Chapter 10

# Phylum Platyhelminthes

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## Chapter Outline

## INTRODUCTION

### General Systematic

The Platyhelminthes historically contain organisms grouped in three major clades, Acoelomorpha, Catenulida, and Rhabditophora, which include the Neodermata (Figure 10.1). The Neodermata (Ehlers, 1985) comprises the group of the parasitic Platyhelminthes: Monogenea (Monopisthocotylea and Polyopisthocotylea), Trematoda (Aspidogastrea and Digenea), and Cestoda (Amphilinidea, Gyrocotylidea and Eucestoda) (Littelwood et al., 1999). They are characterized by the presence of a neodermis (hence the name of this parasitic clade), which is a specialized epidermis formed by a peripheral syncytium with cytoplasmic elongations. Monogenea are normally ectoparasitic on aquatic vertebrates, such as fishes, whereas trematodes and cestodes are exclusively endoparasitic, generally in vertebrates.

The Platyhelminthes, both parasitic and free-living taxa, share a set of characters: they are nonsegmented acolomates without an anus, which lack respiratory and circulatory systems but possess an excretory system. In addition, all are hermaphroditic and asexual (mainly paratomy) and sexual reproduction are present.

The classical characteristics defining the phylum Platyhelminthes are: bilateral symmetry, multiciliated epidermis, absence of an accessory centriolus at the ciliary roots, more

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- Acoelomorpha Ehlers, 1985
  - **Nemertodermatida** Karling, 1940
  - **Acoela** Uljanin, 1870

- Platyhelminthes Minot, 1876
  - **Catenulida** Graff, 1905
  - Rhabditophora Ehlers, 1985
    - Macrostomorpha Doe, 1986
      - **Haplopharyngida** Karling, 1974
      - **Macrostomida** Karling, 1940
    - Trepaxonemata Ehlers, 1984
      - **Polycladida** Lang, 1881
      - Neoophora Westblad, 1948
        - "**Lecithoepitheliata**" Reisinger, 1924
        - **Proseriata** Meixner, 1938
        - Eulecithophora de Beauchamp, 1961
          - **Rhabdocoela** Meixner, 1925
            - Kalyptorhynchia Graff, 1905
            - "Dalyellioida" Bresslau, 1933
            - "Typhloplanoida" Bresslau, 1933
            - Temnocephalida Blanchard, 1849
          - Adiaphanida Noren & Jondelius, 2002
            - **Prolecithophora** Karling, 1940
            - **Tricladida** Lang, 1884
              - Maricola Hallez, 1892
              - Cavernicola Sluys, 1990
              - Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Baguñà, & Riutort, 1998
          - **Neodermata** Ehlers, 1985
            - Monogenea: Polyopisthocotylea + Monopisthocotylea
            - Cestoda: Gyrocotylidea, Amphilinidea + Eucestoda
            - Trematoda: Aspidogastrea + Digenea

**FIGURE 10.1** A diagram showing the Turbellaria groups and their position among the Platyhelminthes. Based on Tyler et al. (2006–2012). Taxa in bold represent traditionally considered orders.

than one cilia in the terminal cell of the protonephridia, stem cells as differentiation cells, presence of a male porus and male copulatory organ, and dorsoventral and anteroposterior polarity (Ehlers, 1985; Ax, 1995). These are shared by all the groups of this phylum, with the exception of the multiciliated epidermis. This character, only present during larval stages in Neodermata, becomes lost at adult stages with the development of the neodermal syncytium.

The transition from a free-living to a parasitic life form involved a series of adaptations, which generated patent differences between the parasitic and free-living taxa of Platyhelminthes. Among several morphological and physiological changes, the formation of the neodermis with perikarya under the surface that effectively protects the external body layer from abrasions or immune reactions of the host is particularly noteworthy. Epidermal ciliary rootlets are also a striking feature that may be useful for anchoring cilia in the epidermis in free-living turbellarians, which retain the ciliated epidermis throughout their life. However, rootlets may not be necessary in neodermatan larvae that lose their cilia following infection. The neodermis also has an important role in nutrient acquisition by increasing the exchange surface area (see Halton, 1997; Tyler and Tyler, 1997; Littlewood et al., 1999).

From an evolutionary point of view, the parasitic classes arose from a primitive free-living flatworm, specifically from Rhabdocoelida (Rieger et al., 1991), or Fecampiida/Urastomidae (symbiotic taxa) or from Neoophora (Rhabdocoela+Prolecithophora) (Littlewood et al., 1999).

Within the free-living Platyhelminthes, i.e., the "Turbellaria," apomorphies, such as the neodermis of the parasitic classes, are not present. Therefore, this taxon is considered paraphyletic (Ehlers, 1985) or polyphyletic (Smith et al., 1986), and hence its name is often written between quotation marks (usually dispensed with later in this manuscript for convenience). Nevertheless, the unique combination of several characters—mainly based on characteristics of the body wall and the position and function of stem cells—produce a grouping of the free-living species (including Acoelomorpha) within the Turbellaria (Tyler and Hooge, 2004; Egger et al., 2007).

Based on their simple body structure ("Bauplan"), Turbellaria has been considered one of the most basal bilateria (Littlewood and Bray, 2001). This hypothesis is supported by recent molecular phylogenetic analyses (Ruiz-Trillo et al., 1999, 2004; Baguñà and Riutort, 2004a,b). Nevertheless, "Turbellaria" has traditionally been considered a class consisting of a heterogeneous group of orders. They are found in all aquatic environments, show a worldwide distribution and can be found in the interstitium of marine, brackish, and freshwater environments, such as gliding on coral reefs, in the water column, or between the roots and leaves of aquatic plants. Most turbellarians are ~1 mm in size, but some orders like Tricladida and Polycladida (the so-called macroturbellaria) can reach body sizes of 0.2–10 cm.

The body shape is variable, spindle-like, vermiform or leaf shaped covered with the characteristic multiciliated epithelium. Sensory cells (e.g., eyes, ciliated pits, and rhabdite rods) and the central nervous system are concentrated in the anterior part, whereas the reproduction organs are located in the posterior part. The position of the pharynx, whether of the simplex, plicatus, or rosulatus types, varies along the main body axis. The oral pore is always ventrally situated, and the intestine is a blind sac without an anus. Excretion occurs through the protonephridial system. Turbellarians are hermaphrodites. The most conspicuous reproductive structures are the different parts of the male copulatory organ that form stylets of different shapes and complexity. The female gonads are paired or unpaired, and the eggs are endolecithal or ectolecithal (for a detailed description organization of Turbellaria, see Figure 10.2).

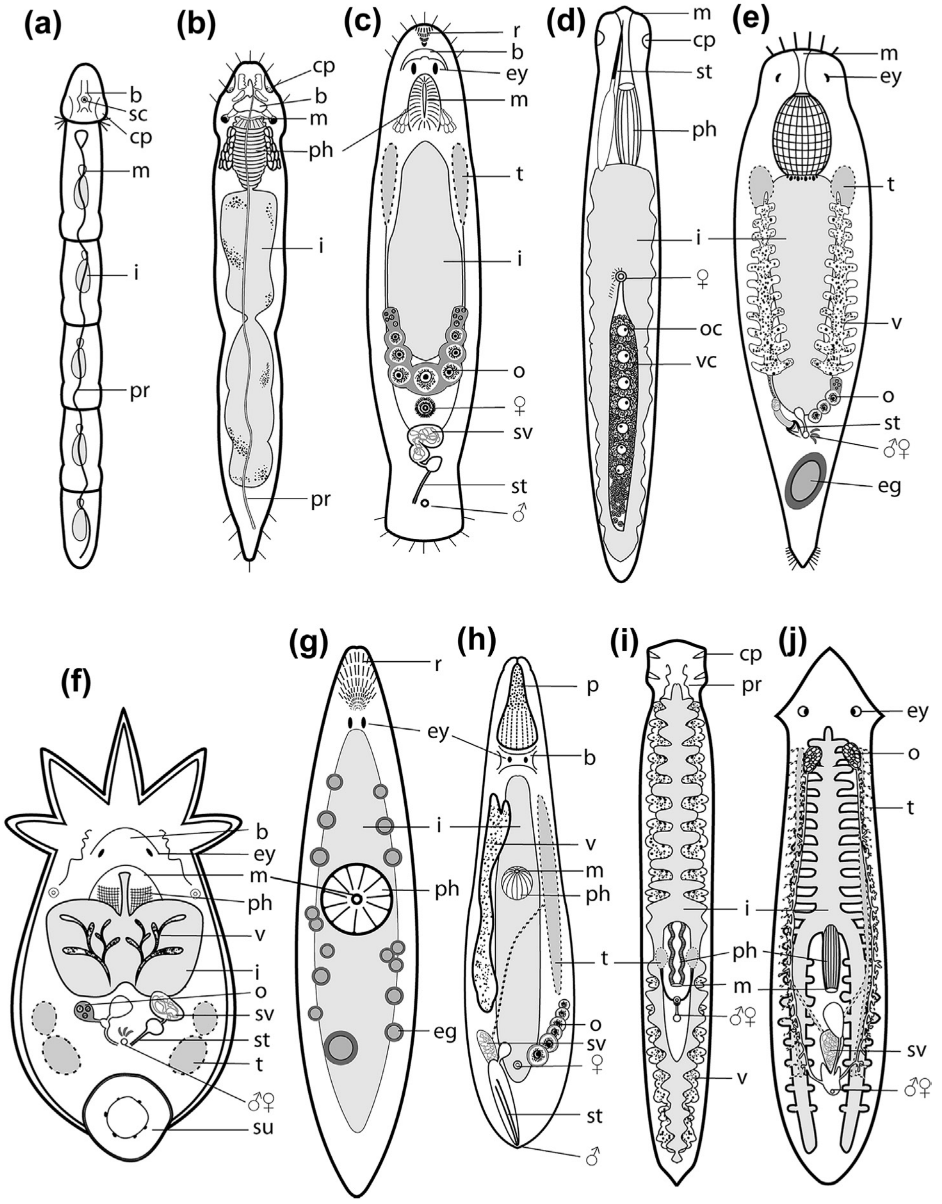

**FIGURE 10.2** Schematic representation of the different orders of Turbellaria: (a) Catenulida, Catenulidae (e.g., *Catenula lemnae*) reproducing asexually, approximately 1–2 mm length; (b) Catenulida, Stenostomidae (e.g., *Stenostomun* sp.), 1 mm length; (c) Macrostomida, Macrostomidae (e.g., *Macrostomum* sp.), 1 mm; (d) "Lecithoepitheliata," Prorhynchidae (*Prorhynchus stagnalis*), ~5 mm; (e) Rhabdocoela, "Dalyellioida," Dalyelliidae (e.g., *Gieysztoria rubra*), ~2 mm; (f) Rhabdocoela, Temnocephalida, Temnocephalidae (e.g., *Temnocephala* sp.), 10 mm; (g) Rhabdocoela, "Typhloplanoida," Mesostomidae (e.g., *Mesostoma* sp.), ~5 mm; (h) Rhabdocoela, Kalyptorhynchia, Polycystididae (e.g., *Gyratrix hermaphroditus*), 2 mm; (i) Bothrioplanida, Bothrioplanidae (*Bothrioplana semperi*), 5 mm; and (j) Tricladida, Continenticola, Dugesiidae (e.g., *Dugesia* sp.), 10–30 mm. Abbreviations: b, brain; cp, ciliated pits; eg, eggs; ey, eyes; i, intestine; m, mouth; o, ovary; oc, oocyte; ph, pharynx; p, proboscis; pr, protonephridial duct; r, rhabdite tracks; sc, statocyst; st, stylet; su, sucker; sv, seminal vesicle; t, testes; v, vitellaria; vc, vitellocyte; ♂, male pore; ♀, female gonopore; ♂♀, hermaphrodite gonopore.

## Phylogenetic Relationships

The nature of the available morphological characters and the relative overall simplicity of body structures make it difficult to resolve the phylogenetic relationship among the "Turbellaria" and to establish their relationship to other groups of invertebrates, such as Eutrochozoa or Gnathostomulida. Recently, the systematic position of Turbellaria has come under dispute again due to recent morphological and molecular discoveries that have allowed a more extensive analysis.

The first nonnumerical cladistic analysis of turbellarians was produced by Karling (1974), in which the evolutionary steps (morphological apomorphies and plesiomorphies) and the relationships between the different orders were showed in two alternative trees. In the 1980s, Ehlers (1985) and Smith et al. (1986) provided a new phylogenetic tree based on morphological characters. These two studies proposed a paraphyletic or polyphyletic status, respectively, of the "Turbellaria," whereas the phylogeny of Karling (1974) suggested a monophyletic status (Figure 10.3).

Molecular techniques currently available for systematic analyses has increased the amount of available data and allowed the analysis of the relationship between higher taxonomic groups. In previous studies, Acoelomorpha were considered to occupy a key position as the most basal bilateria. In addition, Rhabditophora and Catenulida, as sister groups to Acoelomorpha, represent groups that may shed light on the evolutionary processes that occurred during the diversification of bilaterian body forms, and hence play an important role in these studies. Many works that focused on the position of Acoelomorpha and Platyhelminthes as key in the evolution of bilaterians have been carried out during the last two decades. These works include molecular (Riutort et al., 1993; Ruiz-Trillo et al., 1999), morphological (Haszprunar, 1996a,b; Tyler and Hooge, 2004), and combined molecular and morphological analyses (Baguñà and Riutort, 2004a).

In fact, based on recent molecular and morphological discoveries, the controversy about the phylogenetic relationships between the different orders within Turbellaria, as well as phylogenetic relationship with other majors groups, such as Lophotrochozoa (Cook et al., 2004; Garcia-Fernandez, 2005; Jiménez-Guri et al., 2006; Sempere et al., 2007; Philippe et al., 2007; Dunn et al., 2008) or the deuterostomes (Philippe et al., 2011), has attained a crucial stage and no satisfactory hypothesis has been reached.

