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The phylogeny of the 'Typhloplanoida' (Platyhelminthes) inferred from 18S ribosomal DNA sequences

Fylogenie van de 'Typhloplanoida' (Platyhelminthes) op basis van 18S ribosomale DNA sequenties

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Wim WILLEMS

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Wim Willems
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INTRODUCTION

THE WORMS: SHORT HISTORICAL AND TAXONOMICAL OVERVIEW

This study deals with the taxon Typhloplanoida Bresslau, 1933, representatives of which occur in both marine and freshwater environments or even on land, and comprises about 450 species. The name Typhloplanoida is used in two different meanings in literature. Traditionally the taxon Typhloplanoida does not comprise the Kalyptorhynchia Graff, 1905 (see Bresslau, 1933). However, Ehlers (1985) in his seminal work on platyhelminth phylogeny, includes the Kalyptorhynchia within the Typhloplanoida. In this work the name is used in its strictest sense cf. Bresslau (1933), unless it is followed by s.l., indicating that the name is used in its broader sense (cf. Ehlers, 1985).

Currently, eight 'families' of Typhloplanoida are recognised: Byrsophlebidae Graff, 1905, Carcharodopharyngidae Bresslau, 1933, Ciliopharyngiellidae Ax, 1952, Kytorhynchidae Rieger, 1974, Promesostomidae Den Hartog, 1964, Solenopharyngidae Graff, 1882, Trigonostomidae Graff, 1905 (sensu Den Hartog, 1964) and Typhloplanidae Graff, 1905. However, the monophyly of each of them is highly questionable. It is out of the scope of this introduction to give an overview of all species and all possible references on these taxa, and only information pertaining to this work will be given here. As the interrelationships between and within these taxa are unclear, we will treat them in the above mentioned order. An exception is made for the Trigonostomidae, which are treated together with the Promesostomidae for reasons of clarity. Of each of the above mentioned 'families' we will indicate whether 18S rDNA data were available at the start of our PhD.

The relatively small taxon Byrsophlebidae is reviewed by Luther (1936) and more extensively by Karling (1985), who gives an overview of the 10 species known at that time (and an additional unidentified one). Karling (1985) based his detailed morphological account on sectioned material of *Byrsophlebs graffi* Jensen, 1878, *B. lutheri* (Marcus, 1952) Karling, 1985, *Maehrenthalia agilis* (Levinsen, 1879) Graff, 1905, *Maehrenthaliella intermedia* (Graff, 1882) Karling, 1985 and *Parabyrsophlebs coeca* (Luther, 1948) Karling, 1985. Based on this study, Karling (1985) erects two new genera, *Maehrenthaliella* and *Parabyrsophlebs*, and transfers all but one species out of *Maehrenthalia* Graff, 1905 to *Byrsophlebs* Jensen, 1878. Since then, only one new species (*Maehrenthalia americana* Ax and Armonies, 1990) has been described and one other undescribed species has been reported (see Ax and Armonies, 1987, 1990). All representatives are characterised by separate male and female genital pores (Karling, 1985), a feature which is quite rare within rhabdocoels. Molecular data of only one species of Byrsophlebidae, *M. agilis*, are available. Its 18S rDNA sequence is included in Norén & Jondelius (2002).

The family Carcharodopharyngidae contains only one species, *Carcharodopharynx arcanus* Reisinger, 1924, and is characterised by the presence of spines in the tubular prepharyngeal cavity (Reisinger, 1924; Luther, 1963). Only very few individuals of this species were ever observed and no detailed descriptions on the organisation of its genital system are available. The species' relationships with other rhabdocoel taxa are unclear and further study on its anatomy is certainly needed, which will not be straightforward since it appears to be rare, only occuring in muddy habitats in woods of Austria and Finland.

Another enigmatic taxon is the 'family' Ciliopharyngiellidae, which includes only two species, *Ciliopharyngiella intermedia* Ax, 1952 and *C. constricta* Martens & Schockaert, 1981, almost identical in their overall anatomy, but clearly distinguishable by the structure of their stylet (Martens & Schockaert, 1981). Based on the organisation of the female gonads, Ax (1952a) placed it within the Proseriata. Later, Ehlers (1972) placed it within the 'Typhloplanoida', mainly based on the organisation of the male genital system and the structure of the pharynx, which resembles that of some solenopharyngids (see Ehlers, 1972). Also ultrastructural data give contradictory information (Brüggeman, 1985; Sopott-Ehlers, 1997, 1999, 2001) and its position within the Rhabdocoela is still heavily debated.

In 1974 Rieger erected the new typhloplanoid 'family' Kytorhynchidae, which comprises six new species and two unnamed ones, all of them from the northern Atlantic (mainly USA coast). A seventh species *Neokytorhynchus pacificus* Ehlers & Ehlers, 1981 occurs in the Galapagos (Ehlers & Ehlers, 1981) and an unidentified species was collected in Somalia (Schockaert & Martens, 1985). All representatives are characterised by the presence of a rostroterminal invagination, which is clearly different, in both structure and place, of the invagination of some other typhloplanoid representatives (e.g. species of *Trigonostomum* Schmidt, 1852). Moreover, Rieger (1974) suggested the possibility of this invagination to be homologous with the muscular proboscis of the Kalyptorhynchia.

The two largest taxa of marine typhloplanoids are the Promesostomidae Den Hartog, 1964 and the Trigonostomidae Graff, 1905 (sensu Den Hartog, 1964), with about 70 and 90 species respectively. Both were originally considered a single 'family' (e.g. Luther, 1943, 1948), but based on the structure of the female genital system Den Hartog (1964) split the Trigonostomidae Graff, 1905 into Promesostomidae and Trigonostomidae. For the time being, this view is adopted here.

The taxon Promesostomidae originally comprised two 'subfamilies' (see Den Hartog, 1964): Brinkmaniellinae Luther, 1948 and Promesostominae Luther, 1948. A third one, Adenorhynchinae Ax & Heller, 1970, was added later (Ax & Heller, 1970), whereas several taxa (*Paraproboscifer* De Clerck, 1994, *Gaziella* De Clerck & Schockaert, 1995 and *Vauclusia* Willems et al., 2004a) were placed as incertae sedis within the Promesostomidae (see De Clerck, 1994; De Clerck & Schockaert, 1995; Willems et al., 2004a). The monophyly of the Promesostomidae and its subtaxa is highly questionable and their diagnostic characters (see Den Hartog, 1964; Ax & Heller, 1970) are considered to be plesiomorphies (e.g. Karling et al., 1972; Ehlers, 1974; Rieger, 1974; Ehlers & Ehlers, 1981; Armonies & Hellwig, 1987; De Clerck, 1994). Major contributions on Promesostomidae taxonomy, among others, are by Armonies & Hellwig (1987), Ax (1951, 1952c, 1954, 1956a-b, 1959, 1968, 1993, 1994, 1995b), Ax & Ehlers (1973), Ax & Heller (1970), Ehlers (1974, 1980), Ehlers & Ehlers (1981), Ehlers et al. (1994), Ehlers & Sopott-Ehlers (1989a), Luther (1943, 1948), Karling (1967, 1986), Karling et al. (1972), Martens & Schockaert (1981), Marcus (1951, 1952, 1954), Riedl (1954, 1956), Schockaert & Martens (1985) and Willems et al. (2004a, In press a-b).

The Trigonostomidae (sensu Den Hartog, 1964) is characterised by the fact that an efferent and an afferent duct are present in the female system, the so-called double connection. It consists of three subfamilies: Mariplanellinae Ax & Heller, 1970, Paramesostominae Luther, 1948, and Trigonostominae Luther, 1948. The monophyly of the Trigonostomidae and its 'subfamilies' was put in doubt by Luther (1950), Karling et al. (1972) and Karling & Mack-Fira (1973). Major contributions on trigonostomid taxonomy, among others, are by Artois et al. (2000), Ax (1951, 1952b, 1956a, 1959, 1960, 1971, 1995a), Ax & Heller (1970), Den Hartog (1964, 1965, 1966a-b), Ehlers (1974, 1980), Ehlers & Ax (1974), Ehlers & Sopott-Ehlers (1989a), Karling (1978, 1986), Karling et al. (1972), Karling & Mack-Fira (1973), Luther (1943, 1948, 1950, 1962), Riedl (1954) and Willems et al. (2004b, In press a-b). Current molecular data (18S rDNA) for the Trigonostomidae are available for three species: *Mariplanella frisia* Ax & Heller, 1970 (e.g. Littlewood et al., 1999a-b, Norén & Jondelius, 2002), *Trigonostomum penicillatum* (Schmidt, 1857) Micoletzky, 1910 and *Astrotorhynchus bifidus* (McIntosh, 1874) Graff, 1905 (both only in Norén & Jondelius, 2002).

Species of the Solenopharyngidae are characterised by the presence of an elongated pharynx. It is not completely clear whether this pharynx is of the bulbosus-type, because of the presence of very large extracapsular glands, which are normally absent in a pharynx bulbosus. The taxon was reviewed for the first time by Beklemischew (1929) and again by Ehlers (1972), who studied the 12 known species and added another seven. Since then, only four new species were described: *Trisaccopharynx roscoffensis* Ehlers, 1980, *Lenopharynx tubatus* Schockaert & Martens, 1985, *Pilamonila bimascula* Willems et al., 2004 and *Austradenopharynx reynaertsi* Willems et al. (In press). This taxon probably contains much more species, as we found several unknown species during almost all of our sampling expeditions. However, of most of them we only found one or very few individuals and this limited number of individuals, together with their very small size, did not allow formal description. Ehlers (1972) subdivided the taxon in three 'subfamilies' (Anthopharynginae Ehlers, 1972, Lenopharynginae Ehlers, 1972 and Solenopharynginae Ehlers, 1972), which are probably based on plesiomorphic characters (already recognised by Ehlers, 1972). As is shown by the description of *A. reynaertsi* (see Willems et al., In press b) and especially *P. bimascula* (see Willems et al., 2004a), which could not be placed in the current system, a thorough analysis of the taxon based on the study of both known and new organisation types, and critically judging all proposed synapomorphies by Ehlers (1972) is necessary. References to older literature on solenopharyngid species can be found in Beklemischew (1929) and Ehlers (1972).

All typhloplanoid taxa discussed above only comprise marine or brackish water species. The most species-rich taxon within the 'Typhloplanoida', however, consists almost excusively of freshwater species, which are grouped in the 'family' Typhloplanidae. This taxon contains more than 250 known species and its representatives are characterised by the presence of a single ovary. Many species lack hard parts in the male system, which hampers identification. Moreover, in many cases literature concerning species of Typhloplanidae is very old and not very detailed. Luckily enough the works by Graff (1882, 1913) and Luther (1904, 1963) give a comprehensive overview of most of the older literature on the Typhloplanidae (and the other 'families'). Important more 'recent' contributions on Typhloplanidae taxonomy, among others, are by Artois et al. (2004), Ax (1954, 1956a, 1959, 1960), Ehlers & Sopott-Ehlers (1989b), Ferguson & Hayes (1941), Gilbert (1935), Hochberg (2004), Hochberg & Cannon (2002, 2003), Kolasa (1976), Kolasa & Schwartz (1988), Luther (1904, 1946, 1948, 1950), Noreña-Janssen & Faubel (1992) and Willems et al. (In press a). The taxon is subdivided into eight 'subfamilies' by Luther (1963): Ascophorinae Findenegg, 1924, Mesostominae Bresslau, 1933, Olisthanellinae Bresslau, 1933, Opistominae Luther, 1963, Phaenocorinae Wahl, 1910, Protoplanellinae Bresslau, 1933, Rhynchomesostominae Bresslau, 1933, Typhloplaninae Bresslau, 1933. This subdivision of the Typhloplanidae is based on the location of the protonephridia opening and the position of testes and vitellaria, relative to each other (Luther, 1904, 1963). The taxonomic value of these features, which are often very difficult to assess, was already put into doubt by Luther himself (1948). Also several marine taxa were placed within the Typhloplanidae because they have only one ovary (e.g. Luther, 1946; Ax, 1959, Ehlers & Sopott-Ehlers, 1989a). Furthermore, they were placed within the Typhloplaninae as they did not share any of the diagnostic characters with representatives of the other 'subfamilies'. One additional 'subfamily', Cephalopharynginae, was erected by Hochberg (2004), who also gives a cladistical analysis for the Typhloplanidae as a whole. This analysis, however, suffers from a number of shortcomings. Higher-level taxa of the 'family-level' were used as entries in the datamatrix. In this case this is fairly problematic since none of these taxa is proven to be monophyletic, as no prior analyses are available. Furthemore, a very limited number of morphological characters is used (11 in total), all of them from literature. Of at least three characters the phylogenetic value was already seriously doubted by Luther (1948) and Papi (1959). This analysis also suffers from some methodological flaws, including bootstrapping on morphological data (see Sanderson, 1995) and the use of the majority method (i.e. if most members of the family, incl. the type, exhibit a certain character state, that state is scored for the whole 'family'). It is clear that the Typhloplanidae is in great need of taxonomic revision, evaluating all species for their validity and reconsidering morphological characters. For the Typhloplanidae, 18S rDNA sequences are available for four species: *Bothromesostoma personatum* (Schmidt, 1848) Fuhrmann, 1894, *Bothromesostoma* spec., *Mesocastrada* spec. and *Mesostoma lingua* (Abildgaard, 1789) Schmidt, 1848 (e.g. in Katayama et al., 1996; Turbeville et al., 1992; Riutort et al., 1992; Carranza et al., 1997; Littlewood et al., 1999a-b; Norén & Jondelius, 2002).

THE WORMS: SYSTEMATIC POSITION

To understand the systematic position of the Typhloplanoida, the best starting point is Ehlers' (1985) seminal work on the Platyhelminthes (or Plathelminthes: see Ehlers & Sopott-Ehlers, 1995), which was further refined (and contradicted) by several authors, mostly using molecular data (e.g. Riutort et al., 1992, 1993; Rohde et al., 1993; Litvaitis et al., 1994; Katayama et al., 1996; Carranza et al., 1997; Campos et al., 1998; Jondelius, 1998; Littlewood et al., 1999a-b; Litvaitis & Rohde, 1999; Baguñà et al., 2001a; Littlewood & Olson, 2001; review in Baguñà & Riutort, 2004).

In Ehlers' (1985) work, which is mainly based on morphological (incl.ultrastructural) data, the Platyhelminthes are studied for the first time from a cladistical point of view and the main conclusion is that the 'Turbellaria' are paraphyletic, whereas the parasitic groups are united in a clearly monophyletic Neodermata, based on the presence of a neodermis (see Fig. 1). This view is confirmed by recent phylogenetic studies (e.g. Katayama et al., 1996; Carranza et al., 1997; Littlewood et al., 1999a-b; Joffe & Kornakova, 2001; Littlewood & Olson, 2001; Zamparo et al., 2001; Lockyer et al., 2003). Recent evidence even suggests that the Platyhelminthes as a whole is polyphyletic, the Acoela and Nemertodermatida being basal bilaterians (Zrzavy et al., 1998; Ruiz-Trillo et al., 1999, 2002, 2004; Baguñà et al., 2001b; Jondelius et al., 2002; Telford et al., 2000, 2003; but see Tyler (2001) for an alternative view). The Rhabditophora and the Catenulida are found as possible sister-groups within the Lophothrochozoan clade (see review in Baguñà & Riutort, 2004).

In Ehlers' (1985) system the 'Typhloplanoida', the 'Dalyellioida' Bresslau 1933 and the Neodermata are included in the Rhabdocoela. However, since no apomorphies for neither the 'Typhloplanoida' nor the 'Dalyellioida' could be put forward, both groups were regarded as non-monophyletic and their exact position within the Rhabdocoela was left open. However, modern molecular studies showed that the sister group of the Neodermata is not included within the 'Dalyellioida', nor the 'Typhloplanoida', but probably consists of a large clade containing most of the neoophoran subtaxa (Littlewood et al., 1999a-b; Baguñà et al., 2001a; Joffe & Kornakova, 2001; Littlewood & Olson, 2001; Norén & Jondelius, 2002; Lockyer et al., 2003; review in Baguñà & Riutort, 2004). Therefore, the Rhabdocoela is now used by most authors for a group containing the 'Dalyellioida', 'Typhloplanoida', Kalyptorhynchia and Temnocephalida, which is consistent with its most traditional use (and is a synonym of Neorhabdocoela Meixner, 1938).

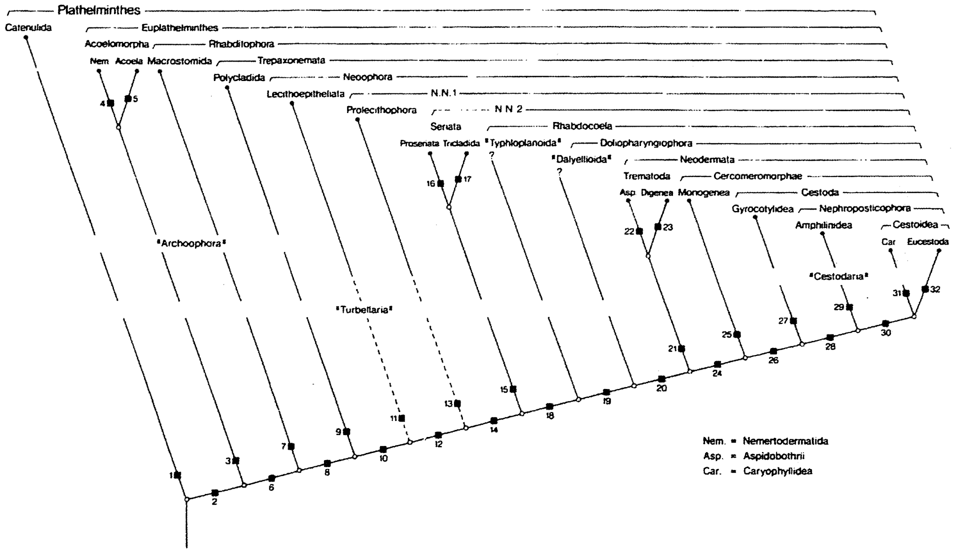

Fig. 1. Cladogram of the Platyhelminthes based on Ehlers (1985).

