6 Amphinomida/Sipuncula*

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6.1 Sipuncula

Introduction

Sipuncula display a unique combination of morphological, anatomical, and developmental traits. They are nonsegmented coelomates with a simple, sac-like trunk and a retractable introvert typically carrying an array of tentacles at its distal end (Fig. 6.1.1). While the monophyly of the Sipuncula is well established, their taxonomic rank is still debatable (see "Phylogeny and Taxonomy" section). Long considered a distinct phylum, the phylum status has recently been called into question by phylogenomic analyses which recognize Sipuncula as an early branch within the annelid radiation (e.g., Weigert *et al.* 2014, 2016). Two major monographs on Sipuncula have been published to date by Cutler (1994) and Stephen and Edmonds (1972). Whereas Stephen and Edmonds (1972) recognized 320 sipunculan species, Cutler (1994) synonymized many and reduced the number to roughly 150.

Several different Anglicized versions of the name Sipuncula have been in usage, including "sipunculoid" and "sipunculid," but we are following Stephen and Edmonds' (1972) compelling arguments for using the term *sipunculan*. Common names for sipunculans are "peanut worms" and, perhaps more appropriately, "star worms." Whereas the name peanut worms refers to the shape of some of these worms when the introvert is withdrawn into the trunk and the occasionally rough texture of the body wall, the name star worms is a literal translation from Japanese *hoshimushi* and refers to the tentacles radiating from the mouth in a star-like pattern (Ruppert *et al.* 2004, Nishikawa 2017).

Sipunculans have recently attracted increased attention in the scientific community as a key taxon to understanding the evolution of segmentation (or loss thereof) (Wanninger *et al.* 2005, Dordel *et al.* 2010) and a model taxon in the field of evolutionary developmental biology, or "evo-devo" (Schulze and Rice 2009, Boyle and Seaver 2010, Boyle and Rice 2014). They are also used as ecological indicator species (Bedini *et al.* 2004, Fonseca *et al.* 2006), as bait (Carvalho *et al.* 2013, Saito *et al.* 2014), as sources of food (Liu and Qiu 2016), and in pharmaceuticals (Fu *et al.* 2016).

Morphology

External morphology

Body regions. The two-partite body of a sipunculan worm consists of a nonsegmented trunk and a retractable introvert. The tip of the introvert carries the mouth, a presumed chemosensory nuchal organ, and an array of tentacles. Recurving, proteinaceous hooks are often present along the introvert, particularly in the distal section (Fig. 6.1.2). These can either be scattered or arranged in rings. In many sipunculan taxa, the structural details and arrangement of the introvert hooks are used as taxonomic characters. Hooks may be simple or ornamented with secondary tips as is often the case in species of *Phascolosoma* Leuckart, 1828 and *Aspidosiphon* Diesing, 1851. The posterior transition between the hook and the epidermis often carries distinctive basal processes. When viewed under light microscopy, the internal structure of the hooks can also be diagnostic for identification purposes (Fig. 6.1.2). The sipunculan body is covered by an epithelium overlain by a cuticle. The body surface may be smooth or form papillae of various types and sizes, which tend to be most prominent at the anterior and posterior ends of the trunk. The anus is typically located near the anterior end of the trunk on the dorsal side. Two nephridiopores are usually present at a similar height as the anus, but on the ventral side, although not always easily detectable externally.

Cuticle. The cuticle covers the outer body surfaces and can form specialized structures such as hooks, spines, scales, and platelets. The cuticle is collagenous and chitin-free (Voss-Foucart *et al.* 1977). Cuticular platelets can reach high densities and merge together. This is particularly noticeable in the Aspidosiphonidae Baird, 1868, which are characterized by hardened shields anterior to the anus (anal shields) and often at the posterior end of the body (caudal shields). In most Aspidosiphonidae, the anal shield is located dorsally and causes the introvert to emerge from the trunk at angle toward the ventral side or forms a calcareous cone covered by a layer of horny material (Rice 1969, Hughes 1974). *Cloeosiphon* Grube, 1868 has a unique and conspicuous anal shield surrounding the base of the introvert and composed of polygonal calcareous plates (Rice 1969).

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* **Note:** The authors of this chapter disagree with this classification based on recent phylogenomic analyses and regard it as very premature.
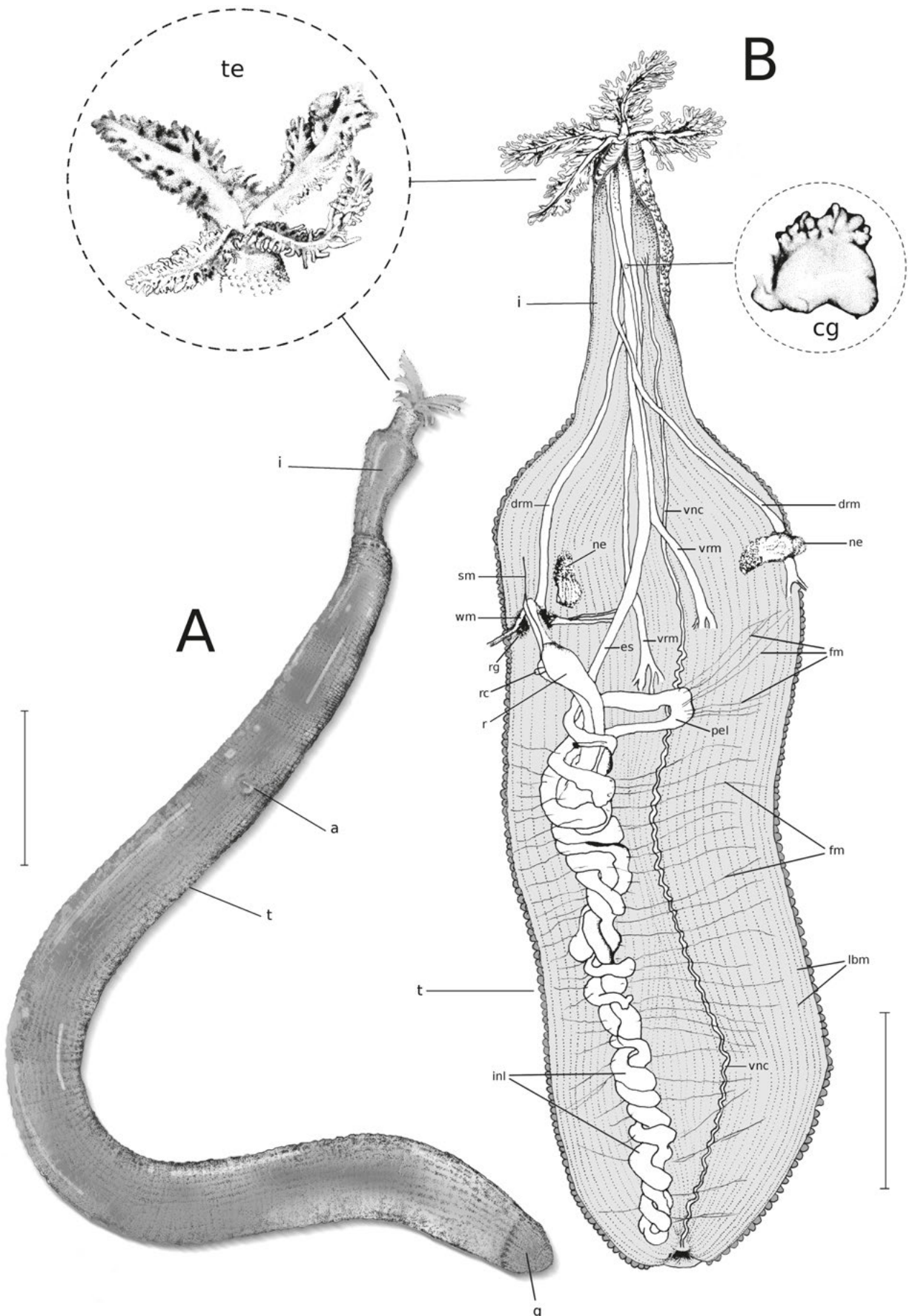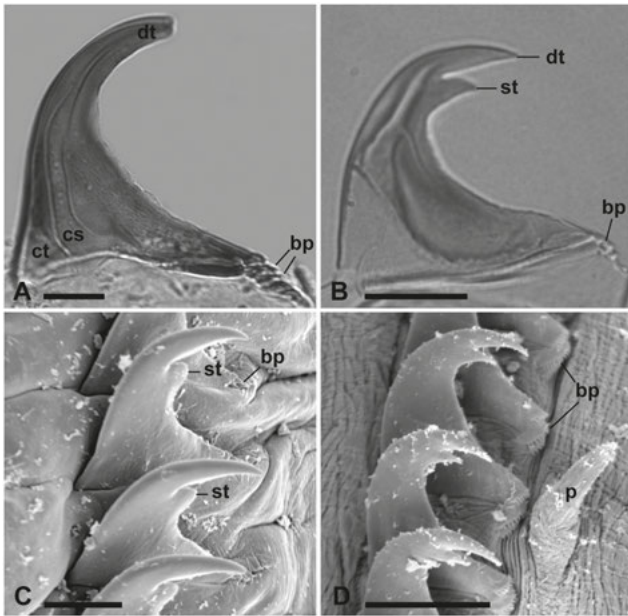
Fig. 6.1.2: Light (LM) and scanning electron (SEM) micrographs of sipunculan introvert hooks. LMs show diagnostic internal structures. A, *Phascolosoma agassizii*, LM (©A. Schulze). B, Compressed, bidentate hook of *Aspidosiphon steenstrupii*, LM (©A. Schulze). C, *Phascolosoma perlucens*, SEM (©A. Schulze). D, *Aspidosiphon fischeri*, SEM (©A. Schulze). bp, basal process; cs, clear streak; ct, clear triangle; dt, distal tip; st, secondary tooth; p, papilla. Scale bars: 20 μm.

Anatomy

Anatomically, the most distinctive characteristics are the coiled intestine, the introvert retractor muscles, and the nephridia (Fig. 6.1.1).

Epidermis and cuticle. The epidermis consists of a single layer of cuboidal cells connected to each other by different types of intercellular junctions (Rice 1993). In postmetamorphic juveniles and adults, the only ciliated epidermal cells are present in the mouth region and on the tentacles. The cilia function to transport food particles into the digestive tract. Many different types of epidermal glands exist in sipunculans, ranging from unicellular to multicellular (Cutler 1994). Epidermal glands are often closely associated with sensory cells forming complex epidermal organs (Andreae 1882, Åkesson 1958, Müller *et al.* 2015). The cuticle is secreted by epidermal supportive cells and covers the outer body surfaces. It is made up of layers of parallel collagen fibers and is free of chitin (Voss-Foucart *et al.* 1977). Its structure corresponds to that of other large polychaetes.

The epidermis is separated from the underlying dermis by a basal lamina. The dermis is formed by connective tissue consisting of collagenous fibers embedding various cell types such as fibrous cells, pigment cells, and amoebocytes (Cutler 1994).

Digestive system. The simple digestive system consists of the mouth, the esophagus, the intestine, a short rectum, and the anus. The mouth region is heavily ciliated (Rice 1993), receiving food particles from the ciliated grooves of the tentacles. The esophagus runs posteriorly from the mouth to the junction between the introvert and trunk. Usually, no discernable stomach is present, although stomach regions have been reported in a few species (Andrews 1890, Cutler 1994). In the trunk, the descending branch of the intestine runs toward the posterior end, then transitions into an ascending branch leading toward the anus. The descending and ascending branches of the intestine wind around each other in a double helix. The degree of coiling can vary from species to species. Whereas the coil is tightly wound in most species, in some cases, the coiling is irregular or loose (Cutler 1994). The rectum is surrounded by a set of wing muscles. A small rectal caecum is present in many species. In *Siphonosoma vastum* (Selenka and Bülow, 1883) and sometimes in *Aspidosiphon laevis* Quatrefages, 1865, multiple rectal caeca are present near the anus. The intestinal coil is fastened to the posterior end of the trunk by a spindle muscle, which also holds the coil together. In the genus *Onchnesoma* Koren and Danielssen, 1875, as well as several *Phascolion* Théel, 1875 species, the anus has shifted anteriorly onto the introvert. This is particularly pronounced in *Onchnesoma steenstrupii* Koren and Danielssen, 1875, where the anus actually lies in close proximity to the mouth, near the tip of the long introvert. The esophagus, the descending branch of the intestine, and the rectum are usually characterized by longitudinal grooves (Rice 1983). The ascending branch of the intestine has a single, ciliated groove that opens into the rectal diverticulum or caecum (Rice 1993).

◄ **Fig. 6.1.1:** Sipunculan morphology, exemplified by *Sipunculus phalloides*. A, External morphology (©M. Anacleto). B, Internal anatomy (©G.Y. Kawauchi and M. Anacleto). a, anus; cg, cerebral ganglion; drm, dorsal retractor muscle; es, esophagus; fm, fixing muscle; g, glans; i, introvert; inl, intestinal loop; lbm, longitudinal muscle band; ne, nephridia; pel, post-esophageal loop; r, rectum; rc, rectum caecum; rg, racemose gland; sm, spindle muscle; t, trunk; te, tentacle; vnc, ventral nerve cord; vrm, ventral retractor muscle; wm, wing muscle (according to Ditadi 1982). Scale bars: 1 cm.

