Hydrobiologia (2008) 595:41–48
DOI 10.1007/s10750-007-9002-8

FRESHWATER ANIMAL DIVERSITY ASSESSMENT

Global diversity of free living flatworms (Platyhelminthes, "Turbellaria") in freshwater

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Abstract This contribution reviews diversity of turbellarian species by biogeographical regions, with comments on species biology. The review draws on the database available at http://www.devbio.umesci.maine.edu/styler/turbellaria. Comparisons between regions suggest that species richness may be at least one order of magnitude higher than the currently reported number of species. In the context of the recent reconstructions of phylogeny of Platyhelminthes based on molecular data, the paper allows inferences as to the history of colonization of freshwaters by turbellarians. Specifically, four, or perhaps six, major invasions of freshwater habitats may have occurred in the Pangean period, each of which gave rise to a monophyletic freshwater taxon. In addition, several occasional invasions by representatives of marine taxa must have taken place.

Keywords Platyhelminthes · Freshwater · Distribution · Phylogeny · History

Guest editors: E. V. Balian, C. Lévêque, H. Segers & K. Martens
Freshwater Animal Diversity Assessment

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Introduction

The taxon Platyhelminthes is traditionally divided into four or five "classes", one of which is the "Turbellaria", characterised by the ciliated epidermis. The other "classes" are all parasites and constitute the monophyletic taxon Neodermata, where, at some stage of their development, the original ciliated epidermis is shed and replaced by a new body lining, the neodermis. The ciliated epidermis is clearly a plesiomorphy, and the "Turbellaria" is thus a paraphyletic assemblage, sometimes referred to as "free-living Platyhelminthes". Since some of them are symbionts, we prefer to use "Turbellaria" (between quotation marks) or the vernacular name turbellarians. The turbellarian database (http://turbellaria.unimaine.edu), compiled and maintained by Tyler and co-workers (2005), lists close to 6,500 species (with a valid name), of which 1/5 have been found in freshwater. Far more turbellarian species are thus known from marine habitats and the marine taxa are more diverse as well.

Platyhelminthes are hermaphrodites, mostly simultaneously male and female, with an internal fertilisation. The reproductive system may be rather complex, especially in the Neoophora (Fig. 1H for an example) where yolk is stored in yolk cells, produced in separate vitellaria, a unique feature in animals. The organisation of the reproductive apparatus and of the digestive system—along with some other morphological characters—have traditionally been the major basis for taxonomy (Fig. 3).

Turbellarians are seldom, if ever, taken into account in biodiversity studies of freshwater habitats, even though they are mostly present in high numbers of species and of individuals. About 1/3 of the freshwater species known are the larger triclads (known as "planarians"). Due to their size (1–5 cm and more) and their "popularity", they have often received more attention than the other taxa. Representatives of the other taxa, only a few millimetres large, must preferably be studied alive for a proper identification. Once fixed, they become opaque and hard, and the internal anatomy, necessary for the identification, can barely be seen under the microscope. Moreover, they contract at fixation and appear as a little sphere that is not even recognised as an animal in a bulk sample. If living material is available, identification is relatively easy. With some training, the major taxa can be recognised and many turbellarians have hard parts in the copulatory organ that provide unambiguous species characters.

Flatworms are bottom dwellers, the triclads often under stones, or live on immersed plants. Only very few species are occasionally found in plankton. Many are heavy predators. Several Dalyelliidae and some Typhloplanidae carry symbiotic algae. The rhabdocoel freshwater flatworms produce dormant and subitaneous eggs (unknown for the other turbellarian taxa), some are viviparous. Several species of temporal waters have been described from individuals that developed in the laboratory from dormant eggs after immersion of sediment (e.g. Artois et al., 2004).

