

Solenogastres, Caudofoveata, and Polyplacophora

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The phylogenetic relationships among the molluscan classes have been debated for decades, but there is now general agreement that the most basal extant groups are the “aplacophoran” Solenogastres (= Neomeniomorpha), the Caudofoveata (= Chaetodermomorpha) and the Polyplacophora. Nevertheless, these relatively small groups, especially the mostly minute, inconspicuous, and deep-water-dwelling Solenogastres and Caudofoveata, are among the least known higher taxa within the Mollusca.

Solenogastres and Caudofoveata are marine, worm-shaped animals. Their body is covered by cuticle and aragonitic sclerites, which give them their characteristic shiny appearance. They have been grouped together in the higher taxon Aplacophora (e.g., Hyman 1967; Scheltema 1988, 1993, 1996; Ivanov 1996), but this grouping is viewed as paraphyletic by others (e.g., Salvini-Plawen 1972, 1980, 1981b, 1985, 2003; Salvini-Plawen and Steiner 1996; Haszprunar 2000; Haszprunar *et al.*, Chapter 2).

SOLENOGASTRES

There are about 240 described species of Solenogastres (Figure 4.1 A–C), but many more are likely to be found (Glaubrecht *et al.* 2005). These animals have a narrow, ciliated, gliding sole located in a ventral groove—the ventral fold or foot—on which they crawl on hard or soft substrates, or on the cnidarian colonies on which they feed (e.g., Salvini-Plawen 1967; Scheltema and Jebb 1994; Okusu and Giribet 2003). Anterior to the mouth is a unique sensory region: the vestibulum or atrial sense organ. The foregut is a muscular tube and usually bears a radula. Unlike other molluscs, the midgut of solenogasters is not divided in compartments but unifies the functions of a stomach, midgut gland, and intestine (e.g., Todt and Salvini-Plawen 2004b). The small posterior pallial cavity lacks ctenidia. The smallest solenogasters measure less than a millimeter in body length (e.g., *Meiomenia swedmarki*, *Meioherpia atlantica*), whereas the largest species are more than 30 cm long (e.g., *Epimenia babai*) and

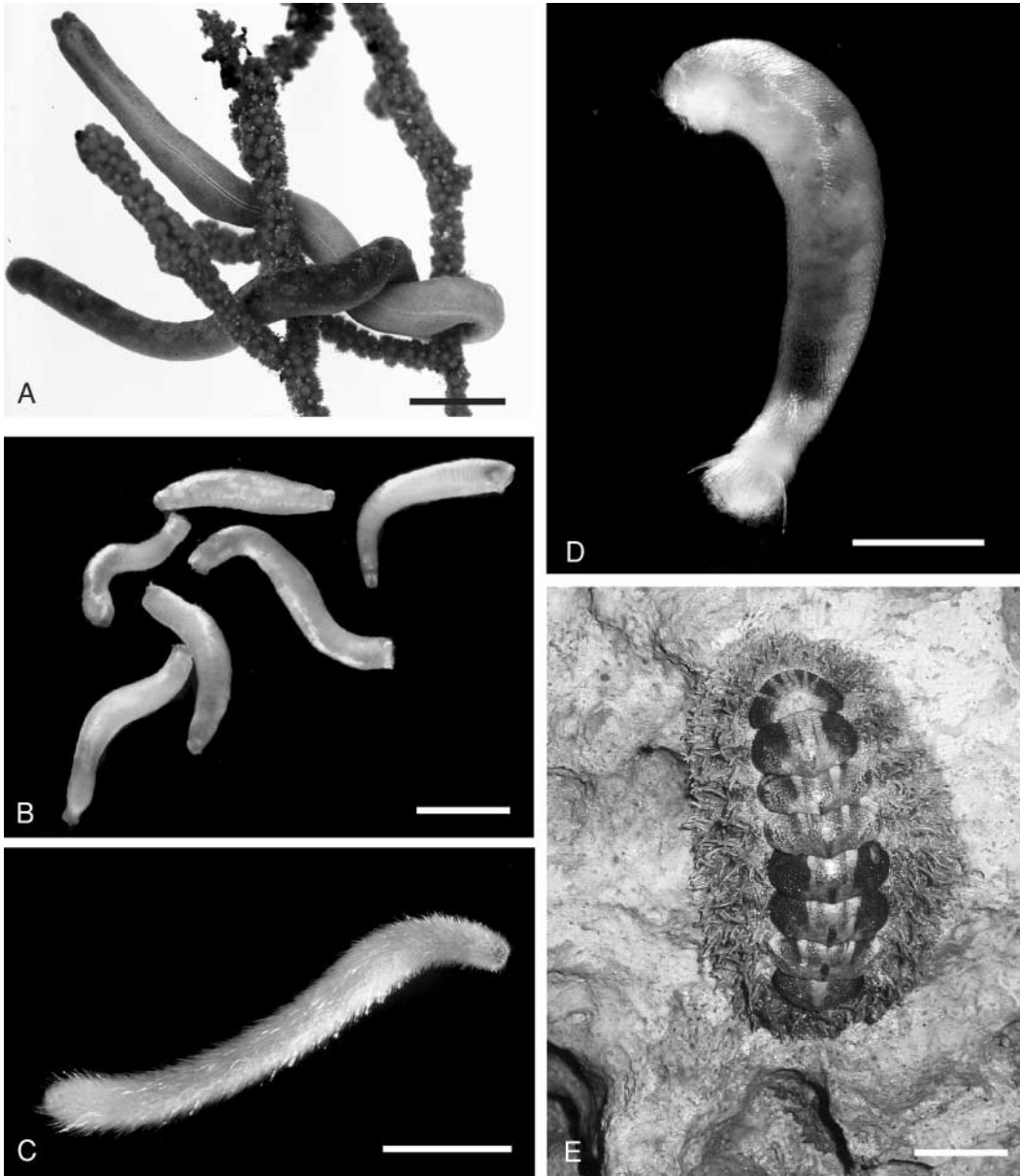


FIGURE 4.1. Living specimens of Solenogastres (A, B, C), Caudofoveata (D), and Polyplacophora (E). (A) *Epimения* n. sp., from Japan, on its gorgonian prey, scale bar: 1 cm; there are blue patches on the dorsal mantle surface. From Okusu 2003. (B) Specimens of *Wirenia argentea* from Galicia (Spain) (micrograph by V. Urganri), scale bar: 1.5 mm. (C) *Biserramenia psammobionta* from Galicia (Spain); note the long epidermal spicules of this interstitial animal (micrograph by V. Urganri), scale bar: 0.25 mm. (D) *Prochaetoderma* sp. from Galicia, Spain; note the terminal knob with fringe of long, pointed sclerites at the posterium (micrograph by V. Urganri), scale bar: 0.5 mm. (E) *Acanthopleura gemmata*, Sulawesi, Indonesia; with long hair-like projections covering the girdle; photo taken in the animal's natural habitat.

often colorful (Okusu 2003). There are a number of overviews of solenogaster morphology (e.g., Thiele 1913; Hoffmann 1930; Hyman 1967; Salvini-Plawen 1971, 1978, 1985) and microscopic anatomy (Scheltema *et al.* 1994), and there are

some comprehensive studies that focused on the histology of the integument (Hoffman 1949) or the histology or physiology of the digestive tract (Baba 1940a; Salvini-Plawen 1967, 1981a, 1988; Scheltema 1981).

CAUDOFOVEATA

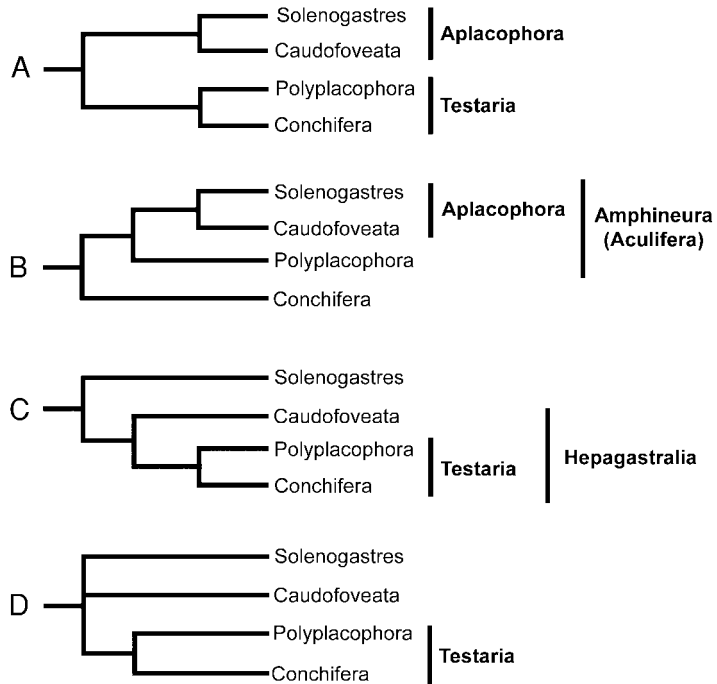
In Caudofoveata, a ventral groove and foot are lacking (Figure 4.1D). The mouth opening is partly or entirely surrounded by an oral shield (foot shield), an area covered by a thick layer of cuticle without sclerites. Caudofoveates are infaunal and feed on detritus or selectively on foraminiferans by burrowing in the mud with their oral shield. They have a muscular foregut bearing a radula, and their posterior midgut is divided into a dorsal tubular region (midgut duct) and a ventral midgut sac. The small, posterior pallial cavity bears a pair of true ctenidia. Caudofoveates range in length from a few millimeters (e.g., *Prochaetoderma raduliferum*; *Falcidens sterreri*) to 14 cm (e.g., *Chaetoderma productum*). Three major body regions are defined: anterium, trunk, and posterium. The latter may consist of a narrow shank and a terminal knob with characteristic elongate sclerites (typical for Prochaetodermatidae). With very few exceptions (e.g., *Chaetoderma rubrum*), caudofoveates are beige to brownish in color. About 120 species have been described so far (Glaubrecht *et al.* 2005). In caudofoveates, aside from the sometimes highly specialized radula, the variation in structure and arrangement of internal organs is limited, and knowledge of internal anatomy is mostly based on older studies (e.g., Wirén 1892; Thiele 1913; Hoffmann 1930; van Lummel 1930; Hyman 1967; Salvini-Plawen 1971, 1975, 1985; Scheltema *et al.* 1994).

