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FORAMATION OF ORGANS AND ORGAN SYSTEMS IN NON-INTENSTIONAL TURBELLARIA

EHTESHAM RABBANI
M.Phil., Roll No.: 142123 Session-2014-15
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, India
E-mail: etheshamrabbanipcs@gmail.com

The Platyhelminthes historically contain organisms grouped in three major clades, Acoelomorpha, Catenulida, and Rhabditophora, which include the Neodermata (Figure 1.1). 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

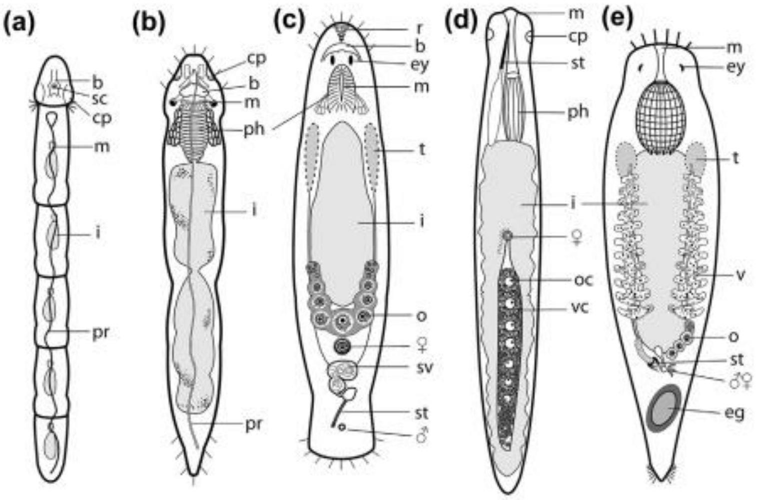
INTRODUCTION

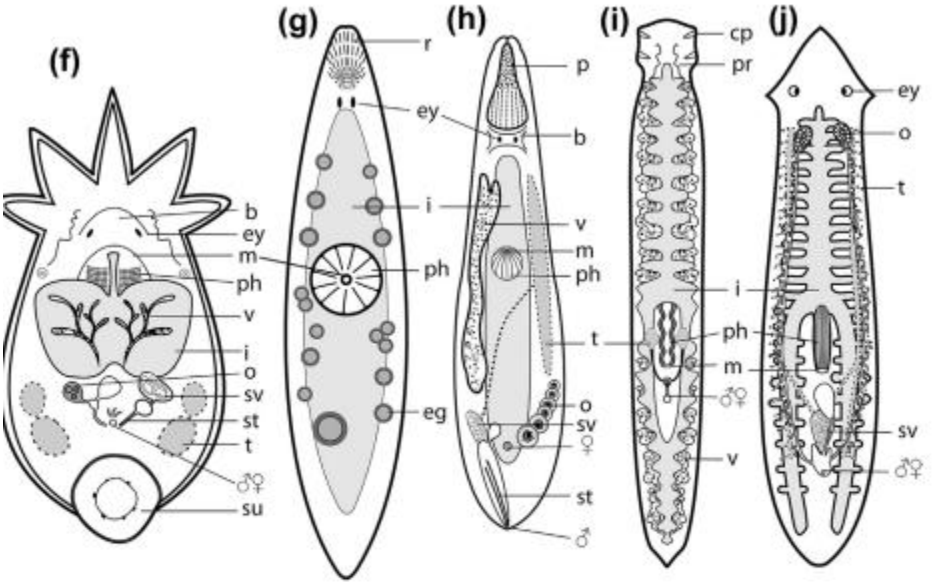
than one cilia in the terminal cell of the protonephridia, stem cells as differentiation cells, presence of a male porus and male copulatory organ, and dorsoventral and anteroposterior polarity (Ehlers, 1985; Ax, 1995). These are shared by all the groups of this phylum, with the exception of the multiciliated 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).