The planarians are known for their tremendous capacity to regenerate, but also other and smaller species of turbellarians are able to regenerate. This regeneration capacity is exclusively due to a reserve of undifferentiated cells, stemcells or neoblasts, which are the only cells able to divide by mitosis, a unique feature in the animal kingdom. Somatic cells do not divide, as in nematodes; they may grow and die and, contary to what happens in nematodes can be replaced by differentiating stemcells. The turbellarians have recently been "discovered" by cell biologists for stemcell research, research on the processes of differentiation and other similar topics.

Other human related issues are accidental invasions, only known for triclads. Invasions of the smaller flatworms must have occurred but are not documented for the reasons explained above. In the first half of the 20th century, *Girardia tigrina* (Girard, 1850) has been introduced in Europe from N. America, while the European *Schmidtea polychroa* (Schmidt, 1861) was introduced in N. America. *Girardia dorotocephala* (Woodworth, 1897) has also undoubtedly been imported in Hawai from the North American continent.

Species diversity and present distribution

Turbellarians can be found in almost all aquatic habitats, marine and freshwater, or in damp terrestrial locations. The Tricladida Terricola (with about 830 species) are exclusively terrestrial. Some 20–25 species of Rhabdocoela have been found in wet terrestrial habitats. They are included in the numbers in Table 1, since some have been found also in fresh water and we suspect that several of the other species may also occur in water bodies.

The number of freshwater species of the various biogeographic regions in fact reflects the scientific activities of the past. In the 19th and 20th century, up to about 1970, the European and Russian continental waters have been investigated rather intensively by e.g. von Graff, Reisinger and Steinböck in Austria, Luther in Finland, Nassonov and Beklemischev in the former USSR, and several other authors. A number of references can be found in Cannon (1986) and in Schockaert (1996). With the on-going research in the Lake Baikal, several species have more recently been added to the list for the Palearctic (see Timoshkin, 2004). Many fewer species have been recorded in North America (see Kenk, 1989; Kolasa, 2000 and the references therein), while the species from South America are mainly known through the activity of Marcus in Brasil in the 1940s and 1950s (see Marcus, 1958 and references in Sluys et al., 2005) and recently of Noreña-Janssen (e.g. Noreña et al., 2005) and Damborenea (for Temnocephalida: Damborenea & Cannon, 2001) in and around Argentina. Records from Africa are all from occasional sampling