Musculature. The introvert retractor muscles connect the tip of the introvert to the interior wall of the trunk. The number of introvert retractor muscles varies from one to four, with four being the most common and most likely ancestral condition (Schulze and Rice 2009). If four introvert retractors are present, they group into a dorsal and a ventral pair, with the ventral pair usually inserting onto the trunk wall posterior to the dorsal pair. The left and right muscle of each pair may be fused together to some degree, but separate insertion points are usually still present.

The body wall musculature consists of an outer layer of circular and an inner layer of longitudinal musculature, sometimes with oblique fibers between them. Both the longitudinal and circular muscles may form a smooth sheath or may be split into distinct, often anastomosing, bands. Other muscles, as mentioned earlier, include the wing muscles surrounding the rectum and anus and the spindle muscle fastening the intestinal coil to the posterior end of the trunk.

Nervous system and sensory organs. The main components of the central nervous system of Sipuncula are the dorsal brain (=cerebral ganglion), a pair of circumesophageal connectives, and a single, ventral nerve cord (Fig. 6.1.1 B). The brain is bilobed and is surrounded by a capsule of connective tissue (Gerould 1938, Åkesson 1958). It is located at the tip of the introvert, directly posterior to the tentacles, but is often withdrawn into a "cerebral pit" (Åkesson 1958). The circumesophageal connectives originate from the lateral margins of the cerebral ganglion. Another pair of nerves branches off from the anterior-lateral margin toward the nuchal organ (Åkesson 1958). At least two pairs of tentacular nerves originate from the circumesophageal connectives near their origin on the cerebral ganglion (Åkesson 1958). Several sensory structures are closely associated with the cerebral ganglion: the cerebral organ, the nuchal organ, and the ocular tubes. The cerebral organ lies at the anterior margin of the cerebral ganglion (Åkesson 1958, Purschke *et al.* 1997). It is nonciliated and contains sensory cells, but its function is unknown (Purschke *et al.* 1997). The nuchal organ is a ciliated patch dorsal to the cerebral organ, which often appears bilobed. Externally, the nuchal organ is surrounded by an incomplete circle of tentacles or it lies outside of the tentacular apparatus. It is richly innervated and consists of sensory cells, as well as ciliary and nonciliary supporting cells (Purschke *et al.* 1997). Sometimes, secretory cells have been reported as well (Åkesson 1958). The function of the nuchal organ is not fully understood but is presumed to be chemosensory

(Rice 1993, Cutler 1994, Purschke *et al.* 1997) and may form a functional unit with the cerebral organ (Åkesson 1958, Purschke *et al.* 1997). The ocular tubes are epidermal invaginations, which extend into the brain. Pigment cells and photoreceptor cells are usually present in the deeper parts of the ocular tube (Åkesson 1958, Rice 1993). Åkesson (1958) describes different levels of complexity in sipunculan eyespots, ranging from simple invaginations to a more complex organization with a refractive body.

The single ventral nerve cord shows no evidence of segmental ganglia in juveniles and adults. It is more or less circular in cross-section and its length and shape can change slightly in different body regions (Åkesson 1958). In *Sipunculus nudus* Linnaeus, 1766, the terminal end of the nerve cord is thickened, and this region has been referred to as a "terminal ganglion" (Åkesson 1958). Generally, the nerve cord is not connected to the ventral body wall, although Åkesson (1958) reports that in the anterior introvert of *Phascolosoma* Leuckart, *Aspidosiphon* Diesing, 1828, and *Siphonosoma* Spengel, 1912, it is connected to the ventral body wall by a mesentery. In other species, only the lateral nerves emerging from the ventral nerve cord connect it to the body wall. The lateral nerves branch off without any regular or opposing pattern. The ventral nerve cord and the sections of the lateral nerves are covered by the epithelial lining of the coelomic cavity (Åkesson 1958).

Body cavity and nephridia. The coelomic spaces in sipunculans fulfill a variety of functions, including hydrostatic support, storage of gametes, excretion, gas exchange, and circulation. Separate circulatory and gas exchange organs are lacking. The large primary coelom fills most of the sipunculan body (Fig. 6.1.1). The smaller tentacular system supplies the tentacles and extends into one or two elongate, blind-ending tubes known as compensation sacs or contractile vessels, which extend along the esophagus. In some cases, the surface of the contractile vessel forms finger or hair-like extensions known as contractile vessel villi. In addition to the primary and tentacular coelom, members of the genera *Sipunculus* Linnaeus, 1766, *Xenosiphon* Fischer, 1947, and *Siphonomecus* Fisher, 1947 have coelomic canals running longitudinally through the dermis layer, apparently facilitating gas exchange over the body surface.

The primary body coelom contains several different types of coelomocytes (Rice 1993). Hemocytes, which carry the respiratory pigment hemerythrin, are the most abundant. Multicellular complexes known as "urns" are unique and distinctive in the coelom of sipunculans. They are ciliated structures that are either attached to

the peritoneum or float freely in the coelomic fluid. Free urns are only known in *Sipunculus* Linnaeus, 1766 and *Phascolosoma* Leuckart, 1828. Urns appear to play a role in waste removal and immune response, as debris and bacteria have been observed to collect in their cilia and mucus (Matozzo *et al.* 2001). Apart from hemocytes and urns, various types of granular and agranular amoeboid cells of uncertain function may be present in the coelom (Rice 1993).

The nephridial system usually includes two elongate sacs located on the anterior ventral side of the trunk, each with its own nephridiopore and a ciliated nephrostome anterior to the nephridiopore. Members of the genera *Phascolion* Théel, 1875 and *Onchnesoma* Koren & Danielssen, 1876 only have a single nephridium. The anterior part of the nephridial sacs can be enlarged to form a bladder-like structure. The nephridial system accomplishes osmoregulation and excretion, and the sacs also temporarily store mature gametes before release. The peritoneal lining of the nephridial sacs includes podocytes, suggesting that ultrafiltration occurs from the primary coelomic cavity into the nephridial sac. The primary urine would then be modified by the epithelial cells that form the inner lining of the nephridial sac before release of the final urine through the nephridiopore.

Reproduction and development

Historical Perspective

Early development in Sipuncula exemplifies the quartet spiral cleavage program observed within several clades of the Spiralia (Lophotrochozoa). Multiple features of the spiralian developmental program are considered highly conserved (Freeman and Lundelius 1992, Henry and Martindale 1999), including a relatively stereotypic pattern of early cleavage symmetries seen in mollusks, nemerteans, polyclad turbellarian flatworms, and annelids, including polychaetes and oligochaetes and, more recently, siboglinids, echiurans, and sipunculans (Struck *et al.* 2011, Weigert *et al.* 2014). Early studies by Selenka (1875) and Hatschek (1883) revealed morphological characteristics of sipunculan development that were distinct from other spiralians, such as the metatroch, used exclusively for locomotion, a terminal organ, and formation of a pelagosphera larva, which is unique among all metazoan larval types. Gerould (1906) produced the most comprehensive study of development with embryos from two sipunculan species including maturation of the egg, external fertilization, cleavage and gastrulation, and larval morphogenesis. Subsequent workers began to characterize the details of gamete formation and morphology, spawning and fertilization, embryogenesis, life history patterns, and metamorphic transitions across a diversity of sipunculan species.

Gametogenesis

Reproductive organs and gametes. Within Sipuncula, there is no evidence of sexual dimorphism and reproduction typically involves dioecious individuals, but there are four known exceptions. One species, *Nephasoma minutum* (Keferstein, 1862), is a protandrous hermaphrodite (Paul 1910, Gibbs 1975); *Themiste lageniformis* (Baird, 1868) can reproduce through facultative parthenogenesis (Pilger 1987); *Aspidosiphon elegans* (Chamisso and Eysenhardt, 1821) can reproduce asexually through budding (Rice 1970); and *Sipunculus robustus* Keferstein, 1865 can reproduce by transverse fission and lateral budding (Rajulu and Krishnan 1969). Male and female gonads are observed as small transient strips or transverse fringes of tissue within a peritoneum on the coelomic wall located posterior to the individual bases of ventral introvert retractor muscles (Paul 1910, Åkesson 1958, Rice 1974, Cutler 1994). Oogonia within ovaries vary in size, exhibiting a sequence of larger cells and more advanced stages of the first meiotic prophase when observed from proximal to distal along the length of the ovary (Gonse 1956a, Rice 1974). As new oogonia divide by mitosis within proximal regions of the gonad, oocytes may extend into the coelom (Gonse 1956b, Rice 1974). When oocytes are shed from the ovary into the coelom, they undergo maturation, growth, and morphological changes in shape that are characteristic for their particular species (Rice 1993). Across species, the extent and sizes of ovaries vary throughout the year relative to spawning season, as do the sizes and numbers of free-floating oocytes within the coelom, although there are typically some eggs within the coelom during the entire year with higher numbers of mature eggs present just prior to spawning (Gonse 1956b, Åkesson 1958, Rice 1966, 1974, 1989, Sawada *et al.* 1968, Gibbs 1975, Pilger 1987). Within Sipuncula, coelomic oocytes also vary in shape, from irregular to spherical or ovoid, and may or may not have follicle cells associated with the egg envelope during growth and maturation within the coelom.

As with oogenesis, spermatogonia differentiate into spermatocytes within the male gonad before release into the coelom. Rice (1974) noted that in at least three species, clumps of spermatocytes exit or "break off from the testis" into the coelom, followed by growth and maturation into spermatids with tails. Based on observations in *Phascolion cryptum* Hendrix, 1975, spermatocytes develop synchronously within the clumps or

"clusters," with their anterior ends connected by intercellular bridges and their tails extending outward to form a morula (Rice 1974, Reunov and Rice 1993). When differentiated, this "primitive" type of spermatozoan of sipunculans consists of the head with a large spherical nucleus surmounted with a rounded or cap-like acrosome, a midpiece with four to five mitochondria, and the standard (9 + 2) arrangement of microtubules comprising the axoneme of an elongate flagellum, or tail (Franzén 1956, 1977, Gibbs 1975). In *Thysanocardia nigra* (Ikeda, 1904), *Themiste pyroides* (Chamberlain, 1920), and *Themiste alutacea* (Grube & Oersted, 1858), free sperm are released from the morula into the coelom when the spermatids have undergone differentiation and may be found within the coelom throughout the year. In *Phascolosoma agassizii* Keferstein, 1866, it is not known how or when differentiated sperm are released within the coelom (Rice 1974). However, sperm flagella are not always active in the coelom prior to entering the nephridia from which individual sperm are spawned into seawater and subsequently become motile (Baltzer 1931, Åkesson 1958, Rice 1974, 1983, Gibbs 1975).

Fertilization mechanisms

Sperm and eggs are released into seawater through broadcast spawning, which is facilitated by extension of the body and elevation of nephridiopores above sediments, crevices or burrows in rubble, the apertures of gastropod shells, and other substrates in which adult worms are found. Although the process of oocyte maturation begins within the coelom, breakdown of the germinal vesicle may not occur until egg cells have entered a nephridium, or until after they exit nephridiopores during spawning events (Gibbs 1975, Rice 1988, 1993). Nephridial uptake of mature oocytes is thought to be a selective process, with the ciliated nephrostome actively sorting large oocytes from immature oocytes and coelomocytes prior to storage and spawning. Mechanisms for the regulatory sorting of mature gametes have been proposed (Gerould 1906, Åkesson 1958, Rice 1975b), and the overall process, including unanswered questions of prespawning mechanisms, has been addressed (Rice 1974, 1989). When released, oocytes are typically in their first meiotic metaphase and maturation is completed in seawater following sperm penetration and the release of two polar bodies, with notable exceptions of hermaphroditism (Gibbs 1975) and parthenogenesis (Pilger 1987). Knowledge of species-specific spawning periods is critical when planning for studies of sipunculan development, as there is notable variation in both the period and the number of periods when mature eggs are available for spawning in the laboratory.