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 ∼1mm in size, but some orders like Tricladida and Polycladida (the so-called macroturbellaria) can reach body sizes of 0.2–10cm.

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 1.2).





Schematic representation of the different orders of Turbellaria

OBJECTIVE OF THIS STUDY

The objective of this study was to contribute to the knowledge of these two turbellarians and to analyze characters that could be used to better establish their phylogenetic position. For Convoluta pulchra, this entailed identifying certain fluorescent non-muscular structures in its epidermis, and for Geonostoma kozloffi this entailed reconstructing the three dimensional morphology of the spermatozoa and the developmental process by which those spermatozoa arise. Both of these cases highlight the importance of basic ultrastructural studies, using fluorescence microscopy and, most importantly, transmission electron microscopy, to address questions of phylogenetics.

GENERAL CHARACTERS:

1. They may be free-living (terrestrial, fresh water or marine) or commensal or parasitic.
2. Their body is soft and dorso-ventrally flattened, may be leaf like or ribbon like.
3. Body shows bilateral symmetry. It shows marked anterior and posterior ends and dorsal and ventral surfaces.
4. Primary germ layers are ectoderm and endoderm. In between the two is present the mesoderm. Thus they show triploblastic organization.

5. Body shows organ-system level of organization in which the cells form different tissues, which join to form different organs, which unite to form different organ systems.

6. They show acoelomate organization in which the body cavity gets filled up with mesenchymal cells.

7. Alimentary canal with anterior or ventral mouth, pharynx and simple or branched intestine. Anus is generally absent. In some forms as in tapeworms alimentary canal is totally absent.

8. In free living forms aerobic respiration occurs through general body surface. In parasitic forms respiration is anaerobic.

9. Circulatory system is absent.

10. Excretory system is represented by flame cells leading into fine tubules which join together to form excretory duct opening by excretory pore.

11. Nervous system is primitive. It shows a pair of anterior ganglia with two longitudinal nerve cords connected by transverse commissures. Thus it is a ladder like system.

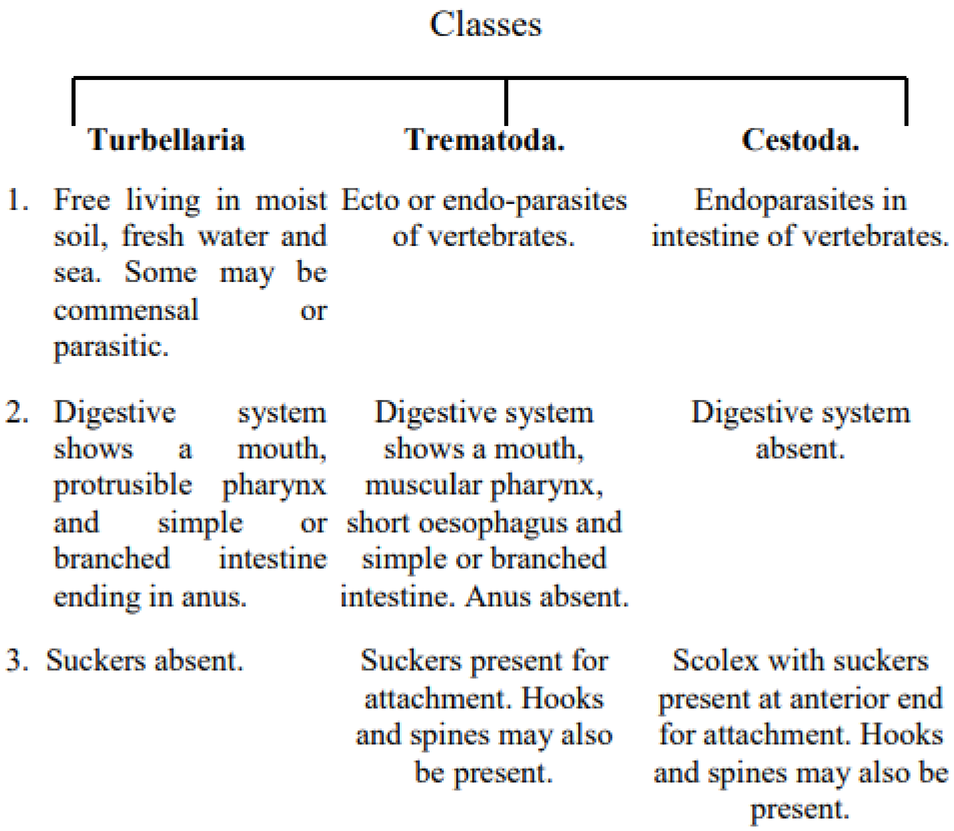
12. Free living aquatic forms show locomotion by epidermal cilia. Parasitic forms show absence of locomotion. Instead they show organs of attachment in the form of suckers, hooks and spines.

