Late Reproductive System Development in Two Cephalaspideans (Gastropoda: Opisthobranchia): *Bulla striata* Bruguière, 1792, and *Acteocina atrata* Mikkelsen & Mikkelsen, 1984

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Abstract. Development of the hermaphroditic reproductive system was investigated in two monaulic opisthobranchs *Bulla striata* Bruguière, 1792 [Bulloidea], and *Acteocina atrata* Mikkelsen & Mikkelsen, 1984 [Philinoidea], representing the two major clades of Cephalaspidea, using histological serial sectioning of growth series. The female glandular masses of the two species are similar, each possessing three major glands (albumen, mucus, membrane) presumably depositing protective layers around fertilized eggs during oviposition. These glands assume similar configurations in the two species, but each gland of *B. striata* intercepts the gonoduct separately at its base, whereas the glands of *A. atrata* have a common point of intersection. The retractile copulatory organs of the two species each include an outer penial sheath, a sperm-storing duct, and a terminal sac. The copulatory organ of *B. striata* is more complex, including a duct-within-a-duct section containing a non-glandular penial extrovert plus a coiled prostatic ejaculatory duct. Fortuitous sectioning of a specimen with its copulatory organ partially everted revealed that while this species has no permanent penial papilla, the penial extrovert everts to form the functional penis. The copulatory organ of *A. atrata* is a simple penial sheath widening into a papillose medial duct and glandular terminal sac; the presence of a penial papilla was unconfirmed, and the function of the organ remains unresolved. Ontogenetic timing in *A. atrata* is protandric, with male ducts and gonadal tissue fully developed (assuming full functionality) before the female components; *B. striata* appears to be a true simultaneous hermaphrodite, with male and female systems maturing concurrently.

INTRODUCTION

Character coding for phylogenetic analysis depends upon having sufficient data to make reasonable assumptions about homologies. In gastropods (the largest and most diverse primary clade of Mollusca), use of reproductive structures in systematic research has been problematic in this regard. This is particularly true in taxa with internal fertilization, in which a variety of structures have evolved to store reproductive products and to protect developing eggs. Ponder & Lindberg (1997:195), in their monumental phylogenetic analysis of gastropods, specifically ignored "accessory reproductive structures such as glands and sperm sacs . . . because of their doubtful homology across major groups." To add to the confusion caused by the sheer structural diversity, gastropod reproductive systems are mosaic structures, in which two or more organogenetic components contribute to the adult system. Many of the terms used to refer to parts of the adult reproductive system are based on assumptions about their ontogenetic origins, which are not documented for most

gastropod species. Uncritical use of such terminology can mask systematically informative data.

This work is part of a larger study to investigate structural homologies within complex gastropod reproductive tracts by analyzing post-larval ontogeny in growth series of selected species. The reproductive system is indeed the only organ system whose ontogeny can be studied in post-larval individuals because it is the last system to develop, and its differentiation is largely post-metamorphic. This has in fact contributed to the lack of data on reproductive system ontogeny because embryological studies generally disregard post-metamorphic processes while morphological studies generally ignore immature stages.

Comparison of reproductive anatomy between heterobranchs and other gastropods has been particularly difficult due to (1) disparate structural names based more on location or sheer assumption than on knowledge of fine structure or function (especially regarding sperm storage sacs and the "female" glands associated with nutritive and protective layers of the egg mass); (2) different anatomical orientations in torted gastropods and "untorted" heterobranchs; and (3) separate male and female tracts in dioecious gastropods versus combined tracts in hermaphroditic heterobranchs.

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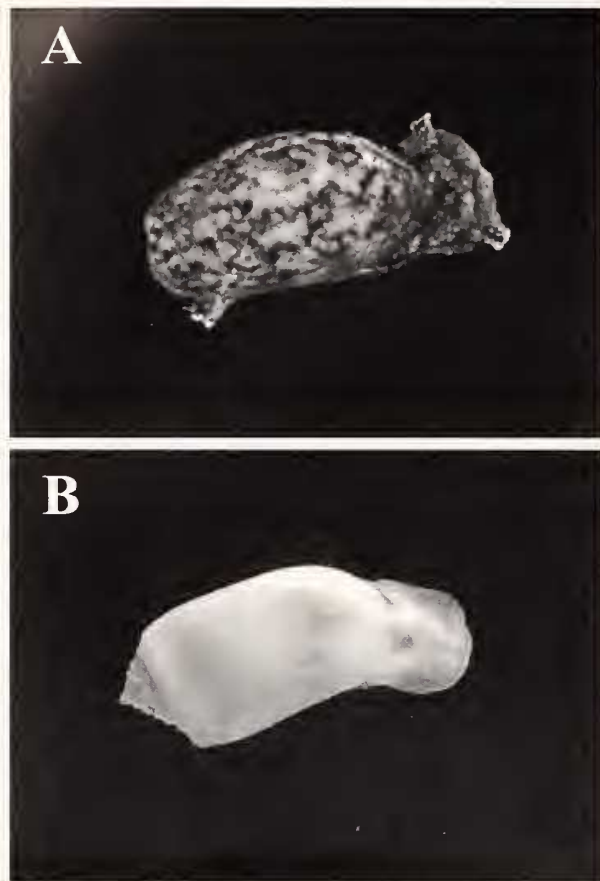
Figure 1. Living animals of species studied herein. A. *Bulla striata* [Bulloidea: Bullidae], (FK-004, 13.0-mm specimen). B. *Acteocina atrata* [Philinoidea: Acteocinidae], (PSM-842, 4.24– mm specimen).

In a recent phylogenetic analysis of Cephalaspidea (traditionally the most primitive living opisthobranchs) and other basal heterobranchs (Mikkelsen, 1996), Cephalaspidea was redefined and found to comprise two major clades at the subfamily level: Bulloidea and Philinoidea. In the latter study, characters of certain structures in the monaulic hermaphroditic reproductive tract (e.g., male "prostate" gland, female glandular mass) were not codable because "gross morphology and cellular configuration appeared to be highly variable [and] homology could not be presumed" (Mikkelsen, 1996:402). The current study focuses on one representative from each of these clades—*Bulla striata* from Bulloidea (Figure 1A), and *Acteocina atrata* from Philinoidea (Figure 1B)—with the immediate goal of comparing adult morphologies and corresponding ontogenetic patterns within Cephalaspidea, and the ultimate aim of contributing to a morphological data set including caenogastropods and members of other gastropod groups.

MATERIALS AND METHODS

Material (Table 1)

Sectioned *Bulla striata* specimens were from: (1) R/V *FLORIDAYS* station FK-163 (10 specimens sectioned, 3.4–11.3 mm; vouchers AMNH 290095), dredged in sandy mud and turtlegrass (*Thalassia testudinum* Banks ex König), with chicken liver sponge (*Chondrilla nucula* Schmidt) and *Dasycladus* green algae, 5.6 ft (1.7 m), off the Florida Bay side of Tavernier, Key Largo, Monroe County, Upper Florida Keys, 25°03.6′N, 80°30.0′W to 25°03.5′N, 80°30.2′W, 12 September 1998, P. M. Mikkelsen & R. Bieler, coll.; (2) R/V *FLORIDAYS* station FK-211 (2 juveniles sectioned, 2.5–3.2 mm; vouchers AMNH 290096), bottom sample in turtlegrass, in an unnamed bay between Shark Key and Big Coppitt Key, Florida Bay side, Monroe County, Lower Florida Keys, 24°36.38′N, 81°39.18′W, 17 April 1999, P. M. Mikkelsen & R. Bieler, coll.; (3) station PSM-767 (1 adult sectioned, 16.5 mm), sieving in shoalgrass (*Halodule wrightii* Ascherson), 40 cm depth, cove near Haulover Canal, northern Indian River Lagoon, Brevard County, central eastern Florida, 28°44.0′N, 80°45.5′W, 10 February 1981, P. S. Mikkelsen et al., coll.; (4) station PMM-931 (1 adult sectioned, 12.5 mm), sieved from sand and turtlegrass, less than 1.0 m depth, "the horseshoe" relic harbor on Florida Bay side of Spanish Harbor Keys, Monroe County, Lower Florida Keys, 24°39′19″N, 81°18′13″W, 24 January 1988, P. M. Mikkelsen & R. Bieler, coll. Additional comparative data were obtained from a sectioned copulatory organ dissected from an adult (30.2 mm) from shallow sand and seagrass, Hobe Sound, Palm Beach County, southeastern Florida, 24 March 1979, HBOM [Harbor Branch Oceanographic Museum, Ft. Pierce, Florida] 065:02023 [utilized by Mikkelsen, 1996]. The live specimen photographed of *B. striata* was from sta. FK-004, seagrass bed on the ocean side of Crawl Key, Monroe County, Florida Keys, 2 October 1994.

All sectioned *Acteocina atrata* specimens (7 specimens sectioned, 1.4–3.1 mm; vouchers AMNH 290097) were sieved from sandy mud in flats off Little Jim Island [LJI], Indian River Lagoon, Ft. Pierce, St. Lucie County, central eastern Florida, August 1989, J. Wise, coll. The live specimen photographed was from sta. PSM-842, muddy sand at the mouth of Turnbull Creek, northern extent of Indian River Lagoon, Volusia County, central eastern Florida, 30 May 1982.

All specimen sizes are expressed as shell length. All animals were fixed in Bouins' fixative or 5% formalin, then transferred to 70–75% ethyl alcohol. All photographs show the specimens in cross-section, from a head-on perspective, so that left and right sides are reversed. Voucher specimens are deposited in the Recent mollusk collection of the Division of Invertebrate Zoology, American Museum of Natural History.

Comparative Anatomy

Data for this project were drawn from histological serial sections of whole individuals of *Bulla striata* and *Acteocina atrata*. After measurement, shells were removed by soaking intact specimens for several hours in 10% acetic acid. Dissecting pins were used to pierce the gizzard region of large specimens of *A. atrata,* to allow the acid to reach the calcified gizzard plates. This step was not necessary for *B. striata,* which has corneous (uncalcified) gizzard plates. The shell-less specimens were then passed through a dehydrating and infiltrating series, and embedded in Paraplast (Oxford Labware, St. Louis, Missouri). They were sectioned either perpendicular to the coiling axis or parallel to it, at 6–10 μm for *B. striata* and 5–8 μm for *A. atrata*. Most sections were stained in Harris' hematoxylin (Vacca, 1985) and counterstained with eosin Y (Humason, 1962)/phloxine (Vacca, 1985) (hereafter H&E). Sections of the adult *B. striata* from PMM-931 and HBOM 065:02023 were stained with Alcian-Blue/Periodic Acid/Schiff's (PAS) trichrome stain. All staining reactions are those of H&E, unless otherwise noted.

Specimens were considered "adult" when both spermatozoa and ova were observed in the gonad. The description of the hermaphroditic system follows the pathway of exiting reproductive products, i.e., from gonad to external genital opening; that of the copulatory organ follows the pathway of entering autosperm, i.e., from external to internal, or from male genital opening to terminal sac.

RESULTS

A summary of the results, indicating the relative timing of appearance and differentiation of various reproductive organs, is given in Table 1. The gross anatomy of the adult reproductive system in *Bulla striata* and *Acteocina atrata* is shown in Figures 2A, B, respectively.

Posterior Gonoduct

In this study, the term posterior gonoduct refers to the gonad (interdigitated with the digestive gland in the apical coil of the snail) and its immediate duct. The duct extends from its origin at the gonad, including the expanded and coiled ampulla (Figures 2A, B [amp]), to a sphincter located just posterior to the female glandular mass (Figures 2A, B [ring surrounding gonoduct]; called sphincter gonoductus by Lemche, 1956). In previous heterobranch literature, the posterior gonoduct has been called the coelomic gonoduct (Ghiselin, 1966; Robles, 1975) or the hermaphroditic duct (Marcus, 1957; Thompson & Bebbington, 1969; Haszprunar, 1985; Gosliner, 1994). Coelomic gonoduct (implying mesodermal origin) is not used here because there is not definitive evidence for the junction site of the ectodermal (female glandular

mass) and mesodermal (gonad) portions of the duct. Without such evidence, a more general term that does not imply a specific ontogenetic origin is preferable. Hermaphroditic duct seems imprecise for this portion alone because the entire gonoduct is hermaphroditic in the sense that both ova and sperm exit the snail via this pathway.

Bulla striata

The gonad of *Bulla striata* is similar to that of *B. gouldiana* (Pilsbry, 1893), as described by Robles (1975). As in the latter species, oogenesis occurs at the outer parts of the gonadal acini, and spermatogenesis toward the inside (Figure 3A). The gonad empties into the ampulla. The ampulla is wide and slightly coiled, and is packed with sperm in the three sectioned adults. The epithelium consists of large, irregularly shaped nonsecretory cuboidal cells, with short cilia. The ampulla extends about one-third to one-half of a shell whorl, and ends directly posterior to the female glandular mass, where there is a funnel-shaped muscular valve here called a sphincter (Figures 2A [ring surrounding gonoduct] and 4A). The sphincter could function to prevent the premature passage of sperm (as suggested by Lemche, 1956), or it could be an artifact generated by the imperfect fusion of mature mesodermal and ectodermal tissues. In each case, the end of the anterior gonoduct overlaps the end of the posterior gonoduct like a sleeve (shown in cross-section of an immature individual, Figure 4B). Just before the sphincter, the ampulla narrows considerably, and the wall becomes more muscular.

In the growth series of immature specimens examined (2.5–11.2 mm), the sphincter develops gradually, first as a weaker version of its mature morphology. Sperm and/or ova are not evident in any of these specimens. The posterior gonoduct is represented only by a narrow uncoiled duct, running straight along the columellar wall and branching into the digestive gland. The epithelium is dense and undifferentiated throughout.