POLYPLACOPHORA

The monophyly of Polyplacophora has been well established (most recently Okusu *et al.* 2003), even if in a recent molecular analysis a species of monoplacophoran appears to be nested within the chitons (Giribet *et al.* 2006). The name Polyplacophora dates back to Gray (1821), but the term Placophora, which was first used by von Ihering (1876), is common, too, especially in German literature. The latter term is also used informally (e.g., Lindberg and Ponder 1996; Parkhaev, Chapter 3) to encompass the Aplacophora, Polyplacophora, and mollusc-

like fossil taxa. The general morphology of Polyplacophora, with some information on histology, was described by Plate (1897, 1901), Hyman (1967), Kaas and Van Belle (1985), and Wingstrand (1985). Information on their microscopic anatomy was compiled by Eernisse and Reynolds (1994). These animals, commonly referred to as chitons, are dorsoventrally flattened, exclusively marine molluscs characterized by the presence of eight dorsal aragonitic shell plates (valves) and a broad ventral ciliated foot (Figures 4.1E, 4.6A). The likewise ventrally positioned head is separated from the foot by a transverse groove. Surrounding the dorsal shell plates—or even completely engulfing them in some species—there is a thick marginal girdle (perinotum) covered by a chitinous cuticle. Embedded in this cuticle are calcium carbonate sclerites (Figure 4.6B), which are only occasionally lacking, and sometimes the cuticle additionally bears corneous processes (e.g., in *Chaetopleura*). The shell plates display a complex morphology and are composed of four layers: properiostracum, tegmentum, articulamentum, and myostracum. The articulamentum projects anteriorly and laterally beyond the tegmentum to form the sutural laminae and insertion plates. The shell plates characteristically bear so-called aesthetes, unique photo- and probably also mechano- and chemosensory organs and in certain taxa ocelli (Figure 4.6C). The head in general lacks eyes and tentacles, but the mouth opening is laterally flanked by mouth lappets. Occasionally (e.g., in the genus *Placiphorella*) precephalic tentacles, which support the animal while feeding, may occur. The mantle cavity or pallial groove surrounds the foot and accommodates the terminal anal papilla, a multitude of laterally positioned ctenidia, the paired osphradium, and lateral sense organs. Chitons have complex muscle systems, including eight paired sets of dorsoventral muscle units that insert at the shell plates, the musculus rectus, which runs longitudinally underneath the shell plates, and a circular enrolling muscle. Usually chitons are grazers with a broad and exceptionally long stereoglossate radula (Figure 4.6D).

FIGURE 4.2. Alternative phylogenies for the placement of Solenogastres, Caudofoveata and Polyplacophora relative to Conchifera. (A) Aplacophora and Testaria as sister groups (after Waller 1998). (B) Aplacophora as Monophylum, Amphineura (Aculifera) sister group to Conchifera (after Ivanov 1996; Scheltema 1996). (C) Solenogastres and Caudofoveata as independent clades with Solenogastres branching earliest (after most parsimonious tree in Salvini-Plawen and Steiner 1996), additionally Caudofoveata grouping with Testaria into Hepagastralia (after Haszprunar 2000). (D) Solenogastres and Caudofoveata as independent clades with unresolved relationship to Testaria (after Salvini-Plawen and Steiner 1996; Salvini-Plawen 2003).



Their diet consists mainly of diatoms, detritus, and encrusting algae, but special feeding habits have been adopted by the carnivorous *Placiphorella* and *Lepidozona* (Latyshev *et al.* 2004), the xylophagous *Ferreiraella* (Sirenko 2004), or the true herbivorous *Stenochiton*. There are about 920 living species (Schwabe 2005), most living in the marine intertidal or sublittoral, with some deep-sea species also known (Kaas *et al.* 1998).

RELATIONSHIPS The two aplacophoran taxa and Polyplacophora were, and still are, considered by most morphologists as basal within Mollusca, preceding the conchiferan radiation, although their relative placement varies between proposed hypotheses (Figure 4.2A–D). In one scheme, Solenogastres and Caudofoveata have been incorporated in the phylum Aplacophora, the sister group of a clade Testaria consisting of Polyplacophora and Conchifera (Waller 1998). Alternatively, based on similarities in their nervous system, Polyplacophora was considered to be the sister group to Aplacophora, the two together forming the Amphineura (von Ihering

1876a, b; Spengel 1881; Hoffmann 1930), while a clade Aculifera was proposed for those groups having a cuticle with sclerites covering at least part of the mantle (e.g., Hatschek 1891; Scheltema 1988, 1996; Ivanov 1996). Other authors have argued that aplacophorans are paraphyletic with respect to a clade Testaria, comprising the remaining molluscs, within which Polyplacophora is a sister taxon to Conchifera (e.g., Wingstrand 1985; Salvini-Plawen 1980, 1985, 1990, 2003; Salvini-Plawen and Steiner 1996). Based on midgut morphology, Haszprunar (2000) additionally defines the clade Hepagastralia for Caudofoveata plus Testaria. A sister group relationship of Polyplacophora with Conchifera is often assumed in studies of conchiferan relationships (e.g., Giribet and Wheeler 2002), but it is also questioned (e.g., Lindberg and Ponder 1996).

Recent discoveries of sclerite-bearing fossils (see following discussion), additional developmental work with new techniques, and recent morphological and molecular studies have shed new light on molluscan origins and the evolution of Solenogastres, Caudofoveata, and

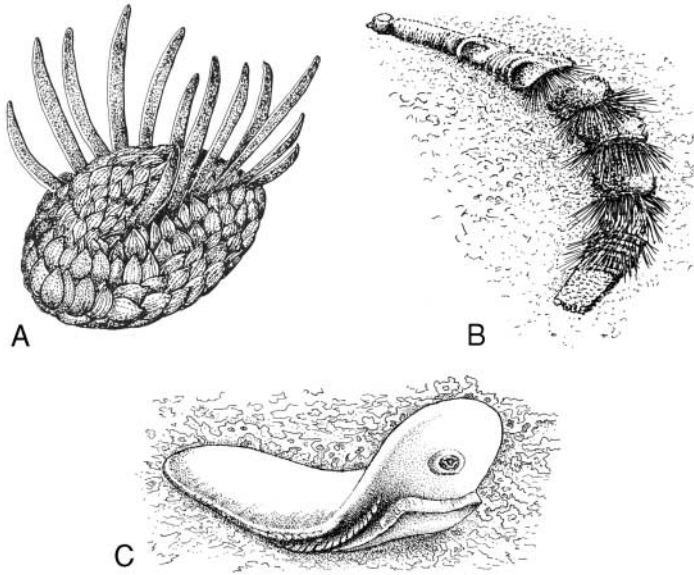


FIGURE 4.3. Fossils interpreted as early molluscs (drawings by C.-O. Schander). (A) *Wiwaxia corrugata* from the Burgess Shale. (B) The Silurian *Acaenoplax hayae*. Note the rows of acicular skeletal elements seen either as aculiferan-like sclerites or as polychaete-like chaetae. (C) The Cambrian *Odontogriphus omalus* from the Burgess Shale.

Polyplacophora. Here, we revisit some old hypotheses and review the newest findings for these fascinating groups.

THE FOSSIL RECORD AND MOLLUSCAN ORIGINS

Molluscan lineages probably extended at least as far back as the base of the Cambrian (543 Mya) (Glaessner 1969; Runnegar and Pojeta 1985; Bengtson 1992) or the Upper Precambrian, if trail-like impressions in the Ediacaran strata are correctly interpreted as traces left by the ventral muscular foot of *Kimberella quadrata*, a limpet-like animal with possibly molluscan affiliations (Fedonkin and Waggoner 1997; see also Parkhaev, Chapter 3). The known polyplacophoran fossil record extends from as early as the Upper Cambrian (Yochelson *et al.* 1965; Runnegar *et al.* 1979; Yates *et al.* 1992; Stinchcomb and Darrough 1995; Sliker 2000). No fossil aplacophorans are known, and thus there is no direct evidence of the time of origin of solenogasters or caudofoveates.

Recently, increasing numbers of problematic sclerite-bearing metazoans from the Early and Middle Cambrian have been discovered and assigned a taxonomic placement close to, or within, Mollusca. These animals have been compared to aplacophorans and polyplacophorans

based on their sluglike appearance, muscular ventral foot, dorsal calcification patterns, gill arrangement, and the possible presence of a radula (e.g., Conway Morris and Peel 1995, Caron *et al.* 2006). Controversies still remain as to which extant Metazoa these fossils are most closely related, but, in any case, they assist in understanding the evolution of external calcification in Mollusca as well as in Metazoa in general.

