of cheilostomes brood embryos in chambers with calcified walls (ovicells), in which the brood cavity is external with respect to the visceral coelom. In species with internal brood sacs formed by noncalcified zooidal walls, the brood cavity is also external with respect to the coelomic cavity of the maternal (egg-producing and brooding) zooid and yet is completely immersed in the maternal zooid and therefore often difficult to recognize (reviewed in Hyman, 1959; Ström, 1977; Reed, 1991; Ostrovsky, 2006a).

Internal brooding is known in both anascan (Suborder Flustrina) and ascophoran (Suborder Ascophora) cheilostomes. Among anascans it has been reported in species assigned to the Flustridae, Candidae, Steginoporellidae, Poricellariidae, Chlidoniidae, and a few other families (see below), and among ascophorans, in species of Adeonidae, Adeonellidae, Watersiporidae, Cryptosulidae, and Eu-

thyrisellidae. In the majority of these bryozoans the presence of internal brood sacs was noticed either in wet colonies containing embryos within the zooids (Waters, 1913; Marcus, 1922; Harmer, 1926; Hastings, 1930, 1943), or when studying anatomical sections (Calvet, 1900; Waters, 1909, 1912, 1913; Mawatari, 1952). For some other species, an internal sac for embryonic incubation is assumed to exist but not proven (Cook, 1979; Cook and Chimonides, 1981). The first observations of internal brooding in cheilostomes were made by Grant (1827) in *Carbasea carbasea*. Grant saw eggs, developing embryos, and larval release, but did not recognize the brood sac, which is still unstudied in this species.

Despite the fairly large number of records of internal brooding, anatomical data are very sparse. The only internal brood sac (pouch or diverticulum of the vestibulum) that has been studied in detail is in the ascophoran *Cryptosula pallasiana* (see Calvet, 1900). Photographs of the sectioned internal sac for brooding were published by Mawatari (1952, figs. 20–38, 44), who studied the ascophoran *Watersipora subtorquata* (as *W. cucullata*). The schematic drawings of internal brooding published by Waters (1909, 1912, 1913) show only the sac wall (see Ostrovsky, 2006a,b, for review and history of the research). Internal brooding has also been recorded in some Romancheinidae (Ascophora) (Hastings 1944, 1964; Eggleston, 1972), and inferred for Bryopastoridae (Flustrina) possessing modified zooids that are possibly brooding zooids (Gordon, 1986; d'Hondt and Gordon, 1999). However, the organs for embryonic incubation have remained unstudied and the anatomy of internal brood chambers is totally unknown in anascan cheilostomes.

Brood chambers are among the most important characters used in bryozoan taxonomy. Since the origin of brooding is intimately connected with acquisition of nonfeeding lecithotrophic larva in cheilostome evolution (Taylor, 1988), it is also important for reconstructing phylogeny and revealing evolutionary trends in these bryozoans. The lack of data on internal brooding has hampered research progress, and polarized opinions have developed that consider internal brooding as either an initial or an advanced stage in the evolution of parental care in cheilostomes (Santagata and Banta, 1996; Ostrovsky, 2002; Ostrovsky and Taylor, 2005a). This controversy cannot be resolved without new data.

Some bryozoans belonging to the families Bryopastoridae, Chlidoniidae, Adeonidae, Euthyrisellidae, and Watersiporidae (*Uscia*) possess large dimorphic zooids that allow their recognition as internal brooders. However, in other families (Flustridae, Watersiporidae, Cryptosulidae) brooding zooids are identical to nonbrooding zooids, and can only be distinguished when holding embryos. Therefore, the study of bleached or infertile colonies can be misleading. For example, *Gontarella gigantea* was interpreted in the original description of Grischenko

et al. (2002) as a member of the nonbrooding family Electridae on the basis of its simple skeletal morphology. However, discovery of internal brooding in an undescribed new species of *Gontarella* (see below) necessitates reevaluation of the taxonomic position of *Gontarella*.

The current article describes for the first time internal brooding in three anascan cheilostomes: *Gontarella* sp., *Biflustra perfragilis*, and *Nematoflustra flagellata*. Based on these results we revise the taxonomic position of the three species studied and hypothesize possible stages in the evolution of internal brooding in the Cheilostomata.

MATERIALS AND METHODS

Colonies of *Gontarella* sp. growing on pectinid shells were collected on 17 July 2003, 3 July 2004, and 3 August 2005 by boat trawl from 5–7 m depth in Akkeshi Bay (43° 01.45′ N, 144° 50.21′ E, eastern Hokkaido, Pacific). Specimens of *Biflustra perfragilis* MacGillivray, 1881 were collected on 13 November 1981 by epibenthic sledge from 59 m depth in the Bass Strait (39° 43.7′ S, 147° 19.6′ E, Bass Strait Survey 160, research vessel "Tangaroa" 81-T-1). Colonies of *Nematoflustra flagellata* (Waters, 1904) were collected on 26 April 2000 by Agassiz trawl from 94 m depth in the Bransfield Strait, Antarctic (63° 04.70′ S, 57° 31.60′ W, Station 158-1, NT XVII/3, research vessel "Polarstern").

Selected specimens were fixed in Bouin's fluid without acetic acid or 70% alcohol. For light microscopy, colonies were decalcified using a 2N solution of hydrochloric acid and gradually dehydrated, embedded in plastic (epoxy resin type TAAB 812), sectioned (3 μm thick) with a glass knife, and stained with Richardson's stain using standard methods. For SEM studies, colonies fixed in alcohol were cleaned in a 7.5% solution of sodium hypochlorite, rinsed, air-dried, and coated with gold. In addition, some colonies fixed in alcohol were dehydrated without bleaching, transferred to hexamethyldisilazane, and air-dried for SEM study (Nation, 1983).