Acteocina atrata

Separate male and female acini in the gonad of *Acteocina atrata* are difficult to distinguish. As Mikkelsen & Mikkelsen (1984) reported, this species has nonplanktic development, with an uncleaved egg diameter at deposition of about 150 μm. Mature ova in the gonad are very large, and can fill the entire whorl width (between the outside body and columellar walls) when viewed in cross-section (Figure 3B). They are characterized by the presence of numerous pink-staining yolk droplets and a large well-defined nucleus and nucleolus that stain pale pink and dark pink, respectively. Their large size relative to the gonoduct suggests that either the ova must distort or the gonoduct must expand to allow egg deposition.

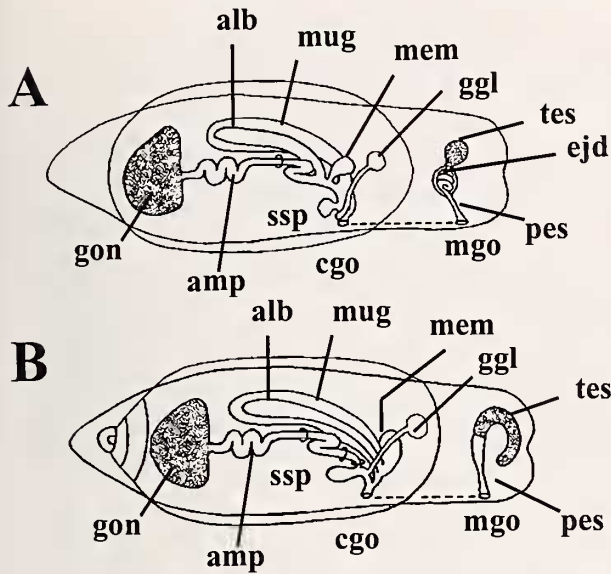The ampulla extends about one-half whorl. The pos-

Table 1

Material examined and ontogenetic development of the reproductive tract. [ad = adult, g = present and glandular, ju = juvenile, n = not present, p = present but nonglandular, x = present, — = not seen or not applicable].

	Bulla striata														*Acteocina atrata*						
Station no. (see text)	FK-211	FK-211	FK-163	FK-163	FK-163	FK-163	FK-163	FK-163	FK-163	FK-163	FK-163	FK-163	PMM-931	PSM-767	LJI	LJI	LJI	LJI	LJI	LJI	LJI
Developmental stage	ju	ju	ju	ju	ju	ju	ju	ju	ju	ju	ju	ad	ad	ad	ju	ju	ju	ju	ad	ad	ad
Shell length (mm)	2.5	3.3	3.4	4.0	5.0	5.4	6.0	7.4	9.0	10.0	11.2	11.4	12.5	16.5	1.4	1.4	1.7	2.0	2.7	3.1	3.2
Copulatory Organ																					
Invagination	x	—	x	x	—	x	—	—	—	—	—	—	—	—	x	x	—	—	—	—	—
Double-walled duct	—	x	—	—	x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Adult epithelia	—	—	—	—	—	—	x	x	x	x	x	x	x	x	—	x	x	x	x	x	x
Female Gland Mass																					
Gametolytic gland duct	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x?	x	x	x	—	x	x
Gametolytic gland	n	p	n	p	p	—	—	p	p	p	g	g	g	g	—	—	g	g	g	g	g
Albumen gland	p	p	p	p	p	p	p	p	p	p	p	g	g	g	p	p	g	p	g	g	g
Mucus gland	p	p	p	p	p	p	p	p	p	p	p	g	g	g	p	p	p	p	g	g	g
Membrane gland	n	p	p	p	p	—	p	p	p	p	g	g	g	g	p	p	p	p	g	g	g
Sperm-storage pouch	x?	x	n	x	x	—	x	x	x	x	p	x	x	x	n?	—	x	x	x	x	x
Posterior Gonoduct																					
Ampulla	—	—	—	n	n	—	n	n	n	n	n	x	x	x	n	x	x	x	x	x	x
Spermatozoa	—	—	—	n	n	—	n	n	n	n	n	x	x	x	x	x	x	x	x	x	x
Ova	—	—	—	n	n	—	n?	n	n?	n	n	x	x	x	—	—	n	n	x	x	x

sexual maturity (*Bulla striata*) · male maturity / female maturity (*Acteocina atrata*)

Figure 2. Diagrammatic gross reproductive anatomy in adult animals. A. *Bulla striata.* B. *Acteocina striata.* Key: alb = albumen gland; amp = ampulla; cgo = common genital opening; ejd = ejaculatory duct of copulatory organ; ggl = gametolytic gland; gon = gonad; mem = membrane gland; mgo = male genital opening; mug = mucus gland; pes = penial sheath of copulatory organ; ssp = sperm-storage pouch; tes = terminal sac of copulatory organ; ring surrounding gonoduct = sphincter.

terior portion of the ampulla is wide, and the contained sperm are oriented in an irregular swirled pattern (Figure 4C). The anterior portion of the ampulla (Figure 2B [amp]) is relatively straight, and ends in a sphincter, as in *Bulla striata,* near the posterior side of the female glandular mass. The ampullar epithelium consists of irregularly shaped cuboidal cells, similar to that in *B. striata.*

In the smallest specimen (1.4 mm), a small amount of mature sperm is visible in the gonad, but no mature ova. The ampulla in this specimen has not fully developed, but is present as a wide duct with nonciliated cuboidal epithelium. The sphincter is also not developed in the smallest specimen, but it is present in all others; no gradual development of the sphincter was noted. Mature ova are present only in the largest animals (2.7–3.1 mm).

Anterior Gonoduct

Traditional names of the glands in the female glandular mass refer to their presumed function, which is difficult to assess and seldom backed by evidence. The female glands assumed present in cephalaspideans—albumen, mucus (= nidamental), and membrane (= capsule, winding)—have been discussed by a number of authors (e.g., Lemche, 1956; Ghiselin, 1966; Thompson, 1976; Hadfield & Switzer-Dunlap, 1984). In the absence of strong evidence about what each contributes to a deposited egg mass, but in the interest of terminological stability, we will refer to them by these conventional names. [A particularly unconventional approach was used by Rudman (1971) in describing the three female glands in *Haminoea zelandiae* (Gray, 1843)—he used "genital gland mass" for the albumen and membrane glands (evidenced from cross-sections, egg pathways, and presumed homology statements), and presented a two part—posterior and anterior "(or pallial)"—mucus gland.]

The terms traditionally applied to exosperm storage pouches—seminal receptacle (= receptaculum seminis, spermatocyst) and bursa copulatrix (= copulatory bursa)—are likewise strongly linked to their presumed function—long- or short-term storage of oriented (embedded) or unoriented sperm, respectively (Fretter & Graham, 1994). These two terms are also heavily linked to location in gastropods—the bursa copulatrix near the common genital opening, and the seminal receptacle more proximal to the gonad near a fertilization chamber. In the two taxa examined here, two pouches occur near the common genital opening. One of these is clearly the gametolytic gland (= spermatheca, ingesting gland), containing degenerating sperm and other reproductive products. The other contains oriented sperm in at least one specimen, but we hesitate to label it "seminal receptacle" because of its location well after the point at which exiting eggs would have received their protective coatings from the female glands. We therefore refer to it generically as a sperm-storage pouch.

The female glands are part of the anterior gonoduct, defined from the sphincter just posterior to the female glandular mass to the common genital opening on the animal's right side. This region is commonly referred to as the pallial gonoduct (e.g., Ghiselin, 1966; Hadfield & Switzer-Dunlap, 1984). That term is not used here because (as with the term 'coelomic gonoduct') the exact location of the transition point between the ectodermal (pallial) and mesodermal (coelomic) gonoduct is unconfirmed. The narrow muscular tube just inside the common genital opening (that presumably receives the copulatory organ of the mating partner) is here called the vagina, in accordance with Marcus (1957) and Gosliner (1994). The wide muscular anterior part of the gonoduct, located posterior to the vagina near the female glands and sperm sacs, is called the vestibule, in accordance with Fretter & Graham (1994).

Bulla striata

The female glandular mass is located on the floor of the pallial cavity posterior to the point where the foregut passes into the posterior body cavity. The main body of the glandular mass is complex, and in mature animals the various parts are difficult to discriminate. The major components of the glandular mass are oriented antero-posteriorly. The anterior gonoduct enters the glandular mass from the rear on the left side, then turns right and tra-
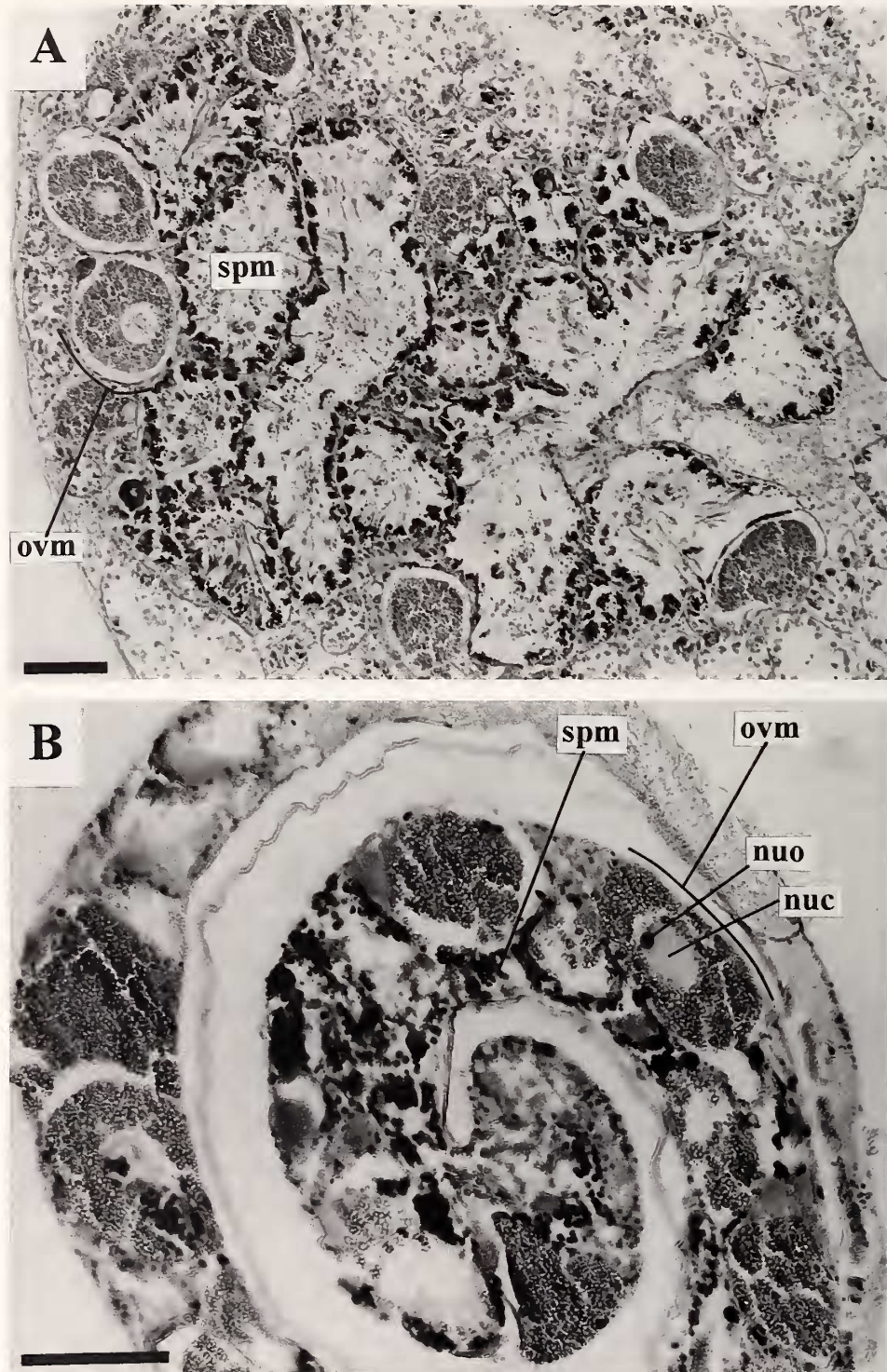
Figure 3.   Cross-sections showing mature gonadal acini. A. Single acinus in *Bulla striata* (FK-163; 11.4-mm specimen). B. Posterior body coil in *Acteocina atrata* (3.2-mm specimen). Scale bars = 100 μm. Key: nuc = nucleus; nuo = nucleolus; ovm = mature ovum; spm = sperm.
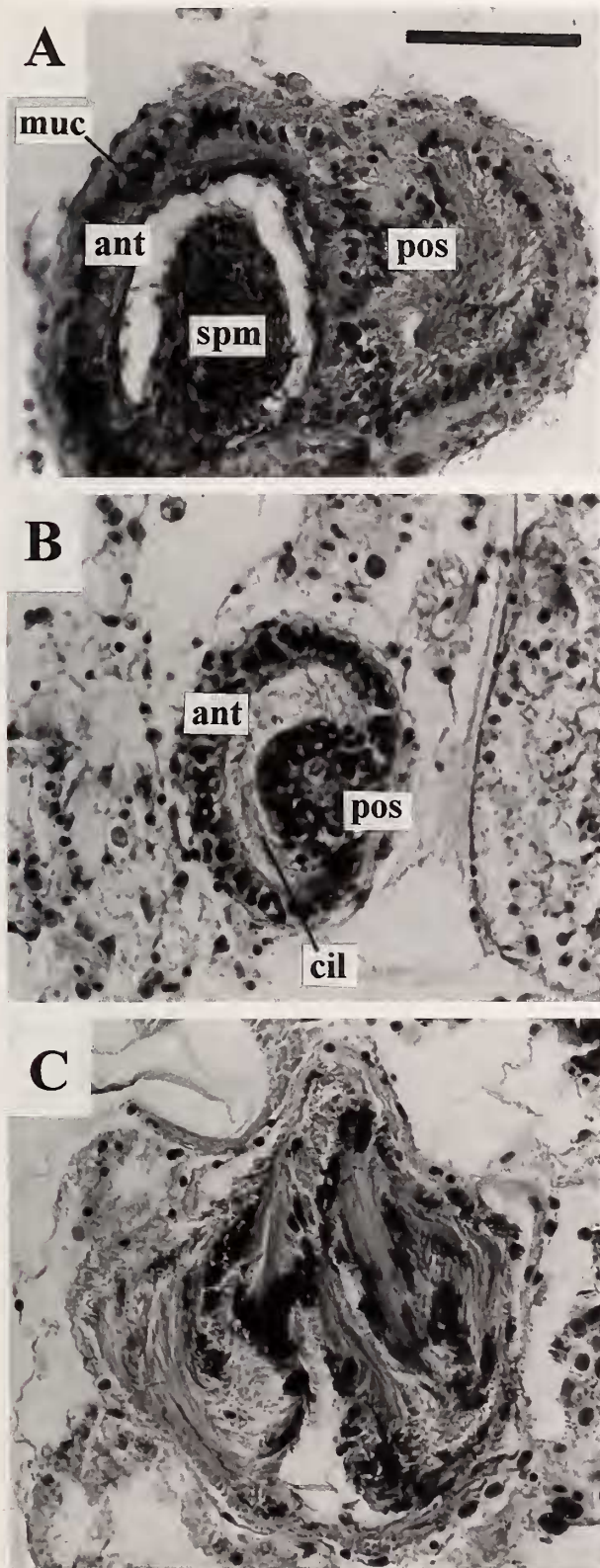
verses within the bases of the glands and into the vestibule.

