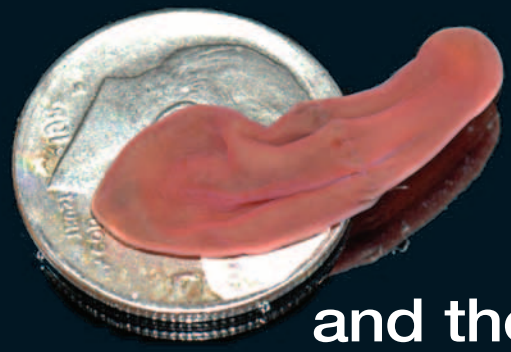


CHAPTER 9

Introduction to the Bilateria and the Phylum Xenacoelomorpha

Triploblasty and Bilateral Symmetry Provide New Avenues for Animal Radiation

Along the evolutionary path from prokaryotes to modern animals, three key innovations led to greatly expanded biological diversification: (1) the evolution of the eukaryote condition, (2) the emergence of the Metazoa, and (3) the evolution of a third germ layer (triploblasty) and, perhaps simultaneously, bilateral symmetry. We have already discussed the origins of the Eukaryota and the Metazoa, in Chapters 1 and 6, and elsewhere. The invention of a third (middle) germ layer, the true **mesoderm**, and evolution of a bilateral body plan, opened up vast new avenues for evolutionary expansion among animals. We discussed the embryological nature of true mesoderm in Chapter 5, where we learned that the evolution of this inner body layer facilitated greater specialization in tissue formation, including highly specialized organ systems and condensed nervous systems (e.g., central nervous systems). In addition to derivatives of ectoderm (skin and nervous system) and endoderm (gut and its derivatives), triploblastic animals have mesodermal derivatives—which include musculature, the circulatory system, the excretory system, and the somatic portions of the gonads. Bilateral symmetry gives these animals two axes of polarity (anteroposterior and dorsoventral) along a single body plane that divides the body into two symmetrically opposed parts—the left and right sides. The evolution of bilaterality also resulted in **cephalization**, the concentration of sensory and feeding structures at a head end. Bilaterians further evolved a **complete gut** (or **through gut**), with a mouth and an anus and excretory organs in the form of protonephridia and metanephridia (except in the phylum Xenacoelomorpha, likely the most primitive living bilaterians).

As noted in Chapter 1, the oldest fossils thought to be bilaterians are from the Ediacaran period—embryos found in the Doushantuo deposits of China, dating 600–580 million years ago. The most recent molecular dating studies also suggest that the origin of the Bilateria was probably in the Ediacaran, about 630–600 million years ago, although some dated trees have estimated the origin even earlier.

Classification of The Animal Kingdom (Metazoa)

Non-Bilateria*
(a.k.a. the diploblasts)
- PHYLUM PORIFERA
- PHYLUM PLACOZOA
- PHYLUM CNIDARIA
- PHYLUM CTENOPHORA

Bilateria
(a.k.a. the triploblasts)
- PHYLUM XENACOELOMORPHA

Protostomia
- PHYLUM CHAETOGNATHA

Spiralia
- PHYLUM PLATYHELMINTHES
- PHYLUM GASTROTRICHA
- PHYLUM RHOMBOZOA
- PHYLUM ORTHONECTIDA
- PHYLUM NEMERTEA
- PHYLUM MOLLUSCA
- PHYLUM ANNELIDA
- PHYLUM ENTOPROCTA
- PHYLUM CYCLIOPHORA

Gnathifera
- PHYLUM GNATHOSTOMULIDA
- PHYLUM MICROGNATHOZOA
- PHYLUM ROTIFERA

Lophophorata
- PHYLUM PHORONIDA
- PHYLUM BRYOZOA
- PHYLUM BRACHIOPODA

Ecdysozoa

Nematoida
- PHYLUM NEMATODA
- PHYLUM NEMATOMORPHA

Scalidophora
- PHYLUM KINORHYNCHA
- PHYLUM PRIAPULA
- PHYLUM LORICIFERA

Panarthropoda
- PHYLUM TARDIGRADA
- PHYLUM ONYCHOPHORA
- PHYLUM ARTHROPODA
  - SUBPHYLUM CRUSTACEA*
  - SUBPHYLUM HEXAPODA
  - SUBPHYLUM MYRIAPODA
  - SUBPHYLUM CHELICERATA

Deuterostomia
- PHYLUM ECHINODERMATA
- PHYLUM HEMICHORDATA
- PHYLUM CHORDATA

*Paraphyletic group

BOX 9A Characteristics of the Phylum Xenacoelomorpha

1. Soft-bodied, dorsoventrally flattened, acoelomate; almost exclusively marine worms
2. Epidermis with unique pulsatile bodies, found in no other Metazoan phylum; cilia of epidermis with distinctive arrangement of microfilaments, with the standard 9+2 arrangement extending for most of the shaft, but microfilament doublets 4–6 fail to reach the end of the cilium (the "xenacoelomorphan cilia")
3. Midventral mouth and incomplete gut (i.e., lacking an anus)
4. Largely lacking discrete organs (e.g., no discrete circulatory system, protonephridia or nephridia, or organized gonads)
5. Cerebral ganglion with a neuropil; with anterior statocysts and a diffuse intraepithelial nervous system
6. With circular and longitudinal muscles
7. Hox and ParaHox genes present (but fewer in number than in other metazoans)
8. With direct development (no larval forms)

Monophyly of the Bilateria is strongly supported by both molecular and morphological analyses. Anatomical synapomorphies of this clade include: presence of a third germ layer (mesoderm), bilateral symmetry, cephalization, and a body with both circular and longitudinal musculature—although there are prominent reversals or losses in all of these characters.

The field of molecular phylogenetics has recently provided us with better resolution of evolutionary relationships among animals. Molecular biology has also led to the expansion of a field called evolutionary developmental biology ("EvoDevo"), which attempts to understand the evolution of the molecular underpinnings of differences in the organization of animal body plans. In large part, understanding the evolution of animal body plans is about unraveling the transition from basal Metazoa (Porifera, Placozoa, Cnidaria, Ctenophora) to the Bilateria, and their subsequent radiation. However, genomic-level research has also shown that there is no simple relationship between genomic/molecular complexity and organismal/developmental complexity, so contrary to earlier assumptions the mere presence of members of conserved gene families (e.g., "segmentation genes") in an organism's genome reveals little about the evolutionary relationships of that organism to other animals. Thus, reconstructing the phylogenetic tree of the animal kingdom using conserved genes and morphological characters is a critical step. Ultimately, phylogenetics and EvoDevo together might allow us to reconstruct the nature of the first bilaterian animal—the so-called **Urbilaterian**.

The Basal Bilaterian

The notion that acoel flatworms are the most primitive living bilaterians has been around for many years. In the past, some workers have even speculated that acoels might be the most primitive living metazoans, having evolved from a ciliate protist ancestry (the "syncytial, or ciliate–acoel hypothesis"). But most biologists have favored some version of the "planuloid–acoeloid hypothesis," which postulated acoels to be the link between diploblasts (via a planula larva) and triploblasts. A third hypothesis, which gained traction for a short while, was that the simplicity of acoels was the result of *loss* of derived features from a more complex ancestor (the "archicoelomate hypothesis").

Recent molecular phylogenetic research strongly suggests that acoelomorph worms (Acoela and Nemertodermatida), perhaps along with worms of the genus *Xenoturbella*, are likely to be the oldest living bilaterian lineages, or perhaps basal deuterostomes—but either way, sharing a great many characteristics in common with an ancestral bilaterian, the so-called hypothetical "Urbilaterian." Acoelomorphs are small, direct-developing (no larval stage), unsegmented, ciliated worms. They have mesodermally-derived muscles (but no coelom, circulatory, or excretory system), multiple parallel longitudinal nerve cords and a centralized nervous system, and a single opening to the digestive cavity. Importantly, research suggests that their mouth—the single gut opening—does not derive from the blastopore (i.e., they are deuterostomous in their development). Thus, the origin of the Bilateria may have been accompanied by an embryological shift in the origin of the mouth from the blastopore (as seen in Cnidaria and Ctenophora) to elsewhere.

Many bilaterian phyla have a larval type that has been described as **primary larvae**. These are ciliated larvae with a characteristic **apical organ**—a true larval organ that disappears, in part or entirely, before or at metamorphosis. The apical organ is a putative sensory structure that develops from the most apical blastomeres during embryogenesis. It does not fit the narrow definition of a ganglion, because it seems to comprise only sensory cells. However, many spiralians develop lateral (cerebral) ganglia in close apposition to the apical organ, and this compound structure was called the "apical organ" in some of the older literature. It appears the apical organ is commonly used in larval settlement, and it is lost once a larva settles (the settlement process typically uses cells around the apical pole). In spiralian protostomes, the apical organ differentiates from the most apical cells (the $1a^1$-$1d^1$ cells in spiral-cleavage terminology). It is highly variable, and

aspects of it are sometimes retained in the adult central nervous system. In the pilidium larva of nemerteans the apical organ is shed at metamorphosis together with the whole larval body, which in some cases is ingested by the emerging juvenile worm.

Deuterostomes are more difficult to interpret, and only in echinoderm and enteropneust (i.e., Ambulacraria) larvae are apical organs clearly present. Apical organs occur in cnidarian larvae, but not in Porifera, thus it has been proposed that the primary larva/apical organ could be a synapomorphy that defines a clade called Neuralia (i.e., Cnidara + Bilateria). In Cnidaria the apical organ consists of a group of monociliated nerve cells—upon settlement, the nervous system becomes reorganized and the larval nerve net is lost, with development of a new adult nerve net. Recent gene expression studies have demonstrated that the apical pole of cnidarians and the apical pole of bilaterians are probably homologous. Apical organs are apparently absent in Ecdysozoa and Chordata (except, perhaps, in the nonfeeding amphioxus larva).

Protostomes and Deuterostomes

Early in the evolution of bilaterians there was a split into two major lineages, which have long been called Protostomia and Deuterostomia. These groups were named over 100 years ago, and they were long defined on the basis of embryological principles. In protostomes the blastopore (the position in the embryo that typically gives rise to endodermal tissues) was said to give rise to the mouth ("protostome" = mouth first). Typically in deuterostomes, the blastopore gave rise to the adult anus, the mouth thus forming secondarily at a different location ("deuterostome" = secondary mouth). In both lineages, the blastopore sits at the vegetal pole of the embryo when gastrulation begins.

As molecular phylogenetic discoveries have reshuffled the animal phyla among the protostome and deuterostome lineages, a new view of embryological patterns has emerged. In the Deuterostomia (now defined as the phyla Echinodermata, Hemichordata, and Chordata), the blastopore *does* consistently give rise to the anus, and the mouth forms secondarily. But among the Protostomia, gastrulation is now known to be much more variable. In fact, we have learned that in protostomes, while the anus usually does form secondarily, the blastopore does not always give rise to the mouth, especially among animals in the large clade known as Spiralia (annelids, molluscs, nemerteans, and others). Even within the clade Ecdysozoa we now know deuterostomy can occur. For example, both nematomorphans and priapulans have deuterostomous development, with the blastopore giving rise to the anus (at the vegetal pole) and the mouth arising at the animal pole. Gene expression studies have shown that, in *Priapulus caudatus*, typical metazoan foregut and hindgut gene expressions accompany this development, and the hindgut/posterior markers *brachyury* (*bra*) and *caudal* (*cdx*) are expressed as the anus emerges from the blastopore. Continuing developmental work on crustaceans is revealing that most species probably also express a form of deuterostomy. And in Chaetognatha gastrulation occurs by invagination of the presumptive endoderm, leaving no blastocoel—the blastopore marks the eventual posterior end of the animal, and both mouth and anus form secondarily, thus also a deuterostome-like development. In fact, evidence is accumulating that mouth formation from oral ectoderm (in the animal hemisphere), typical of deuterostomy, may be ancestral in both protostomes and deuterostomes, and perhaps in Bilateria itself.

So we see that, although the names Protostomia and Deuterostomia are still used for the two main clades of Bilateria, the names themselves are no longer perfectly descriptive—they are **legacy names**. It has been suggested that new names should be coined for these two large clades, but as yet there has been no agreement on what these names might be. The largest animal phyla belong to Protostomia—Arthropoda (over a million described living species) and Mollusca (nearly 80,000 described living species)—as do the smallest animal phyla (Micrognathozoa and Placozoa, one described species each; Cycliophora, two described species), although several undescribed species are known to exist in these small phyla.

Today, the groups Protostomia and Deuterostomia constitute clades based mostly on molecular phylogenetic evidence, and morphological and developmental synapomorphies defining these two clades remain ambiguous. A probable synapomorphy of the Protostomia, as it is now constituted, is a central nervous system with a dorsal cerebral ganglion that usually has circumesophageal connectives to a pair of ventral nerve cords. Probable synapomorphies of the Deuterostomia are a trimeric body coelom condition and pharyngeal gill slits, at least primitively (trimery is lacking in the phylum Chordata, gill slits are absent in extant echinoderms but may have been present in some extinct, basal echinoderms). Although still somewhat controversial, the position of the phylum Xenacoelomorpha (acoels, nemertodermatids, and *Xenoturbella*) appears to be basal within Bilateria, this group not aligning strongly with either protostomes or deuterostomes (Box 9A).

The Protostomia contains 24 phyla, five of which still remain enigmatic in terms of their phylogenetic alignment: Chaetognatha, Platyhelminthes, Gastrotricha, Rhombozoa, and Orthonectida. Some molecular evidence suggests all of these but Chaetognatha probably belong in the clade known as Spiralia, and in fact Platyhelminthes and Rhombozoa do seem to show spiral cleavage (and some evidence suggests

Chaetognatha might also have spiral cleavage). Recent studies have suggested that Chaetognatha may be the sister group to Spiralia. Gastrotrichs have a unique, but non-radial embryogenesis, and the embryology of Orthonectida, Cycliophora, and Micrognathozoa is not yet known. Bryozoa and Brachiopoda clearly do not have spiral cleavage. Thus, we do not yet know if spiral cleavage is a synapomorphy of the clade that bears its name—i.e., "Spiralia" is another legacy name. It may eventually be shown that all of these phyla comprise a single clade and are descendants of a spirally-cleaving ancestor, making this cleavage pattern a valid synapomorphy for the group known as Spiralia. If spiral cleavage does prove to be a synapomorphy for Spiralia, its absence in some phyla would be viewed as the product of secondary modifications to the embryological process. Recall that in spiral cleavage, the 4d cell (also known as the mesentoblast) gives rise to most of the mesoderm, called endomesoderm. Most spiralians also generate some mesoderm from micromeres of the second or third quartet that are primarily responsible for ectoderm formation (thus it is called ectomesoderm); this commonly gives rise to larval musculature.

Some spirally-cleaving animals have a unique larval type, called the trochophore larva (e.g., Mollusca, Annelida, Nemertea, and possibly some others), and the clade name "Trochozoa" has been proposed for those phyla, although this clade gets very mixed support in molecular trees and might be paraphyletic. In addition, recent phylogenomic work suggests that these "trochophore phyla" may comprise a sister group to the Lophophorata (Phoronida, Bryozoa, Brachiopoda), and perhaps also including Entoprocta, as a larger grouping known as the Lophotrochozoa. Although DNA sequence data support the clade Spiralia, no unambiguous morphological synapomorphies that might define it have been identified. The phylogenetic relationships of the spiralian phyla remain to be sorted out, and so far their deep ancestry has defied clear resolution. However, two clades within Spiralia do seem to be well supported, and we treat these as chapters in this book; these are the clades Gnathifera (phyla Gnathostomulida, Micrognathozoa, Rotifera) and Lophophorata (phyla Phoronida, Bryozoa, Brachiopoda).