RESULTS

In all three species studied zooids containing either large eggs or single embryos (never more than one) were found in groups, three or more generations proximally of the colony growing edge. Occasionally they formed ill-defined bands transverse to colony growth direction. Note that in the absence of seasonal observations our data on polypide recycling during brooding are provisional.

Nematoflustra flagellata

Embryonic incubation occurs in the internal brood sac, which is immersed in the distal part of the visceral coelom of the maternal autozooid (Figs. 1A,C, 5A). The distal part of the brood sac is situated close to the transverse wall between the brooding zooid and its distal neighbor. Brooding zooids differ externally from nonbrooding zooids in possessing an ooecial plug or vesicle that forms a recognizable fold in the distal part of the cuticular frontal wall (Fig. 1C, arrows). The zooidal operculum rests on the base of the ooecial vesicle that, in turn, plugs the entrance to the brood chamber. The sac communicates
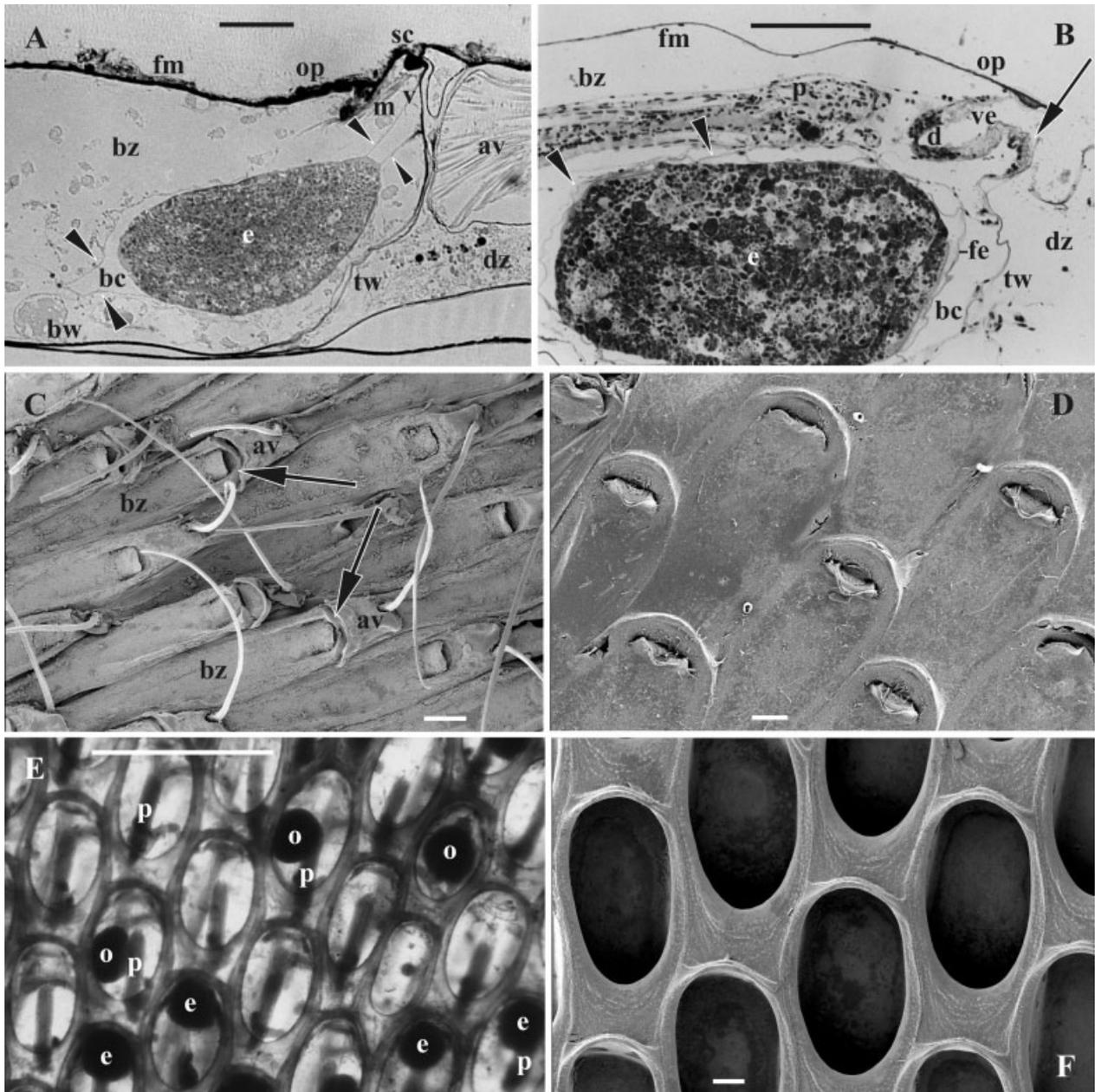
Fig. 1. **A,C:** *Nematoflustra flagellata*; **B,D–F:** *Biflustra perfragilis*. **A,B:** Longitudinal sections through the brooding zooids with an early embryo in the internal sac (walls of the brood sacs are shown with arrowheads; in **B** the communication passage of the brood sac is shown with an arrow). LM. **C,D:** General views of air-dried, unbleached colony fragments (arrows in **C** show the ooecial vesicles present only in brooding zooids). SEM. **E:** Wet colony with brooding and nonbrooding autozooids. LM. **F:** Bleached colony fragment. SEM. av, avicularium; bc, brood cavity; bz, brooding zooid; bw, basal wall; d, diaphragm; dz, distal zooid; e, embryo; fe, fertilization envelope; fm, frontal membrane; m, muscular bundles of the ooecial vesicle; o, oocyte; op, operculum; p, polypide; sc, sclerite of the ooecial vesicle; tw, transversal wall; v, ooecial vesicle; ve, vestibulum. Scale bars = 100 μm in **A,B,D,F;** 200 μm in **C;** 1 mm in **E.**

directly with the exterior; it does not open into the introvert. There is a large sclerite—a cuticular thickening—in the upper part of the vesicle (Figs. 1A,C, 5A). A group of muscles attaches to the sclerite, their lower ends being anchored to the basal wall behind the proximal end of the brood sac. The contraction of these muscles apparently leads to de-

formation of the ooecial vesicle, causing the brood sac to open during oviposition and larval release.