The Middle Cambrian *Wiwaxia corrugata* from the Burgess Shale (Figure 4.3A) was considered “strikingly similar” to molluscs on the basis of body shape and the radular-like feeding apparatus (Conway Morris 1985). This radular-like structure was interpreted as homologous to the radula of an extant solenogaster *Helicoradomenia* (Scheltema 1998, Scheltema *et al.* 2003). Analyses of *Wiwaxia*’s sclerites, however, have led to doubts as to its molluscan affinity (Butterfield 1990, 1994; Watson Russel 1997) because the solid wiwaxiid sclerites were originally chitinous and had longitudinal ornamentation on their dorsal side, much like chrysopetalid polychaete paleae (specialized chaetae). The most recent and, so far, the most comprehensive study of the phylogenetic placement of *Wiwaxia*, was carried out by Eidbye-Jacobsen (2004). He found “no characters that could indicate any close relationship with Polychaeta or Annelida.”

Thus, a molluscan affinity of *Wiwaxia* again seems plausible.

The Cambrian *Odontogriphus omalus* (Figure 4.3C) was originally interpreted as a lophophorate or with a possible connection to some Cambrian conodonts (Conway Morris 1976). This interpretation was based on a single specimen in rather poor condition. Newly discovered specimens from the Burgess Shale allowed a reinterpretation of this fossil, revealing several characters with possible molluscan affinity (Caron *et al.* 2006a; but see also Butterfield 2006 and Caron *et al.* 2006b). *Odontogriphus* was a dorsally-ventrally compressed, elongated animal with an oval body up to 12 mm long. It had a muscular foot lined by simple gills and a ventral mouth with a radula strikingly similar to that of *Wiwaxia*. There is also indications of a pair of salivary glands. It lacked a mineralized shell and sclerites. It was most likely a bacterial grazer feeding on the cyanobacterium *Morania*. The general shape of the body is suggestive of the Precambrian fossil *Kimberella* as well as the Cambrian *Wiwaxia*.

Another fossil repeatedly referred to as a mollusc is the Early Cambrian *Halkieria* from northern Greenland. The first entire articulated halkieriid to be discovered, *Halkieria evangelista*, is described as a long flat animal with a ventral creeping sole, dorsal sclerites, and two terminal valves (Conway Morris and Peel 1990, 1995). Although it possesses several mollusc-like characters, Conway Morris and Peel (1995) placed *Halkieria* either as the sister group to Annelida or within Brachiopoda. Halkieriid sclerites are filled with phosphate, leading to speculation that they originally were filled with tissue and formed by “external mineralization of a protruding organic template,” like polychaete chaetae (Butterfield 1990; Bengtson 1992; Conway Morris and Peel 1995). The mode of sclerite formation was thought by Bengtson (1992, 1993) to be in contrast to that in aplacophorans and polyplacophorans, where sclerites are produced by invagination of a single cell or, in chitons, by invaginated groups of cells (Haas 1981; Eernisse and Reynolds 1994). However, there

are certain hollow aplacophoran sclerites that form around an organic template protruding from the sclerite-producing cell (Hoffman 1949; Okusu 2002). Moreover, the terminal valves and sclerites of *Halkieria* may have been aragonitic and thus similar in their mineralogical composition to those of aplacophorans and polyplacophorans (Eernisse and Reynolds 1994; Vinther and Nielsen 2005). Similarities between the three distinct types of sclerites in halkieriids (siculates, cultrates, and palmates) and wiwaxiids (ventro-laterals, upper-laterals, and dorsals) and the zones of sclerites in aplacophorans and polyplacophorans have been noted (Conway Morris and Peel 1990; Bengtson 1992; Conway Morris and Peel 1995; Scheltema 1998). Scheltema and Ivanov (2002) also suggested that the serially clustered siculate sclerites in *Halkieria* are homologous to the seven transverse regions devoid of sclerites seen in a solenogaster postlarva. Most recently, Vinther and Nielsen (2005) convincingly demonstrated the molluscan affinity of *Halkieria*. They stressed a number of similarities between *Halkieria* and Polyplacophora, such as the overall morphology and sclerite arrangement, but the terminal valves of *Halkieria* are different from polyplacophoran valves and more similar to conchiferan shells in lacking a tegmentum layer and pore canals. The hollow siculate sclerites also show resemblance to those in the fossil polyplacophoran *Echinochiton* (Pojeta *et al.* 2003). Consequently, the new class Biplacophora for molluscs with two shell plates and a covering of sclerites was introduced (Vinther and Nielsen 2005), and the class Coeloscleritophora, a taxon that used to unify a number of fossils with hollow sclerites (Bengtson and Missarzhevsky 1981), was declared to be polyphyletic.

Intrepretations of *Halkieria* as a mollusc have raised further questions regarding the evolution of shell and sclerites among molluscs. It has been suggested that polyplacophoran and halkieriid valves are formed through coalescence of calcium carbonate sclerites (Pojeta 1980; Salvini-Plawen 1985; Eernisse and Reynolds 1994). The extant chiton, *Acanthochitona*, and

the fossil ?coeloscleritophoran? *Maikhanella*, both have valves with scaly sculptures that appear to be composed of merged neighboring sclerites (Bengtson 1992). Other spicule-bearing fossils, however, lack those sculptures. The valves (shells) of *Maikhanella* have been suggested to grow by marginal accretion and their sclerites by interposition, just as occurs in some molluscs and was suggested for halkieriids (Bengtson 1992; Conway, Morris, and Peel 1995), but new findings on a Recent vetigastropod, *Vacerrina kesteveni*, show that a scaly shell-surface may be a calcified periostracal sculpture (Ponder *et al.* 2007). Scheltema (1998) doubts that chiton valves are formed by coalescence of calcium carbonate sclerites and points out that seven transverse regions devoid of sclerites in a solenogaster postlarva (see preceding paragraph) may be homologous to the chiton larval shell fields. If chiton valves are not formed by coalescence of sclerites, they may have originated simply through modification of spicular calcification mechanisms (Carter and Hall 1990), necessitating only a simple step in the evolution of a shell from sclerites (Scheltema and Schander 2006).

The exceptionally well-preserved Silurian *Acaenoplax hayae* (Figure 4.3B) was thought to be related to aplacophorans (Sutton *et al.* 2001a, b, 2004). *Acaenoplax* is a vermiform fossil with about 18 iterated rows of ridges bearing needle-shaped sclerites similar to those in annelids, seven dorsal calcareous plates similar to those in chitons, a single posterior ventral plate, and posterior gills (Sutton *et al.* 2001a, b). Its seven dorsal valves and single ventral valve have been interpreted to be homologous with valves 1–6 and 8 of chitons and with the seven dorsal transverse regions free of sclerites in an aplacophoran postlarva (Scheltema and Ivanov 2002; see preceding paragraphs). Although this may seem to corroborate the Aculifera hypothesis, this placement was challenged by Steiner and Salvini-Plawen (2001), who suggested that an annelid affinity of *Acaenoplax* was just as likely because of its lack of explicit molluscan characters and overall similarity to some Recent tube-dwelling annelids.

Hoare and Mapes (1995) discussed the Devonian problematic taxon *Strobilepis* from the Moscow Formation in New York (United States) and introduced a new Carboniferous (Pennsylvanian) problematic genus *Diadeloplax*, from the Gene Autry Formation in Oklahoma (United States). These two genera were placed in the new family Strobilepidae and new class Multiplacophora whose phylum assignment remained uncertain. They noted that multiplacophorans characteristically have 12 plates that have diverse shapes and, at least in part, lack bilateral symmetry, and that small auxiliary plates are always associated with larger intermediate plates. The recent discovery of an exceptionally well-preserved specimen of another multiplacophoran, *Polysacos vickersianum*, from the Carboniferous of Indiana (United States), enabled a more accurate reconstruction of the body plan of this group (Vendrasco *et al.* 2004). The animal is very similar to a chiton in body shape and bears 17 shell plates and a lateral fringe of spines. Both plates and spines are most likely homologous to polyplacophoran valves, and the valves are articulated as in modern chitons. The oldest multiplacophoran fossils are Devonian and thus much younger than the oldest chitons (see following discussion). Vendrasco *et al.* (2004) place the multiplacophorans as an order within the Polyplacophora, implying an early divergence from the eight shell plate plan in Polyplacophora. It is possible that changes in the number and patterning of shell plates involved only small changes in homeobox genes, analogous to the changes that have occurred in the relative number of vertebrae in modern snakes (Cohn and Tickle 1999; Wiens and Slingluff 2001). Although it has been shown that homeobox genes are involved in the patterning and formation of modern chiton shell plates (Jacobs *et al.* 2000), details have not yet been investigated.

The oldest polyplacophoran fossils are known from the Upper Cambrian (Yates *et al.* 1992), and since then, with exception of multiplacophorans, their general body plan and valve morphology did not change significantly. This

is confirmed by findings of numerous complete articulated specimens, such as *Glaphurochiton concinnus* from the Carboniferous of Illinois (United States) (Yochelson and Richardson 1979). Fossil plates, however, show that the occurrence of microaesthete structures must be interpreted as a post-Paleozoic innovation (Hoare 2000).