13. Body is mostly hermaphroditic with complex reproductive system; female reproductive system shows peculiar vitelline glands which produce yolk.

14. Fertilization is generally cross and internal.

15. Development may be direct in free living forms or indirect occurring in endoparasites showing complex life cycles involving many larvae and hosts.

Classification of Phylum Platyhelminthes Phylum Platyhelminthes



Classes

Turbellaria	Trematoda.	Cestoda.
1. Free living in moist soil, fresh water and sea. Some may be commensal or parasitic.	Ecto or endo-parasites of vertebrates.	Endoparasites in intestine of vertebrates.
2. Digestive system shows a mouth, protrusible pharynx and simple or branched intestine ending in anus.	Digestive system shows a mouth, muscular pharynx, short oesophagus and simple or branched intestine. Anus absent.	Digestive system absent.
3. Suckers absent.	Suckers present for attachment. Hooks and spines may also be present.	Scolex with suckers present at anterior end for attachment. Hooks and spines may also be present.

Class Turbellaria Order : Acoela eg. Convoluta

Order : Rhabdocoela eg. Microstomum

Order : Alloecoela eg. Plagiostomum

Order : Tricladida eg. Dugesia (fig 1.4)

Order : Polycladida eg. Leptoplana

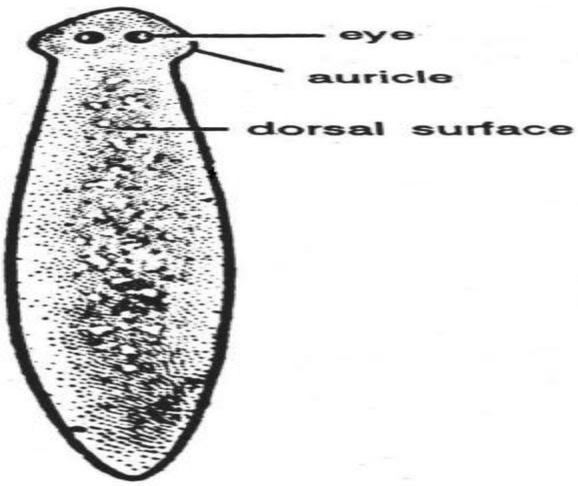


Fig.1.4 Dugesia. External Features

Class Trematoda

Order : Monogenea eg. Polystomum

Order : Digenea eg. Fasciola

Order : Aspidogastrea eg. Aspidogaster

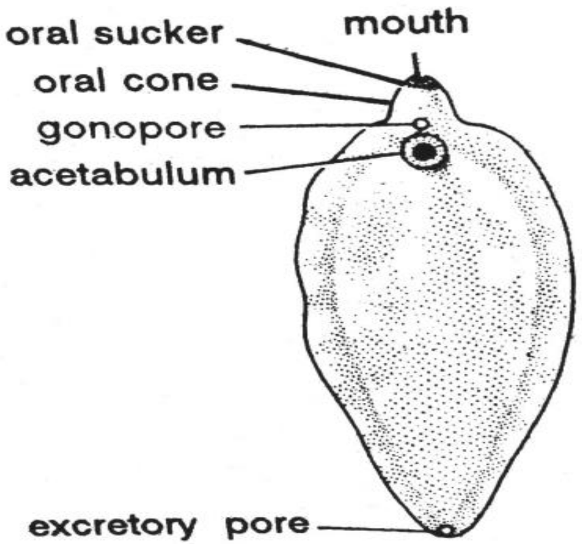


Fig.1.5 Fasciola