The mature multilayered egg envelope typical of most sipunculan species consists of three discernible layers (outer, middle, and inner) when imaged with a transmission electron microscope (Sawada *et al.* 1968, Rice 1993). Microvilli extend from cortical cytoplasm to the surface of the egg within pore canals, passing through all three layers of the envelope (Sawada *et al.* 1968, Rice 1975b). Cytochemical analyses of the egg envelope indicate that the inner layer contains mucoprotein, the middle layer is primarily protein, and the outer layer is an acid mucopolysacharide (Gonse 1956b, Rice 1974, 1975b). Sipunculan egg envelopes vary in thickness from 2 µm in *Golfingia margaritacea* (Sars, 1851) to 10 µm in *Phascolosoma agassizii* (see Sawada *et al.* 1968, Rice 1974). The variation in thickness of these envelopes reflects both the composition and geometry of egg shapes that range from spherical to lens shaped or ovoid across the clade (Fig. 6.1.3). For review and appreciation of the diversity of egg size, shape, and dimension, see Rice (1989, Table 1). During fertilization, species-specific sperm initially attach to the outer egg envelope by an acrosomal filament and then digest a canal through the envelope, leaving an entry hole that is visible for several days following a fertilization event. For eggs that have a jelly coat surrounding the envelope, the acrosomal filaments of sperm can be extensive, with some measuring up to 50 µm in length in order to reach the envelope surface (Rice 1966, 1975b). Chemical analyses of gamete recognition proteins or envelope digestion by acrosomal proteins and associated enzymes have not been performed for any sipunculan species. Once inside, the male pronucleus increases in size and migrates toward the center of the egg. The female pronucleus is located toward the animal pole in spawned eggs. Meiotic divisions reduce the diploid number of chromosomes in the egg from 20 to 10 and generate two or three haploid polar bodies (Fig. 6.1.3 B, C, F) that are extruded outside the egg envelope at the animal pole to complete the process of egg maturation (Gerould 1906, Rice 1975b, 1988, 1989). Following meiosis and polar body release, male and female pronuclei fuse in the region between the center of the egg and the animal pole to form the zygotic nucleus, followed by the formation of the cleavage asters, mitosis, and cytokinesis of the zygote.

Development. In all sipunculan species observed, division of the zygote exhibits an unequal, holoblastic, spiral cleavage program of early development (Selenka 1875, Gerould 1906, Åkesson 1958, 1961, Rice 1967, 1975a, Boyle and Seaver 2010). The alternating orientation of cleavage divisions and resulting topology of embryonic blastomeres around the egg axis during embryogenesis
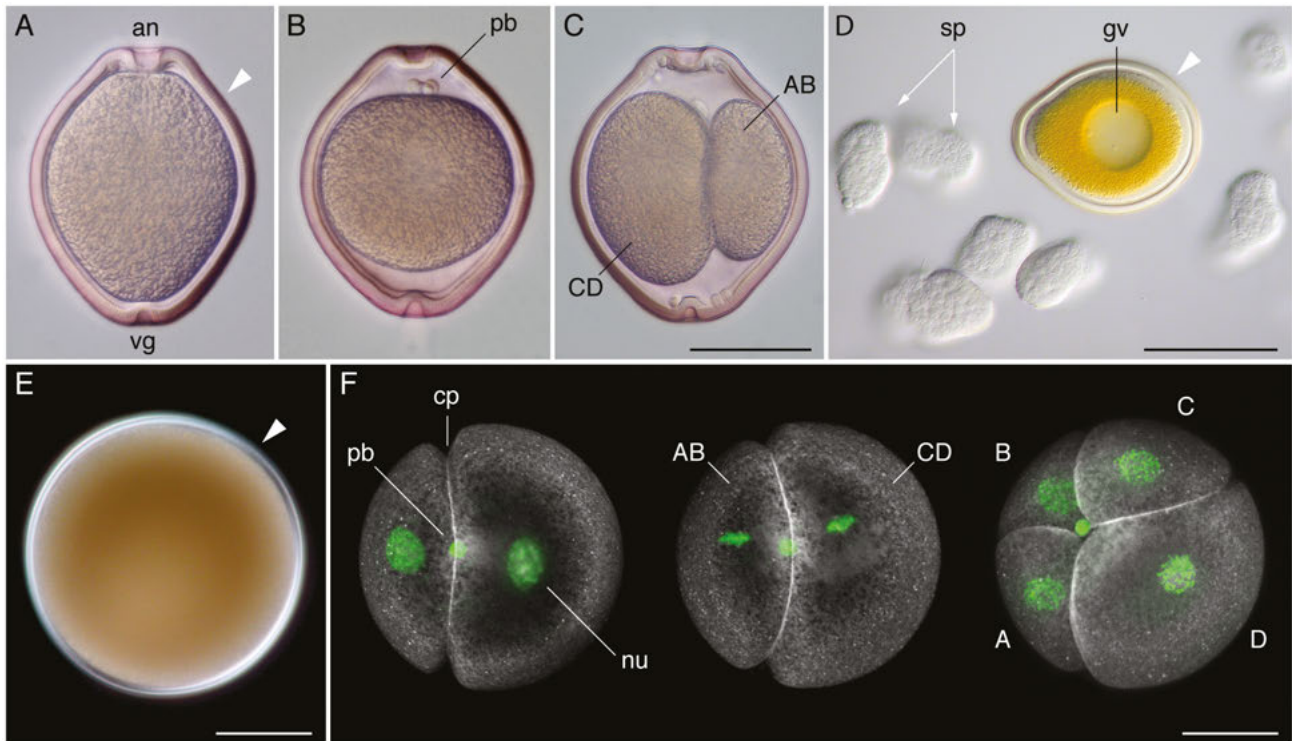
Fig. 6.1.3: Egg morphology and cleavage symmetry in early stages of sipunculan development. A–C, *Phascolosoma perlucens*. A, Ovoid oocyte with a thick egg envelope (arrowhead) and distinct animal and vegetal pole morphologies (©M.J. Boyle). B, Fertilized egg internally rounded up with three polar bodies extruded at the animal pole (©M.J. Boyle). C, First mitotic division showing unequal spiral cleavage into CD and AB blastomeres. Note the asymmetry and relative size differences of the blastomeres (©M.J. Boyle). D, *Phascolosoma* sp. Ovoid coelomic oocyte with large germinal vesicle. Clusters of coelomic spermatocytes were added to oocytes on a microscope slide (©M.J. Boyle). E, F, *Themiste lageniformis*. E, Spherical oocyte with egg envelope (arrowhead) (©M.J. Boyle). F, Confocal laser scanning micrographs of first and second mitotic divisions. Green, DNA; grayscale, cytoskeleton; egg envelope is not visible in these micrographs. From left to right: first mitotic division with unequal cleavage; nuclear position shift of metaphase chromosomes prior to second cleavage of AB and CD blastomeres; animal pole view of second mitotic division with unequal cleavage most notable between the C and D blastomeres (©M.J. Boyle). Abbreviations: an, animal pole; cp, cleavage plane; gv, germinal vesicle; nu, nucleus; pb, polar body; sp, spermatocytes; vg, vegetal pole. All scale bars = 50 μm.

resemble the quartet pattern observed in polyclad turbellarians, most mollusks, and the annelids (including echiurans) (Costello and Henley 1976). As with these other taxa, quartet spiral cleavage in sipunculans is most obvious during the third round of cleavage divisions, when each of four macromeres divides to produce one of four animal blastomeres, collectively referred to as the first quartet of micromeres. However, unlike annelids and mollusks, each of which exhibits both equal and unequal blastomere divisions within their clades, and the nemerteans and echiurans with predominantly equal cleavage, embryogenesis in Sipuncula (Fig. 6.1.3 C, F) proceeds exclusively through a series of unequal cleavage divisions (Boyle and Rice 2014). Another distinct feature of early sipunculan development includes the production of a first quartet of micromeres that are equal to or larger in size than their respective parent macromeres in the A, B, and C quadrants of the eight-cell embryo,

which is not seen in the D-quadrant (Rice 1985b). This condition is found in nemerteans, although only in a few polychaete species, and is not observed in mollusks or polyclads (Boyer *et al.* 1998, Henry and Martindale 1999, Boyle and Rice 2014). In Sipuncula, these larger first quartet micromeres contain relatively large volumes of yolk granules that will subsequently provide nutritional resources to developing larvae or juvenile worms (Gerould 1906). Daughters of the first quartet (1a–1d) will give rise to the large "girdle" of prototroch cells (Fig. 6.1.4 C–E) in sipunculan trochophore larvae (Åkesson 1958, Rice 1975a, 1988). In indirect developers, the blastula is ciliated and motile within the water column and may include an apical tuft of cilia (Fig. 6.1.2 C). Gastrulation is typically achieved by epiboly of animal micromeres and, in some species, also by the invagination of vegetal macromeres and micromeres that generate endodermal and mesodermal precursor cells, respectively. In
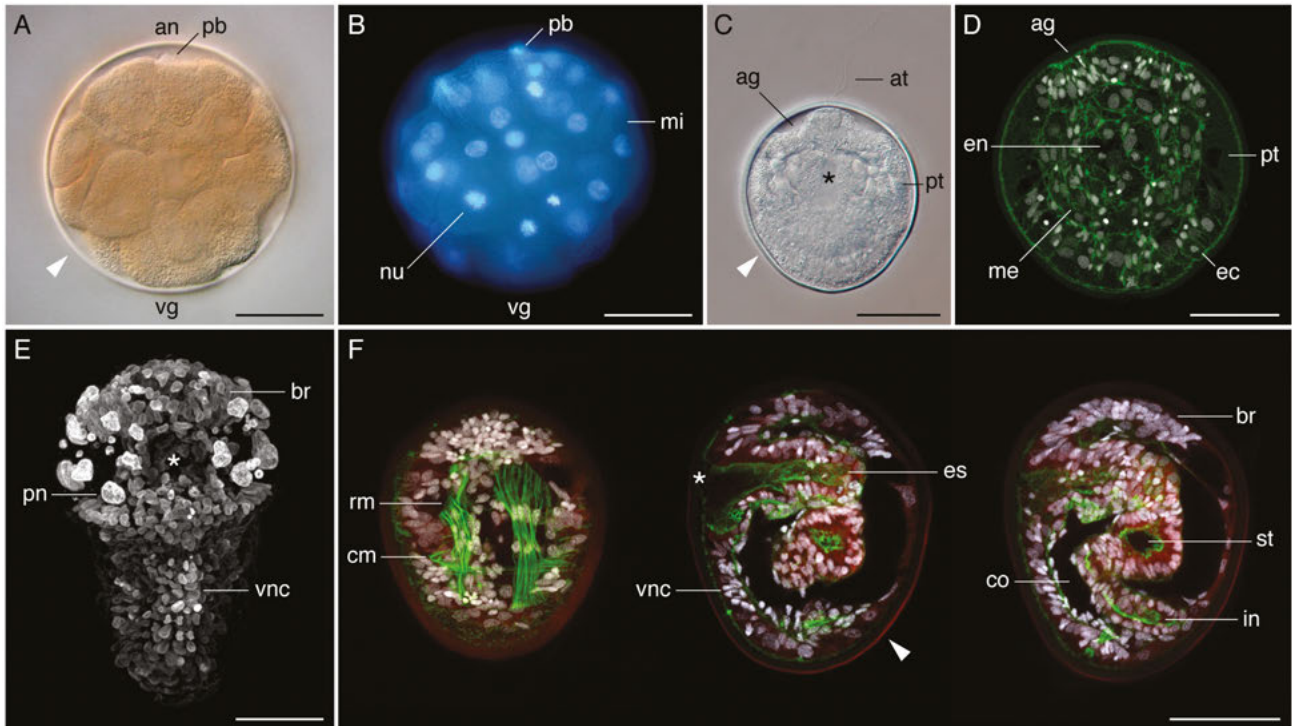
Fig. 6.1.4: Common characters of development in lecithotrophic and planktotrophic sipunculans. A, B, *Themiste lageniformis*. A, Gastrula stage embryo with an egg envelope (arrowhead) surrounding blastomeres with high yolk content, a polar body, and macromeres extended at the vegetal pole (©M.J. Boyle). B, Same embryo as in A showing DNA in cell nuclei (nu), including large pre-trochal micromeres in the episphere (©M.J. Boyle). C, *Nephasoma pellucidum*. Trochophore larva with apical tuft passing through the egg envelope (arrowhead) and apical grooves at the anterior end, with large prototrochal micromeres and stomodeum (asterisk). D, *Phascolion cryptum* (©M.J. Boyle); confocal laser scanning micrograph (CLSM); grayscale, DNA; green, cytoskeleton. The view is through a central z-stack region of the embryo showing germ layers, apical grooves, and large prototrochal micromeres (nonciliated) in direct development (©M.J. Boyle). E, *Themiste lageniformis*. CLSM of hatching cone-shaped stage with conspicuously large prototrochal nuclei (pn) surrounding the stomodeum. Posterior end shows a developing ventral nerve cord (©M.J. Boyle). F, *Nephasoma pellucidum*. CLSM; grayscale, DNA; green, F-actin; red, acetylated tubulin-like immunoreactivity. All views are lateral with anterior to the top and ventral to the left. Series of three z-stacks moving deeper through the embryo from left to right, showing circular and retractor muscles, ventral nerve cord, brain, coelom, stomach, and intestine, all within the egg envelope (arrowhead), which stretches during metamorphosis of the trochophore larva (see trochophore in C) (©M.J. Boyle). ag, apical groove; an, animal; at, apical tuft; br, brain; cm, circular muscle; co, coelom; ec, ectoderm; en, endoderm; es, esophagus; in, intestine; me, mesoderm; mi, micromere; nu, nucleus; pb, polar body; pn, prototrochal nucleus; pt, prototroch cell; rm, retractor muscle; st, stomach; vg, vegetal; vnc, ventral nerve cord. An asterisk marks the position of the stomodeum. All scale bars = 50 µm