From the sphincter, the anterior gonoduct becomes narrow and ciliated, with mucoidal cells interspersed in the epithelium. This duct runs anteriorly nearly to the wall of the pallial cavity, then turns posteriorly along the glandular mass for a long distance. It loops and passes back anteriorly on the left side of the gland. Its epithelium becomes entirely mucoidal. The albumen gland lumen opens into the duct close to the posterior part of the loop. This loop of duct is equivalent to the post-ampullar duct of Robles (1975).

The three large glands of the female glandular mass have different staining reactions. The two largest components of the mature mass are the albumen and mucus glands. The albumen gland (Figure 2A [alb]) is the first one encountered by exiting eggs in the gonoduct. It is very long, almost equal in length to the visceral mass, but shorter than the mucus gland partially surrounding it. The albumen gland is many cell layers in thickness. Its cells stain pale purplish red, primarily from the eosin and phloxine stains. In the specimen stained with PAS, the cells are bright magenta in color. This gland is a diverticulum, emptying into the gonoduct, rather than comprising or surrounding the gonoduct, as depicted by Marcus (1957:fig. 5). The lumen of the gland is narrow and densely ciliated (Figures 5A, B).

The mucus gland (Figure 2A [mug]) is longer than the albumen gland, and is composed of larger cells (Figure 5B [mug]). It wraps around the albumen gland over most of its length, but its wide base is to the right of the albumen gland. As a result, its lumen is distal to that of the albumen gland where it contacts the main channel of the gonoduct (Figure 2A [mug]). The cells of the gland stain pale purplish white, primarily with hematoxylin. In the specimen stained with PAS, the cells stain pale purple, with some regions turquoise (suggestive of mucus). The lumen is ciliated; one wall has a pale red-staining columnar epithelium with central nuclei, the other side is stained like the rest of the gland. Farther posteriorly, close to the albumen gland, there is a region with smaller cells that stains more strongly with hematoxylin, and appears pale purple in sections. The anteriormost region of the mucus gland, near the common genital opening, has distinctly smaller cells.

The lumen of the anterior gonoduct at this point becomes very complex and is difficult to follow in sections. Part of the duct opens into a thick-walled gland on the

Figure 4.   Portions of the posterior gonoduct. A. Tangential section of fully developed sphincter of *Bulla striata* (PSM-767;

←

16.5-mm specimen). B. Cross-section of immature sphincter of *B. striata* (FK-163; 11.2-mm specimen). C. Sperm (in swirled pattern) in ampulla of *Acteocina atrata* (2.7-mm specimen). Scale bars = 50 μm. Key: ant = anterior gonoduct; cil = cilia; muc = mucus cell; pos = posterior gonoduct; spm = sperm.
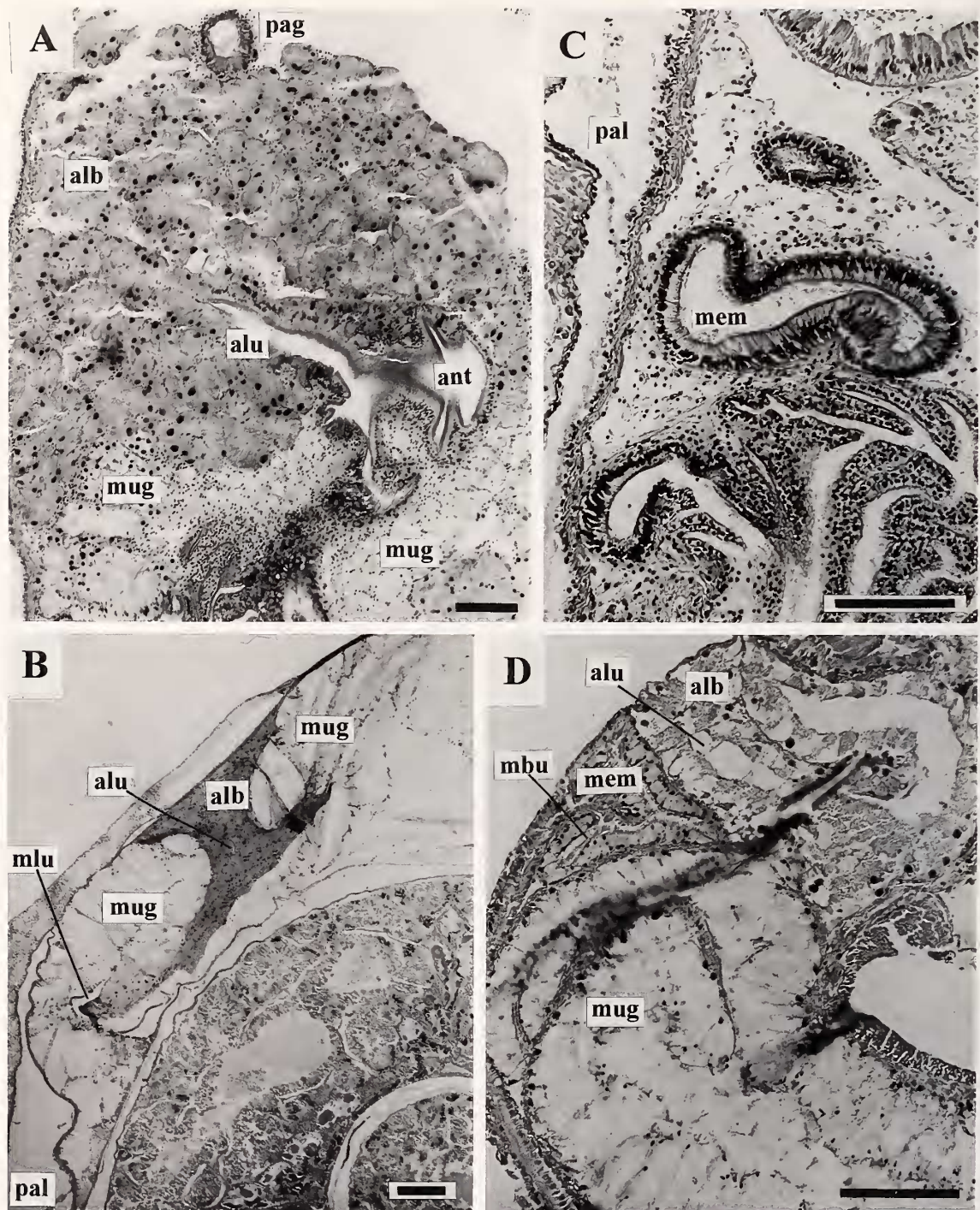
Figure 5. Female glandular mass. A. Cross-section of albumen gland in *Bulla striata,* at its junction with the gonoduct (PSM-767, 16.5-mm specimen). B. Cross-section of posterior ends of albumen and mucus glands in *B. striata,* showing the former nested in the latter (PSM-767, 16.5-mm specimen). C. Cross-section of membrane gland in immature *B. striata* (PSM-767, 16.5-mm specimen). D. Cross-section of female glandular mass in *Acteocina atrata* (2.7-mm specimen). Scale bars = 100 μm (A, C, D); 200 μm (B). Key: alb = albumen gland; alu = lumen of albumen gland; ant = anterior gonoduct; cil = cilia; mbu = lumen of membrane gland; mem = membrane gland; mlu = lumen of mucus gland; muc = mucus cell; mug = mucus gland; pag = posterior end of anterior gonoduct; pal = pallial cavity; pos = posterior gonoduct; spm = sperm.

anterior left side of the glandular mass, which is S-shaped in cross-section. Gosliner (1979) referred to a small gland at this position in *Acteocina canaliculata* (Say, 1826) as the membrane gland, and we use this term here. The epithelium of this gland has tall, pale-purple-staining columnar cells, with long cilia and basal nuclei (Figure 5C [mem]). There are no subepithelial glandular tissues associated with it. The gland is a diverticulum, but its body lies extended toward the pallial cavity instead of toward the posterior alongside the albumen/mucus glands. At the blind end of the gland, the right wall becomes nonglandular, and has a columnar ciliated epithelium.

In the same region, the anterior gonoduct enters the dense network of narrow folds or tubules that makes up the anteriormost part of the glandular mass, and turns right toward the common genital opening. The walls in this region are thin. The epithelium is cuboidal, with a few mucus cells; the presence of cilia could not be confirmed. The main part of the anterior gonoduct in this region also has a ciliated cuboidal epithelium. The sperm-storage pouch and the gametolytic gland (see below) open here, nearby each other on the posterior side of the glandular mass.

The sperm-storage pouch (Figure 2A [ssp]) is a small diverticulum. Its walls consist of narrow columnar cells with central nuclei. In the largest specimen (16.5 mm), the lumen contains mucus and a few oriented sperm with their heads embedded in the epithelial wall (Figure 6A [spm]). The outside wall is muscular.

The duct of the gametolytic gland passes anteriorly from the anterior gonoduct into the pallial/pericardial wall, alongside a posterior extension of the pericardium (which ends along the body wall near the glandular mass, slightly to its left). The gland is muscular in mature animals, with a tall, dense epithelium. The terminal sac of the gametolytic gland (Figures 2A, 6B [ggl]) is located adjacent to the pericardium in the pallial cavity roof. In mature specimens, this is spherical with thin walls. The epithelium is composed of tall columnar cells with central nuclei. Its lumen in the largest specimen (16.5 mm) is filled with yellow- and pink-staining material, but no obvious sperm, as is present in the other adult specimens (11.4 and 12.5 mm).

Just before the common genital opening, the anterior gonoduct branches into two vestibular chambers (Figure 7A [vec]). One chamber leads to the vagina and common genital opening. The other is a blind chamber next to the body wall that communicates with the right side of the mucus gland, which is located adjacent to the anterior side of the gonoduct. Both of these chambers are muscular. The epithelia are similar, and consist of dense ciliated columnar cells with central nuclei. Some regions along the vestibular wall have yellow-staining particles in the cells.

One adult (11.4 mm) has a large amount of sperm in the anterior gonoduct. In this animal, the sperm is found in the gametolytic gland and in the vestibule, between the vagina and the densely folded region on the anterior left side (Figure 7B [spm]). None is present in the sperm-storage pouch (Figure 7B [ssp]). The gonoduct wall is markedly distended by the large amount of sperm.

In one of the small specimens (3.4 mm; Figure 8A), the glandular mass is partially differentiated, and the posterior gonoduct is present. The glandular mass in this animal consists of small sacs representing the albumen and mucus glands. The gametolytic gland duct and the coiled section of duct anterior to the sphincter are also present. The base of the glandular mass near the common genital opening is a simple oval chamber, which splits posteriorly into the mucus gland to the left of the opening and the albumen gland to the right (Figure 8A [mug, alb]). Both glands are U-shaped in cross-section at the base. The albumen gland becomes round in cross-section posterior to where the two glands diverge, and becomes nested within the 'U' of the mucus gland (Figure 8B, shown in a slightly larger specimen). The epithelia are undifferentiated and densely nucleated. The posterior gonoduct, as it extends away from the glandular mass, has a less dense epithelium.

In small specimens, the gametolytic gland is a small darkly nucleated hollow sphere at the end of its duct. In progressively larger specimens, the lumen enlarges and bulges into the pericardial lumen. The epithelia of the gametolytic gland and its duct consist of low cuboidal cells, and the wall is nonmuscular. In the smallest animals, both the main part of the glandular mass and the gametolytic gland duct open into the pallial cavity at the same place, and they are only narrowly connected to each other. Thus it appears that they might develop as independent invaginations from the same site on the pallial wall, but this was not positively confirmed.