The other main protostome clade, Ecdysozoa, contains 8 phyla (and about 80% of animal species diversity) that all molt their cuticle at least once during their life history. The Ecdysozoa comprise three well-supported clades: Panarthropoda (Tardigrada, Onychophora, Arthropoda), Nematoida (Nematoda, Nematomorpha), and Scalidophora (Priapula, Kinorhyncha, Loricifera), the latter supported mostly by morphological data. The phylogenetic relationships of these three clades have not yet been determined, so they appear as an unresolved trichotomy in our tree of the Metazoa (Chapter 28). Morphological evidence suggests that Nematoida and Scalidophora are sister groups, and they share a number of morphological similarities (e.g., a circumoral collar-shaped brain composed of a ring neuropil with anterior and posterior concentrations of cell bodies). However, these morphological similarities might be plesiomorphic within Ecdysozoa, and most molecular analyses place Nematoida as a sister group to Panarthropoda. The internal relationships of the scalidophoran phyla also remain unclear. The most recent work on Panarthropoda suggests Onychophora may be the sister group to Arthropoda, and Tardigrada the sister group to those. Unlike the Spiralia, the Ecdysozoa can be defined by unambiguous morphological synapomorphies, including their three-layered cuticle that can be molted, a process regulated by ecdysteroid hormones in those groups where this is known. The cuticle consists of a proteinaceous exocuticle and an endocuticle with chitin or collagen, with the epicuticle forming from the apical zone of the epidermal microvilli. Ecdysozoans also lack external epithelial cilia, lack a primary larva or ciliated larva, and none of them has spiral cleavage. This clade was discovered in one of the first, pioneering studies using molecular sequence data (Aguinaldo et al. 1997).

The other great bilaterian clade, Deuterostomia, is quite small, comprising fewer than 100,000 living species in only three phyla: Echinodermata, Hemichordata, and Chordata. Although only a "side-branch" in the tree of life, we tend to give this clade exaggerated importance because, of course, it is the lineage to which we humans (and other vertebrates) belong. As noted above, this clade was originally defined largely on the basis of deuterostomous embryology. However, we now know that deuterostomous development occurs throughout its sister lineage, the Protostomia, leaving us with few definitive morphological or developmental features defining the Deuterostomia. However, as noted above, a trimeric body coelom and pharyngeal gill slits, at least primitively, may eventually be proven to be synapomorphies for the Deuterostomia. Deuterostomes also appear to possess a unique developmental gene, called *Nodal*.

Within the deuterostome clade, recent morphological and molecular work (and also Hox gene motifs) suggests that echinoderms and hemichordates are sister groups, constituting a clade called Ambulacraria, and this is the sister group to the phylum Chordata. If this assessment is correct, it means the features shared between chordates and hemichordates (long thought to comprise a sister group), such as gill slits, may have indeed been ancestral within Deuterostomia, but lost in the echinoderm line (and also in some hemichordate lineages), as suggested by the putative presence of gill slits in some extinct echinoderms. Gill slits in Deuterostomia have been shown to be homologous based on their gene expression patterns. Several

deuterostome animals with gill slits are known from the fossil record, although it is not yet certain whether these belong to basal urochordates, to basal echinoderms, or to their own extinct lineages. Another feature shared between the Hemichordata and Chordata is the stomochord/notochord, long viewed as homologues. It is now thought that these structures might have had much earlier origins and may or may not be homologous, or that a group of vacuolated cells in ancestral Deuterostomia gave rise to these structures independently in hemichordates and chordates. Within Chordata, Urochordata is the sister group to Vertebrata (a clade known as Olfactores), and Cephalochordata is the sister group to those. There is some evidence that a fourth group, the genus *Xenoturbella* (or even the whole clade Xenacoelomorpha) might be near the base of the deuterostome line, but opposing evidence suggests *Xenoturbella* is more likely allied with the Acoelomorpha as an ancestral bilaterian clade, the view we follow in this book.

Deuterostomia is an ancient lineage, and dated phylogenetic trees (using fossils to date branching points) suggest the ancestral line existed well into the Precambrian. The oldest definitive fossil of Deuterostomia is a 530-million-year-old creature called *Yunnanozoon*, from the lower Cambrian Chengjiang biota of Yunnan Province, China, although the affinities of yunnanozoans are still uncertain.

The classification of Metazoa used in this book is shown in the box at the start of Chapters 6 through 27. You will notice that phyla are listed under clade names (most of which lack formal nomenclatural ranking). You will also notice that within these clades, there is often little phylogenetic structure indicated. This is because much of the branching pattern of the tree of life still remains to be discovered. Genomic data are still lacking for many groups, and in other cases data are available for only one or two species. Expanded taxonomic sampling, additional genomic data sets, and new analysis techniques should resolve the remaining questions of animal phylogeny over the coming decade.

Phylum Xenacoelomorpha

The acoels and the nemertodermatids have had a long journey. They were initially viewed as the most primitive living platyhelminths (true flatworms), due to their simple anatomy, and in fact, were thought by many to be the most primitive living Bilateria because most workers placed the phylum Platyhelminthes at the base of the bilaterian tree. As ultrastructural work revealed increasing complexity, opinion shifted, and from the 1960s to the turn of the century these worms, together known as the Acoelomorpha, were widely regarded not as primitive, but as secondarily reduced platyhelminths. However, as multigene phylogenetic analyses have begun to explore these small soft-bodied worms, it has become evident that they are indeed primitive bilaterians (perhaps diverging even before the protostome–deuterostome split), and not members of the phylum Platyhelminthes at all. Thus, the pendulum has swung 180 degrees, as is known to happen in phylogenetics. A growing knowledge base and new technologies can lead to major shifts in our understanding of life. In addition, molecular phylogenetics has shown a close relationship between the acoel and nemertodermatid worms, which is further supported by their unique ciliary rootlet system, perhaps the early cleavage pattern (i.e., the horizontal orientation of the second, asymmetric cleavage plane), and several other features described below.

Even more recently, another genus of small marine worms, *Xenoturbella*, was found to be allied closely with the Acoelomorpha, and a new phylum name was created to house these three worm groups—Xenacoelomorpha. The phylum currently contains about 400 species, two in the subphylum Xenoturbellida and 398 in the subphylum Acoelomorpha (mostly in the class Acoela). All described species are small, flattened, marine worms with an incomplete digestive system (i.e., lacking an anus) and lacking discrete excretory systems (however, there is an undescribed xenoturbellid species reported to be several centimeters in length).

DNA sequence analyses have suggested that Acoelomorpha are basal bilaterians and are likely the sister group of Xenoturbellida. Analyses have been divided on whether Xenoturbellida are deuterostomes or basal bilaterians, but the latter idea seems to have stronger support. However, the high evolutionary rate of analyzed genes in Acoelomorpha might be creating long-branch attraction problems and further studies are needed. Thus, although we recognize the phylum Xenacoelomorpha, and treat Acoelomorpha and Xenoturbellida as subphyla, it is possible that these two groups will eventually again be separated, with Acoelomorpha being placed at the base of the Bilateria, and Xenoturbellida within the Deuterostomia. We discuss each of the three curious worm groups (Acoela, Nemertodermatida, Xenoturbellida) separately below.

In addition to the molecular phylogenetic data that support an Acoela–Nemertodermatida sister group relationship, both groups have unique epidermal bodies that represent degenerating ciliated cells, the **pulsatile bodies** (and a type of pulsatile body also occurs in the xenoturbellids). These epidermal bodies are unknown from any other metazoan phylum. In Acoela, the cilia are retained in vacuoles prior to digestion, whereas in nemertodermatids the cilia appear to be lost before resorption begins. The musculature of acoels and

nemertodermatids is also strikingly similar, yet different in some key aspects; acoels have a grid of orthogonal musculature with mainly ventral diagonal musculature, and a muscular posterior pharynx in what may be basal species. More derived acoels have more complex layers of diagonal muscles. Nemertodermatids seem to have an orthogonal grid and well-developed diagonal muscles throughout the body, but no evidence of a muscular pharynx. These anatomical features are described below.

In addition to pulsatile bodies, both Acoela and Nemertodermatida (and *Xenoturbella*) lack discrete excretory systems, the presence of which unites all other Bilateria, and their cerebral ganglion has a neuropil (i.e., it can be considered a true brain, but see below). Furthermore, they share a unique pattern of neurotransmitter activity, body-wall musculature, and mode of embryonic development. Hox and ParaHox genes are present in both groups, although these are not strictly similar. Both taxa appear to have the beginning of the extended central Hox set.

Although initially considered to be a turbellarian flatworm, the unusual anatomy of *Xenoturbella bocki* quickly distinguished it from platyhelminths, as well as from the Acoelomorpha. Phylogenetic (and even some morphological) studies initially linked *Xenoturbella* to deuterostomes. Sequences of Hox genes in *X. bocki* also suggested it could be a basal deuterostome with a reduced Hox gene complement. Additional work using the entire mitochondrial genome of *Xenoturbella* showed links with deuterostomes. However, the lack of typical deuterostome characteristics suggested that *Xenoturbella* might belong at the very base of the deuterostome tree. Other phylogenetic analyses, including nuclear genes from *X. bocki*, also suggested that *Xenoturbella* might be closely tied to the clade known as Ambulacraria (Echinodermata and Hemichordata). If these relationships are correct, developmental evidence of structures common to other Ambulacraria should exist, including gill slits, endostyle, and enterocoelic coelom formation. However, such evidence has not been found (although studies have been frustrated by the fact that *Xenoturbella* ova are very yolky, which obscures observation of early cleavage).

By 2009, large-scale molecular phylogenetic studies had begun to move *Xenoturbella* even further down the animal tree, suggesting it is sister to the Acoelomorpha (Acoela + Nemertodermatida), at the base of the Bilateria. The anatomical data seemed to agree with this linking, and it was eventually suggested that the three groups together warranted phylum status, the Xenacoelomorpha. Acoels have only three Hox genes (one each of the anterior, central, and posterior groups). Nemertodermatids have only two (a central and a posterior group). *Xenoturbella* has one anterior, two (or three) central, and one posterior gene. Platyhelminths, on the other hand, have an almost complete Hox cluster. The most recent phylogenetic studies on Acoelomorpha and *Xenoturbella* are still conflicting, plagued by long-branch attraction and small taxon sampling issues. Although we accept the phylum Xenacoelomorpha in this edition of *Invertebrates*, we recognize that the relationships of these three worm taxa are still subject to modification.

CLASSIFICATION OF PHYLUM XENACOELOMORPHA

Generally small, flattened or cylindrical, acoelomate marine worms with anterior statocysts, diffuse intraepithelial nervous system, midventral mouth, incomplete gut (i.e., lacking an anus), unique pulsatile bodies (unknown from any other Bilateria), and largely lacking discrete organs (e.g., without a discrete circulatory system, nephridia, or organized gonads). Cilia of epidermal cells with distinctive arrangement of microfilaments wherein the standard 9+2 arrangement extends for most of the ciliary shaft, but toward the end, microfilament doublets 4 through 7 end, leaving doublets 1–3 and 8–9, which continue to the end of the cilium. These **xenacoelomorphan cilia** are not known in any other animal phylum (although very similar cilia have been described from the pharynx of some enteropneust hemichordates). With both circular and longitudinal muscles. With direct development and no distinct larval forms. Two subphyla, Acoelomorpha and Xenoturbellida.

SUBPHYLUM ACOELOMORPHA The union of Acoela and Nemertodermatida as sister taxa is based on molecular phylogenetic evidence, as well as anatomical data. Both groups: lack discrete excretory systems (present in all other Bilateria), have cerebral ganglia with a neuropil, share a unique pattern of neurotransmitter activity and unique body-wall musculature, and go through a distinctive mode of embryonic development. Hox and ParaHox genes are present in both groups, although these are not strictly similar.

CLASS ACOELA Acoels lack a permanent digestive cavity. The pharynx, when present, is simple, leading to a solid syncytial or cellular endodermal mass. With a unique anterior statocyst containing one statolith, and biflagellate sperm with 2 flagella whose axonemes are incorporated into the sperm cell; endolecithal ova; without epithelial basal lamina, or discrete excretory or circulatory systems. Small (1–5 mm) worms, common in marine and brackish-water sediments; a few are planktonic or symbiotic. (e.g., *Actinoposthia*, *Amphiscolops*, *Antigonaria*, *Conaperta*, *Convoluta*, *Convolutriloba*, *Daku*, *Diopisthoporus*, *Eumecynostomum*, *Haplogonaria*, *Hofstenia*, *Isodiametra*, *Myopea*, *Oligochaerus* [with freshwater species], *Paratomella*, *Philactinoposthia*, *Polychoerus*, *Praesagittifera*, *Proporus*, *Solenofilomorpha*, *Symsagittifera*, *Waminoa*)

CLASS NEMERTODERMATIDA Interstitial or endosymbiotic marine worms possessing a ciliated, glandular epidermis and an anterior statocyst generally containing two

statoliths; a proboscis with extensible filaments is present in some species; mouth may be present or absent; pharynx never present; gut cavity with small and relatively occluded, but with true epithelium and gland cells; uniflagellate sperm; with endolecithal ova; with limited basal lamina beneath the epidermis. One genus (*Meara*) contains species that are symbionts in sea cucumbers. (e.g., *Ascoparia*, *Flagellophora*, *Meara*, *Nemertinoides*, *Nemertoderma*, *Sterreria*)

SUBPHYLUM XENOTURBELLIDA Two described species, *Xenoturbellida bocki* and *X. westbladi* (but others are known to exist, and the species-level differences between the two described species have been questioned). Despite its simple body plan, *X. bocki* is a relatively large worm, reaching 4 cm in length, and some undescribed species may exceed that size. Xenoturbellids have a humplike structure in the anterior third of the body but lack other structural organs (other than a statocyst) and possess only a diffuse nervous system. These worms live in holes on sandy coastlines or deeper offshore muds and are specialized to eat molluscs.

Class Acoela

Acoels are mostly minute, marine or brackish-water, sediment- or surface-dwelling worms. They range in size from less than a millimeter to about a centimeter in length. Those inhabiting interstitial habitats are generally long and slender, whereas those inhabiting surfaces tend to be more disc shaped, broad, and flat. Swimming species are cylindrical with tapered ends, or occasionally enrolled sides. Epiphytic species are usually cone shaped with ventrally enrolled sides that may give the appearance of trailing "fins." A few species of acoels have also been found in the gut of echinoderms, in fresh water, and in hydrothermal vents. (Figures 9.1A–H)

Acoela lack a distinct internal body cavity—they are acoelomate (as are the other members of the phylum Xenacoelomorpha). Acoels also lack a structural gut, and this was actually the basis of the name Acoela. Instead, they possess a multinucleated mass (a syncytium) that phagocytizes ingested food particles (Figure 9.2). Larger species often supplement their nutritional requirements with endosymbiotic algae, which can contribute to the bright coloration seen in many (Figure 9.3A). Acoels living in the guts of other animals often have symbiotic bacteria inhabiting their epidermis.

Acoels possess both circular and longitudinal muscles. Their nervous system consists of an array of paired longitudinal nerve cords with a concentration of anterior sensory cells and a cerebral commissure (the "brain") (Figure 9.4). The anterior statocyst with a single statolith is distinctive in acoels and (along with simple, light sensitive eyes in a few species) appears to assist in maintaining the animal's orientation (Figure 9.1A–H). They lack sclerotized structures other than those associated with genitalia, although some species manufacture crystalline spicules in the parenchyma. They also possess aberrant, complex, biflagellate sperm that vary in the structure of the usual 9+2 arrangement of microtubules possessed by many metazoans. Acoels have direct development and exhibit no distinct larval forms.