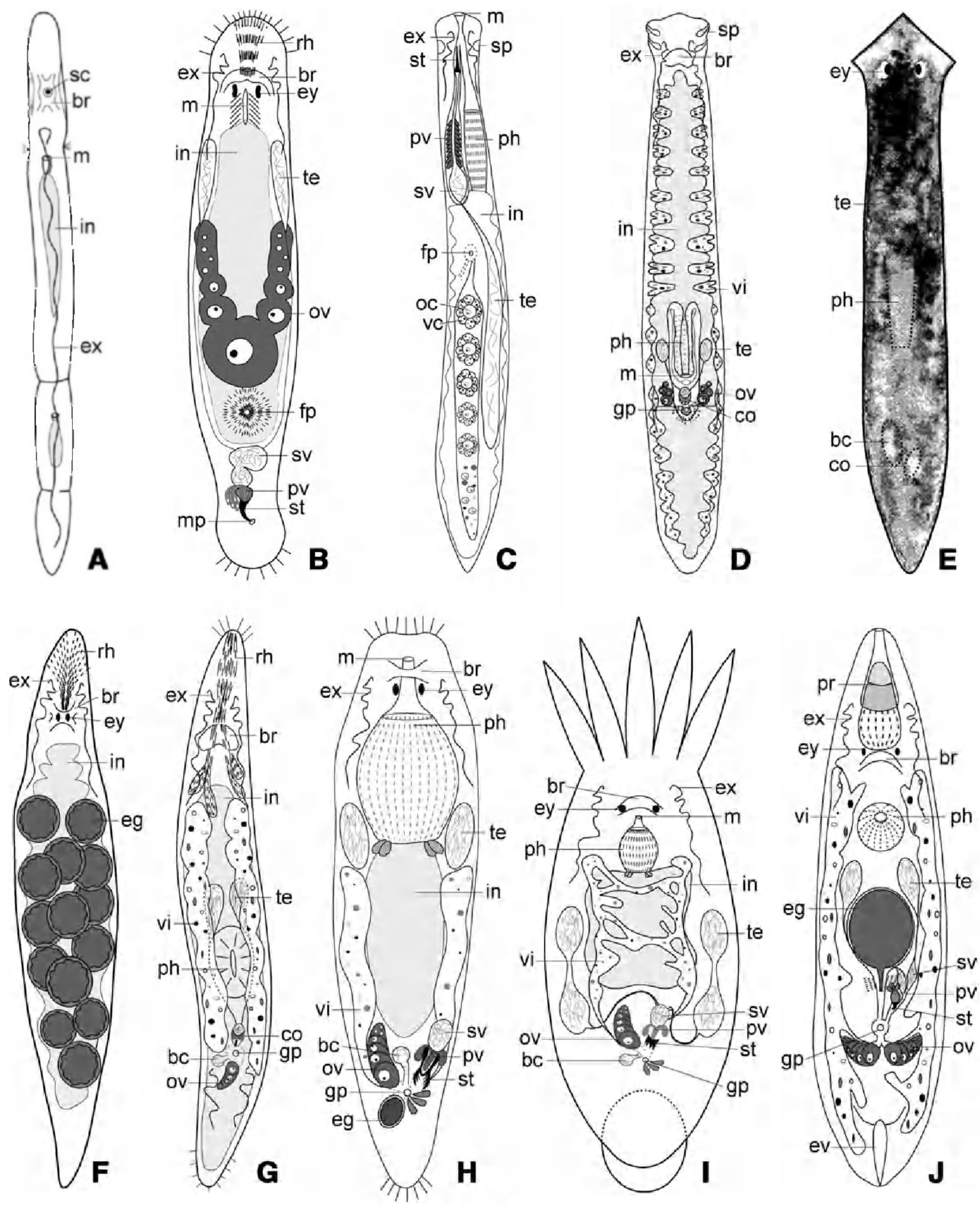


Fig. 1 Some representatives of the major freshwater taxa, as seen alive—(**A**) *Catenula lemnae*, ±1 mm (Catenulida) reproducing asexually—(**B**) *Macrostomum* spec., 1–2 mm (Macrostomida); note the absence of vitellaria ("archoophoran" organisation)—(**C**) *Prorhynchus stagnalis*, ±5 mm (Lecithoepitheliata) the vitellocytes form a follicle around the ovocytes; the male pore is combined with the mouth—(**D**) *Bothrioplana semperi*, ±5 mm (uncertain taxonomic position)—(**E**) *Dugesia* spec., 10–50 mm; position of some structures can be seen—(**F**) *Mesostoma lingua*, ±5 mm (Mesostomidae) with the uterus filled with dormant eggs—(**G**) *Olisthanella* spec., ±1 mm (Mesostomidae)—(**H**) *Microdalyellia* spec. 1–3 mm (Dalyelliidae)—(**I**) *Temnocephala* spec. ±10 mm (Temnocephalida: in the Temnocephalida the number of tentacles ranges from 2 to 10)—(**J**) *Opistocystis goettei* ±2 mm (Eukalyptorhynchia) Abbreviations: bc: bursa copulatrix, br: brain, co: copulatory organ, eg: egg (in uterus), ev: excretory vessel, ex: excretory canal (protonephridium), ey: eye, fp: female pore, gp: common male and female genital pore, in: intestine, m: mouth, mp: male pore, oc: ovocyte, ov: ovary, ph: pharynx, pr: proboscis, pv: prostate vesicle, rh: rhabdite tracks, sc: statocyst, sp: sensory pits, st: stylet, sv: seminal vesicle, te: testis, vc: vitellocyte, vi: vitellarium