Smith and Hoare (1987) divided the Polyplacophora into three subclasses: Paleoloricata, Phosphatoloricata, and Neoloricata. Later (Sirenko 1997) followed Bergenhayn (1955) and Van Belle (1983) in accepting two lineages within the Polyplacophora: Paleoloricata and the more derived, articulamentum-bearing Neoloricata (or Loricata in Sirenko 1997). All extant chitons belong to Neoloricata, whereas fossil forms are classified within both groups. In the Neoloricata there are Cenozoic and Mesozoic taxa, while only Paleoloricata are known from the Paleozoic. Sirenko (1997) recognized four orders, including five suborders and 14 families from the Paleozoic. Hoare (2000) suggested minor changes in the system but otherwise accepted Sirenko's conclusions. Nevertheless, a few problems with uncertain affiliations to Polyplacophora still exist, such as *Luyanhaochiton* from the Lower Cambrium of China (Hoare 2000; see also Parkhaev 2007, Chapter 3).

DEVELOPMENT

Studies on the early embryology and development of aplacophoran molluscs are rare, and thus comparisons with other molluscan classes remain difficult (for review see Verdonk and Van den Biggelaar 1983; Buckland-Nicks *et al.* 2002). Knowledge of the development of Solenogastres is restricted to a few species, whereby the early studies of Pruvot (1890), Heath (1918), and Baba (1938, 1940b) were only recently added to by Okusu's (2002) work on the embryogenesis and development of *Epimenia babai*. This description of early embryogenesis revealed that cleavage is spiral, unequal, and holoblastic. Solenogastres are hermaphrodites with internal fertilization and

have free-swimming, lecithotrophic larvae with an enlarged swimming test (pericalymma) with differing numbers of rows of ciliated prototrochs. The apical test of *E. babai* larvae is completely ciliated with an apical tuft and a single prototroch composed of compound cilia (Figure 4.4A). It is lost during metamorphosis. The pericalymma test is often regarded as homologous to the enveloping test of protobranch bivalve larvae as well as to the velum of bivalve and gastropod veliger larvae (for review see Nielsen 2004). Homology of these structures remains uncertain, and either they are interpreted as similarly modified apical structures evolved from a basic trochophore specialized in swimming (Jaegersten 1972; Nielsen 1987, 2004) or the pericalymma test is seen as a primitive trait within the Mollusca (Salvini-Plawen 1972, 1980, 1988; Chaffee and Lindberg 1986). The trunk region of the larvae is unciliated and gives rise to definitive ectodermal structures, such as cuticle, epidermis, and epidermal sclerites. No external metameric iteration can be found at any stage, and there is no evidence of protonephridia.

Earlier findings (Nielsen 1995, 2004) have been recently supported by a thorough study on *Chaetoderma* employing electron microscopy and fluorescence staining of musculature (Nielsen *et al.* 2007). This study shows lecithotrophic (pseudo-)trochophore larvae with a prototroch and a telotroch and a pair of protonephridia. In the later stages, a ventral suture and seven dorsal transverse rows of spicules are present.

Chiton embryos, as studied to date, undergo equal cleavage in a typical spiralian pattern (Heath, 1899; Grave, 1932; Van den Biggelaar, 1996). The resulting trochophore larvae are lecithotrophic and possess a unique prototroch composed of two to three irregular rows of differentially ciliated trochoblasts, as shown for *Chiton polii* (see Kowalevsky 1883), *Ischnochiton rissoi* (see Heath 1899), *Lepidopleurus asellus* (see Christiansen 1954), and *Chaetopleura apiculata* (see Henry *et al.* 2004). The free-swimming larval stage ranges from a few minutes to a few days. After settlement, the

apical tuft and the prototroch may persist for a while. Metamorphosis starts with a dorsoventral flattening of the body. A detailed summary of larval development in chitons was presented by Buckland-Nicks *et al.* (2002).

The relationship of larval shell formation with expression of the *engrailed* gene has been reported in various molluscs (Wray *et al.* 1995), as has the expression of this gene in cells adjacent to the shell fields in chiton larvae (Jacobs *et al.* 2000).

A first cell lineage study (Henry *et al.* 2004) pointed out that polyplacophoran epidermal sclerites arise from different, if overlapping, sets of cells than the shell plates and the conchiferan shell, an important finding for consideration of the evolution of molluscan shells. The same study demonstrated that the larval ocelli of *Chaetopleura apiculata* develop post-trochally from a unique set of cells not seen in other spiralian.

Detailed investigations of myogenesis using fluorescent markers during the early development of chitons showed that serial muscle structures and dorsal shell plates do not develop simultaneously (Friedrich *et al.* 2002; Wanninger and Haszprunar 2002). This indicates that hypotheses indicating a sister taxon relationship between molluscs and other segmented protostomes such as Annelida, based on the serial repetition of organs (e.g., Götting 1980; Ghiselin 1988; Nielsen 1995), are not supported.

PHYLOGENY AND SYSTEMATICS

SISTER GROUP RELATIONSHIPS

Although a number of attempts have been made to resolve molluscan phylogeny using both morphological and molecular sequence data, there has not yet been any consensus on the position of aplacophoran taxa and Polyplacophora within Mollusca (see Figure 4.2).

One problem with most phylogenetic studies is the lack of a representative taxon sampling for the basal clades (Ghiselin 1988; Winnepenninckx *et al.* 1994, 1996; Rosenberg *et al.* 1997;

Lydeard *et al.* 2000; Giribet and Wheeler 2002). There are only a few molecular analyses that have included representatives of Solenogastres (Okusu 2003; Okusu *et al.* 2003; Passamanek *et al.* 2004; Giribet *et al.* 2006) and Caudofoveata (Winnepenninckx *et al.* 1994; Okusu 2003; Okusu *et al.* 2003; Passamanek *et al.* 2004; Giribet *et al.* 2006). Obtaining DNA sequence data has been challenging for aplacophoran taxa because they are difficult to collect and because of contamination issues in Solenogastres (Okusu and Giribet 2003). In an investigation of molluscan phylogeny using large-subunit and small-subunit nuclear rRNA sequences of 33 molluscan taxa, including a solenogaster, a caudofoveate, and four chitons, neither the Aculifera hypothesis nor the Testaria hypothesis is supported (Passamanek *et al.* 2004). In this study, Polyplacophora does not emerge as a basal clade, and it groups only in some of the analyses with Solenogastres and never with Caudofoveata. A recent analysis of five genes and gene fragments from 101 species representing all molluscan classes shows Solenogastres and Caudofoveata as independent clades near the base of the tree but Polyplacophora as more derived and forming a clade (Serialia) with Monoplacophora (Giribet *et al.* 2006).

Recent attempts to study chiton phylogenetic relationships using several combined genes (Okusu *et al.* 2003) resulted in a well-resolved phylogeny of chitons but could not resolve the placement of chitons relative to Solenogastres, Caudofoveata, and Conchifera.

The notion of a basal position of Solenogastres, Caudofoveata, and Polyplacophora was recently supported by Lundin and Schander's studies on the ultrastructure of locomotory cilia in Solenogastres (2001b), Caudofoveata (1999), and Polyplacophora (2001a). These cilia are of the common metazoan type, with paired ciliary rootlets orientated at almost 90° to each other and without an accessory centriole. Such paired ciliary rootlets do not occur in gastropods, bivalves, and monoplacophorans (Lundin and Schander 2001b), nor in scaphopods (Lundin and Schander, unpublished data).

SYSTEMATICS AND PHYLOGENY OF APLACOPHORAN MOLLUSCS

Histology has been the standard method used for species identification and classification in aplacophorans, mostly because of their small size, lack of a shell, and often poor preservation of sclerites in non-buffered fixatives. Thus, the morphological and histological data available for Solenogastres and Caudofoveata are surprisingly detailed compared to other molluscan taxa. External characters are sufficient for a species diagnosis in many caudofoveates but only in relatively few solenogasters. However, the addition of internal hard-part characters (radula and copulatory stylets), usually allows identification of members of both groups (Scheltema and Schander 2000), but knowledge of anatomical and histological characters is of great importance for systematics and phylogenetic analyses.

In both Solenogastres and Caudofoveata, classification is based on comprehensive publications by Salvini-Plawen (1975, 1978). Some recent additions have been made and doubts on the monophyly of certain clades raised (e.g., Scheltema 1999), but the general concepts remain unchallenged.

SOLENOGASTRES Solenogaster higher classification uses external characters, such as types of sclerites (solid elements versus hollow elements, flat scales versus rimmed or trough-like elements), thickness of the cuticle, and general characteristics of the lateroventral foregut glands. Four orders were recognized by Salvini-Plawen (1978) (see also Figure 4.8):

Pholidoskepia: Cuticle is thin, sclerites are scales in one layer, lateroventral foregut glands are either endoepithelial (no glandular duct) or with duct and exoepithelial gland cells (e.g., Wirenidae, Dondersiidae, Lepidomeniidae).

Neomeniamorpha: Cuticle is thin; sclerites are scales, massive acicular elements, rimmed, trough-like, and harpoon-shaped elements; no lateroventral foregut glands present (e.g., Neomeniidae, Hemimeniidae).

Sterrofustia: Cuticle is thick, sclerites are solid acicular or scalelike elements, lateroventral foregut glands are diverse (e.g., Phyllomeniidae, Imeroherpiidae).