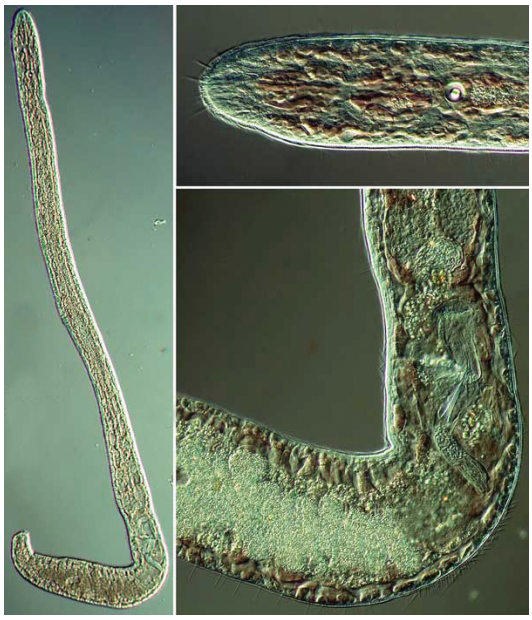
Acoels were first described at the turn of the nineteenth century from northeast Atlantic coastlines. These and other early descriptions placed the Acoela within the turbellarian Platyhelminthes, and distinguished major subtaxa on the basis of the female reproductive system. Later revisions in the middle of the twentieth century established over 20 families, and most of the nearly 400 described species were based primarily on details of male copulatory structures. Similarities in internal anatomy, epidermal ciliation, and the appearance of epidermal "pulsatile bodies" led to combining Acoela with another turbellarian group, Nemertodermatida, as the Acoelomorpha.

The lack of hard anatomical features in these worms led workers to studies of microscopic ultrastructure using scanning and transmission electron microscopy, including investigations of muscle fiber orientation and structure (which distinguished several major lineages), sperm morphology, and spermatogenesis (which identified biflagellate sperm and unusual patterns of microtubules within sperm acrosomes), as well as neuroanatomy. Studies increased in number near the end of the twentieth century as the diversity of habitats investigated increased, including anoxic sulfide sands. 18S and 28S rRNA, mitochondrial DNA, and myosin heavy chain type II nucleotide sequences have all placed Acoelomorpha outside of the Platyhelminthes. Further systematic refinements within major acoel clades (notably the polyphyletic family Convolutidae), and developmental analyses, have corroborated genetic results that place acoels outside the Platyhelminthes. Much taxonomic revision is still underway, and about 9 to 20 families are currently recognized, depending on whose schema is followed.

Both molecular phylogenetics and EvoDevo research provide evidence that acoels likely lie at the base of the bilaterian tree. For example, the pattern of expression of *ClEvx* (a gene responsible for sensory specificity brain neurons) anterior and posterior to the statocyst in hatchling acoels is more similar to that found in cnidarians than it is to more derived bilaterians. Other studies indicate that *brachyury* (*bra*) and *goosecoid* (*gsc*), genes associated with the formation of the acoel mouth, are also expressed during mouth development in protostomes as well as deuterostomes, suggesting that acoel and bilaterian mouths are homologous. Studies of neural development and structure in the acoel *Symsagittifera* show that genes associated with brainlike structures are present, suggesting that such genetic machinery was in place in the Urbilaterian ancestor (if indeed acoels represent such an ancestor). The overall primitiveness of Acoela appears to also be reflected in their lack of a clearly differentiated gut or

(A)

(B)



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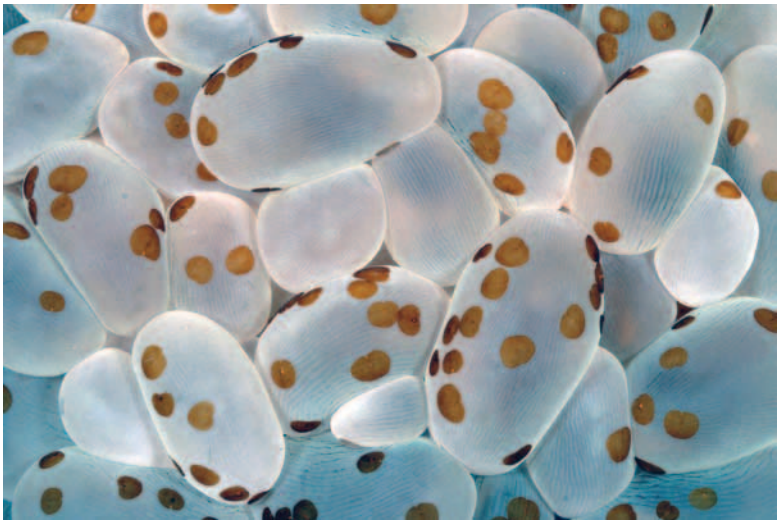

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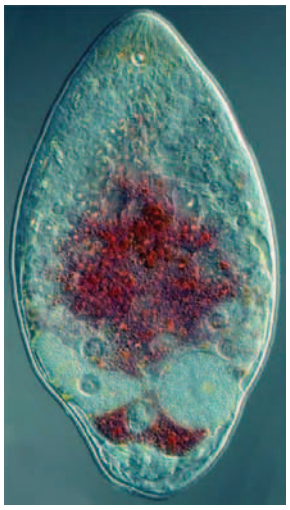

(H)

◀ **Figure 9.1 Acoela.** (A) *Diopisthoporus lofolitus* (Diopisthoporidae). (B) *Paratomella rubra* (Paratomellidae). (C) Color variation in 33 specimens of *Hofstenia miamia* (Hofsteniidae) from the Caribbean. (D) *Philactinoposthia novaecaledoniae*, living specimen (Dakuidae). (E) *Waminoa* sp. (Convolutidae) on bubble coral (*Plerogyra sinuosa*). (F) *Daku riegeri* (Dakuidae). (G) *Eumecynostomum evelinae* (Mecynostomidae). (H) *Paramecynostomum diversicolor*.

excretory system, unencapsulated gonads, absence of ciliary or rhabdomeric eyes, lack of a basal lamina under the epidermis, and absence of a larval stage.

The Acoel Body Plan

Body Wall and External Appearance

Most acoels are tiny, just a few millimeters long. The smallest species tend to be interstitial, feeding on bacteria and organic particulates available on the surfaces or between the spaces of the sediments they inhabit. Infaunal species tend to be more elongate (Figure 9.1A,B,F,H). Larger species usually inhabit the surfaces of rocks, large algae, or cnidarians (Figure 9.1C–E,G). These more rapidly moving species are often predatory, gliding quickly on their ciliated surfaces and capturing prey with a raptorial "hood" that consists of lateral extensions of the body.

Large-bodied species, reaching lengths of 4–5 m, in some families (e.g., Convolutidae) have anterior ocelli (Figure 9.1G), whereas small-bodied species tend to lack these. Larger-bodied species also often have photosynthetic endosymbionts under their epidermis (Figures 9.1E and 9.3A–E). Endosymbiotic algae are contained within the bodies of many large species of acoels, and this association probably evolved more than once—both zoochlorellae and zooxanthellae have been identified among the various species. Algae are usually obtained during feeding by juvenile worms, but in some species can also be transmitted within oocytes by parents to their offspring (vertical transmission). In *Heterochaerus langerhansi*, the dinoflagellate *Amphidinium klebsii* resides below the epidermis and has been shown, using radioactively labeled carbon and nitrogen, to receive these substances from its host in the forms of CO_2 from respiration and excreted ammonia from protein metabolism. The rate of transfer is light dependent.

It has been suggested that acoel body pigmentation may have several contexts. One might be to provide protection from UV radiation for their symbiotic photosynthetic protists. A second context might be to provide cryptic coloration, either to make acoels inconspicuous to visual predators, or possibly to make them less visible to prey. *Hofstenia* species, also known as "panther worms," are highly polymorphic in dorsal pigmentation, with diverse patterns of dappling and striping of brown, yellow, and white colorations (Figure 9.1C).

The acoel body is completely covered with cilia, which may or may not also line the mouth and entrances to reproductive structures. The epithelium lacks a basal lamina (extracellular matrix, or ECM). Early researchers identified **pulsatile bodies** embedded within the epidermis of acoels (and nemertodermatids), which later proved to be clumps of ciliated cells in the process of being resorbed and replaced by the epidermis.

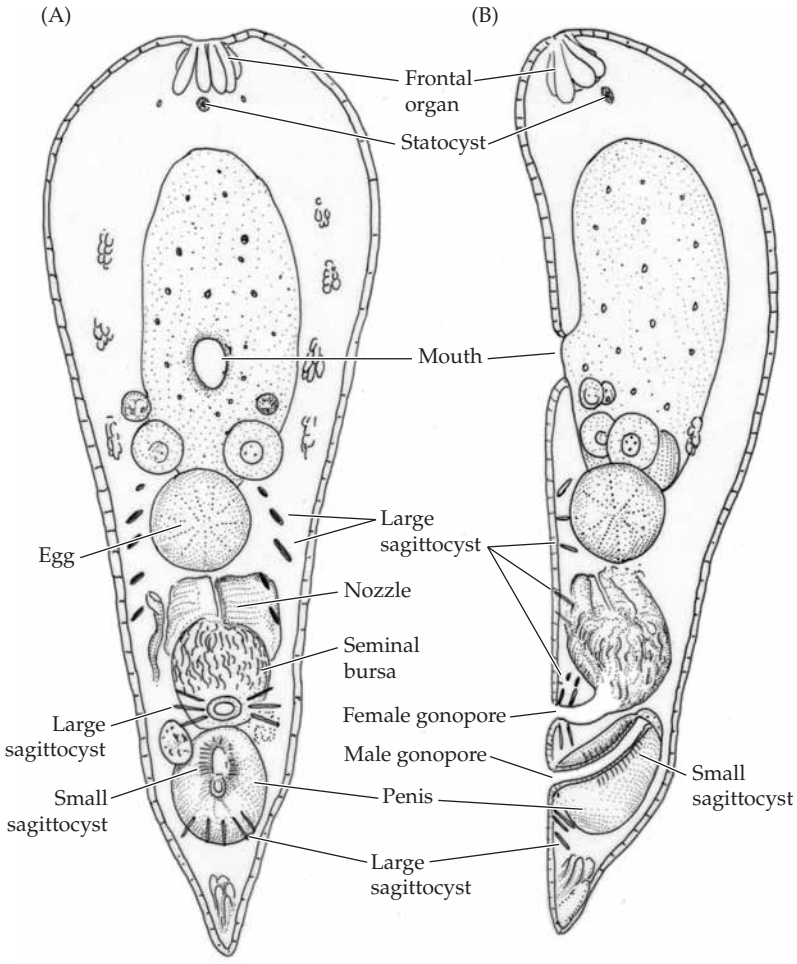

Figure 9.2 The anatomy of *Praesagittifera shikoki* (Acoela). (A) dorsal view, (B) lateral view.

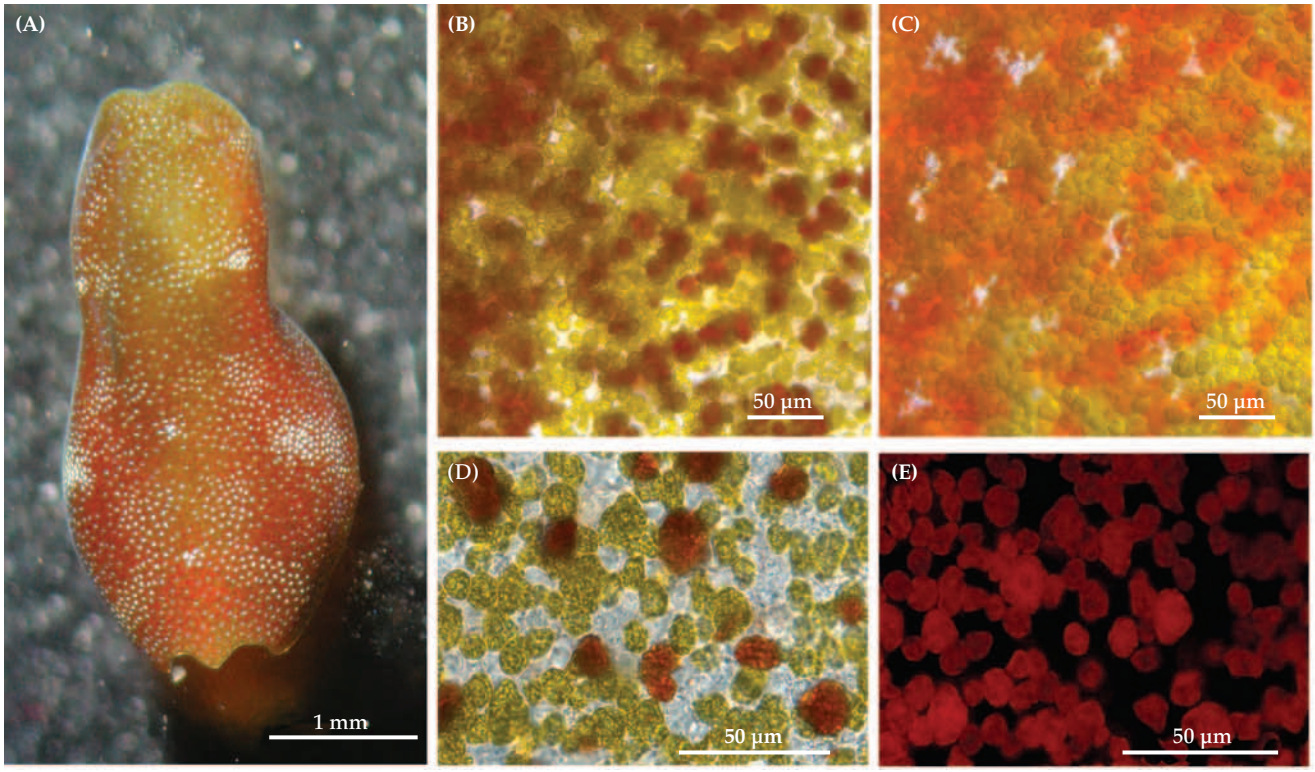

Figure 9.3 Bright coloration in *Convolutriloba longifissura* (Acoela). (A) Whole body (dorsal view). (B–E) Close-up views of the dorsal surface of *C. longifissura* showing endosymbiotic algae (B,D) transmission light; (C) incident light; (E) epifluorescent light (blue excitation). Note that (B,C) and (D,E) are paired images.

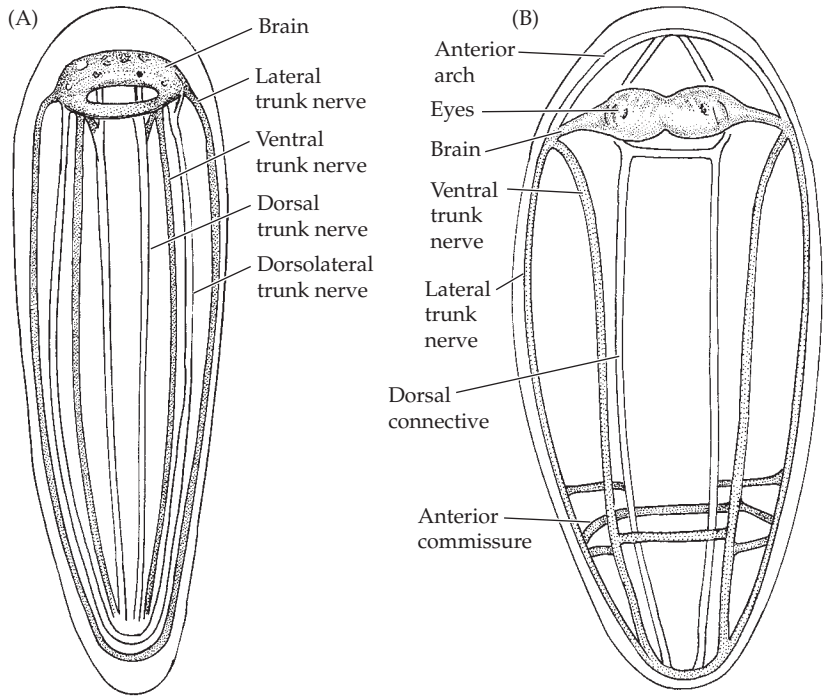

Figure 9.4 Comparison of the central nervous systems of (A) an acoel (*Actinoposthia beklemishevi*) and (B) a free-living flatworm (*Gievzstoria expedita*).