Phascolosoma species that have been studied, invagination produces a narrow archenteron within their embryos, as also found in *Sipunculus nudus* (Hatschek 1883, Rice 1988). On the anterior end of all sipunculan embryos, there is an apical plate of ectoderm from which marginal cells ingress internally to form an apical groove around the anterior-most plate of cells (Fig. 6.1.4 C, D). The ingressing cells are a source of ectomesoderm that gives rise to the two sets of ventral and dorsal introvert retractor muscles characteristic of all sipunculan juvenile worms (Gerould 1906). These cells are thought to also derive from first quartet micromeres and likely represent a source of ectomesoderm unique to sipunculans and that has not been designated as ectomesoderm in cell-lineage or cell-fate map studies

of mollusks, polychaete annelids, nemerteans, or polyclads (Boyer *et al.* 1998, Maslakova *et al.* 2004, Hejnol *et al.* 2007, Meyer and Seaver 2010). Mesodermal bands appear to derive from endomesoderm precursor cells of the D-quadrant, and the macromere lineages of the A–D quadrants contribute to the intestine, both of which are typical developmental patterns observed throughout most spiralian groups (Gerould 1906). Notably, a second source of ectomesoderm contributes to circular muscles (Gerould 1906). This second ectomesoderm is posterior to the level of oral ectoderm and is on midlateral sides of developing trochophores. Considerable cell proliferation is observed in this region, and it is thought to act as a main center for longitudinal growth, and thus, there is no prominent region of terminal growth in Sipuncula

that is analogous to the posterior growth zone (PGZ) facilitating prepygidial segment formation among polychaete annelids (Åkesson 1958, Wanninger *et al.* 2005, Boyle and Rice 2014). For a more comprehensive treatment of early sipunculan development, the following resources should be reviewed: Åkesson (1958, 1961), Boyle and Rice (2014), Boyle and Seaver (2010), Rice (1967, 1973, 1975a,b, 1989).

Larval forms and metamorphosis. Within Sipuncula, development proceeds through four recognized life history pattern categories: (I) direct development from a fertilized egg to a crawling juvenile worm; (II) indirect development with a trochophore larva; (III) indirect development with a trochophore larva, followed by a lecithotrophic pelagosphera larva; and (IV) indirect development with a trochophore larva followed by a planktotrophic pelagosphera larva (Table 1 life histories). All sipunculan trochophores are lecithotrophic (nonfeeding). These life history patterns are distinct from one another in their species-specific developmental and ecological characteristics (Table 2). With the exception of category II, trochophore larvae undergo a metamorphosis to the pelagosphera, which then undergoes a second metamorphosis

Tab. 6.1: Developmental life history patterns in the Sipuncula

DIRECT DEVELOPMENT *lecithotrophic*
  (I) Egg ⟶ Worm

INDIRECT DEVELOPMENT *lecithotrophic*
  (II) Egg ⟶ Trochophore ⟶ Worm
 (III) Egg ⟶ Trochophore ⟶ Pelagosphera ⟶ Worm

INDIRECT DEVELOPMENT *lecithotrophic/planktotrophic*
 (IV) Egg ⟶ Trochophore *lecitho.* ⟶ Pelagosphera *plankto.* ⟶ Worm

Table 1 is from Boyle and Rice (in press), Courtesy Smithsonian Institution.

Tab. 6.2: Developmental characteristics of species-specific life history patterns in the Sipuncula

	Direct development (I) *Phascolion cryptum*	**Indirect lecithotrophy (III)** *Themiste alutacea*	**Indirect planktotrophy (IV)** *Nephasoma pellucidum*
Egg size (dia)	136 μm[1]	138 μm[1]	105 μm[2]
8-cell blastomeres (A,B,C quadrants)	micromeres > macromeres[1]	micromeres > macromeres	micromeres = macromeres[3]
Yolk reserves	high, cellular and coelomic	high, cellular and coelomic	moderate, primarily cellular
Prototroch cells	large, non-ciliated	large, ciliated	small, ciliated
Trochophore larva	no trochophore	present, ~20 hpf	present, ~48 hpf[3]
Apical tuft	absent	present	present
Circular muscles ~30 hpf	distinct circular muscle bands	non-distinct circular muscle bands	circular muscle bands not detected
Retractor muscles ~30 hpf	distinct ventral and dorsal fibers	diffuse ventral and dorsal fibers	retractor muscles not detected
Pelagosphera larva with metatroch	no pelagosphera, crawling worm	lecithotrophic, ~30 hpf[1] crawling-swimming larva	planktotrophic, ~72 hpf[1] swimming larva
Terminal organ	absent	absent[1]	present[3]
Circular muscles ~3.0 dpf	thick bands of A-P	thick bands of A-P	thin bands at anterior
Retractor muscles ~3.0 dpf	extend length of A-P	extend length of A-P	extend 2/3 of A-P additonal musculature
Functional gut	one week[1]	two weeks[1]	3 days[3]
Juvenile worm	one week[1]	four weeks[1]	~ six weeks[3]

1. Rice 1975a; 2. Rice 1975b; 3. Schulze and Rice 2009. Referenced and non-referenced characteristics are from laboratory observations.
Abbreviations: dia, diameter; hpf, hours post fertilization; dpf, days post fertilization; A-P, anterior-posterior axis.

Table 2 is from Boyle and Rice (in press), Courtesy Smithsonian Intstitution.
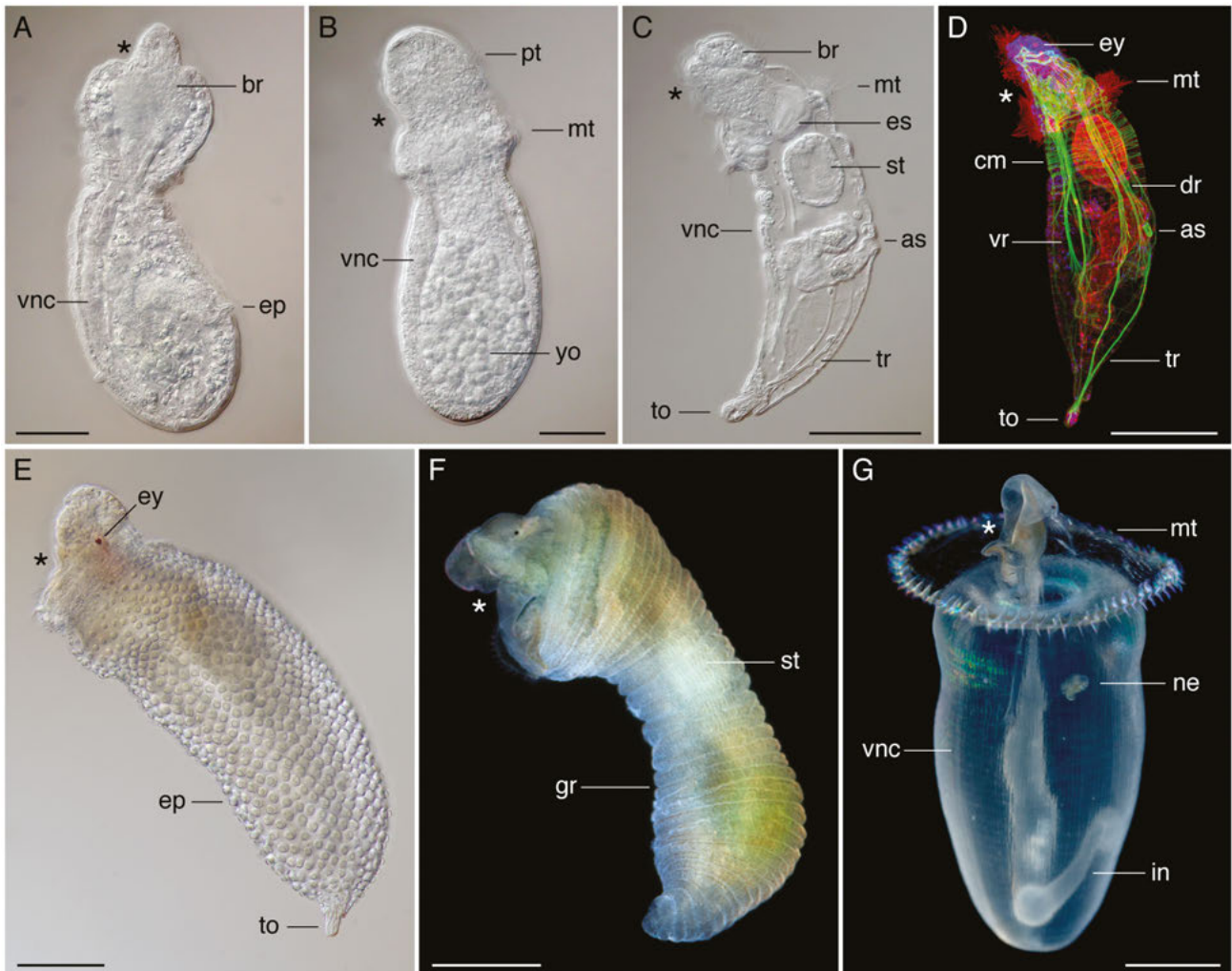
Fig. 6.1.5: Morphological characters among the predominant dispersal stages of sipunculans. A–C, Differential interference contrast (DIC) micrographs of each specimen after 3 days of development. A, *Phascolion cryptum*. Direct development showing a crawling vermiform stage (©M.J. Boyle). B, *Themiste alutacea*. Indirect development showing a lecithotrophic pelagosphera larva (©M.J. Boyle). C, *Nephasoma pellucidum*. Indirect development showing a planktotrophic pelagosphera larva (©M.J. Boyle). D, *Nephasoma pellucidum*. CLSM of a 4-day pelagosphera larva; green, F-actin; red, acetylated tubulin-like immunoreactivity; blue, DNA. Labeling reveals circular and retractor muscle pairs, eyes, metatrochal cilia, functional digestive system, and the terminal organ system with a pair of retractor muscles and adhesive organ (©M.J. Boyle). E, *Phascolosoma* sp. white papillated pelagosphera larva from the Caribbean of Panama, scale bar = 100 μm (©M.J. Boyle). F, *Siphonosoma* sp. transverse groove pelagosphera larva from the Florida Current of the Gulf Stream, scale bar = 200 μm (©M.J. Boyle). G, *Sipunculus* sp. large, smooth transparent pelagosphera larva from Florida Current of the Gulf Stream, scale bar = 400 μm (©M.J. Boyle). The larger pelagosphera larvae in F and G are considered teleplanic larvae that may spend weeks to months in the open ocean. All specimens (A–G) are oriented anterior to the top and ventral to the left side. as, anus; br, brain; cm, circular muscle; dr, dorsal retractor; ep, epidermal papilla; ey, eye; es, esophagus; gr, grooves; in, intestine; mt, metatroch; ne, nephridia; pt, prototroch; st, stomach; to, terminal organ; tr, terminal retractor; vnc, ventral nerve cord; vr, ventral retractor; yo, yolk. An asterisk marks the position of the stomodeum or mouth. Scale bars in A–D = 50 μm. A–C, From Boyle and Rice (in press), courtesy of the Smithsonian Institution.

to a juvenile sipunculan. Both larval forms are distinct from one another, and the pelagosphera is unique within Metazoa. Specific characteristics of the sipunculan pelagosphera (Fig. 6.1.5) include two pairs of internal retractor muscles, a postoral metatrochal band of cilia used exclusively for locomotion, a terminal organ with distinct retractors and adhesive properties, and a U-shaped

digestive system with descending and ascending regions of the intestine (Rice 1976, 1985b, Ruppert and Rice 1983, Boyle and Rice 2014). There have been a number of studies on sipunculan metamorphosis, including histological and behavioral descriptions, as well as attempts to describe culture and follow the development of the long-lived, teleplanic pelagosphera larvae (Fig. 6.1.5 F, G) from the

open ocean (Hall and Scheltema 1975, Rice 1981, Scheltema and Rice 1990). Additionally, several studies have focused specifically on the development of the sipunculan musculature (Wanninger *et al.* 2005, Schulze and Rice 2009, Kristof *et al.* 2011), digestive system (Boyle and Seaver 2010), nervous system (Åkesson 1958, Wanninger *et al.* 2005, Kristof *et al.* 2008), and life history evolution (Rice 1985b, Boyle and Rice in press, Schulze *et al.* in press). Regarding myogenesis, it was suggested that circular ring-like muscle bands develop synchronously along the anterior-posterior axis, and there is no evidence of a PGZ during elongation of the larvae of *Phascolion strombus* (Montagu, 1804) (see Wanninger *et al.* 2005). Furthermore, Schulze and Rice (2009) suggest that although the final number of retractor muscles may vary in adult worms, four retractors are present during the development of species with contrasting life histories and therefore likely represent the ancestral condition. The first gene expression studies for a member of Sipuncula showed that gut-specific transcription factors, known to be conserved in patterning and specification of the digestive system across metazoans (Boyle *et al.* 2014), are expressed subregionally along the gut during development in *T. lageniformis* and *P. cryptum* (Boyle and Seaver 2010, Boyle and Rice 2014). This work should be followed up with experiments characterizing the expression patterns of additional "gut-specific" genes to evaluate similarities and differences between sipunculans and other spiralian models during gut formation. Regarding the development of the central nervous system, one confocal imaging study of serotonin-like immunoreactivity indicated that there may be transient segmental organization along the developing ventral nerve cord in *P. agassizii* (see Kristof *et al.* 2008). However, this interpretation is questionable and should be reinvestigated since developmental chronology, as presented in that study, is not replicable. Other than one interpretation by Kristof *et al.* (2008), the conclusion from more than a century of studies on sipunculan development is that there is no evidence of segmentation during the formation of any tissues or organ systems derived from the primary germ layers (ectoderm, endoderm, mesoderm) within any stages (embryos, larvae, juvenile worms) of the four recognized life history patterns (Hatschek 1883, Gerould 1906, Åkesson 1958, 1961, Hyman 1959, Clark 1969, Rice 1973, 1985b, 1993, Wanninger *et al.* 2005, Schulze and Rice 2009, Boyle and Rice 2014). Relatively recently, the molecular phylogenetic hypotheses of sipunculan genera (Kawauchi *et al.* 2012) and families (Lemer *et al.* 2015) have provided additional support for the relationship of Sipunculidae as the sister clade to all remaining families (Fig. 6.1.6), further supporting an inference of planktotrophic pelagosphera larvae within the ancestral sipunculan life history pattern, as previously suggested from morphological, systematic, and developmental studies (Rice 1985b, Boyle and Rice in press).