All molecular studies mentioned above agree on the monophyly of the Rhabdocoela, but the relations within the taxon, however, are far from resolved as only very few rhabdocoel sequences were used (max. 20 in Norén & Jondelius, 2002). Being part of the Rhabdoela, the 'Typhloplanoida' are greatly undersampled in all foregoing 18S rDNA studies. Therefore only some general comments on their relationships within the Rhabdocoela are given in what follows (based on Littlewood et al., 1999a-b; Joffe & Kornakova, 2001; Norén & Jondelius, 2002): 1) The Kalyptorhynchia, traditionally included within the 'Typhloplanoida' (see Ehlers, 1985), are considered monophyletic and they probably form the sister group of all other rhabdocoels. 2) There is no support for a monophyletic 'Typhloplanoida', however, only very few species are included (max. 7 in Norén & Jondelius, 2002). 3) There is some evidence for a freshwater clade containing two freshwater 'families', Dalyelliidae ('Dalyellioida') and Typhloplanidae ('Typhloplanoida') and perhaps the Temnocephalida ('Dalyellioida'). No information as such is available for the relationships within the 'Typhloplanoida', but it is evident from former molecular results that the 'Typhloplanoida' as a whole are probably not monophyletic because some clades contain both former dalyellioid and typhloplanoid species (e.g. Littlewood et al., 1999a; Norén & Jondelius, 2002).

Apart from these molecular studies, two morphological analyses of the Rhabdocoela exist (Jondelius & Thollesson, 1993; Zamparo et al., 2001) in which a number of typhloplanoid taxa are excluded. However, these analyses suffer from a number of methodological flaws and shortcomings, which will be discussed in greater detail in the general discussion. In short, both studies only use a limited number of characters and represent higher-level taxa ('families') by examining only a few of its members, which may bias the analysis. Almost all taxa in the analyses are also not proven to be monophyletic, and therefore cannot be used as terminal taxa in an analysis.

PHYLOGENETIC NOMENCLATURE

In the taxonomical studies (see Appendix: papers I-VII) and during the naming of new clades in our phylogenetic analysis, it became apparent that the Linnean system and with it, the Code of Zoological Nomenclature (International Commission on Zoological Nomenclature - hereafter ICZN - 1999), suffers from several shortcomings if used on the results of a cladistical analysis. These problems arise from both the rank-based hierarchical nature of the Linnean system and its use of a type and a category to define (names of) taxa. These problems were already recognised in the early days of cladistics (Hennig, 1966, 1969; Ghiselin, 1966; Hull, 1966; Griffiths, 1974a-b, 1976) and were further studied in recent years by, among others, Ax, 1984, 1995c; De Queiroz, 1988, 1992, 1997a; De Queiroz & Gauthier, 1990, 1992, 1994; Ereshefsky, 1994, 2001, 2002; Härlin, 1998, 2003; Härlin & Sundberg, 1998; Pleijel & Härlin, 2004. It is not the purpose of this introduction to elaborate on these problems; to name just a few: redundancy of taxon names, proliferation of categories, lumping and splitting of taxa. We refer to the above mentioned literature (and the references therein) and the intelligible introduction on phylogenetic nomenclature by Artois (2001; unpublished PhD-thesis).

An alternative, which abandons categorical ranks and in which taxon names are defined by reference to a phylogenetic tree, has been worked out by De Queiroz& Gauthier (1990, 1992, 1994), some of its principles already stated earlier (e.g. Griffiths, 1974a-b, 1976; Ghiselin, 1984; Rowe, 1987; de Queiroz, 1988). The rules of this system have been formalised in a draft code, the Phylocode (available at http://www.ohio.edu/phylocode), of which the official implementation is tentatively scheduled for 2006 (see Laurin & Cantino, 2004). Phylogenetic nomenclature has been criticized by several authors since its proposal, but even more since the release of the draft version of the Phylocode in spring 2000 (e.g. Lidén & Oxelman, 1996; Lidén et al., 1997; Dominguez and Wheeler, 1997; Benton, 2000; Jørgensen, 2000; Nixon & Carpenter, 2000;

Stuessy, 2000, 2001; Forey, 2001, 2002; Berry, 2002; Blackwell, 2002; Keller et al., 2003; Schuh, 2003). However, most of these critiques, if not all, were rebutted or shown to be based on misconceptions about the PhyloCode (see Lee, 1996a, 1999a-b, 2001; Schander, 1998; de Queiroz, 1997b, 2000; Cantino, 2000; De Queiroz& Cantino, 2001a-b; Bryant & Cantino, 2002; Pleijel & Rouse, 2003; Pleijel & Härlin, 2004). Moore (1998) and Langer (2001) provide various arguments for and against phylogenetic nomenclature. A good overview of studies, which applied PhyloCode principles experimentally, is given by Pleijel and Rouse (2003).

The most important difference between the Phylocode and the current taxonomical codes (Lapage et al., 1992; ICZN, 1999; Greuter et al., 2000) is the use of explicit definitions of clade names in terms of common descent, i.e. names are explicitly applied to a common ancestor and its descendants (Ghiselin, 1995), in contrast to the implicit definitions under the current codes (see De Queiroz & Cantino, 2001b), which take the form 'the family A-idae is the taxon of the family-level with A-us as its type'. De Queiroz& Gauthier (1990) originally proposed three ways to define a name phylogenetically (see Fig. 2).

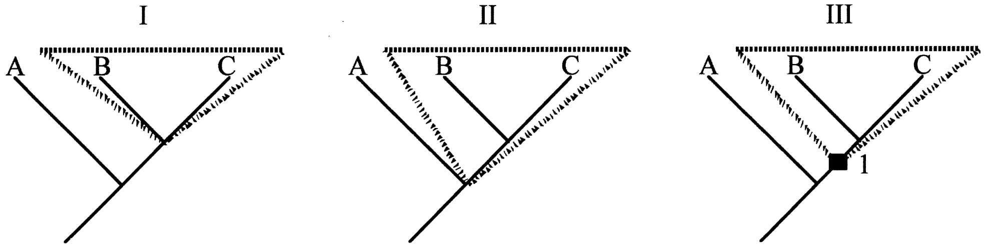

Fig. 2. Phylogenetic definitions. I. Node-based definition. II. Stem-based definition. III. Apomorphy-based definition.

Node-based definitions take the form 'the most recent common ancestor of A and B and all of its descendants', or without specifically referring to a common ancestor: 'the least inclusive clade containing A and B'. Stem-based definitions are formulated as 'all species sharing a more recent common ancestor with A than with C' or 'the most inclusive clade containing A but not C'. The third type, apomorphy-based definitions, take the form 'all species that stem from the first species to possess character 1 synapomorphic with that in A'.

Altough, there are several proponents of the Phylocode who are strongly in favour of this third type of definition (e.g. Lee, 1996b, 2001; Pleijel, 1999; De Queiroz& Cantino, 2001b), it is often argued that apomorphy-based definitions should be avoided because they suffer from several problems (review in Pleijel & Rouse, 2003), e.g. character-coding ambiguity and optimization ambiguity (e.g. Bryant, 1994; Schander & Thollesson, 1995; Sereno, 1999; Forey, 2001, 2002). Furthermore, if apomorphies are to be used in phylogenetic definitions, one should have a list of apomorphies available. This is generally not the case if the phylogeny on which the names will be based is the result of the analysis of molecular data.

It is argued by Schander & Thollesson (1995) that stem-based definitions can be ambiguous and refer to non-existing clades. This is, however, only the case if more than one internal specifier is used and thus can easily be circumvented by using only one internal specifier as pointed out by Lee (1996a). It is furthermore argued that stem-based definitions are especially useful when the sister group relationship of the taxon is known, but its internal relationships are unresolved (Sundberg & Pleijel, 1994; Schander, 1998). Another reason for using stem-based instead of node-based definitions is the fact that with stem-based definitions new species that, after analysis, appear at the base of the clade, are automatically included.

Given the lack of morphological data, the lack of clear apomorphies, the poorly supported internal relationships, the high number of new species and high probability of discovering new taxa, stem-based definitions are the most appropriate type of definition to use within the studied group.

OBJECTIVES

The main objective of this study is to elucidate the relationships of and within the 'Typhloplanoida', based on molecular data. Therefore a large number of species currently placed within the 'Typhloplanoida' and the Kalyptorhynchia, of which the sister taxon could be a part of the typhloplanoids, were collected, whereas also other taxa ('Dalyellioida' and Proseriata) were sampled, initially to function as possible outgroups. Additional molecular data from GenBank of both typhloplanoid and outgroup taxa were used to complete the molecular datamatrix. The major questions that we tried to answer were the following:

1. Is the 'Typhloplanoida' monophyletic?
2. Is there a 'freshwater clade' consisting of the Typhloplanidae ('Typhloplanoida') and the Dalyelliidae ('Dalyellioida')? What is their relationship with the third (symbiotic) freshwater taxon, the Temnocephalida?
3. What are the relationships of the various typhloplanoid 'families'?
4. What is the position and the sister taxon of the Kalyptorhynchia?
5. What is the position of the enigmatic taxon *Ciliopharyngiella* Ax, 1952?

The description of new taxa found during the collection of taxa for DNA analysis is an important secondary objective, as data on new species can alter our insights into the phylogeny of a whole group.

Originally this study was started with the idea to combine morphological and molecular data, using a total-evidence approach, as advocated by Littlewood et al. (1998). However, during preliminary work we encountered two major problems, which hampered the compilation of a morphological data matrix. As the 'Typhloplanoida' is a very large taxon, grouping species which are enormously diverse in the construction of their genital system, it is extremely difficult to make the primary homology assessments, a problem that is even magnified when outgroups are considered (e.g. Proesriata). Furthermore, data on the detailed internal organisation of a large number of species are lacking (e.g. *Carcharodopharynx arcanus;* a vast number of representatives of the Typhloplanidae) and literature is in many cases insufficient. Secondly, material of a large number of typhloplanoid species is unavailable or does not exist.

THE METHODS

This section deals with the methodology used in the phylogenetic analysis of the 'Typhloplanoida'. One method in particular, Bayesian inference, deserves a detailed explanation, as it is very complex and as it is only very recently used within the discipline of systematics, but is already very popular (see for instance Shoemaker et al., 1999; Lewis, 2001; Huelsenbeck et al, 2000, 2001; Archibald et al., 2003; Holder & Lewis, 2003).

Following the section on Bayesian analysis, all other methods, including collection of animals, PCR-protocols, etc. are given.

BAYESIAN INFERENCE

Bayesian analysis is a statistical technique wich is only recently applied into the field of phylogenetic systematics and therefore its properties are not discussed in literature as detailed as for instance for the parsimony and the maximum-likelihood technique. In 1996 the idea of using Bayesian inference in phylogenetic studies was proposed independently by three groups (Rannala & Yang, 1996; Mau, 1996; Li, 1996). The basics of the method were explained in several papers in the following years (Mau and Newton, 1997; Yang and Rannala, 1997; Larget and Simon, 1999; Mau et al., 1999; Newton et al., 1999; Li et al., 2000). The foundation of Bayesian statistics is formed by a formula, which is called Bayes' theorem:

$$P[H \mid D] = \frac{P[D \mid H] \text{ x } P[H]}{P[D]}$$

in which $P[H \mid D]$ is the posterior probability (i.e. the probability of the hypothesis given the data), $P[D \mid H]$ is the likelihood (i.e. the probability of the data given the hypothesis), $P[H]$ is the prior probability (i.e. the unconditional probability of the hypothesis) and $P[D]$ is the unconditional probability of the data, which can be obtained by calculating the sum of the product $P[D \mid H]$ x $P[H]$ over all hypotheses ($=\Sigma_H P[D \mid H] \text{ x } P[H]$). This in fact is a normalizing constant which ensures that the sum of the posterior probabilities is 1.

To explain this terminology and what can be calculated with this formula a very simple example is given. Suppose someone is tossing coins. He knows that 10% of the coins are biased to turn up heads 80% of the time, and therefore 90% of the coins is true (i.e. not biased). Suppose the person is asked to blindly pick a coin and then asked to answer the question what the probability is that his coin is a biased one. Since the only information he has, is that 10% of the coins are false, his obvious answer will be 0.1, but if he is allowed to toss the coin 5 times and then asked to answer the same question, he would be able to revise his estimate based on the expected distribution of outcomes from true and biased coins, and the expectation of the initial proportion of true and biased coins. Suppose he observes the following result (with H for heads and T for tails): HHTHH. This result is called X. The likelihood or probability of that result (the data) given that the coin is true (the hypothesis) is:

$$P[X \mid \text{True coin}] = 0.5^5 = 3.125 \text{ x } 10^{-2}$$

The probability of the same result given a biased coin is:

$$P[X \mid \text{Biased coin}] = 0.8^4 \text{ x } 0.2 = 8.192 \text{ x } 10^{-2}$$

Furthermore, prior probabilities of a biased and an unbiased coin are 0.1 and 0.9 respectively. The fact that an unbiased coin has an odds ratio giving heads or tails of 0.5/0.5 and a biased coin of 0.8/0.2 is the model. The posterior probability that the coin is biased, given the data (HHTHH) is given by:

$$P[\text{Biased coin} \mid X] = \frac{P[X \mid \text{Biased coin}] \text{ x } P[\text{Biased coin}]}{(P[X \mid \text{Biased coin}] \text{ x } P[\text{Biased coin}]) + (P[X \mid \text{True coin}] \text{ x } P[\text{True coin}])}$$

or

$$P[\text{Biased coin} \mid X] = \frac{8.192 \text{ x } 10^{-2} \text{ x } 0.1}{(8.192 \text{ x } 10^{-2} \text{ x } 0.1) + (3.125 \text{ x } 10^{-2} \text{ x } 0.9)} = 0.236$$

Similarly, the posterior probability that the coin is unbiased can be calculated:

$$P[\text{Unbiased coin} \mid X] = \frac{3.125 \text{ x } 10^{-2} \text{ x } 0.9}{(3.125 \text{ x } 10^{-2} \text{ x } 0.9) + (8.192 \text{ x } 10^{-2} \text{ x } 0.1)} = 0.774$$

So, we used the observations during coin tossing to update our prior belief having a biased coin from a probability of 0.1 to a probability of 0.236. In other words, given the date it becomes more probable that we have a biased coin. Suppose we tossed the coin 10 times and observed 10 heads, the posterior probability that we tossed a biased coin would be almost equal to 1, completely reversing our prior beliefs. Similarly, another choice of prior could alter the posterior probability dramatically. It is obvious that both the observed data and the prior probability play key roles in Bayes' theorem and especially the choice of a credible prior in most cases is a problem (see further). In most cases no prior information is available and then a so-called flat prior is used, which in fact is nothing more than giving an equal prior probability to all hypotheses. In the above example this would be equal to give the prior a value of 0.5.

How can this very simple problem be translated to the field of phylogenetic inference? First of all, the data are formed by the aligned sequences, whereas all possible trees for this dataset are regarded as the possible hypotheses. From this it should be clear that the calculation of, for instance P[D], becomes very complicated, as it should be calculated as the sum of $P[D \mid H]$ x $P[H]$ over all possible trees. The number of possible rooted bifurcating trees for 50 species for instance is equal to $2.75292 \text{ x } 10^{76}$ (see Felsenstein, 1978a).

Bayes' theorem adapted for calculating the posterior probability of the *i*th phylogenetic tree (T_i) given an alignment of DNA sequences (X) is:

$$f[\mathrm{T_i} \mid \mathrm{X}] = \frac{f[\mathrm{X} \mid \mathrm{T_i}] \times f[\mathrm{T_i}]}{\sum_{\mathrm{j}=1}^{\mathrm{B}(s)} f[\mathrm{X} \mid \mathrm{T_i}] \times f[\mathrm{T_i}]}$$

$$\text{with } f[\mathrm{X} \mid \mathrm{T_i}] = \int_v \int_m f[\mathrm{X} \mid \mathrm{T_i}, v, m] \times f[v, m] \, \mathrm{d}v \, \mathrm{d}m$$

The likelihood ($f[\mathrm{X} \mid \mathrm{T_i}]$) of a certain tree is given by a double integration of the function describing the tree over all combinations of branch-lengths (v) and substitution-model parameter values (m). The function $f[v, m]$ in the likelihood formula is the prior distribution of branch lengths and substitution parameters. The prior for phylogenetic trees ($f[\mathrm{T_i}]$) is usually set to 1/B(s), in which B(s) is the number of all possible trees for s taxa (terminals). This is in fact a 'flat' prior, giving all possible trees an equal prior probability. Other priors are possible (e.g. as in Yang & Rannala, 1997). As in the coin tossing example, the summation in the denominator is over all possible hypotheses. In the case of phylogenetic inference these hypotheses are all trees (B(s)) that are possible for s terminal taxa (formula's in Felsenstein, 1978a). Calculating the posterior probability in this way involves a summation over all possible trees and, for each tree, integration over all combinations of branch-lengths and substitution-model parameter values. Since it is impossible to calculate the posterior probabilities analytically, another statistical method is needed to draw samples from the posterior distribution in such a way that the actual posterior distribution is approximated. The most useful method to achieve this is by drawing a very large number of interdependent samples, a method which is called the Markov Chain Monte Carlo method or MCMC (see Metropolis *et al.*, 1953; Hastings, 1970; reviewed in Gilks et al., 1996). It is out of the scope of this PhD to explain this method in detail, but in short the algorithm starts with a certain tree (mostly chosen ad random). Then a new tree is built up (step 1) by stochastically perturbing the topology of the current tree or one of the parameter values. This new tree is then either accepted or rejected (Step 2) with a probability described by Metropolis *et al.* (1953) and Hastings (1970). When the posterior probability of the new tree is higher than that of the current tree, the new tree is automatically accepted. However, when the posterior probability of the new tree is lower than that of the current tree, the new tree is only accepted when the ratio of the posterior probabilities of the new tree over the current tree is higher than a random number, randomly drawn between 0 and 1. If the new tree is accepted, then the chain starts over again (back to step 1): perturbing the tree topology, a branch length or a substitution parameter. If the new tree is rejected the chain starts over from the original tree. This process is called one generation. A typical Markov chain is run for several millions of generations and sampled every hundred generations. Practically this means that for a chain run for 5,000,000 generations 50,001 trees are sampled. The proportion of the time any single tree is visited during the course of the chain is an approximation of its posterior probability (Tierney, 1994). As the chain is usually started from a point in the distribution with low posterior probability (a random tree), it will take some time before the chain produces a reasonable approximation of the posterior distribution. This initial part of the chain ('burn-in') has to be discarded. A Markov chain can get stuck on a local optimum (a peak in the tree landscape) and is then said to mix poorly. To resolve this problem Geyer (1991) proposed Metropolis coupled MCMC (MCMCMC or $(MC)^3$). In this method several chains are run simultaneously, one of which is a 'cold' chain, the others are 'heated chains'. The 'cold' chain samples from the posterior distribution of interest or 'cold' distribution (= $f[T_i | X]$), whereas the others are sampling from 'heated' distributions (= $f[T_i \mid X]^{\beta}$), where the heat is applied to the *i*th chain with:

$$\beta = \frac{1}{1 + (i\text{-}1) \text{ x } t}$$

in which t is the heating parameter. If $i = 1$ then $\beta = 1$ and $f[T_i \mid X]^{\beta} = f[T_i \mid X]$. This is the 'cold chain'. The effect of the heating is to lower peaks and to fill in valleys in the tree distribution landscape, decreasing the overall difference between both. In this way the 'heated' chains move more readily among the available isolated hilltops (= local optima of the posterior distribution), because the new proposal is very likely to be accepted. At regular intervals the 'cold' chain and one of the 'heated' chains are swapped. The swap is accepted or not using the same Metropolis-Hastings algorhitm, described above. If the swap is accepted the 'cold' chain can leap over a valley to another hilltop. Inference is only based on the cold chain, sampling a large number of trees, which can be summarised in one single majority rule consensus tree (Larget & Simon, 1999). The proportion in which every branch is present in the posterior distribution of the trees is then interpreted as a measure of confidence that the branch is true (but see further). The combination of estimating trees and obtaining measurements of support is a major advantage of the Bayesian approach over other phylogenetic inference methods.