## Distribution and Diversity

### Geographical Distribution

Some turbellarians are cosmopolitan, while others have restricted distributions. The European fauna is the best known with more than 800 described species (Noreña, 2012). The faunas for part of the USA (150 species; Kolasa and Tyler, 2010) and South America, mainly for Brazil (over 100 species in South America; Noreña et al., 2003; Tyler et al., 2006–2012) are also known. Within the Neotropical Region, the turbellarians of southeastern Brazil (mainly in the state of São Paulo) are the best known. From this region, representatives of the orders Prolecithophora, Lecithoepitheliata, Catenulida, Macrostomida, Proseriata, and Rhabdocoela (Dalyellioida, Typhloplanoida, Kalyptorhynchia) are well known and are found in various inland bodies of water. This knowledge was achieved in the mid-twentieth century by Ernesto Marcus (1943–1954) and Eveline du Bois-Reymond Marcus (1951–1958). Unfortunately, the type localities of most of the described species are currently in poor condition or have completely disappeared due to the advance of urbanization.

The distribution of microturbellarian species is a difficult if not impossible topic to cover adequately, as most of the information is local, and large geographic areas remain unexplored. A new discovery or record can radically change the observed distribution patterns. As mentioned above, the best-known region in South America is Brazil, where species appear to extend along the Tietê River basin, but have also been found periodically along the Río de la Plata (e.g., Brusa et al., 2003; Noreña et al., 2005; Damborenea et al., 2007). Likewise, species recorded in the Amazon basin, mainly belonging to the orders Catenulida and Rhabdocoela (Noreña et al., 2006; Damborenea et al., 2011), show a Neotropical distribution that extends along the three main basins: the Orinoco, Amazon, and Paraná.

Some groups of Temnocephalidae (Rhabdocoela) have a notogeic distribution (South America + Australia + New Zealand) (Damborenea and Cannon, 2001). In Asia, the planarians are mainly known from Japan, whereas microturbellarians are nearly unknown. Only a scarce number of species belonging to Dalyellioida or the genera *Macrostomum* (Macrostomida) or *Gyratrix* (Rhabdocoela) are known in China (Wang and co-authors from 2004 to 2008). Some microturbellarians from distinct regions of Africa (contributions of Young, Artois, and other collaborators between 1974 and 2004) and Australia (reports of Hochberg and Cannon from 2001 to 2004) are also known.

*Gyratrix hermaphroditus* belonging to Kalypthorhynchia (Rhabdocoela) has been registered worldwide, from Alaska to Antarctica, and in a variety of environments, from freshwater to marine (Ax and Armonies, 1990; Curini-Galletti and Puccinelli, 1998; Artois et al., 2000).

The role of so-called invasive or immigrant species is another interesting topic related to the distribution patterns of Platyhelminthes. Some marine or freshwater species can migrate to freshwater or marine areas, respectively.

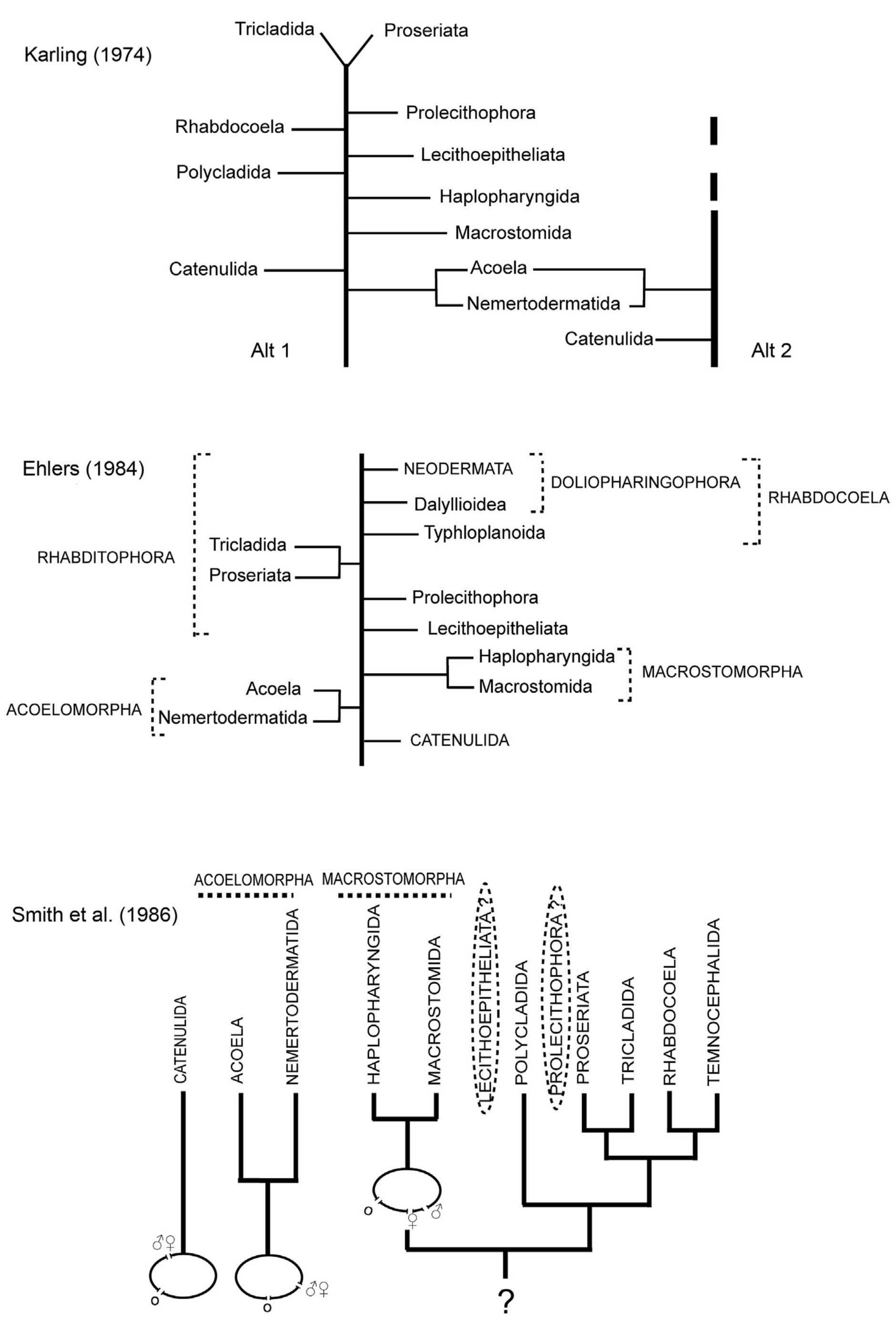

**FIGURE 10.3** Phylogenetic trees based in morphological characters.

Marine species, like *Archimonotresis limnophila* (Prolecithophora) and *Coelogynopora biarmata* (Proseriata), can colonize areas with a high freshwater content. Within the order Proseriata, species like *Philosyrtis rauli, Itaspiella parana* (Noreña et al., 2005), *Itaipusa graefei, Pseudosyrtis fluviatilis* and *Pseudosyrtis neiswestnovae* are characteristically found in freshwater environments, but they belong to a historically strict marine genera and families. Among these potential freshwater invaders, the largest representation is found within the Order Rhabdocoela, with species belonging mainly to the genera *Castrada* (Typhloplanidae) and *Gieysztoria* (Dalyelliidae) (Ax, 2008).

For species with a brackish or brackish-tolerant distribution, two main geographic regions have been described that consist of overlapping species. The first is located along the coasts of the North Atlantic Ocean and extends from east to west, with an observed decrease of overlapping species in the south. The second region extends from the

North Atlantic Ocean through the Mediterranean basin to the Black Sea (Ax, 2008).

### Species Diversity and Abundance

The number of freshwater species currently described is approximately 1300 (Schockaert et al., 2008). This diversity mainly comprises species of the orders Catenulida, Macrostomida, Rhabdocoela, Tricladida, Lecithoepitheliata, and Prolecithophora. Perhaps the most characteristic freshwater order of turbellarians is Catenulida. Catenulids are represented in inland waters with many species (more than 80) grouped into four of the five families of the order; the fifth family, Retronectidae, comprises marine specimens, with the exception of *Myoretronectes paranaensis*, which is, presently, the only recognized freshwater representative of this family.

The continental Macrostomida has two families with ~20 species, most of which are in the genus *Macrostomum*. However, the order Rhabdocela contains the most widely distributed and abundant continental turbellarians, with representatives of different suborders (Kalyptorhynchia, "Dalyellioida," "Typhloplanoida," and Temnocephalida). Tricladida, with two families, also has many species distributed worldwide.

The remaining orders, Acoela and Proseriata, contain freshwater species, but in low representation compared with the number of marine species. Only three species of the order Acoela are known from freshwater environments, *Oligochoerus limnophilus*, *Oligochoerus* sp., and *Limnoposthia polonica*; all are known only from the continental waters of Europe. The order Proseriata contains some freshwater species. The best known and most widely distributed is *Bothrioplana semperi* (Bothrioplanidae), with nearly a cosmopolitan distribution. *Otomesostoma auditivum* and *Otomesostoma arovi* (Otoplanidae) are two other remarkable freshwater representatives within this order.

Studies on the abundance or species richness of freshwater turbellarians are scarce and limited by locality, e.g., Europe (Young, 1977, 2001), North America (Kolasa and Tyler, 2010), and sporadically in South America (Noreña-Janssen, 1995). This paucity in research and information has one main reason: in general, microturbellarians are of secondary importance as consumers in stream ecosystems, with the exception of the predatory triclads, which can appear in greater numbers. Nevertheless, their abundance is closely related to temperature, reproduction phases, and competition among other species. Studies by Heitkamp (1978) showed that *Phagocata vitta* reproduces by fissipary, and that maximum abundance and rates of reproduction are reached in winter (December and January in the Northern Hemisphere). *Polycelis nigra*, on the other hand, could be the absolute dominant triclad species (densities of up to 800 individuals/0.1 m$^3$, after Heitkamp, 1978). This abundance is caused by two intense reproductive periods in spring and autumn, together with optimal temperatures and food conditions. The competition between certain species is partly influenced by these factors. For example, *Dugesia tigrina* is competitive to *P. nigra* at temperatures of about 20 °C, and *Dendrocoelum lacteum* is found in lower densities in the presence of *P. nigra*, likely due to interspecific food-competition (Heitkamp, 1978; also see Young, 2001).

In fact, most of the studies examining abundance and species richness of turbellarians have been undertaken in marine or brackish areas of Europe (e.g., Müller and Faubel, 1993; Noreña et al., 2007).

## GENERAL BIOLOGY

The bauplan or body organization of turbellarians is simple, but essential for its relationship with the environment and for its biology. The main structures that comprise the body organization are described below (see also Figure 10.4).

### Body Wall, Epidermis, and Sensory Structures

#### External Epithelial, Basal Membrane, and Cell Connections

The body surface is covered by a layer of mucus (carbohydrate complex) or glycocalix. The glycocalix forms a layer between the microvilli of the apical cell membrane and is quite well developed in all Platyhelminthes. Acoelomorpha and some Catenulida (Retronectidae) show a dense glycocalix between the microvilli (Ehlers, 1985; Rieger et al., 1991; Tyler and Hooge, 2004). This substance, together with other epidermal products (primarily rhabdoid proteins), is secreted by various types of glands. Generally, the cell bodies of these glands are embedded in the parenchyma and reach the surface through a thin channel between the epidermal cells. These gland secretions provide a shield that covers the body surface, which is important during locomotion. Many species show an abundance of glands on the ventral surface, and its secretion forms a "creepsole" that allows the planarian to slide on the substrate.

No known species of Platyhelminthes generate a cuticle as is observed in higher organisms.

Acoelomorpha lack a basal membrane; instead they form a basal cellular matrix. In addition, it has been described for some Catenulida that the basal matrix is discontinuous between the epidermal cells and underlying muscle (Tyler and Hooge, 2004), thus lacking a uniform basal membrane.

In contrast, in Rhabdocoela, the basal membrane is a well-developed bilayered lamina (lamina densa), and a reticular lamina fiber (lamina fibroreticularis) set is present, which is sometimes involved in the formation of the rigid structures of the female genital system (Gevaerts et al., 1995). Exceptions to this model can be found within the

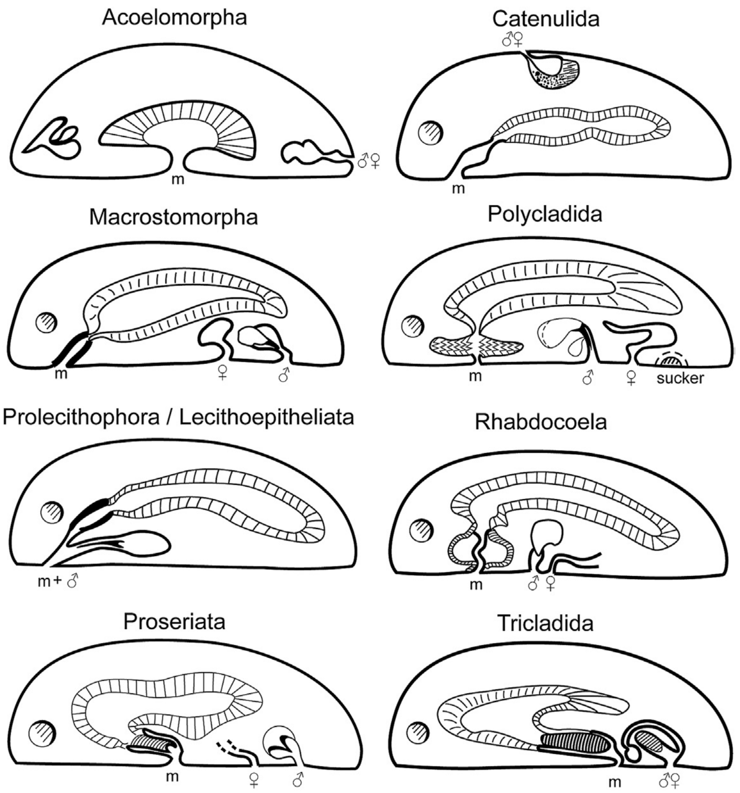

**FIGURE 10.4** General scheme of the position of the mouth and genital pores in the main taxa. Abbreviations: m, mouth; ♂ male gonopore; ♀ female gonopore; ♂♀ hermaphrodite gonopore.