Table 1 Number of species recorded in the various biogeographical regions

Taxon	PA	NA	NT	AT	OL	AU	PAc	ANT	TOT	OBS	>1
Acoela	2								2	2	0
Catenulida	36	36	45	10	1	1			90	129	30
Macrostomida	43	26	3	14	2	1			84	89	5
Polycladida					1				1	1	0
Lecithoepitheliata	20	4	4	3		3		1	31	35	2
Proseriata[a]	6	1	3	1				1	11	12	1
Prolecithophora	12	2			1	1			12	12	0
Dalyellioida	98	28	25	13	1	3			159	168	10
Typhloplanoida	233	56	13	19	4	10		1	307	336	26
Temnocephalida	18		20	1	3	56			98	98	0
Kalyptorhynchia	82	2	1	1	1	1			82	88	2
Tricladida	238	66	36	23	23	40	2	3	426	431	3
Total	788	221	150	85	36	116	2	5	1,303	1,404	79
% obs. of total obs.	56.2	15.8	10.7	6.1	2.6	8.3	0.1	0.4	–	–	5.6

TOT: number of species; OBS: total number of observations of those species; >1: number of species observed in more than one region

[a] Including *Bothrioplana semperi* Hofsten, 1907. PA: Palaearctic; NA: Nearctic; NT: Neotropical; AT: Afrotropical; OL: Oriental; AU: Australasian; PAc: Pacific & Oceanic Islands; ANT: Antarctic

campaigns (see Marcus, 1955; Young, 1976); virtually nothing is known of the Oriental region, except some records of triclads, one prolecithophoran and the only known freshwater polyclad, *Limnostylochus borneensis* (Stummer-Traunfels, 1902); of the Australian region only the Temnocephalida and Tricladida are relatively well known (see Sewell & Cannon, 1998; Sluys & Kawakatsu, 2001). In some areas almost only triclads have been studied, as in Japan by Kawakatsu and the Japanese "school" (cf. Kawakatsu, 1991).

The number of species known today in each region is listed in Table 1 and Fig.2 (following the traditional taxonomy: see below). Questionable species, i.e. species we consider insufficiently described or impossible to identify with the existing data, are not included in the counts.

Of the 1,403 records of turbellarian species, 56% were in the Palaearctic, 16% in the Nearctic and 28% in the rest of the world. All together 1,303 different species were recorded. Only 79 species were observed in more than one region, representing 5.6% of the observations and 6.1% of the species. Of those, 16 have been found in three or more regions, 10 of which are Catenulida, difficult to identify for various reasons.

The number of representatives of each genus ever found in each region is given in Table 2 and Fig.2. Species of 181 genera, or 46%, occur in the Palaearctic, 16% in N. America and 37.5% in the rest of the world. To classify the Palearctic species, taxonomists need one genus for every 5.8 species, in North America 4.7 species/genus, in the Neotropic area 4.5 species/genus, 4 in Australia, but one genus for every 3 species in Africa and even less in the other regions. This is of course due to the fact that completely new organisation types are found in those areas which have been studied the least, and the more species get known, the few genera are "needed" and "created" to contain these species. This puts a strong bias in the conclusions when numbers of genera are used as a measurement for biodiversity. Interesting considerations about the pitfalls of measuring biodiversity-using categories above the species level (taxonomic surrogacy) can be found in Bertrand et al. (2006).

Phylogeny

The first comprehensive phylogenetic approach to platyhelminth relationships, based on morphological characters (including ultrastructure) and life histories, was published by Ehlers (1985). The old turbellarian "orders" and "suborders" are now at the same "level" as the former parasitic "classes" (Fig. 3), but