Summarising the methodology of Bayesian inference, the method aims at estimating the posterior probability distribution for a parameter (such as a tree), given the data, a model of character evolution, and prior probabilities on parameters in the model. The prior is then updated in the light of the data to give the posterior updated belief in, e.g., a tree. To achieve this interference is made about a particular parameter in the model (such as the tree), while integrating (marginalizing) over the uncertainty in all other parameters (= marginal estimation).

Bayesian inference can be done using the program MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; available for free from http://morphbank.ebc.uu.se/mrbayes) or others (like BAMBE), which, however, can only implement a limited number of simple substitution models.

The use of Bayesian statistics in phylogenetic inference is still controversial (see Huelsenbeck et al., 2002 for an overview) and the four main problems are:

1) Bayesian inference, as maximum likelihood, makes use of specific models of nucleotide substitution (overview in Swofford et al., 1996: 434) to allow calculation of the probabilities of nucleotide changes that are needed to compute the likelihood. Basically these models for nucleotide substitution consist of a 4x4 rate matrix, in which the elements represent the rate of change from one nucleotide to another. Within these models two types of parameters are included, rate parameters describing the rates of change and frequency parameters, which correspond to the frequencies of the bases A, C, G and T. The most general model available at the moment is the GTR model (general time reversible model; Lanave et al., 1984; Rodríguez et al., 1990), in which 6 rate parameters (one for each possible nucleotide substitution) and 4 frequency parameters are included. All other models are special cases of the GTR model, because there are, for instance only two rate parameters: one for transitions and one for transversions. As for all methods using substitution models, the results of a Bayesian analysis are strongly dependent upon the correctness of the chosen model (see Sullivan & Swofford, 1997; Cunningham et al., 1998; Posada & Crandall, 2001; Buckley, 2002; Suzuki et al., 2002; Erixon et al., 2003; Lemmon & Moriarty, 2004). However, several strategies exist to test which model best fits the data (review in Posada & Crandall, 2001). Likelihood-ratio tests generally outperform other methods (Posada & Crandall, 2001) and can be implemented by the program MODELTEST (Posada & Crandall, 1998; available for free at http://darwin.uvigo.es/software/modeltest.html).

2) As explained earlier the Markov chain is typically started at a random point in tree space, resulting in a very low posterior probability. Therefore it will take time to reach stationarity, which is usually monitored by examining plots of ln likelihood values. These ln likelihood plots are, however, rather unreliable for deciding about convergence to stationarity (Gilks et al., 1996). For example, it is possible that a chain oscillates around what appears to be a stable likelihood value for many generations but then suddenly starts to climb again. In that case the chain has not converged on a stable likelihood value. There is, however, no objective method to decide for how long a chain must be run to converge on a stable value. It is therefore appropriate to monitor many or all of the parameters (apart from the likelihood value) to decide about convergence (see Huelsenbeck et al., 2002).

3) A third problem concerning Bayesian analysis is formed by the posterior probability values. As Huelsenbeck et al. (2002) already recognise based on empirical observation, posterior probabilities are usually consistently higher than bootstrap support values calculated using parsimony or maximum likelihood. This concern is now confirmed by several simulation studies, which all agree that posterior probabilities are generally misleadingly high in comparison with bootstrap (Suzuki et al., 2002; Wilcox et al., 2002; Alfaro et al., 2003; Douady et al., 2003; Erixon et al., 2003) and jackknife values (Simmons et al., 2004), both considered to be too conservative.

4) The last and probably the most important, but least studied problem is formed by the sensitivity of a Bayesian analysis to the utilised priors. In his new handbook on phylogenetic systematics, Felsenstein (2004) gives a number of theoretical examples in which the priors could seriously influence the posterior probability. However, no empirical data are available on this issue and Huelsenbeck et al. (2002) argue that the influence of prior beliefs on the posterior decreases with an increasing amount of data. It is, however, a fact that the prior, expressing the uncertainty about the parameters before observing the data, is subjective.

A more theoretical objection against Bayesian inference, and against statistical methods for phylogenetic inference in general, is their use of explicit knowledge about the process of evolution, in the form of substitution models. This is in contrast with maximum parsimony which is based on 'Occam's razor'. This philosophical principle states that one should prefer those hypotheses that require the least ad-hoc explanations (fewer assumptions). Maximum parsimony, in contrast with likelihood methods, is the simplest description of the data and minimizes the requirement of ad hoc hypotheses of homoplasy (see Farris, 1983 for an elaborate discussion). In contrast, 'likelihoodists' explain deviations from the most parsimonious solution by means of the model, which is supposed to be a correct reflection of how the evolution has proceeded, but what happens if the used model is false?

Bearing all the above mentioned problems and critiques on Bayesian inference in phylogeny in mind, why should one bother to do the effort? The use of likelihood based methods can be justified by the possible occurence of the long-branch attraction effect (Felsenstein, 1978b). This phenomenon (also called the Felsenstein zone), whereby branches with large numbers of characters evolving in parallel, group together in a maximum parsimony approach, is the result of multiple nucleotide substitutions at a particluar site (Felsenstein, 1978b). All likelihood based methods are said to be less susceptible to long-branch attraction, as these methods are corrected for multiple substitution events. Apart from Bayesian inference, this is also achieved in maximum likelihood, but the use of $(MC)^3$ makes Bayesian inference vastly faster, which in itself can be used as a criterion in favor of Bayesian analysis over maximum likelihood. The former method has yet a second advantage over maximum likelihood. In the latter method bootstrapping has to be performed if a meaurement of clade confidence is wanted. Combining bootstrapping with maximum likelihood is, again, very time-consuming.

For reasons stated above both maximum parsimony and Bayesian inference were used in inferring the relationships of and within the 'Typhloplanoida'.

OVERVIEW OF THE OTHER METHODS

Taxon sampling

The sequences of 41 specimens of 39 rhabdocoel species (24 'Typhloplanoida' species including *Ciliopharyngiella constricta*, 1 'Dalyellioida' species and 14 Kalyptorhynchia species; see Tab. 1). The specimens were collected in both freshwater and marine habitats. Marine specimens were extracted from the sediment or from algae using the $MgCl_2$-decantation method, whereas the freshwater specimens were collected by the oxygen depletion method (see Schockaert, 1996).

	Species	Acc. no.
PROSERIATA	*Archimonocelis oostendensis* Martens & Schockaert, 1981	**AY775732**
	Cirrifera sopottehlersae Noldt & Jouk, 1988	**AY775733**
	Coelogynopora axi Sopott, 1972	**AY775734**
	Pseudomonocelis ophiocephala (Schmidt, 1861) Meixner, 1943	**AY775735**
	Pseudomonocelis ophiocephala §	**AY775736**
RHABDOCOELA - Kalyptorhynchia	*Acrorhynchides robustus* Karling, 1931	**AY775737**
	Gyratrix hermaphroditus Ehrenberg, 1831	**AY775739**
	Karkinorhynchus bruneti Schilke, 1970	**AY775740**
	Mesorhynchus terminostylus Karling, 1956	**AY775741**
	Phonorhynchus helgolandicus (Metschnikow, 1863) Graff, 1905	**AY775742**
	Polycystis naegelii Kölliker, 1845	**AY775743**
	Proschizorhynchus triductibus Schilke, 1970	**AY775744**
	Schizochilus caecus L'Hardy, 1963	**AY775745**
	Schizochilus choriurus Boaden, 1963	**AY775746**
	Schizochilus marcusi Boaden, 1963	**AY775747**
	Schizorhynchoides caniculatus L'Hardy, 1963	**AY775748**
	Stradorhynchus terminalis Willems et al.	**AY775738**
	Thylacorhynchus ambronensis Schilke, 1970	**AY775749**
	Zonorhynchus seminascatus Karling, 1956	**AY775750**
RHABDOCOELA - 'Typhloplanoida'	*Castrada lanceola* (Braun, 1885) Luther, 1904	**AY775751**
	Castrada luteola Hofsten, 1907	**AY775752**
	Castrada viridis Volz, 1898	**AY775753**
	Castrada sp.	**AY775775**
	Ciliopharyngiella constricta Martens & Schockaert, 1981	**AY775754**
	Ciliopharyngiella constricta §	**AY775755**
	Einarella argillophyla Luther, 1948	**AY775756**
	Einarella argillophyla §	**AY775757**
	Gaziella sp.	**AY775776**
	Litucivis serpens Ax & Heller, 1970	**AY775758**
	Mesostoma lingua (Abildgaard, 1789) Schmidt, 1848	**AY775759**
	Mesostoma thamagae Artois et al., 2004	**AY775760**
	Olisthanella truncula (Schmidt, 1858) Luther, 1904	**AY775761**

	Phaenocora unipunctata Oersted, 1843	**AY775762**
	Promesostoma justinei Willems et al. §	**AY775763**
	Proxenetes flabellifer Jensen, 1878	**AY775764**
	Proxenetes puccinellicola Ax, 1960	**AY775765**
	Proxenetes quadrispinosus Den Hartog, 1966	**AY775766**
	Proxenetes simplex Luther, 1948	**AY775767**
	Proxenetes trigonus Ax, 1960	**AY775768**
	Ptychopera plebeia Beklemischev, 1927	**AY775769**
	Ptychopera westbladi Luther, 1943	**AY775770**
	Strongylostoma elongatum Hofsten, 1907	**AY775771**
	Styloplanella strongylostomoides Findenegg, 1924	**AY775772**
	Trigonostomum denhartogi (Karling, 1978) Willems et al., 2004	**AY775773**
	Trisaccopharynx westbladi Karling, 1940 §	**AY775774**
RHABDOCOELA - 'Dalyellioida'	*Castrella truncata* (Abildgaard, 1789) Hofsten, 1907	**AY775777**

Tab. 1. Overview of new sequences added to GenBank with their accession numbers (§: sequences provided by Littlewood and Webster).

The specimen of *Mesostoma thamagai* Artois et al., 2004 was collected after inundating a sediment sample, containing the resting propagules of this species from an ephemeral rock pool in Botswana (for details: see Artois et al., 2004).

The animals were starved for several hours up to one day to prevent DNA contamination by gut contents. Specimens were fixed in 96% ethanol and stored at 4°C until DNA extraction.

Two undescribed species are included in the analysis, but to avoid creating nomina nuda, they are not given a species name here. *Gaziella* sp. from the eastern Mediterranean is clearly identifiable as a species of *Gaziella* De Clerck and Schockaert, 1995. However, lack of material prevents its formal description. A second species, *Castrada* sp. from northern Sweden, could not be identified at the species level, but certainly belongs to the taxon *Castrada* Schmidt, 1861 (sensu Luther, 1963). The sequence of *Arrawarria inexpectata* was already used by Littlewood et al. (1999b) and therein named *Arrawarria* n.gen..

Apart from the new rhabdocoel sequences, five proseriate species were sequenced (Tab. 1). Additional sequences (19 rhabdocoel and 99 non-rhabdocoel sequences) were extracted from GenBank (Tab. 2), using Blast search (Altschul et al., 1997). Species of which more than one sequence was available are numbered (e.g. *Geocentrophora baltica* 1, *G. baltica* 2).

	Species	In model	Acc. no.
MACROSTOMIDA	*Paromalostomum fusculum* Ax, 1952		**AJ012531**
HAPLOPHARYNGIDA	*Haplopharynx rostratus* Meixner, 1938	M	**AJ012511**
LECITHOEPITHELIATA	*Geocentrophora baltica* 1 (Kennel, 1883)	M	**AF167421**
	Geocentrophora baltica 2	M	**AF065417**
	Geocentrophora sp.	M	**U70079**
	Geocentrophora sphyrocephala De Man, 1876	M	**D85089**
	Geocentrophora wagini Timoshkin, 1984	M	**AJ012509**
PROSERIATA - Lithophora	*Archiloa rivularis* de Beauchamp, 1910	M	**U70077**
	Archimonocelis crucifera Martens & Curini-Galletti, 1993		**AJ270151**
	Archimonocelis staresoi Martens & Curini-Galletti, 1993		**AJ270152**
	Archotoplana holotricha Ax, 1956	M	**AJ243676**
	Calviria solaris Martens & Curini-Galletti, 1993		**AJ270153**
	Coelogynopora gynocotyla Steinböck, 1924	M	**AJ243679**
	Monocelis lineata (Müller, 1774) Oersted, 1844	M	**U45961**
	Otoplana sp.	M	**D85090**
	Parotoplana renatae Ax, 1956	M	**AJ012517**
PROSERIATA - Unguiphora	*Nematoplana coelogynoporoides* Meixner, 1938	M	**AJ012516**
	Polystylophora novaehollandiae Curini-Galletti, 1998		**AJ270161**
BOTHRIOPLANIDA	*Bothrioplana semperi* Braun, 1881	M	**AF051333**
ADIAPHANIDA - Fecampiidae	*Kronborgia isopodicola* Blair & Williams, 1987	M	**AJ012513**
ADIAPHANIDA - Genostomatidae	*Ichthyophaga* sp.	M	**AJ012512**
ADIAPHANIDA - Urastomidae	*Urastoma cyprinae* 1 (Graff, 1882) Graff, 1903	M	**AF065428**
	Urastoma cyprinae 2	M	**AF167422**
	Urastoma sp.	M	**U70085**
ADIAPHANIDA - Prolecithophora	*Allostoma neostiliferum* Karling, 1993	M	**AF167420**
	Cylindrostoma fingalianum 1 (Claparède, 1861) Levinsen, 1878	M	**AF065415**
	Cylindrostoma fingalianum 2	M	**AF051330**
	Cylindrostoma gracilis Westblad, 1955	M	**AF065416**
	Euxinia baltica Meixner, 1938	M	**AF167418**
	Plagiostomum cinctum Meixner, 1938	M	**AF065418**
	Plagiostomum ochroleucum Graff, 1882	M	**AF065419**
	Plagiostomum striatum Westblad, 1956	M	**AF065420**
	Plagiostomum vittatum 1 (Frey & Leuckart, 1847) Jensen, 1883	M	**AF051331**
	Plagiostomum vittatum 2	M	**AF065421**
	Plicastoma cuticulata Brandtner, 1934	M	**AF065422**
	Protomonotresis centrophora Reisinger, 1924	M	**AF167419**
	Pseudostomum gracilis Westblad, 1955	M	**AF065423**
	Pseudostomum klostermanni (Graff, 1874) Graff, 1913	M	**AF065424**
	Pseudostomum quadrioculatum (Leuckart, 1847) Graff, 1911	M	**AF065425**
	Reisingeria hexaoculata Westblad, 1955	M	**AF065426**

	Scleraulophorus cephalatus Karling, 1940	M	**AF167423**
	Ulianinia mollissima Levinsen, 1879	M	**AF065427**
	Vorticeros ijimai Togawa, 1918	M	**D85094**
ADIAPHANIDA - Tricladida	*Artioposthia triangulata*1 (Dendy, 1895) Graff, 1896	M	**AF033038**
	Artioposthia triangulata 2	M	**AF033044**
	Artioposthia triangulata 3	M	**Z99945**
	Australoplana sanguinea (Moseley, 1877) Winsor, 1991	M	**AF033041**
	Australoplana sp.	M	**AF050434**
	Baikalobia guttata (Gertsfeldt, 1858) Kenk, 1930	M	**Z99946**
	Bdelloura cnadida (Girard, 1850) Girard, 1852	M	**Z99947**
	Bipalium kewense Moseley, 1878	M	**AF033039**
	Bipalium sp.	M	**X91402**
	Bipalium trilineatum Stimpson, 1857	M	**D85086**
	Caenoplana caerulea Moseley, 1877	M	**AF033040**
	Caenoplana sp.	M	**AF048765**
	Crenobia alpina (Dana, 1766) Kenk, 1930	M	**M58345**
	Cura pinguis (Weiss, 1909) Kenk, 1974	M	**AF033043**
	Dendrocoelopsis lactea Ichikawa & Okugawa, 1958	M	**D85087**
	Dugesia iberica Gourbault & Benazzi, 1979	M	**M58343**
	Dugesia japonica 1 Ichikawa & Kawakatsu, 1964	M	**AF013153**
	Dugesia japonica 2	M	**D83382**
	Dugesia ryukyuensis Kwakatsu, 1976	M	**AF050433**
	Dugesia subtentaculata (Draparnaud, 1801) De Vries, 1986	M	**AF013155**
	Ectoplana limuli (Ijima & Kaburaki, 1916) Kaburaki, 1917	M	**D85088**
	Girardia tigrina 1 (Girard, 1850)	M	**AF013156**
	Girardia tigrina 2	M	**AF013157**
	Microplana nana Mateos, Giribet & Carranza, 1998	M	**AF033042**
	Microplana scharffi (Graff, 1896)	M	**AF050435**
	Neppia montana (Nurse, 1950) Ball, 1974	M	**AF050432**
	Newzealandia sp.	M	**AF050431**
	Phagocata sibirica (Sabussow, 1903) Kenk, 1974	M	**Z99948**
	Phagocata sp.	M	**AF013150**
	Phagocata ullala Sluys, Ribas & Baguñà, 1995	M	**AF013149**
	Platydemus manokwari Beauchamp, 1962	M	**AF048766**
	Polycelis nigra (Müller, 1774)	M	**AF013151**
	Polycelis tenuis Ijima, 1884	M	**Z99949**
	Procerodes littoralis (Ström, 1768) Hallez, 1893	M	**Z99950**
	Schmidtea mediterranea 1 (Benazzi et al., 1975)	M	**M58344**
	Schmidtea mediterranea 2	M	**U31084**
	Schmidtea mediterranea 3	M	**U31085**
	Schmidtea polychroa 1 (Schmidt, 1861)	M	**AF013152**
	Schmidtea polychroa 2	M	**AF013154**
	Romankenkius libidinosus Sluys & Rohde, 1991	M	**Z99951**
	Uteriporus sp.	M	**AF013148**
NEODERMATA	*Aspidogaster conchicola* Baer, 1827		**AJ287478**
	Caryophyllaeides ergensi Scholz, 1990		**AF286979**
	Dasyrhynchus pillersi Southwell, 1929		**AJ287496**
	Diphyllobothrium stemmacephalum Cobbold, 1858		**AF124459**
	Echinococcus granulosus (Batsch, 1786) Rudolphi, 1805		**U27015**
	Echinostoma caproni Richard, 1964		**L06567**