The sac itself is an oval invagination of the distal-most part of the noncalcified frontal wall of the maternal zooid. It consists of a main chamber and a short neck leading to the outside. The wall of the sac is thin and easily deformed, comprising a cuticle and
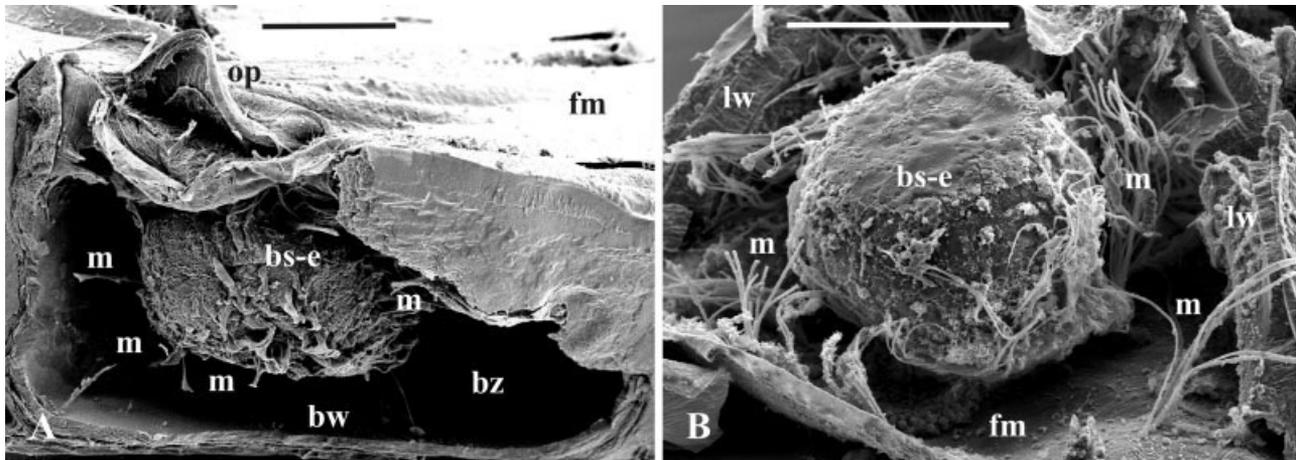
Fig. 2. Embryos in internal brood sacs (dehydrated specimens air-dried after hexamethildisilazane). SEM. **A:** *Biflustra perfragilis.* **B:** *Gontarella* sp. (upside-down, basal wall removed). bs-e, brood sac with embryo; bz, brooding zooid; bw, basal wall; fm, frontal membrane; m, muscular bundles of the brooding sac; op, operculum; lw, lateral wall. Scale bars = 100 μm in **A;** 200 μm in **B.**

flat cells of the epidermal and peritoneal layers. Several groups of muscles possibly serve to extend the brood sac during larval release and perhaps during oviposition as well. These muscles attach to the lower and lateral walls of the main chamber of the sac in both its distal and proximal parts. Their opposite ends are anchored onto the transverse, basal, and lower part of the lateral cystid walls (Figs. 1A,C, 5A).

The polypide degenerates during brooding but regeneration has not been observed. Early embryos found in the studied material reached 200 × 400 μm in diameter on average and were yellow in color.

Biflustra perfragilis

Brooding occurs in an internal sac for embryonic incubation that is immersed in the distal part of the maternal autozooid, below the tentacle sheath. The distal part of the sac adjoins the transverse wall (Figs. 1B,E, 2A, 5B), and embryos touch this wall in some instances.

The thin-walled brood sac is an oval invagination of the distalmost part of the noncalcified frontal wall of the maternal zooid and is easily deformed. When not containing an embryo it is almost completely flattened, is positioned between the polypide and the basal wall, and extends for more than half of the cystid length. The sac consists of a main chamber connecting with the exterior via a short passage that resembles a neck, as in *Nematoflustra*. The passage ends with an opening, located close to the distal edge of the zooidal operculum and independent of the introvert. This opening is plugged by a fold in the frontal wall of the maternal zooid resembling an ooecial vesicle. The cuticle of the fold is thickened but lacks a sclerite. Numerous muscular bundles attach to the wall of this fold (Fig. 5B) with their

opposite ends anchored onto both the upper wall of the brood sac and the lateral cystid walls. They are presumed to serve in expansion of the opening of the sac during oviposition and larval release.

The brood sac has a thin cuticular wall and flattened epithelial layers to which several muscle bands are attached. These muscles extend between the lower and lateral walls of the main chamber of the sac in both its distal and proximal parts and the transverse, basal, and lateral cystid walls (Figs. 2A, 5B). They are clearly involved in the expansion of the brood sac.

Brooding zooids are superficially identical to nonbrooding zooids (Fig. 1D–F). The majority of zooids containing embryos possess functional polypides. However, a few were observed with regenerating polypide buds and brown bodies (Fig. 1E).