Cavibelonia: Cuticle is thick, sclerites are hollow acicular elements, additional solid elements may occur, lateroventral foregut glands are diverse and include tubular glands with intraepithelial glandular cells (e.g., Pararrhopaliidae, Rhopalomeniidae, Simrothiellidae, Epimeniidae).

Solenogaster phylogenetics still struggles with the great diversity of hard-part as well as soft-body characters among the families and with the lack of a general concept as to the plesiomorphic character states. Most phylogenetic analyses based on morphology (e.g., Scheltema and Schander 2000) have included only a limited number of taxa. A recent comprehensive study of solenogaster phylogeny based on morphological characters included all genera (Salvini-Plawen 2003). Although poor resolution was obtained, Cavibelonia was monophyletic and derived (see also Salvini-Plawen 2004), whereas Pholidoskepia emerged from a basal polytomy. Handl and Todt (2005) discussed the evolution of foregut glands in solenogasters and the so-called *Wirenia*-type lateroventral foregut glands (Figure 4.4B, a), without a duct or lumen, seen in the pholidoskepien Gymnomeniidae, were considered to be the most primitive extant type. *Pararrhopalia*-type glands (Figure 4.4B, c) occur in some Pholidoskepia and Cavibelonia taxa, while certain gland types (e.g. *Helicoradomenia*-type, Figure 4.4B, d; *Simrothiella*-type, Figure 4.4B, e) occur in Cavibelonia only.

Due to the ontogenetic change from solid sclerites to hollow needles seen in some species, the hollow epidermal sclerites are considered derived, thus ruling out Cavibelonia as a basal clade. Hollow needles, however, also occur in the Acanthomeniidae, a taxon closely related to pholidoskepien taxa, such as the Dondersiidae (Salvini-Plawen 2003; see also Scheltema 1999, Handl and Salvini-Plawen 2001). Thus the

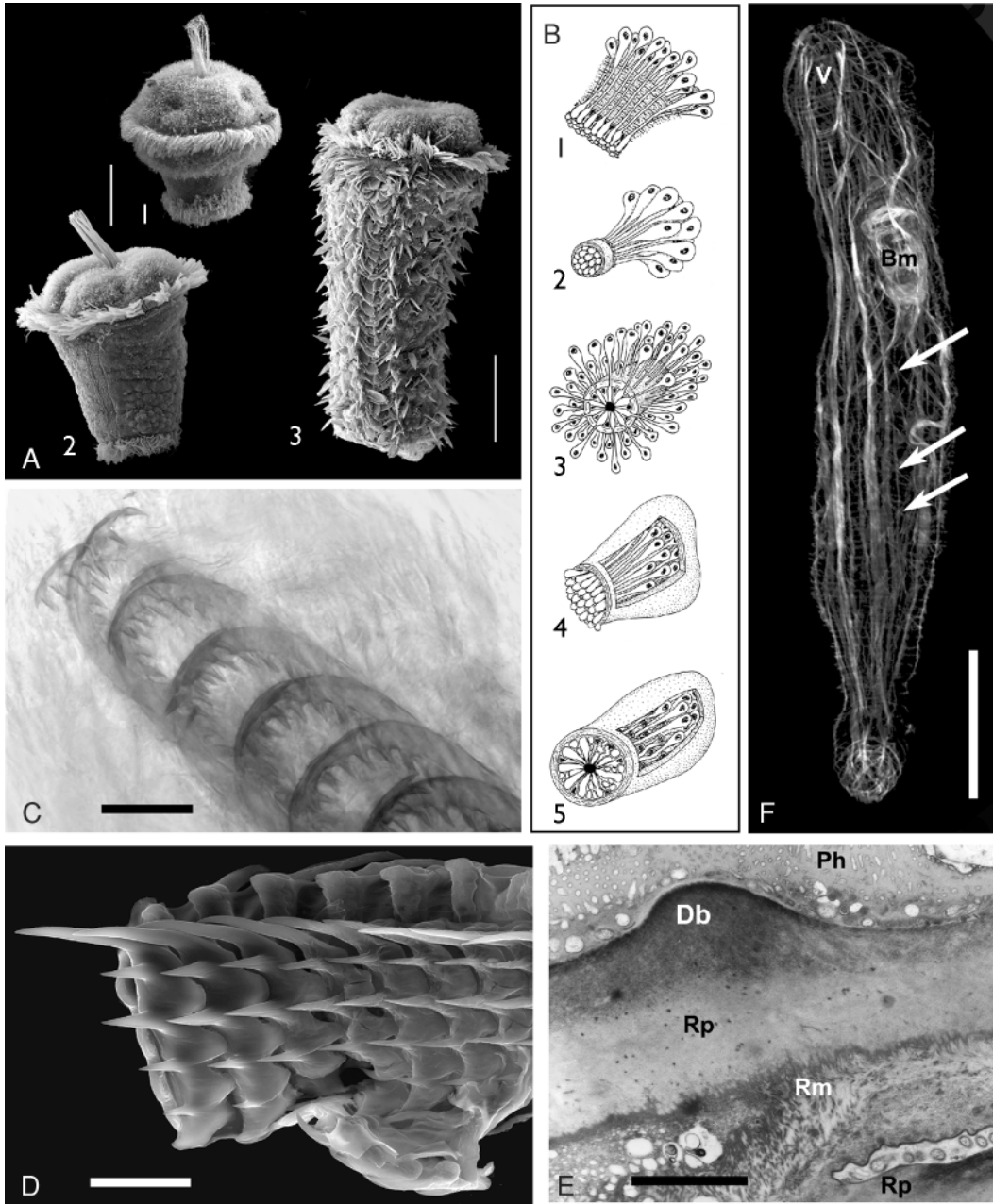


FIGURE 4.4. Solenogastres and Caudofoveata, development and important characters. (A) Larvae of *Epimения babai* (Solenogastres) during the completion of metamorphosis, 1: 4–6 days old, 2, 3: 9–12 days old; scale bar: 100 µm. From Okusu 2002. (B) Examples for lateroventral foregut glands of Solenogastres, 1: *Wirenia*-type, 2: *Meioherpia*-type, 3: *Pararrhopalia*-type, 4: *Helicoradomenia*-type, 5: *Simrothiella*-type. (C) Radula of *Scutopus robustus* (Caudofoveata), light micrograph; scale bar: 50 µm. (D) Part of the right half of a radula of *Helicoradomenia* sp. (Solenogastres), scanning electron micrograph; scale bar: 20 µm. (E) Ultrathin section of a radular plate of *Helicoradomenia acredema*, transmission electron micrograph; scale bar: 20 µm. (F) Confocal scanning micrograph of Alexa-phalloidin stained *Meioherpia atlantica*, the arrows indicate spiral muscle fibers of the body wall; Bm = buccal musculature; V = vestibulum; scale bar: 0.1 mm.

homology of certain hollow sclerites may be questioned (Salvini-Plawen 2003).

Attempts toward a phylogeny of solenogasters by means of molecular methods has been hampered by technical problems (see Okusu and Giribet 2003), but refined techniques and intensified efforts should provide results in the near future.

CAUDOFOVEATA This taxon is less diverse than Solenogastres, with only three or four families recognized, which are based on characters of the radula, mouth-shield, and body shape (Salvini-Plawen 1975, but see Ivanov 1981) (see Figure 4.8):

Limifossoridae: Radula is bipartite, of several transverse rows, without lateral supports; body is homogeneously shaped; mouth shield is disk- or U-shaped posterior of mouth opening, or paired lateral to mouth opening.

Prochaetodermatidae: Radula is bipartite, in several transverse rows, with ventral and lateral supports; posterior body is tail-shaped, mouth shield is paired lateral to mouth opening.

Chaetodermatidae: Radula is generally represented by only one pair of teeth, with large ventral and lateral supports; body is homogeneously shaped or posterior body is tail-shaped; mouth shield is U-shaped posterior to mouth opening or encircling mouth opening.

An additional family, Scutopodidae, was introduced by Ivanov (1981) but was rejected by Salvini-Plawen (e.g., 1992), who included *Scutopus* within Limifossoridae.

There are no modern phylogenetic analyses published for Caudofoveata. *Scutopus* and *Psilodens* are probably the most basal genera because some species have traces of a retained ventral suture innervated from the ventral nerve cords, as well as primitive radular (distichous pairs of teeth with median denticles; Figure 4.4C) (Salvini-Plawen 1975, 1985, 1988) and midgut configuration (Scheltema 1981; for *Psilodens* see Salvini-Plawen 1988, 2003). In contrast, Chaetodermatidae have a highly derived radula,

usually a single pair of teeth with prominent lateral and ventral supports, and the stomach has a gastric shield. The radula of Prochaetodermatidae appears to represent an intermediate state (Salvini-Plawen and Nopp 1974; Salvini-Plawen 1975; fig. 6, and slightly modified in 1988: fig. 1), but the phylogenetic relationship between Prochaetodermatidae and the other families is not well resolved (see Figure 4.8).

MORPHOLOGICAL CHARACTERS Over the last few decades, modern techniques, such as scanning and transmission electron microscopy, have provided new insights into the morphology and histology of solenogasters and caudofoveates and helped to further define characters valuable for systematics and phylogeny. Some recent studies are summarized as follows.

Haszprunar (1986, 1987) supported the homology of the dorsoterminal sense organ (DTS) in Solenogastres and Caudofoveata with the usually paired osphradia of chitons and higher molluscs but suggested an independent origin of the unpaired condition of the DTS in the two aplacophoran taxa.