The anterior gonoduct differentiates relatively early. In the 5.0-mm specimen, the common genital opening is located a short distance to the right of the main body of the glandular mass (instead of immediately anterior to it), indicating that the vestibule has started to develop. The future glandular regions posterior to the vagina have thicker, cuboidal epithelia. The walls of the vagina and glandular mass are still nonciliated. In the 7.4-mm specimen, the anterior wall of the glandular mass has a few folds, and the vestibular and vaginal walls are ciliated. The first glandular tissues in the gonoduct appear in the membrane gland, as shown in Figure 5C.

As the anterior gonoduct develops, it changes shape. This change is particularly apparent in the position of the common genital opening. In the smallest animals, the differentiating glandular mass and the gametolytic gland duct open at the same place (Figure 9A), along the pallial wall adjacent to the anterior tip of the digestive gland. Continuing development of the glandular mass effectively carries the gametolytic gland duct away from the body
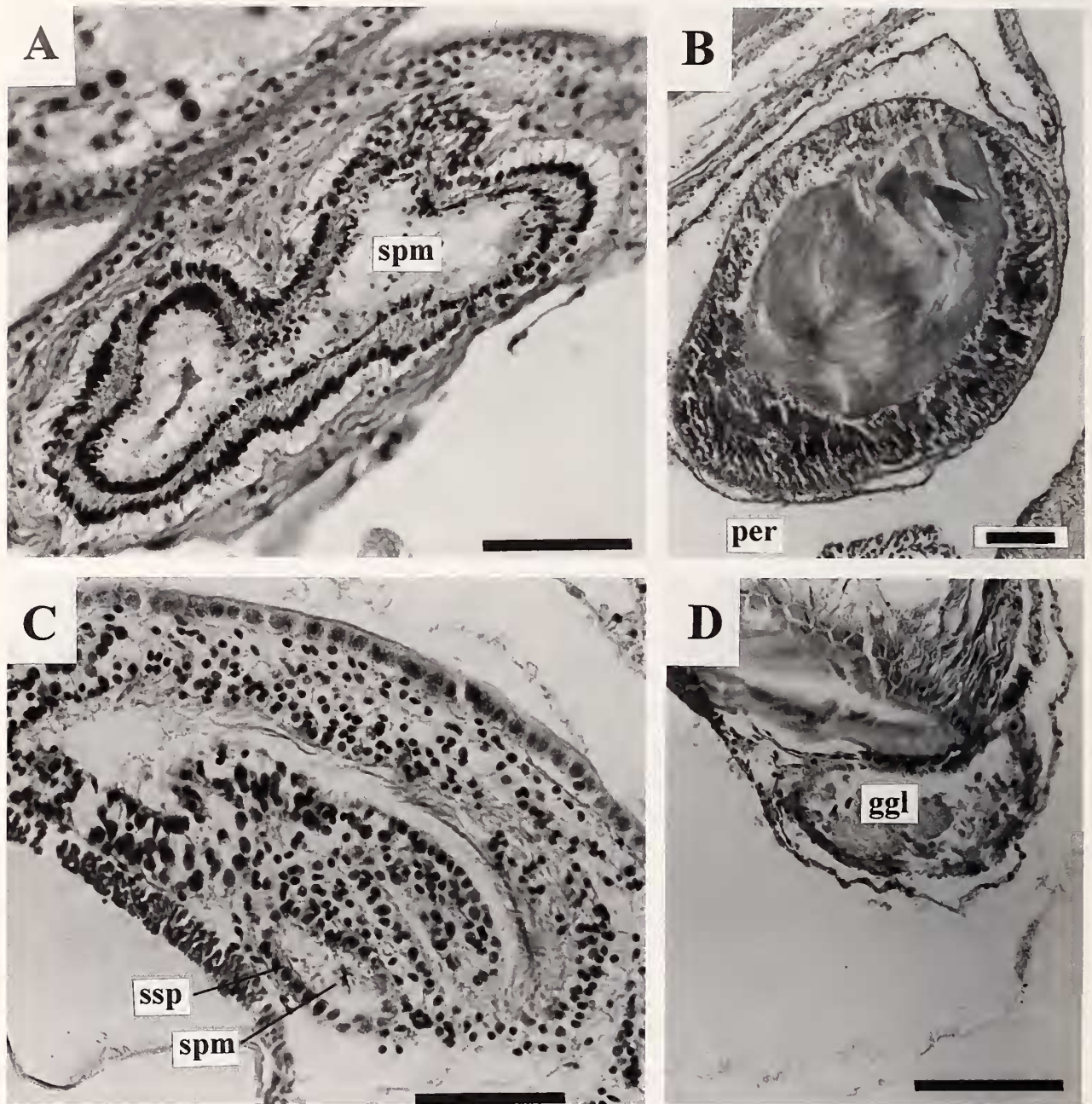
Figure 6. Sperm-storage pouches and gametolytic glands. A. Tangential section of sperm-storage pouch containing sperm in *Bulla striata* (PSM-767; 16.5-mm specimen). B. Tangential section of gametolytic gland in *B. striata* (FK-163; 11.4-mm specimen). C. Cross-section of gonoduct showing sperm-storage pouch containing sperm in immature *Acteocina atrata* (2.0-mm specimen). D. Tangential section of gametolytic gland in *A. atrata* (3.1-mm specimen). Scale bars = 50 μm (A, C); 100 μm (B, D). Key: ggl = gametolytic gland; per = pericardium; spm = sperm; ssp = sperm-storage pouch.

wall, and in larger animals it opens into the ventral side of the gonoduct near the sperm-storage pouch (Figure 8C). The common genital opening is shifted progressively to the right from its original location, as described in the previous paragraph (Figures 9B, C). As a result, the common genital opening and gametolytic gland duct in ad- vanced juvenile and adults are some distance apart, in different regions of the gonoduct. The mucus gland also changes shape with ontogeny. The base is narrow in the smallest animals, but it broadens as the vestibule devel- ops, and it remains connected to a large portion of the vestibule roof.
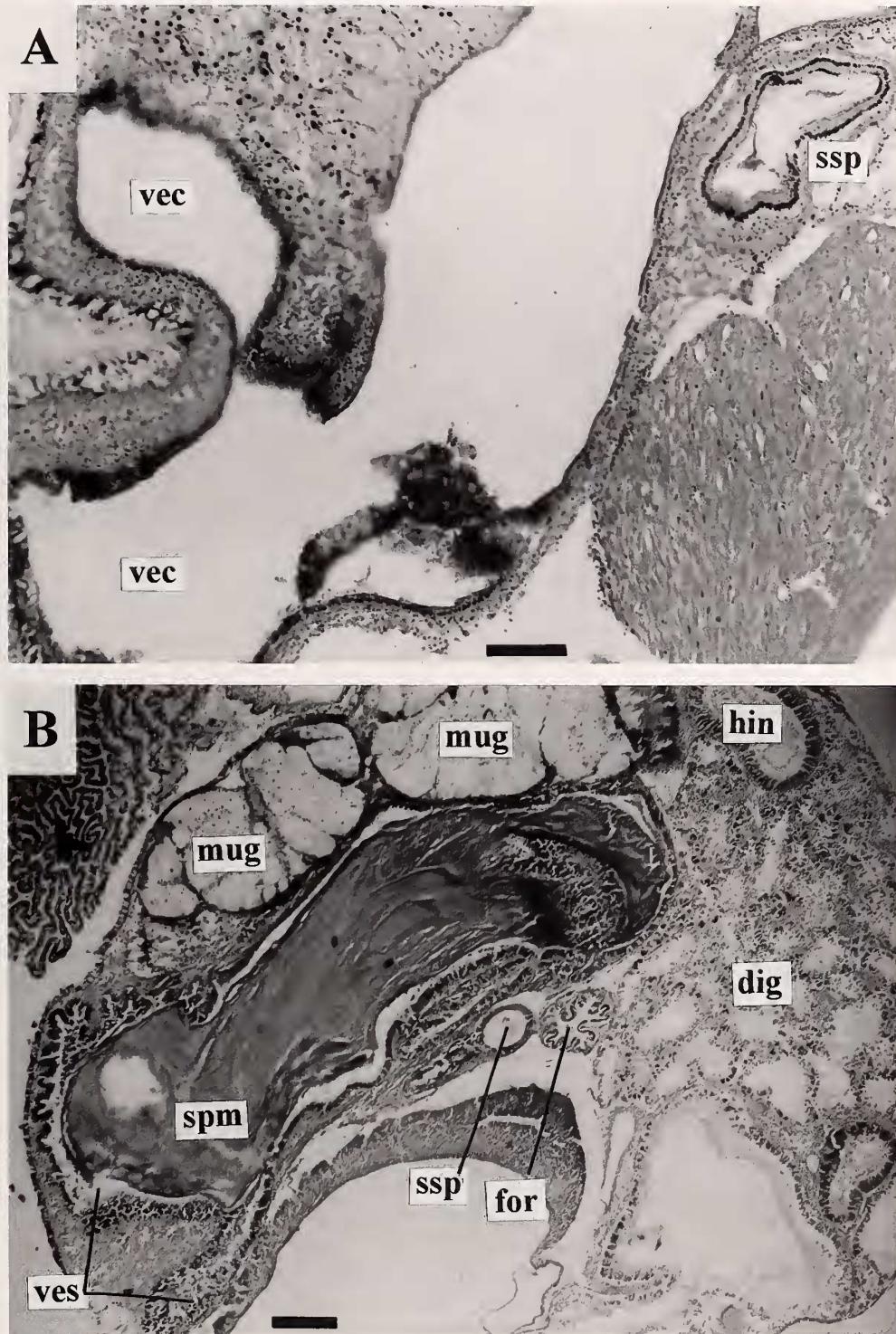
Figure 7.   Vestibule in *Bulla striata*. A. Tangential section of vestibular chambers (PSM-767; 16.5-mm specimen). B. Sperm in anterior gonoduct and vestibule (FK-163, 11.4-mm specimen). Scale bars = 100 μm (A); 200 μm (B). Key: dig = digestive gland; for = foregut; hin = hindgut; mug = mucus gland; spm = sperm (in anterior gonoduct); ssp = sperm-storage pouch; vec = vestibular chambers; ves = vestibule.
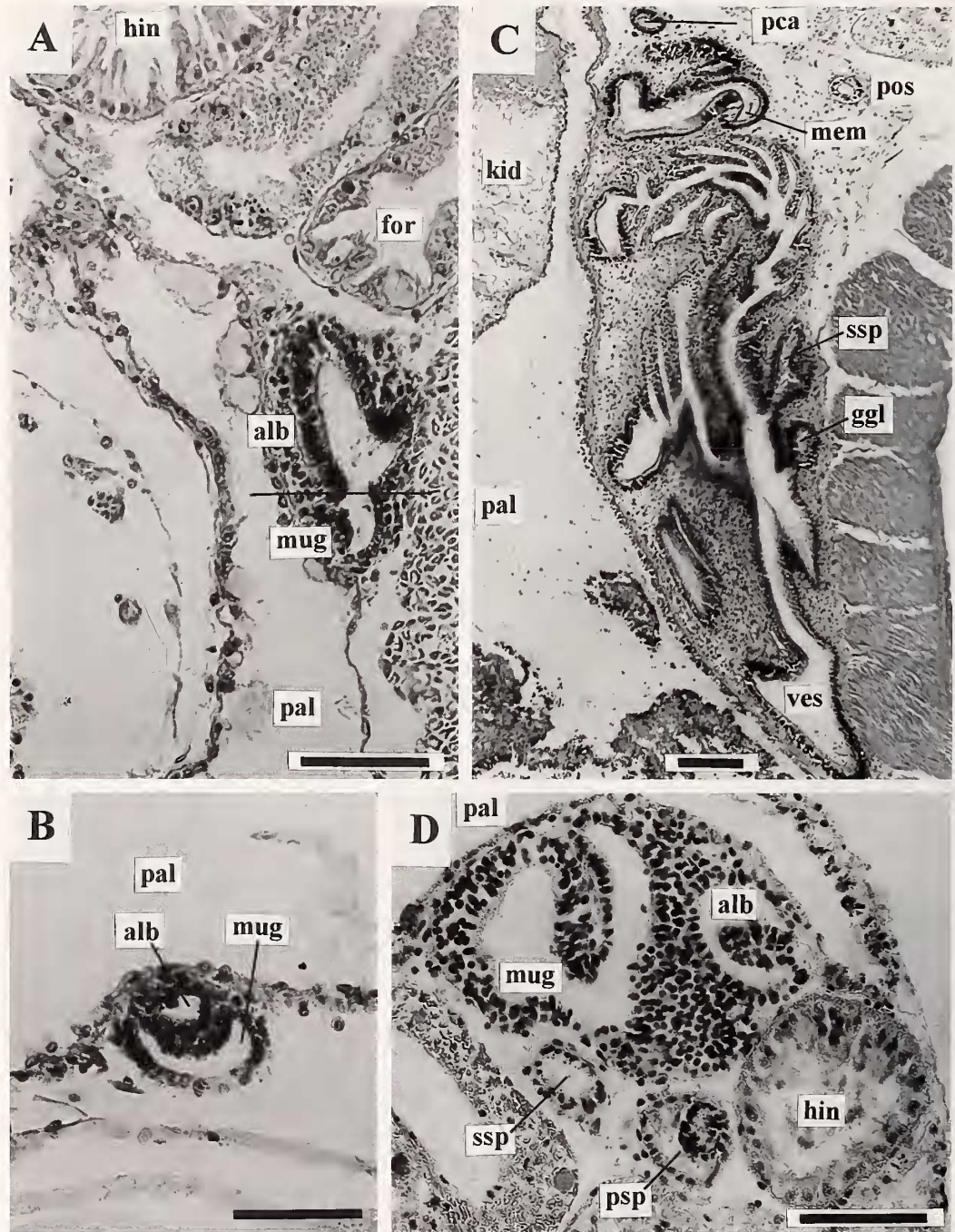
Figure 8. Developing anterior gonoduct. A. Cross-section of the base of the mucus and albumen glands in juvenile *Bulla striata* (FK-163; 3.4-mm specimen). B. Cross-section of albumen and mucus glands in juvenile *B. striata* (FK-163; 4.0-mm specimen). C. Overview of developing anterior gonoduct in *B. striata* (FK-163; 11.2-mm specimen). D. Posterior region of anterior gonoduct of *Acteocina atrata* (1.7-mm specimen). Scale bars = 50 μm (A, B, D); 100 μm (C). Key: alb = albumen gland; for = foregut; hin = hindgut; kid = kidney; mem = membrane gland; mug = mucus gland; pal = pallial cavity; pca = posterior coil of anterior gonoduct; pos = posterior gonoduct; psp = posterior sphincter; ssp = sperm-storage pouch; ves = vestibule.
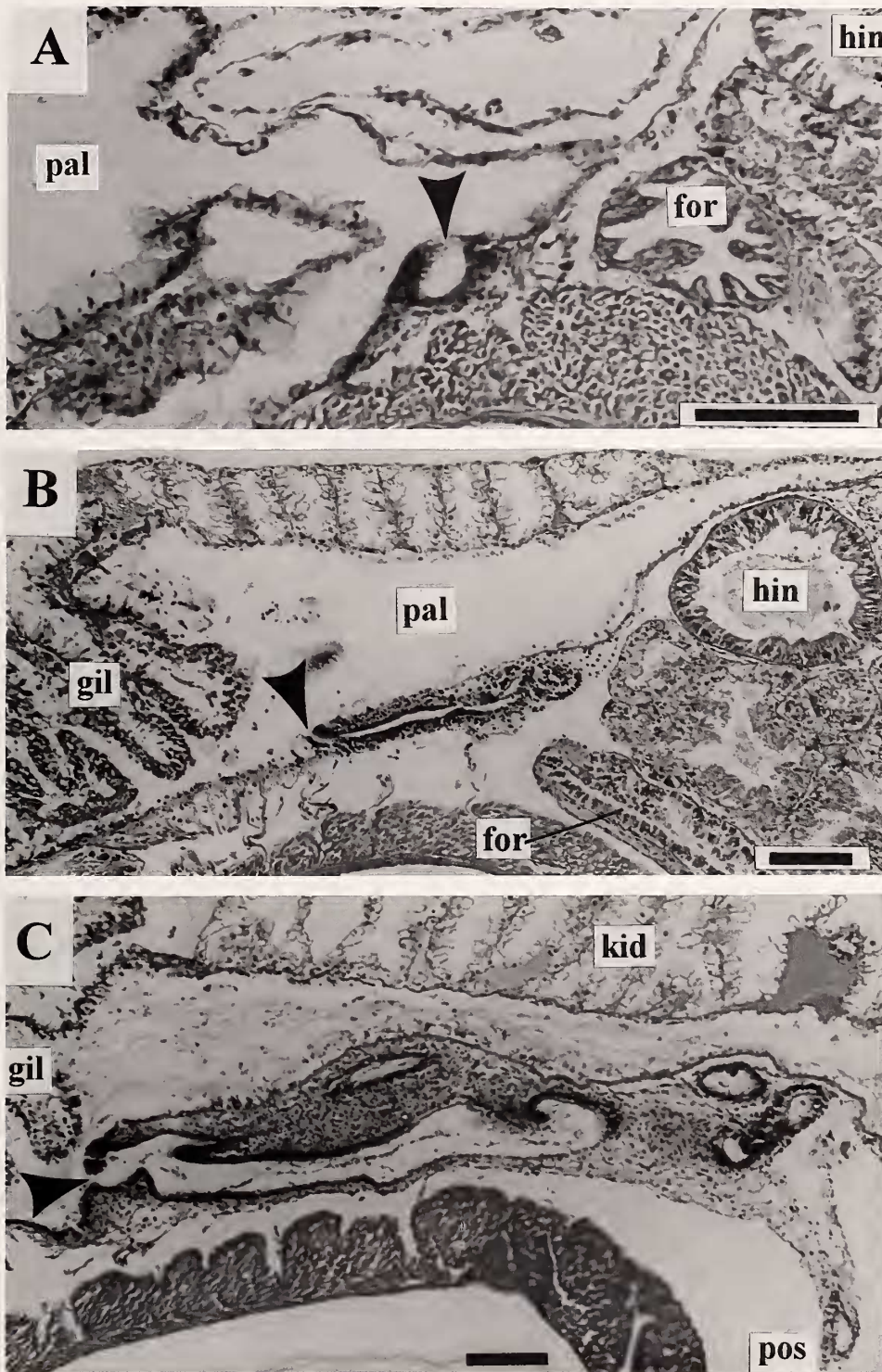
Figure 9. Developing glandular mass in *Bulla striata*, showing location of common genital opening relative to the foregut, hindgut, kidney, and gill. A. 3.4-mm specimen. B. 5.0-mm specimen. C. 7.4-mm specimen. Scale bars = 100 μm. Key: gil = gill; for = foregut; hin = hindgut; pal = pallial cavity; pos = posterior gonoduct; arrow = common genital opening.