Embryos found in the studied material were surrounded by a fertilization envelope (Figs. 1B, 5B), reached 185 × 310 μm in diameter, and were yellow in color.

Gontarella sp.

This undescribed species is clearly congeneric with the type and only previously known species of *Gontarella*, *G. gigantea*. It differs, however, in having smaller zooids (generally less than 1 mm long) and lacking a beaded mural rim (Fig. 3A,B).

Brooding occurs in a special internal sac for embryonic incubation located in the distal half of the maternal autozooid. The proximal end of the embryo is positioned roughly at the center of the zooid, sometimes proximally and sometimes distally of mid-length but never at its far distal end (Fig. 4A–E). Externally, brooding zooids are indistinguishable from nonbrooding zooids, save for the presence of embryos visible through the semitransparent
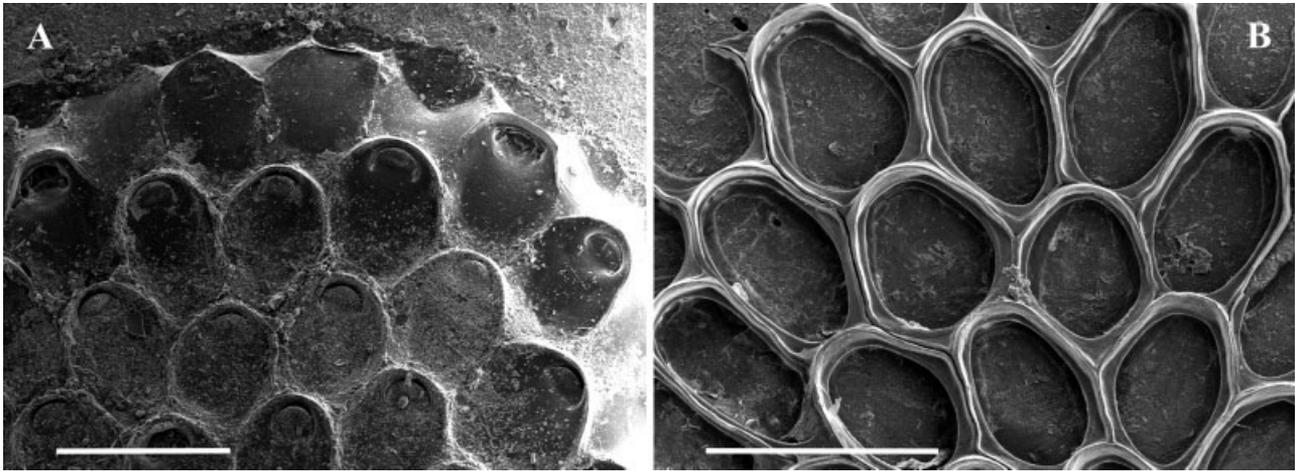
Fig. 3. **A,B:** *Gontarella* sp. General view of unbleached (**A**) and bleached (**B**) colony fragments. SEM. Scale bars = 1 mm.

frontal membrane (Fig. 4A,B,D). The brood sac consists of a main chamber connecting with the exterior via a narrow neck that is considerably longer than in *Nematoflustra*. The neck ends with an opening, located close to the distal edge of the zooidal operculum and independent of the introvert. This opening is closed by a fold of the distalmost part of the noncalcified frontal wall of the maternal zooid comparable with an ooecial plug, and adjoining the cuticular lip in the upper part of the transverse wall (light stippling in Fig. 5C, compare with Fig. 4E,F). Although the cuticle of the fold is rather thick, it lacks a distinct sclerite. Numerous thin muscular bundles attach to the wall of this fold or to the neck of the brood sac (Figs. 2B, 4E, 5C). Their opposite ends are anchored onto the transverse, basal, and lateral cystid walls, and also onto the frontal membrane, presumably serving to expand the neck during oviposition and larval release. No fibers were found attached to the main chamber wall except for funicular strands.

The thin-walled brood sac is an oval invagination of the distalmost part of the frontal wall of the maternal zooid and is easily deformed. When not containing an embryo, it is completely flattened, positioned just above the basal wall, and extends for up to two-thirds of cystid length (Fig. 4F). Its wall consists of a thin cuticle and underlying epidermal and peritoneal epithelia.

Polypide recycling occurs during brooding (Fig. 4B,C). The polypide degenerates some time after oviposition and a new one begins to grow (Fig. 4D). However, additional observations are needed to ascertain whether regeneration is completed before or after larval release.

Early embryos found in the studied material reached up to 217 × 330 μm in diameter and were orange in color (Fig. 4A,B,D). Some were seen to be surrounded by a fertilization envelope.

DISCUSSION
Taxonomic Position of *Gontarella* sp. and *Biflustra perfragilis*

The presence of internal brood sacs clearly shows that neither *Gontarella* sp. nor *Biflustra perfragilis* are malacostegans. Both species possess large macrolecithal eggs and embryos and, thus, coronate larva. They lack the malacostegan cyphonautes larvae previously inferred to have been present in *Gontarella* (Grischenko et al., 2002). Overall zooidal and colonial morphology as well as the existence of internal embryonic incubation favor placement of these two cheilostomes into the neocheilostome family Flustridae instead. As currently understood, this family includes forms with either hyperstomial (*Klugeflustra*) or, in most species, endozooidal ovicells, as well as some internal brooders (*Carbasea*, *Nematoflustra*) (Hayward, 1995; Hayward and Ryland, 1998). Note that in having external brood sacs, "*Carbasea*" *indivisa* is probably not a flustrid (see Stach, 1938).