In the two recognized larval types in Sipuncula, the trochophore and pelagosphera, there are morphological, functional, and behavioral transitions to a subsequent species-specific life history stage (Gerould 1906, Åkesson 1958, Rice 1978). Those transitions have been described as a first and second metamorphosis (Jägersten 1963, 1972, Rice 1985b). Whether the first or second metamorphic transition occurs depends upon the category of indirect development (II–IV) exhibited by a particular life history pattern, as well as the degree of morphogenesis required to reach the juvenile sipunculan stage. Although embryos are often ciliated and motile, in many species, there appears to be a definitive trochophore stage that undergoes a series of changes to the lecithotrophic or planktotrophic pelagosphera larval stage. Prior to metamorphosis, the top-shaped trochophore has an apical tuft of cilia on the anterior end; a dorsolateral pair of reddish colored eyes; a broad double band of multiciliated, yolk-rich prototroch cells; and a uniformly thick egg envelope surrounding the entire larva (Fig. 6.1.4 C, F).

During the first metamorphosis, multiple larval-specific changes take place. The development of circular, longitudinal, and introvert retractor muscles and their contraction and extension within the body, in addition to somatic cell proliferation, cause a stretching and thinning of the egg envelope and elongation of the larva in the post-trochal region along the antero-posterior axis (Åkesson 1958). Concurrently, the coelomic cavity expands, the prototroch is reduced to a narrow semicircular band of cilia on the dorso-posterior of the pretrochal head region, a distinct metatrochal band of cilia develops around the thorax between the head and future trunk (Fig. 6.1.5 B, C, D), and a terminal organ with adhesive properties and a designated pair of retractor muscles develop at the posterior end of the larva (Rice 1976, Ruppert and Rice 1983). This emerging pelagosphera larva also develops a broad field of cilia lining the ventral-anterior head and surrounding a stomodeum anterior to the metatroch, both open to the exterior and functional in planktotrophic life history stages (Fig. 6.1.5 C, D). The metatroch is now the primary organ of locomotion in the pelagosphera, and the high yolk content previously stored within the band of large prototroch cells has been transferred into the coelom to nourish growth of this second larval stage (Gerould 1906, Åkesson 1958, Rice 1976, 1985b, 1988). Internally, there is one pair of metanephridia, an elaborate centralized

nervous system, and a U-shaped gut with the rectum and anus typically positioned on the dorsal-anterior of the larval trunk (Fig. 6.1.5 C, D, G). Furthermore, the egg envelope of the trochophore is liberated from the epispheric or head region in many species, and posteriorly, it is transformed into an "extensible and flexible cuticle" of the trunk in pelagospherae (Rice 1976). The variability and extent of these and other metamorphic changes between trochophore and pelagosphera stages are species specific and particular to lecithotrophic or planktotrophic forms. For species without a pelagosphera stage, metamorphosis of the trochophore generates a crawling juvenile worm. Development to a planktotrophic larva also entails the formation of a complex feeding morphology for growth and survival within a pelagic environment, especially among species with the comparatively larger forms of inferred long-duration teleplanic pelagosphera larvae found within major surface currents circulating around and across much of the world's open ocean (Hall and Scheltema 1975, Rice 1981, Boyle and Rice in press, Rice *et al.* in press). For example, planktotrophic pelagospherae possess a protrusible buccal organ supported and operated by extensive pharyngeal musculature, and the combined apparatus of a grooved ciliated lip with a lip pore and two to four lobate secretory glands, which, collectively, are most likely involved in handling food particles and the conspicuous behavior of "tasting" or sampling substrates in connection with settlement. These anterior feeding-associated structures are closely aligned along the esophagus, which in planktotrophic larvae leads to a well-defined stomach region that connects posteriorly to an intestinal section of the digestive system (Fig. 6.1.5 C, G). Planktotrophic larvae may also vary in the number and pattern of their dorsal eyespots, pigmentation of the body and head, arrangement of body-wall muscles, and both the morphology and presence or absence of papillae extending from larval cuticle (Fig. 6.1.5 A, E), with this last feature correlating with a range of papillated structures that have been characterized on the juvenile and adult worms that they will develop into (Rice 1975a, 1981, 1988).

The second metamorphic transition is from the pelagosphera larva to a juvenile sipunculan (Jägersten 1972, Rice 1985b). Lecithotrophic pelagospherae have a considerable resource of yolk to facilitate growth and metamorphosis, which in all pelagosphera larvae includes the loss of prototrochal and metatrochal ciliary bands, morphogenetic rotation of the mouth and esophagus from ventral-anterior to an apical position, and the formation of tentacle buds surrounding the mouth, or surrounding a nuchal sensory organ with the mouth positioned outside the developing crown of ciliated tentacles. The second metamorphosis of planktotrophic species is more complex and involves the reduction and disappearance of associated feeding structures (buccal organ, lip gland), including loss of a defined stomach region with elongation of the body, formation and growth of descending and ascending loops of the intestine, transformation of the larval cuticle with sensory papillae, secretory papillae, and, in many but not all species, the formation of hooks in different arrangements on the anterior introvert. Additionally, it is not known whether the larval eyes are retained or if juvenile eyes develop independently; however, the eyes are now located internally on the dorsal side of the brain, which is positioned dorsally along the esophagus and internal to the tentacular crown. Another mystery involves the final morphology and/or transformation and function of the larval terminal organ within the adult. In species where such changes have been observed in the laboratory, the second metamorphic transition occurs over a period of 1 to 5 days, although specific, generic, and familial characters become distinguishable over periods of weeks to months (Åkesson 1958, Rice 1976, 1988). Furthermore, some characters such as hooks or particular retractor muscles may be absent in adult stages, while some epidermal structures become distinct or fully functional only in adult stages (Rice 1976, Schulze and Rice 2009, Boyle and Rice 2014, Rice *et al.* in press).

Overall, when following sipunculan development in each of the four life history patterns (I–IV), and by comparison with closely related taxa in Annelida or among other members of the spiralian superclade, there are a number of sipunculan-specific characters that distinguish this group of coelomate marine worms. The sipunculan character complex includes unequal spiral cleavage in all species studied, conspicuously large first quartet micromeres, introvert retractor muscles derived from 1q ectomesoderm, mid-body growth zone, all trochophore larvae being lecithotrophic, a unique pelagosphera larva, metatroch with exclusive locomotor function, U-shaped gut with middorsal anus, a terminal organ with apparent adhesive properties, and no segmental rudiments in embryonic, larval, or juvenile life history stages (Rice 1985b, Boyle and Rice 2014).

Although there are extensive comparative observations and a broad range of descriptive studies on sipunculan reproduction and development, there is very little information about the blastomeric or even germ-layer origins of multiple sipunculan-specific characteristics. To date, no cell-lineage or intracellular fate map has been constructed for a member of Sipuncula. These resources will be required for any critical understanding of the origins of a sipunculan character complex or

any of the many other features characteristic of their ontogeny. Therefore, any inferences of homology appropriately based upon cellular and germ-layer-specific studies tracing the cellular origins of particular tissues and organ systems are currently unavailable to allow direct comparison of developmental or functional morphology between sipunculan worms and other members of Spiralia (e.g., metatroch, buccal organ, tentacles). When such studies are eventually published, there already is in place an impressive breadth of comparative literature across multiple species-specific life history patterns, from first cleavage through second metamorphosis of sipunculan development. Attempts to permeabilize and inject sipunculan embryos are underway but remain a considerable challenge due to their complex multilayered egg envelope and its mostly unknown biochemical composition. Once such technical challenges are overcome, a delivery system will be available to pursue functional genomics through multiple molecular tools. Previously, gene expression studies have been performed (Boyle and Seaver 2010, Boyle and Rice 2014) and have been now verified to work in four species with distinctly contrasting life history patterns. Comparative developmental transcriptome sequencing and genome sequencing and assembly have been completed. With genomic sequence resources, in combination with working protocols for gene expression by in situ hybridization, a number of studies, focused on when and where developmental genes pattern and build the sipunculan body plan, should be pursued. Interesting targets include the sipunculan hox code, nervous and digestive system architecture, the elaborate musculature of feeding and introvert retraction, and the locomotory-related ciliation and neural regulation of the metatroch. Moreover, when and where are the so-called segmentation genes expressed during the development of nonsegmented sipunculans currently considered to be among the basal branches of the overtly segmented and extremely diverse radiation of worms within the annelid tree of life? There are insightful experiments to be performed on sipunculan developmental models and a rich history of in-depth morphological and taxonomic studies to guide future interpretations of new research efforts on these under-represented marine worms.

Biology and ecology

Sipuncula generally lead a cryptic life but can reach high local abundances (e.g., Rice *et al.* 1983). They have been reported from the intertidal zone to the abyss and all latitudes (Cutler 1994). Most species appear to have wide environmental tolerances (Cutler 1994). The Indo-Pacific *Phascolosoma arcuatum* (Gray, 1828), which inhabits mangrove swamps, is particularly tolerant to air exposure and salinity fluctuations and has been referred to as semi-terrestrial (Green 1975).

Some species, such as *Sipunculus* spp., burrow into sand or mud, sometimes 50 cm or more below the sediment surface (Voss-Foucart *et al.* 1977, Romero-Wetzel 1987, Cutler 1994, Shields and Kedra 2009, Maiorova and Adrianov 2010) and can be major contributors to bioturbation of the sediment (Graf 1989, Li *et al.* 2015). Other species are common in roots or holdfasts of submerged vegetation, sponges, algal mats, or bivalve aggregations. *Phascolosoma turnerae* Rice, 1985 was first reported from submerged wood (Rice 1985a) and is also abundant in areas of hydrocarbon or methane seepage, where it inhabits sediment at the base of tubeworm (siboglinid) aggregations (MacAvoy *et al.* 2005). *Phascolosoma saprophagicum* Gibbs, 1987 was discovered in the crevices of a whale bone recovered from 800-m depth off the coast of New Zealand (Gibbs 1987). Several species, in particular *Phascolion* spp., inhabit abandoned gastropod shells, polychaete tubes, or foraminiferan tests. Sipunculans also commonly bore into hard substrates, including coral rubble, mollusc shells, calcareous rock, or sandstone, and contribute to the bioerosion of these materials (Stearley and Ekdale 1989, Glynn and Manzello 2015), sometimes with detrimental effects (Antonelli *et al.* 2015).

Most sipunculans are presumed to be semisessile in the juvenile and adult stage, and their locomotion is largely limited to the introvert. Introvert extension and retraction are achieved by antagonistic interaction between the body wall musculature and the introvert retractor muscles. Contraction of the body wall musculature increases the hydrostatic pressure in the main body coelom, causing the eversion of the introvert (Zuckerkandl 1950). Retraction is achieved by contraction of the introvert retractor muscles.

Little is known about the normal activity patterns of sediment burrowing species. The small *Nephasoma lilljeborgi* (Danielssen & Koren, 1880) forms complex networks of burrows up to at least 30 cm deep and rapidly moves organic matter to the subsurface (Shields and Kedra 2009). Large species, such as *Sipunculus* spp., make almost straight vertical burrows up to 1 m deep (Maiorova and Adrianov 2010). When removed from the sediment, the worms can very quickly rebury themselves by using a combination of peristaltic movement and digging with the introvert.

The paucity of morphologically diagnostic features in sipunculans makes species delineation a difficult task.