	Fasciola gigantica Cobbold, 1856		**AJ011942**
	Fasciola hepatica Linnaeus, 1758		**AJ004969**
	Gyrodactylus rhodei Zitnan, 1964		**AJ567670**
	Multicotyle purvisi Dawes, 1941		**AJ228785**
	Phyllobothrium lactuca Van Beneden, 1850		**AF286999**
	Troglocephalus rhinobatidis Young, 1967		**AJ228795**
	Udonella caligorum Johnston, 1835		**AJ228796**
RHABDOCOELA - 'Dalyellioida'	*Anoplodium stichopi* Bock, 1925	M	**AF167424**
	Graffilla buccinicola Jameson, 1897	M	**AJ012521**
	Microdalyellia rossi (Graff, 1911) Gieysztor, 1938	M	**AJ012515**
	Provortex balticus (Schultze, 1851) Graff, 1882		**AJ312268**
	Provortex tubiferus Luther, 1948		**AJ312269**
	Pterastericola australis Cannon, 1986	M	**AJ012518**
RHABDOCOELA - 'Typhloplanoida'	*Astrotorhynchus bifidus* (McIntosh, 1874) Graff, 1905		**AJ312270**
	Bothromesostoma personatum (Schmidt, 1848) Fuhrmann, 1894	M	**M58347**
	Bothromesostoma sp.	M	**D85098**
	Maehrenthalia agilis (Levinsen, 1879) Graff, 1905		**AJ312273**
	Mariplanella frisia Ax & Heller, 1970	M	**AJ012514**
	Mesoscastrada sp.	M	**U70081**
	*Mesostoma lingua**	M	**AJ243682**
	Trigonostomum penicillatum Schmidt, 1857		**AJ312275**
RHABDOCOELA - Kalyptorhynchia	*Arrawarria inexpectata* Willems et al.	M	**AJ243677**
	Cheliplana cf. *orthocirra*	M	**AJ012507**
	Diascorhynchus rubrus Boaden, 1963	M	**AJ012508**
	*Gyratrix hermaphroditus**	M	**AJ012510**
	*Phonorhynchus helgolandicus**		**AJ312274**
RHABDOCOELA - Temnocephalida	*Temnocephala* sp. 1	M	**AJ012520**
	Temnocephala sp. 2	M	**AF051332**

Tab. 2. List of all additional flatworm species used in this study with their GenBank accession number (*: species of which a new sequence is also included, see Tab. 1). Numbered species names: two different sequences extracted from GenBank. Species used to construct the probability model (see further) are indicated with 'M'.

In total 164 sequences are included in the analyses, 62 of them from species today considered Rhabdocoela. *Paromalostomum fusculum, Haplopharynx rostratus* and five sequences of four species of Lecithoepitheliata (*Geocentrophora* sp., *G. wagini, G. baltica* 1, *G. baltica* 2 and *G. sphyrocephala*) were used as outgroups.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from entire specimens using the DNeasy Tissue Kit (Qiagen) following the manufacturer's protocol.

The complete 18S rDNA gene, approximately 1800 bp long, was amplified using the primers TimA and TimB (see Tab. 3). Thermal cycling was started with an initial denaturation of 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 90 s with a final extension of 8 min at 72°C. Using nested PCR (with the same cycling profile), the 1100 bp closest to the 5′ end of the 18S rDNA gene were amplified using TimA and 18S 1100R, while the 1200 bp closest to the 3′ end were amplified with the primers 18S 600F and TimB, which gave approximately a 500 bp overlap. All PCR reactions were performed in 25 µl, using Promega PCR Core System I. These reactions, containing 0.2 µM of the respective forward and reverse primer, 1 mM of each dNTP, one-tenth volume of *Taq* DNA Polymerase buffer 10X, 1.5 mM $MgCl_2$ and 1.25 U of *Taq* DNA Polymerase, were carried out on an Eppendorf Mastercycler Gradient. The PCR results (5 µl) were verified on a 1% agarose gel, stained with ethidium bromide. PCR products were purified with the Qiaquick PCR Purification Kit from Qiagen and stored at 4°C.

Sequencing was done by the Genetic Service Facility of VIB (Flanders Interuniversity Institute for Biotechnology), using TimA, TimB and six internal primers (see Table 3) on an ABI 3730 DNA Analyzer (Applied Biosystems) with the ABI PRISM BigDye Terminator cycle sequencing kit. The sequences were verified by forward and reverse comparisons using Chromas v. 1.45 (freeware from http://www.technelysium.com.au/index.html).

Primer	Used in	Primer sequence	Reference
Tim A	PCR/Sequencing	5'-AMCTGGTTGATCCTGCCAG-3'	Norén and Jondelius (1999)
Tim B	PCR/Sequencing	5'TGATCCATCTGCAGGTTCACCT-3'	Norén and Jondelius (1999)
600F	PCR	5'-GGTGCCAGCAGCCGCGGT-3'	Modified after Norén and Jondelius (1999)
1100R	PCR	5'-GATCGTCTTCGAACCTCTG-3'	Norén and Jondelius (1999)
18S4FB	Sequencing	5'-CCAGCAGCCGCGGTAATTCCAG-3'	Norén and Jondelius (1999)
18S4FBK	Sequencing	5'-CTGGAATTACCGCGGCTGCTGG-3'	Norén and Jondelius (1999)
18S5F	Sequencing	5'-GCGAAAGCATTTRYCHAGDA-3'	Modified after Norén and Jondelius (1999)
18S5FK	Sequencing	5'-THCTDGRYAAATGCTTTCGC-3'	Modified after Norén and Jondelius (1999)
18S7F	Sequencing	5'-GCAATAACAGGTCTGTGATGC-3'	Norén and Jondelius (1999)
18S7FK	Sequencing	5'-GCATCACAGACCTGTTATTGC-3'	Norén and Jondelius (1999)

Tab. 3. Primers used in PCR- and sequencing reactions.

Three rhabdocoel species of which 18S rDNA sequences are available in GenBank (*Gyratrix hermaphroditus*, *Mesostoma lingua* and *Phonorhynchus helgolandicus*; see Tab. 2: *) are also represented by a new sequence as an additional check on the sequence's quality. For some of the specimens, both PCR reactions and sequencing were performed in Dr Littlewood's laboratory by Dr Bonnie Webster (indicated in Tab. 1 by §) using protocols outlined in Littlewood et al. (2000).

All new sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov). Their accession numbers can be found in Tab. 1.

Alignment

A prior alignment of 94 sequences of rhabditophoran flatworms (see Tab. 2, indicated with 'M') based on secondary structure was downloaded from the SSU rRNA database (http://www.psb.ugent.be/rRNA; see also Wuyts et al., 2004). This initial alignment was used to create a model with the hmmbuild option in HMMER 2.3.2 (http://hmmer.wustl.edu/; see also Eddy, 1998). This model contains probability parameters, which are estimated from the observed frequencies of residues and transitions in the initial multiple sequence alignment. With this probability model, HMMER was used to create a profile alignment (hmmalign option) of the new sequences (see Tab. 1) and 24 additional sequences from GenBank, mainly Neodermata sequences (see Tab. 2) against the initial alignment. This resulted in an alignment of 3522 base positions, which was edited using MacClade 4.06 (Maddison and Maddison, 2003). In total, 1729 positions of the alignment were deleted because they had a gap in all except for one or two of the species. These positions would not influence the parsimony analysis, but would have resulted in a larger amount of computational effort in the Bayesian analyses. Moreover the longer blocks of deleted nucleotides only appeared in the neodermatan species.

Phylogenetic analyses

The final data matrix consists of 1793 unambiguously aligneable base positions for 164 species and was analysed using maximum parsimony and Bayesian inference.

Prior to the analyses, base composition (% GC content) was calculated using PAUP 4.0 b10 (Swofford, 2003) to account for possible base compositional bias.

Parsimony analysis (with gaps treated as missing data) was performed using both PAUP* 4.0 b10 and TNT 1.0 (Goloboff et al., 2001), the former in combination with PAUPRat (Sikes & Lewis, 2001). PAUPRat implements the parsimony ratchet (Nixon, 1999), making the tree search more efficient. In PAUP* the Rat search was performed once and the settings were as follows: nchar=1793, random seed=0, nreps=200, pct=15 (default; ideally between 5 and 25%, see Nixon, 1999), wtmode=uniform, terse. For all the heuristic searches performed by PAUPRat, the default settings were used. In TNT the Rat-search was repeated 20 times (as recommended in the PAUPRat manual: Sikes and Lewis, 2001), each with 500 iterations and all other settings the same as above.

We employed several different approaches to control for rate heterogeneity effects that may affect the results of a parsimony analysis: Bayesian inference; evaluation of pairwise distances and removal of possible 'long branches'.

Bayesian inference (Rannala & Yang, 1996; Mau & Newton, 1997; Yang & Rannala, 1997; Mau et al., 1999; Larget & Simon, 1999) was performed in MrBayes 3.0 b4 (Huelsenbeck & Ronquist, 2001) under the general time-reversible model (GTR; Rodríguez et al., 1990), with discrete gamma-distributed rate variation among sites (Yang, 1993, 1994) with four categories, and allowing for invariant sites (Gu et al., 1995; Waddell & Steel, 1997). This model was chosen by MODELTEST 3.06 (Posada & Crandall, 1998) as the model of DNA evolution that best fitted the data. Five independent runs, each with 2,000,000 generations and four chains (default temperature), sampled every 100 generations, are performed. Branch lengths were saved. An additional run with 10,000,000 generations was also done to ensure that the analysis was running long enough to converge on a stable lnL value. As already suggested by Huelsenbeck et al. (2002) different parameters can converge at different rates. After plotting of both ln-likelihood and tree length values against the generation number the burn-in value was chosen in function of both parameters converging on a stable value. Two 95% majority rule consensus trees were computed: one summarising the results of four out of five runs (see further) of 2,000,000 generations and one summarising the results of the single 10,000,000 generations run.

The occurrence of long-branch attraction (see Felsenstein, 1978b) was additionally tested by omitting the four taxa with the largest mean pairwise distance compared to all other taxa from the parsimony analysis in PAUP*: *Graffilla buccinicola* Jameson, 1897, *Plagiostomum ochroleucum* Graff, 1882, *Udonella caligorum* Johnston, 1835 and *Vorticeros ijimai* Graff, 1899.

All analyses were run on two x86 AMD 2800+ CPUs.

Clade support

Clade support was assessed by calculating jackknife values (Lanyon, 1985; Siddall, 1995), Bremer support values (Bremer, 1988, 1994) and Bayesian posterior probabilities (see above for references).

Bremer support (Bremer, 1988, 1994) was calculated using TreeRot v2 (Sorenson, 1999). This program generates a command file for PAUP*, which consists of the constraint statement for each node and the commands to search for the shortest tree, incompatible with this node. The constrained searches to determine the Bremer support indices were done using the parsimony ratchet (parameters).

For estimating nodal support in parsimony analysis jackknifing was preferred to bootstrapping for its computational efficiency (see Farris, 1998), and was performed with Xac (Farris, 1997); cut-off frequency of 50%, character deletion frequency e^{-1}, 1000 replicates, 3 random additions and branch swapping enabled. For Bayesian analysis nodal support was estimated by determining posterior probabilities with MrBayes.

A 50% majority-rule consensus tree of all trees sampled in four out of five independent Bayesian analyses (excluding the burn-ins; see further) was computed in PAUP*. Only posterior probabilities (PP) higher than 95 were considered, as PP values less than this are very often an overestimate for nodal support (Suzuki et al., 2002; Alfaro et al., 2003; Douady et al., 2003; Erixon et al., 2003; Simmons et al., 2004).

Monophyly of the Typhloplanoida (sensu Bresslau, 1933) and the Typhloplanoida s.l. (Typhloplanoida + Kalyptorhynchia; sensu Ehlers, 1985) was tested by constructing two constraint trees in MacClade: one with a monophyletic Typhloplanoida, the other with a monophyletic Typhloplanoida s.l. A PAUPRat search in PAUP*, with 200 iterations was run and Templeton (Templeton, 1983) and Winning sites (Prager & Wilson, 1988) tests were performed with PAUP* to determine whether these constraint solutions were significantly different from the most parsimonious solution. All trees sampled in the Bayesian analysis (post burn-in) were filtered using both constraint trees.

RESULTS

TAXONOMICAL ACCOUNT

In total, 43 new species (29 typhloplanoids, 12 polycystidid kalyptorhynchs and 2 dalyellioids) are described (overview in Tab. 4), most of them collected from the southern hemisphere, the rest from the Mediterranean Sea. The species of Polycystididae were described in my graduate thesis and more elaborately studied by Artois (2001; unpublished PhD-thesis), which includes a cladistical analysis of this taxon based on morphological data. The descriptions can be found in seven papers (or manuscripts; see list below), which are included as an appendix.

List of papers

I. **Willems. W.R.**, Artois T.J., Vermin W.A., Schockaert E.R., 2004. Revision of *Trigonostomum* Schmidt, 1852 (Platyhelminthes, Typhloplanoida, Trigonostomidae) with the description of seven new species. *Zoological Journal of the Linnean Society* 141: 271-296.

II. **Willems. W.R.**, Artois T.J., Vermin W.A., Backeljau T., Schockaert E.R., 2004. Reports on the free-living Platyhelminthes from Australia: Typhloplanoida, with the description of three new taxa. *Zoological Science* 21: 333-341.

III. Artois T., **Willems W.**, De Roeck E., Jocqué M., Brendonck L., 2004. Freshwater Rhabdocoela (Platyhelminthes) from ephemeral rock pools from Botswana, with the description of four new species and one new genus. *Zoological Science* 21: 1063-1072.

IV. **Willems W.R.**, Artois T.J., Vermin W.A., Backeljau T., Schockaert E.R. 'Typhloplanoida' (Platyhelminthes: Rhabdocoela) from the Indian Ocean, with the description of six new taxa. *Journal of Natural History*. (In press a; page proofs included)

V. **Willems W.R.**, Artois T.J., Backeljau T., Schockaert E.R. Typhloplanoida (Platyhelminthes: Rhabdocoela) from New Caledonia and eastern Australia, with the description of six new taxa. *New Zealand Journal of Zoology*. (In press b; manuscript included)

VI. **Willems W.R.**, Artois T.J., Schockaert E.R. Report on the Polycystididae (Kalyptorhynchia, Rhabdocoela) from Australia, with the description of 12 new taxa. (Manuscript in preparation)

VII. **Willems W.R.**, Artois T.J., Backeljau T., Schockaert E.R. Five new species of *Promesostoma* (Platyhelminthes, Rhabdocoela, Promesostomidae), with a general discussion on this species-rich taxon. (Manuscript in preparation)

	SPECIES NAME	PAPER
'Typhloplanoida' - Promesostomidae	*Brinkmaniella australiensis*	II
	Coronhelmis cuypersi	V
	Coronhelmis novaecaledoniae	V
	Kymocarens kanakorum	V
	Promesostoma alexanderi	VII
	Promesostoma calcareum	VII
	Promesostoma corsicum	VII
	Promesostoma justinei	VII
	Promesostoma lincolni	VII
	Vauclusia conica	II
Solenopharyngidae	*Austradenopharynx reyneartsi*	V
	Pilamonila bimascula	II
Trigonostomidae	*Gandalfia bilunata*	IV
	Mahurubia clava	IV
	Parapharyngiella involucrum	IV
	Poseidoplanella halleti	IV
	Trigonostomum australis	I
	Trigonostomum franki	I
	Trigonostomum galapagoensis	I
	Trigonostomum nataschae	I
	Trigonostomum tori	I
	Trigonostomum spinigerum	I
	Trigonostomum watsoni	I
Typhloplanidae	*Aegira annabellae*	IV
	Castrada trispina	IV
	Kaitalugia falcata	V
	Kaitalugia lydieae	V
	Mesostoma thamagai	III
	Syringoplana kolasai	III
Kalyptorhynchia - Polycystididae	*Alchoides alchoides*	VI
	Alchoides dittmanni	VI
	Ametochus gehrkei	VI
	Arrawarria inexpectata	VI
	Cincturorhynchus monaculeus	VI
	Duplexostylus winsori	VI
	Duplexostylus rowei	VI
	Paraustrorhynchus caligatus	VI
	Polycystis australis	VI
	Stradorhynchus caecus	VI
	Stradorhynchus terminalis	VI
	Triaustrorhynchus armatus	VI
'Dalyellioida' - Dalyelliidae	*Gieysztoria faubeli*	III
	Gieysztoria isoldeae	III

Tab. 4. Overview of newly described species, with an indication of their systematic position, according to 'classical' taxonomy.