The structure of the sac for embryonic incubation in both *Gontarella* sp. and *Biflustra perfragilis* is similar to that of *Nematoflustra flagellata*. In fact, the only major differences are the absence of a sclerite in the "ooecial vesicle" of *Gontarella* sp. and *B. perfragilis*, and the length of the neck of the brood sac. Minor differences exist in the size of the brood sac, the position of its opening relative to the zooidal operculum, and the size and number of muscle bundles. Species of *Gontarella* have encrusting colonies, whereas the vast majority of Flustridae form erect colonies with flexible branches. However, encrusting flustrids are known, for instance, species of *Hincksina*. Similarly, *B. perfragilis* can be provisionally reassigned to the Flustridae. Although its true generic identity remains uncertain pending a more complete revision
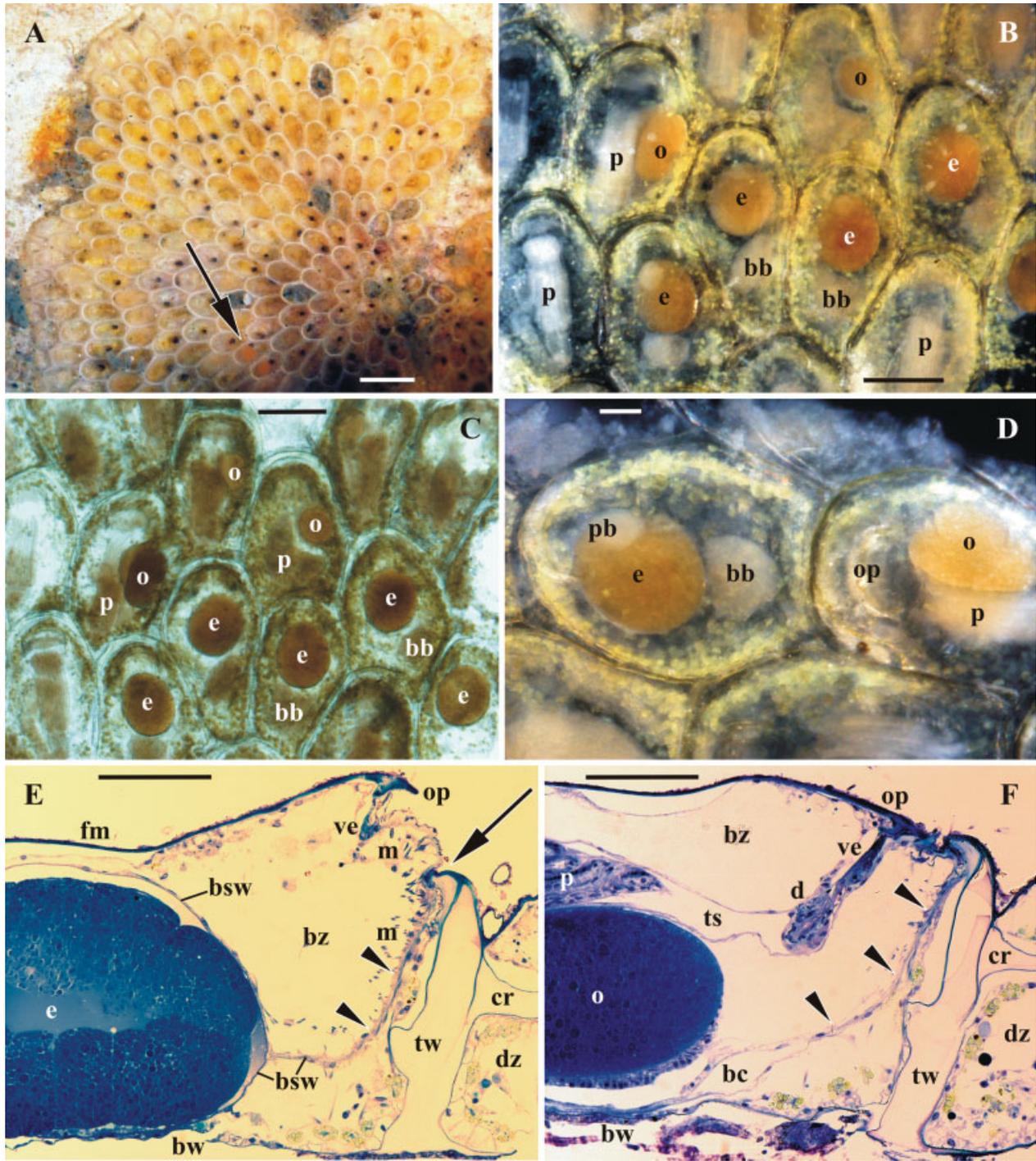
Fig. 4. *Gontarella* sp. **A:** General view of living colony (arrow shows two brooding zooids with embryos). LM. **B–D:** Decalcified colony fragments with brooding zooids containing oocytes and embryos at different developmental stages. LM. **E,F:** Longitudinal sections through brooding zooids with an early embryo in the internal sac (**E**) and macrolecithal oocyte in the ovary and empty sac (**F**) (walls of the neck of the brood sac shown with arrowheads, opening of the brood sac shown with an arrow). LM. bb, brown body; bc, brood cavity; bsw, wall of the brood sac; bz, brooding zooid; bw, basal wall; cr, cryptocyst; d, diaphragm; dz, distal zooid; e, embryo; fm, frontal membrane; m, muscular bundles of the brooding sac; o, oocyte; op, operculum; p, polypide; ts, tentacle sheath; tw, transversal wall; ve, vestibulum. Scale bars = 1 mm in **A**; 300 μm in **B,C**; 100 μm in **D–F**.

of this and similar species, it may perhaps fit provisionally within an enlarged definition of *Gontarella*.