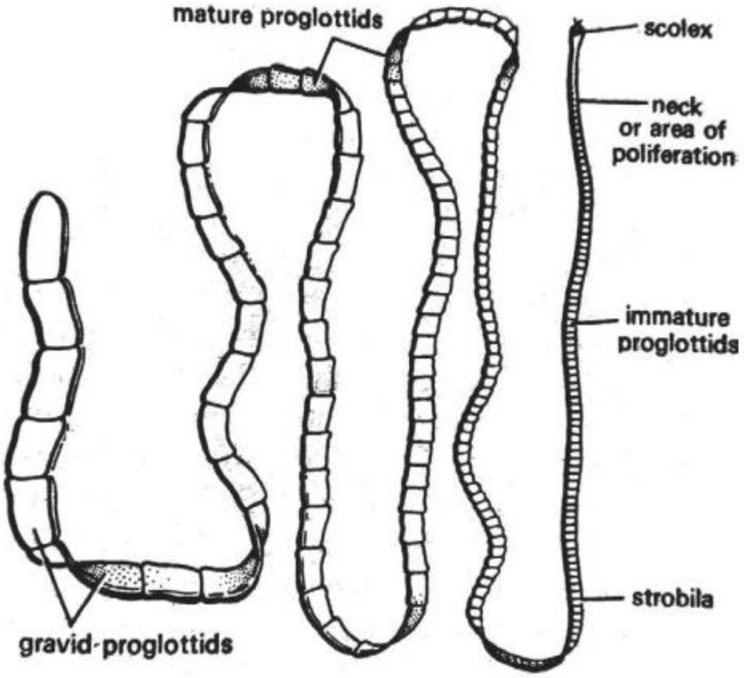


Fig.1.6 Taenia solium

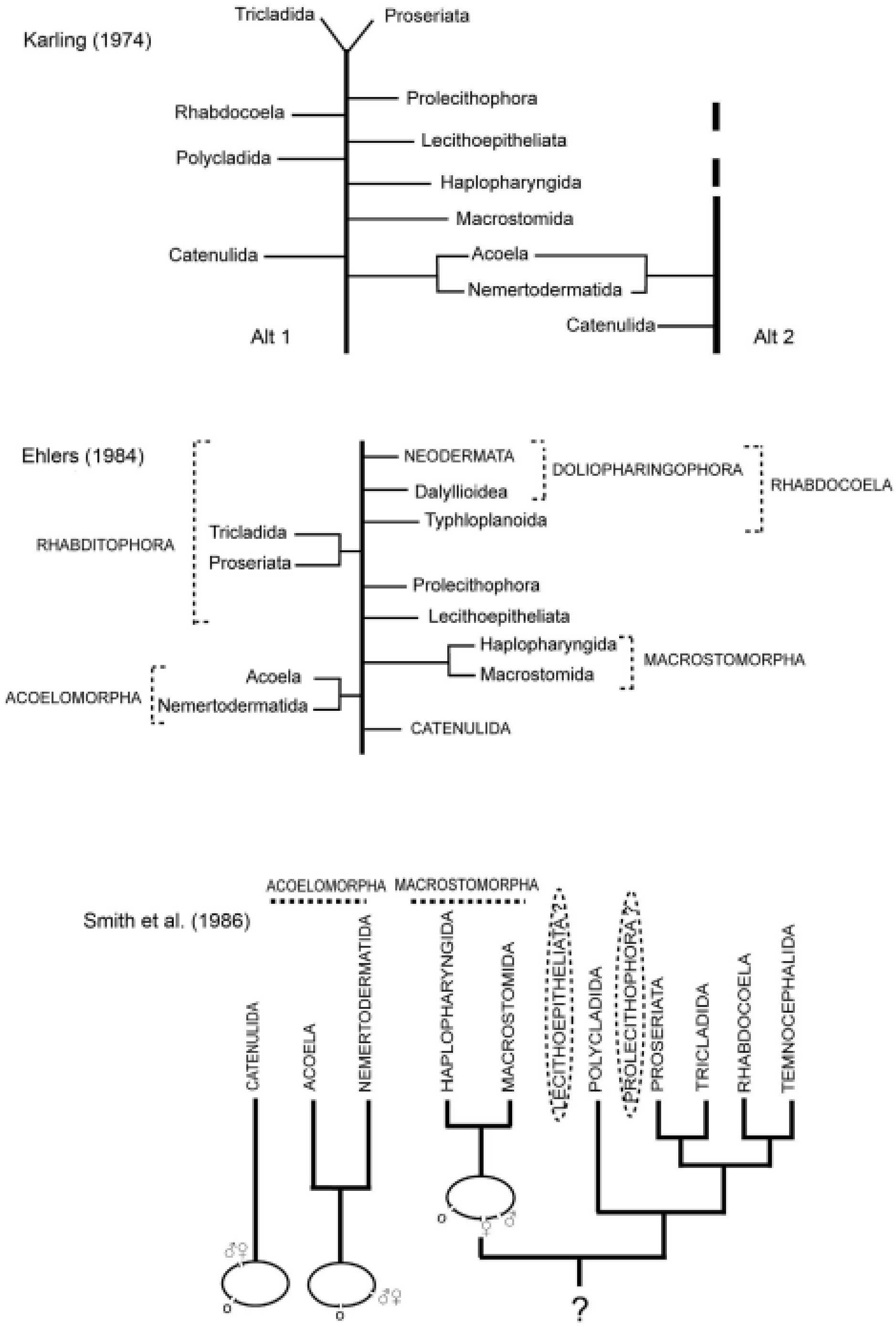
Phylogenetic Relationships The nature of the available morphological characters and the relative overall simplicity of body structures make it difficult to resolve the phylogenetic relationship among the "Turbellaria" and to establish their relationship to other groups of invertebrates, such as Eutrochozoa 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 1.7). 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; CuriniGalletti 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.



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 (NoreñaJanssen, 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/0.1m3, 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).