Macrostomorpha, where the basal membrane is an irregular extension of the general extracellular matrix between muscle and other sub-epidermal cells (Rieger et al., 1991; Tyler and Hooge, 2004). The basal membrane of some Rhabditophora, for instance Kalyptorhynchia, is thickened and serves as skeletal support (Rieger and Doe, 1975).

Although in invertebrates the morphological structure of septate junctions shows considerable differences—thus allowing for comparative analyses (Tyler and Hooge, 2004)—the presence of true gap and septate junctions, and true spot, hemi- or desmosomes in free-living Platyhelminthes was recently questioned by Tyler and Hooge (2004).

### *Cilia*

One of the hallmarks of Turbellaria is the presence of cilia in the epidermis (Figure 10.5). However, the development of these cilia and their distribution on the body surface is variable, depending on lifestyle differences.

The standard type of locomotion for Platyhelminthes is by cilia found on its multiciliated epidermis, which is characterized by locomotory cilia without centrioles. This characteristic is considered as an autapomorphy of the taxon (Ehlers, 1985; Tyler, 1984).

Within the free-living Platyhelminthes, the density of the locomotory cilia varies from six cilia per cell in Rhabditophora to two to three cilia per cell in Catenulida. In addition, Catenulida present a characteristic disposition of basal corpuscles similar to the monociliar epidermis of Gnathostomulida (Sörensen et al., 2006). This type of low ciliated epidermis of Catenulida covers the whole surface with the exception of the pharynx, where there are a greater number of cilia.

Cilia are anchored in roots, and the morphology of these root systems as well as their distribution vary considerably among Platyhelminthes. Acoela build a net-like root system, in which the fibrous extensions of the rostral roots connect the different cilia of the same cell. This character state is considered an autapomorphy of Acoela and Nemertodermatida (Ehlers, 1985; Baguñà and Riutort, 2004a). In all other turbellarians, each cilium is attached to two roots located in the surface membrane. In Catenulida (Ehlers, 1985) and Macrostomida (Smith et al., 1986), the ciliary roots are located opposite to each other, one pointing rostrally and the other caudally. Their distribution on the body surface is considered relatively primitive (Ehlers, 1985), and Smith et al. (1986) suggested that these structures are homologous in Catenulida and Macrostomida. In other Platyhelminthes, the radicular distribution of the cilia is, apart from slight variations, rostral and perpendicular to the body wall.

Ciliary structure varies somewhat among groups of turbellaria. Within Rhabditophora, the cilia show a continuous

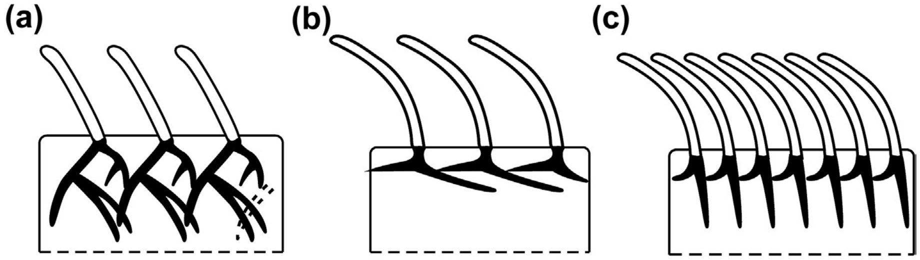

**FIGURE 10.5** Cilia: (a) Acoela; (b) Catenulida; and (c) Rhabditophora.

narrowing of the axoneme, as is also observed in other Metazoa. In Acoelomorpha and Catenulida, the axoneme shows a different pattern. Each epidermal cilium in Acoela has a typical 9+2 axonemal pattern that abruptly narrows near its distal tip (Tyler, 1979; Ehlers, 1985). For the cilia typically found in Catenulida, a progressive unilateral stepwise reduction of the double tubules into single filaments toward the distal end has been described (Ehlers, 1985). The structure of the cilia in Acoela and Catenulida represents a morphological character important for the classification of species in both groups (Lundin, 1997). Furthermore, these characters are considerate primitive (Ehlers, 1985) and support the basal position of Acoelomorpha and Catenulida (Rieger, 1978).

### Other Epidermal Structures

Rod-shaped rhabdites, which are found in the epidermal cells or below the epidermis, are characteristic for Platyhelminthes (Smith et al., 1982; Baguñà and Riutort, 2004). These structures may have a protective function or serve as mucus production sites for ciliary gliding (Martin, 1978). Three kinds of rhabdites have been recognized in Platyhelminthes: the Macrostomum type, the lamellated type, and the Triclad type, which are considered homologous (Smith et al., 1982). In addition, rhabdite-like structures were described from Acoelomorpha and Catenulida. These so-called "false rhabdites" (rhabdoids) of Catenulida are not homologous to the rhabdites of Rhabditophora (Smith et al., 1982). This fact indicates a phylogenetically distance of these groups to Rhabditophora (Smith et al., 1982; Rieger et al., 1991). The bodies of the glandular cells that produce the rhabdites can be found in the parenchyma (adrenal glands) or in the epidermis (dermal glands), although often the difference between the two types is difficult to recognize. The specific role of rhabdites has been difficult to establish. Proposed functions include repulsion from possible predators, prey capture, cocoon formation, removal of metabolic products and mucus production for ciliary-mediated gliding during locomotion.

A duo-gland adhesive organ has a secretory function. It is composed of three different cell types: the epidermal anchor and two adhesive glandular cell types, which are the thick grain-secreting and the fine grain-secreting cells (Ehlers, 1985). The presence and use of this organ varies among basal groups. Catenulida and Acoelomorpha lack a duo-gland organ (symplesiomorphy after Ehlers, 1985), but Proseriata, a mainly intertidal group, use the secretion of these glands for adhesion to the substrate.

The frontal organ complex is a simple structure consisting of an anterior concentration of glandular cells, which shows a terminal opening into pores. Several functions have been proposed for this complex (e.g., fixation, defense, or generation of a locomotory film), although its function not exactly known. The concentration of frontal glands can be found in Rhabditophora (except Dalyellioida and Kalyptorhynchia) and Acoela, but not in Catenulida (Rieger et al., 1991; Ehlers, 1992; Haszprunar, 1996a).

### Musculature

The body musculature (Figure 10.6(a)) of free-living Platyhelminthes is composed of muscular cells, or myocytes. The presence of three relatively well-developed muscular layers is characteristic and includes an external circular muscular layer, an internal longitudinal layer and a diagonal muscle layer located in between the external and internal layers.

Differences of this muscular disposition occur sporadically, generally in species with voluminous bodies, such as in Polycladida and Tricladida, in some Rhabdocoela (Mesostominae, see Noreña-Janssen and Faubel, 1992), and exceptionally in *Symsagittifera roscoffensis* (Acoela) (Dörjes, 1968; Ehlers, 1985). In these groups, a fourth layer, the dorsoventral muscle layer, is frequently present.

In general, the smooth musculature is considered an ancestral character for Platyhelminthes, and therefore, a plesiomorphic character for these taxa (Ehlers, 1985).

## Parenchyma

### Organization and Structure of the Parenchyma

The parenchyma in turbellarians is a connective tissue that fills all of the free spaces not occupied by the intestine and reproductive organs. This tissue is composed of cells and an extracellular matrix (ECM). The ECM is absent or very scarce in the primitive groups of Platyhelminthes, Acoela, Nemertodermatida, and Catenulida. In the remaining groups, we can find small traces of parenchyma all the way to well-developed parenchyma tissue, as in Tricladida and Polycladida. The ECM is a basal substance with fibers of a collagenous nature. The limits of the parenchyma are sometime complex to define because extracellular components can be found inserted in the epidermal or gastrointestinal basal lamina.

### Cell Types and Musculature of the Parenchyma

Rieger et al. (1991) distinguished three cells types occupying the parenchyma of turbellarians: insunk bodies of epithelial cells, stem cells, and "true" parenchymal cells of various types.

The insunk bodies comprise cells that come from other organs and tissues (e.g., digestive system, reproductive organs and nervous system).

The stem cells comprise the neoblasts (see section below on Regeneration) and the epidermal replacement cells, which apparently originate from the neoblasts. Neoblasts are pluripotent cells (Agata, 2008) that are responsible for: (1) normal cell replacement; (2) cell substitution during regeneration; and

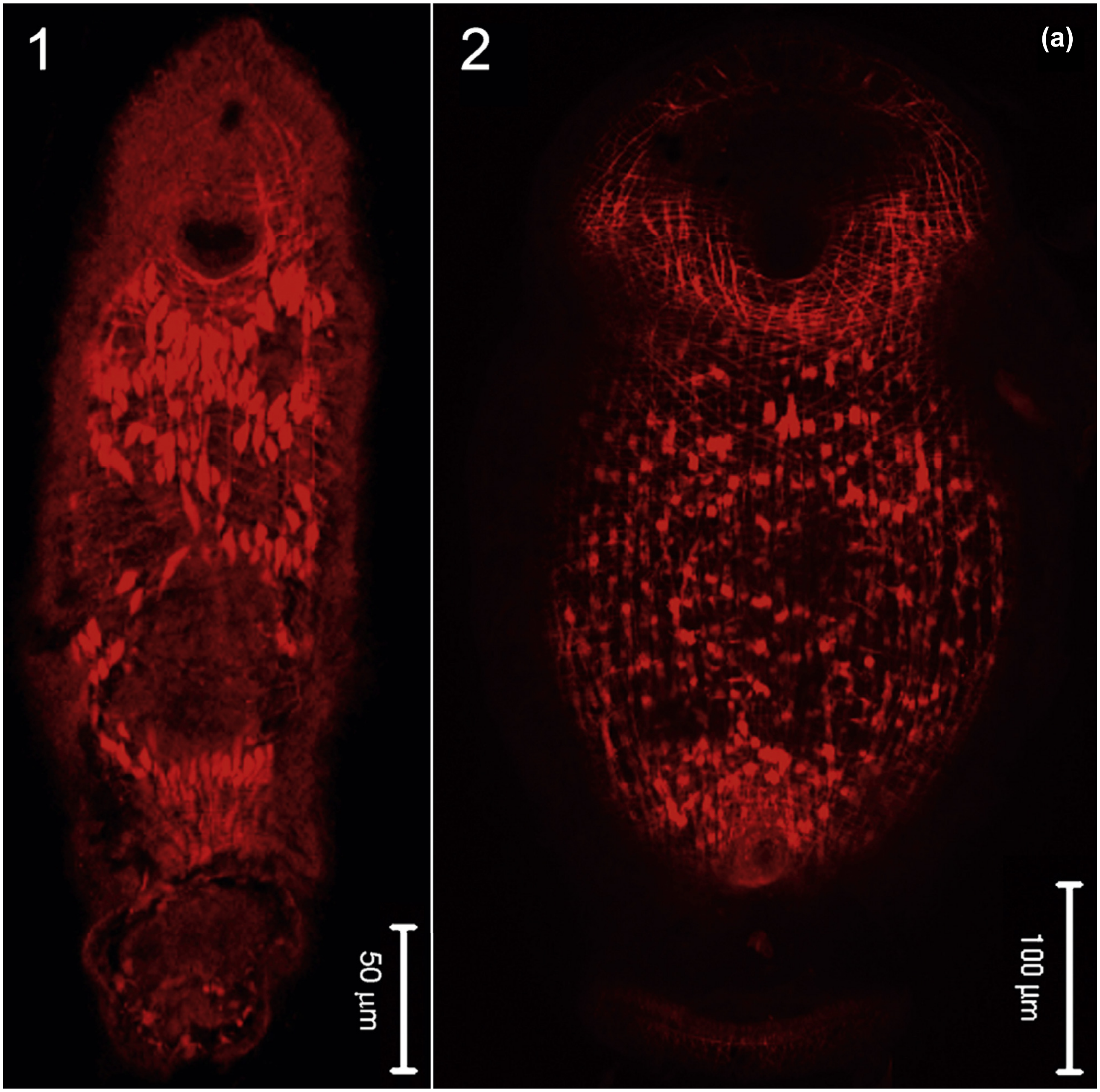

**FIGURE 10.6** Images of: (a) whole-mounts of *Stenostomum* sp. (1) and *Macrostomum platensis* (2) labeled with rhodamine-phalloidin showing the muscle arrangement.

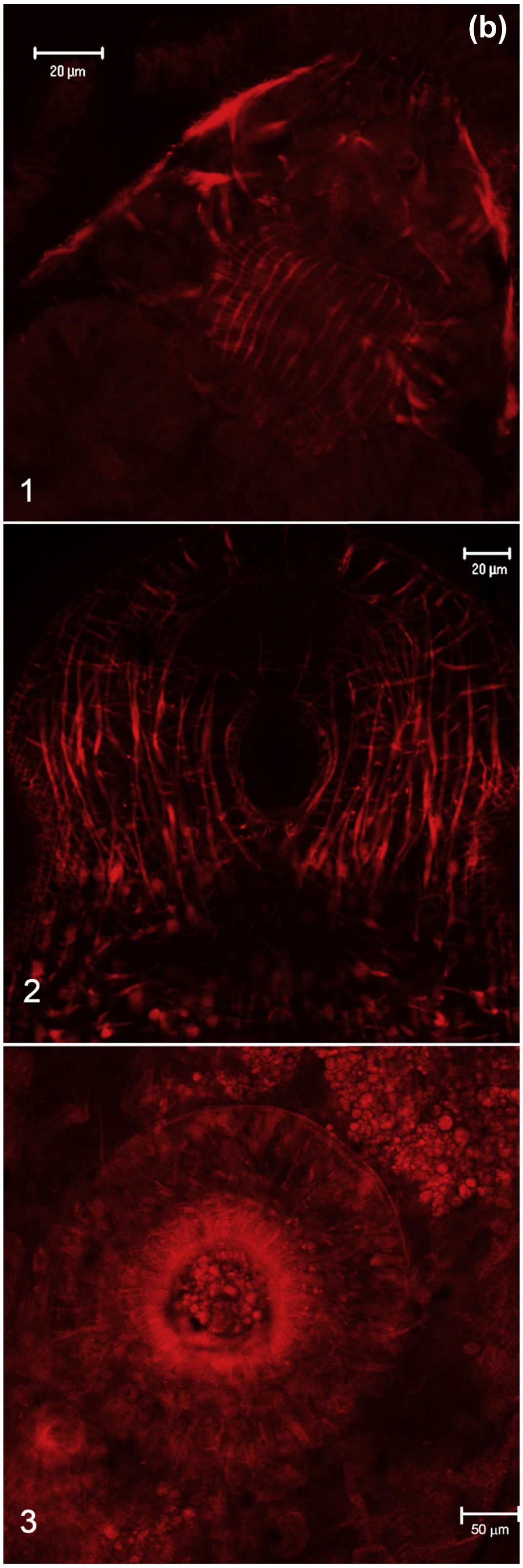

**FIGURE 10.6 Cont'd** (b) rhodamine-phalloidin labeled showing pharyngeal muscles of *Stenostomum* sp. (1), *Macrostomum platensis* (2), and *Mesostoma ehrenbergii* (3).