PHYLOGENETIC ANALYSIS

Sequence data

Within the alignment the length of the 18S rDNA fragment varied between 1308 bp (*Dugesia iberica*) and 1792 bp (*Archimonocelis crucifera*, *Cirrifera sopottehlersae* and *Coelogynopora axi*). The GC content varied between 40.4% (*Dugesia mediterranea* 3) and 51.7% (*Echinococcus granulosus*) with an average of 45.3%. Remarkably, all neodermatans have a high GC content, ranging from 47.1% to 51.7%, whereas most of the triclads have a rather low value ranging between 40.7% and 47.4%. Representatives of the Rhabdocoela had a GC content which is scattered over almost the whole range, from 42.3% in *Astrotorhynchus bifidus* to 48% in *Olisthanella truncula*. Since the difference between these extremes (within the Rhabdocoela) was lower than the 8-10% value, commonly assumed to be the maximum value at which a biasing effect of compositional heterogeneity can occur (e.g. Galtier & Gouy, 1995), it is unlikely that GC bias forms a problem in our data set.

Parsimony analysis

Of the 1793 unambiguously alignable base positions in the final alignment, 468 sites were constant and 245 were parsimony-uninformative, resulting in 1080 parsimony-informative characters.

The analysis in PAUP* combined with PAUPRat generated 26 trees of 14822 steps (CI = 0.180; RI = 0.678; RC = 0.122). However, only seven MP trees were topologically distinct; the strict consensus of these is depicted in Fig. 3 and 3bis.

Bayesian inference

Four out of five independent runs of 2,000,000 generations (Tab. 5: nos I, II, IV, V) converged rather quickly, however with different values for the burn-in according to ln-likelihood and treelength values (see Tab. 5). The third run (no. III, see Tab. 5) did not converge, and therefore the results of this run were not used when computing the overall consensus tree for the 2,000,000 generations runs.

Run	Burn-in (LnL)	Burn-in (TL)	LnL
I	110000	**250000**	-67346.772
II	**150000**	**150000**	-67347.648
III	**1400000**	1370000	/
IV	**240000**	200000	-67343.042
V	140000	**165000**	-67344.184
VI	100000	**150000**	-67345.293

Tab. 5. Burn-in (with respect to ln-likelihood and treelength values) and mean ln-likelihood values of six independent MrBayes-runs. Run III failed to converge: LnL plot showed plateaus after 150,000; 850,000; 1,060,000; 1,400,000 generations; TL plot after 250,000; 870,000; 1,030,000 and 1,370,000 generations (I-V: 2M generations, VI: 10M generations; burn in value used in computing consensus tree is indicated in bold)

The single 10,000,000 generations run (Tab. 5: no. VI) converged after a similar number of generations in comparison to runs I, II, IV and V.

The likelihood values around which the ln-likelihood curve oscillated after reaching stability are given in Tab. 5. Both resulting consensus trees (one for runs I, II, IV and V and one for run VI) were identical to the strict consensus of the seven most parsimonious trees in Fig. 3 and 3bis. Certainly, congruence of this nature indicates strength of phylogenetic signal as the methods use completely different algorithms. The analysis, in which the taxa with the largest mean pairwise distance were excluded, resulted in a tree of 14022 steps with the same overall topology as that of the tree depicted in Fig. 3 and 3bis.

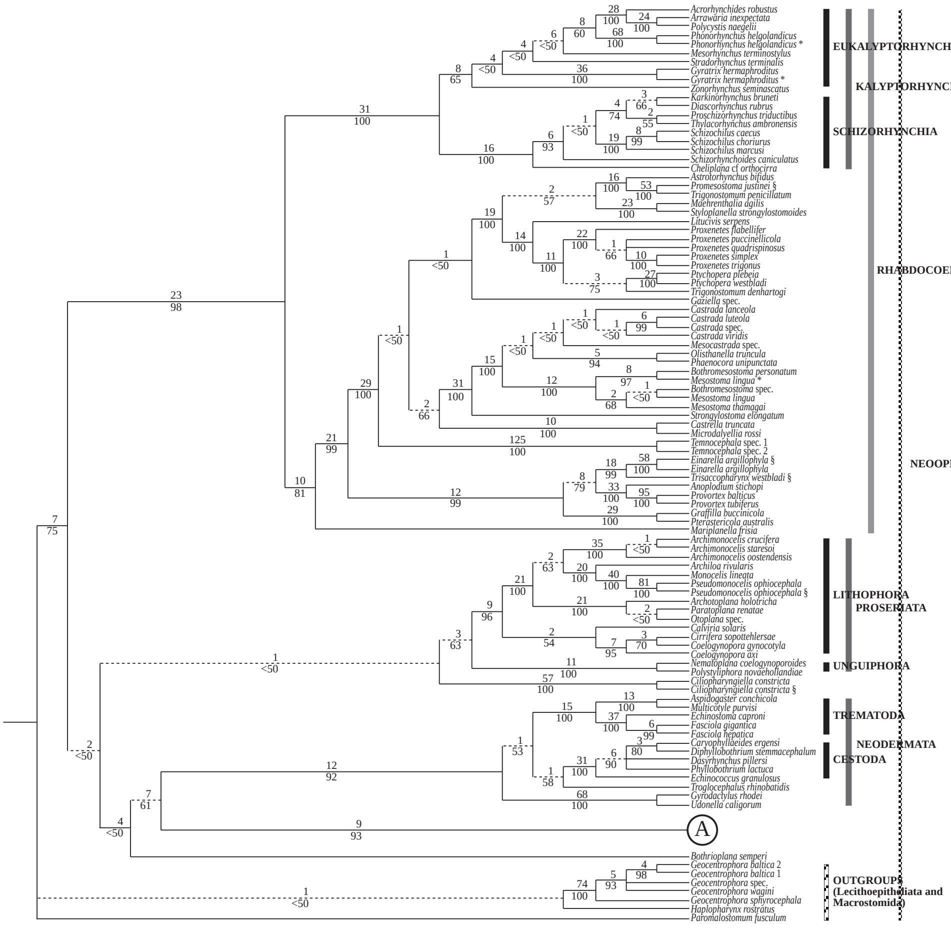

Fig. 3. Strict consensus of seven most parsimonious trees of 14822 steps (CI = 0.180; RI = 0.678; RC = 0.122) obtained in PAUP* combined with PAUPRat (200 iterations). Bremer support values are indicated above each clade; jackknife values beneath each clade. Clades with a posterior probability ≤95% in the Bayesian analysis are indicated as dashed lines. Present taxonomic positions are indicated on the right. (§ and *: see Tab. 1 and 2). The Adiaphanida (A) are depicted in Fig. 3 bis.

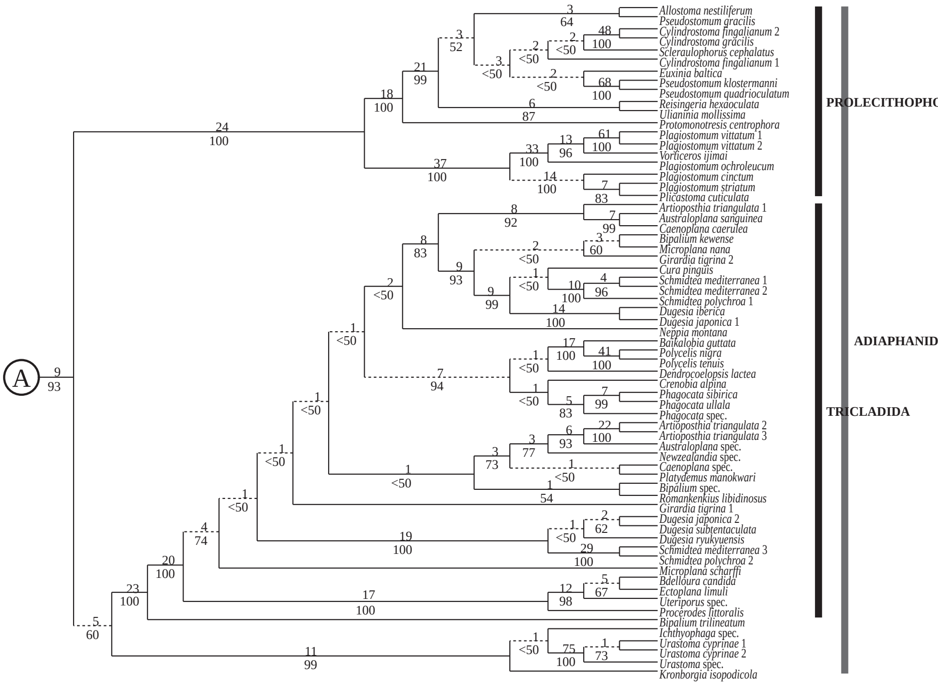

Fig. 3 bis. Adiaphanida (A). Strict consensus of seven most parsimonious trees of 14822 steps (CI = 0.180; RI = 0.678; RC = 0.122) obtained in PAUP* combined with PAUPRat (200 iterations). Bremer support values are indicated above each clade; jackknife values beneath each clade. Clades with a posterior probability ≤95% in the Bayesian analysis are indicated as dashed lines. Present taxonomic positions are indicated on the right. (§ and *: see Tab. 1 and 2).

Clade support

In Fig. 4 all three measures of clade support are compared. Clades with jackknife and PP values <50% are not included in the graphs. From Fig. 4A, it appears that for clades with Bremer values >10, the jackknife support was >80%. The posterior probabilities were exceptionally high compared to the jackknife (Fig. 4B) and the Bremer support (Fig. 4C) values.

These results confirm that PP values may be misleadingly high (see also Suzuki et al., 2002; Alfaro et al., 2003; Douady et al., 2003; Erixon et al., 2003; Simmons et al., 2004), even with a low jackknife and Bremer support.

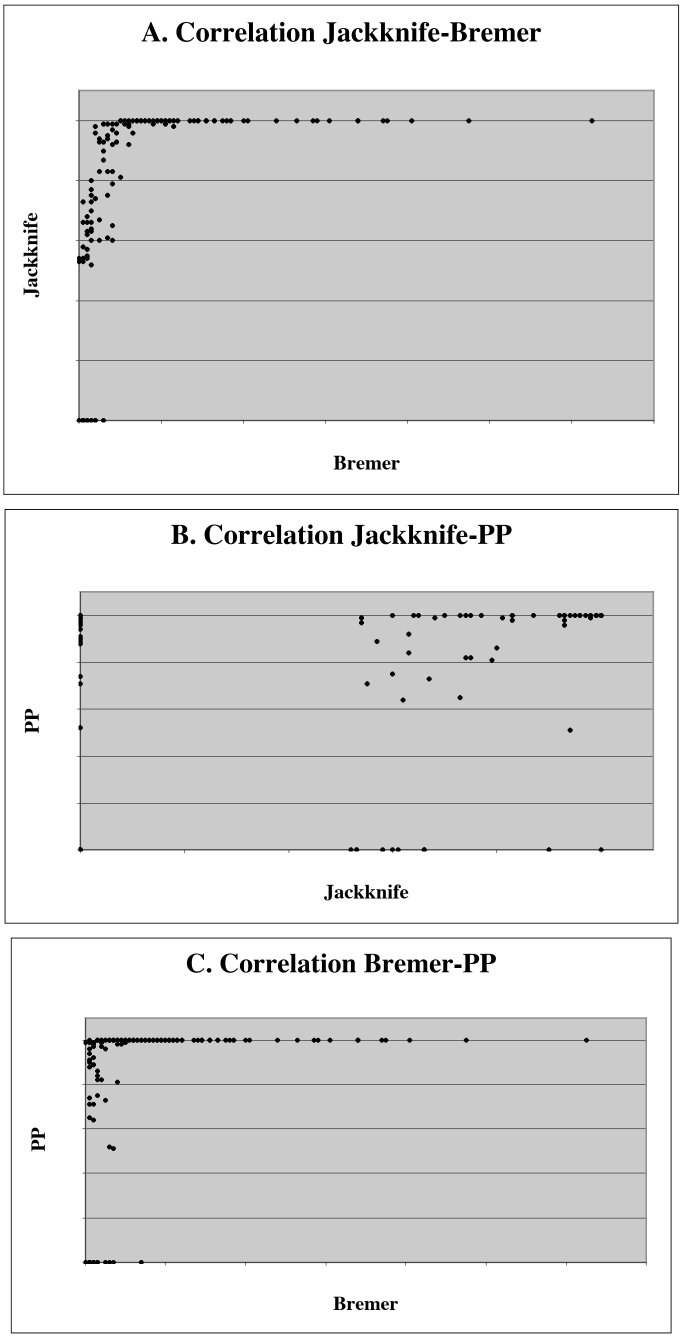

Fig. 4. Comparison of Bremer support, jackknife and posterior probability values. Correlation between jackknife and Bremer support values (A), jackknife values and posterior probabilities (B), and Bremer support values and posterior probabilities (C).

Tree topology

The tree depicted in Fig. 3 shows jackknife and Bremer values on each clade, whereas clades with low PP values (<95%) are shown as dashed lines. When clades with jackknife <90% are collapsed and only the major taxa are considered, the tree looks as in Fig. 5, and it becomes clear that sister group relations of the major (ingroup) neoophoran taxa are far from being resolved. They form a large polytomy consisting of the Rhadocoela, the Lithophora, the Unguiphora, the Neodermata and the Adiaphanida (= Prolecithophora + Tricladida + 'turbellarian Revertospermatida') and two isolated species, *Ciliopharyngiella constricta* and *Bothrioplana semperi*. The Rhabdocoela is clearly monophyletic (with high support values: Bremer support 23; jackknife 98%) and its sister group cannot be indicated as yet.

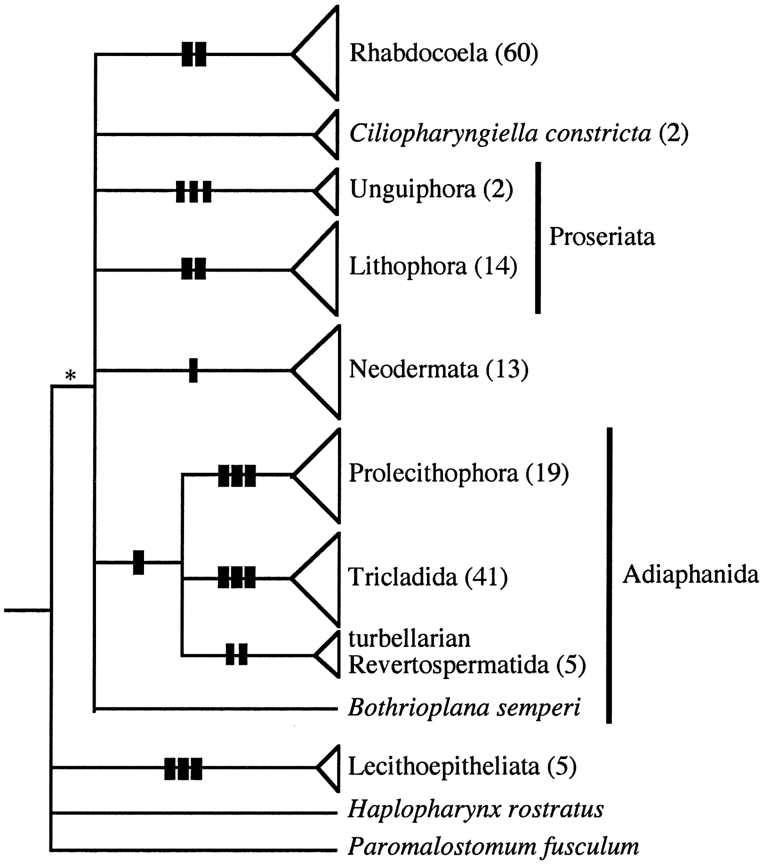

Fig. 5. Summary of results based on strict consensus of seven most parsimonious trees with the major taxa under consideration. Only clades with jackknife values >90% are indicated. Clades with one rectangle have a jackknife support >90, two rectangles represent a support >95%, three a support of 100%, the clade with * has a support of 75%. Number of sequences used are indicated between parentheses following taxon names.

A 90% majority rule consensus tree summarizing the parsimony jackknife analysis of the Rhabdocoela is shown in Fig. 6.

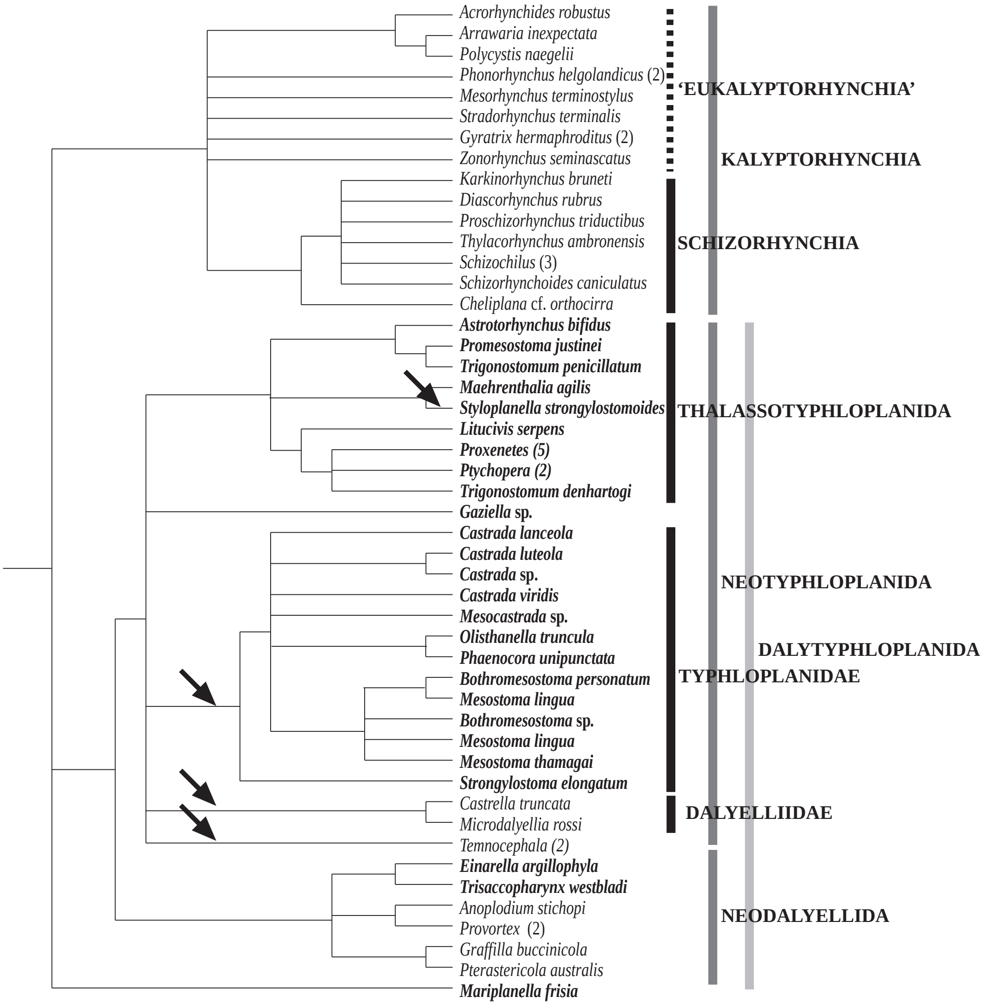

Fig. 6. Rhabdocoela. Strict consensus of seven most parsimonious trees obtained in PAUP* combined with PAUPRat. Clades with jackknife values <90% are collapsed. The number between parentheses indicate the number of specimens used in case of a species name and the number of species used in case of a genus name. Freshwater clades are indicated with an arrow. 'Typhloplanoida' are in bold. 'Dalyellioida' are underlined.