Acteocina atrata

The female glandular mass of *Acteocina atrata* is similar to that of *Bulla striata* in several respects; however, the path that the anterior gonoduct takes is quite different. Instead of passing through the mass as in *B. striata*, the gonoduct enters the glandular mass near the common genital opening. All of the glands empty into this same common area.

In this species, the posteriormost portion of the anterior gonoduct immediately following the sphincter has a cuboidal epithelium and is heavily ciliated. It becomes glandular, and forms a loop posterior to the glandular mass, following which there is a second sphincter; this portion is ciliated like the previous one. The epithelium here is cuboidal and is stained light blue. Anteriorly, the gonoduct is nonglandular once again, then enters the base of the sperm-storage pouch following a third sphincter (presumably to prevent the posterior transport of sperm past the pouch). The gonoduct and sperm-storage pouch have thin muscular walls and cuboidal epithelia (Figure 6C). The sperm-storage pouch (Figure 2B [ssp]) is semiserial, in other words, it has separate entrance and exit ducts and is part of the pathway, but is in the form of a diverticulum to one side (cf. two out of three specimens of *Acteocina culcitella* discussed by Gosliner, 1979). Anteriorly, the gonoduct opens broadly into the vestibule.

The vestibule splits posterior to the common genital opening. One portion is the anterior end of the gonoduct, directed ventrally. The other heavily ciliated portion is directed dorsally below the pallial wall, and receives the ducts of the three female glands. The largest and anteriormost is the mucus gland, opening into the vestibule roof. It is histologically similar to the mucus gland in *Bulla striata*, with large cells staining very light blue with hematoxylin (Figure 5D [mug]). The posterior lumen of the gland is U-shaped in cross-section and has short cilia. The albumen and mucus gland walls in this species appear to be about one cell thick, perhaps because of the small adult size.

The membrane gland, smaller and to the left of the mucus gland, has a Y-shaped lumen in cross-section (Figure 5D [mem]). Its epithelial cells are smaller, stain purplish blue, and have short dense cilia. This stains similar to the S-shaped membrane gland in *Bulla striata*, occupies a similar position, and is also relatively small; it is therefore here considered homologous.

The albumen gland lies to the left of the other two glands. It has large epithelial cells with basal nuclei and short cilia, partially stained red with eosin/phloxine (Figure 5D [alb]). As in *Bulla striata*, the posterior part of the albumen gland is located within the "U" of the mucus gland, although their bases are widely separated in this species.

The gametolytic gland of *Acteocina atrata* (Figure 6D [ggl]) is similar to that in *Bulla striata*. Its terminal sac is adjacent to the pallial wall and pericardium. Its walls are thick, composed of large pink-staining cuboidal cells. In larger specimens, the gland is filled with pink-staining secretions and other purplish-gray material. The gametolytic gland duct is located along the pallial wall adjacent to the gizzard, and enters into the anterior fork of the gonoduct close to the sperm-storage pouch.

Development of the anterior part of the female glandular mass in this species is similar to that in *Bulla striata* in that the glands are present (but undifferentiated) early, and the common genital opening in the smallest specimens is in a different location than at maturity. In the smallest specimen (1.4 mm), the anterior glandular mass is a triangular array of sacs, and the anterior gonoduct passes through the glands rather than being separated from them as in adults. The U-shaped mucus gland is the longest, on the right side of the mass, with the common genital opening lying at its anterior center. A second undifferentiated sac on the left side is probably the incipient albumen gland, and a smaller undifferentiated sac between these two could be the membrane gland. There is no sperm-storage pouch in the smallest specimen. The 1.7-mm specimen has a fully differentiated glandular mass (Figure 8D) with the common genital opening on the right side, and glandular tissue in the anterior gonoduct. The 2.0-mm specimen has a very small amount of sperm in the base of the sperm-storage pouch (Figure 6C [spm]).

Copulatory Organ

The copulatory organ of a cephalaspidean (sometimes called the penis in its entirety, e.g., Gosliner, 1994) opens to the exterior on the right side of the head, in the lateral groove between the cephalic shield and the foot. This leads to an outer duct, here called the penial sheath [after Thompson, 1976; also called penial sac by Lemche (1956), Ghiselin (1966), and Hadfield & Switzer-Dunlap (1984), or male vestibulum by Marcus (1957); Marcus (1957) and Robles (1975) confoundingly labeled the outer wall of the duct-within-a-duct sections the "penial sheath."]. The sheath contains the penial papilla [after Gosliner, 1994; also called glans penis by Lemche (1956) and penis by Thompson (1976) and Mikkelsen (1996)] that sometimes is equipped with hardened spines or ridges [e.g., in *Scaphander* spp. (Marcus, 1974); *Haminoea elegans* (Gray, 1825) (see Marcus, 1958a)]. This in turn leads to a penial extrovert, an ejaculatory duct, and a terminal sac [called spermatic bulb by Hadfield & Switzer-Dunlap (1984) and "root of the penis" by Marcus (1957)]. The gross form, histological structure, and histochemistry of the so-called prostatic gland across Opisthobranchia is especially variable, and has even been implicated in spermatophore formation in Haminoeidae and Runcinidae (Ghiselin, 1966; Hadfield & Switzer-Dunlap, 1984). It is doubtful that all such structures (including

those of caenogastropods) are homologous (Mikkelsen, 1996). Although glandular tissue associated with the copulatory organ might be considered prostatic (e.g., producing sustaining fluid for stored spermatozoa), the terms "prostatic gland" or "prostate" are not used here to avoid the implication of homology with so-labeled organs in other gastropods.

Bulla striata

The adult copulatory organ of *Bulla striata* (Figure 2A [right]) lies relatively loose within the cephalic haemocoel, anchored by a few muscle bundles, and in most specimens coiled dorsally around the buccal mass. Histologically, it is composed of four distinct parts. (1) The penial sheath (Figure 10A) is a longitudinally folded duct leading from the male genital opening. On its wall is a longitudinal gutter delimited by tissue flaps (not seen by Marcus, 1957) and continuous with the external sperm groove running along the animal's right side to the common genital opening. The cross-section of this duct is generally H-shaped through most of its length, and its longitudinal folding renders it expandable to accommodate the everting penis. The walls are muscular, with a partially mucoidal epithelium. (2) At the internal end of the folded duct is the narrowed opening into the next section—a point which has been called the "penial papilla" (Marcus, 1957) (Figure 10B [pep]). This is not a permanent structure, varies in extent, and (as will be described below) does not form the tip of the penis upon eversion. The opening leads to the penial extrovert (so called for reasons that will be obvious below), a wide, longitudinally folded region (Figure 10C) with a non-mucoidal epithelium. Posteriorly, this region is irregularly folded and densely ciliated. The penial extrovert and the penial sheath are embedded within the body wall. The penial extrovert and the following section have a double-walled (duct-within-a-duct) structure; the internal and external walls of the duct are muscular. (3) Also within the double-walled region is the ejaculatory duct, which is a tightly coiled glandular tube (Figure 10D), smaller in diameter than the previous. The epithelium consists of columnar, pale red-staining cells; in the adult stained with PAS, the particles visible in these cells stain magenta. This portion contains stored endosperm in some specimens. (4) Behind the coiled ejaculatory duct, the separated inner and outer walls coalesce, and the copulatory organ ends as a muscular blind sac (Figure 10E), wider in diameter than the double-walled region, and with an epithelium similar to but denser than that of the ejaculatory duct.

The largest adult examined (16.5 mm) is preserved with the penis partially everted to the external opening (Figures 11A, B). From this animal, it is evident that the "penis" is formed of an eversion of the penial extrovert (the second part of the copulatory organ) described above. In its retracted state, the walls of this portion are folded, and thus are clearly expandable in diameter. The coiled ejaculatory duct in this specimen lies close but not immediately behind the penial tip, and is still somewhat coiled. This duct is narrow in diameter, with non-folded epithelium, and thus appears not expandable. Almost all of the endosperm in this specimen are in the ejaculatory duct rather than in the terminal bulb.