(3) gonadal stem cells. Neoblasts are small cells with high nucleocytoplasmatic ratios, numerous free ribosomes, chromatoid bodies, a prominent nucleolus, few mitochondria, small (or absent) endoplasmatic reticulum, and a characteristic pattern of heterochromatin with fine clumps distributed through the nucleus (Rieger et al., 1991; Ladurner et al., 2008). Neoblast have been studied most extensively in triclads, but neoblast-like cells have been reported for catenulids, macrostomids, acoels, and polyclads. In the latter orders, such cells differ from the neoblasts of triclads, however, by lacking chromatoid bodies. In addition, their pluripotency has been experimentally demonstrated in only a few cases. Parasitic groups show morphological similar neoblasts, as the triclads (Ladurner et al., 2008).

"True" parenchymal cells can be grouped into three categories: (1) fixed parenchymal cells, or large highly ramified cells with a clear cytoplasm; (2) chordoid cells with vacuoles; and (3) cells filled with small granules (sometimes pigment cells). True parenchymal cells have probably evolved independently at various times during the evolutionary history of Acoelomorpha, Rhabditophora, and perhaps in Catenulida (Ehlers, 1985).

The parenchymal musculature includes dorsoventral and transverse muscles. They are mainly developed in large turbellarians, like Tricladida and Polycladida. Other internal musculatures of the parenchyma are associated with the oral and genital pores, as well as the pharynx, copulatory apparatus, gut, and some sensory organs, such as tentacles.

### *Functions of the Parenchyma*

Generally, the parenchyma serves as a substrate for the organization and functions of the epithelial layers. In addition, it houses the neoblasts and is, therefore, essential for all developmental and regenerative processes (Rieger et al., 1991) (see below). It is generally accepted that most of the cellular and fibrous (fibrillar skeletal) elements of the ECM play a role in the biomechanics of the acoelomate body plan. Fixed parenchymal cells are thought to also function in nutrient storage and distribution or in regulating the intracellular ionic concentrations between parenchymal cells and the various other cells types connected by gap junctions.

## Regeneration

The structural changes accompanying regeneration (Figure 10.7) comprise three stages: (1) wound closure; (2) formation of the blastema and proliferation of cells; and (3) neoblast differentiation and morphogenesis (Rieger et al., 1991).

Wound closure occurs by a rapid contraction of body wall muscles, followed by the active accumulation of undifferentiated cells below the wound epidermis to form a blastema. For subsequent epithelial cell proliferation and distribution, microfilaments are involved, as well as

**FIGURE 10.7** *Dugesia benazzi* after regenerating the anterior part of the body.

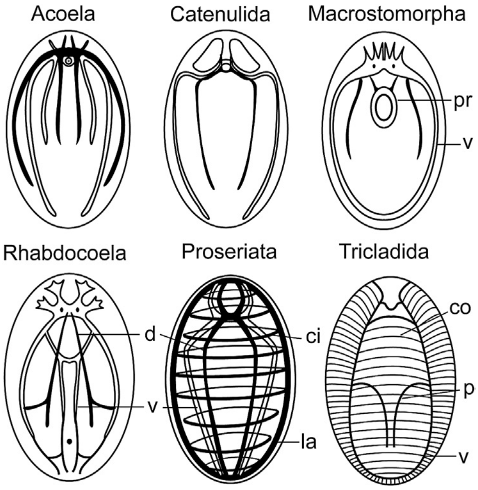

**FIGURE 10.8** Schematic representation of the development of the nervous system in the main taxa. Abbreviations: ci, circular cord; co, commeasure d, dorsal cord; la, lateral cord; p, pharynx cord; pr, pharynx ring; v, ventral cord.

rhabdite slime, the basement membrane and underlying fixed parenchymal cells.

The morphological stages of differentiation of neoblasts into various cell types have been described mainly for triclads. Among microturbellarians, a similar process of wound closure has been described for regeneration in *Macrostomum* (Ladurner et al., 2008), Catenulida, and Rhabditophora (Egger et al., 2007). In these cases, the wound is initially covered by a thin layer of stretched epithelial cells that are joined to one another by cell junctions. Regeneration in Catenulida differs from what has been described for triclads in that no blastema appears; instead the stem cells form two types of regenerative cells. Regeneration processes in rhabdocoela leads to the formation of pads of cells of embryological nature, comparable to a "true" blastema (Egger et al., 2009).

It seems that regeneration capacity may be closely linked to asexual reproduction such that groups that exclusively undergo sexual reproduction (e.g., Typhloplanoida) have lost the capacity for regeneration. For additional literature on this subject, see Pfister et al., 2008; Gaviño and Reddien, 2011.

## Neural System

### Central Nervous System

The central nervous (neural) system of Platyhelminthes (Figure 10.8) includes a central system (cerebral ganglion), an orthogonal system of major nerve cords (for another terminology see Reuter et al., 1998), a peripheral system (submuscular, subepidermal pharyngeal nerve ring, intestinal and genital nerve plexus), and minor cords (lateral nerve cords) (Reuter et al., 1998). A neuropil (cephalic differentiation) as commonly defined can be identified in Catenulida and Rhabditophora, but Acoela lack a neuropil; therefore, the acoelan brain may not show sufficient similarities with the central nervous system of Rhabditophora to allow a comparison (Kotikova and Raikova, 2008). A differentiated intestinal plexus appears only in those taxa with a well-developed intestine; therefore, it is absent in Acoela and Nemertodermatida. Reuter et al. (1998) proposed that the main nerve cords of Platyhelminthes (main longitudinal nerve cords) are homologous structures.

Regarding the cerebral ganglion, clear differences exist among the taxa. In Acoela and Nemertodermatida, it is a nervous cell cluster around the statocyst. The posterior and anterior nerve fibers form a peripheral plexus. This type of nervous cluster can be found in Cnidaria, therefore is considered as a plesiomorphic character state in free-living Platyhelminthes (Haszprunar, 1996b). The cerebral ganglion of the Catenulida is generally lobed, except in Chordariidae, where it is oval. The formation of cerebral lobes is considered an autapomorphy of Catenulida (Ehlers, 1985). Short nerve cords stretch from the lobes toward the pharynx, and the posterior and anterior regions form the peripheral plexus (Sterrer and Rieger, 1974). In Rhabditophora (Proseriata, Haplopharyngida, and Prolecithophora), the cerebral ganglion is surrounded by a capsule. This feature is absent in some families of the Proseriata (Bothrioplanidae, Monotoplanidae, Otomesostomidae) and Prolecithophora (Protomonotresidae, Hypotrichinidae, Urastomidae, Baicalarctiidae, Multipeniatidae, Plagiostomidae). Ehlers (1985) considered these exceptions a case of convergence. On the other hand, the cerebral ganglion of the Tricladida is a spongy nervous mass with numerous lagoons (Baguñà and Ballester, 1978). A bi-lobed cerebral ganglion with a peripheral nervous plexus is present in the

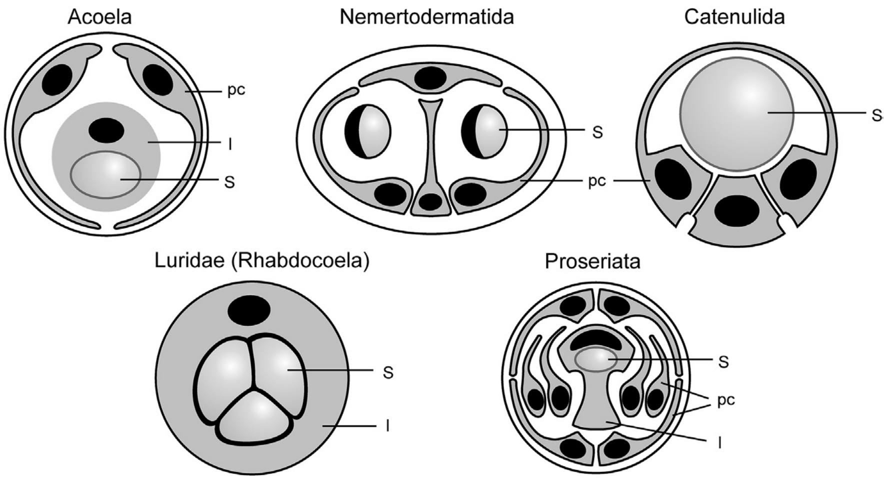

**FIGURE 10.9** Different types statocysts. Abbreviations: pc, parietal cell; s, statolyte; l, lithocyte.

remaining taxa. The development of the peripheral plexus depends, in some cases, on the development of the basal lamina and body muscular layers. Taxa with basal lamina and well-developed musculature (e.g., Macrostomida and Polycladida) have three plexuses: submuscular, subepithelial, and epidermal (Ehlers, 1985), while the remaining taxa include only the sub or infra-epithelial plexus.

In some Platyhelminthes, several types of polarized and unpolarized synapses are found. Unpolarized synapses exist in some Rhabdocoela and in Acoela, whereas polarized synapses are more frequent in presumably less primitive flatworms.

Two points should be considered regarding the evolution of the nervous system of the free-living Platyhelminthes. First, nervous system differences between groups are strongly linked to nutrition, locomotion, and body size (Baguñà and Ballester, 1978). And second, the presence of a peripheral nervous system is a primitive character (plesiomorphy) inherited from the common ancestor of the Platyhelminthes (Ehlers, 1985).

### *Sensory Elements*

Sensory elements associated with the brain include statocysts (Catenulida) (Figure 10.9), photoreceptors (Rhabdocoela), and refractile bodies (*Stenostomum* sp.). Also, all turbellarians have epidermal sensory receptors distributed on the surface or concentrated in specific areas which mainly function as chemoreceptors. These include the auricles of Tricladida, the sensory pits of Stenostomidae, or the ciliate grooves of Catenulidae.

Statocysts are present in Catenulida (except Stenostomidae), Acoela, Nemertodermatida, and Proseriata (except Bothrioplanidae and Nemertoplanidae), while all other Platyhelminthes orders lack this structure (except the genus *Lurus* of the order Rhabdocoela). These statocysts differ in the number and disposition of the parietal cells and in the number of statoliths. Generally, Catenulida have one statolith and three parietal cells, while Acoela have one statolith and two parietal cells. In Proseriata, one statolith, six parietal cells, and one lithocyte per statocyst is observed. Interestingly, Hofstenidae (Acoela) and Luridae (Rhabdocoela) have two or more statoliths and four parietal cells (Rohde et al., 1993), similar to what is observed in Nemertodermatida. Ax (1963) and Karling (1974) agreed that the ancestral state is represented by the presence of a statocyst. However, Karling proposed the presence of several statoliths as the plesiomorphic state, while Ax favored the presence of only a single statolith. Ehlers (1985) contradicted this view by suggesting that statocysts were not present in ancestral species of the Platyhelminthes. However, the two statoliths (and four parietal cells) of the Nemertodermatida, the two parietal cells in Acoela, and the nearly triangular shape of the statocysts in Proseriata are autapomorphic characters for these taxa (Ehlers and Sopott-Ehlers, 1990; Ferrero and Bedini, 1991).

The majority of the light-sensitive structures in Turbellaria are pigment cup ocelli situated proximal to the basal membrane or the musculature (Polycladida). The number and size of the pigment cups vary widely along the entire group (Rieger et al., 1991).

## Digestive Tract

The structure of the digestive system was the basis of earlier classifications, but it is still useful for the recognition of higher taxa, with the exception of the Acoelomorpha. In Acoelomorpha, the lack of a gut-like structure is compensated for a central digestive syncytium. The digestive system or gut of the remaining no parasitic Platyhelminthes is a blind sac without an anus and is organized as described below (see Figures 10.4 and 10.10).

### *Oral (Mouth Opening)*

Two distinct character states can be defined, the posterior-ventral and the anterior-ventral position of the mouth opening.