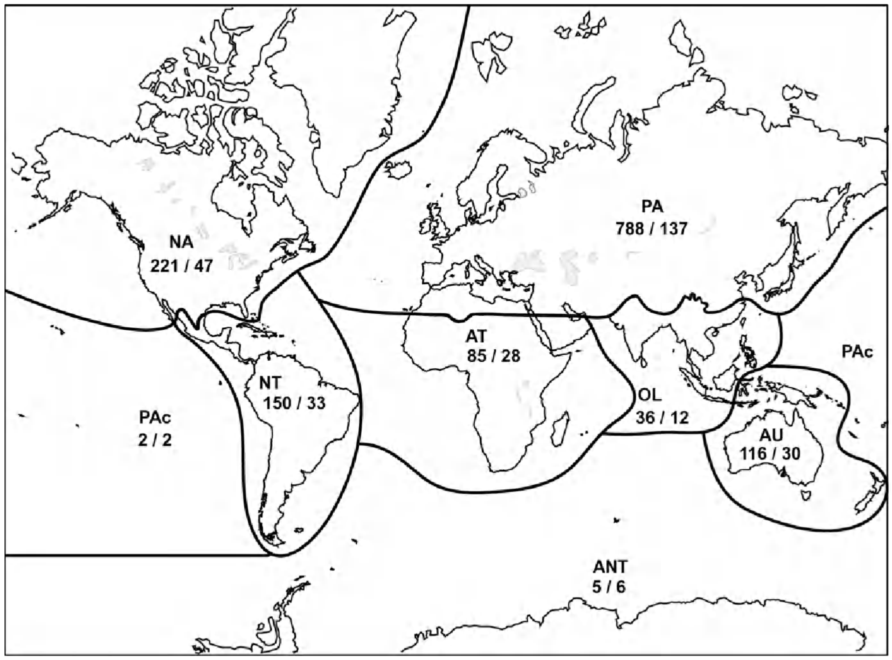


Fig. 2 Species and genus distribution of freshwater platyhelminth per zoogeographic region (species number/genus number). PA—Palaearctic, NA—Nearctic, NT—Neotropical, AT—Afrotropical, OL—Oriental, AU—Australasian, Pac—Pacific & Oceanic Island, ANT—Antarctic

Table 2 Number of genera of which representatives were recorded in the various biogeographical regions

Taxon	PA	NA	NT	AT	OL	AU	PAc	ANT	TOT	OBS
Acoela	2								2	2
Catenulida	9	6	8	5	1	1			10	30
Macrostomida	4	2	2	1	2	1			4	12
Polycladida					1				1	1
Lecithoepitheliata	3	2	3	2		1		1	3	12
Prolecithophora	5	1			1	1			8	8
Proseriata[a]	5	1	3	1				1	9	11
Dalyellioida	14	6	3	4		3			16	30
Typhloplanoida	37	15	6	10	2	2		1	42	73
Temnocephalida	5		1	1	1	10			15	18
Kalyptorhynchia	20	2	1	1	1	1			20	26
Tricladida	33	12	6	3	3	10	2	3	51	72
Total	137	47	33	28	12	30	2	6	181	295
% obs. of total obs.	46.4	15.9	11.2	9.5	4.1	10.2	0.7	2.0	–	–
# species observed	788	221	150	85	36	116	2	5	1303	1404
# species/# genera	5.8	4.7	4.5	3.0	3.0	3.9	1.0	0.8	7.2	–