Mucus-producing **frontal organs**, that superficially resemble those of flatworms but are probably not homologous in structure, occur in most families. The ciliated epidermis of acoels also bears **rhabdoid glands** distributed over the body. The **rhabdoids** themselves are composed of mucopolysaccharides and are chemically as well as structurally distinct from the rhabdites of free-living flatworms, although their role in producing mucus to assist ciliary gliding appears to be similar. Most members of the family Sagittiferidae also have **sagittocysts** (Figure 9.5), complex needle-shaped secretory products (5–50 µm long) that are ejected with force in prey capture or for defense, and probably also to assist in sperm transfer during copulation (perhaps by perforating the partner's epidermis). Each sagittocyst arises from a **sagittocyte**, which is surrounded by tightly spiraled muscle filaments that expel the sagittocyst upon contraction (Figure 9.6).

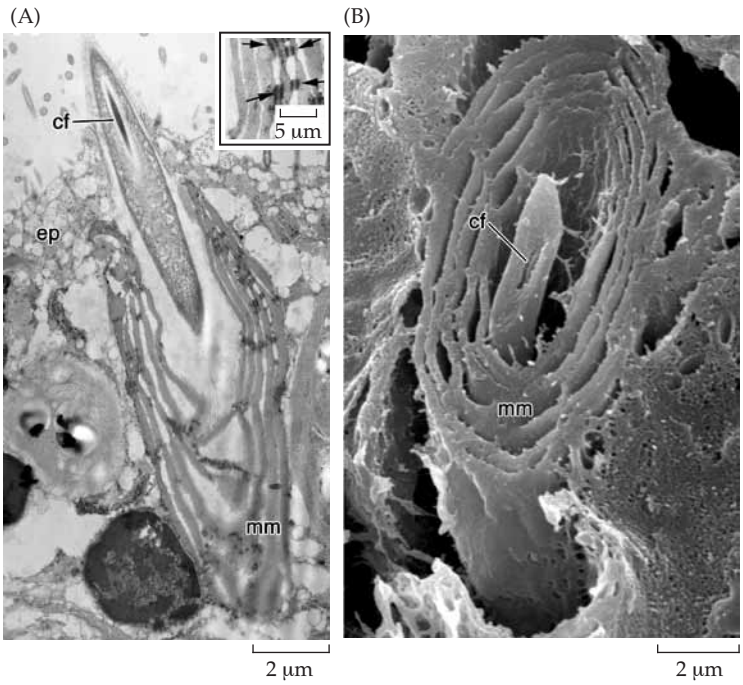

Figure 9.5 TEM images of a *Convolutriloba longifissura* (Acoela) sagittocysts. (A) A sagittocyst (cf = central filament of sagittocyst) during extrusion from the muscle mantle (mm) and penetration of the epidermis (ep). Inset shows higher magnification of the muscle mantle; arrows indicate the location of desmosomes linking the mantle layers. (B) Close-up of the cut surface of a sagittocyst within the muscle mantle.

The position of the mouth in acoels is highly variable. In families thought to be primitive, the mouth opens at the posterior end of the animal and leads to a distinct pharynx (Figure 9.1A). Other families have anteroterminal mouths, although most acoel mouths open midventrally (Figures 9.2 and 9.7B). Both a circulatory system and an excretory system, even in the form of protonephridia, are lacking in the Acoela. Male and female reproductive organs are visible through the body wall of smaller acoels (Figures 9.1A,F,H). In larger species they may protrude from the body surface (Figures 9.7D, 9.12B).

Body Musculature, Support, and Movement

The mesodermally derived musculature of acoels provides the primary means of support, whereas the body cilia (assisted by body muscles) provide for their gliding movement. The shape of the cilia is distinctive, having a marked shelf at the tip where doublets 4–7 terminate. The rootlet system that connects the cilia is also unique. Two lateral rootlets project from each cilium and connect to the tips of the adjacent cilia. From a caudal rootlet, two bundles of fibers project to join the kneelike bend of those same adjacent rootlets. Epidermal cilia of acoels beat in a coordinated fashion to create metachronal waves that move from anterior to posterior.

Abundant dorsoventral muscles serve to flatten the body, and muscles in the body wall generate bending, shortening, and lengthening movements (Figures 9.7A–E and 9.8A–G). The body wall musculature includes circular, diagonal, longitudinal, crossover,

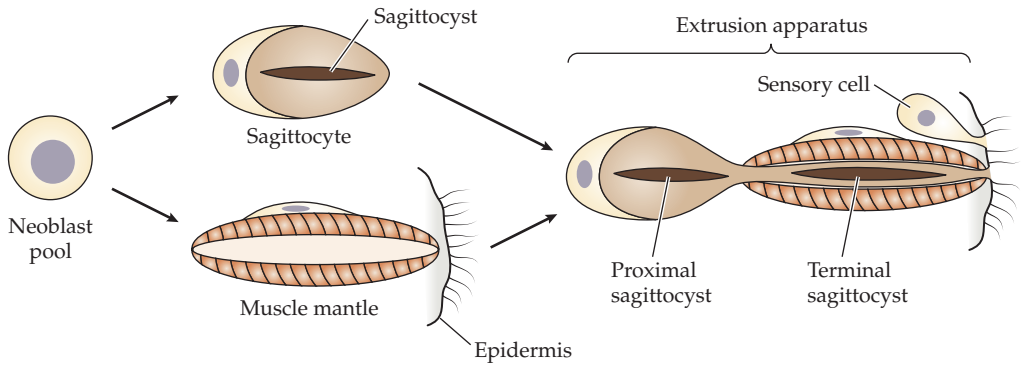

Figure 9.6 Formation and differentiation of sagittocytes and their muscle mantle from neoblast cells in Acoela. See text for description.

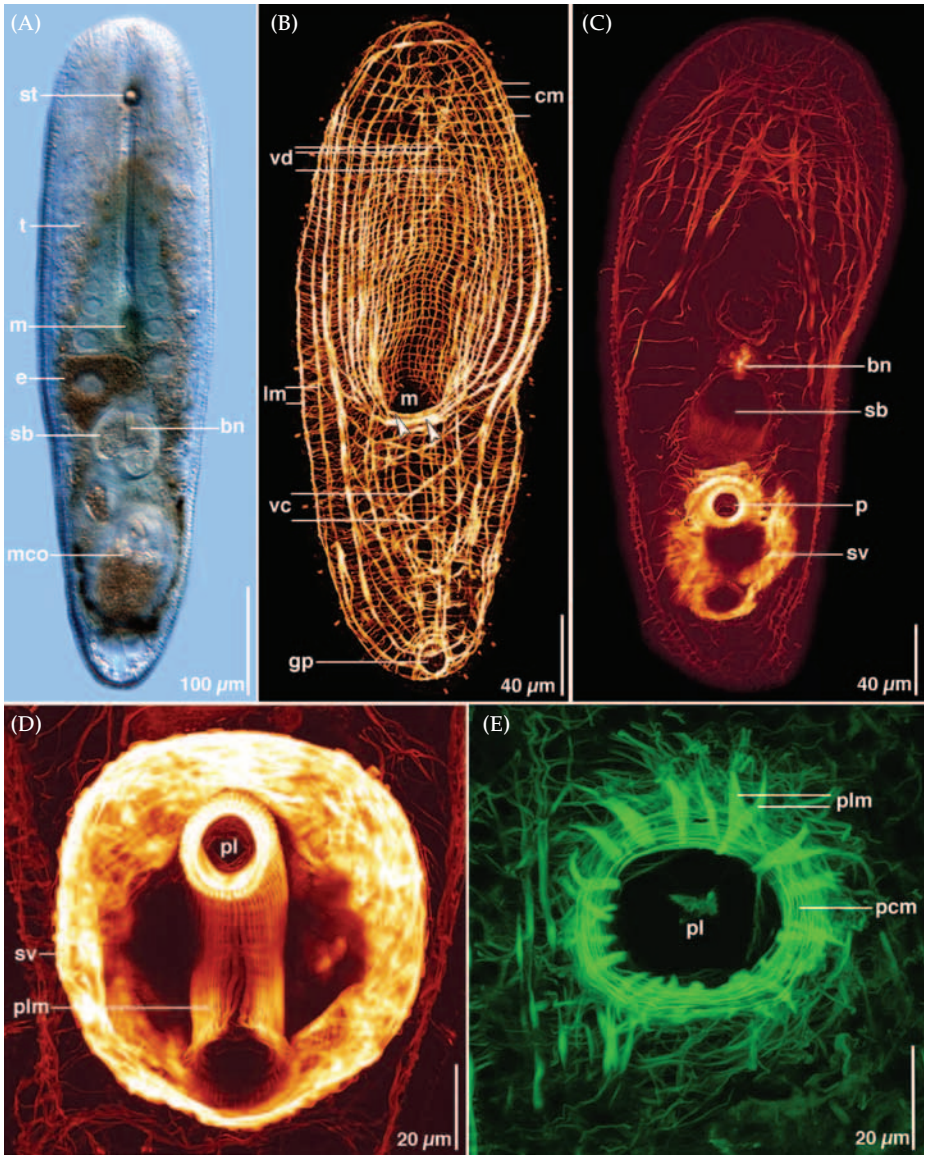

Figure 9.7 Musculature of acoels. (A) Whole mount of living specimen of *Isodiametra earnhardti*. (B) Ventral body-wall musculature of *Haplogonaria amarilla*. (C) Parenchymal musculature of *I. divae*, showing portions of copulatory organs. (D) Male copulatory organ of *I. divae*, showing musculature of seminal vesicle and invaginated penis. (E) Penis musculature of *Convoluta henseni*. Projections of musculature in whole-mount specimens of acoels stained with Alexa-488-labeled phalloidin and viewed using CLSM. bn: bursal nozzle; cm: circular muscle of body wall; e: egg; gp: gonopore; lm: longitudinal muscle of body wall; m: mouth; mco: male copulatory organ; p: penis; pcm: circular muscle of penis; pl: penis lumen; plm: longitudinal muscle of penis; sb: seminal bursa; st: statocyst; sv: seminal vesicle; t: testes; vc: ventral crossover muscle; vd: ventral diagonal muscle.

spiral, and even U-shaped muscles. Species lacking a pharynx appear to have specialized, complicated ventral musculature to compensate for the lack of a muscular food-moving structure and this allows body movements to force food through the mouth.

Nutrition, Excretion, and Gas Exchange

As juveniles, most acoels appear to feed on protists, including unicellular algae such as diatoms. Smaller species may continue this diet throughout their lives, whereas larger species (e.g., *Convoluta convoluta*) are often predaceous, hunting minute crustaceans but also feeding on larval molluscs and other worms. Smaller protists are captured as acoels glide over them with the syncytial gut extruded through the mouth such that it engulfs food with "amoeboid"-like movements. Larger prey are grasped with the anterior margin of the body and entrapped with mucus before being pressed toward the mouth. Swimming prey may also be rapidly captured and ingested, whereas dead material seems to be actively avoided.

Some acoels possess a pharynx, in some cases known as a **pharynx simplex** (Figure 9.1A), and this structure is variable among the families. In some cases the pharynx is a flexible, tube-shaped structure that can be everted from the mouth. The pharynx is anchored by musculature attaching to circular muscles within the body wall. In the larger, predaceous species, there is no oral sphincter but several layers of circular muscles interspersed with oblique and longitudinal muscles extend throughout the protrusible structure, which is attached to the body wall by densely packed muscles (Figure 9.7B,C). In cases where no distinct pharynx exists, muscle fibers encircle the mouth to form a sphincter.

Ingested prey is enclosed within vacuoles that drift within the digestive syncytium, and food is completely

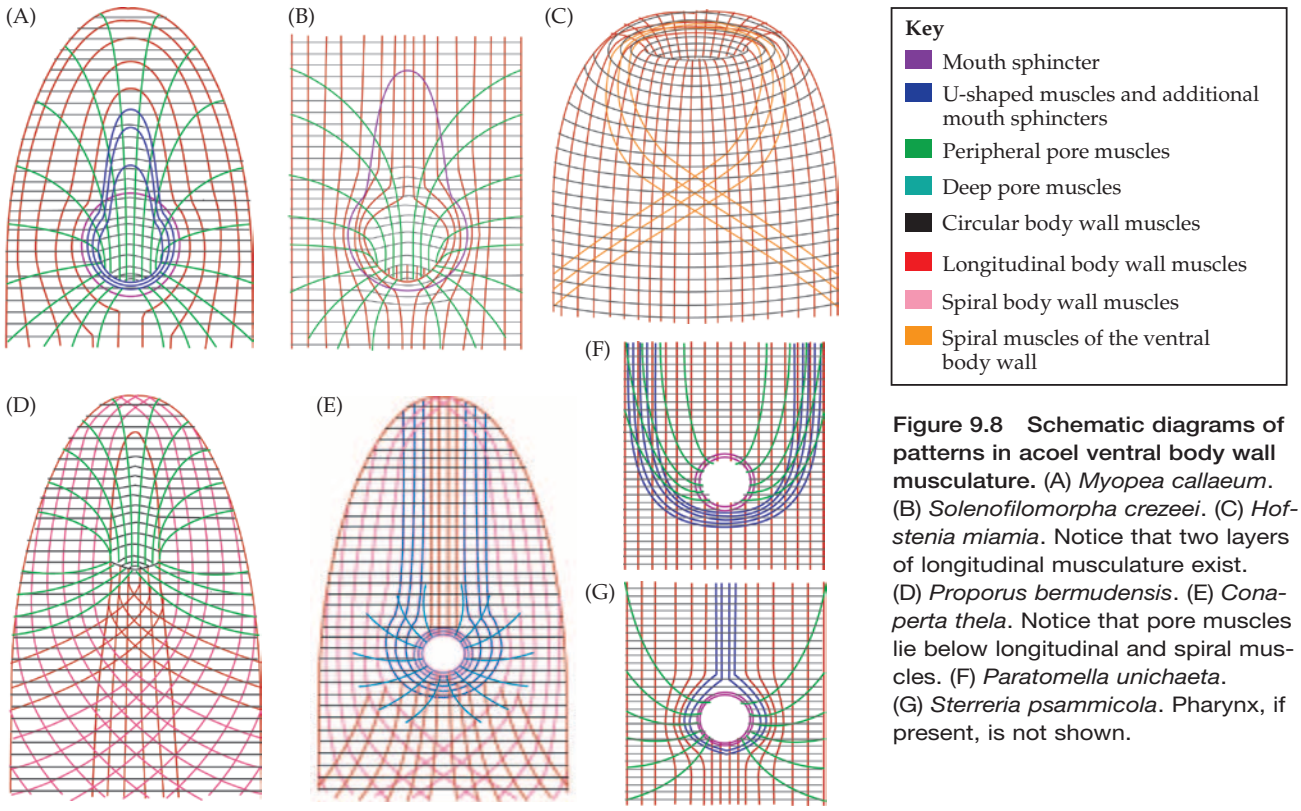

Figure 9.8 Schematic diagrams of patterns in acoel ventral body wall musculature. (A) *Myopea callaeum*. (B) *Solenofilomorpha crezeei*. (C) *Hofstenia miamia*. Notice that two layers of longitudinal musculature exist. (D) *Proporus bermudensis*. (E) *Conaperta thela*. Notice that pore muscles lie below longitudinal and spiral muscles. (F) *Paratomella unichaeta*. (G) *Sterreria psammicola*. Pharynx, if present, is not shown.

absorbed within 18 to 24 hours. The exoskeletons of hard-bodied prey such as crustaceans are voided through the mouth. Fat globules and occasional glycogen vacuoles stored within cells appear to be the primary forms of food reserve. A number of acoel species associate with corals (including *Waminoa* and several species of *Convolutriloba*). These associations appear to primarily benefit the acoels who likely feed on mucus produced by these cnidarians (Figure 9.1E). It has been suggested that the syncytial digestive system of acoels might be an extreme state of the condition seen in nemertodermatids, which have a small, relatively occluded gut lumen (and a remnant of a gut lumen is evident in the acoel *Paratomella rubra*).