In both solenogasters and caudofoveates, the mantle sclerites exhibit extraordinary variability in size and shape, but certain sclerite types are characteristic at higher taxonomic levels (e.g., the hollow hooklike elements of Pararrhopaliidae). Information on sclerite thickness can be gained by the use of cross-polarized light or by scanning electron microscopy (Scheltema and Ivanov 2000, 2004). Because they vary according to their location, sclerites should be sampled from standardized body regions for taxonomic purposes (e.g., Scheltema 1976, 1985; Scheltema and Ivanov 2000).

Scheltema *et al.* (2003), in a review of the radula of basal molluscs, presented a theory on the nature of the primitive molluscan radula. Like Eernisse and Kerth (1988), she argued that the most basal type was the distichous or bipartite radula with rows of paired radular plates. This type is present in the solenogaster genus *Helicoradomenia* (Figure 4.4D) and the caudofoveate genus *Scutopus* (Figure 4.4C). In

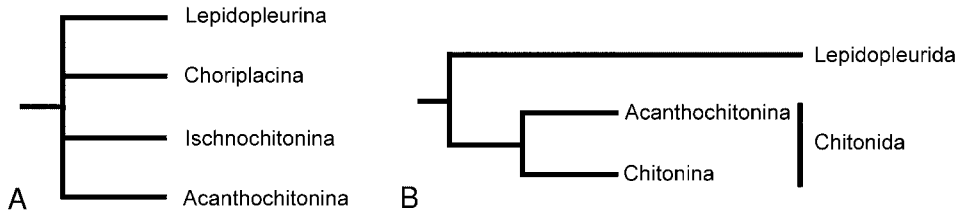


FIGURE 4.5. Alternative phylogenies for Recent Neoloricata. (A) Unresolved tree based on Kaas and Van Belle (1994) and Kaas *et al.* (1998). (B) Phylogenetic tree after Sirenko (1993, 1997).

contrast, Salvini-Plawen (1988, 2003; see also Sirenko and Minichev 1975), suggested that the monoserial radula type, consisting of rows of single teeth, was the most primitive. Wolter (1992) showed that radular formation in aplacophoran groups is like that in higher molluscs, although each tooth is continuous with the underlying membrane, there being no separate tooth base as in chitons or conchiferans (Figure 4.4E) and there is no subradular membrane. The radula is basically composed of a chitin-rich organic matrix (Peters 1972; Salvini-Plawen and Nopp 1974; Wolter 1992) with deposition of minerals (caudofoveates: Cruz *et al.* 1998; solenogasters: C. Todt, personal observation). As in chitons (see following), such studies promise additional phylogenetic characters.

In solenogaster systematics, foregut glands are among the most important characters, especially the multicellular lateroventral and dorsal glands (Salvini-Plawen 1972, 1978). Handl and Todt (2005) clarified the foregut gland terminology and modified Salvini-Plawen's (1978) classification system of the lateroventral glands. In addition, a number of ultrastructural studies showed the complexity of multicellular foregut glands, which are composed of up to five different types of glandular cells and nonglandular supporting cells (Todt and Salvini-Plawen 2004a, 2005; Todt, in press).

Attempts to apply modern fluorescence techniques to study musculature (Figure 4.4F) and nervous systems in Solenogastres are under way, and preliminary results have been presented as conference contributions (D. Eheberg and G. Haszprunar, R. Croll, and R. Hochberg, personal communication).

POLYPLACOPHORA SYSTEMATICS AND PHYLOGENY

Until recently, the higher classification of Polyplacophora has remained unsettled (Bergenhayn 1955; Smith 1960; Van Belle 1983; Eernisse 1984; Sirenko 1993, 1997; Buckland-Nicks 1995). Traditionally, classifications were based primarily on the morphology of shell plates (valves), spicules, and perinotum processes (e.g., Smith 1960; Van Belle 1983; Kaas *et al.*, 1998), the shell and spicules being the only characters available for fossil chitons (Smith 1960; Van Belle 1983). Of the four layers of the shell plates (properiostracum, tegmentum, articulamentum, myostracum) two are of highest taxonomic relevance: the often colorful and sculptured tegmentum and the articulamentum, which underlies the tegmentum and also forms the insertion plates (see previous discussion). All extant species (order Neoloricata) have been divided into three suborders (e.g., Bergenhayn 1930; Smith 1960; Kaas and Van Belle 1985; Van Belle 1983, 1985). Gowlett-Holmes (1987) reestablished the monotypic Choriplacina (for *Choriplax grayi*), and her proposal was followed by others (Kaas and Van Belle 1994; Kaas *et al.* 1998) (Figure 4.5A).

Lepidopleurina: Articulamentum may have unslit insertion plates or none; tegmentum is well developed; perinotum is narrow to wide, dorsally covered with elongate scale-like spicules, ventrally either naked or with scales.

Choriplacina: Articulamentum is well developed with large, unslit insertion plates; tegmentum is reduced; perinotum

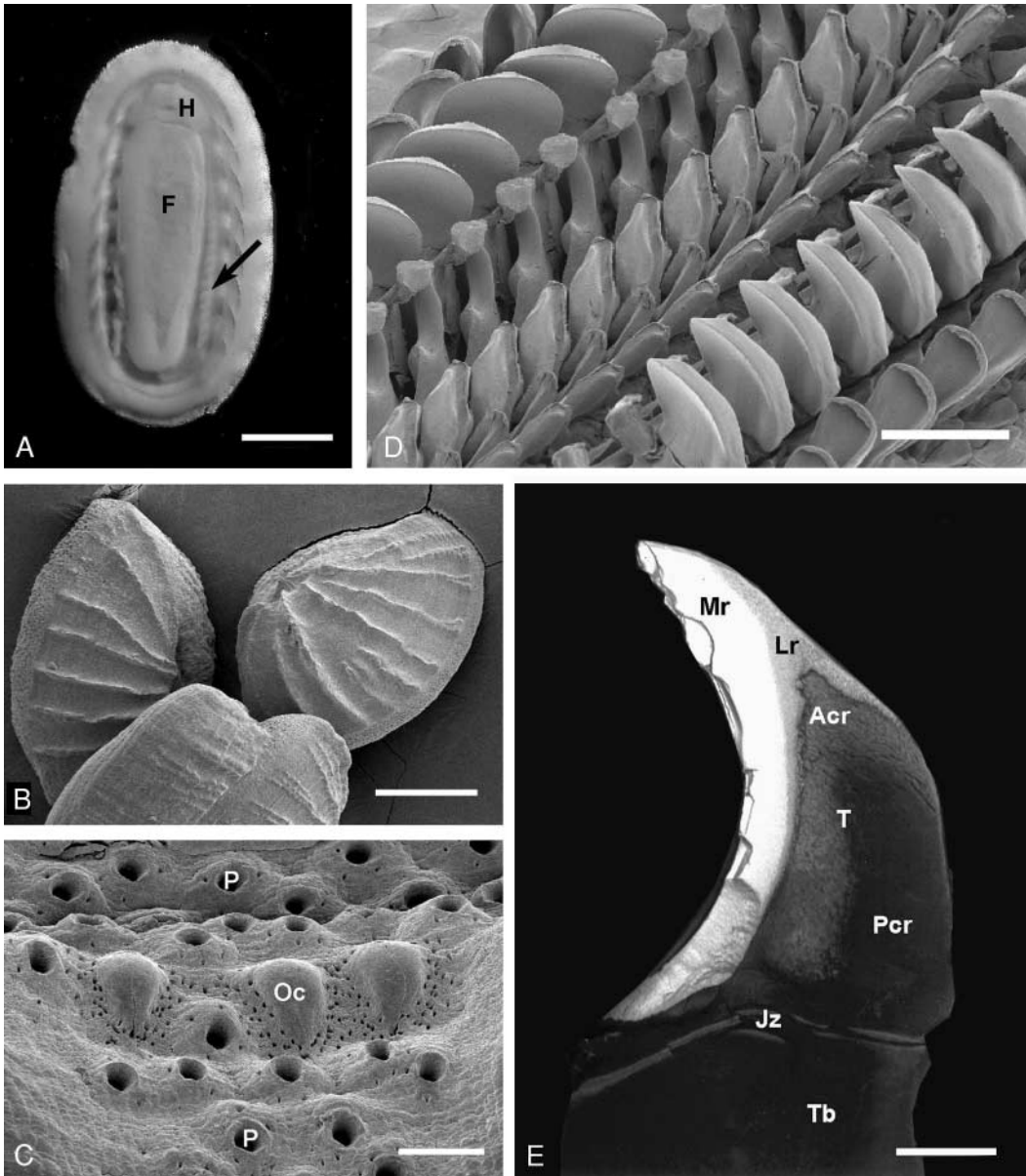
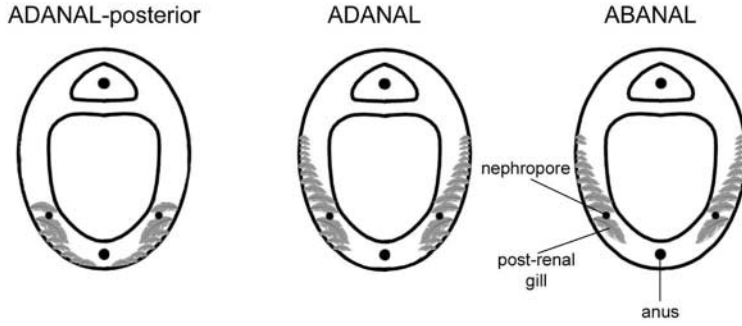
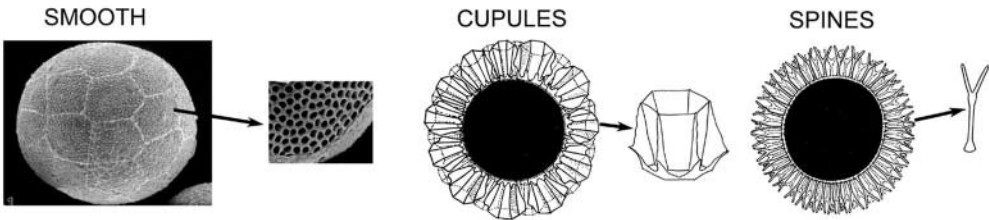