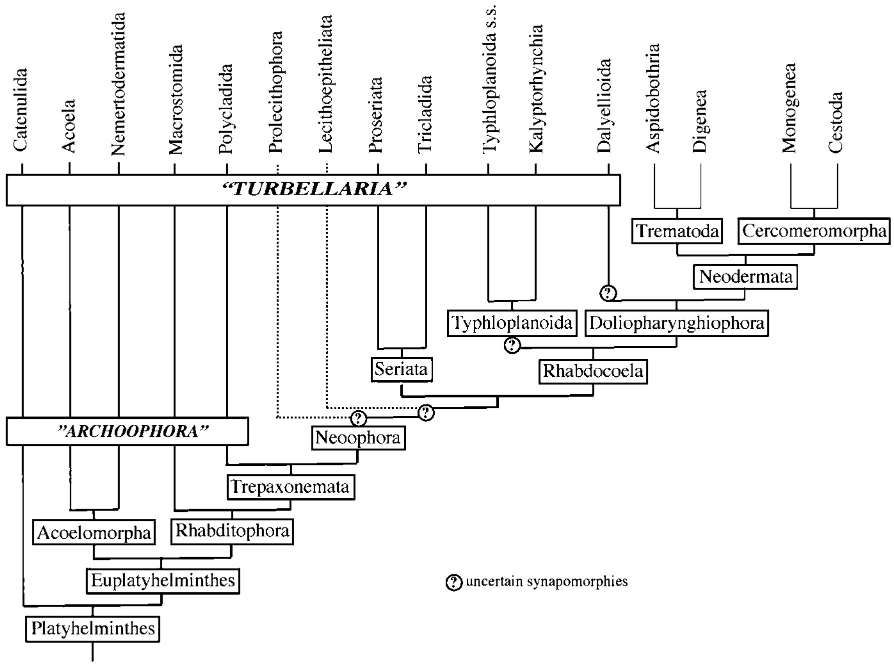
TOT: number of genera; OBS: total number of observations of those genera; >1: number of genera observed in more than one region. PA: Palaearctic; NA: Nearctic; NT: Neotropical; AT: Afrotropical; OL: Oriental; AU: Australasian; PAc: Pacific & Oceanic Islands; ANT: Antarctic

[a] Including *Bothrioplana semperi* Hofsten, 1907

some important uncertainties remained. In Table 1 we have used these "classic" taxa since these are the names found in the existing literature.

New views on flatworm phylogeny are being developed, based on DNA-sequences. The Platyhelminthes may not be monophyletic and the

Fig. 3 Phylogenetic relationships of the major platyhelminth taxa according to Ehlers (1985)



Acoelomorpha may not belong to the same clade as the Catenulida + Rhabitophora (see Ruiz-Trillo et al., 2004 and references therein). However, since only two species of acoels have been found in continental waters, we are not considering this matter further.

The phylogenetic relationships of the Platyhelminthes, as they seem to emerge from molecular data, are represented in Fig. 4. This cladogram is a combination of the cladograms of several authors who used various methods of tree building and to assess the support of the clades. Of the marine Retronectidae and Gnosonesimida there are currently no sequences known, and the monophyly of the Catenulida and of the Lecithoepitheliata, as they are defined today on morphological grounds, is still to be confirmed. The monophyly of the freshwater representatives in both these taxa is, however, highly supported. The support for the taxon Trepaxonematida is rather weak and the sistergroup of the Neodermata is still unclear, but is certainly not the "Dalyellioidea", a taxon that even does not seem to exist! The taxon "Typhloplanoidea" does not seem to exist either, and the representatives of these two old groups are distributed over a number of new taxa. Despite remaining questions about flatworm phylogeny, some conclusions on the history of the freshwater turbellarians can be made.

The taxa in bold in the top row in Fig. 4 are all monophyletic and exclusively found in continental habitats. Five out of these six taxa have representatives on all continents, suggesting that their ancestors invaded Pangea before it broke up. There have been at least four major and independent invasions of freshwater: (1) the freshwater Catenulida (i.e. all catenulids except the Retronectidae), (2) the Prorhynchida, (3) the Continenticola (=Tricladida Paludicola + Tricladida Terricola) and (4) Dalyelliidae + Typhloplanidae + Temnocephalida (if their common ancestry is confirmed; if not, then there must have been one or two more independent invasions). The Temnocephalida, ecto-symbionts on crayfish and atyid shrimps, considered a Gondwana taxon by Cannon & Joffe (2001), may have originated later than the other two taxa, but probably not from brackish water "dalyellioids", since these marine species are members of the Neodalyellida which have no close relationship with the Temnocephalida.