The small size of acoels is sufficient to allow them to eliminate waste nitrogen and carbon dioxide, as well as obtain oxygen from the surrounding water, without a need for specific excretory or circulatory systems. Food vacuoles evidently serve to move materials from the digestive syncytium to other cells within the body.

Nervous Systems and Sense Organs

The central nervous system of acoels usually includes an anteriorly located cluster of large commissures and a few cell bodies that form a paired ganglia system with what some workers consider to be a minute neuropil (though it is quite rudimentary compared to other metazoans). Arising from this are three to five pairs of longitudinal nerve cords connected by an irregular network of transverse fibers (Figure 9.9). Typically there are single or paired dorsal nerve cords, and paired lateral and ventral cords. Peripheral neurons connect to epidermal sensory cells and to anterior light-sensitive cells that serve as simple eyes. There is no indication that the eyes have ciliary or rhabdomeric elements, and they are probably simple pigment cells with refractive inclusions and up to three nerve cells to relay the stimulus. This organization contrasts markedly with that of platyhelminths, where the brain consists of a comparatively dense ganglionic mass, the nervous system is primarily developed ventrally, and the nerve cords form an orthogonal nervous system composed of eight orthogons largely developed laterally and ventrally (Figure 9.4). Although organized as a bilobed structure, the acoel "brain" lacks the dense ganglionic cell mass (neuropil) seen in the Platyhelminthes.

The acoel statocyst is a fluid-filled, proteinaceous spherical capsule, 10–30 μm in diameter, surrounding a single retractile statolith (Figure 9.1A–H). The statolith appears to be a single spherical cell. The capsule enclosing the statolith comprises two unciliated cells. Behavioral observations indicate that acoels are capable of precise geotactic orientation, suggesting that movements of the statolith within the statocyst are detectable by the animal. Three pairs of muscle fibers insert into the membrane of the statocyst, evidently

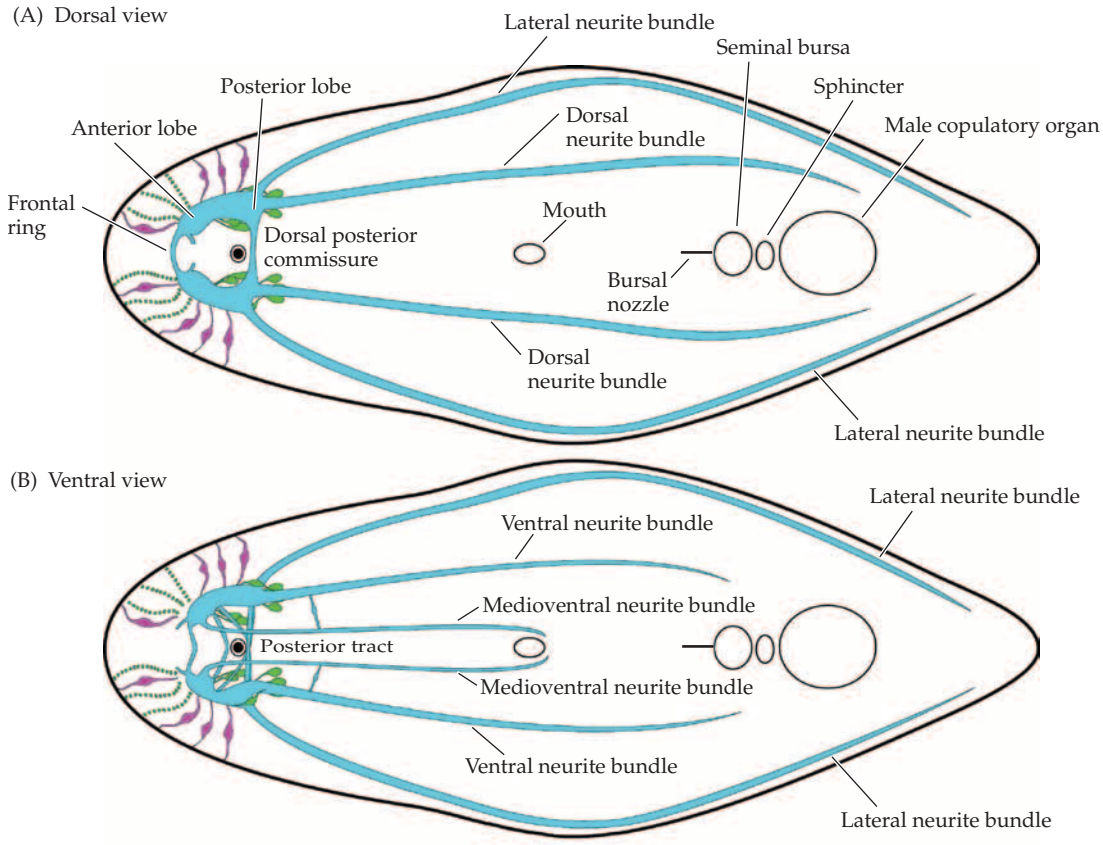

Figure 9.9 Diagram of the nervous system of *Isodiametra pulchra* (Acoela) revealed using nervous tissue-specific staining (green and magenta colors denote separate types of neural tissue in the bilobed acoel brain; cyan color is the central nervous system).

assisting in maintaining its position. While the cerebral commissure is closely associated with the statocyst, specific innervation of the structure is difficult to clearly identify, although a small nerve cushion created by two nerve bundles insert on the capsule and a cell body located at the ventral pole may be responsible for detecting deformation of statocyst fluid. Alternatively, positional information may be conveyed by the stretching of muscle fibers surrounding the statocyst. While statocysts appear in other metazoans, including cnidarians, ctenophores, platyhelminths, annelids, and others, statolith movement within the statocyst in these taxa is generally detected by cilia along the internal surface of the statocyst. The lack of these modifications within the Acoela appears to be unique.

Reproduction and Development

Acoels are capable of both sexual and asexual reproduction, and have considerable ability to regenerate cells through the actions of multipotent, mesodermally derived, neoblastlike cells. These structures were originally described in the Platyhelminthes, but analogous (or homologous) cells appear in the Acoela as well. These cells replace damaged or missing body components and appear to have few limitations in how they are able to repair or replace tissues, particularly epidermal cells.

Three distinct forms of asexual reproduction have been documented within the Acoela: transverse fission, longitudinal fission, and budding (Figure 9.10). Although

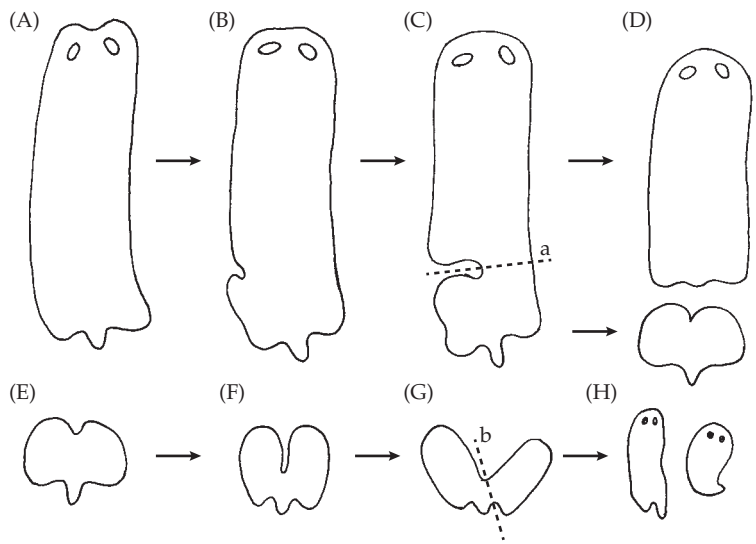

Figure 9.10 Modes of asexual reproduction in *Convoluta longifissura* (Acoela). (A) Intact animal. (B–D) Transverse fission; lower element of (D) shows "butterfly" stage preceding transversion fission (E–H).

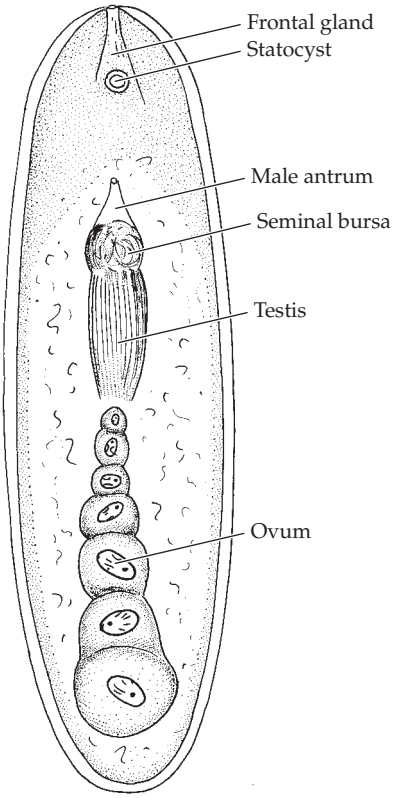

Figure 9.11 Internal organization of *Antigonaria arenaria* (Acoela).

capable of asexual reproduction, and while often found in large local abundances, acoels are not known to markedly increase their numbers asexually under natural conditions (as seen in many other asexual organisms), except perhaps in the family Paratomellidae.

Most acoels are simultaneous hermaphrodites (Figure 9.11), although some (e.g., all members of the family Solenofilomorphidae) are protandrous. Ovaries and testes may be paired or unpaired, with testes usually dorsal and ovaries more ventral (Figure 9.12A). In some species a single mixed gonad exists. In no cases are the gonads saccate—that is, the germ cells are not lined or discretely separated from the surrounding parenchyma.

Genitalia are usually visible near the posterior end of the animal. The penis is a muscular and glandular, or needle-like structure, often with multiple stylet-like elements (Figure 9.12B). Male intromittent organs, regardless of form, can be retracted into a seminal vesicle. During copulation the penis is everted through the gonopore that typically lies in a distinct antrum, or vestibule on the body surface. A separate female gonopore exists in some species. In others, the female pore connects directly to the male pore. In still others, no external female opening exists and insemination is hypodermic. Most, but not all acoels have the vagina positioned anterior to the penis.

A **seminal bursa** may exist that appears to receive sperm from mating partners either during copulation or after hypodermic insemination, and a sclerotized **bursal** or **vaginal nozzle** or sphincter regulates the

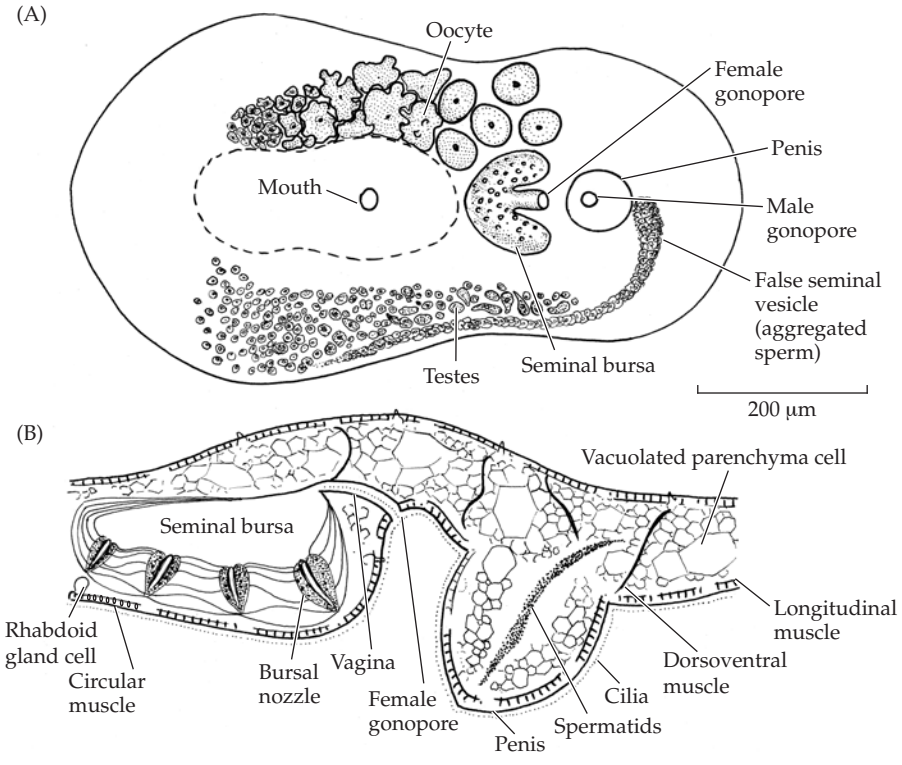

Figure 9.12 Acoela. Reproductive anatomy of *Polychoerus gordoni*. (A) Dorsal view; note in this species as in other Convolutidae, male and female gonads are paired, but are shown singly here. (B) Sagittal view of female and male reproductive anatomy.

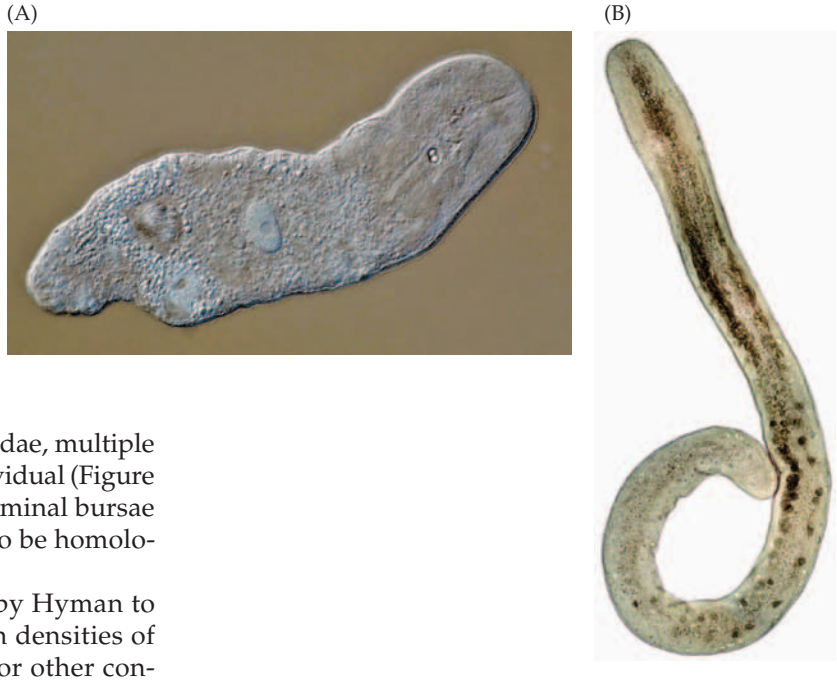

Figure 9.13 Nemertodermatids. (A) *Flagellophora apelti*. (B) *Sterreria* sp.

passage of sperm to eggs. These are among the few sclerotized structures in these soft-bodied worms, and they were considered important in early taxonomic treatments of Acoela. Like many persistent terms in invertebrate zoology, the name "nozzle" was coined by Libbie Hyman who thought these structures resembled the nozzle of a hose. In certain Convolutidae, multiple bursal nozzles may exist in the same individual (Figure 9.12B). While highly variable in form, seminal bursae and their associated structures appear to be homologous among all acoels.