The Rhabdocoela shows a trichotomy formed by *Mariplanella frisia*, the clearly monophyletic Kalyptorhynchia and a clade containing all 'Dalyellioida' and all 'Typhloplanoida' (excl. *M. frisia* and *Ciliopharyngiella constricta*), which we will call the Dalytyphloplanida (see Definitions of clade names). There is some support for a clade formed by the Dalytyphloplanida and *M. frisia* (Bremer 10; jackknife 81% - see Fig. 3).

Within the Kalyptorhynchia there is strong support for a monophyletic Schizorhynchia. Within both the Kalyptorhynchia and the Schizorhynchia the internal relationships are unresolved. The sister taxon of the Schizorhynchia is still uncertain, but is with our data not formed by the Eukalyptorhynchia. Within the Dalytyphloplanida there are two highly supported clades, which we will call the Neodalyellida and the Neotyphloplanida (see Definitions of clade names). In the Neodalyellida we find all marine 'dalyellioids' (including the symbionts: *Anoplodium stichopi, Pterastericola australis* and *Graffila buccinicola*) and two marine typhloplanoids: *Trisaccopharynx westbladi* (Solenopharyngidae) and *Einarella argillophyla* (Promesostomidae). The Neotyphloplanida consists of a polytomy with *Gaziella* sp. and four clades: the (freshwater) Temnocephalida Blanchard, 1849, the (freshwater) Dalyelliidae Graff, 1905, the (freshwater) Typhloplanidae Graff, 1905, and the Thalassotyphloplanida (all marine typhloplanoids + the freshwater *Styloplanella strongylostomoides*, so far included in the Typhloplanidae; see Definitions of clade names).

Within the Typhloplanidae, the Mesostominae Luther, 1963 (with the taxa *Mesostoma* Ehrenberg, 1837 and *Bothromesostoma* Braun, 1885) is monophyletic. None of all other 'subfamilies' within the Typhloplanidae emerges as monophyletic.

The Thalassotyphloplanida contains *Maehrenthalia agilis* (Byrsophlebidae), *Styloplanella strongylostomoides* (Typhloplanidae) and several representatives of the 'families' Promesostomidae and Trigonostomidae. The two latter taxa, however, do not appear to be monophyletic. Remarkably, both representatives of *Trigonostomum* are not related, one of them forming a clade with *Astrotorhynchus bifidus* and *Promesostoma justinei*, whereas the other one is included in a clade with all other Trigonostominae and a promesostomid (*Litucivis serpens*).

DEFINITIONS OF CLADE NAMES

New clade names are defined following the rules of phylogenetic nomenclature (de Quieroz & Gauthier, 1990, 1992, 1994), which can be found in the draft Phylocode (available at http://www.ohio.edu/phylocode). However, only clades with high support (jackknife >95%; see also Tab. 6 in the Discussion) and for which we think naming is useful, are defined. To avoid nomenclatural instability, only taxa of which the original type is included in the analysis receive a converted name. For example, the taxon Typhloplanidae, which is well supported, does not receive a converted name, as *Typhloplana viridata* (Abildgaard, 1789) Luther, 1904 is not included in the analysis. Converted names are indicated with n.c.c. (nomen cladi conversum), whereas new clade names receive the indication n.c.n. (nomen cladi novum) following article 9.3 of the Phylocode. Both converted and new clade names can be found in Fig. 6, except for the name Rhabdocoela, which can be found in Fig. 3.

Rhabdocoela Ehrenberg, 1831 n.c.c. (stem-based): the most inclusive clade containing *Polycystis naegelii* Kölliker, 1845 but not *Ciliopharyngiella constricta* Ax, 1952, *Monocelis lineata* (Müller, 1774) Oersted, 1844, *Nematoplana coelogynoporoides* Meixner, 1938, *Fasciola hepatica* Linnaeus, 1758 and *Pseudostomum quadrioculatum* (Leuckart, 1847) Graff, 1911.

Kalyptorhynchia Graff, 1905 n.c.c. (stem-based): the most inclusive clade containing *Polycystis naegelii* Kölliker, 1845 but not *Mariplanella frisia* Ax & Heller, 1970 and *Provortex balticus* (Schultze, 1851) Graff, 1882.

Schizorhynchia Meixner, 1928 n.c.c. (stem-based): the most inclusive clade containing *Schizochilus marcusi* Boaden, 1963 but not *Polycyctis naegelii* Kölliker, 1845.

Dalytyphloplanida n.c.n. (stem-based): the most inclusive clade containing *Provortex balticus* (Schultze, 1851) Graff, 1882 but not *Mariplanella frisia* Ax & Heller, 1970 and *Polycystis naegelii* Kölliker, 1845.

Neodalyellida n.c.n. (stem-based): the most inclusive clade containing *Provortex balticus* (Schultze, 1851) Graff, 1882 but not *Proxenetes flabellifer* Jensen, 1878.

Neotyphloplanida n.c.n. (stem-based): the most inclusive clade containing *Proxenetes flabellifer* Jensen, 1878 but not *Provortex balticus* (Schultze, 1851) Graff, 1882.

Thalassotyphloplanida n.c.n. (stem-based): the most inclusive clade containing *Proxenetes flabellifer* Jensen, 1878 but not *Castrada lanceola* (Braun, 1885) Luther, 1904 and *Castrella truncata* (Abildgaard, 1789) Hofsten, 1907.

GENERAL DISCUSSION

The 'Typhloplanoida' together with the Kalyptorhynchia, the Temnocephalida and the 'Dalyellioida' traditionally form the taxon Rhabdocoela (see Introduction). However, no molecular phylogenetic study at present deals exclusively with this taxon and only very few (e.g. Littlewood et al., 1999a-b; Baguñà et al., 2001a; Joffe & Kornakova, 2001; Littlewood & Olson, 2001; Norén & Jondelius, 2002; Lockyer et al., 2003) include more than five representatives of the Rhabdocoela. All these studies are based on 18S rDNA sequences, except for those by Littlewood et al. (1999b) and Lockyer et al. (2003), which also include data on 28S rDNA, but which are mainly focussing on the parasitic Neodermata. At the moment, the study of Norén and Jondelius (2002), which includes 20 species of Rhabdocoela, seven of them typhloplanoids, has the most extensive taxonomic sampling within the Rhabdocoela. Our study and the above mentioned studies find a monophyletic Rhabdocoela, albeit with varying nodal support (bootstrap values of <50-77% in Baguñà et al., 2001a; Joffe & Kornakova, 2001; Littlewood et al., 1999a-b; Littlewood & Olson, 2001; jackknife value of 98-99% in the present study and in Norén & Jondelius, 2002). Monophyly of the Rhabdocoela is however, still not supported by a clear morphological apomorphy. All rhabdocoels have a 'pharynx bulbosus', which they share with the parasitic neodermatans, some prolecithophorans and lecitoepithelians. Therefore it is possible that the 'pharynx bulbosus' has originated more than once, and that the homologies should be reconsidered carefully (as suggested by Joffe, 1987). Another possible apomorphy could be found in the ultrastructure of the protonephridial system. In all rhabdocoels the terminal cell has a single row of longitudinal ribs (see Rohde, 2001). However, this construction is also found in some other taxa (Lecitoepitheliata and Prolecithophora; see Littlewood et al., 1999a; Rohde, 2001). A second possible apomorphy based on protonephridial ultrastructure is the lack of a terminal perikaryon, whereas the flame bulb is continuous with the proximal canal cell and without a junction (Type C of Watson & Schockaert, 1997; see also Rohde, 2001). Moreover, Watson (2001) also proposed the presence of a dense heel on the basal bodies during spermiogenesis as a possible apomorphy for the Rhabdocoela. However, this feature is lost in several taxa within the Rhabdocoela (all kalyptorhynchs, except for some shizorhynchs).

The sister group of the Rhabdocoela cannot be indicated with the present results, as its forms part of a polytomy, also including the well supported Adiaphanida, Neodermata, Lithophora and Unguiphora and two isolated species, *Ciliopharyngiella constricta* and *Bothrioplana semperi* (see Fig. 5). Former studies found some support for a sister group relationship of the Rhabdocoela with the Adiaphanida (Littlewood et al., 1999a-b; Baguñà et al., 2001a; Joffe & Kornakova, 2001; Littlewood & Olson, 2001; Norén & Jondelius, 2002; Lockyer et al., 2003). However, with bootstrap and jackknife values around 50%, none of these studies are convincing and with poorly supported clades collapsed, all cladograms would show the same polytomy as in our study (see Fig. 5).

One of the members of the polytomy, *Ciliopharyngiella constricta*, was formerly placed within the Rhabdocoela and even within the 'Typhloplanoida' (see Ehlers, 1972), but its taxonomic position has been heavily debated based on morphological and ultrastructural data (see the Introduction) and now it appears that the molecular data do not completely solve the problem either.

Within the Rhabdocoela several good supported clades (see Fig. 6 and Tab. 6) can be recognised and are formally named (see Definitions of clade names). All above mentioned molecular studies, except for Norén & Jondelius (2002), include too few species to allow detailed comparison with our results. Therefore, our cladogram will be compared with that of Norén & Jondelius (2002) and the discussion will mainly focus on the recognition of possible morphological apomorphies for well supported clades. An overview of the support for these clades and the (possible) apomorphies is given in Tab. 6. A rather large number of morphological aspects concern ultrastructural data, which should be interpreted with caution, as only few taxa have been sampled for homologous characters and therefore are highly fragmentary. However, good reviews of the current knowledge on sperm and protonephridia ultrastructure with an overview of all available literature are given by Watson (2001) and Rohde (2001) respectively. Several contributions on the ultrastructure of other organs (e.g. male and female atrial system, eyes, epidermis) exist (e.g. Brüggeman, 1985; Rohde et al., 1987; Sopott-Ehlers, 1996, 1997; Sopott-Ehlers & Ehlers, 1997), but are even more fragmentary than those on spermatology and protonephridia.

	Jackknife (%)	Bremer support	PP (%)	Possible apomorphies
Rhabdocoela	98	23	100	pharynx bulbosus (?); terminal cell of protonephidia with single row of ribs (?); type C protonephridia (?); dense heel in sperm (?)
Kalyptorhynchia	100	31	100	proboscis; incorporation of axonemes in sperm
Schizorhynchia	100	16	100	split proboscis; loss of one axoneme in sperm
Dalytyphloplanida	99	21	100	presence of small dense granules, an axonemal spur, a group of longitudinal microtubules in the sperm and a fine connection between nuclear and plasma membranes (?)
Neodalyellida	99	12	100	none
Neotyphloplanida	100	29	100	none
Thalassotyphloplanida	100	19	100	none

Tab. 6. Overview of support for newly defined clades.

For the monophyletic Kalyptorhynchia two clear apomorphies can be indicated: the presence of a muscular proboscis and the incorporation of the axonemes within the sperm body during spermiogenesis (see Ehlers, 1985; Watson, 2001). Within the Kalyptorhynchia, a split probocis and the loss of one axoneme during spermiogenesis (see Watson, 2001) characterises all representatives of the Schizorhynchia. As the Eukalyptorhynchia is still unresolved, a possible sister group for the Schizorhynchia cannot be indicated.

The sister taxon of the Kalyptorhynchia is still unclear, as it forms a polytomy with the Dalytyphloplanida and *Mariplanella frisia* in all phylogenetic studies based on 18S rDNA. However, the present study shows a relatively high support for a clade uniting *M. frisia* with the Dalytyphloplanida (see Fig.3: jackknife: 81%). Therefore, this clade probably is the sister group of the Kalyptorhynchia.

The taxa 'Dalyellioida' and 'Typhloplanoida' are clearly not monophyletic in our analysis, as suggested in earlier molecular studies (e.g. Littlewood et al., 1999a-b; Norén & Jondelius, 2002) and based on spermatological data (see Watson, 2001). The non-monophyly of the 'Typhloplanoida' is also corroborated by our constrained analyses. Instead, the 'Typhloplanoida' and the 'Dalyellioida', together with the temnocephalids, form a well supported 'mixed clade', the Dalytyphloplanida (see Fig. 6). The presence of small dense granules, an axonemal spur and a group of longitudinal microtubules (originating from a particular manner of flagellar rotation) in the sperm (see Littlewood et al., 1999a; Watson, 2001) are possible apomorphies for the Dalytyphloplanida. However, these features are secondarily lost within some thalassotyphloplanids (in all species formerly included in the Trigonostomidae, which were studied by Watson (2001); see further) and within the neodalyellids. It is possible that the loss of these features is synapomorphic for a subclade of the Thalassotyphloplanida and independently also for a subclade of the Neodalyellida. With the present data, which are still scarce for both 18S rDNA and spermatology, we therefore refrain from giving any possible relationships within the Neodalyellida and the Thalassotyphloplanida. Within the Dalytyphloplanida there is a clear sister group relationship between the Neodalyellida and the Neotyphloplanida, both of which are well supported clades (see Tab. 6).

A clade that coincides with our Neodalyellida was also found by Norén & Jondelius (2002). Our analysis clearly shows that, apart from the marine free-living (*Provortex* species) and symbiotic (*Anoplodium stichopi, Pterastericola australis* and *Graffilla buccinicola*) dalyellioids, included by Norén & Jondelius (2002), two former typhloplanoid taxa also are part of the Neodalyellida: the promesostomid *Einarella argillophyla* and the solenopharyngid *Trisaccopharynx westbladi*.

The Neotyphloplanida consists of a polytomy, including three large freshwater taxa (Temnocephalida, Typhloplanidae, Dalyelliidae), *Gaziella* sp. and the Thalassotyphloplanida. Littlewood et al. (1999 a-b) and Joffe & Kornakova (2001; but see also further) suggest that a freshwater rhabdocoel clade may exist

(Typhloplanidae + Dalyelliidae + Temnocephalida). We did not find such a clade (see arrows in Fig. 6), but there is some support for a clade consisting of the Typhloplanidae and the Dalyelliidae (see Fig. 3: jackknife: 66%). However, with only two species included in the analysis, the dalyelliids (as with the temnocephalids) are very poorly sampled. Based only on our molecular data, all we can say is that the Typhloplanidae (excl. *Styloplanella strongylostomoides*) and the Dalyelliidae, and probably the Temnocephalida, are each monophyletic freshwater taxa.

There is strong evidence from morphological data that the freshwater ectosymbiotic Temnocephalida is monophyletic, characterised by a split or splayed proximal end of the sperm shaft (Watson, 2001). Also Joffe et al. (1998) and Cannon & Joffe (2001) give several possible apomorphies (e.g. multisyncytial epidermis, posterior adhesive organ, ring-shaped protonephridial canals). A single ovary was considered to be characteristic for the Typhloplanidae, but *S. strongylostomoides* nor *M. frisia*, both with a single ovary, do not belong to the Typhloplanidae. Paired or unpaired gonads have always been considered of low taxonomic significance (see De Clerck & Schockaert, 1995). A morphology based cladistical analysis of the Typhloplanidae was performed by Hochberg (2004), but for reasons mentioned in the introduction (very small number of characters, errors in primary homology assessment; methodological flaws) these results are not considered here.

Within the Thalassotyphloplanida, which consists of marine species and one freshwater typhloplanoid species (*Styloplanella strongylostomoides*) representatives of the former typhloplanoid 'families' Trigonostomidae, Promesostomidae and Byrsophlebidae are found. The double connection in the female system has always been considered an apomorphy for the Trigonostomidae (see Ax & Heller, 1970; Ehlers, 1974; but put in doubt by Karling et al., 1972). As in our analysis, *Trigonostomum penicillatum* and *Mariplanella frisia*, both with a double connection, were not found in the same clade as the other trigonostomids (*T. denhartogi*, five *Proxenetes* species, two *Ptychopera* species; see Fig. 6). Watson (2001) states that the Trigonostomidae (observations on *Beklemischeviella contorta* (Beklemischew, 1927) Luther, 1943; *Ceratopera* sp.; *Proxenetes fasciger* Ehlers, 1974; *Ptychopera westbladi* (Luther, 1943) Den Hartog, 1964; *Trigonostomum setigerum* Schmidt, 1852) are 'remarkable for the uniformity of their sperm' (sic). Trigonostomid sperm is characterised by the absence of axonemes, the presence of basal bodies in a diplosomal formation during spermiogenesis (disappearing later) and the presence of a tightly woven microvillus network that may originate from the cytophore (Watson, 2001 and pers. comm.).

Some other studies on rhabdocoel interrelationships exist, two of them based on morphological characters (Jondelius & Thollesson, 1993; Zamparo et al., 2001), the other one is based on qualitative molecular markers (Joffe & Kornakova, 2001).

Both morphological studies suffer from a number of shortcomings. First, both studies include only a relatively small number of characters (21 and 89 respectively), which were extracted from literature and not checked on real specimens. Secondly, some primary homology assessments of Jondelius & Thollesson (1993) are erroneous (see Littlewood et al., 1999a). The same is also true for the data of Zamparo et al. (2001). For example, Zamparo et al. (2001) assigned the character state 'cirrus present' to all Promesostomidae and Trigonostomidae, whereas almost all representatives of these taxa have a stylet. Furthermore, higher-level taxa (family-level) are used as entries in the data matrix, but to score these taxa, only a very limited number of species are considered. Many high-level taxa, however, are highly diverse, and representing them by only a few representatives may bias any analysis. The studies of Jondelius & Thollesson (1993) and Zamparo et al. (2001) also do not include a number of taxa because they consider them to be non-monophyletic. Yet, there is a theoretical problem with this decision: how can one decide whether a taxon is monophyletic prior to the analysis?