The discovery of internal brooding in the three anascans studied here shows that caution must be exercised when assigning anascans with simple skeletal morphologies and lacking ovicells to the Malacostegina. Some of these species, both Recent and fossil, may instead be internal brooders that
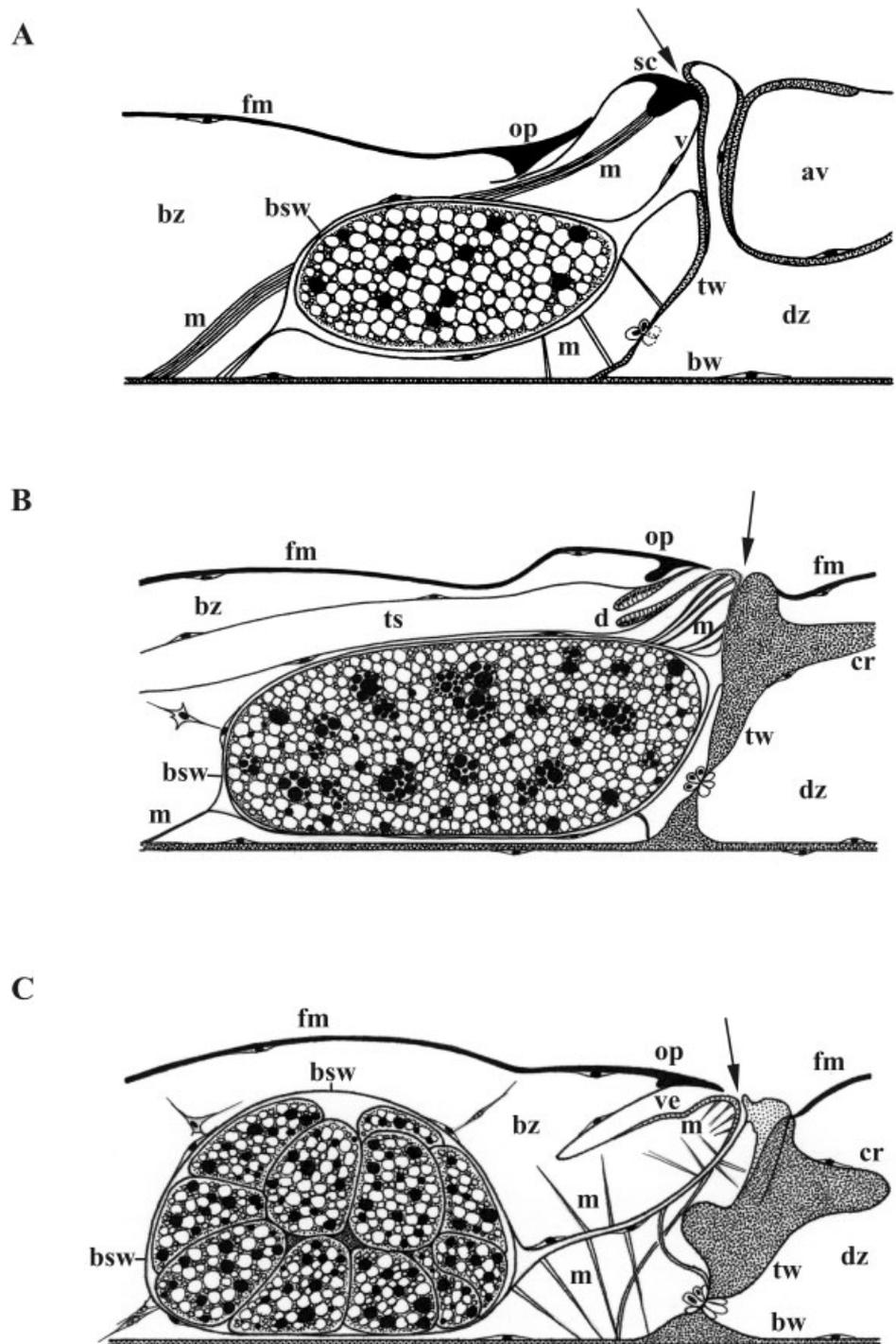
Fig. 5. Schematic longitudinal sections through the brooding zooids with embryos in the internal sac (opening of the brood sac shown with an arrow). **A:** *Nematoflustra flagellata.* **B:** *Biflustra perfragilis.* **C:** *Gontarella* sp. av, avicularium; bsw, wall of the brood sac; bz, brooding zooid; bw, basal wall; cr, cryptocyst; d, diaphragm; dz, distal zooid; fm, frontal membrane; m, muscular bundles of the ooecial vesicle and brood sac; op, operculum; sc, sclerite of the ooecial vesicle; ts, tentacle sheath; tw, transverse wall; v, ooecial vesicle; ve, vestibulum.

belong to neocheilostome families such as the Flustridae. One means of distinguishing internally brooding neocheilostomes from malacostegans is by the presence of avicularia of conventional morphology in neocheilostomes. Avicularia are rare in malacostegans (e.g., Liu, 1992) and when present tend to resemble the B-zooids of *Steginoporella*. This criterion can be applied successfully to both *Nematoflustra flagellata* and *Biflustra perfragilis*, but not to *Gontarella* sp. from Akkeshi Bay, in which avicularia are lacking. *Biflustra perfragilis* also shows similarities to the encrusting genus *Sinoflustra*, currently assigned to the Flustridae, which has avicularia like those of *B. perfragilis* but possesses distinctive spines marginal to the opesia (see Liu and Yang, 1995).