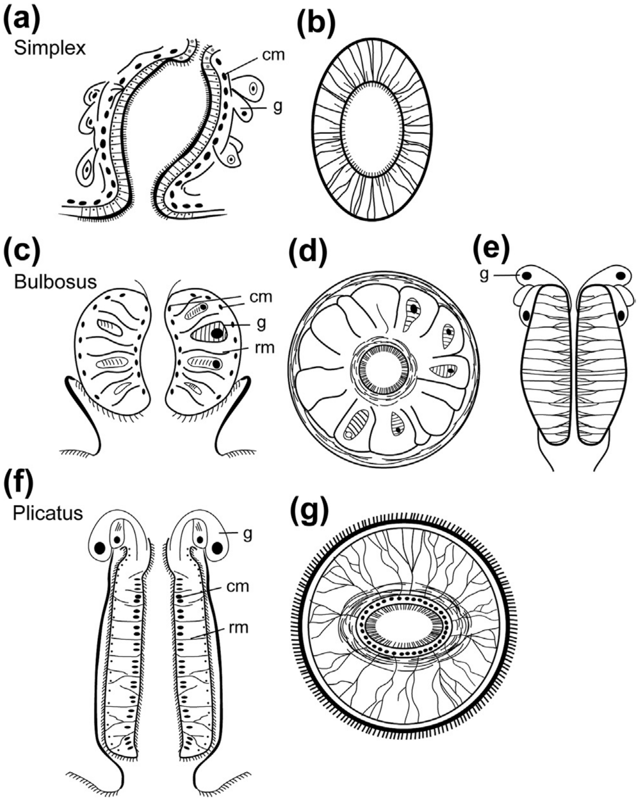

**FIGURE 10.10** Different types of pharynx: (a) median longitudinal section of type simplex pharynx; (b) transversal section of type simplex pharynx; (c) median longitudinal section of type bulbosus-rosulatus pharynx; (d) transversal section of type bulbosus pharynx; (e) median longitudinal section of type bulbosus-doliiformis pharynx; (f) median longitudinal section of type plicatus pharynx; and (g) transversal section of type plicatus pharynx. Abbreviations: cm, circular muscle fibers; g, gland cells; rm, radial muscle fibers.

The mouth of Acoela and Nemertodermatida open to the posterior-ventral site of the body. Generally the oral pore of Platyhelminthes is located ventrally. Catenulida, Macrostomorpha, and Prolecithophora show a ventro-frontal, or in some cases, a nearly completely frontal (*Microstomum lineare*) position of the mouth opening. The mouth in Rhabditophora is medial-ventral or sometimes posterior-ventral (e.g., in several polyclads, Proseriata and Tricladida), with exceptions found among Lecithoepitheliata (e.g., *Prorhynchus stagnalis*), which show a common oral and genital frontal pore.

## Intestine

In general, the gut or intestine of turbellarians consists of a blind sac, formed by a layer of glandular cells (cells of Minot or "granular clubs") and ciliated cells with vacuoles or phagocytes (Ehlers, 1985). This blind-sac gut of the turbellarians surely proceeds from the common ancestor of the Bilateria. The possible variations to this model are the intestinal branching of Tricladida and Polycladida considered as a secondary formation.

On the other hand, Acoelomorpha do not have an intestine; therefore, no intestinal glands are present, and the central body tissue is thought to adopt the digestive functions. The intestine of Nemertodermatida is described as a syncytial intestine with (Cannon, 1986) or without (Ehlers, 1985) lumen. Characteristic of Nemertodermatida are the glandular cells near the oral opening.

Another difference in free-living Platyhelminthes can be found in the ciliated layer of the intestinal cells (phagocytes). While Catenulida, Macrostomida, and Polycladida have ciliated intestinal cells, Tricladida, Rhabdocoela, Lecithoepitheliata, Prolecithophora (although, some species do have cilia), and Proseriata lack these kind of intestinal cells.

## Pharynx

Within the free-living Platyhelminthes (except Nemertodermatida), several distinct types of pharynges are known (Figures 10.6(b) and 10.10), and their detailed morphology is considered important for phylogenetic studies in each of the groups (Ehlers, 1985).

The simplest structure within the pharynx complex is the pharynx simplex. This type of pharynx is an ectodermal invagination. The epithelium lining the pharyngeal lumen is frequently ciliated; muscle and gland development are variable. The possession of a pharynx simplex allows the ingestion of whole prey. The pharynx simplex has evolved convergently in several taxa, such as in Catenulida, Acoela, Macrostomida, and Haplopharyngida. All these taxa, except Acoela, present pharyngeal glands. A pharynx simplex is also present in the larvae of Polycladida, substituted by a more muscular pharynx of the plicatus type in the adult.

The next level of complexity, the pharynx plicatus, appears within Proseriata, Tricladida, and Polycladida. Among these taxa, clear differences exist: the "ruffled" pharynx of Polycladida and the "tubular" pharynx of Proseriata and Tricladida are possibly autapomorphic characters of both groups (Karling, 1974). This is a very mobile pharynx with a great capacity for protrusion. The pharynx, for instance, can pierce the integument of a prey and then suck the tissue of the prey into the flatworm's body.

A bulbosus pharynx is characteristic of the order Rhabdocoela, wherein the pharyngeal cavity is reduced and only the end of the pharynx may be protruded. The glands can be found between the muscles of the pharynx or posteriorly. The bulbosus pharynx is considered an autapomorphy for this group and has convergently evolved into different pharynx types, such as rosulatus (Typhloplanidae), doliiformis (Dalyelliidae), and barrel-like forms (Phaenocorinae).

Lecithoepitheliata and Prolecithophora have a variabilis pharynx that can be considered as an intermediate state among the previously described pharynxes. The pharynx

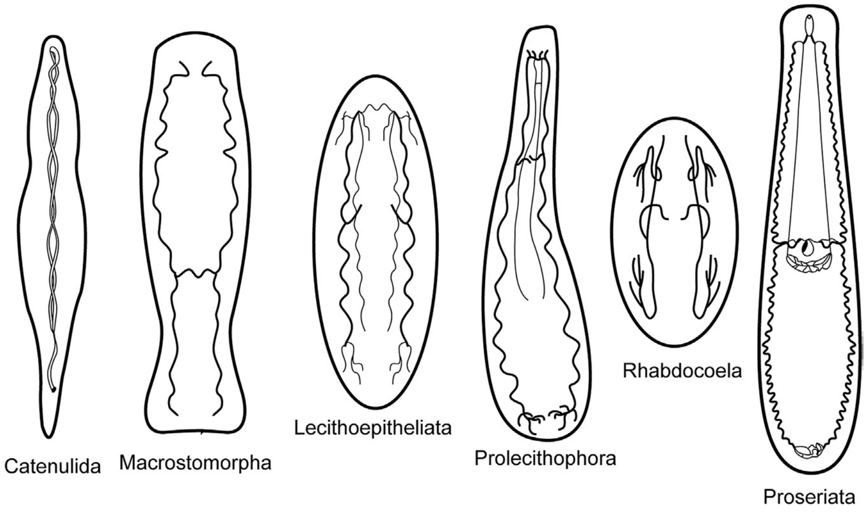

**FIGURE 10.11** Patterns for different excretory systems.

variabilis can be bulbous or coniform, similar to the pharynx plicatus or simplex, but with a more developed musculature.

## Osmoregulatory and Excretory Systems

The organization of the internal medium includes removing excretory products and regulating the ionic composition, or osmoregulation. In turbellarians, excretion is achieved through the epithelial lining of the gut, the body wall and the protonephridial system (Hertel, 1993) (Figure 10.11).

Osmoregulation is essential in freshwater environments and is performed through the protonephridial system that is, consequently, more developed in freshwater species compared to marine species. The protonephridia are formed by channels with terminal flame cells. Flame cells filter the excretion products from the parenchyma to the nephridian tubes. The final step of the excretion process occurs through the protonephridial pores (one or two), located in the body surface or in the pharynx pouch. Different morphological patterns of this basic structure are observed within the turbellarians. Nevertheless, the cell number and morphology of terminals channels are characteristic of high-ranking taxa.

With the exception of Acoela and Nemertodermatida, the free-living Platyhelminthes use protonephridia as an osmoregulation organ. The lack of protonephridia in Acoela and Nemertodermatida is caused by a secondary reduction (Ax, 1963; Ehlers, 1985). Masses of digestive tissues are thought to have adopted the osmoregulatory function in Acoelomorpha; therefore, this is considered an autapomorphic character for Acoelomorpha (Ehlers, 1985). In free-living Platyhelminthes, paired protonephridial organs are present; a single (unpaired) protonephridial system exists only in Catenulida. Karling (1974) proposed two possible situations for the ancestral state of this character: (1) absence of protonephridia; or (2) presence of diffuse or primitive protonephridia.

## Reproductive System and Development

### Reproductive Organs and Gametes

Separation of male and female gonads is commonly found in the free-living Platyhelminthes. Ehlers (1985) considered it a plesiomorphic state, already present in ancestral bilaterians. The gonad types (Figure 10.12) are associated with the mode of development of the oocytes and vitellocytes (endolecithal and ectolecithal eggs). Common or hermaphrodite gonads are only found in Retronectidae (Catenulida), Acoela, and Nemertodermatida. In Retronectidae, the gonads are clearly delineated (Sterrer and Rieger, 1974), while in Acoela, the gonads or germinal tissues are spread within the parenchyma. This type of gonad represents the primitive state, according to Ehlers (1985). The other taxa show separate gonads, except some species of Prolecithophora (e.g., *Prolecithoplana lutheri*), which form diffuse reproductive tissue, as an aggregation of male and female germinal cells that do not form clearly defined gonads.

Female gonads, or ovaries, are present in Catenulida, Acoela, Nemertodermatida, Macrostomida, Lecithoepitheliata, and Polycladida, and these germinal tissues show structural similarities with adjacent nutritional tissues (vitellaria). Among these groups, Polycladida are exceptional as they can form ovaries secondarily (Karling, 1974; Ehlers, 1985). All the other taxa have ovaries in which the germinal and nutritional tissues are separated. To date, it is difficult to determine whether the acquisition of separate germinal tissues has a single origin in Platyhelminthes or has evolved independently multiple times (Ehlers, 1985).

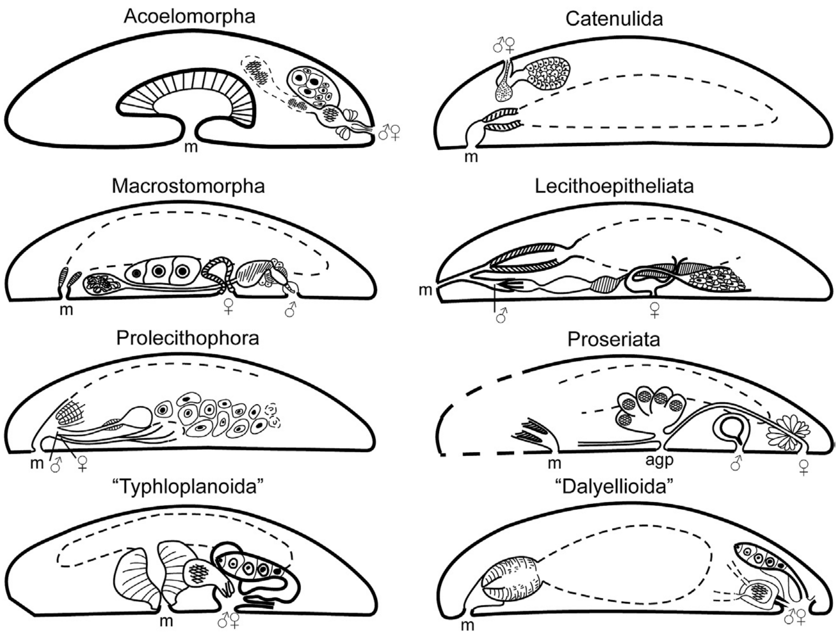

**FIGURE 10.12** Diagrammatic representation of the reproductive systems. Abbreviations: agp, accessory genital pore; m, mouth; ♂ male gonopore; ♀ female gonopore; ♂♀ hermaphrodite gonopore.

In free-living Platyhelminthes the presence of a gonopore is permanent, except in Acoela where the gonopore can be reabsorbed following the reproductive phase or degenerates completely due to aging (A. Faubel, personal communiation). The lack of a female genital pore is characteristic for Catenulida, Acoela, and Nemertodermatida. In animals that lack a female genital pore, a break in the body wall is necessary for egg liberation, with the consequent death of the "mother". This missing female genital pore has been considered a primitive character state (Karling, 1974; Ehlers, 1985). The presence of separate male and female genital pores has only been found in Rhabditophora (Ehlers, 1985). In some taxa, the acquisition of a female genital pore is combined with the development of genital structures in the uterus. This can be found in some families of Rhabdocoela (e.g., Mesostomidae and Dalyelliidae) and Polycladida, in which a part of the oviduct carries out the uterus function. A female genital pore is located either anterior to (Macrostomida) or posterior to the male genital pore (Haplopharyngida, Lecithoepitheliata, Prolecithophora, Proseriata, Rhabdocoela, Tricladida, and Polycladida).

The position of the genital pore (or pores) in relation to the position of the oral pore is an important phylogenetic character within Platyhelminthes (Ehlers, 1985). Karling (1974) proposed that the ancestral state for Bilateria is a ventrally located oral pore and a caudo-ventral or terminal male genital pore. In a proposed next evolutionary step, the male pore moves toward the dorsal region (autapomorphy of Catenulida) or toward the ventral region; in both cases, the oral pore is ventrally located. Some exceptions are found within Lecithoepitheliata and Prolecithophora, where some families (Gnosonesimidae (Lecithoepitheliata), Baicalarctiidae, Ulianinidae, Multipeniatidae, and Plagiostomidae (Prolecithophora)) form a single pore in which the male gonads and the oral pore are merged.

The male copulatory organ can be "naked" or "unarmed" (without rigid structures) as in Catenulida, Nemertodermatida, Tricladida, and in some families of Acoela, or it can be "armed" (with hard sclerotized structures) as in the remaining groups. Within the taxa with a sclerotized male copulatory organ, the hard structures usually come in the form of stylets, thorns or spines (e.g., Macrostomida, Polycladida, Proseriata, and Rhabdocoela [Dalyelliidae], and in some Acoela) or as an inner cover of the *ductus ejaculatorius* (Mesostominae [Rhabdocoela]). Previously, it was thought that these structures or reinforcements were of cuticular origin (Luther, 1955); now it is considered either the result of intracellular differentiation (Ehlers, 1985) or basement membrane differentiation (Rieger et al., 1991). The form and structure vary between groups and are used for species identification and phylogenetic analysis.