Apart from their molecular analysis, Joffe & Kornakova (2001) propose certain short indels in the 18S rDNA fragment as possible homologies for certain taxa even if they are only one nucleotide long. This methodology is highly questionable for two main reasons: (1) it is highly dependent on the alignement and (2) only very few species were used to represent each higher-level taxon. Furthermore, Joffe & Kornakova (2001) state that 'short indels (one to two nucleotides) may appear independently and are therefore unreliable' (sic). However, Joffe & Kornakova (2001) postulated such short indels to be homologous for several clades, which are also found in our analysis: a one nucleotide long indel for the Typhloplanidae, a three nucleotides long indel for a clade consisting of the Dalyelliidae, Typhloplanidae and Temnocephalida (the 'freshwater' clade) and a two nucleotides long indel for a clade consisting of the Dalyelliidae, Typhloplanidae, Temnocephalida, Pterastericolidae and Graffillidae. Only for a clade consisting of the Pterastericolidae and the Graffillidae Joffe & Kornakova (2001) found four different long indels, one of which includes a five to six nucleotides long insertion. This clade could coincide with our Neodalyellida, as Joffe & Kornakova (2001) did not use sequences of any marine dalyellioid.

CONCLUDING REMARKS

1. The Rhabdocoela (Kalyptorhynchia + 'Typhloplanoida' + 'Dalyellioida') is monophyletic, but its sistertaxon cannot be indicated yet.

2. Monophyletic 'Typhloplanoida' and 'Dalyellioida' do not exist (also indicated by sperm morphology). Their representatives form 'mixed' clades

(Dalytyphloplanida, Neodalyellida, Neotyphloplanida and Thalassotyphloplanida).

3. The Kalyptorhynchia is monophyletic; also supported by the muscular proboscis and sperm morphology. Its sister taxon is the Dalytyphloplanida (+ *M. frisia*?).

4. The Schizorhynchia is monophyletic; also supported by the split proboscis and sperm morphology.

5. The freshwater Dalyelliidae and Typhloplanidae (and probably also the Temnocephalida) are monophyletic. None of the other 'families', so far included in the 'Dalyellioida' and the 'Typhloplanoida' emerge as monophyletic.

6. A clade of all freshwater Rhabdocoela does not appear so far, but denser sampling of the Dalyelliidae and of the Temnocephalida is needed.

7. The relationships of the taxon *Ciliopharyngiella* are still unresolved.

REMAINING PROBLEMS: THE FUTURE

Finding answers on the initial questions is not the only result of this analysis. As in most scientific studies a number of new problems and questions arises:

1. What are the relationships within the Kalyptorhynchia and within the Schizorhynchia?

2. What are the relationships within the Thalassotyphloplanida, in which none of the classic 'families' is found?

3. What are the relationships within the Neodalyellida?

4. What are the relationships of the three freshwater taxa within the Rhabdocoela (Typhloplanidae, Dalyelliidae and Temnocephalida)? Is there a 'freshwater' clade?

5. What is the sistertaxon of the Rhabdocoela?

Most, if not all, of these problems could be resolved by a much denser sampling of the Eukalyptorhynchia, the Schizorhynchia and the Dalytyphloplanida. Ideally, morphological aut- and synapomorphies should be searched for in order to better characterise the clades. To resolve the sister group relationships of the Rhabdocoela next to denser sampling also the use of other molecular markers is needed.

The molecular results can be used as a starting point for cladistical analyses based on morphology, which focus on parts of our cladogram.

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NEDERLANDSTALIGE SAMENVATTING

Inleiding

Dit werk behandelt de fylogenie van de Typhloplanoida Bresslau, 1933, een zeer soortenrijk taxon van vrijlevende platwormen ('Turbellaria'). Het taxon omvat een 450-tal soorten, die voorkomen in zowel mariene, brakwater- als limnische habitats. De naam Typhloplanoida is in de literatuur in twee verschillende betekenissen gebruikt. In de traditionele betekenis worden de Kalyptorhynchia (gekenmerkt door een gespierde proboscis) niet tot de Typhloplanoida gerekend. Ehlers (1985) doet dit echter wel en wanneer in deze betekenis gebruikt zal de afkorting s.l. (sensu lato) toegevoegd worden.

De Typhloplanoida worden momenteel ingedeeld in acht 'families', waarvan van geen enkele de monofylie aangetoond is: Byrsophlebidae Graff, 1905, Carcharodopharyngidae Bresslau, 1933, Ciliopharyngiellidae Ax, 1952, Kytorhynchidae Rieger, 1974, Promesostomidae Den Hartog, 1964, Solenopharyngidae Graff, 1882, Trigonostomidae Graff, 1905 en de Typhloplanidae Graff, 1905. Voor alle families wordt een overzicht van de belangrijkste beschrijvende literatuur gegeven en tevens worden de belangrijkste diagnostische kenmerken vermeld. De huidige systematische positie van de 'Typhloplanoida' wordt geschetst, vertrekkend van de eerste fylogenetische analyse van de Platyhelminthes door Ehlers (1985; zie Fig. 1), die gebaseerd was op morfologische (vnl. ultrastructurele) kenmerken en in mindere mate op embryologie en levenscycli. In dit systeem zijn de vrijlevende platwormen of 'Turbellaria' parafyletisch, terwijl de parasitaire Neodermata duidelijk monofyletisch zijn. De 'Typhloplanoida', alsook de 'Dalyellioida' werden in dit systeem aan de basis geplaatst van de Neodermata, hoewel beider monofylie betwijfeld werd en het zustertaxon van de Neodermata onduidelijk bleef. De Kalyptorhynchia werden door Ehlers in de 'Typhloplanoida' geplaatst. Uiteraard zijn sinds 1985 belangrijke aanvulligen op dit systeem aangebracht door het gebruiksvriendelijker worden van moleculaire technieken en het verbeteren van cladistische analysemethoden.

De interne verwantschappen van de 'Typhloplanoida' zijn echter nog grotendeels onopgelost, vermits recente moleculaire studies voornamelijk gericht zijn op: 1) oorspronkelijk basale taxa binnen de Platyhelminthes en 2) de relaties van en binnen de Neodermata en het ontstaan van parasitisme. Het is dan ook niet verwonderlijk dat tot nog toe de Rhabdocoela, die de 'Typhloplanoida', 'Dalyellioida', Kalyptorhynchia en Temnocephalida omvatten, nauwelijks aan bod kwamen in moleculair fylogenetische studies. De voornaamste resultaten betreffende de Rhabdocoela uit voorgaand moleculair onderzoek zijn: 1) de Kalyptorhynchia zijn monofyletisch en vormen waarschijnlijk de zustergroep van alle andere Rhabdocoela; 2) de 'Typhloplanoida' zijn inderdaad niet monofyletisch; 3) er is mogelijk een 'zoetwaterclade' die de drie grote zoetwatertaxa behorend tot de Rhabdocoela verenigt (Typhloplanidae, Dalyelliidae en Temnocephalida).

Tijdens het beschrijven van nieuwe soorten (zie Appendix: Artikels I-VII) werd duidelijk dat het traditionale Linneaanse systeem, en daarbij de traditionele nomenclatorische codes, te lijden hebben van een aanzienlijk aantal problemen wanneer dit systeem gebruikt wordt op de resultaten van een cladistische analyse. Daarom werd tijdens het benoemen van clades in ons cladogram gebruik gemaakt van een alternatieve methode, die categorische rangen weert en waarbij taxon namen gedefinieerd worden door te refereren naar een fylogenetische boom. De drie verschillende soorten definities (zie Fig. 2) worden kort besproken en een verantwoording voor het gebruik van 'stem-based' definities wordt gegeven.

Doelstellingen

De hoofddoelstelling van dit onderzoek is het nagaan van de relaties van en binnen de 'Typhloplanoida' aan de hand van moleculaire data (18S rDNA). Daarom werd een zo groot mogelijk aantal soorten behorend tot de 'Typhloplanoida' en de Kalyptorhynchia verzameld. Tevens werden ook andere taxa verzameld die gebruikt werden als 'outgroup' en werd de bekomen datamatrix aangevuld met bestaande sequenties uit GenBank. De belangrijkste vragen die we trachtten te beantwoorden zijn:

1. Is de 'Typhloplanoida' monofyletisch?
2. Vormen de drie grote zoetwatertaxa binnen de Rhabdocoela (Typhloplanidae, Dalyelliidae en Temnocephalida) één monofyletisch taxon?
3. Wat zijn de relaties van de verschillende typhloplanoide subtaxa (= 'families')?
4. Wat is de relatie van de Kalyptorhynchia met de andere taxa?
5. Wat is de positie van het enigmatische taxon *Ciliopharyngiella*?

Het beschrijven van nieuwe soorten, die opdoken tijdens het verzamelen van taxa voor DNA-analyses, werd een belangrijke nevendoelstelling van deze studie.

Oorspronkelijk werd met dit onderzoek gestart vanuit een 'total-evidence' benadering, m.a.w. er werd getracht morfologische en moleculaire kenmerken te combineren in het uitzoeken van de relaties binnen de 'Typhloplanoida'. Dit werd echter bemoeilijkt door het ontbreken van materiaal voor heel wat taxa, die bovendien nog moeilijk te verzamelen waren. Daarenboven was het vaststellen van primaire homologieën in zo een divers en slecht gekend taxon bijzonder moeilijk, zo niet onmogelijk. Bijgevolg werd dan ook geopteerd enkel een moleculaire analyse uit te voeren en achteraf te trachten de clades te karakteriseren met morfologische data uit de literatuur, wat slechts ten dele mogelijk bleek.

Methodes

Een uitgebreide bespreking van de methodes, gebruikt voor het bekomen van de sequentiedata en het verwerken van deze data in een fylogenetische analyse vallen buiten het bestek van deze samenvatting. Alle gebruikte methodes, met protocols, referenties en eventuele verantwoordingen voor het gebruik van bepaalde methodes kunnen in dit hoofdstuk teruggevonden worden. Bovendien wordt de basis van de Bayesiaanse analysemethode, die gebruikt werd naast de parsimoniemethode, uitgelegd. Deze statistische methode is immers erg complex en heeft slechts zeer recent zijn intrede gedaan in het fylogenetisch onderzoek.

De fylogenetische analyse werd uitgevoerd met behulp van parsimonie en Bayesiaanse methoden, terwijl drie verschillende waarden ('Bremer support', Jackknife en posterieure probabiliteiten) berekend werden die een idee geven van de ondersteuning van de clades in het cladogram.

Resultaten

De belangrijkste resultaten kunnen als volgt samengevat worden en bestaan uit twee delen.

Het taxonomisch gedeelte is een kort overzicht van zeven beschrijvende artikels, die toegevoegd werden als appendix. Daarin worden in totaal 43 nieuwe soorten beschreven (zie Tab. 4), waarvan 29 'Typhloplanoida', 12 Polycystididae (Kalyptorhynchia) en twee 'Dalyellioida'.

De fylogenetische analyse omvat in totaal 60 soorten Rhabdocoela en 104 soorten behorend tot alle belangrijke platwormtaxa. Zesenveertig nieuwe 18S rDNA-sequenties werden gedeponeerd in GenBank (zie Tab. 1). De uiteindelijke datamatrix bestond uit 164 taxa en 1793 karakters. In het resulterende cladogram (zie Figs 3 en 5), dat voor beide methoden (parsimonie en Bayesiaanse analyse) identiek is, zijn de Rhabdocoela duidelijk monofyletisch, hoewel een mogelijke zustergroep niet gevonden werd. Binnen de Rhabdocoela (zie Fig. 6) vormen de monofyletische Kalyptorhynchia het zustertaxon van alle andere rhabdocoelen, vanaf hier Dalytyphloplanida genoemd. De Dalytyphloplanida omvat alle vertegenwoordigers van de voormalige 'Dalyellioida' en 'Typhloplanoida', maar geen van beide blijkt monofyletisch en er duiken dus zogenaamde 'mengclades' op. Geen van de 'families' uit de traditionele taxonomie is monofyletisch en binnen de Dalytyphloplanida vormen twee 'mengclades' (elk omvatten ze zowel 'Dalyellioida' als 'Typhloplanoida') elkaars zustergroep: de Neodalyellida en de Neotyphloplanida. De Neotyphloplanida bestaan uit een polytomie die gevormd wordt door de Temnocephalida, de Dalyelliidae, de Typhloplanidae, *Gaziella* sp. en een clade die alle mariene 'Typhloplanoida' (= Thalassotyphloplanida) omvat. De positie van het enigmatische taxon *Ciliopharyngiella* blijft onzeker.

Definitie van clade namen

Als resultaat van de fylogenetische analyse werden tevens drie bestaande (Rhabdocoela, Kalyptorhynchia, Schizorhynchia) en vier nieuwe namen (Dalytyphloplanida, Neodalyellida, Neotyphloplanida, Thalassotyphloplanida) gedefinieerd volgens de regels van de Phylocode. De geconverteerde namen worden aangeduid met n.c.c. ('nomen cladum conversum'), terwijl de nieuw gedefinieerde namen aangeduid worden met n.c.n. (nomen cladum novum'). Alle definities zijn 'stem-based'. De taxa kunnen gevonden worden in de cladogrammen in Figuren 3 en 6.

Rhabdocoela Ehrenberg, 1831 n.c.c.: de meest omvattende clade die *Polycystis naegelii* Kölliker, 1845 bevat, maar niet *Ciliopharyngiella constricta* Ax, 1952, *Monocelis lineata* (Müller, 1774) Oersted, 1844, *Nematoplana coelogynoporoides* Meixner, 1938, *Fasciola hepatica* Linnaeus, 1758 en *Pseudostomum quadrioculatum* (Leuckart, 1847) Graff, 1911.

Kalyptorhynchia Graff, 1905 n.c.c.: de meest omvattende clade die *Polycystis naegelii* Kölliker, 1845 bevat, maar niet *Mariplanella frisia* Ax & Heller, 1970 en *Provortex balticus* (Schultze, 1851) Graff, 1882.

Schizorhynchia Meixner, 1928 n.c.c.: de meest omvattende clade die *Schizochilus marcusi* Boaden, 1963 bevat, maar niet *Polycyctis naegelii* Kölliker, 1845.

Dalytyphloplanida n.c.n.: de meest omvattende clade die *Provortex balticus* (Schultze, 1851) Graff, 1882 bevat, maar niet *Mariplanella frisia* Ax & Heller, 1970 en *Polycystis naegelii* Kölliker, 1845.

Neodalyellida n.c.n.: de meest omvattende clade die *Provortex balticus* (Schultze, 1851) Graff, 1882 bevat, maar niet *Proxenetes flabellifer* Jensen, 1878.

Neotyphloplanida n.c.n.: de meest omvattende clade die *Proxenetes flabellifer* Jensen, 1878 bevat, maar niet *Provortex balticus* (Schultze, 1851) Graff, 1882.

Thalassotyphloplanida n.c.n.: de meest omvattende clade die *Proxenetes flabellifer* Jensen, 1878 bevat, maar niet *Castrada lanceola* (Braun, 1885) Luther, 1904 en *Castrella truncata* (Abildgaard, 1789) Hofsten, 1907.

Discussie

In de algemene discussie wordt getracht een aantal van de clades te karakteriseren aan de hand van morfologische, de meeste ultrastructurele kenmerken (vnl. op basis van spermatologie). Deze EM-data moeten echter met de nodige voorzichtigheid benaderd worden, vermits de gegevens fragmentarisch zijn. Een overzicht van de ondersteuning op basis van onze moleculaire gegevens wordt gegeven in Tab. 6., samen met een aantal (mogelijke) apomorfieën.

Voor het taxon Rhabdocoela (sterk ondersteund door moleculaire data) kon geen duidelijke apomorfie gevonden worden, hoewel verschillende karakters mogelijke apomorfieën vormen, zoals bvb. de 'pharynx bulbosus', die echter ook voorkomt bij de parasitaire neodermaten, sommige prolecithophoren en de lecithoepitheliaten. Enkele andere mogelijke apomorfieën, op basis van ultrastructureel onderzoek van zowel sperma als protonephridiën zijn voorhanden.

De Kalyptorhynchia, die het zustertaxon van alle andere Rhabdocoela (= Dalytyphloplanida) vormt, heeft als apomorfie de aanwezigheid van een proboscis en de incorporatie van de axonemata tijdens de spermiogenese. De vertegenwoordigers van de Schizorhynchia, een deeltaxon van de

Kalyptorhynchia, bezitten een gespleten proboscis en sperma waarin één van de axonemata verloren ging.

De taxa 'Typhloplanoida' en 'Dalyellioida' zijn duidelijk niet monofyletisch en vormen samen met de Temnocephalida het taxon Dalytyphloplanida. Mogelijke apomorfieën kunnen gevonden worden in de ultrastructuur van het sperma. Voor geen enkel ander taxon dat gedefinieerd werd kon een duidelijke morfologische apomorfie gevonden worden.

Conclusies

1. De Rhabdocoela (Kalyptorhynchia + 'Typhloplanoida' + 'Dalyellioida') is monofyletisch, maar haar zuster taxon werd nog steeds niet gevonden.
2. Zowel de 'Typhloplanoida' als de 'Dalyellioida' is niet monofyletisch. Vertegenwoordigers van beide taxa vormen zogenaamde 'mengclades'.
3. Het taxon Kalyptorhynchia is monofyletisch, wat ondersteund wordt door duidelijke apomorfieën (proboscis, ultarstructuur sperma).
4. Het taxon Schizorhynchia is monofyletisch, wat ondersteund wordt door duidelijke apomorfieën (gespleten proboscis, ultarstructuur sperma).
5. De zoetwater taxa Dalyelliidae en Typhloplanidae (en waarschijnlijk ook het taxon Temnocephalida) zijn monofyletisch. Van geen enkele andere 'familie' uit de traditionele taxonomie kon de monofylie aangetoond worden.
6. Een zogenaamde 'zoetwaterclade', bestaande uit alle zoetwater-rhabdocoelen (Dalyelliidae, Typhloplanidae en Temnocephalida) komt niet voor in ons cladogram.
7. De relaties van het taxon *Ciliopharyngiella* zijn nog steeds onopgelost.