In its early stages (some of the 2.5–5.4 mm specimens), the developing copulatory organ of *Bulla striata* is represented by a long, narrow invagination anterior to the buccal mass (Figure 12A). The wall is thick and densely nucleated overall. The epithelium is cuboidal, unciliated, and also very densely nucleated. The end of the interior lumen is glandular, with pale-red-staining secretory cells. The organ opens anteriorly into a wide chamber, which communicates with the exterior.

In the 5.0-mm specimen, the copulatory organ is slightly longer, although it still does not extend posteriorly to the level of the buccal mass. The double-walled region has differentiated, and the duct ends as a terminal bulb connected to the outer wall (Figure 12B). The epithelia of both interior and exterior ducts are still low, dense, and undifferentiated, except for the end of the terminal bulb. This has a tall, secretory epithelium, with some mucoidal cells and a few dark-red-staining cells interspersed with the paler-red-staining cells present in the smaller individuals.

The 3.3-mm specimen, in spite of its small size, is in a more advanced state of development than those previously discussed. The copulatory organ lies dorsal and to the right of the buccal mass, but is not coiled around it. The penial sheath is only weakly folded. The double-walled region has developed, but the epithelium is undifferentiated, so that the two mature sections of this part (penial extrovert, ejaculatory duct) are not distinguishable. Approximately midway through the inner duct, there is a short section that is U-shaped in cross-section (Figure 12C, arrow). This could correspond to the junction of the penial extrovert and ejaculatory duct of the mature organ. The terminal sac is relatively narrow, about half the width of the double-walled sections (Figure 12D [tes]).

Acteocina atrata

The adult copulatory organ in *Acteocina atrata* (Figure 2B [right]) opens to the exterior on the right side of the head, and coils around the buccal mass as in *Bulla striata*. It is relatively simple in gross morphology, but is extremely long, coiling extensively within the anterior body cavity. External and dorsal to the male genital opening, there is a small smooth purple-staining area along the body wall that is consistently present but of unknown nature (mucoidal? cuticular?) and function. Histologically, the copulatory organ has three distinct portions. (1) The outermost penial sheath is similar to that in *Bulla*
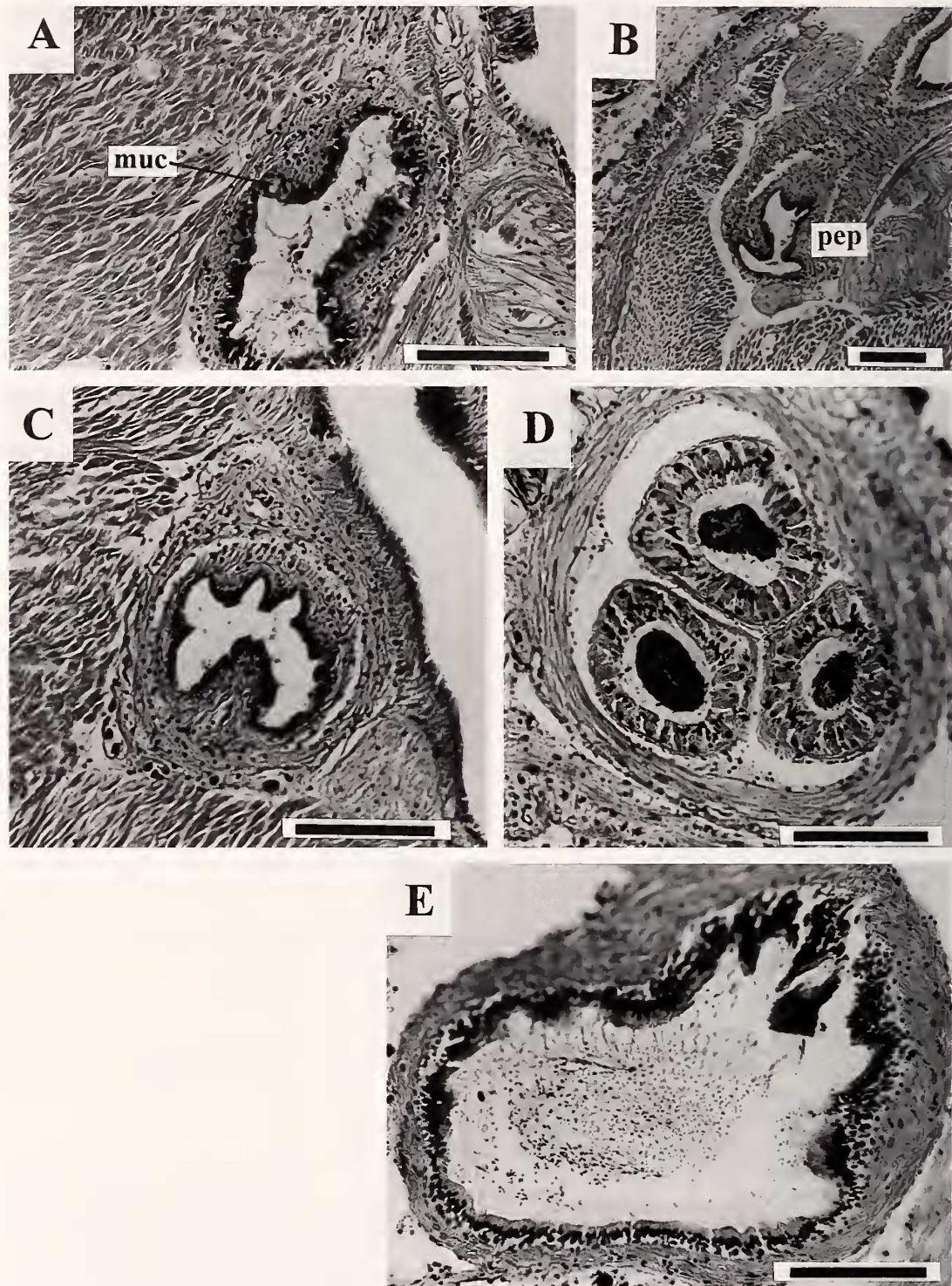
Figure 10.   Copulatory organ of *Bulla striata*. A. Cross-section of penial sheath (FK-163; 11.2-mm specimen). B. Tangential section of retracted copulatory organ, showing "penial papilla" (FK-163; 7.4-mm specimen). C. Cross-section of penial extrovert (FK-163; 11.2-mm specimen). D. Cross-section through coiled (prostatic) ejaculatory duct (PSM-767; 16.5-mm specimen). E. Tangential section of terminal sac (PSM-767; 16.5-mm specimen). Scale bars = 100 μm. Key: muc = mucus cells; pep = "penial papilla."
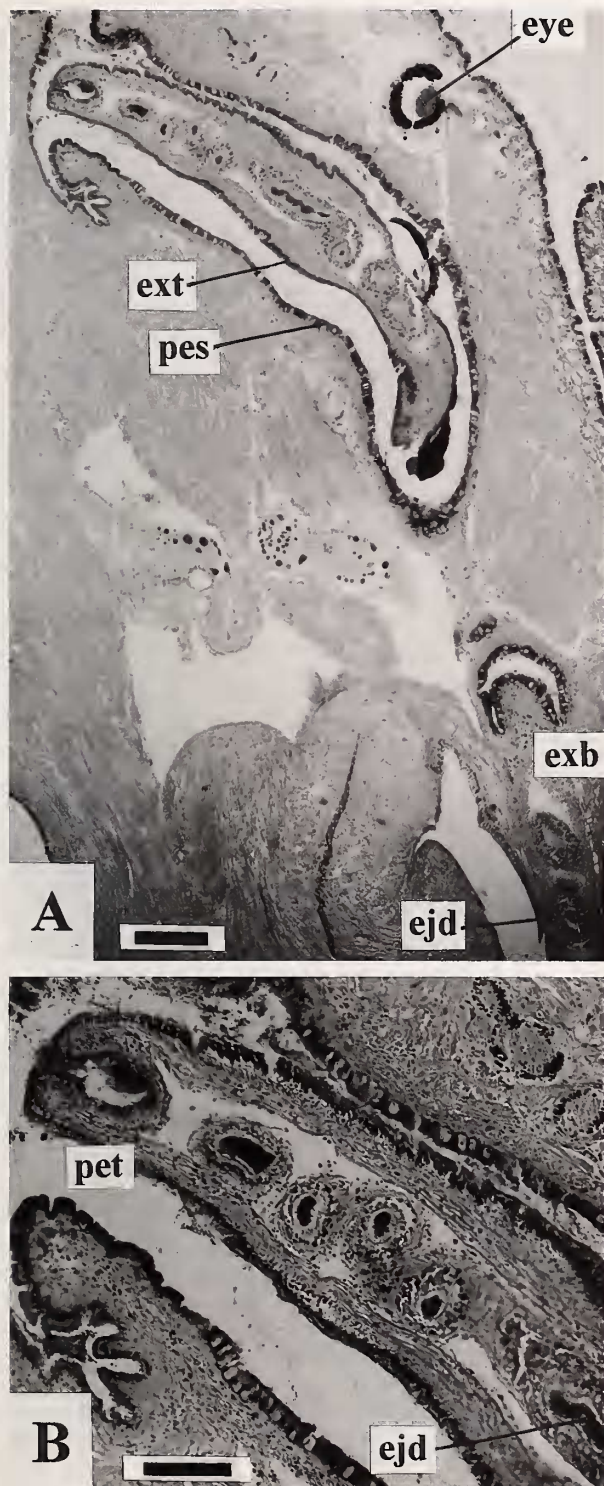
Figure 11.   A. Partially everted penis in *Bulla striata* (PSM-767; 16.5-mm specimen). B. Same, penial tip (enlarged). Scale bars = 100 μm (B); 200 μm (A). Key: ejd = ejaculatory duct; ext = extrovert (wall); exb = base of extrovert; eye = right eye; pes = penial sheath (wall); pet = penial tip.

striata, in being H-shaped in cross-section and somewhat muscular, but it is not mucoidal (Figure 13A). The walls become irregularly folded as one proceeds interiorly, and grade without sharp demarcation into the (2) wider medial section. No definitive penial papilla is present at this junction in all seven specimens sectioned for this study. The epithelium of the medial section is unusual; the wall itself is thin and flat, but has large papillae composed of distinct clumps of tall cells with central nuclei (Figures 13B [pap], C [med]). These become still larger internally, where small amounts of stored endosperm are present in some specimens. The terminus of the copulatory organ is (3) an elongated sac, delimited from the medial section by a slight constriction, and with thick, secretory, non-folded walls (Figure 13C [tes]). The epithelial cells are ciliated and stained red, with large basal nuclei; near the blind end, the sac narrows slightly, and there are no cilia.

The copulatory organs of juvenile *Acteocina atrata* are very similar to those of juvenile *Bulla,* at a stage before the double-walled region develops. The smallest animal (1.4 mm) has an elongated invagination that ends just anterior to the buccal mass (Figure 13D). The epithelium is cuboidal and unciliated, with large, dark nuclei. There are no glandular epithelia evident in the copulatory organ of this juvenile. The other 1.4-mm juvenile is in a more advanced stage of copulatory organ development, with some glandular tissue present.

DISCUSSION

Ontogeny of the Cephalaspidean Gonoduct

The heterobranch gonoduct, like that of caenogastropods, develops from several ontogenetic components, including both ectodermal and mesodermal tissues. In opisthobranchs, there are generally three components, including the male copulatory organ, the female glandular mass, and the posterior gonoduct (Moor, 1983). The copulatory organ and glandular mass develop as ectodermal invaginations, and the gonad develops from mesodermal tissues.

Bulla striata and *Acteocina atrata* are consistent with this general pattern. The copulatory organ develops as an invagination of the ectodermal body wall. The ectodermal glandular mass and mesodermal posterior gonoduct are already formed and continuous in the smallest specimens examined in this study, so the point of fusion between these components could not be definitively demonstrated. However, the smallest specimens of *B. striata* showed a distinct change in the structure of the gonoducal epithelium, which is otherwise undifferentiated, just behind the glandular mass in the region where the sphincter develops. This supports the hypothesis that the adult sphincter represents the point of fusion between the different parts of the duct.