Another supposed malacostegan that may in fact be a neocheilostome is *Electra bellula*. According to

Marcus (1938), zooids of *E. bellula* contain only one large mature egg at a time. In contrast, all true malacostegan species with planktotrophic larvae produce many small eggs. It is very probable that the bryozoan studied by Marcus is an internal brooder, with some of the apparent eggs being embryos.

Brooding and Polypide Recycling

Degeneration of the polypide in maternal zooids is common in ctenostome brooders (reviewed in Ström, 1977). This happens when incubation occurs in the introvert, making lophophore extension impossible. It is also known to occur in the cheilostome family Epistomiidae, which has intraovarial brooding (Marcus, 1941; Dyrynda, 1981; Dyrynda and King, 1982). Polypide degeneration in some cheilostome internal brooders could be explained by the necessity of leaving sufficient space for a large embryo that sometimes occupies the greater part of the visceral coelom, as in *Steginoporella* (Cook, 1964, 1968a). In contrast, Calvet (1900, fig. 42) depicted an unmodified vestibulum and tentacle sheath in a brooding zooid of *Cryptosula pallasiana*. Because the embryo is relatively small, the polypide probably does not degenerate during incubation in this species. Polypide degeneration, however, does occur in *Watersipora subtorquata* (see Mawatari, 1952), with regeneration beginning only after release of the larva that occupies a large part of the maternal zooid. In both of these ascophoran species, the internal brood sac opens into the vestibulum, which does not degenerate. Because the entrance to the brood sac is independent of the introvert, larval release in *Nematoflustra flagellate*, *Biflustra perfragilis*, and the Akkeshi *Gontarella* sp. also does not depend on the presence of a functional polypide.

Evolution of Internal Brooding in the Cheilostomata

The three types of brood sacs described here can be considered as a morphoseries representing successive stages in the evolution of internal brooding. Anatomically, the internal sac for embryonic brooding in *Nematoflustra flagellata*, with its large ooecial vesicle and sclerite with muscles, is similar to a flustrid endozooidal ovicell, except that in endozooidal ovicells the brooding cavity is immersed in the distal zooid whose frontal wall is continuous with the ectooecium (Vigelius, 1884a,b; Levinsen, 1893, 1894, 1909; Calvet, 1900; Ostrovsky, unpubl. data). Reduction of the ooecium and proximal displacement of the brooding cavity is the first hypothetical step in the evolution of an internal brood sac. The next step is the reduction of the sclerite of the ooecial vesicle, with further proximal migration of the main chamber and consequent lengthening of the neck, to give the condition seen in *Biflustra perfragilis* and

the Akkeshi species of *Gontarella*. These changes would be accompanied by appropriate modification of the muscular system.

The most primitive ovicells in cheilostomes are constructed of modified mural spines (Ostrovsky and Taylor, 2004, 2005a). Their subsequent evolution entailed reduction in spine number and spine fusion. Another trend was immersion of the brooding cavity (Ostrovsky and Taylor, 2005b), which was achieved in some taxa by lowering the ovicell floor toward the basal wall of the distal zooid. This inevitably led to a decrease in size of the ooecial fold, culminating in the evolution of endozooidal ovicells. Evolution of internal sacs for incubation appears to be a subsequent stage (see above).

The existence of related, sometimes even congeneric, species with well-developed ooecia, vestigial ooecia, or lacking ooecia was first noted by Levinsen (1909). Harmer (1926) subsequently wrote that such a trend characterizes several "lines of Cheilostome evolution" (p. 405), an issue discussed further by Hastings (1964). Analysis of the literature supports the multiple evolution of immersed brooding cavities accompanied by reductions and losses of the ooecial fold.

Vestigial ooecia are well known among Calloporidae. Of particular note are *Aplousina filum* and *A. gigantea* (see Cook, 1968b), which are reminiscent of *Gontarella* in zooidal morphology and size but possess immersed ovicells with small cap-like ooecia. Although the majority of calloporids have hyperstomial or prominent ovicells, *Crassimarginatella* (see Hastings, 1945, 1964; Cook, 1968b; Winston, 1984; Tilbrook et al., 2001), *Caulorhamphus* (Mawatari and Mawatari, 1981; Dick and Ross, 1988), *Vibracellina*, *Cymulopora* (Winston and Håkansson, 1986), and *Cranosina* (Osburn, 1950; Chimonides and Cook, 1994) include or consist exclusively of species with vestigial ooecia and immersed brood cavities. Hastings (1964) and Cook (1968b) found both prominent and vestigial ooecia within the same colony fragments of *A. gigantea* and *Crassimarginatella spatulifera*. This shows that the degree of brood cavity immersion can vary within a family, a genus, or even a colony. Cook (1968b:141–142) described three types of ovicells within *Aplousina,* noting that the brood cavity is placed in the proximal part of the distal zooid in the first type, but in the distal part of the maternal zooid in the second and third types. Ostrovsky (2006a) differentiated these variants as endozooidal and immersed, respectively.

A similar variability in brooding organs can be inferred for the related family Antroporidae which possesses immersed ovicells with vestigial ooecia (Osburn, 1950; Cook, 1968b; Mawatari and Mawatari, 1981; Tilbrook, 1998; Tilbrook et al., 2001; Tilbrook and Grischenko, 2004). Complete reduction of the ooecium is as yet unknown in calloporids and antroporids.