TURBELLARIANS AS MEMBERS OF THE MEIOBENTHOS

The term ‘meiobenthos’ (meiofauna) was introduced by Mare (1942) to indicate those benthic metazoans smaller than the ‘macrobenthos’ but larger than the ‘microbenthos’. In practice the meiofauna consists of animals which pass through a 0 .5 -2 mm sieve but are retained on a sieve of 0.1-0.04 mm mesh width (McIntyre, 1969; Coull & Bell, 1979; Platt, 1981). However, many turbellarian species are larger than 2 mm (even up to more than 1 cm) and are nevertheless considered meiofaunal elements. In a recent study, Warwick (1984) compared life strategies of several taxa in different marine benthic communities and concluded that animals larger than ±45 fig dry weight (macrofauna) have a planktonic development, disperse in the larval stage, have a continuous growth throughout life with a generation time of more than one year, and feed in indiscriminate fashion on particles (but often selected on the basis of particle size). Species smaller than ±45 fig dry weight (meiofauna) have no planktonic development, disperse in the adult stage, have an asymptotic growth to a maximum body size with a generation time of less than one year, and feed by selection on size but also on shape or quality of the particles. From these observations, meio- and macrofauna can be redefined on the basis of biological criteria and of body size. Except for some triclads and polyclads all marine benthic turbellarians fit very well into this new definition, and some of them must be considered 'large meiobenthos! Two factors - among others - characterize the benthic habitat: grain size of the sediment and water movement (dynamism); and these are related to each other to some extent. Grain size and packing of the grains determine the space available between grains for the so called interstitial meiofauna (Coull & Bell, 1979). The coarser the sand, the larger the interstices and vice versa. In a very fine sediment, such as mud, animals move by displacement of the sediment and so constitute burrowing meiofauna. Intertidal animals are small and/or elongate and exhibit a number of adaptive features (Remane, 1933; Wilson, 1935; Swedmark, 1964); there seems to be a relation between body shape and grain size (Wieser, 1959), but there is, of course, a limit: in mud-living meiofauna body size and shape are not related to grain size (Coull & Bell, 1979). Sandy bottoms, especially on beaches exposed to wave action, are mostly dynamic environments. Muddy bottoms, found in estuaries, lagoons, saltmarshes and sublittoral places, are much less dynamic. Turbellarians are not very abundant in muddy bottoms: we find a lower diversity, as in other meiofauna taxa, and a lower density, contrary to many other meiofaunal taxa (see below). In a sandy habitat, both diversity and density of turbellarian species are high (see also below) and all orders are represented. They often share a number of characteristics: they are haptic and may be quite contractile or move fast by muscular action, some of them have a tail appendage, many have long sensory bristles, a chordoid structure can be present, and some are extremely elongate. All these characteristics are considered adaptations to the interstitial way of life or to the dynamism of the environment or to both (Remane, 1933; Hyman, 1951; Ax, 1963, 1966; Boaden, 1968; Bush, 1968). The functional significance of these features has been discussed by Ax (1963, 1966) and Bush (1968). Some of these characteristics, such as adhesive papillae, sensory bristles, and body musculature, are well developed in Otoplanidae which is the dominating metazoan group in the swash zone, the most dynamic zone of the beach and called the Otoplana zone by Remane (1933). As with other interstitial meiofaunal groups, there seems to be a relation between grain size of the sand and body length of the turbellarians. On the beaches of the island of Sylt (Germany) with coarse sand (400-500 fim\ Schmidt, 1968) Proseriata is the dominating group, with many representatives of the Monocelididae, Coelogynoporidae, and Nematoplanidae reaching up to more than 1 cm in length (Sopott, 1973; Noldt & Wehrenberg, 1984). On the other hand at Robin H ood's Bay in fine sand (Gray & Rieger, 1971) and at the Belgian coast also in fine sand (Martens, 1984) Neorhabdocoela up to ± 2 mm (Dalyellioida and Kalyptorhynchia, respectively) dominate the turbellarian fauna (together with the Acoela). According to Douglas (1984), there might be a relation between body size and grain size in the acoel Convoluta roscoffensis.

CONCLUSIONS

Increased taxon sampling of Trigonostominae, including four new species, in a comprehensive molecular phylogeny inferred from 18S and 28S rRNA sequences allowed us to demonstrate inter- and intrageneric relationships within this group. This phylogenetic context enabled us to reassess homology statements on some key morphological traits associated with the stylet and the afferent system. Our results show that (1) species of Ceratopera and Messoplana cannot be clearly separated based on morphological and molecular data leading to the suppression of the latter genus in favour of Ceratopera; (2) traditional species groups within Trigonostomum are partly supported by molecular data, while traditional species groups within Proxenetes are not; (3) the presence of a mantle accompanying the stylet proper reflects the plesiomorphic condition of the Trigonostominae; (4) the spermatic duct and seminal receptacle in Ptychopera is inferred to be homologous with (parts of) the bursal appendage and the insemination duct in other genera; and (5) the ancestral bursal appendage resembled a simple, slightly sclerotized tube. Further taxon sampling and additional molecular markers will be necessary to more fully resolve the phylogeny of Trigonostominae and refine our inferences about the evolution of their highly complex reproduction structures.

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