Other heterobranchs display similar developmental patterns. In *Tritonia hombergi* Cuvier, 1803 [Sacoglossa:
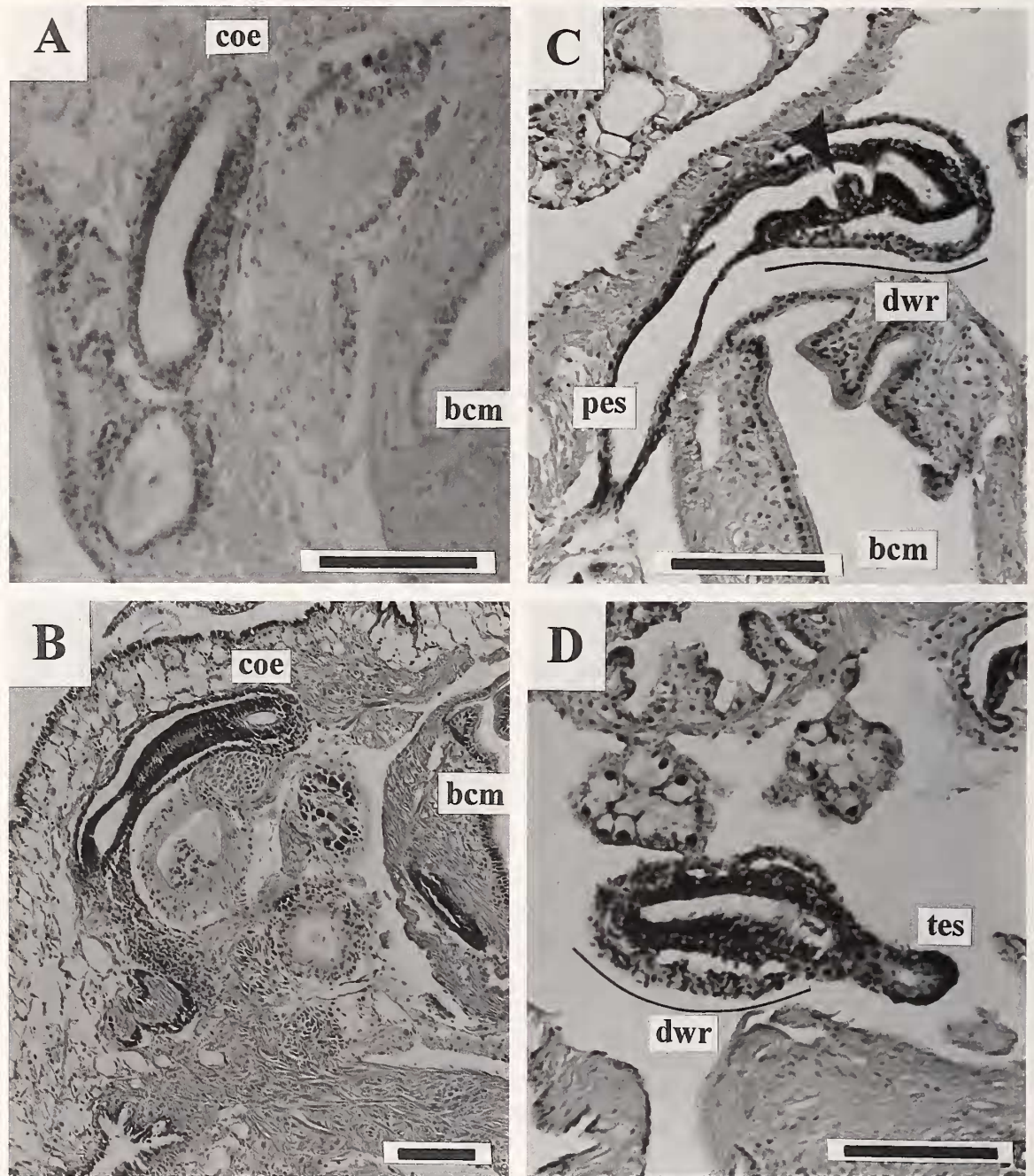
Figure 12. Development of copulatory organ of *Bulla striata*. A. Tangential section, invagination stage (FK-163; 3.4-mm specimen). B. Tangential section, double-walled duct stage (FK-163; 5.0-mm specimen). C. Tangential section, advanced double-walled duct stage, showing partial differentiation of inner duct (FK-211; 3.3-mm specimen). D. Tangential section, advanced double-walled duct stage, showing small terminal sac (FK-211; 3.3-mm specimen). Scale bars = 100 μm. Key: bcm = buccal mass; coe = end of copulatory organ; dwr = double-walled region; pes = penial sheath; tes = terminal sac; arrow = possible junction of extrovert and ejaculatory duct.
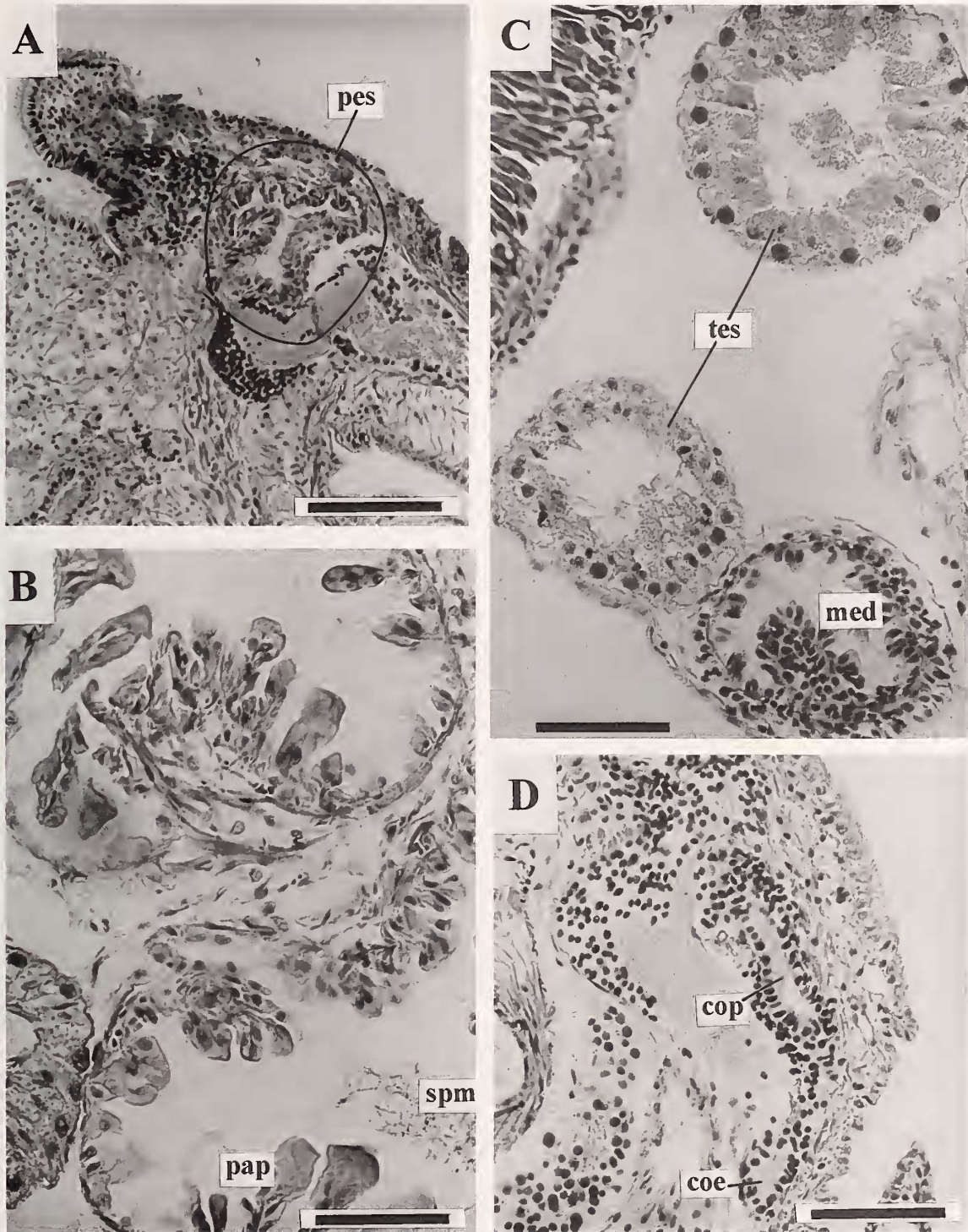
Figure 13.   Copulatory organ of *Acteocina atrata*. A. Cross-section of the penial sheath (3.2-mm specimen). B. Tangential section of medial region, containing sperm (3.1-mm specimen). C. Cross-section of medial region and terminal sac (2.0-mm specimen). D. Tangential section of developing copulatory organ, invagination stage (1.4-mm specimen). Scale bars = 50 μm (B–D); 100 μm (A). Key: coe = end of copulatory organ; cop = copulatory organ; med = medial region; pap = papillae; pes = penial sheath; spm = sperm; tes = terminal sac.

Tritoniidae], the anterior parts of the gonoduct appear as a single invagination of the body wall, and fuse with the rudiment of the posterior gonoduct directly posterior (Thompson, 1962); a sphincter or valve is present in this location in adults (Thompson, 1961). Pulmonates (and many opisthobranchs) are more complex as adults because of the independent development of male and female parts of the gonoduct, but appear to display similar development. Fraser (1946) found three ontogenetic components in the reproductive system of *Lymnaea stagnalis appressa* Say, 1821 [Pulmonata: Lymnaeidae], including two ectodermal components forming the male and female ducts, respectively.

Lemche (1956) included some discussion of gonoduct development in *Cylichna cylindracea* (Pennant, 1777) in the form of a description of the gonoduct in a single subadult specimen of unknown size. The reproductive anatomy of this specimen differs little from that of the adults examined. Glandular tissues are starting to differentiate, particularly in the posterior loop of the anterior gonoduct, the gametolytic gland (= spermatocyst of Lemche) and in the albumen gland. The mucus gland (= nidamental gland of Lemche) has not started to develop secretory tissues. *C. cylindracea* shows similar developmental characteristics to subadults of the two species studied here, but more data will be needed to compare other stages.

Caenogastropods, the sister group to heterobranchs (Ponder & Lindberg, 1997), also have two or more reproductive system components, but these differ from those of heterobranchs. The posterior gonoduct is very similar in the two groups. The heterobranch ampulla is functionally and histologically similar to the seminal vesicle in male caenogastropods, and could be homologous. Ponder & Lindberg (1997) reached a similar conclusion, however noted that the seminal vesicle is modified or lacking in many basal heterobranchs. The development of the copulatory organ from a separate *anlage* occurs in some heterobranchs, including the species examined here, similar to the independent initiation of development of the penis and its duct seen in some caenogastropods, e.g., *Littorina saxatilis* (Olivi, 1792) [Littorinidae], discussed by Guyomarc'h-Cousin (1976), and *Crepidula adunca* Sowerby, 1825 [Calyptraeidae], described by Moritz (1939). The differentiation of the anterior gonoduct differs most strongly between heterobranchs and caenogastropods. In heterobranchs, it develops from a single ectodermal *anlage,* unless it is connected to the copulatory organ, in which case the two portions develop separately and fuse (e.g., *Lymnaea*; Fraser, 1946). In caenogastropods, the same region can develop from two or more *anlagen,* especially if both ectodermal and mesodermal components are included, such as is the case in *Viviparus viviparus* Linnaeus, 1758 [Viviparidae] (see Drummond, 1902).

The location of the common genital opening in both species examined in this study shifts markedly during ontogeny, from an original medial position near the foregut and pericardium to a more lateral position on the right side of the gizzard. The change in location is related to the development of the vestibule, and could (like "detorsion" in these animals) be related to differential growth and lateral migration of the pallial cavity (Brace, 1977; Mikkelsen, 1996). It is tempting to suggest that the original position of the opening is primitive because it is similar to the location of the pallial gonoduct opening in caenogastropods. Insufficient data are available to address this issue, but if this is not related to an evolutionary change in position, then some other reason will be necessary to explain the shift.

Comparison of *Bulla striata* and *Acteocina atrata*

The adult reproductive anatomy of *Bulla striata* and *Acteocina atrata* differs most strikingly in the morphology and development of the copulatory organ. *B. striata* has a very complex copulatory organ, and its function, in light of the partially everted specimen discussed herein, is relatively clear. The copulatory organ of *A. atrata* appears relatively simple (although the epithelium of the medial section is unique), and the mechanism of its function is not obvious. Developmentally, the copulatory organs of the two species are similar only during the earliest stage, when they consist of undifferentiated invaginations. Otherwise, the only histological similarity between them is the eosinophilic glandular tissues in the ducts and terminal sac, and in the general morphology of the penial sheath. Ghiselin (1966) stated that opisthobranch prostatic glands can be recognized in part by their eosinophilic secretions (probably consisting mostly of protein), which is consistent with the tissues observed in these two species. It is reasonably assured that in *B. striata* and *A. atrata* these tissues are prostatic (without calling them prostatic glands) and are homologous, in spite of their gross morphological differences.

Another major difference between *Bulla striata* and *Acteocina atrata* is the relative involvement of the gonoduct in the mature glandular mass. In *B. striata,* the anterior gonoduct travels through the glandular mass and intercepts each gland separately at its base. In *A. atrata,* the gonoduct is entirely free of the three large glands on a single diverticulum [also noted in this genus (as *Cylichnella*) by Gosliner, 1979]. In the smallest specimen of *A. atrata* examined, however, the gonoduct does pass through the glandular mass.

The two species display different organogenetic timing (Table 1). In *Acteocina atrata,* different parts of the reproductive system develop more or less contemporaneously. This species appears to be protandric in the sense that it is anatomically able to function as a male before it can function as a hermaphrodite. This is in agreement with Gosliner (1994) who considered protandry the typical condition in opisthobranchs. Sperm production and

maturation of the copulatory organ begins before egg production and maturation of the glandular mass, perhaps because of the investment required by the egg's large size and nutrient load. Immature animals (i.e., the 2.0-mm specimen) also had small amounts of sperm in the anterior sperm-storage pouch. In *Bulla striata,* the copulatory organ matures much earlier than the glandular mass, but sperm production in the gonad does not appear to do so.

Within the series of sectioned *Bulla striata,* individuals from different populations appeared to develop at different rates. The two smallest specimens, from station FK-211, were at the same stage of organogenesis as individuals nearly twice as large from FK-163. This difference could be seasonal; FK-211 was made in April at the transition between cool spring and hot summer, while FK-163 was made in September in the hottest part of the summer.

The overall structure of the female glandular mass is similar in the two species, although not identical. The similar histology and early development of the albumen and mucus glands are striking, particularly in the nesting of the albumen gland within the 'U' of the mucus gland. This is also apparent in members of *Cylichna* (see Lemche, 1956). The membrane glands in *Bulla striata* and in *Acteocina atrata,* although differently shaped, appear homologous due to similar locations, structures, and staining properties. Ghiselin (1966) reported that nudibranch egg membranes stain less strongly with hematoxylin than do their mucus layers. Thus the mucus glands in sections might be expected to stain darker than membrane glands. They did not in this study, leaving some doubt in the application of the labels and function of these glands.