The shape of the sperm of free-living Platyhelminthes is frequently described as filiform. Observed differences mainly concern the cilia. Whereas Catenulida, Macrostomida and Prolecithophora show aciliar (unciliated) sperm (Rieger, 1978), the other taxa, with exception of Nemertodermatida, show biciliar sperm. Nemertodermatida are characterized by the presence of mono-ciliar sperm (Tyler and Rieger, 1975). Exceptions occur in all groups. For instance, the sperm of some species of Nemertodermatida have two cilia. Catenulida produce transitory cilia during some phases of spermatogenesis. Also, some species of Prolecithophora (e.g., *Pseudostomum quadrioculatum*) have sperm without cilia (Ehlers, 1981).

Differences in the internal structures of the microtubule arrangement in the cilia of these sperm are observed among taxa. The 9+1 pattern is found in most of the taxa (Lecithoepitheliata, Proseriata, Rhabdocoela, Tricladida and Polycladida), whereas the internal structure is 9+2 for Nemertodermatida. Remarkably in Acoela, three types of microtubule arrangements can be found: 9+0, 9+1 or 9+2 (Hendelberg, 1977).

## Reproductive Types

The free-living Platyhelminthes are hermaphrodites, and reproduction can be either sexual or asexual. During sexual reproduction, internal fertilization occurs by auto- or exogamy. The main types of asexual reproduction are paratomy (chain-like transverse fission where individuals are formed before breaking), architomy (division of the organisms in two and each fragment develops the missing organs), fragmentation (several fragments are formed due to muscle contractions and each of them originates a new individual) and parthenogenesis (reproduction without fertilization). Paratomy is usually regarded as the plesiomorphic state, belonging to a complex of ancestral characters of Platyhelminthes (Rieger et al., 1991; Ehlers, 1992). Paratomy is common in Catenulida, but is also present in Acoela, Macrostomida, Proseriata and Tricladida.

Asexual reproduction by parthenogenesis is less common than paratomy and appears to have evolved independently in two lineages: Catenulida (Borkott, 1970) and Tricladida (Weinzierl et al., 1999). The Tricladida can also reproduce asexually by fragmentation. In architomy and fragmentation, each fragment develops the missing parts. The processes involved in these types of reproductive modes are comparable to the regenerative processes that lead to the formation of a blastema and postblastema (Baguñà et al., 1989) (see section on regeneration above).

Most Platyhelminthes can reproduce asexually or sexually (Egger et al., 2007). In accordance with the currently accepted phylogenetic relationship among the major groups of Platyhelminthes, an evolutionary process by which a gradual substitution of asexual reproduction by the dominant mode of sexual reproduction has been proposed (Karling, 1974; Ehlers, 1985). Remarkably in this context, there is a clear positive correlation between asexual reproduction and regeneration capacity, such that taxa with highly efficient regenerative abilities mainly reproduce asexually by paratomy (Egger et al., 2007).

## Development

Most free-living Platyhelminthes undergo a monophasic life cycle, or direct development. The presence of larvae has been detected in only two distantly related groups: Catenulida (*Rhynchoscolex simplex*, Luther's larvae) and Polycladida (Müller larvae, Götte larvae). The Müller and Götte larvae resemble the ciliated trochophore larvae (mainly pilidium) of Eutrochozoa, thus allowing comparative analysis and deduction of phylogenetic relationships (Nielsen, 2005). The presence of this biphasic life cycle in Catenulida and Polycladida is due to isolated and independent evolutionary events. Therefore, the monophasic life cycle of Turbellaria is considered the ancestral state and not a convergent reduction of the biphasic life cycle (Ehlers, 1985).

The free-living Platyhelminthes can produce two types of eggs: endolecithal and ectolecithal. Each egg type is closely related with a particular type of female gonad. While endolecithal eggs are conceived inside the whole gonad (or ovary), organisms that produce ectolecithal eggs have separate gonads (germar and vitellaria). The ovary was likely derived from the last common ancestor of bilaterians and can, therefore, be considered as a plesiomorphic character state. In contrast, the differentiation process was probably a single event common to all taxa in the taxa with separated gonads (germar and vitellaria) (Ehlers, 1985).

Direct developers are usually oviparous and lay eggs with thick shells that allow embryos to resist adverse conditions (e.g., low temperatures and drought). Other species (mainly typhloplanoids) have thin-shelled eggs that can hatch quickly during the spring and summer seasons. Generally, the number of embryos per egg is one; however, some species can hold more than one embryo per egg (e.g., *Baicalellia evelinae* has two embryos in each egg). When more than two embryos per egg are present, they are then called "capsules" (e.g., *Plagiostomum evelinae* with four embryos). These capsules attach to solid, protected substrates until hatching.

Recently, Martín-Durán and Egger (2012) presented a thorough overview of the embryonic development of the different groups of free-living Platyhelminthes, including Catenulida, Macrostomorpha, Polycladida, Lecithoepitheliata, Proseriata, Bothrioplanida, Rhabdocoela, Fecampiida, Prolecithophora, and Tricladida, and discussed their "bauplan" from a phylogenetic point of view. Platyhelminthes are considered the most basal bilaterians and are placed within the Spiralia. It is possible, however, that the transition from radial to bilateral symmetry is not reflected in the embryological processes of Platyhelminthes. Their study is essential for understanding their own development and the diversification of bilaterians. The basal groups, such as Polycladida, shows quartet spiral pattern during cleavage stages, whereas the remaining taxa diverged considerably from this pattern and develop different strategies to give rise to the characteristic body plan of the mature animals. A comparative study of developmental diversity of Platyhelminthes will greatly clarify different evolutionary processes, such as the evolution of different types of cleavage, apparition of the oral-aboral pore or the dorso–ventral axis, evolution of direct and indirect development, and differentiation and specialization of organ systems.

## GENERAL ECOLOGY AND BEHAVIOR

### Habitat Selection

Freshwater turbellarians can be found in headwater streams to large rivers and in wetlands to large lakes where they are part of the bottom surface and interstitial benthos (Figure 10.13(a) and (b)). For instance, some species of Catenulida (*Myoretronectes paranaensis*) and Proseriata (*Otoplana rauli*) are found at the bottom of the large Paraná and Uruguay Rivers (Noreña-Janssen and Faubel, 1996; Noreña et al., 2005). They also can be found in very small streams and brooks where they can be especially important predators in the absence of fish (Carroll, 2009). They can also be found in or on different types of substrates (e.g., moss, vascular plants, wood snags, detritus, gravel, or sand) in the littoral areas of rivers (Noreña et al., 2005, 2007). The presence of turbellarians in these habitats is determined by the structure of the substrate and the breadth and type of ecological micro-niches (Young, 2001). Littoral species of lotic and lentic environments, inhabitants of plants roots, leaves, and surface sediments (i.e., the first cm) and are subject to disturbances during the different seasons due to the changes in the water level (Schwank, 1981) (Figure 10.13(b)). This fact causes great oscillations in the abundance dynamics of a species. Interstitial species, in contrast, are not greatly affected by weather or seasonal oscillations. The constancy of interstitial populations has been observed in both freshwater and marine environments.

Larger forms, such as planarians (order Tricladida), can be found under stones in rivers or lakes. They can also live in association with the marginal vegetation (floating or rooted) in the littoral areas. The floodplains have a great number of water bodies associated with the main river and its drainage pattern. Within this ecosystem, the exchange between ponds and the river is either periodic or continuous, thus allowing reproduction and development within lentic habitats and the distribution through lotic environments. In these habitats, Rhabdocoels are the most abundant (mainly represented by Dalyelliidae and Typhloplanidae) along with some Tricladida and Catenulida.

Rhabdocoelida (Protoplanellinae, Typhloplanidae) can be found in particular environments as part of the community associated with the water film of mosses (Van Steenkiste et al., 2010) and decomposing leaves or detritus.

Most of the freshwater turbellarians produce eggs enclosed in cocoons or capsules that provide resistance to extreme changes in environmental conditions, such as in temperature, drought or ice (Young, 1974, 1977).

Some species are ectoparasitic, commensal, or symbiotic (e.g., Pterastericolidae, Umagillidae, and Temnocephalidae) and live in association with other invertebrates (e.g., snails, crabs, bugs, and other insect larvae) or with vertebrates (turtles). In particular, they live in cavities or on surfaces open to the environment, such as in the gills or mantle cavities, or on the surfaces of shells.

For the distribution of the microturbellarians, especially from the interstitial environments, two environmental factors appear to be fundamental: substrate type and the salinity. Sandy, sandy-gravel, and sandy-stony sediments appear to be the preferred substrate for most species. On the other hand, the first few centimeters of muddy bottom is also an adequate habitat for microturbellarians; but in these cases, the presence of green algae or periphyton is also required (Noreña et al., 2007). It appears that the interstitium can be considered as a refuge area and migration medium (Karling, 1974).

Turbellarians in general show a remarkable degree of ecological or biological adaptation that allows this group to have high dispersal potential and colonize other areas. Therefore, several cosmopolitan species are found within this taxon.

### Food Web Role in the Ecosystem

Some turbellarians are predators (e.g., planarians and some microturbellarians) of other invertebrates present in their environment, although, they can also feed on algae and other microorganisms. The Catenulida has a pharynx simplex and is well represented in inland waters where it mainly plays a predatory role. Generally the principal preys are other members of the microbenthos and plankton, such

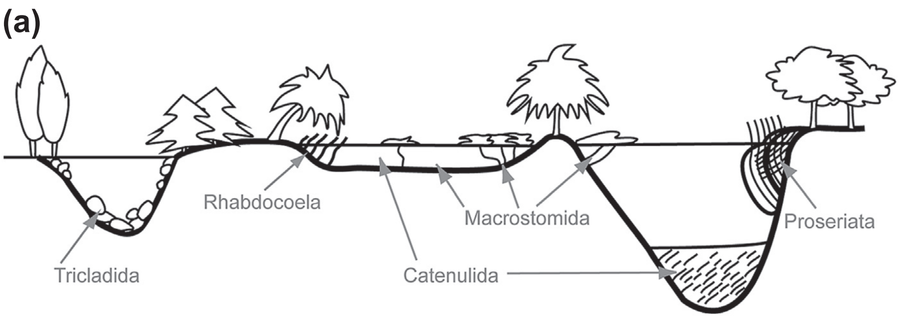

**FIGURE 10.13** Characteristic habitats for free-living Platyhelminthes. (a) Diagrammatic scheme of the continental waters.

**FIGURE 10.13** Characteristic habitats for free-living Platyhelminthes. (b) Characteristic lotic habitats.

as ciliates, amebae, rotifers, ostracods, and even smaller congeners. The family Macrostomidae, which also uses a pharynx simplex, is a well-known predator. Curiously, the genus *Macrostomum*, or "big mouth" in Latin, possesses a small oral porus, and its prey is limited to the size of the mouth. Therefore, the principal food resources are amebae and small rotifers and ostracods. In contrast, the genus *Microstomum*, or "small mouth," shows a very dilatable oral porus and pharynx that allow them to capture large objects, such as entire midge (Diptera, Chironomidae) larvae. The ingested objects can occupy the entire volume of the intestine, where the soft body parts are digested and the hard parts (e.g., chitinous cover) are expelled. Within Rhabdocoela are well known predatory forms, such as *Mesostoma ehrenbergii* and *Mesostoma lingua* (Typhloplanoida) that feed on a variety of planktonic and benthic fauna, such as mosquito larvae. Different strategies for capturing prey have been observed within typhloplanids (Blaustein and Dumont, 1990; Tranchida et al., 2009); in general, traps and threads of mucus are used for capture. Sometimes the microturbellarians are suspended by their mucous thread in the water or behind cover while waiting to attack suitable prey. *M. lingua* produces a poisonous neurotoxin enclosed in the mucus that causes the paralysis of prey. This temperature-dependent toxin is delivered through contact or diffusion (Case and Washino, 1979; Schwartz and Hebert, Dumont and Carels, 1987; Tranchida et al., 2009). However, the presence of neurotoxins in Platyhelminthes is uncommon. For instance, neurotoxins could not be found in *M. ehrenbergii*. In this case, the observed paralysis in prey is likely due to "playing possum" (Young, 2001) or the restriction of movement due to the amount of secreted mucus. Kalyptorhynchia (Rhabdocoela) use a proboscis to capture prey. *Gyratrix hermaphroditus* (Kalyptorhynchia) and *Prorhynchus stagnalis* (Lecithoepitheliata) hunt with the stylet of the male copulatory organ. In the case of *Gyratrix*, prey are first immobilized with the proboscis and then killed with the stylet (Kolasa and Tyler, 2010).

In general, digestion in turbellarians involves extracellular and intracellular phases. Extracellular digestion is enzymatic and takes place in the pharynx and gastrodermis, which acts to reduce the size of food particles. The small particles are then phagocytized by the gastrodermal cells (intracellular phase). The storage of nutrients occurs in the gastrodermal cells in the form of lipids, although in triclads, lipids are rapidly metabolized aerobically after ingestion (Rieger et al., 1991).

Supplementary energetic inputs can be obtained via symbiosis with algae (zoochlorellae). This relationship between algae and turbellarians is mainly observed in Rhabdocoela and Acoela (Kolasa and Tyler, 2010). The zoochlorellae are swallowed, phagocytized, and then placed under the body wall.

## Ectosymbiosis

Some freshwater turbellarians developed particular life strategies, such as Temnocephalida (Rhabdocoela), which live in association with aquatic invertebrates (e.g., crustaceans, molluscs, insect larvae) and rarely on freshwater turtles. This group has important morphological differences from other turbellarians. For instance, the epidermis has a constant number of syncytial plates without locomotory cilia. Most of the genera show anterior tentacles and a posterior sucker or adhesive disc used to attach to the host. The pharynx is doliiformis, and the intestine is a blind sac. Temnocephalida can be found either within the gill cavities or on the surface of the host; however, egg deposition occurs on the surface. Using water flows generated by the host, they capture algae and small crustaceans, among other prey.