Copulatory behavior was observed by Hyman to occur during daylight hours when high densities of individuals are maintained in aquaria or other confined spaces. Individuals approach one another and exchange quick touches or "nips" of the anterior ends. Larger individuals appear to initiate copulation, which proceeds after both individuals roll into a ball, and then unroll with their genitalia firmly engaged. Individuals mutually insert their penes into their partner's female gonopore and direct sperm and seminal fluid into the female bursa.

Acoel sperm are distinctively biflagellate, with the two axonemes of the flagella incorporated into the cell body (a condition also seen in Platyhelminthes). Several well-defined patterns exist in acoel sperm morphology, and these seem to be phylogenetically informative. Combined studies of 18S rRNA sequence data and sperm morphology have revealed remarkable concordance between these two sources of data. Seminal bursae and bursal nozzle complexity also appears to correlate with variation in sperm morphology.

Oocytes in the gonads of acoels give rise to endolecithal eggs. Fertilization is always internal, and zygotes are released either through the mouth, via the female gonopore, or through a rupture created in the epidermis by the growing embryos. Zygotes may be brooded or protected by encapsulation, but are usually deposited singly and undergo direct development. Eggs appear to be laid primarily at night, in flat gelatinous masses.

Embryonic development is direct, and the cleavage pattern of acoels appears to be a unique "**spiral duet**" cleavage program that is different from any other metazoan (although this has been questioned). Nemertodermatids, while exhibiting duet cleavage in the 4-cell stage, do not exhibit this spiral duet pattern, and their cleavage pattern is distinct from the Spiralia. It has been suggested that the acoel spiral duet-cleavage pattern might have evolved from an ancestral spiral quartet cleavage typical of the Spiralia. As in spiral quartet cleavage, the first horizontal cleavage is unequal and so produces micromeres, but it occurs at the two-cell stage instead of the four-cell stage, so the micromeres appear as duets instead of quartets. One is tempted to call this "bilateral cleavage," rather than spiral cleavage, and it is quite different from that of all other spiral-cleaving Metazoa. So far as is known, acoel embryos generate only endomesoderm, whereas most spiral-cleaving animals tend to also produce some ectomesoderm. Internal tissues arise either by delamination or by immigration of cells that form the ectoderm and mesoderm. By the time gastrulation is complete, the embryo has a layered appearance, with an outer epidermal primordium, and a middle layer of progenitor cells of muscles and neurons, while the innermost cells are those that will develop into the digestive syncytium. Endomesoderm forms from both of the third duet macromeres at the vegetal pole, whereas the mouth forms anteriorly as 1a micromere descendants expand around the posterior pole. An anus never forms.

Class Nemertodermatida

Nemertodermatida comprise a few dozen species of marine worms described from several locations around the world, including the Swedish coast (their original discovery site), the Mediterranean, Adriatic, North and Caribbean Seas, and the east coast of North America. Like acoels, nemertodermatids were once classified in the phylum Platyhelminthes. Nearly all known species are free-living, usually in fine sand, mud or gravel; however, species in the genus *Meara* are symbionts in

the foreguts of holothurian echinoderms (sea cucumbers). Nemertodermatids range in length from a few millimeters to nearly a centimeter. They can be leaf shaped or narrow and elongated, and they may creep over the substratum or swim with serpentine movements. Their bodies are densely covered with locomotory cilia, and as a group they are easily recognized by an anterior statocyst containing two statoliths in separate chambers (Figure 9.13A,B)—although a few reports of one to four statoliths also exist in the literature. Some species possess an eversible proboscis associated with feeding (oddly, some of these species lack a distinct mouth) with numerous branches that extend anteriorly "like a witch's broom" (Figure 9.14).

The epidermal cells of nemertodermatids lack a true basal lamina but are connected to underlying muscle cells and to each other via a narrow extracellular matrix. Septate junctions between epidermal cells are lacking. As in the acoels, old or damaged ciliated epidermal cells are withdrawn into the body and resorbed, creating temporary structures called pulsatile bodies (Figure 9.15). Also as in acoels, species inhabiting the guts of other animals often have symbiotic bacteria inhabiting their epidermis (Figure 9.16). The form of the mouth and gut vary among species from temporary structures to a porelike opening and a narrow intestinal lumen, although a complete gut is lacking (there is no anus, nor is there a discrete pharynx). They lack discrete circulatory or excretory structures. Asexual reproduction has not been reported. Male sexual structures may consist of a simple, ciliated invagination of the epidermis or an eversible penis; seminal vesicles may be present. Where these structures are lacking, sperm appear to simply be ejected from the

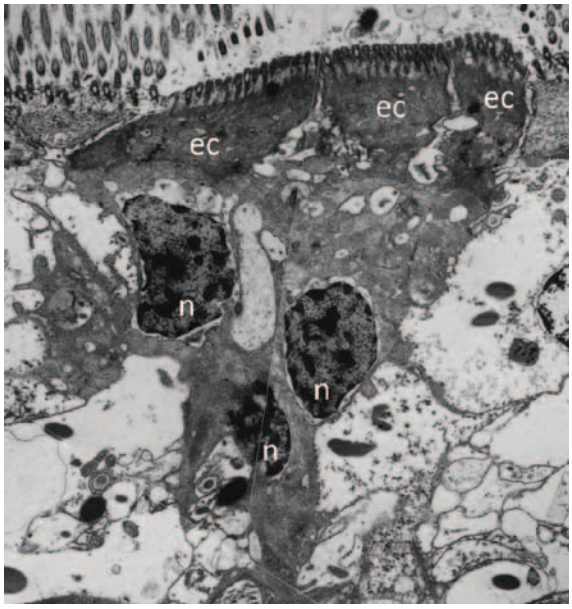

Figure 9.15 TEM micrograph showing a cross section the epidermis of the nemertodermatid, *Meara stichopi*. Three ciliated epidermal cells (ec), presumably worn or damaged and bearing only the dark stubs of locomotory cilia, are being compacted and withdrawn into the integument to be dissolved; the three dark structures are the epidermal cell nuclei (n).

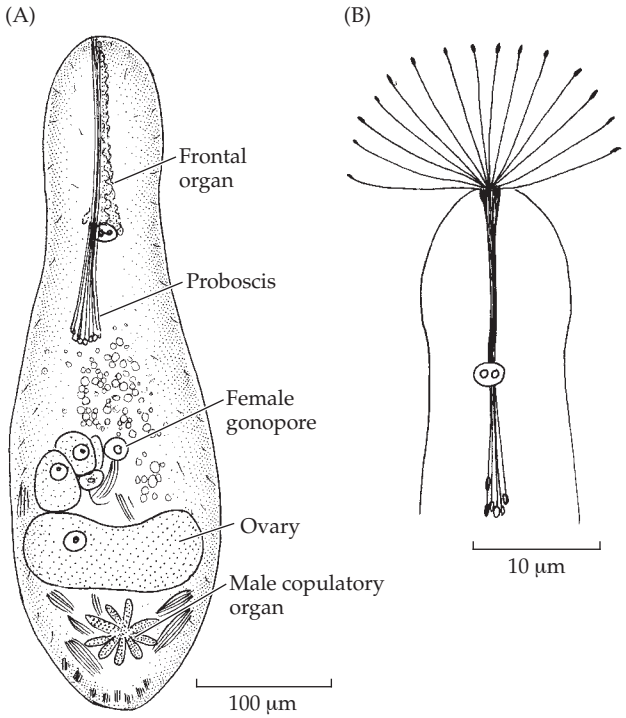

Figure 9.14 Nemertodermatids. *Flagellophora apelti*. (A) Dorsal view of mature specimen. (B) Protruded proboscis.

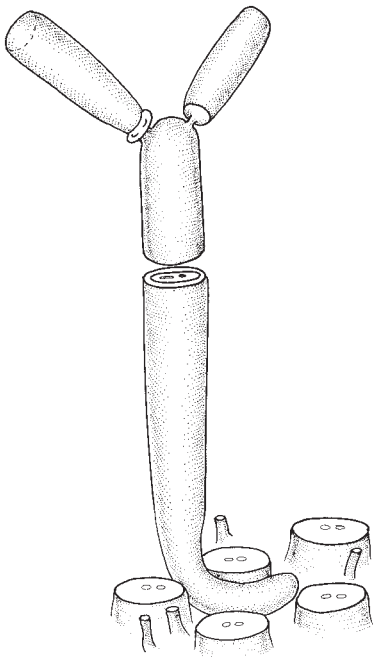

Figure 9.16 Nemertodermatida. Y-shaped elongated symbiotic bacteria associated with the epidermis of *Meara stichopi*.

male antrum. The female gonopore with an associated bursa is located dorsally in most species. Mature eggs are released through the mouth. Duet cleavage and direct development are similar to that observed in acoels (Figure 9.20).

The first described nemertodermatids was classified within the acoel Platyhelminthes by Otto Steinböck in 1930. Long before similar hyperbolic statements by now-deposed dictators, Steinböck, a colorful individual known to express himself in double-spaced capital letters with exclamation marks for emphasis, announced his discovery as "the mother of all turbellarians," possessing a "novel, two-stoned statocyst, an unusually thick and gland-rich epidermis, a peripheral nervous system, and a mixed, lacunar gonad without accessory organs." Going Steinböck one better, in 1940 Tor Karling removed the Nemertodermatida from the Acoela and other-than-turbellarians altogether because of their well-formed intestinal lumen, a structure lacking in acoels. The Acoela and Nemertodermatida were combined as sister taxa within the Platyhelminthes in 1985 with Ulrich Ehlers' recognition of the taxon Acoelomorpha, primarily based on ciliary structures. Additional work on Nemertodermatida has proceeded slowly because specimens are difficult to come by and because many characters can be highly variable within populations.

The relationship of *Meara stichopi* to its echinoderm hosts is poorly understood, but does not appear to be parasitic—hosts do not appear to be harmed by the presence of the worms. In fact, the relationship could be mutualistic, as nematodes have been found within the guts of endosymbiotic *Meara*. Symbiotic species of both *Meara* and *Nemertoderma* are known to possess elongated, y-shaped symbiotic bacteria (Figure 9.16). In *Meara*, these symbionts are found primarily on the ventral side of the host's body. The y-shape of the bacteria has been suggested to represent the mode of asexual reproduction because appendages are found only on certain bacterial cells. Ultrastructural studies indicate that bacteria occur only on the outside surface of their worm hosts, suggesting that the association between bacteria and host does not represent infection.

The Nemertodermatid Body Plan

General Body Structure

In general, nemertodermatids are small. The endosymbiotic *Meara stichopi* is usually less than 2 mm in length, free-living *Nemertoderma* average about 3 mm in length, and a few "giant" nemertodermatids grow to nearly one centimeter in length. Most individuals are colorless to yellow or red, but pigmentation can be variable within populations.

The epidermis of most species appears to contain numerous bottle-shaped mucous glands. Overall, the epidermis resembles that of nemertean worms, which led to the namesake "Nemertodermatid." In the genus *Nemertoderma*, these glands are more abundant at the apical pole, forming an anterior gland complex with separated, outwardly directed gland openings or necks. However, these openings are not grouped together in a regular way at an apical pore and thus do not form a "frontal organ" as has been described in Platyhelminthes. Nevertheless, the structures are sufficiently similar to that of turbellarians that earlier authors considered them to be homologous with the frontal organ structures of flatworms.

Cell and Tissue Organization

The epidermis of nemertodermatids is entirely ciliated. The cells are connected by an intracellular terminal web—a stratified structure composed of a closely woven inner layer of intensely staining fibrils overlain with more loosely packed fibrils, which bulges at the cell borders. Epidermal cells are joined apically by belt-like adherens junctions (belt-desmosomes) called **zonula adherens**. Interspersed among the cells are the necks of various glands and sensory receptors, particularly in the anterior region of the animal. The necks of glands appear to have associated muscular rings that may regulate the flow of gland contents (Figure 9.17E).

The ciliary rootlet structure is similar in Acoela and Nemertodermatida, one of the primary reasons workers grouped these two taxa together (as the Acoelomorpha). The rootlets of nemertodermatids include a rostrally-oriented rootlet and a caudally-oriented rootlet. In their original description, *Meara stichopi* was reported to possess "restitution cells," that appeared to contain ciliary structures in the process of being resorbed. Indeed, these cells represent structures similar to the pulsatile bodies reported in acoels, wherein worn cells are encapsulated and transported to the digestive tract for resorption (Figure 9.15). However, this feature is distinct in the nemertodermatids because the cilia detach from their basal apparatus before encapsulation, eliminating their ability to pulsate, causing some researchers to refer to them as "degenerating epidermal bodies."

Support and Movement

As in the acoels, body wall musculature of nemertodermatids has been extensively investigated using phalloidin-staining procedures.[1] Like most acoels, body musculature in nemertodermatids consists of outer circular and inner longitudinal muscle layers. Diagonal musculature typically is also present and varies among species, with fibers forming connec-

[1]Phalloidin is a naturally occurring toxin in the death cap mushroom (*Amanita phalloides*). Its toxicity is due to its ability to stabilize actin filaments within cells, and this attribute has led to it being widely used (fluorescently labeled) in research to visualize filamentous actin, such as muscle fibers.