Different degrees of ooecial reduction in immersed ovicells have been described in Bugulidae, especially *Bugula* and *Camptoplites* (Robertson, 1905; Harmer, 1926; Hastings, 1943; Osburn, 1950; Ryland, 1962; Bobin and Prenant, 1963; Prenant and Bobin, 1966; Ryland and Hayward, 1977, 1992; Soule et al., 1995; Hayward, 1995). Moreover, two bugulid genera—*Himantozoum* (Hastings, 1943; Gordon, 1986; Hayward, 1995) and *Caulibugula* (Harmer, 1926)—include species with vestigial ooecia but in others ooecia are totally absent. This is also the case for *Farciminellum* (Farciminariidae), *Beania* (Beaniidae), *Menipea* (Candidae), and *Tetraplaria* (Tetraplariidae) (Harmer, 1926, 1957; Hastings, 1943; Osburn, 1950; Gordon, 1984, 1986; Zabala and Maluquer, 1988; Hayward, 1995).

The position of the embryo in some illustrations of immersed ovicells (*Aplousina major*, Cook, 1968b, text-fig. 8; *Bugula gautieri*, Ryland, 1962, fig. 1D) suggests the presence of a deep invagination of the distalmost part of the noncalcified frontal wall of the maternal zooid, effectively, a prototype brood sac. This is supported by data on *Antropora tincta*, where Hastings found such a sac with associated muscles below the vestibulum and proximally of the vestigial ooecium (1930, pl. 5, fig. 17). Although Hastings did not show its structure in sections, the similarity of this immersed ovicell with internal brood sacs is clear.

There are also a number of cheilostome families in which ovicells are usually present but are either vestigial (ooecia) or even lacking in some genera. These include *Desmacystis* and *Oshurkovia* in Umbonulidae (Hastings, 1944, 1964; Eggleston, 1972; Gordon and Grischenko, 1994; Grischenko and Mawatari, 2005), *Arctonula* in Romancheinidae (Kluge, 1975; Gordon and Grischenko, 1994; Hayward and Ryland, 1999), and *Odontoporella* (Hippoporidridae) (Canu and Bassler, 1929; Osburn, 1950; Prenant and Bobin, 1966; Ryland and Hayward, 1977; Gordon, 1989; Hayward, 1995). The Microporidae contains genera with well-developed ooecia (*Micropora*, *Mollia*, *Apiophragma*, etc.), vestigial ooecia (*Roselliana*), and without ooecia (*Calpensia*, *Ogivalia*, *Microporina*) (Prenant and Bobin, 1966; Hayward and Ryland, 1998). Recent Onychocellidae possess vestigial ooecia, possibly lacking altogether in some species of *Smittipora* (d'Hondt and Gordon, 1999), whereas most Cretaceous species have well-developed ooecia. A trend from hyperstomial to endozooidal ovicells was noted in Upper Cretaceous Onychocellidae by Voigt (1991). In the Poricellaridae, internal brooding characterizes modern species but the oldest species from the Upper Cretaceous have ovicells and the loss of these structures apparently occurred during the Tertiary (Cheetham, 1968). Ooecia, as a rule, are absent in Exechonellidae but vestigial ooecia occur in some species (Gordon, 1984). In contrast, the vast majority of Cribrilinidae possess ooecia, although they are unknown in *Jullienula* (as *Lyrula* in Osburn, 1950). Lang (1921) listed species of Upper Cretaceous cribrimorphs with endozooidal ovicells, the oldest dating back to the Cenomanian, which is very early in the evolutionary history of ovicells.

The wide taxonomic scatter of endozooidal and immersed ovicells as well as internal brooding supports a hypothesis of repeated and independent evolution of these brooding methods within the Neocheilostomatina. Fossil evidence points to internalization of brooding commencing as early as the Cenomanian and continuing in different clades through to the present day.

But why has there been a trend towards internalization of brooding in cheilostome bryozoans? Comparing pairs of species from genera with both ovicellate and internally brooding species, Hastings (1964) was unable to find any correlation with depth or geographical or climatic distribution. Eggleston (1972) noted that internal brooders are mainly littoral inhabitants, inferring that their embryos may be better protected from drying during low tides. While it is clear that immersion of the brooding cavity could serve for better protection of the embryos, strong reduction or even loss of the skeletal cover (ooecium) in the forms with internal brood sacs may have the opposite effect, making the embryos more vulnerable. In addition to Eggleston's hypothesis, which cannot explain internal brooding in subtidal species, several alternative ideas may be suggested to explain the internalization of brooding among cheilostomes:

1) As formation of a prominent ooecium and associated tissues undoubtedly expends energy, reduction of the ooecium could minimize this outlay, freeing resources for somatic growth.
2) Internal brooding cavities may be more spacious than ovicells, allowing embryos of larger size to be brooded, in turn resulting in larger, better provisioned larvae with a greater competence to recruit successfully.
3) Internal brooding may be an evolutionary response to the evolution of predators that feed on embryos held in the relatively exposed or acleithral ovicells.

Santagata and Banta (1996) suggested that brooding in the introvert was the initial stage in the evolution of parental care in cheilostomes. The geological record does not support this suggestion: evidence for internal brooding typically appears late in the fossil record, from the mid-Eocene onwards, and the well-known internal brooders *Watersipora* and *Cryptosula* both first appear in the Upper Miocene. Instead, the primitive mode of brooding occurred on the colony surface in hyperstomial ovicells formed from cages of modified mural spines belonging to the zooid distal of the maternal zooid (Taylor and McKinney, 2002; Ostrovsky and Taylor, 2005a). Internal brooding is better interpreted as an advanced trait,

the final stage in the transition from hyperstomial to endozooidal ovicells to immersion of the brooding chamber that has occurred independently in several groups of cheilostomes. A future challenge will be to use phylogenetic analyses and data from the fossil record to establish how many times internal brooding has evolved in cheilostomes, when in geological time the transition was made in different clades, and what selective factors were responsible.

ACKNOWLEDGMENTS

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