The geographical distribution of this group is also restricted. In particular, species belonging to the Scutariellidae (with an anterior oral pore and tubular pharynx) are only found in the Northern Hemisphere, along the southern areas of Eurasia. The remaining families are restricted to the Southern Hemisphere, and most of the genera are endemic to specific areas, e.g., the genera *Temnocephala* in the Neotropical Region and *Dactylocephala* in Madagascar.

## Physiological Constraints

The distribution pattern of the free-living Platyhelminthes depends mainly on oxygen content of the water and food availability. Other factors that influence the presence and success of the turbellarian fauna are temperature, salinity, and substrate type (e.g., grain size, granulometry). Benthic turbellarians are normally present in oxygenated areas of sand and mud sediments. Low oxygen levels are a clearly limiting factor for the distribution and the survival of the organisms. Therefore, turbellarians are considered bioindicators for a variety of environmental factors, mainly oxygen. Nevertheless, oxygen adaptability or delimitation is a species-specific characteristic, rarely applicable to a whole family or genus. For example, in certain species of the genus *Phaenocora*, effective resistance to low oxygen levels has been observed. Respiration rates (or oxygen rates) also appear related to body shape and the presence or absence of zoochlorellae (Kolasa and Tyler, 2010).

Temperature mainly influences development and reproduction of turbellarians, as well as other general metabolic processes. In polyclads, the influence of temperature on development is clearly related to the mode of development. In direct-developing polyclads, temperature changes have less pronounced effects on developmental time than in polyclads with larval stages in the live cycle; for these taxa, an increase of a few degrees can produce a drastic reduction on developmental time (up to 50%) (Gammoudi et al., 2012). Similar effects can be observed in *Phaenocora unipunctata* and *Phaenocora typhlops* (Typhloplanidae), where hatching rates increase with rising temperatures (Young, 2001). Temperature also has a significant effect on reproduction. Egg plate (ovoposition), hatching time, and the number of embryos or hatched juveniles, all depend primarily on temperature rather than food.

The salinity tolerance is also a critical limiting factor for the distribution of flatworms. Some species are nearly exclusive to limnetic habitats (e.g., *Microdalyellia tennesseensis, Castrella truncate, Phaenocora unipunctata* (Rhabdocoela), *Prorhynchus stagnalis, Geocentrophora baltica, G. sphyrocephala, Bothrioplana semperi* (Proseriata), and most species of Catenulida), but they can also be found in brackish environments. Some species previously described for marine and brackish-marine environments (e.g., *Nematoplana nigrocapitula* and *Proschizorhynchus triductibus*) can also be found in limnetic habitats; but in these cases, it is probably an "alien" species (Noreña et al., 2007).

Changes in salinity are important for marine turbellarians and for inhabitants of brackish environments, although the latter show a high adaptation range for salinity and can tolerate strong fluctuations. Recent studies demonstrated that moderate changes in salinity (0.5%) delayed hatching for about one day (Gammoudi et al., 2012), while larger variations in salinity led to a developmental deceleration of several days with frequent deformations, or completely aborted development (Ballarin and Galleni, 1984).

Other ambient factors, such as light, have moderate influence in the different taxa. Triclads are photonegative, but rhabdocoels (mainly species with zoochlorellae as symbionts) are photopositive (Kolasa and Tyler, 2010). In general, inhabitants of the interstitial and benthic areas of lotic and lentic environments are photophobic, whereas species that inhabit vegetative meadows, shores and littoral areas are, in general, positively phototactic. Response to light is an adaptation to the ecological niche.

# COLLECTING, CULTURING, AND SPECIMEN PREPARATION

## Collecting

Methods for collecting turbellarians from continental waters vary depending on body size and type of microhabitat. Larger-sized specimens, such as planarians, can be manually collected in the benthos, under rocks or fallen trunks where they are protected. Planarians living on floating vegetation are manually captured by washing the roots or leaves of the plants on which they inhabit. Planarians can also be caught using night traps made with receptacles with narrow openings and bait, such as liver.

In the case of microturbellarians, the collection methods vary depending on whether the species are associated with floating vegetation, or are benthic or more rarely planktonic species. Microturbellarians associated with vegetation can be sampled using nets with a mesh size less <100 µm; nets with a larger mesh size (150 μm) can be used for environments with more organic matter in suspension. You then need to use a stereomicroscope to manually separate out the microturbellarians. For benthic forms, collect sediment using a variety of methods depending on the type of sediment (e.g., gravel, sand, or mud) and then observe the animals through a stereomicroscope. The loss of oxygen in the sediment forces the microturbellarian to surface and away from the sediment in search of oxygen. For planktonic forms, collect and concentrate the turbellarians using traditional methods for zooplankton sampling and then observe the microturbellarians with a stereomicroscope for the extraction of microturbellarians.

## Culturing

In general, turbellarians are difficult to maintain in culture, with some species being more difficult than others. Tricladida are one of the easier organisms to cultivate. It is possible to obtain multiple generations by keeping individuals at temperatures of 10–17 °C. Some species of *Dugesia* and *Schmidtea* reproduce asexually if the water temperature exceeds 20 °C. Food for cultured turbellarians generally consist of liver parts (preferably chicken or beef). The aquarium water needs to be changed daily after feeding. Using mineral water with salt percentages similar to the environment where individuals were caught is best. Aquariums must remain clean and free of algal or bacterial colonies.

Some Mesostominae (Typhloplanidae, Rhabdocoela), such as *M. lingua* or *Mesostoma ehrenbergii*, can be easily maintained with mineral water. Midges (Chironomidae) (Tranchida et al., 2009) or water fleas (Cladocera), preferably alive, can be used as food sources. In addition, these worms can tolerate a wide range of temperatures and can, therefore, be maintained at room temperature.

*Catenula lemnae* and *Stenostomum* spp. (Catenulida) are also generally easy to cultivate. A glass vessel or aquarium filled with pond or lagoon water and "sowed" with detritus, grasses, or other substrates can be used. If the "sowed" material contained catenulids, their presence is typically observable between 24 and 48 h following introduction into the aquarium. Cultures of these fragile worms require long periods of light and an ideal temperature range of 18–20 °C, although they can tolerate temperatures up to 25 °C. *Stenostomum* and *Catenula* preferentially eat algae, rotifers, and small planktonic invertebrates. Therefore, the aquarium water should only be filled and not completely changed.

Providing cultures with an adequate food source is one of the biggest challenges for successful cultivation. The turbellarians are very selective predators. Currently, *Macrostomum lignano* cultures are fed with diatoms. Under these conditions, these cultures can be viable for years.

Overall, for any type of culture is very important to keep the tanks or containers clean and to maintain water conditions as natural (original) as possible (e.g., pH, salinity and temperature).

## Specimen Preparation

In vivo observations are very important for the study of turbellarians since certain structures are only visible in living specimens. Once collected, the specimens will need to undergo different treatments, depending on the group, for subsequent identification and preservation. In the case of the larger planarians, external characteristics are easily observable under stereomicroscope. Specimens should then be fixed (in Steinman's or Bouin fixative) for later histological analysis. In the case of microturbellarians, they should be observed in vivo with a microscope and by increasing the pressure applied to the microscope cover slip. Some specimens are also fixed and preserved in polyvinyl lactophenol, which makes them more transparent, thus allowing the observation of sclerotized diagnostic structures (e.g., spines, stylets, and cirrus). Specimens can be fixed in a variety of conventional fixation fluids (e.g., Bouin, 5% formaldehyde, absolute alcohol) prior to being processed for histological analysis of the internal structures of diagnostic importance.

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## REFERENCES

Agata, K., 2008. Chapter 4: Stem cells in planarians. In: Bosch, T.C.G. (Ed.), Stem Cells. From *Hydra* to Man. Springer Science+Business Media B.V, pp. 59–74.

Artois, T., Vermin, W., Schockaert, E., 2000. Rhabdocoela (Platyhelminthes) from the Weddell Sea (Antarctica) with the description of eight new species. Belg. J. Zool. 130, 103–110.

Ax, P., Armonies, W., 1990. Brackish water Plathelminthes from Alaska as evidence for the existence of a boreal brackish water community, with circumpolar distribution. Mikrofauna Mar. 6, 7–109.

Ax, P., 1963. Die Ausbildung eines Schwanzfadens in der interstitiellen Sandfauna und die Verwertbarkeit von Lebensformcharakteren fur die Verwandtschaftsforschung (Mit Beschreibungen zweier neuer Turbellarien). Zool. Anz. 171, 51–76.

Ax, P., 1995. Das System der Metazoa I. Ein Lehrbuch der phylogenetischen Systematik. Gustav Fischer, Stuttgart, Jena, New York. 226 pp.

Ax, P., 2008. Plathelminthes aus Brackgewässern der Nordhalbkugel. Akademie der Wissenschaft und der Literatur Mainz, Franz Steiner Verlag, Stuttgart. 696 pp.

Baguñà, J., Ballester, R., 1978. The nervous system in planarians: peripheral and gastrodermal plexuses, pharynx innervation, and the relationship between central nervous system structure and the acoelomate organization. J. Morphol. 155, 237–252.

Baguñà, J., Riutort, M., 2004a. The dawn of bilaterian animals: the case of acoelomorph flatworms. BioEssays 26, 1046–1057.

Baguñà, J., Riutort, M., 2004b. Molecular phylogeny of the Platyhelminthes. Can. J. Zool. 82, 168–193.

Baguñà, J., Saló, E., Auladell, C., 1989. Regeneration and pattern formation in planarians—III. Evidence that neoblasts are totipotent stem cells and the source of blastema cells. Development 107, 77–86.

Ballarin, L., Galleni, L., 1984. Preliminary report on the effects of salinity on the early development of *Stylochus mediterraneus* (Turbellaria: Polycladida). Int. J. Invertebr. Reprod. Dev. 7, 259–262.

Blaustein, l, Dumont, H.J., 1990. Typhloplanid flatworms (*Mesostoma* and related genera): mechanisms of predation and evidence that they structure aquatic invertebrate communities. Hydrobiologia 198, 61–77.

Borkott, H., 1970. Geschleichtliche Organisation, Fortpflanzungsverhalten und Ursachen der sexuallen Vermehrung von *Stenostomum sthenum* nov. spec. (Turbellaria, Catenulida). Mit Beschreibung von 3 neuen *Stenostomum*-Arten. Z. Morphol. Tiere 67, 183–262.

Brusa, F., Damborenea, C., Noreña, C., 2003. A new species of *Gieysztoria* (Platyhelminthes, Rhabdocoela) from Argentina and a kinship analysis of South American species of the genus. Zool. Scr. 32, 449–457.

Cannon L.R.G., 1986. Turbellaria of the world. A guide to families and genera.Brisbane: Queensland Museum, 136 pp.

Carroll, T.M., 2009. Resource Pulses and Spatial Subsidies in Ozark Karst Springs: Effects on Community Structure and Food Webs (Ph.D. dissertation). University of Kansas, Lawrence, KS.

Case, T.J., Washino, R.K., 1979. Flatworm control of mosquito larvae in rice fields. Science 206, 1412–1414.

Cook, C.E., Jiménez, E., Akam, M., Saló, E., 2004. The Hox gene complement of acoel flatworms, a basal bilaterian clade. Evol. Dev. 6, 154–163.

Curini-Galletti, M., Puccinelli, I., 1998. The *Gyratrix hermaphroditus* species complex (Kalyptorhynchia: Polycystidae) in marine habitats of eastern Australia. Hydrobiologia 383, 287–298.

Damborenea, C., Brusa, F., Noreña, C., 2007. New free living Dalyelliidae (Platyhelminthes, Rhabditophora) from Buenos Aires province in Argentina, with their stylets ultrastructure. Zool. Sci. 24, 803–810.

Damborenea, C., Brusa, F., Almagro, I., Noreña, C., 2011. A new species of *Stenostomum* (Platyhelminthes, Catenulida) from the Peruvian Amazon Basin and a phylogenetic analysis of Neotropical congeners. Invertebr. Syst. 25, 155–169.

Damborenea, M.C., Cannon, L.R.G., 2001. On neotropical *Temnocephala* (Platyhelminthes). J. Nat. Hist. 35, 1103–1118.

Dörjes, J., 1968. Die Acoela (Turbellaria) der deutschen Nordseeküste und ein neues System der Ordnung. Z. Zool. Syst. Evol. 6, 56–452.

Dumont, H.J., Carels, I., 1987. Flatworm predator (*Mesostoma* cf. *lingua*) releases a toxin to catch planktonic prey *(Daphnia magna)*. Limnol. Oceanogr. 32, 699–702.

Dunn, C., Hejnol, A., Matus, D., Pang, K., Browne, W., Seaver, E., Smith, S., Touse, G., Obst, M., Edgecombe, G., Sorensen, M., Haddok, S., Schmidt-Rhaesa, A., Okusu, A., Kristensen, R., Wheeler, W., Martindale, M., Giribert, G., 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452, 745–750.

Egger, B., Gschwentner, R., Hess, M.W., Nimeth, K.T., Adamski, Z., Willems, M., Rieger, R., Salvenmoser, W., 2009. The caudal regeneration blastema is an accumulation of rapidly proliferating stem cells in the flatworm *Macrostomum lignano*. BMC Dev. Biol. 9, 41.

Egger, B., Gshwentner, R., Rieger, R., 2007. Free-living flatworms under the knife: past and present. Dev. Genes Evol. 217, 89–104.

Ehlers, U., Sopott-Ehlers, B., 1990. Organization of statocysts in the Otoplanidae (Plathelminthes): an ultrastructural analysis with implications for the phylogeny of the Proseriata. Zoomorphology 109, 309–318.

Ehlers, U., 1981. Fine structure of the giant aflagellate spermatozoon in *Pseudostomum quadrioculatum* (Leuckart) (Platyhelminthes, Prolecithophora). Hydrobiologia 84, 287–300.

Ehlers, U., 1985. Das Phylogenetische System der Plathelminthes. Gustav Fischer Verlag, Stuttgart, New York. 317 pp.

Ehlers, U., 1992. Frontal glandular and sensory structures in *Nemertoderma* (Nemertodermatida) and *Paratomella* (Acoela): ultrastructure and phylogenetic implications for the monophyly of the Euplathelminthes (Plathelminthes). Zoomorphology 112, 227–236.