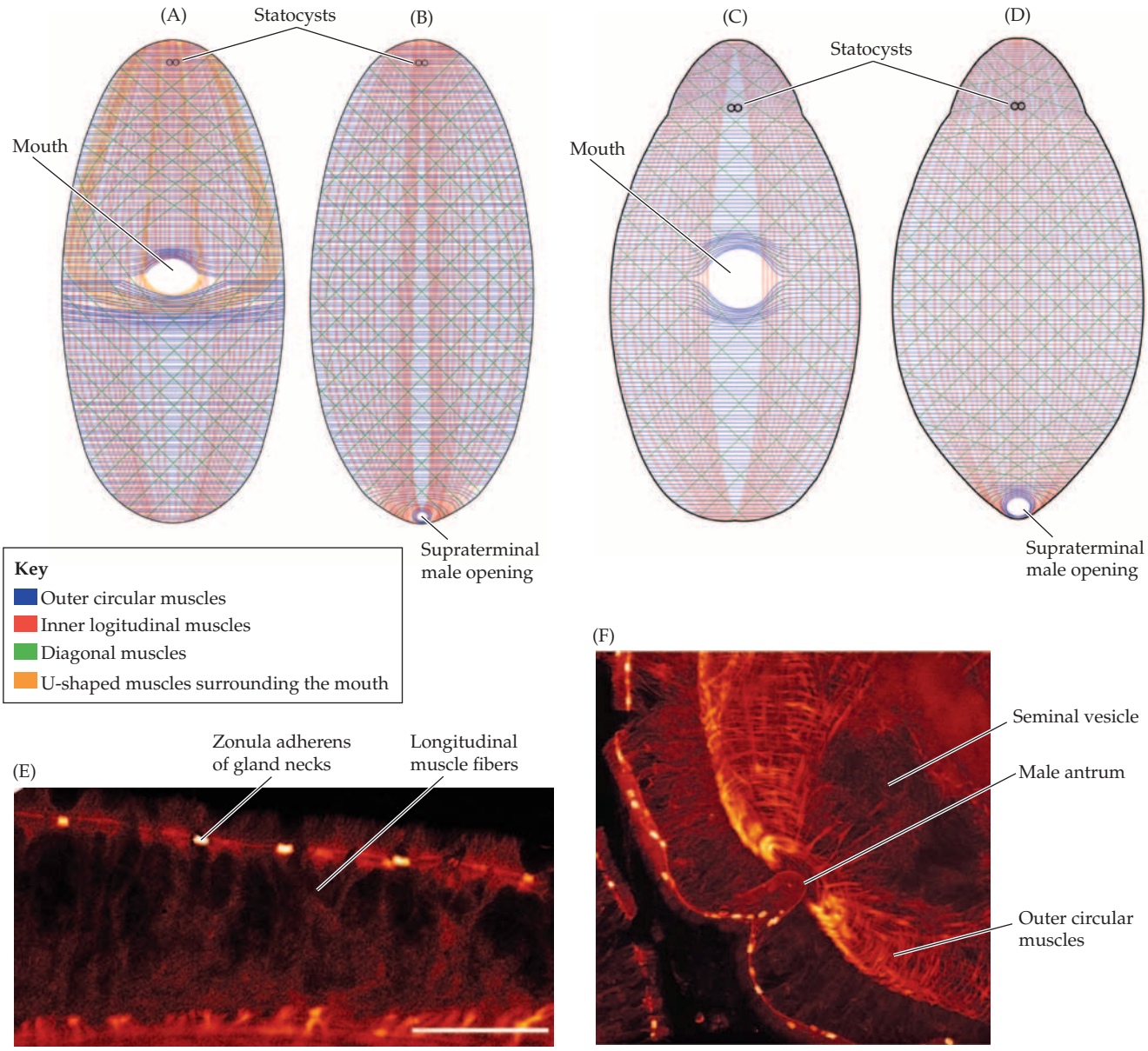

Figure 9.17 Schematic diagrams (A–D) and phalloidin-enhanced micrographs (E,F) of nemertodermatid musculature. (A) Ventral and (B) dorsal views of *Meara stichopi* (graphic showing muscle patterns). (C) Ventral and (D) dorsal views of *Nemertoderma westbladi* (graphic showing muscle patterns). Outer circular muscles (blue); inner longitudinal muscles (in red); diagonal muscles (in green); U-shaped muscles surrounding the mouth (in orange) on ventral side. (E) Lateral view of epidermis in *Nemertoderma westbladi*, showing longitudinal muscle fibers beneath circular ones in central space. Above this are two thin stained layers, the lower layer corresponding to the intracellular web, the upper layer corresponding to microvilli of the epidermal surface. The zonula adherens of the gland necks appear as brightly stained areas at this level. (F) Posterior body region of *Nemertoderma westbladi*, with invagination of body wall to form the male antrum; finer musculature of the seminal vesicle is visible in open space.

tions between layers in some (e.g., *M. stichopi*; Figure 9.17A,B) and forming distinct layers in others (e.g., *N. westbladi*; Figure 9.17C,D). Musculature surrounding the mouth also varies, being best developed in species with a permanent mouth. Musculature is also well developed around permanent genital openings (e.g., *M. stichopi*), but less so in species with transient genital orifices (e.g., *N. westbladi*).

The opening of the male gonopore and its associated antrum appear as an invagination of the entire body wall, and musculature associated with the seminal vesicle consists of a thin layer, present only in individuals with mature male organs (Figure 9.17F). Parenchymal muscles may also be present in individuals in all life stages, forming a three-dimensional network throughout parenchymal tissue. The statocyst is

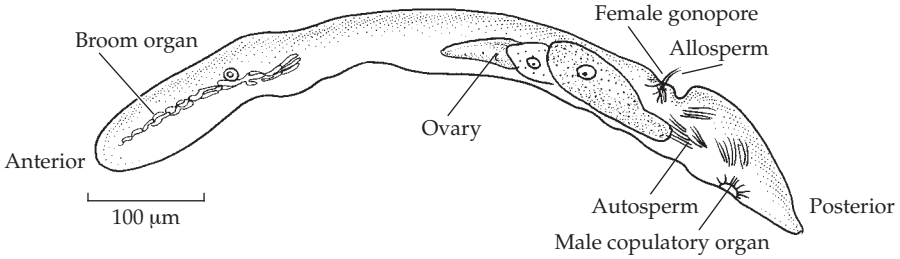

Figure 9.18 Nemertodermatida. Diagram of *Ascoparia* sp., showing location of dorsal female gonopore and subterminal male copulatory organ.

supported by muscles that attach dorsoposteriorly and anterolaterally to other body wall musculature. Nemertodermatids move by creeping on their ciliated surfaces or, in more elongated species, by undulating their bodies in a serpentine way.

Nutrition, Excretion, Gas Exchange

The gut of nemertodermatids has only a single opening, like that of cnidarians and other Xenacoelomorphs. However, unlike acoels, the nemertodermatid gut is not syncytial and instead contains a well-defined intestinal lumen. In some species, a cone of gut tissue has been reported to protrude and retract like a tongue to collect food particles. However, no known nemertodermatid possesses a structure recognizable as a muscular pharynx. Other species appear to lack a mouth altogether. In such species (e.g., *Flagellophora*), an anterior **broom organ** is reported although this structure does not seem to be directly connected to the gut. Instead it seems to consist of a bundle of up to 30 glands whose necks are protrusible through a canal at the anterior end of the body (Figure 9.14B). When opened, the broom organ appears to possess distal ends that are slightly swollen and possibly adhesive. Some researchers suggest that the lack of a mouth may represent an ancestral condition and that the mouth of nemertodermatids is a transient structure that appears during a limited part of postembryonic life, with the duration of persistence dependent upon the species.

Meara stichopi inhabits the foregut of the holothurian *Parastichopus tremulus*, a species common on Scandinavian coastlines, and appears to feed on detritus as well as upon nematodes within the guts of its host. Free-living species have been found with comparatively large turbellarians and nematodes within their guts.

As in acoels, the small body sizes of nemertodermatids allow them to eliminate waste nitrogen and carbon dioxide, as well as obtain oxygen from the surrounding water, without a need for discrete excretory or circulatory systems.

Nervous System

The nervous system of nemertodermatids is still poorly understood. Immunoreactivity studies to the neurotransmitter serotonin (5-hydroxytrypamine; 5-HT), and the regulatory neuropeptide FMRFamide, have shown considerable variation in responses in the species examined. In *Meara stichopi*, 5-HT reactivity reveals a subepidermal nerve net and two, loosely organized longitudinal nerve bundles along the length of the animal. In *Nemertoderma westbladi*, 5-HT reactivity shows a two-ringed, anterior commissure, with the rings converging near the statocyst, and connected by thin fibers. Two lateral fibers extend longitudinally from the commissure, as does a delicate curtain of evenly spaced finer longitudinal fibers that become indistinct caudally. FMRFamide immunoreactivity follows the same pattern as 5-HT reactivity in *M. stichopi* and *N. westbladi*. These results suggest that the nemertodermatid nervous system is distinct from the bi-lobed ganglionic brain and orthogon peripheral nervous systems of Platyhelminthes (i.e., paired longitudinal ventral nerve cords connected by a regular pattern of transverse commissures). Nemertodermatid central nervous systems are also distinct from the commissural brains of acoels (i.e., symmetrical commissural fibers with few cell bodies and 3–5 pairs of radially arranged longitudinal nerve cords, irregularly connected with transverse fibers).

Reproduction and Development

The reproductive anatomy and natural history of nemertodermatids is not well studied, and only a few species have been examined in this regard. The male gonopores in nemertodermatids appear to open dorsally (or supraterminally) and are associated with a muscular male antrum. In fully mature specimens, a muscular seminal vesicle and often a male copulatory organ may also evert either posteriorly or slightly dorsally (Figure 9.18). Female genitalia, if present, are located dorsally. *Flagellophora* seem to have a deep, well-defined invagination that may represent a female gonopore (Figure 9.14).

In *Meara stichopi*, follicular testes occupy most of the preoral part of the body. The ovary occupies the postoral part of the body and often contains one or more large ova within the posterior body region. The male intromittent organ opens terminally to slightly supraterminally in this species.

In general, Nemertodermatida have a 9+2 arrangement of microtubules in their uniflagellate sperm, a condition distinct from the variable microtubule arrangement and biflagellate condition of acoel sperm. Many field-collected nemertodermatids contain two types of sperm. **Autosperm** (sperm produced by the individual in which they are found) in *M. stichopi* are filiform, about 45–60 pm long, and under phase contrast microscopy show indistinct divisions of individual

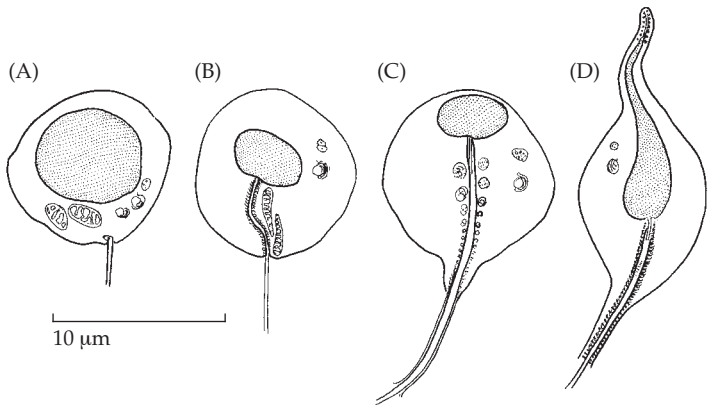

Figure 9.19 Nemertodermatida. Stages in spermatogenesis in *Meara stichopi*. (A) In early stages of spermatogenesis, the nucleus is large, with heterogeneous electron density; two mitochondria are visible. A single flagellum begins to form from a basal body situated near the cell membrane. (B) The nucleus shrinks and becomes homogenous in density. The mitochondria begin to elongate, and the basal body and associated fibers move into the cytoplasm toward a depression forming in the nucleus. Microtubules coil around the flagellar channel. (C) The mitochondria coil around the flagellar channel and a sheath grows from the cell to surround the proximal flagellum. (D) The cell and nucleus elongate to form the head of the spermatozoon. The mitochondria form tighter whorls and wander into the length of the flagellar sheath.

sperm into head, middle piece, and tail, as is typical of more derived spermatozoan forms within Metazoa (Figure 9.19). Some sperm appear to be coiled, corkscrew-fashion, over half of their length and are nonmotile within the animal producing them. **Allosperm** (sperm not produced by the individual in which they are found) are distinctive because they tend to be uncoiled and motile within the bodies of recipient individuals.

The pioneering investigator of acoels and nemertodermatids (what is now called Acoelomorpha), Einar Westblad, noticed that development of female reproductive structures seemed to precede that of male structures indicating that some nemertodermatids are protogynous. On the other hand, other authors have noted that "male maturity seems to precede female maturity," or they have specifically stated that individuals are protandrous. In *N. westbladi*, individuals were found to have matured as males, females, and hermaphrodites, with no clear evidence of either protandry or protogyny. Mature females contained a single large egg but also contained up to 10 ova, and allosperm were found in only a few individuals. In *Ascoparia neglecta*, an elongate species with no actual mouth in mature individuals, although male and female pores are visible, and individuals possess a male copulatory organ, allosperm appear to be contained in vacuoles near the vagina.

Many species appear to lack female genitalia, yet are found to contain autosperm that is clearly contained within male reproductive structures, as well as allosperm that appear to have been introduced to the individual housing it. Individuals bearing allosperm appear to include mature as well as immature individuals raising the possibility of sperm storage and sperm competition among individuals. Taken together, there appears to be great diversity in reproductive life history among nemertodermatids. Adults in some species appear to be smaller than juveniles suggesting that maturing individuals may cease to feed and complete their life history using stored food reserves or other resources.

Copulation has not been observed in any nemertodermatids. However, earlier workers suggested that worms may simply press their posterior ends together long enough for spermatids to get through the epithelium of the recipient worm. Since male structures are located subterminally, this behavior would have to occur with the recipient positioned somewhat dorsally, or an individual transferring sperm would have to undergo dorsiflexion to accomplish impregnation. In species lacking female structures, insemination appears to be hypodermic. Oviposition is accomplished by flexing the body into a dorsi-convex shape followed by protrusion of the circumoral area of the body to force individual eggs out of the mouth.

Development in nemertodermatids is similar to that of the Acoela. The first cleavage division is holoblastic (Figure 9.20). The second division results in the formation of micromeres and macromeres. In later 4-cell stages, the micromeres shift slightly clockwise (dexiotropic), resembling spiral cleavage, but this shift does not occur until well after the division has taken place. Nemertodermatids do not have the unique

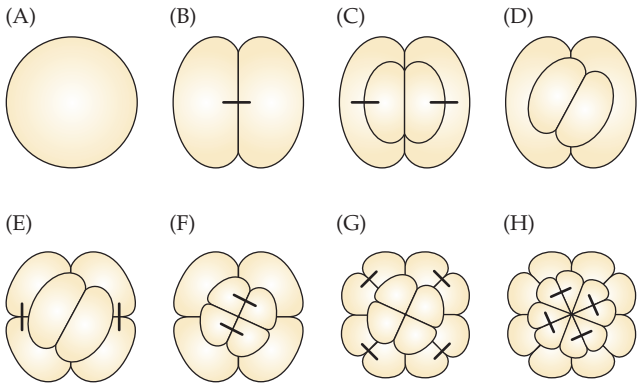

Figure 9.20 Diagram of duet cleavage in Nemertodermatida (*Nemertoderma westbladi*), viewed from the animal pole. Lines indicate cleaved relationships among cells. (A) Uncleaved zygote. (B) 2-cell stage. (C) Early 4-cell stage with micromeres oriented radially. (D) Late 4-cell stage with micromeres shifted. (E) 6-cell stage. (G) 12-cell stage with 8 macromeres and 4 micromeres. (H) 16-cell stage with 8 macromeres and 8 micromeres.

spiral duet-cleavage program seen in Acoela. Instead, cleavage starts out radial, but then takes place in a duet pattern, the micromeres shifting clockwise to produce a spiral-like pattern. The 4-cell divisions involve both macromeres, resulting a 6-cell embryo, followed by another division by the micromeres to yield an 8-celled embryo. This alternating pattern is followed until the 16-cell stage, similar to what is known in acoels as "duet cleavage," although in acoels, the first division involves a counterclockwise (levotropic) shift of the micromeres rather than the clockwise shift documented for *N. westbladi*. Nevertheless, the form of duet cleavage is similar in both taxa, suggesting to some researchers that this trait is ancestral in the Acoelomorpha.

Postembryonic development in *N. westbladi* appears to follow three life history phases. Hatchlings are nearly round; only slightly longer than wide (250 × 200 μm). These individuals grow into juveniles, which are bottle shaped and may reach nearly 1mm in length, but possess no discernable sexual structures. Mature specimens may be variable in size (averaging 450 μm in length), and possess a slightly more elongated shape as well as visible male copulatory organs and a small pointed tip at the posterior end formed by the male gonopore.

Subphylum Xenoturbellida

The free-living marine creatures known as xenoturbellids comprise just two described species, *Xenoturbella bocki* (Figure 9.21 and chapter opener photo) and *X. westbladi*, although several undescribed species are known to exist. They are delicate, ciliated worms with a very simple body plan, and are recognizable by their body furrows, the **horizontal furrows** (= side or anterolateral furrows), and a **ring furrow** (= equilateral furrow), the latter nearly crossing the animal's midline (Figure 9.22A). The furrows contain what has been interpreted as high concentrations of sensory cells, so they are presumed to be sensory structures. Like acoels and nemertodermatids, xenoturbellids possess a diffuse, basi- or intraepithelial nervous system, they use a statocyst for orientation, have circular and longitudinal muscles, and a mid-ventral mouth. Also like acoelomorphs, *Xenoturbella* lack a complete gut, organized gonads, excretory structures, coelomic cavities, or a well-developed brain.