FIGURE 4.6. Polyplacophora; characters relevant for taxonomy and systematics. (A) Specimen of Ischnochitonidae, ventral view showing head (H), foot (F), and gills (arrow); scale bar: 2 mm. (B–D) Scanning electron micrographs of *Acanthopleura* spp. provided by L. Brooker. (B) Three girdle scales from *A. loochooana*, note the sculptured surface; scale bar: 100 μ m. (C) Section of the lateral region of an intermediate valve of *A. brevispinosa* showing three ocelli and numerous apical and subsidiary pores of aesthetes; scale bar: 50 μ m. (D) Radula of *A. echinata*, scale bar: 400 μ m. (E) Back-scattered electron image of ground and polished resin-infiltrated major lateral tooth of *A. spinosa* composed of tooth base (Tb) and tooth proper (T) fused at a distinct junction zone (Jz); brightness of tooth compartments varies according to mineral contents: magnetite region (Mr), lepitocroite-region (Lr), anterior cusp region (Acr), posterior cusp region (Pcr); scale bar: 50 μ m (micrograph by L. Brooker).

A Gill placement



B Egg hull sculpture



C Sperm morphology

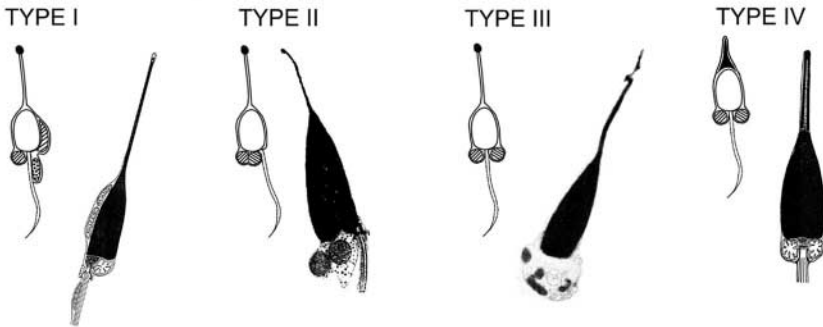


FIGURE 4.7. Polyplacophora, characters important for phylogeny illustrating variations in gill placement (A), egg hull sculpture (B), and sperm morphology (C). A, from Okusu (2003), B, two left hand figures from Buckland-Nicks and Hodgson (2000), others from Sirenko (1993), C, schematic drawings from Okusu *et al.* (2003), others from Buckland-Nicks and Hodgson (2000). For further information see text.

is wide and fleshy, appears naked, dorsally with randomly distributed minute spicules.

Ischnochitonina: Articulamentum is well developed, generally with slits in all valves; teeth of insertion plates are pectinated or smooth; number of slits in the first valve generally higher than five; perinotum has

various types of elements (scales, hairs, spicules).

Acanthochitonina: Articulamentum is well developed with insertion plates in all valves; number of slits in the first valve does not exceed five; teeth of insertion plates never pectinated; perinotum wide and fleshy,

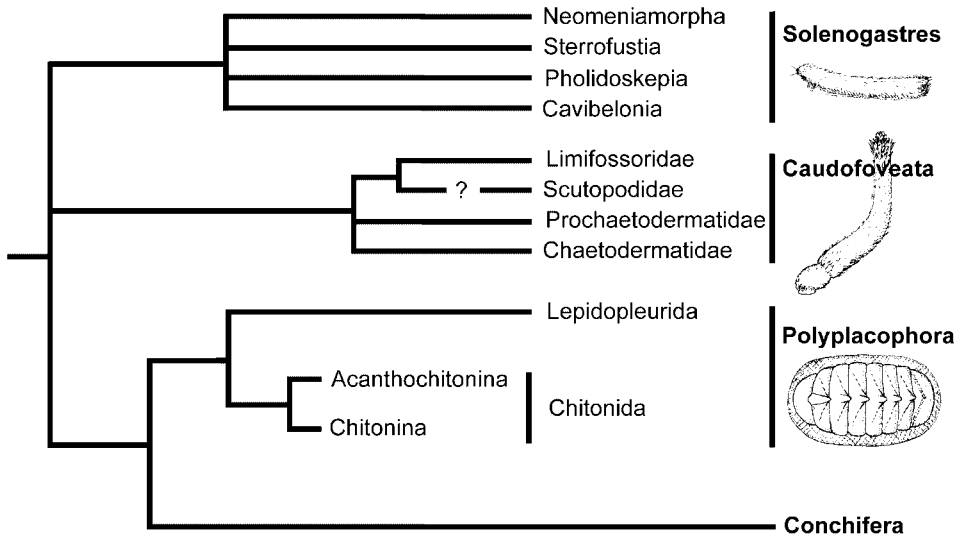


FIGURE 4.8. Tree diagram summarizing major clades within Solenogastres, Caudofoveata, and Polyplacophora with regard to recent knowledge. Within Caudofoveata, families are given as the highest taxonomic level because there are no orders defined. Note the lack of resolution in many positions and on different levels.

generally with spicules of different size, never scaly.

Some of the diagnostic characters in the preceding list have been criticized as being inappropriate for the higher classification of chitons. Although the tegmental structure of shell plates is of taxonomic relevance at the specific level (Haas 1972), the nature of the articulamentum is of interest for higher classification and reflects an evolutionary trend (Sirenko 1997). Therefore, an undeveloped articulamentum lacking insertion plates in the terminal and intermediate valves and with short and mainly unconnected apophyses is seen as the basal condition, and is still present in a few extant chitons (e.g., *Leptochiton*). The derived condition with either slit (e.g., *Ischnochiton*) or unslit insertion plates (e.g., *Choriplax*) is more common. Insertion plates with smooth teeth (e.g., *Ischnochiton*) are considered to be more primitive than those with pectinated teeth (e.g., *Chiton*).

Russell-Hunter (1988) discussed the importance of gill placement in chiton phylogeny, and recent work has shown a correlation among

egg hull type, sperm morphology, and gill placement (Eernisse 1984; Sirenko 1993; Buckland-Nicks 1995; Okusu *et al.* 2003) (Figure 4.7). Chiton eggs have hull processes that are primarily secreted by the egg (Richter 1986) and seem to direct sperm to localized areas during fertilization (Buckland-Nicks 1993). The processes are typically either cup-shaped or spiny (Figure 4.8B) and show species-specific differences (Pearse 1979). Chitons with elaborate egg hulls also have sperm with asymmetrically arranged mitochondria and a long filamentous anterior extension of the nucleus (Pearse 1979), which has a reduced acrosomal vesicle at its tip (Type I and II sperm *sensu* Buckland-Nicks *et al.*, 1990; Buckland-Nicks 1995) (Figure 4.8C). The ctenidia are positioned in characteristic numbers and arrangements along each side of the foot within the pallial cavity (for example, see Kaas and Van Belle 1985; fig. 3), even if variations in the exact number of ctenidia within species occur (Plate 1897, 1899, 1901). During ontogeny, the first ctenidial pair to appear is post-renal (immediately behind the nephridiopore) (Pelseneer 1899). In certain chitons, ctenidia are added exclusively anterior to the

post-renal gill pair (abanal type), while in others they are added anteriorly and posteriorly (Ernissse 1984; Sirenko 1993; Ernissse and Reynolds 1994).

Sirenko (1993, 1997) updated the former classifications (Thiele 1909–1910; Bergenhayn 1930; Smith 1960; Van Belle 1983) and divided extant chitons into two orders, Lepidopleurida and Chitonida, the latter having two suborders, Chitonina and Acanthochitonina (Figure 4.5B). This is consistent with Buckland-Nicks' (1995) phylogenetic analysis, using 25 characters scored from egg hull, sperm, shell valves and ctenidia, of 10 polyplacophoran families (25 species examined in total), with two aplacophorans as outgroup taxa.

Lepidopleurida: Valve characters are

presumably primitive, without slits in the insertion plates; ctenidia are adanal and restricted to the posterior region; sperm are ectaquasperm; eggs are smooth with extraordinary thick egg hulls.

Chitonida: Valve characters are presumably derived, with either slit or unslit insertion plates extending laterally into the girdle; ctenidia are of adanal or abanal type, always with a space between them and the anus papilla; sperm have a filamentous extension of the nucleus and reduced acrosome; there are elaborate egg hull processes.

The Chitonida was further divided into two suborders:

Chitonina: Ctenidial placement is adanal; a posterior extension of midpiece alters sperm shape; spiny, narrow-based egg hull projections; ocelli occur in some genera (e.g., *Onithochiton*).