The invasion history of the Macrostomida cannot be deduced from this cladogram yet. All freshwater Macrostomida are members of the Macrostomidae and Microstomidae. However, *Macrostomum* and *Microstomum* species are found almost equally abundant in marine and in freshwater habitats and

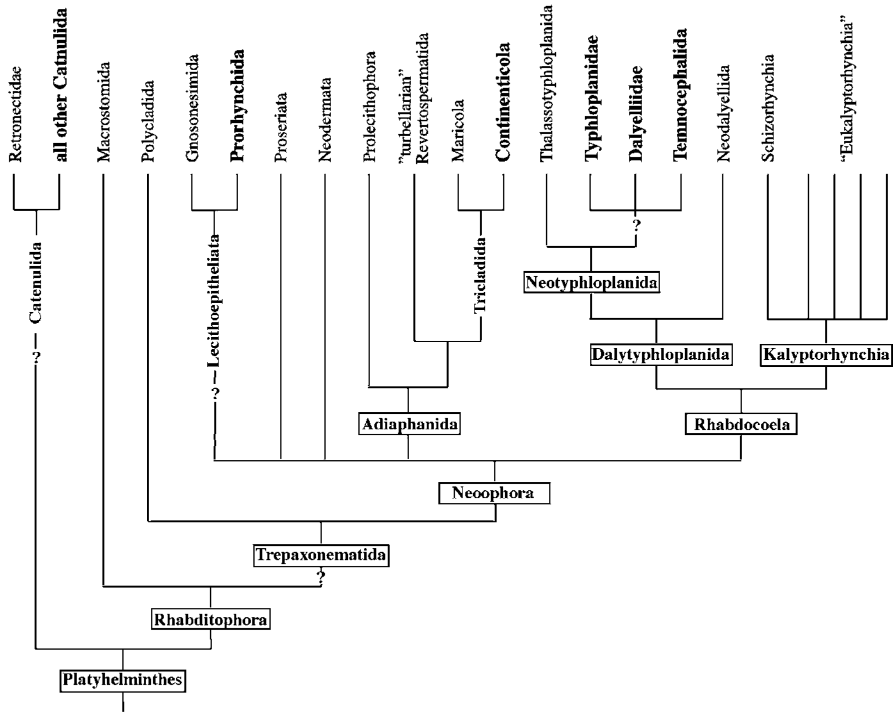


Fig. 4 Phylogenetic relationships of the Platyhelminthes as they appear from 18S rDNA. All named clades are strongly supported, except where indicated by "?". (combined from Baguna et al., 2001; Joffe & Kornakova, 2001; Norén & Jondelius, 2002; Willems et al., 2006)

on all continents. Also the biogeographic history of the freshwater "Eukalyptorhynchia" remains unresolved: representatives of the different "families" (as they are defined today) occur in freshwater.

In the other taxa with representatives in freshwater, independent invasions must have occurred: one single species of Polycladida, some Thalassotyphloplanida and Neodalyellida, and 11 species of Proseriata with different species in the different parts of the world. They all occur in the freshwater zone of rivers and canals connected to the sea.

Conclusions

The number of freshwater flatworms known in the various regions reflects the scientific activity of the past. Although relatively many scientists have been active in Europe, there are still many areas and habitats that have been sampled very poorly, such as the temporary waters around the Mediterranean Sea. Except for lake Baikal and environs, only some old and very scattered data are available for Asia. With these facts in mind, and making a very cautious estimate, the number of species in the Palaearctic must be about 5–10 times higher than known today. Is it realistic then, that only 200–100 or even fewer species occur in the other regions? Certainly not, and without exaggeration it can be said that the number of species of freshwater flatworms is at least one magnitude larger than what is known today.

In view of the above, it is evident that on the basis of such scant and unbalanced information, not many considerations can be made on the distribution of species and higher taxa. Nor can areas with high species richness or endemism be indicated, except perhaps the Baikal Lake with its high sympatric speciation processes.

More sampling in the different parts of the world and further phylogenetic analyses will certainly tell us more about the distribution and the origin in time and space of the freshwater Platyhelminthes. More (young) zoologists should therefore be trained to identify and to describe flatworms. Turbellarians have the reputation to be "difficult" to identify, but they are not, provided one knows how to study them.

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