However, unlike acoelomorphs, xenoturbellids possess simple spermatozoa, similar to those seen in externally fertilizing species. Also, muscle layers are connected by extensive interdigitations among the layers of cells as well as longitudinal muscles that are exceptionally robust. Their nervous system, while diffuse, is concentrated along its sensory furrows. Xenoturbellids also are generally larger in size, reaching up to 4 cm in length.

Sixten Bock (1884–1946), the great Swedish platyhelminth specialist, was collecting along the Swedish coast near The Sven Lovén Centre for Marine Sciences (then known as the Kristineberg Marine Station) in 1915 when he came across an odd-looking "flatworm." Bock never got around to identifying the creature, but Einar Westblad, another great platyhelminth specialist did, initially considered it an archoophoran turbellarian, along with similar specimens he had collected near Scotland and Norway. Eventually, in 1949, Westblad described the original specimens as *Xenoturbella bocki*, after its collector. The creatures caused immediate controversy because of their distinctive appearance. In 1999 the second species was described, and named after Westblad as *Xenoturbella westbladi*. By this time people were beginning to wonder about how unique these two "flatworms" were. The name *Xenoturbella* means "strange turbellarian" because, while they

Figure 9.21 *Xenoturbella bocki*. Live specimen, from 80 m depth, off the west coast of Sweden.

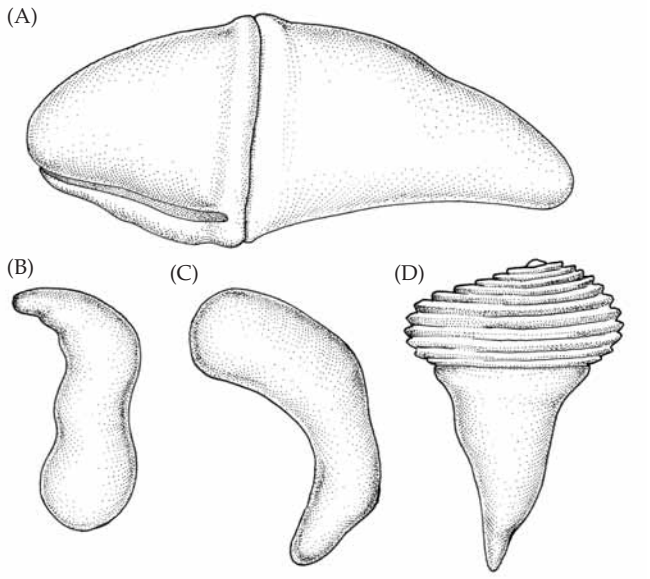

Figure 9.22 Line drawings of *Xenoturbella bocki* from live specimens. (A) Dorsolateral view showing the horizontal and ring furrows. (B,C) Lateral views of specimens moving by ciliary gliding (arrows indicate direction of movement). (D) Animal with contracted anterior end after exposure to $MgCl_2$.

resembled acoelomorphs overall, their epidermis was reminiscent of hemichordates, and their statocyst seemed similar to that of certain holothurians.

As morphological evidence accumulated on *Xenoturbella*, their relationship to flatworms began to be doubted, and by the late 1950s most researchers agreed that *Xenoturbella* was not a flatworm, but there was little consensus about what these animals actually were. Opinions on their identity ranged from considering them "among the coelenterates" to placing them as a sister taxon to the enteropneusts.

Then, in the late 1990s, analysis of ribosomal RNA on what appeared to be developing oocytes and embryos in some specimens led to the conclusion that *Xenoturbella* was in fact a highly degenerate mollusc, possibly some form of shell-less bivalve. However, subsequent investigations showed that these samples had been contaminated with gut contents containing mollusc DNA. Subsequent DNA studies suggested *Xenoturbella* might be a highly degenerate deuterostome, near the base of the deuterostome line or perhaps closely related to echinoderms and hemichordates (the clade known as Ambulacraria). Continued molecular phylogenetic studies have suggested that *Xenoturbella* is closely tied to acoels and nemertodermatids, and thus the new phylum name Xenacoelomorpha was created to house these three odd, primitive worms. While we accept this classification for this book, it is clear that the final resolution of *Xenoturbella* phylogenetic relationships is yet to be settled.

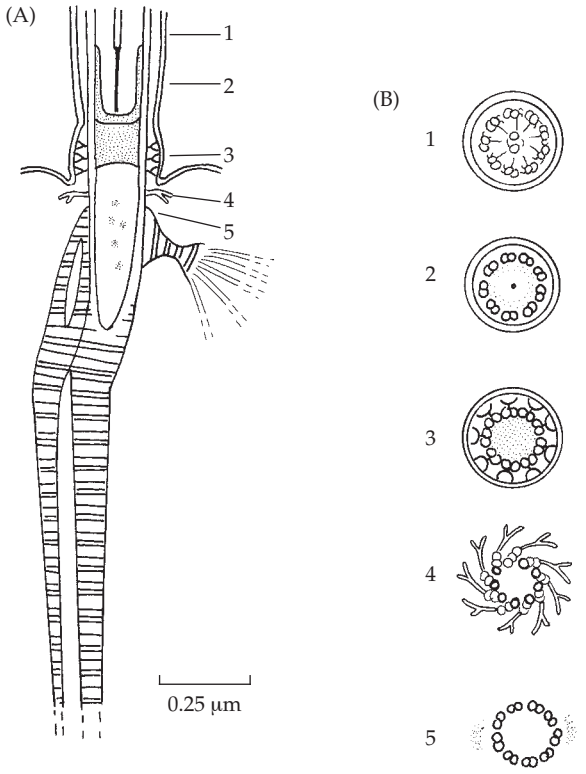

Figure 9.23 Diagram of the basal part of the cilium, basal body, and ciliary rootlets of *Xenoturbella bocki*. (A) Longitudinal median section of basal part of cilium. (B, 1–5) Cross sections of basal part of cilium and the basal body, showing the position of the microtubules at different levels. (1) Basal part of cilium. (2) Cup-shaped structure at the base of cilium. (3) Dense aggregation of granules and champagne-glass structures in the upper part of the basal body. (4) Centriolar triplet part of the basal body with winglike projections (the "alar sheets"). (5) Lower part of the basal body.

The Xenoturbellid Body Plan

General Body Structure

Most specimens of *Xenoturbella* are ovoid in shape, with a flattened ventrum and a length of 4 cm or less. These worms can be quite active and capable of considerable changes in shape (Figure 9.22B–D). The anterior region of most individuals is slightly lighter in color, and the horizontal furrows extend posteriorly, on either side, from the head end. Approximately midway down the body, these furrows nearly intersect with a ring furrow. The nervous system appears to be concentrated in these areas, suggesting a sensory function to these structures.

The epidermis of *X. bocki* consists of a layer of tall columnar cells with nuclei situated basally. These cells are densely ciliated, and are interspersed with unciliated or monociliated gland cells and ciliary receptors, the latter being most numerous in the horizontal furrows. The cilia themselves are attached to epidermal cells by several structures (Figure 9.23A). Each cilium ends in a basal body whose protruding **basal foot** has microtubules that extend into the epidermal cell. Two ciliary rootlets project from the basal body deeper into the epidermal cell; the thinner one, located on the same side of the cilium as the basal foot, projects straight into the cell, whereas the thicker rootlet has a knee-like bend. The cilia each have a distinctive arrangement of microfilaments in which the standard 9+2 arrangement extends for most of the ciliary shaft length, but near the end microfilament doublets 4–7 abruptly end, leaving only doublets 1–3 and 8–9 to continue on to the end of the cilium (Figure 9.23B). This "shelf" arrangement of microtubules is also present in Nemertodermatida and Acoela but is unknown in other known metazoan taxa (Figure 9.24).

The basal region of the epidermis houses the cell processes of the multiciliary cells, supporting cells, and a prominent intraepidermal nerve layer. The cell membrane of adjacent epidermal cells intermingle with each other, but tight couplings between the membranes of adjacent extensions do not appear to exist. However, where the cytoplasmic protrusions are shorter, they show a regular arrangement as if the two cells were held together by a zipper, but tight junctions,

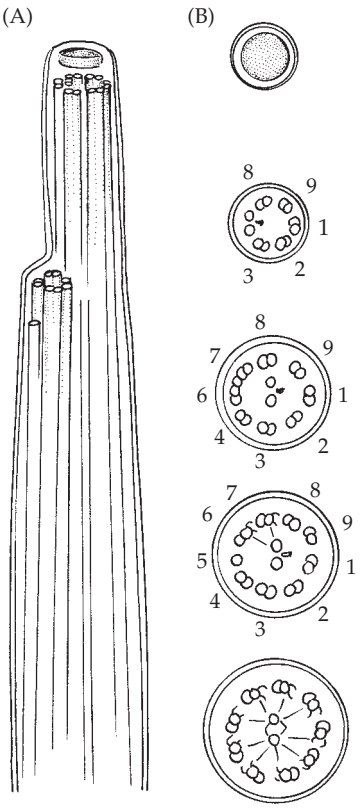

Figure 9.24 Diagram of the configuration of axonemal fibers within the distal shafts of epidermal cilia in *Xenoturbella bocki*. (A) Lateral view of the distal shaft showing the "shelf" located approximately 1.5 μm from the cilium tip. (B) Transverse sections of the cilium along its length; a 9+2 arrangement of axonemal fibers begins at the cilium base but microtubule doublets 4–7 end at the shelf.

desmosomes or gap junctions between cells have yet to be identified.

A number of workers have noted the similarities in both ciliary roots and ciliary tips in *Xenoturbella*, acoels, and nemertodermatids. Like these other groups, *Xenoturbella* is capable of withdrawing and resorbing worn out epithelial cells, yet there are differences in the character of this process. Whereas nemertodermatids do not withdraw still-motile ciliary cells, the withdrawn epidermal cells in *Xenoturbella* assume an orientation perpendicular to that of the other cells and retain some motility.

Support and Movement

Xenoturbella possess a highly muscular body wall (Figure 9.25A). An outer circular muscle layer surrounds a well-developed inner layer of longitudinal muscles, and with radial musculature extending from the gastrodermis to the outer circular layer of muscle cells (Figure 9.25B). The longitudinal layer of muscles is substantial, and individual muscle cells consists of a number of isolated fibers that when viewed in cross-section resemble a monolayered rosette.

No specialized parenchyma cells exist between the epidermis and the gastrodermis. However, all muscle cells tend to have numerous and well-defined cytoplasmic extensions with extensive mutual interdigitation. Tight attachment of adjacent cell membranes does not appear to exist, but connections resembling the zonula adherens in acoels and nemertodermatids are present, as is a fibrous subepidermal layer up to 5 μm thick. The extensive connections between muscle cells observed in *Xenoturbella* has been said to be reminiscent of hemichordates.

Xenoturbella inhabit marine mud bottoms at 20–120 m depth and move by ciliary gliding, without requiring modification of the body profile. The ventral surface is richly supplied with epidermal glands and moving animals leave behind a trail of mucus. While capable of considerable variation in body configuration due to powerful circular and longitudinal muscles, in most circumstances animals do not require such gymnastics in their basic activities.

Nutrition, Excretion, and Gas Exchange

Feeding by *X. bocki* occurs when individuals open their simple mouth and protrude their unciliated foregut. Extrusion of this structure appears to take place as a result of contractions of the surrounding body wall musculature, with relaxation of these muscles resulting in foregut retraction. The gut is cellular, but unciliated. Considerable attention has focused on the gut contents of *Xenoturbella*. Examination of mitochondrial DNA (cytochrome *c* oxidase subunit I sequence data) in the gut contents of *Xenoturbella* suggests that they feed primarily on bivalve prey, possibly in the form of eggs and benthic larvae. Such specificity suggests that these worms may be specialized predators, a hypothesis supported by the results of stable isotope studies indicating high ratios of N_{15} to N_{14} (3.42) characteristic of most predators. Two species of endosymbiotic bacteria have been described from the gut of *X. bocki*. Researchers have suggested that these bacteria might assist in nitrogen detoxification (given excretory organs are lacking) or might supply growth factors or chemical defenses to their hosts. Discrete excretory structures have not been described for *Xenoturbella*.

Nervous System and Sense Organs

The nervous system of *Xenoturbella* is a diffuse intraepithelial net without much of an anterior concentration and most researchers are reluctant to call this a brain. This arrangement is similar to that seen in some acoels and nemertodermatids, although the latter do have a small anterior concentration of neurons (larger than that of xenoturbellids). The sensory furrows of xenoturbellids appear to have slightly greater concentrations

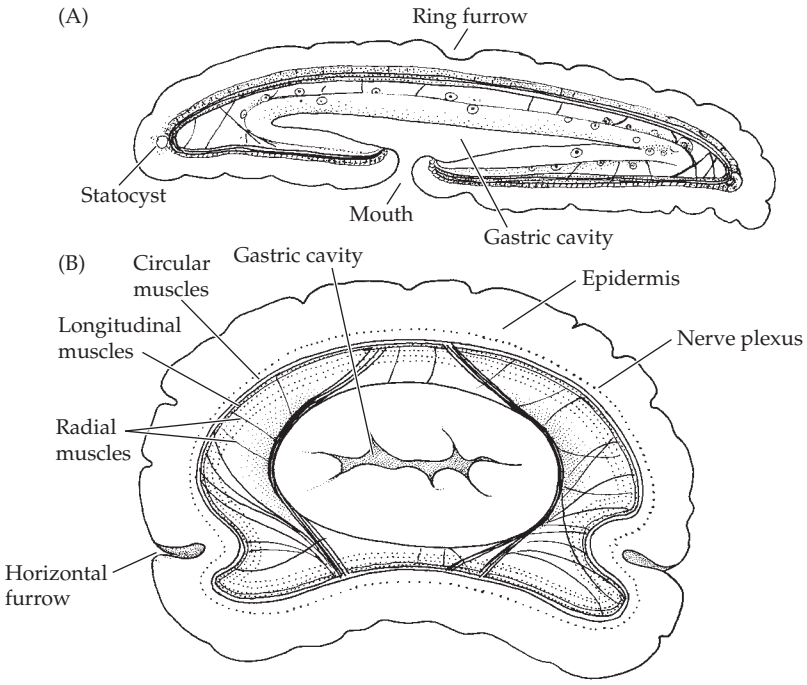

Figure 9.25 Internal morphology of *Xenoturbella bocki*. (A) Transverse section anterior to the mouth showing the gastric cavity. (B) Schematic diagram of longitudinal section anterior to mouth showing orientation of circular, longitudinal and radial muscles. Scale bars = 0.1 cm.

of neurons than other parts of their bodies. Like acoelomorphs, *Xenoturbella* have an anterior statocyst (Figure 9.25B), but the arrangement of muscles and neurons associated with this structure differs in that it appears to be embedded within the nerve net rather than specifically supplied with connecting commissures.

Reproduction and Development

Xenoturbellids are simultaneous hermaphrodites producing relatively large diameter, yolky eggs. Neither well-developed ovaries nor testes have been observed in adult individuals. In particular, male gonads appear to consist simply of a layer of male sex cells surrounding the gut. Sperm develop in clumps and appear to be of a "primitive" type, usually associated with external fertilization, wherein spermatids possess a small conical acrosome and a single flagellum. There are no copulatory organs and gametes appear to be spawned either through the gut or mouth opening. Although *Xenoturbella* has been said to have direct development, as in acoelomorphs, recent work has suggested the "hatching stage" might be called a larva; these are elongate/ovoid, swimming with a rotating motion with uniform ciliation, and have an apical tuft of cilia that are 20–30 μm in length. No mouth or blastopore has been seen in the larva.

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