Of the two sperm sacs associated with the female glandular mass, the gametolytic gland is almost certainly homologous in the two species; however, it is interesting to note that the point of entry into their respective gonoducts is different. In juvenile *Bulla striata* and in *Acteocina atrata* of all sizes, the gametolytic gland duct enters the gonoduct anteriorly. During development in *B. striata,* however, the entrance of the duct is moved to the posterior vestibular wall.

Homology of the Cephalaspidean Gonoduct

Homology of the various components of the cephalaspidean reproductive tract relative to those of other gastropods is a clear priority in heterobranch phylogenetics, but is still elusive. Ghiselin (1966:fig. 1C) diagrammed the reproductive system of a hypothetical ancestral opisthobranch for comparison with idealized gonochoric prosobranchs as presented by Fretter & Graham (1994).

Ghiselin's (1966) hypothetical organization claimed the presence of a pallial prostate in the ancestral opisthobranch (undoubtedly in reference to the once-cephalaspidean *Acteon,* now considered a lower heterobranch; Mikkelsen, 1996). The so-called prostatic glands of the male portion of the reproductive tract are especially problematic for homologous considerations. Prostatic glands of caenogastropods (also of the former cephalaspideans *Acteon* and *Hydatina*; Mikkelsen, 1996) are pallial, along an internal spermiduct leading to the base of the penis, and there is often additional secretory epithelium in the penial duct. Penial and pallial prostates develop very differently, although both are ultimately derived from ectodermal tissues. Although the penial prostatic glands of *Bulla striata* and *Acteocina atrata* are probably homologous, their relationship to similarly labeled structures in other gastropods will require detailed study.

Hadfield & Switzer-Dunlap (1984:234) commented that the gonoduct of bulloideans is more similar to that of anaspideans than to that of philinoideans. This statement would be phylogenetically interesting to corroborate, in view of the close relationship of the cephalaspidean Bulloidea to the Anaspidea (Mikkelsen, 1996). However, comparison of the reproductive system of *Aplysia* (see Thompson & Bebbington, 1969) does not offer strong encouragement. Like most cephalaspideans, *Aplysia* has a proximal seminal receptacle, absent in both of the species examined herein. The distal sperm-storage pouch (near the common genital opening) in these two species is similar to a distal "seminal receptacle" in *Umbraculum* [Notaspidea] (Schmeckel, 1985:fig. 7), and a distal "exogenous sperm sac" in some members of Aglajidae [Cephalaspidea: Philinoidea] (Rudman, 1974). In these last two examples, all of the components of the female glandular mass join the gonoduct near the vestibule, as is true in *Acteocina atrata* studied here. This area can justifiably be called the "fertilization chamber" in such species.

As in *Acteocina, Bulla striata* examined here has a sperm-storage pouch near the common genital opening. The pouch in the largest specimen (16.5 mm) contained a small amount of embedded oriented sperm, but we have avoided the label "seminal receptacle" for reasons explained earlier. Whether these pouches are homologous with each other, with the similarly located seminal receptacles of *Umbraculum* or Aglajidae, or with the proximal receptacles of any of the caenogastropods, is unknown. Histological characterization of the tissue walls in the two species examined here (relative to the cellular definitions by Schmekel, 1971) was inconclusive. The pouch of *Acteocina atrata* is semiserial with the anterior gonoduct, differs histologically, and did not contain oriented sperm in any of the sectioned specimens. Gosliner (1979) found "semiserial or serial" to vary in several species of *Acteocina,* so this difference does not seem informative.

Gosliner (1994:320) claimed that a seminal receptacle was "clearly absent" in a number of opisthobranch taxa, including *Bulla.* This statement might be more reflective of position than of histological structure—*Bulla striata* has no pouch on the pallial gonoduct where the receptacle "should be" (i.e., between the ampulla and the female

glandular mass; Ghiselin, 1966; Gosliner, 1994). The presence of a seminal receptacle within the tissues of the female glandular mass was reported by Robles (1975) and Mikkelsen (1996); no such structures were observed in this study. So-called seminal receptacles, defined either by location or by the presence of oriented sperm, also bear homology questions in caenogastropods and neritopsines (Ponder & Lindberg, 1997).

Potential homology between the heterobranch gametolytic gland and the caenogastropod bursa copulatrix has been mentioned by several authors (e.g., Ghiselin, 1966; Hadfield & Switzer-Dunlap, 1984; Gosliner, 1994; Mikkelsen, 1996). Such a relationship is supported by this study by the initial location of the gametolytic gland duct at the common genital opening in *Bulla striata*, similar to the location of the bursa in caenogastropods.

Cephalaspideans are well known for depositing fertilized eggs in the laboratory, long after a mating event could have occurred, suggesting that they are well equipped for sperm storage. One specimen of *Bulla striata* examined here suggests a possible scenario. This adult (11.4 mm) contained sperm in the anterior gonoduct but none in the sperm-storage pouch. It is thus likely that the small sperm-storage pouch near the opening is not a bursa copulatrix, and that the anterior gonoduct itself is used for short-term storage of exosperm after mating. The sperm-storage pouch, by evidence of the oriented sperm in one large specimen, seems to be for long-term storage. But whether these are exosperm or endosperm is unknown.

The homologies of the female reproductive glands in heterobranchs and caenogastropods are unclear. Heterobranchs are thought to have three female glands, putatively responsible for producing the three layers of the egg coatings: albumen, membrane, and mucus, from egg to exterior (Ghiselin, 1966). Caenogastropods have two female glands, the albumen and capsule glands. [It must be stressed that although "egg capsules" and even "capsule glands" are occasionally described in opisthobranchs (e.g., Rudman, 1971; Robles, 1975; Mikkelsen & Mikkelsen, 1984), opisthobranch egg capsules are without doubt not chemically identical or homologous to the hardened external egg capsules common in caenogastropods.] The staining reactions of these can differ considerably from one investigation to another, in large part because researchers rarely use the same histological methods. Additionally, neogastropod reproductive glands often have many differently staining regions within the same mass of glandular tissue (Fretter, 1941; Houston, 1976). Albumen glands are present in both caenogastropods and heterobranchs, but whereas heterobranch albumen glands produce mostly neutral carbohydrates (Ghiselin, 1966), albumen of caenogastropods consists of proteins and mucopolysaccharides (Fretter & Graham, 1994). Thus analogous glands have different products and staining properties in addition to different morphologies, and their homologies across higher gastropod taxa are doubtful.

Functional Morphology

It is a well established fact that the male copulatory organ of cephalaspids (and most other opisthobranchs) is retractile (also called protrusible; Hadfield & Switzer-Dunlap, 1984; Gosliner, 1994; and others). However, we have little real evidence on how this organ everts to transfer sperm to the mating partner. Some data are available from Marcus (1974:figs. 63, 64), who figured the retracted and everted copulatory organ of *Scaphander darius* Marcus & Marcus, 1967. The fortuitous sectioning during this study of an adult *Bulla striata* with a partially everted copulatory organ, coupled with sections of the organ in full retraction, here provides some of the best functional data for cephalaspids.

The adult copulatory organ in *Bulla striata* was described briefly and figured by Marcus (1957:395, fig. 6). Those of *B. solida* Gmelin, 1791 (Marcus, 1976:fig. 10) and *B. gouldiana* (Marcus, 1961:fig. 4) are comparable. The specimens examined here agree reasonably well with previous interpretations, although some of the terminology (most of which implies function) is misleading. The longitudinally folded outermost duct, here called the penial sheath, is appropriately labeled once the penis has fully everted, although (contrary to Marcus, 1957) there is no permanent penial papilla in *B. striata*. Marcus (1957) also labeled the coiled ducts in *B. striata* the prostatic gland, and it is true that this portion has secretory epithelium and stores sperm. However, it has no subepithelial glandular tissue, and it could be argued that it is not a "gland" per se. The terminal bulb is similarly secretory and also appears capable of storing sperm. It could by this evidence be considered an expanded extension of the prostatic gland. To be conservative, however, we continue to label it separately here. It is consistently as wide as the double-walled region in adult *B. striata*, but is only a tiny terminal caecum in immature specimens. Tiny end bulbs were also described on the copulatory organs of *B. gouldiana* [see Bergh, 1901 (as *B. ampulla* var. *nebulosa* Gould in A. Adams, 1850); Marcus, 1961; Robles, 1975) and *B. solida* (see Marcus, 1976)]. Marcus (1961) claimed the end bulb was sometimes absent in *B. gouldiana*, which led Ghiselin (1966) to surmise that its full expression might only occur during breeding condition. It is equally possible that the diminutive bulbs described by earlier authors were all based on immature animals.

Penial eversion in *Bulla striata* is probably accomplished by a combination of muscular action and hydrostatic pressure—the longitudinal/circular muscles of the walls of the copulatory organ contract to force fluid (contained between the inner and outer ducts of the double-walled sections) outward. The laterally attached penial
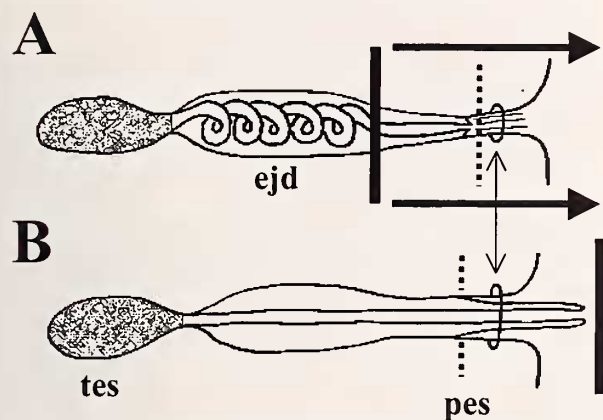
Figure 14. Diagrammatic copulatory organ of *Bulla striata*, showing retraction (A) and eversion (B). Key: ejd = ejaculatory duct; pes = penial sheath; tes = terminal sac; dashed line = base of everted penis; ring = diameter of penial sheath, showing expansion during penial eversion; solid line = penial tip.

retractor muscle(s) could also play a role. It is the longitudinally folded non-mucoidal penial extrovert that "turns inside out" to become the functional penis. This is similar to eversion in *Scaphander darius,* although the "turning" is one-sided, as evidenced from the figures of Marcus (1974:figs. 63, 64), resulting in a lateral open groove. In *Bulla,* the penial sheath expands to accommodate the everting structure (and might itself also evert), but it does not alone "serve as a penis" as explained by Hadfield & Switzer-Dunlap (1984:234). The "penis" of *B. striata* when fully everted has an outer wall composed of the "inside out" penial extrovert, and an inner duct of the uncoiled (prostatic) ejaculatory duct (Figure 14). The ultimate penial tip is the junction between these two sections.

The copulatory organ of *Acteocina atrata* differs markedly from that of *Bulla striata,* and cannot function in the same way. It does not have the double-walled, duct-within-a-duct construction, which would allow hydrostatic pressure to operate, nor does it have a coiled duct capable of extending. The structure as a whole is not as muscular as that of *B. striata,* but there are some longitudinal and circular muscles associated with the penial sheath and medial sections. It does have a longitudinally folded penial sheath, which appears capable of expanding when some part of the structure is everted. A similar configuration was figured by Marcus (1958a:fig. 32, as *Tornatina*) for *A. candei* (d'Orbigny, 1941) and by Marcus (1977:fig. 53) for *A. inculta* (Gould, 1855), albeit with slightly more definitive penial papillae. No penial papilla was confirmed in *A. atrata,* although its presence has been previously noted in this and other philinoideans (e.g., Marcus, 1958b, 1974, 1977; Mikkelsen, 1996). From our data and Marcus' (1958a; 1977) figures, it seems possible that the penial sheath "turns inside out"

to allow the medial section to push outward through its lumen. This is the mechanism supposed by Marcus (1958a). The medial section would thus be called the ejaculatory duct, although its unusual epithelium precludes homology with the ejaculatory duct of *Bulla.* It is also unknown whether or not the medial section also "turns inside out." A terminal sac is certainly present [contrary to Hadfield & Switzer-Dunlap (1984) who claimed that members of Philinoidea (including *Acteocina*) lack a spermatic bulb], although its function is uncertain. It is similarly extremely long in *A. eximia* (Baird, 1863) (Marcus, 1977:fig. 58). Further study of reproductive structure and function in Acteocinidae is warranted.

The routes of sperm and ova travelling through the mature gonoduct of the two species studied here are not completely clear; however, the morphological differences between the two species imply different pathways. Ghiselin (1966) noted that the products of the three female glands must be applied to exiting eggs in the order that they are found in the egg mass, i.e., albumen, membrane, mucus. This order was confirmed by the sequential intersections of the three glands with the pallial gonoduct in *Bulla striata.* The glands of *Acteocina atrata* have a common duct, so it is more difficult to interpret in this case. In *B. striata,* it appears likely that the albumen gland functions as a diverticulum because its lumen is quite small and is probably not capable of accommodating inward and outward tracts of ova. Robles (1975) found this to be the case in *B. gouldiana,* in which eggs passed through the membrane and mucus glands (but not through the albumen gland). Ghiselin (1966) noted that this had been also demonstrated in Anaspidea and Sacoglossa, the two closest sister groups of Cephalaspidea (Mikkelsen, 1996). The mucus gland of *B. striata,* on the other hand, has an extremely wide opening. The lumens of all three glands (albumen, mucus, membrane) are ciliated in both species. Schmekel (1985) noted that eggs pass through the membrane and mucus glands of all opisthobranch taxa thus far studied. Living animal studies, or techniques for preserving specimens in the act of mating and/or egg deposition (e.g., Robles, 1975) will be necessary to confirm the exact pathways.

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