Ferrero, A.E., Bedini, C., 1991. Ultrastructural aspects of nervous-system and statocyst morphogenesis during embryonic development of *Convoluta psammophila* (Turbellaria, Acoela). Hydrobiologia 227, 131–137.

Gammoudi, M., Noreña, C., Tekaya, S., Prantl, V., Egger, B., 2012. Insemination and embryonic development of some Mediterranean polyclad flatworms. Invertebr. Reprod. Dev. 56, 272–286.

Garcia-Fernandez, J., 2005. Hox, ParaHox, ProtoHox: facts and guesses. Heredity 94, 145–152.

Gaviño, M.A., Reddien, P.W., 2011. A Bmp/Admp regulatory circuit controls maintenance and regeneration of dorsal-ventral polarity in planarians. Curr. Biol. 21, 294–299.

Gevaerts, H., Moens, J.B., Martens, E.E., Schockaert, E.R., 1995. Hard parts in the female system of *Syndesmis longicanalis* (Platyhelminthes, Rhabdocoela, Umagillidae) are basement membrane derivatives. Invertebr. Biol. 114, 279–284.

Halton, D.W., 1997. Nutritional adaptations to parasitism within the Platyhelminthes. Int. J. Parasitol. 27, 693–704.

Haszprunar, G., 1996a. Plathelminthes and Plathelminthomorpha paraphyletic taxa. J. Zool. Syst. Evol. Res. 34, 41–48.

Haszprunar, G., 1996b. Chapter 1: The Mollusca: coelomate turbellarians or mesenchymate annelids? In: Taylor, J. (Ed.), Origin and Evolutionary of the Mollusca. Oxford University Press, Oxford, pp. 1–28.

Heitkamp, U., 1978. Abundanzdynamik und Reproduktionsphasen limnophiler Trikladen (Turbellaria) aus stehende Kleingewässern. Hydrobiologia 65, 49–57.

Hendelberg, J., 1977. Comparative morphology of turbellarian spermatozoa studied by electron microscopy. Acta Zool. Fenn. 154, 149–162.

Hertel, L.A., 1993. Excretion and osmoregulation in the flatworms. Trans. Am. Microsc. Soc. 112, 10–17.

Jiménez-Guri, E., Paps, J., García-Fernàndez, J., Saló, E., 2006. Hox and ParaHox genes in Nemertodermatida, a basal bilaterian clade. Int. J. Dev. Biol. 50, 675–679.

Karling, T.G., 1974. Chapter 1: On the anatomy and affinities of the Turbellarian orders. In: Rise, N., Morse, P. (Eds.), Biology of the Turbellaria. McGraw-Hill Book Company, New York, pp. 1–16.

Kolasa, J., Tyler, S., 2010. Chapter 6: Flatworms: Turbellarians and Nemertea. In: Thorp, J.H., Covich, A.P. (Eds.), Ecology and Classification of North American Freshwater Invertebrates. Academic Press, Elsevier, London, pp. 143–161.

Kotikova, E.A., Raikova, O.I., 2008. Architectonics of the central nervous system of Acoela, Platyhelminthes, and Rotifera. J. Evol. Biochem. Physiol. 44, 95–108.

Ladurner, P., Egger, B., De Mulder, K., Pfister, D., Kuales, G., Salvenmoser, W., Schärer, L., 2008. Chapter 8. The stem cell system of the basal flatworm *Macrostomum lignano*. In: Bosch, T.C.G. (Ed.), Stem Cells. From Hydra to Man. Springer Science +Business Media B.V, pp. 75–112.

Littlewood, D.T.J., Bray, R.A. (Eds.), 2001. Interrelationships of the Platyhelminthes. Taylor & Francis, London, New York.

Littlewood, D.T.J., Rohde, K., Bray, R.A., Herniou, E.A., 1999. Phylogeny of the Platyhelminthes and the evolution of parasitism. Biol. J. Linn. Soc. 68, 257–287.

Lundin, K., 1997. Comparative ultrastructure of the epidermal ciliary rootlets and associated structures in species of the Nemertodermatida and Acoela. Zoomorphology 117, 81–92.

Luther, A., 1955. Die Dalyelliiden (Turbellaria Neorhabdocoela) Eine Monographie. Acta Zool. Fenn. 87, 1–337.

Martin, G.G., 1978. A new function of rhabdites: mucus production for ciliary gliding. Zoomorphologie 91, 235–248.

Martín-Durán, J.M., Egger, B., 2012. Developmental diversity in free-living flatworms. EvoDevo 3, 7.

Müller, D., Faubel, A., 1993. The "Turbellaria" of the River Elbe Estuary. A faunistic analysis of oligohaline and limnic areas. Arch. Hydrobiol./Supplement Band 75, 363–396.

Nielsen, C., 2005. Trochophora larvae: cell lineages, ciliary bands and body regions. Other groups and general discussion. J. Exp. Zool. Part B: Mol. Dev. Evol. 304, 401–447.

Noreña, C., 2012. Fauna Europaea: Platyhelminthes, Turbellaria. Version 2.4, http://www.faunaeur.org.

Noreña, C., Damborenea, C., Brusa, F., Escobedo, M., 2006. Free-living Platyhelminthes of the Pacaya-Samiria National Reserve, a Peruvian Amazon floodplain. Zootaxa 1313, 39–55.

Noreña, C., Brusa, F., Faubel, A., 2003. Census of "Microturbellarians" (Free-living Platyhelminthes) of the zoogeographical regions originating from Gondwana. Zootaxa 146, 1–34.

Noreña, C., Damborenea, C., Brusa, F., 2005. New freshwater interstitial Otoplanidae (Platyhelminthes, Proseriata) from Paraná and Uruguay rivers, South America. J. Nat. Hist. 39, 1457–1468.

Noreña, C., Damborenea, C., Faubel, A., Brusa, F., 2007. Composition of meiobenthic Platyhelminthes from brackish environments of the Galician and Cantabrian coasts of Spain with the description of a new species of *Djeziraia* (Polycystididae, Kalyptorhynchia). J. Nat. Hist. 41, 1989–2005.

Noreña-Janssen, C., Faubel, A., 1992. Revision of the subfamily Mesostominae, Rhabdocoela, Platyhelminthes. Mittl. Hambg. Zool. Mus. Inst. 89, 7–47.

Noreña-Janssen, C., Faubel, A., 1996. *Myoretronectes paranaensis* n. gen. et sp., a new freshwater genus of the family Retronectidae (Turbellaria, Catenulida) from the Paraná, Argentina. Hydrobiologia 330, 111–118.

Noreña-Janssen, C., 1995. Studies on the taxonomy and ecology of the Turbellaria (Plathelminthes) in the floodplain of the Paraná River (Argentina). II. Taxonomy and ecology of the Turbellaria. Arch. Hydrobiol./Supplement Band 107, 211–262.

Pfister, D., De Mulder, K., Hartenstein, V., Kuales, G., Borgonie, G., Marx, M., Morris, J., Ladurner, P., 2008. Flatworm stem cells and the germ line: Developmental and evolutionary implications of macvasa expression in *Macrostomum lignano*. Dev. Biol. 319, 146–159.

Philippe, H., Brinkmann, H., Martinez, P., Riutort, M., Baguñà, J., 2007. Acoel flatworms are not Platyhelminthes: evidence from phylogenomics. PloS One 2, e717.

Philippe, H., Brinkmann, H., Copley, R., Moroz, L., Nakano, H., Poustka, A., Wallberg, A., Peterson, K., Telford, M., 2011. Acoelomorph flatworms are deuterostomes related to *Xenoturbella*. Nature 470, 255–258.

Reuter, M., Raikova, O.I., Gustafsson, M.K.S., 1998. An endocrine brain? The pattern of FMRF-amide immunoreactivity in Acoela (Plathelminthes). Tissue Cell 30, 57–63.

Rieger, R.M., Doe, D.A., 1975. The proboscis armature of Turbellaria-Kalyptorhynchia, a derivative of the basement lamina? Zool. Scr. 4, 25–32.

Rieger, R.M., 1978. Multiple ciliary structures in developing spermatozoa of marine Catenulida (Turbellaria). Zoomorphologie 89, 229–236.

Rieger, R.M., Tyler, S., Smith III, J.P.S., Rieger, G., 1991. Chapter 2: Turbellaria. In: Harrison, F.W., Bogitsh, B.J. (Eds.), Microscopic Anatomy of Invertebrates. Platyhelminthes and Nemertinea, vol. 3. Wiley-Liss, New York, NY, pp. 7–140.

Riutort, M., Field, K.G., Raff, R.A., Baguñà, J., 1993. 18S rRNA sequences and phylogeny of Platyhelminthes. Biochem. Syst. Ecol. 21, 71–77.

Rohde, K., Watson, N.A., Faubel, A., 1993. Ultrastructure of the statocyst in an undescribed species of Luridae (Platyhelminthes: Rhabdocoela: Luridae). Aust. J. Zool. 41, 215–224.

Ruiz-Trillo, I., Riutort, M., Littlewood, D.T.J., Herniou, E.A., Baguñà, J., 1999. Acoel flatworms: earliest exant bilaterian metazoans, not members of Platyhelminthes. Science 283, 1919–1923.

Ruiz-Trillo, I., Riutort, M., Fourcade, H.M., Baguñà, J., Boore, J.L., 2004. Mitochondrial genome data support the basal position of Acoelomorpha and the polyphyly of the Platyhelminthes. Mol. Phylogen. Evol. 33, 321–332.

Schockaert, E.R., Hooge, M., Sluys, R., Schilling, S., Tyler, S., Artois, T., 2008. Global diversity of free living flatworms (Platyhelminthes, "Turbellaria") in freshwater. Hydrobiologia 595, 41–48.

Schwank, P., 1981. Turbellarien, Oligochaeten and Archianneliden des Breitenbachs und anderer oberhessischer Mittelgebirgsbäche. I. Lokalgeografische Verbreitung und die Verteilung der Arten in den einzelnen Gewässer in Abhängigkeit vom Substrat. II. Die Systematik und Autökologie der einzelen Arten. Arch. Hydrobiol. Suppl. 62 (1–85), 86–147.

Schwartz, S.S., Hebert, P.D.N., 1986. Prey preference and utilization by *Mesostoma lingua* (Turbellaria, Rhabdocoela) at a low arctic site. Hydrobiologia 135, 251–253.

Sempere, L.F., Martinez, P., Cole, C., Baguñà, J., Peterson, K.J., 2007. Phylogenetic distribution of microRNAs supports the basal position of acoel flatworms and the polyphyly of Platyhelminthes. Evol. Dev. 9, 409–415.

Smith III, J.P.S., Tyler, S., Thomas, M.B., Rieger, R.M., 1982. The morphology of Turbellarian rhabdites: phylogenetic implications. Trans. Am. Microsc. Soc. 101, 209–228.

Smith, J., Tyler, S., Rieger, R.M., 1986. Is the Turbellaria polyphyletic? Hydrobiologia 132, 13–21.

Sörensen, M.V., Sterrer, W., Giribert, G., 2006. Gnathostomulid phylogeny inferred from a combined approach of four molecular loci and morphology. Cladistics 22, 32–58.

Sterrer, W., Rieger, R.M., 1974. Chapter 4: Retronectidae – a new cosmopolitan marine family of Catenulida (Turbellaria). In: Riser, N.W., Morse, M.P. (Eds.), Biology of the Turbellaria. McGraw- Hill Book Company, New York, London, Sydney, pp. 63–92.

Tranchida, M.C., Maciá, A., Brusa, F., Micieli, M.V., García, J.J., 2009. Predation potential of three flatworm species (Platyhelminthes: Turbellaria) on mosquitoes (Diptera: Culicidae). Biol. Control 49, 270–276.

Tyler, S., Schilling, S., Hooge, M., Bush, L.F., 2006–2012. Turbellarian Taxonomic Database. Version 1.7, http://turbellaria.umaine.edu.

Tyler, S., Hooge, M., 2004. Comparative morphology of the body wall in flatworms (Platyhelminthes). Can. J. Zool. 82, 194–210.

Tyler, S., Rieger, R.M., 1975. Uniflagellate spermatozoa in *Nemertoderma* (Turbellaria) and their phylogenetic significance. Science 188, 730–732.

Tyler, S., Tyler, M.S., 1997. Origin of the epidermis in parasitic Platyhelminths. Int. J. Parasitol. 27, 715–738.

Tyler, S., 1979. Distinctive features of cilia in metazoans and their significance for systematic. Tissue Cell 11, 385–400.

Tyler, S., 1984. Ciliogenesis in embryos of the acoel turbellarian *Archaphanostoma*. Trans. Am. Microsc. Soc. 103, 1–15.

Van Steenkiste, N., Davison, P., Artois, T., 2010. *Bryoplana xerophila* n. g. n. sp., a new limnoterrestrial microturbellarian (Platyhelminthes, Typhloplanidae, Protoplanellinae) from epilithic mosses, with notes on its ecology. Zool. Sci. 27, 285–291.

Weinzierl, R.P., Beukeboom, L.W., Gerace, L., Michiels, N.K., 1999. Spatial and ecological overlap between coexisting sexual and parthenogenetic *Schmidtea polychroa* (Tricladida; Platyhelminthes). Hydrobiologia 392, 179–185.

Young, J.O., 1974. The occurrence of diapause in the egg stage of the life-cycle of *Phaenocora typhlops* (Vejdovsky) (Turbellaria: Neorhabdocoela). J. Anim. Ecol. 43, 719–731.

Young, J.O., 1977. An ecological study of *Phaenocora unipunctata* (Oersted) (Turbellaria Rhabdocoela): population dynamics. Acta Zool. Fenn. 154, 105–118.

Young, J.O., 2001. Keys to the Freshwater Microturbellarians of Britain and Ireland, with Notes on Their Ecology. Scientific Publication No. 59. Freshwater Biological Association, Ambleside. 142 pp.

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