A number of species have only been reported from their type localities. Some of these have been synonymized with other, more widespread, species or are now regarded *Incertae Sedis* or *Species Inquirenda* (Cutler 1994). The majority of sipunculan species, however, have large reported distribution ranges and some are referred to as "cosmopolitan." This view of generally wide geographic distributions is supported by the existence of pelagic larval stages (pelagosphera), which can potentially cross large swaths of ocean to disperse to remote areas (Hall and Scheltema 1975). On the other hand, over the past two decades, molecular data have challenged the notion of cosmopolitan sipunculan species by revealing significant genetic differences among geographically separated populations, leading to the detection of "cryptic" (i.e., morphologically unrecognizable but genetically distinct) or "pseudo-cryptic" (i.e., morphologically and genetically distinct but lumped together in the taxonomic literature) species. These include *Apionsoma misakianum* Ikeda, 1904 (Staton and Rice 1999), *Sipunculus nudus* Linnaeus, 1766 (Kawauchi and Giribet 2014), *Phascolosoma perlucens* Baird, 1868 (Kawauchi and Giribet 2010), *Phascolosoma agassizii* Keferstein, 1866 (Schulze *et al.* 2012, Johnson *et al.* 2016, Johnson and Schulze 2016), *Themiste pyroides* (Chamberlin, 1919) (Schulze *et al.* 2012), and *Thysanocarida nigra* (Ikeda, 1904) (Schulze *et al.* 2012). These newly detected species have not, as yet, been formally described.

Three feeding modes are dominant among sipunculans: nonselective deposit feeding, surface grazing, and suspension feeding. In sediment-dwelling species, the intestine is usually filled with particles that seem to resemble the surrounding sediment in composition and grain size (Edmonds 1962), suggesting nonselective ingestion of sediment and subsequent digestion of organic particles, including detritus and meiofaunal organisms. Species that burrow into or inhabit the crevices of hard substrates, such as coral rubble or mollusc aggregations, generally have a long introvert, often with numerous hooks and a relatively small tentacular apparatus. These species tend to extend their introverts from the opening of the burrow and scrape bacteria and microalgae off the surface using their introvert hooks (Cutler 1994). Members of the genus *Themiste* Gray, 1828 are suspension feeders and possess elaborate branched tentacles for food capture (Pilger 1982, Adrianov *et al.* 2006). A few additional species, such as *Antillesoma antillarum* Grube, 1858, members of the genus *Thysanocardia* (Fisher, 1950), and possibly *Sipunculus* spp., also have large tentacles and may use them for suspension as well as surface deposit feeding (Jumars *et al.* 2015).

Sipunculans are commonly recovered in the stomach contents of bottom-feeding fish (reviewed in Cutler 1994), some crustaceans (Woods and McLay 1996, Cristo 1998), octopods (Villegas *et al.* 2014), and gastropods. Remarkably, among gastropods, the diet of members of the gastropod family Mitridae consists exclusively of sipunculans (Taylor 1984, 1989). Sipunculans have also occasionally been recovered in the stomach contents of olive ridley sea turtles (Bjorndal 1996) and gray whales (Ferguson *et al.* 2015).

Phylogeny and taxonomy

Phylogeny

The evolutionary origin and phylogenetic relationships of sipunculans have long been mysterious and contentious (for a review, see Saiz-Salinas 2018). Linnaeus (1766) placed *S. nudus,* still one of the most referenced sipunculan species today, into the "Vermes Intestina," a group that included a number of worm-like taxa without appendages, such as *Fasciola*, *Lumbricus, Hirudo,* and others. Apparently, due to a superficial resemblance to sea cucumbers, Lamarck (1816) regarded them as echinoderms without appendages. Quatrefages (1847) proposed the concept of "Gephyrea" (literally meaning bridge) to include a number of superficially similar groups that he considered a bridge between segmented worms and echinoderms. Apart from sipunculans, this group also included holothurians, echiurans, and priapulids. Although Sedgwick (1898) recognized "Sipunculoidea" as a separate phylum, the Gephyrea concept lingered in the scientific literature for over a century. Hyman (1959: 611) finally put the concept to rest by recognizing that "...adopting the concept of Gephyrea offers an easy way of disposing of three groups of very uncertain affinities. But as all modern students of these groups are agreed that there is no close relationship between them *the name and the concept of the Gephyrea must be obliterated from zoology*" (emphasis by original author). Although she assumed a close relationship between annelids and sipunculans, Hyman treated sipunculans as a distinct phylum, which she named "Sipunculida." The name was later changed to "Sipuncula" by Stephen (1964).

Embryonic development, in particular the occurrence of spiral cleavage (Gerould 1906), clearly places the Sipuncula in the Spiralia/Lophotrochozoa clade, and their monophyly is uncontested (Maxmen *et al.* 2003, Schulze *et al.* 2005, 2007, Kawauchi *et al.* 2012, Lemer *et al.* 2015). However, it still remained unclear what the sister taxon to the Sipuncula is. Embryological data such as the

presence of a "molluscan cross" in the 64-cell stage initially seemed to point to a sister group relationship with molluscs (Scheltema 1993), but in light of more recent cell lineage studies (Maslakova *et al.* 2004), the concept of the molluscan vs. the annelid cross is no longer supported. Several phylogenetic and phylogenomic studies have placed sipunculans in the annelid radiation (Boore and Staton 2002, Telford *et al.* 2005, Dunn *et al.* 2008, Hejnol *et al.* 2009, Mwinyi *et al.* 2009, Struck *et al.* 2011), most notably near the base of the annelid tree as the sister taxon to Amphinomidae (e.g., Weigert *et al.* 2014, 2016). However, this placement is not supported by cladistic and probabilistic analyses based on combined data from fossil and extant taxa (Parry *et al.* 2014, 2016). The fossil record, although sparse, shows a remarkable conservation of the basic sipunculan morphology since the Cambrian (Huang *et al.* 2004, Muir and Botting 2007).

Taxonomy

Higher classification was recently revised based on multigene phylogenetic (Kawauchi *et al.* 2012) and phylogenomic analyses (Lemer *et al.* 2015). The new classification scheme differs in several respects from older ones (Stephen and Edmonds 1972, Cutler 1994), although some of the family names were retained. The new classification recognizes six families, but no higher taxonomic ranks (Fig. 6.1.6).

Sipuncula Stephen, 1964
Family Sipunculidae Rafinesque, 1814
Type genus: *Sipunculus* Linnaeus, 1766
Synonyms: Sipunculidae Baird, 1868:77 [partim]; Stephen and Edmonds 1972:19 [partim]; Cutler and Gibbs 1985:166 [partim]; Gibbs and Cutler 1987:47 [partim]; Cutler, 1994:24 [partim]
Diagnosis: Large sipunculans, commonly 100–200 mm long, with introvert shorter than the trunk. Oral disk with tentacles surrounding a central mouth. Hooks absent; prominent triangular papillae arranged irregularly, covering most of the length of the introvert. Trunk cylindrical and covered externally by swollen rectangular "mini pillows." Longitudinal and circular muscle layers of the inner trunk divided into distinct bands (Longitudinal muscle bands, LMBs and Circular muscle bands, CMBs, respectively). Functional pore present between each pair of overlapping longitudinal and circular bands, allowing direct contact between coelomic fluid and the skin to facilitate gas exchange. Pore opens in a closed space (sac) or into parallel longitudinal canals limited in length to the width of one circular muscle band (coelomic extensions), extending through most of the trunk length. Thread-like muscle (spindle muscle) extends through the center of gut coil from anterior to posterior; not attached to the wall of the posterior trunk. Two pairs (two dorsal and two ventral) of introvert retractors muscles extend from the head to the anterior trunk wall. Intestinal coil attached to body wall along most of its length by many connective tissue strands (fixing muscle). A pair of nephridia opening anterior or posterior to the anus.

Genus *Sipunculus* Linnaeus, 1766 (Fig. 6.1.7 A)
Type species: *Sipunculus nudus* Linnaeus, 1766
Synonyms: *Syrinx* Bohadsch, 1761:63–97, *Sipunculus* Linnaeus, 1766:1078; Quatrefages, 1865b:613; Selenka *et al.*, 1883:88; Stephen and Edmonds, 1972:21; E. Cutler and Cutler, 1985b:232
Diagnosis: Large species with trunk longer than 50 mm in adults. Introvert shorter than trunk (5–30%). Two pairs of stemlike tentacles with ventral pair shorter than dorsal pair. Well-defined longitudinal muscles bands between 20 and 50 in number. Coelomic extensions in body wall in the form of canals between longitudinal muscle bands extending along most of the trunk. Two smooth contractile vessels without villi. Esophagus with long and loose loop before entering intestine coil (postesophageal loop). Rectal caecum present. Nephridiopores opening anterior to the anus.

Genus *Xenosiphon* Fisher, 1947 (Fig. 6.1.7 B)
**Type species:** *Sipunculus mundanus* var. *branchiatus* Fischer, 1865
Synonyms: *Xenosiphon* Fisher, 1947:360; 1954a:311; Stephen and Edmonds, 1972:38; E. Cutler and Cutler, 1985b:228
Diagnosis: Large species. Introvert shorter than trunk. Tentacular crown consisting of many stemlike tentacles of the same size around mouth. Between 29 and 37 well-defined longitudinal muscles bands. Coelomic extension in the body wall consist of short, diagonal canals limited in length by the limit of one CMB. An extra pair of thin muscles (protractor muscles) attaches near the brain. Gut without a postesophageal loop. Spindle muscle arises on the ventral wall of the rectum; not attached to the trunk wall posteriorly. Nephridiopores opening posterior to anus.

Family Golfingiidae Stephen & Edmonds, 1972
Type species: *Golfingia* Lankester, 1885
Synonyms: Sipunculidae Baird, 1868:77[partim]; Stephen and Edmonds, 1972:19 [partim]; Cutler and Gibbs 1985:166 [partim]; Gibbs and Cutler, 1987:47, Cutler:24 [partim]
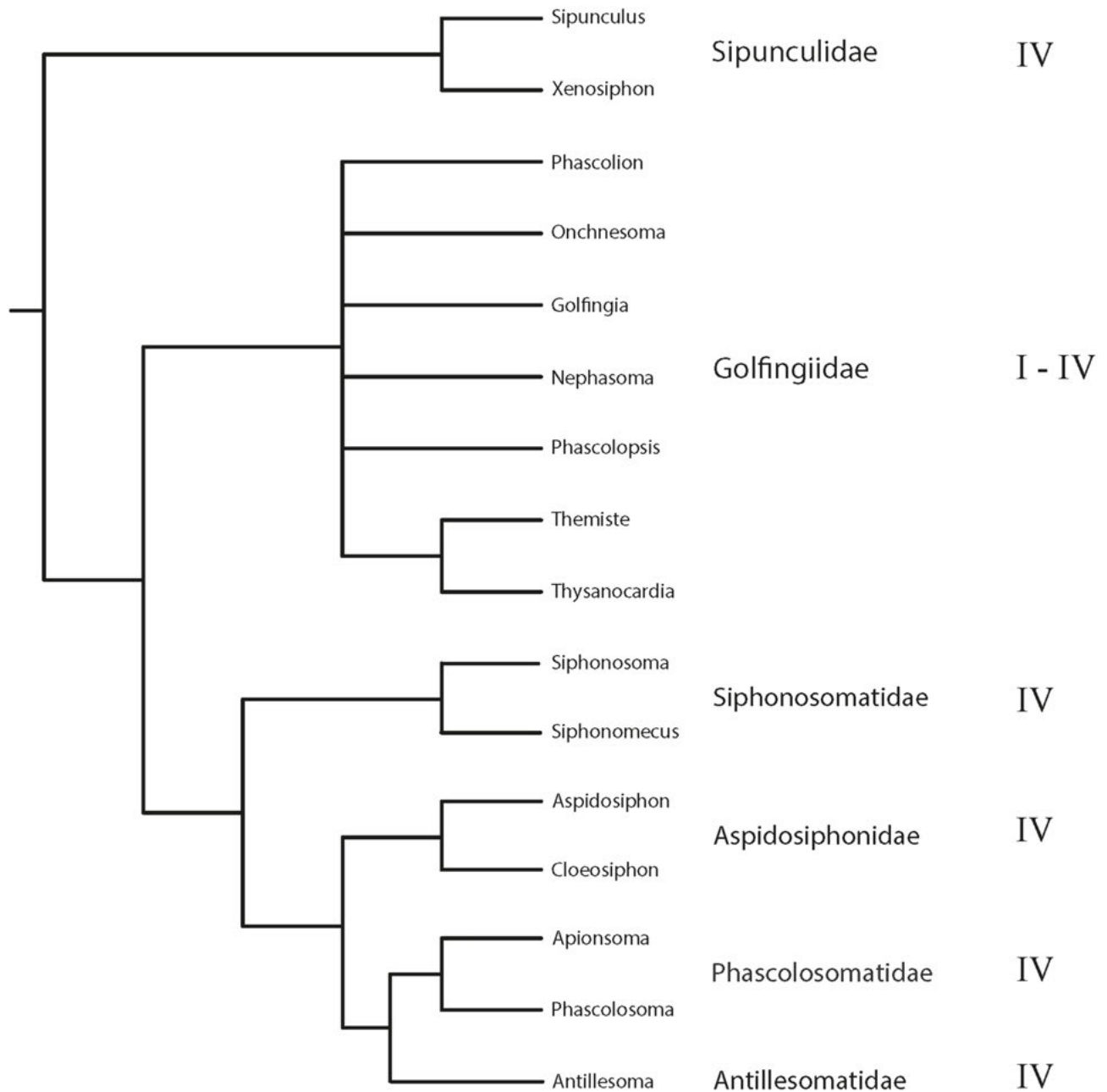
Fig. 6.1.6: Phylogeny and distribution of developmental life history patterns within Sipuncula. Sipunculan genera are located at the terminal branch tips. Six recognized sipunculan families (Sipunculidae, Golfingiidae, Siphonosomatidae, Aspidosiphonidae, Phascolosomatidae, and Antillesomatidae) are placed to the right of the clades of related sipunculan genera that are found within each of the six families. Roman numerals are placed to the right of the six families to designate the developmental life history patterns found within each family. In all but one family, a life history including planktotrophic larval development (IV) is the exclusive pattern observed for species in that family, with few exceptions (see text). Within Golfingiidae, all four developmental patterns (I, II, III, and IV) have been characterized. The cladogram topology combines two phylogenetic hypotheses (Kawauchi *et al.* 2012, Lemer *et al.* 2015). Modified from Boyle and Rice (in press), courtesy of the Smithsonian Institution.