Acanthochitonina: Ctenidial placement is abanal; the overall sperm shape differs from the preceding groups (for detailed descriptions see Buckland-Nicks 1995); egg hull with broadly based cupules that are not spiny; ocelli are absent.

This classification of Polyplacophora is corroborated by a recent molecular phylogenetic analysis of chiton relationships (Okusu *et al.* 2003), which included representatives of 28 species belonging to 13 families based on the combination of five genes (18S rRNA, 28S rRNA, 16S rRNA, COI, and histone 3). The resulting topology supports the two lineages, Lepidopleurida and Chitonida, but refutes monophyly of many classical taxonomical groups *sensu* Kaas and Van Belle. Okusu *et al.* (2003) further showed a strong correlation of egg hull morphology with the molecular phylogenetic trees. The study showed Lepidopleurida to be the more basal clade and Chitonida was divided into three lineages: taxa with simply round to weakly hexagonal cupules of the egg hull, abanal gills, and type I sperm (clade A in Okusu *et al.* 2003: fig. 8); taxa with egg hulls with strongly hexagonal cupules with flaps, abanal gills, and type I sperm (clade B in Okusu *et al.* 2003: fig. 8); and taxa with various shapes of spiny egg hulls, adanal gills, and type II sperm (clade C in Okusu *et al.* 2003, fig. 8; Chitonoidea *sensu* Sirenko 1997).

A number of additional characters, useful for systematics and phylogeny at different levels, have been investigated over the past few decades, and are summarized in the following paragraphs.

The position and morphology of osphradia vary among chiton taxa and may also be useful phylogenetically. According to ultrastructural data (Haszprunar 1986, 1987), a true osphradium is present only in Chitonida, while the more basal Lepidopleurida show branchial and lateral sense organs that do not appear to be homologous. However, some (if not all) genera of Lepidopleuridae have dark pigmentation under the mouth lappets, which may represent a true, anteriorly positioned osphradium (E. Schwabe, personal observation).

The occurrence and distribution of other sensory elements, including various types of aesthetes and ocelli in the shell plates (e.g., Fischer and Renner 1979; Currie 1992), as well

as so-called ampullary cells and FMRF-amide-positive¹ neurons situated anteriorly underneath the apical ciliary tuft in chiton larvae (Haszprunar *et al.* 2002; Voronezhskaya *et al.* 2002), also appear to reflect phylogenetic relationships.

Radular characters have been used for chiton classification in the past (e.g., Thiele 1893, 1909–1910) but since then have been shown to be too homoplastic at the deeper levels (Eernisse 1984; Sirenko 1993, 1997; Eernisse and Reynolds 1994; Buckland-Nicks 1995; Okusu *et al.* 2003). Nevertheless, they are valuable at certain taxonomic levels (e.g., Bullock 1988; Saito 2004). Saito (2004), for example, points out that selected radular characters within the Cryptoplacoidea correlate with a reduction of the tegmentum within that group. Morphometric data such as the ratio of radular length to total body length, length of the radular cartilages to total radular length, and number of radular teeth rows to radular length may also be of phylogenetic relevance (E. Schwabe, personal observation). There is a wealth of data on radular mineralization in chitons (e.g., Macey *et al.* 1994; Macey and Brooker 1996; Macey *et al.* 1996; Lee *et al.* 1998; Brooker *et al.* 2003; Wealthall *et al.* 2005) and, according to Brooker and Macey (2001), specific traits in radular biomineralization can also be of systematic importance. With the help of light and scanning electron microscopy as well as energy-dispersive spectroscopy (see Figure 4.6E), they showed that iron levels in the teeth of some species only recently merged into *Acanthopleura* by Ferreira (1986) differ considerably from the traditional members of this taxon, including its type species.

Interesting information is also available on the karyotypes of chitons. Yum (1993) provided cytogenetic data for eight species and thus extended Nakamura's (1985) list to 22. The diploid chromosome number in chitons ranges

from 12 to 26, with Acanthochitonidae showing a higher variability, ranging from 16 to 24, while Chitonidae are more uniform, ranging from 24 to 26 and all Ischnochitonidae are 24. In Ischnochitonidae, the chromosome arm morphology is meta- or submetacentric only, while additional telo- or subtelocentric arm morphologies occur in other chiton taxa.

The oxygen-binding protein hemocyanin has been found in chitons, cephalopods, protobranch bivalves, and gastropods. As its origin is calculated to be Precambrian it has been explored for its potential to resolve molluscan evolution (Lieb and Markl 2004). The importance of this protein for a species-level phylogeny and as a marker for evolutionary studies was demonstrated for basal gastropods by Streit *et al.* (2006), and attempts to reveal chiton phylogenetic relationships by means of this new molecular approach are in progress (B. Lieb, personal communication).

ADAPTIVE RADIATIONS

The most outstanding innovations of early molluscs in comparison to their putative predecessors, and to other extant spiralian with similar lifestyles, are the differentiation of a dorsal mantle completely covered in cuticle and sclerites, a ciliated ventral foot for locomotion, and the development of the radula as an effective feeding apparatus.

A protective cover composed of sclerites (scleritome) can be found in many of the earliest known putative molluscs (*Wiwaxia*, *Halkieria*) as well as in all three basal groups of extant molluscs (with additional shell plates in Polyplacophora) and thus may be viewed as a symplesiomorphy of modern Mollusca (most recently by Scheltema and Schander 2006). It is interesting to note, however, that the earliest fossils presumably belonging to the molluscan lineage (*Kimberella*, *Odontogriphus*) did not possess any shell or scleritome at all, indicating that these structures were derived within early molluscs. The evolutionary advantage of a scleritome composed of numerous small sclerites, such as in

1. FMRF-amide, a molluscan cardio-excitatory neurotransmitter, is a tetrapeptide composed of phenylalanine (F), methionine (M), arginine (R), and phenylalanine (F) residues, with the terminal acid group converted to an amide group.

solenogasters and caudofoveates, is obviously protection against predators and not so much against the physical impacts of tides and water currents. This probably accounts for aplacophorans being largely restricted to more sheltered habitats such as deep-water soft sediments and sublittoral hard bottoms. The very few shallow-water species (mostly Solenogastres) occur in coral reefs or are part of the subtidal meio-benthos. In contrast, many chitons inhabit the rocky intertidal, where they withstand strong physical forces protected by their tough cuticle and shell plates and are kept in place by their broad, highly muscular foot.

The model archimollusc of textbooks typically resembles a chiton or untorted limpet in body shape, and the vermiform shape of aplacophorans is usually viewed as a derived feature (Salvini-Plawen 1972, 1985, 2003; Scheltema 1993, 1996) or sometimes a plesiomorphic one (Haszprunar 2000; Haszprunar *et al.*, Chapter 2). The expansion along the longitudinal body axis may be explained as an adaptation to epizoism (Solenogastres) or burrowing (Caudofoveata) (Salvini-Plawen 1972; but see Scheltema 1996 for a contrasting view). The complete reduction of a foot in caudofoveates, combined with the appearance of a mouth shield, is generally seen as connected to their burrowing lifestyle.

The radular morphologies of the three clades discussed herein, reflect divergent feeding habits. Based on fossil evidence, the most primitive radula (*Wiwaxia*, *Odontogriphus*) was used for algal mat grazing (Caron *et al.* 2006). Some authors argue that the primitive radula was used for either shoveling in detritus or grabbing large food items and was a broad structure consisting of several rows of wide, sclerotized teeth with denticles, the individual teeth connected by a flexible cuticle (e.g., Salvini-Plawen 2003; Scheltema *et al.* 2003). This type of radula is found in some caudofoveates (*Scutopus*; Figure 4.4C) and solenogasters (*Helicoradomenia*; Figure 4.4D). From this state pincer-like structures for picking up individual diatoms evolved within caudofoveates, while multiple rows of distichous hooks

with long and pointed denticles and a variety of other radular morphologies adapted for carnivory were developed in solenogasters. The extremely long radular ribbon of all modern chitons, which bears multiple sclerotized and sturdy teeth in part impregnated with metals, is, in contrast, a specialized tool for grazing on hard substrates.

GAPS IN KNOWLEDGE

As shown above, recent research in the fields of palaeontology, ultrastructure, and molecular biology has led to a better understanding of basal molluscs, their biology, and internal relationships. Although modern approaches, such as selective staining techniques for nervous tissues and musculature, *in situ* hybridization combined with tracing of gene expression in development, and multigene approaches for phylogenetic analyses, have already brought a wealth of important knowledge about Polyplacophora, such investigations are still largely lacking for the aplacophoran taxa. In Polyplacophora, however, information about more taxa needs to be added to the existing data matrices to strengthen phylogenetic concepts. This includes morphological data, such as sperm and egg hull structure, chromosome numbers, and radula characters, as well as molecular data. Comparative investigations of sense organs, excretory organs, and larval characters are needed to clarify the usefulness of these characters for phylogeny. The same is true for protein coding sequences, such as hemocyanin or ribosomal protein coding sequences, revealed by expressed sequence tag (EST) projects or selective analysis. For the aplacophoran taxa we still lack molecular studies that include a representative set of taxa. Even though our knowledge of the morphology of Solenogastres and Caudofoveata is extensive, the homology of certain characters between these taxa (mouth shield, vestibulum, foot; regions of the gonopericardial tract) and Polyplacophora (midgut regions; excretory system) is not yet well established. Moreover,

additional cladistic analyses based on morphological characters are needed for both the aplacophoran taxa.

ACKNOWLEDGMENTS

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