Golfingiidae Stephen and Edmonds, 1972:77 [partim]; Cutler and Gibbs 1985:167 [partim]; Gibbs and Cutler, 1987:50, Cutler:60 [partim]

Themistidae Cutler and Gibbs, 1985:167 [partim]; Gibbs and Cutler, 1987:53, Cutler:140 [partim]

Phascolionidae Cutler and Gibbs, 1985:166 [partim]; Gibbs and Cutler, 1987:51, Cutler:107

Diagnosis: Small to medium sipunculans (trunk no longer than 200 mm). Introvert equal to or shorter than the trunk. Tentacles surround the central mouth. Hooks
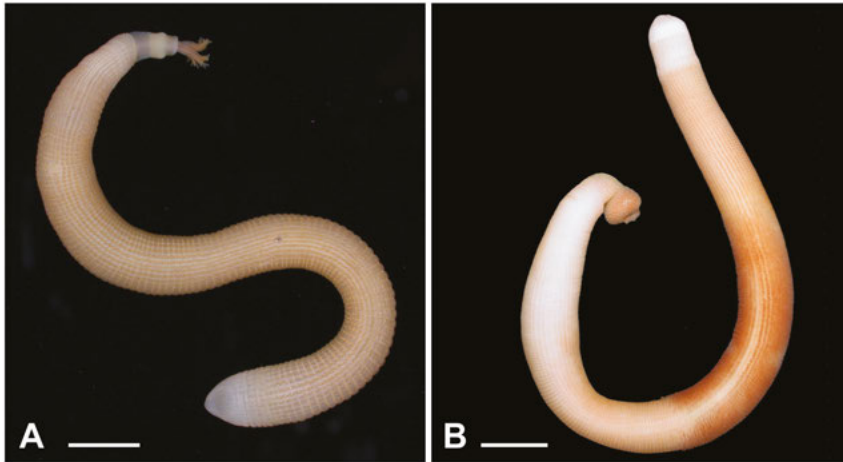
Fig. 6.1.7: Sipunculidae. A; *Sipunculus nudus* (©G.Y. Kawauchi). B, *Xenosiphon branchiatus* (©G.Y. Kawauchi). All scale bars = 10 mm.

may be deciduous, absent, or simple when present, not sharply curved and generally scattered, but sometimes arranged in rings. Tentacles organized in a close-set array of digitiform, filiform, leaflike, or stemlike extensions of the oral disk or reduced to lobes. External trunk wall generally smooth or with papillae, the latter usually small in diameter and height. In small worms (few millimeters), trunk wall sometimes transparent or translucent, but specimens larger than 20 mm with opaque trunk wall (often yellow or whitish). Longitudinal musculature of inner trunk continuous, except in *Phascolopsis*. One to four retractor muscles. Contractile vessel simple or with numerous villi. One or two nephridia. Nephridiopore opening anterior or posterior to the anus.

Genus *Golfingia* Lankester, 1885
Type species: *Golfingia macintoshii* Lankester, 1885 (= *Sipunculus vulgaris* sensu Blainville, 1827)
Synonyms: *Golfingia* Lankester, 1885a:469
Golfingia (*Golfingia*) Fisher, 1950a:549
Golfingia (*Dushana*) Murina, 1975c:1085
Themiste (*Stephensonum*) Edmonds, 1980:19
Centrosiphon Shipley, 1903:173; Edmonds, 1980:19
Diagnosis: Small- to medium-sized worms (very few exceed 30 mm in length). Introvert equal to or shorter than trunk (65–100% of trunk length, only one species up to 200%). Tentacles a series of digital circumoral tentacles, whose number and complexity increase with age within species of this genus. Hooks when present commonly small (20–40 μm) and scattered. Body wall with continuous muscle layer. Four retractor muscles. Contractile vessel without villi. Spindle muscle attached terminates posteriorly within gut coil. Two nephridia open anterior to the anus.

Genus *Nephasoma* Pergament, 1940
Type species: *Nephasoma marinki* Pergament, 1940 (= *Onchnesoma glaciale* Danielssen and Koren: E. Cutler and Murina, 1977; = *Phascolosoma lilljeborgii* Danielssen and Koren: Gibbs, 1982).
Synonyms: *Nephasoma* Pergament, 1940:1; N. Cutler and Cutler, 1986:548
Golfingia (*Phascoloides*) Fisher, 1950:550; Stephen and Edmonds, 1972:131
Diagnosis: Small- to medium-sized worms, with trunk less than 50 mm in length. Introvert commonly shorter than trunk. Tentacular crown around mouth commonly with flattened, digitate tentacles, shorter and lobate tentacles or reduced in size and numbers. Hooks, when present, generally scattered. Longitudinal musculature of inner trunk continuous. Two retractor muscles, often partially fused. Contractile vessel without villi. Spindle muscle not attached to the posterior end of the trunk. Two nephridia usually open at the level of the anus but can vary opening anterior and posterior to the anus.

Genus *Onchnesoma* Koren and Danielssen, 1875
Type species: *Onchnesoma steenstrupii* Koren and Danielssen, 1875
Synonyms: *Onchnesoma* Koren and Danielssen, 1875:133; 1877:142; Selenka *et al.*, 1883; Théel, 1905:13; Stephen and Edmonds, 1972:161; E. Cutler and Cutler, 1985a:840
Diagnosis: Small specimens with trunk less than 10 mm long. Introvert always longer than trunk. Anus on distal half of the introvert. Tentacle crown with few tentacles (less than 10) and sometimes reduced in size or completely absent. Trunk with papillae and/or radiating folds of skin (keels) on posterior end. Hooks absent. Longitudinal musculature of inner trunk continuous. Retractor muscles almost completed fused forming a single retractor

column. Contractile vessel, when visible, without villi. Spindle muscles apparently absent. Anus always opening on the distal half of the introvert. Only one nephridium opening posterior to the anus.

Genus *Phascolion* E. Cutler and Gibbs, 1985
Type species: *Sipunculus strombus* Montagu, 1804
Synonyms: *Phascolion* Théel, 1875b; Selenka *et al.*, 1883:41; Stephen and Edmonds, 1972:164
Diagnosis: Small- to medium-sized worms (less than 50 mm in length). Common inhabitants of empty gastropod shells. Introvert one-half to four times the trunk length. Tentacular crown around mouth with tentacles varying from only few lobes, broad digitiform, slender digitiform, or reduced in number and with dendritic pattern. Hooks present or absent. Longitudinal musculature of inner trunk continuous. In most species, trunk with unique holdfast papillae forming dark, horny protein structures, shaped like a U, V, or broken O. Holdfast papillae distributed around the trunk, usually in midregion, and probably functioning as anchors in the protective shelter. Retractor muscles fused into dorsal and ventral pair, giving the appearance of only one or two muscles. Degree of fusion and relative size define subgenera. Intestinal coil loose and without axial spindle muscle. Anus positioned 20–95% of the distance toward distal tip of introvert or at anterior end of trunk. One nephridium (generally the right one) usually opening posterior to the anus.

Genus *Phascolopsis* (Fisher, 1950)
Type species: *Sipunculus gouldii* (Portalés, 1851)
Golfingia (*Phascolopsis*) Fisher, 1950:550
Phascolopsis Stephen, 1964:459; Stephen and Edmonds, 1972:74; E. Cutler, 1986:460
Diagnosis: Medium to large worms. Introvert about one-third of trunk length. Tentacular crown consisting of many filiform peripheral tentacles. Trunk up to 150 mm long (commonly 50–100 mm in length). Hooks deciduous and only present in very young worms up to 3 or 4 cm long. Longitudinal musculature of the inner trunk gathered into anastomosing bands; circular muscle layer continuous. Four introvert retractor muscles. Contractile vessel without villi. Spindle muscle not attached to the posterior end of trunk. Two nephridia open slightly anterior to the anus.

Genus *Themiste* Gray, 1828 (Fig. 6.1.8 A)
Type species: *Themiste hennahi* Gray, 1828
Synonyms: *Themiste* Gray, 1828:8; Baird, 1868:98; Stephen, 1965:58; Stephen and Edmonds, 1972:193

Fig. 6.1.8: Golfingiidae and Siphonosomatidae. A, *Themiste alutacea* (©A. Schulze), scale bar = 5 mm. B, *Thysanocardia catharinae* (©G.Y. Kawauchi), scale bar = 20 mm. C, *Siphonosoma cumanense* (©G.Y. Kawauchi), scale bar = 20 mm.

Dendrostomum Grube and Oersted, 1858:118; Quatrefages, 1865b:629

Dendrostoma Keferstein, 1865b:438; Selenka *et al.*, 1883:83

Diagnosis: Small to large sized worms (4 to 400 mm). Introvert shorter than trunk. Tentacles surrounding mouth with unique dendritic branching patterns emerging from a few individual stems. Hooks are present or absent in adults. Inner trunk wall with continuous muscle layer. Four introvert retractor muscles. Contractile vessel with short villi or long tubules. Spindle muscle not attached posteriorly to trunk. Two nephridia open posterior to the anus.

Genus *Thysanocardia* (Fisher, 1950) (Fig. 6.1.8 B)

Type species: *Phascolosoma procerum* Möbius, 1875

Synonyms: *Golfingia* (*Thysanocardia*) Fisher, 1950a:551; Stephen and Edmonds, 1972:120;

Thysanocardia Gibbs *et al.*, 1983:295

Diagnosis: Small to medium size worms (adult specimens with trunk less than 70 mm). Introvert longer than trunk. Tentacular crown consisting of up to 26 festoons with many tentacles around the mouth and up to 30 tentacles enclosing the nuchal organ. Hooks present in very young worms but absent in adults. Longitudinal musculature of inner trunk continuous. Two introvert retractor muscles. Contractile vessel with many distinct short villi. Spindle muscle not attached to posterior end of trunk. Two nephridia.

Family Aspidosiphonidae Baird, 1868

Type genus: *Aspidosiphon* Diesing, 1851

Synonyms: Aspidosiphonidae Baird, 1868: 100 [partim]; Stephen and Edmonds, 1972:215; Cutler and Gibbs:166; Gibbs and Cutler, 1987:55; Cutler, 1994: 199

Loxosiphonidae Baird, 1868: 103

Diagnosis: Generally small worms (5–30 mm). Introvert usually longer than trunk protruding at an angle of 45–90° ventral to the main axis of the trunk. Tentacles encircling nuchal organ. Three types of hooks may be present (compressed uni- and bidentate, pyramidal, and conical) in a combination of two or three of them. Outer trunk wall with some kind of cuticular structure at one or both ends (anal and caudal shield). Inner trunk wall usually as a continuous muscle layer (some species with anastomosing bands). Two introvert retractor muscles. Contractile vessel, when visible, without villi. Spindle muscle attached to posterior end of trunk. Two nephridia usually open posterior to the anus.

Genus *Aspidosiphon* Diesing, 1851 (Fig. 6.1.9 A–C)

Type species: *Aspidosiphon muelleri* Diesing, 1851

Synonyms: *Aspidosiphon* Diesing, 1851:67–68

Pseudaspidosiphon Baird, 1868: 102 [partim]

Diagnosis: Small- to medium-sized worms (most species are less than 40 mm). Introvert usually longer than or as long as trunk and protruding from ventral margin of anal shield. Hooks recurved and present in numerous rings but can be scattered or absent in some species. Tentacular crown generally with very short finger tentacles enclosing the dorsal nuchal organ. Trunk with hardened structure composed of a horny protein (anal shield) or by a subcuticular calcareous conical structure. Inner-body wall either with continuous muscle layer or in anastomosing bands, sometimes ill-defined. Two introvert retractor muscles, often almost completed fused. Contractile vessel smooth or with villi. Spindle muscle attached to posterior end of trunk. Two nephridia.

Genus *Cloeosiphon* Grube, 1868

Type species: *Loxosiphon aspergillus* Quatrefages, 1865

Synonyms: *Loxosiphon* Quatrefages, 1865 [partim]

Cloeosiphon Grube, 1868a:48–49.–Selenka *et al.*, 1883:126.– Stephen and Edmonds, 1972:297

Diagnosis: Medium-sized worms. Introvert longer than trunk, protruding from the center of anal shield. Unique anal shield composed of calcareous plates with a pineapple skin appearance. Hooks recurved and present in numerous rings. Tentacular crown enclosing dorsal nuchal organ. Inner trunk wall with continuous muscle layer. Two introvert retractor muscles, often almost completed fused. Contractile vessel with no villi. Spindle muscle attached posteriorly. Two nephridia.

Family Phascolosomatidae Stephen & Edmonds, 1972

Type genus: *Phascolosoma* Leuckart, 1828

Synonyms: Sipunculidae Baird, 1868:77 [partim]

Phascolosomatidae Stephen and Edmonds, 1972:269 [partim]; Gibbs and Cutler 1987:54 [partim]; Cutler, 1994:156 [partim]

Diagnosis: Small to medium sized worms (trunk up to 12 cm in length). Introvert usually of equal size or much longer than trunk. Hooks recurved, usually with internal structures or series of basal spinelets. Hooks typically closely packed in regularly spaced rings. Short tentacles surrounding the nuchal organ in an arc. Outer trunk wall with obvious papillae covering the whole body, concentrated at anterior and/or posterior end of trunk. Internally longitudinal muscle subdivided into anastomosing bands or a thinner continuous layer. Four introvert retractor muscles. Contractile vessel smooth but may be large with
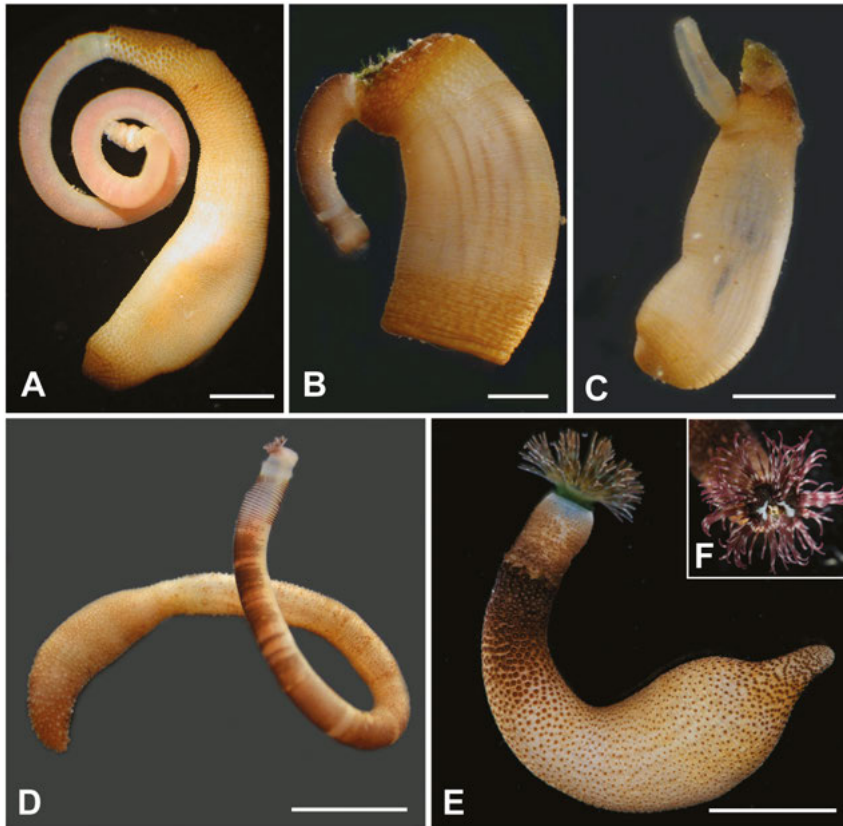
Fig. 6.1.9: Aspidosiphonidae, Phascolosomatidae and Antillesomatidae. A, *Aspidosiphon parvulus* (©G.Y. Kawauchi), scale bar = 2 mm. B, *Aspidosiphon steenstrupii* (©A. Schulze), scale bar = 5 mm. C, *Aspidosiphon cristatus* (©A. Schulze), scale bar = 2 mm. D, *Phascolosoma nigrescens* (©A. Schulze), scale bar = 10 mm. E, *Antillesoma antillarum* (©G. Y. Kawauchi), scale bar = 10 mm. F, *Antillesoma antillarum* tentacular crown (©G.Y. Kawauchi).

bulbous pouches or swelling in some species. Spindle muscle attached or not posteriorly. Two nephridia, uni or bilobed.

Genus *Phascolosoma* Leuckart, 1828 (Fig. 6.1.9 D)
Type species: *Phascolosoma granulatum* Leuckart, 1828
Synonyms: *Phascolosoma* Leuckart, 1828:22; Fisher, 1950a:551; Stephen and Edmonds, 1972:270; Gibbs and Cutler, 1987:54; N. Cutler and Cutler, 1990:691
Phascolosomum Diesing, 1851:63 [partim]
Phymosomum Quatrefages, 1865b:621
Phymosoma Selenka *et al*. 1883:54
Physcosoma Selenka, 1897:460
Diagnosis: Introvert length commonly equal to trunk. Hooks recurved with internal structures organized in rings at distal tip of introvert. Tentacular crown consisting of fewer than 30 tentacles surrounding the nuchal organ. Inner trunk wall in continuous or occasionally anastomosing bands. Contractile vessel without bulbous pouches or swelling, not true villi. Spindle muscle attached posteriorly. A pair of unilobed nephridia.

Genus *Apionsoma* Sluiter, 1902
Synonyms: *Apionsoma* Sluiter, 1902:42; E. Cutler *et al.*, 1984:299; Gibbs and Cutler, 1987:55

Golfingia (*Apionsoma*) Murina, 1975d: 1748; E. Cutler, 1979:382
Golfingia (*Mitosiphon*) Fisher, 1950a:550; Stephen and Edmonds, 1972:113; E. Cutler, 1973:139
Golfingia (*Phascolana*) Wesenberg-Lund, 1959a:183; Stephen and Edmonds, 1972:116
Diagnosis: Small specimens commonly less than 20 mm in trunk length. Introvert much longer than trunk. Hooks recurved, organized in rings with series of accessory spinelets at the bases. Longitudinal musculature of inner trunk in bands or continuous. Contractile vessel without bulbous pouches or swelling, not true villi. Spindle muscle unattached or attached posteriorly. A pair of bilobed nephridia.

Family Siphonosomatidae Kawauchi *et al.*, 2012
Type genus: *Siphonosoma* Spengel, 1012
Sipunculidae Baird, 1868:77 [partim], Stephen and Edmonds 1972:19 [partim], Cutler and Gibbs 1985:166 [partim, Gibbs and Cutler, 1987:47 [partim], Cutler, 1994:24 [partim]
Diagnosis: Medium to large worms (trunk >50 mm). Introvert much shorter than trunk. Hooks, if present, in rings; likewise, prominent conical papillae. Oral disc bearing tentacles arranged around the mouth. Inner trunk wall

with small irregular sac-like coelomic extensions. Circular and longitudinal muscle layers in anastomosing, sometimes indistinct, bands. Two to four introvert retractor muscles. Contractile vessel with or without villi. Spindle muscle attached posteriorly. Two nephridia.

Genus *Siphonosoma* Spengel, 1912 (Fig. 6.1.8 C)

Siphonosoma Spengel, 1912:264.–Stephen and Edmonds, 1972:43.–E. Cutler and Cutler, 1982:748

Siphonosoma (*Dasmosiphon*) Fisher, 1950b:805.–Stephen and Edmonds, 1972:44

Siphonosoma (*Siphonosoma*) Fisher, 1950b:805.–Stephen and Edmonds, 1972:58

Siphonosoma (*Hesperosiphon*) Fisher, 1950b:805.–Stephen and Edmonds, 1972:43

Diagnosis: Usually large specimens with trunk longer than 50 mm in adults. Introvert shorter than trunk, with conical papillae, and sometimes hooks, arranged in rings. Coelomic extensions in body wall, irregular and saclike. Anastomosing longitudinal muscles, sometimes divided into distinct bands. Two pairs of retractor muscles. Contractile vessels, with or without villi. A pair of nephridia.

Genus *Siphonomecus* Fisher, 1947

Type species: *Siphonosoma australe* (Keferstein, 1865)
Siphonomecus Fisher, 1947:363.–Stephen and Edmonds, 1972:73.–E. Cutler, 1986:490

Diagnosis: Introvert shorter than trunk, with hooks and conical papillae in rings. Tentacular crown around mouth. Coelomic extensions in body wall. Circular and longitudinal muscle layers assemble into anastomosing bands. A pair of retractor muscles. The contractile vessel with villi. Spindle muscle attached posteriorly. A pair of nephridia.

Family Antillesomatidae Kawauchi *et al.*, 2012

Type genus: *Antillesoma* Stephen and Edmonds, 1972
Phascolosomatidae Stephen and Edmonds, 1972:269 [partim]; Cutler and Gibbs 1985:166; Gibbs and Cutler, 1987:51 [partim]; Cutler 1994:156 [partim]

Diagnosis: Medium-sized worms with trunk up to 80 mm in length. Introvert shorter than trunk. Few hooks present only in small individuals (less than 10 mm in length). Oral disc bearing numerous tentacles (30–200) in adults, enclosing nuchal organ. Body wall with longitudinal muscle layer gathered into anastomosing bands. Contractile vessel with many villi. Two pairs of introvert retractor muscles, the lateral pair often extensively fused. Spindle muscle attached posteriorly. Two nephridia.

Genus *Antillesoma* (Stephen and Edmonds, 1972)

(Fig. 6.1.9 E, F)

Types species: *Antillesoma antillarum* (Grube and Oersted, 1858)

Synonyms: *Phascolosoma* (*Antillesoma*) Stephen and Edmonds, 1972:277; E. Cutler, and Cutler, 1983:176

Antillesoma E. Cutler, and Cutler, 1985:163; Gibbs and Cutler, 1987:55

Diagnosis: Small- to medium-sized worms (up to 80 mm long). Introvert variable in length, but often about equal to trunk. Numerous slender digitiform tentacles (>30 in adults), arranged around nuchal organ. No hooks in adults. Introvert and trunk covered by dark brown papillae. Inner trunk wall with longitudinal muscle layer divided into anastomosing bands. A pair of introvert retractor muscles, but each lateral pair may be considerably fused, appearing as one muscle on each side of the nerve cord, with a small bifurcation near the origin. Contractile vessel with distinct digitiform villi. Spindle muscle attached posteriorly. Two nephridia.

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Alexandra Kerbl and Katrine Worsaae

6.2 Lobatocerebridae Rieger, 1980

Introduction

Lobatocerebridae is an exclusively meiofaunal annelid family with sizes of 1–3 mm in length and 40–110 μm in diameter, comprising an elongated head and a cylindrical, unsegmented body (Fig. 6.2.1 A, B). It contains only two described species: *Lobatocerebrum psammicola* Rieger, 1980 and *Lobatocerebrum riegeri* Kerbl, Bekkouche, Sterrer & Worsaae, 2015 from the shallow waters of the West Atlantic and Red Sea, respectively, and one unde-scribed species (*L*. sp. 1) from fine silty sediment at 800 m depth off Beaufort, North Carolina (Rieger 1980).

Lobatocerebridae have elongated, cylindrical, entirely ciliated bodies, which appear slightly greenish in light microscopes (LMs) due to the glandular epidermis (Fig. 6.2.1 B, F, G). They lack the usual characteristics of annelids such as distinct segmentation, parapodia, chaetae, or nuchal organs. The body may be divided into a head and a trunk region; a distinct prostomium and a pygidium are lacking. The head carries a ventral mouth opening and a large brain and is not clearly separated posteriorly from the uniform trunk or anteriorly from the elongated rostrum, which makes up 20–30% of the total body length. The distinct lobular, large brain with two frontal and four posterior lobes embracing the central neuropil is visible in LM and has prompted the family name (Fig. 6.2.1 B) (Rieger 1980, 1981, 1988, 1991, Kerbl *et al*. 2015). They are hermaphrodites, and the single male gonopore opens mid-dorsally, whereas lateral openings of the seminal receptacles are found in the posterior region of the body, anterior to the dorsal, subterminal anus (Fig. 6.2.1 A, B, D, E) (Rieger 1980, 1981, Kerbl *et al*. 2015).