Resterende problemen

Als bij elk onderzoek doken ook hier een aantal nieuwe vragen op. In dit geval ligt de belangrijkste oorzaak bij het feit dat voor een aantal clades duidelijk te weinig soorten gebruikt werden om een betere resolutie van de verwantschappen toe te laten. De belangrijkste vragen die zich aandienen zijn:

1. Wat zijn de verwantschappen binnen de Kalyptorhynchia en binnen de Schizorhynchia?
2. Wat zijn de relaties binnen de Thalassotyphloplanida?
3. Wat zijn de relaties binnen de Neodalyellida?
4. Wat zijn de relaties tussen de drie grote zoetwatertaxa binnen de Rhabdocoela?
5. Wat is het zustertaxon binnen de Rhabdocoela?

Vooral voor het beantwoorden van de vragen 1 tot 4 moet het aantal soorten in toekomstige analyses uitgebreid worden. De vragen 1 en 5, en gedeeltelijk ook vraag 3 behoorden eigenlijk niet tot het oorspronkelijke opzet van dit onderzoek.

APPENDIX: ARTICLES AND MANUSCRIPTS

I

Zoological Journal of the Linnean Society, 2004, **141**, 271–296. With 11 figures

Revision of *Trigonostomum* Schmidt, 1852 (Platyhelminthes, Typhloplanoida, Trigonostomidae) with the description of seven new species

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A morphological and taxonomic account of the genus *Trigonostomum* is provided. All known species are discussed and briefly re-described where necessary. Seven new species are described: ***T. franki*** from Curaçao, Florida (USA), the East African Coast and New Caledonia, ***T. nataschae*** from the French sub-Antarctic island Kerguelen, ***T. spinigerum*** from New Caledonia and ***T. watsoni*** from the Australian East Coast and New Caledonia. ***T. tori*** and ***T. galapagoensis***, both formerly enclosed in *T. setigerum*, are considered new species, while ***T. australis*** also belongs to the same species group. *Proxenetes denhartogi* is transferred to *Trigonostomum*. Based on a comparison of old and new material, *T. marki* is synonymized with *T. penicillatum*, while *T. prytherchi* and *T. divae* are regarded as junior synonyms of *T. lilliei*; *T. intermedium* and *T. quadrifolium* are considered synonyms of *T. coronatum*. Three species are considered *species inquirendae*: *T. brunchorsti*, *T. piriforme* and *Marinellia lingulifera*. Similarities and differences of the 17 valid species are discussed and summarized in an identification key. © 2004 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2004, **141**, 271–296.

ADDITIONAL KEYWORDS: identification key – morphology – phylogenetic nomenclature – taxonomy – Trigonostominae.

INTRODUCTION

The genus *Trigonostomum* was introduced by Schmidt (1852) for a single species, *T. setigerum*. Thirty years later, Graff (1882) changed the name to *Hyporhynchus* and added a new species, *H. coronatus*. He also brought three previously described species – *Kylosphaera armata* Jensen, 1878, *Orcus venenosus* Uljanin, 1870 and *Vortex penicillatus* Schmidt, 1857 – into the genus and considered *Spiroclytus nisus* Schmidt, 1857 and *S. euryalus* Schmidt, 1857 to be synonyms of *Hyporhynchus setigerus*. The number of species grew rapidly in the late 19th and early 20th centuries (Uljanin, 1870; Pereyaslawzewa, 1893; Attems, 1897; Graff, 1905, 1911a, b, 1913) most of them described as belonging to *Hyporhynchus*, although Graff (1905) changed *Hyporhynchus venenosus* (Uljanin, 1870) into *Hyporcus venenosus*. Eventually, Meixner (1924b) re-introduced the first name *Trigonostomum* Schmidt, 1852, and also considered *Woodsholia lilliei* Graff, 1911, to be a species of *Trigonostomum*.

When changing the name to *Hyporhynchus* in 1882, Graff placed the genus within the subfamily Pseudorhynchina of the family Proboscida Carus, 1863. All members of this 'family' were characterized by the presence of a 'proboscis', a muscular frontal organ for capturing prey. In 1905, Graff changed the name Proboscida to Kalyptorhynchia, enclosing four families: Trigonostomidae, Schizorhynchidae, Polycystididae and Gyratricidae. However, in a detailed study of the proboscis of several Kalyptorhynchia, Meixner (1924b, 1926) showed that the 'proboscis' of *Trigonostomum* is by no means the same structure as that found in the other Kalyptorhynchia. He therefore transferred the Trigonostomidae from the Kalyptorhynchia to the Euliporhynchia Graff, 1905, which also included the families Proxenetidae Graff 1908, Byrsophlebidae Graff, 1905 and Typhloplanidae

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Luther, 1904. Two decades later, Luther (1948) grouped the Proxenetidae within the Trigonostomidae. Den Hartog (1964) split this taxon again, this time into Promesostomidae and Trigonostomidae, based on the structure of the female genital system: the Trigonostomidae are characterized by female gonads connected with the genital atrium by two ducts. Within the Trigonostomidae Den Hartog (1964) recognized two taxa, Trigonostominae and Paramesostominae, while a third, Mariplanellinae, was added by Ax & Heller (1970). The Trigonostominae comprises 11 genera, the phylogenetic relationships of which were described by Ax (1971), who considered *Marinellia lingulifera* Riedl, 1954 to be the sister taxon of *Trigonostomum*.

Due to the detailed studies by Graff (1913) and Meixner (1924b), the general anatomy of *Trigonostomum* is well known. The anatomy of a number of other representatives of the family, in particular that of the genital system, has been thoroughly described by Luther (1943). In the morphological section we summarize the essentials of the anatomy of representatives of *Trigonostomum*.

Not all described species are easy to recognize, often due to inaccurate descriptions and poor figures, especially in the older literature. The main purpose of this revision is to elucidate the species' identities and synonyms. A neotype is designated for each of the following species – *T. armatum*, *T. breitfussi*, *T. coronatum*, *T. lilliei*, *T. mirabile*, *T. penicillatum*, *T. setigerum* and *T. venenosum* – as a name-bearing type was never designated for any of them. Data for these species can be found in the taxonomy section, and all conditions for considering the designation of a neotype are fulfilled (ICZN, 1999: Art. 75). One new combination (*T. denhartogi*) and seven new species are added, three of them resulting from the analysis of the polymorphic '*T. setigerum*'. Since their anatomy is uniform, the descriptions of the separate species focus on the hard parts of the copulatory organ and the bursal appendage, with additional remarks where needed. A separate section provides a comparison of the species and the taxonomic conclusions resulting from this analysis, and includes an identification key, replacing the differential diagnoses.

Some preliminary indications of relationships within *Trigonostomum* are mentioned, but we refrain from discussing in depth possible phylogenetic relationships both within the taxon and with other taxa within the Trigonostomidae. A cladistic analysis of the Typhloplanoida, based on morphological and molecular characters, is currently in progress. Consequently, each diagnosis given now should not be seen as a phylogenetic definition (De Queiroz & Gauthier, 1990, 1992, 1994). However, as, in our view, species are fundamentally different from higher taxa because they cannot be monophyletic in a Hennigian sense (see Nixon & Wheeler, 1990), we propose for every species an alternative to the Linnean binomen, which can be used within the framework of phylogenetic nomenclature. This alternative species name consists of a praenomen and epithet, connected with a hyphen, and is unchangeable (see Cantino *et al.*, 1999; Artois, 2001). In the taxonomy section, the species are listed in alphabetical order.

MATERIAL AND METHODS

The zoological collection of Limburgs Universitair Centrum (LUC), Diepenbeek, Belgium, contains very rich material of both new and known *Trigonostomum* species from many parts of the world, collected by the authors (Australia, New Caledonia, Curaçao, Florida, East Africa, Zanzibar, French Mediterranean coast, Greece, Sardinia, Sweden, Kerguelen, and the Weddell Sea) and by some earlier collaborators of the Diepenbeek group: Corsica (Dr P. Martens), Kenya (Dr P. Jouk and Mr G. De Clerck). Material from the Swedish Museum of Natural History (SMNH) and of the Smithsonian Institution (SI-NMNH) was also at our disposal. Of the following species no material is available: *T. brunchorsti* Graff, 1905, *T. intermedium* (Attems, 1897) Graff, 1913, *T. marki* Graff, 1911, *T. piriforme* (Pereyaslawzewa, 1893) Graff, 1905 and *Marinellia lingulifera* Riedl, 1954.

Animals were extracted from sediment or from algae using the $MgCl_2$ decantation method (see Schockaert, 1996), studied while live and mounted in lactophenol. If sufficient specimens were available, some were fixed in hot Bouin's solution, embedded in paraffin and serially sectioned. The 4 µm-thick sections were stained with Heidenhain's iron haematoxylin using eosin as a counterstain. Camera lucida drawings of the hard parts were made with Nomarski phase contrast microscopy on a Reichert Polyvar microscope. Drawings without a scale are freehand. Measurements of the (inner) stylet and the bursal appendage are taken along the axis, unless indicated otherwise in the text. The length of the whole copulatory organ is given as a top–bottom measurement; it is not measured for species where the organ is coiled as the spires can spread, causing a great degree of variability in such a measurement. The positions of the gonopore and organs are expressed in percentages of the total body length (distance from the anterior tip of the body).

All material (including type material), except that from Australia and that belonging to other institutions, is deposited in LUC. The type material of the Australian species (*T. australis* and *T. watsoni* spp. nov.) is deposited in the collection of the Queensland Museum, Brisbane, Australia.

GENERAL MORPHOLOGY

Mature *Trigonostomum* species are 0.5–1.5 mm long, mostly uncoloured with a whitish-yellowish hue. Some individuals of species of the *T. setigerum* species group have a spot of black reticulated epidermal pigment (reflecting white under incident light; Fig. 8I) and a mid-dorsal pigment stripe of a subepidermal brown pigment (Fig. 8B, G); other individuals may have a spot on the head (Fig. 8A). There is always one pair of eyes, but sometimes each eye is divided into two parts, giving the impression that there are two pairs of eyes (Fig. 8A–B, I). At the rostral tip two bundles of rhabdites are present, the glands (Fig. 1: rg) which are found in front and next to the pharynx. Rostrally there is also a tuft of sensory bristles (Fig. 1: ac). The epidermis is cellular, completely ciliated, and contains many rhabdites.

Trigonostomum species have a 'proboscis', which is an antero-ventral integumental invagination with a highly developed system of retractors and dilators, described by Meixner (1924b). In the live animal it is triangular (Fig. 1: i) and not always clearly visible in a mounted specimen. Retractors (Fig. 2: rr) insert on the dorsal and lateral body wall, causing the invagination. The opening of the invagination can be enlarged by several dilators, inserting on the ventral body wall in front of it (Fig. 2: dil1) and near the mouth (Fig. 2: dil2), probably with the help of integument retractors (Fig. 2: ir), with the origin on the ventro-caudal side of the opening and insertion on the dorsal body wall. The 'proboscis' can be evaginated by contraction of the strong circular muscles of the body wall in the frontal part of the animal, causing a local rise in the internal body pressure. A bundle of eosinophilic glands enters near the opening (Fig. 2: ig). The epithelium of the invagination appears to be glandular and contains a few small nuclei. In *Marinellia lingulifera*, there seems to be a delimited muscle mass behind the invagination (Riedl, 1954).

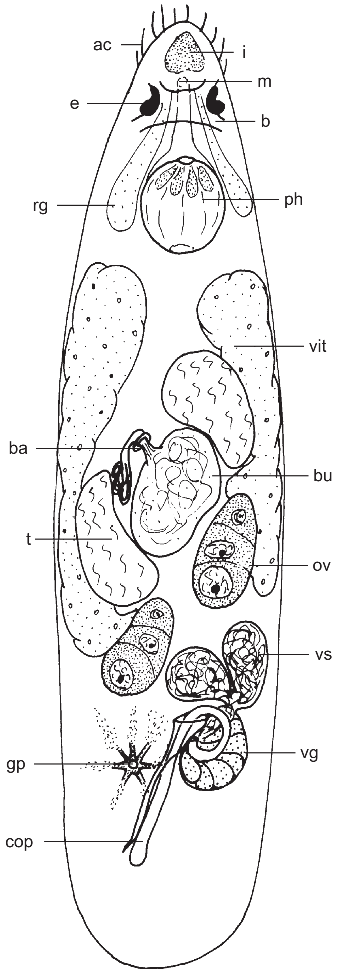

Figure 1. Habitus of live animal (*Trigonostomum venenosum*). *Abbreviations*: ac, apical tuft of cilia; b, brain; ba, bursal appendage; bu, bursa; cop, copulatory organ; e, eye; gp, common genital pore; i, antero-ventral invagination; m, mouth; ov, ovary; ph, pharynx; rg, rostral rhabdite glands; t, testis; vg, prostate vesicle; vit, vitellary; vs, seminal vesicle.

The pharynx (Fig. 1: ph; Fig. 2) is of the so-called 'bulbosus'-type and is situated in the first half of the body, highly inclined forwards and almost parallel to the ventral body wall. In section it is more or less barrel-shaped. The mouth is close to the anterior invagination, but never combined with it, though this impression may be given in highly contracted animals (Graff, 1911b; Meixner, 1924b). The pharynx cavity is tubiform and muscular at its distal end (with inner circular and outer longitudinal muscles), and lined with a low, anucleated epithelium with short cilia. The pharynx lumen has a low epithelium with only a few nuclei. The distal rim of the pharynx is devoid of cilia. The internal musculature of the pharynx bulb consists of 13 very thick outer circular muscle fibres (in *T. franki* and *T. setigerum*) and a continuous layer at the proximal and distal ends (Fig. 2: ecm), while circular (Fig. 2: icm) and longitudinal (Fig. 2: ilm) muscles around the lumen are much weaker. In the longitudinal sense, there are 11 radial muscles (of which the first is stronger than the other ten) and a fan-like group at the proximal end (Fig. 2: rm). In cross-section, there are 24 such radial muscles, insert-

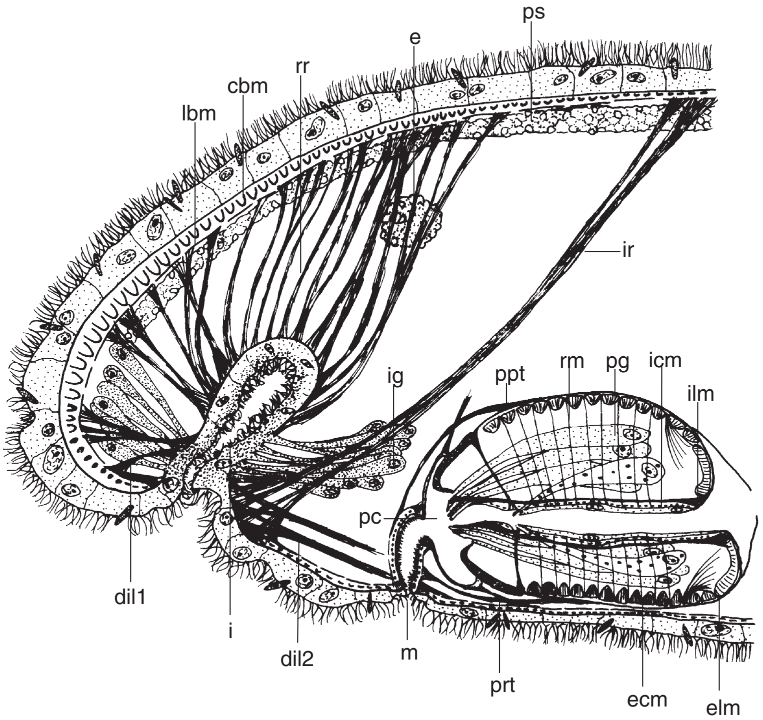

Figure 2. Reconstruction on sagittal sections of the rostral body end from the left hand side (***T. franki* sp. nov.**). *Abbreviations:* cbm, circular body muscles; dil1–2, dilators of opening of antero-ventral invagination; e, eye; ecm, external circular pharyngeal muscles; elm, external longitudinal pharyngeal muscles; i, antero-ventral invagination; icm, internal circular pharyngeal muscles; ig, invagination glands; ilm, internal longitudinal pharyngeal muscles; ir, integument retractors; lbm, longitudinal body muscles; m, mouth; pc, prepharyngeal cavity; pg, pharynx glands; ppt, pharynx protractors; prt, pharynx retractors; ps, pigment stripe; rm, radial pharyngeal muscles; rr, retractors of antero-ventral invagination.

ing on the pharynx lumen between 24 longitudinal muscles. Fine-grained eosinophilic glands enter the pharyngeal lumen at its distal end and coarse-grained basophilic ones more proximally. There are 16 of each.

The gonads are paired. The testes are positioned mid-body, just behind the pharynx. Ovaries and vitellaria are not separated, forming a pair of so-called 'ovovitellaria'. The vitellarian parts extend at both sides of the body from the level of the pharynx, and the ovaries are positioned slightly in front of the gonopore, situated at about 80%. The common genital atrium (Fig. 3: cga) is lined with a high, nucleated epithelium and surrounded by outer longitudinal and inner circular muscles, forming a sphincter at the porus.

The seminal vesicles (Fig. 3: vs) are paired, lined with a low epithelium without nuclei, and surrounded by spirally running muscles. Both vesicles narrow and join to form the short seminal duct (Fig. 3: sd), which enters the prostate vesicle (Fig. 3: vg) and runs axially through it, surrounded by mainly eosinophilic and some basophilic prostate glands, all parts of which are inside the vesicle. In some live individuals extracapsular parts of these glands can be seen. Relatively strong circular muscles surround the prostate vesicle. The male atrium, where the hard parts of the copulatory organ lie, is surrounded by an inner circular and an outer longitudinal muscle sheath, and enters the common genital atrium dorsally.

The copulatory organ proper consists of a stylet, surrounded by a second, very thin-walled stylet, called the 'mantle' by Ax (1971). The organ can thus be considered as double-walled with an inner 'stylet' (Fig. 4: s) and an outer 'mantle' (Fig. 4: mt), as shown in the theoretical diagram in Figure 4A.

In the species of the first group (see comparison of species, below) the mantle is attached to one side of

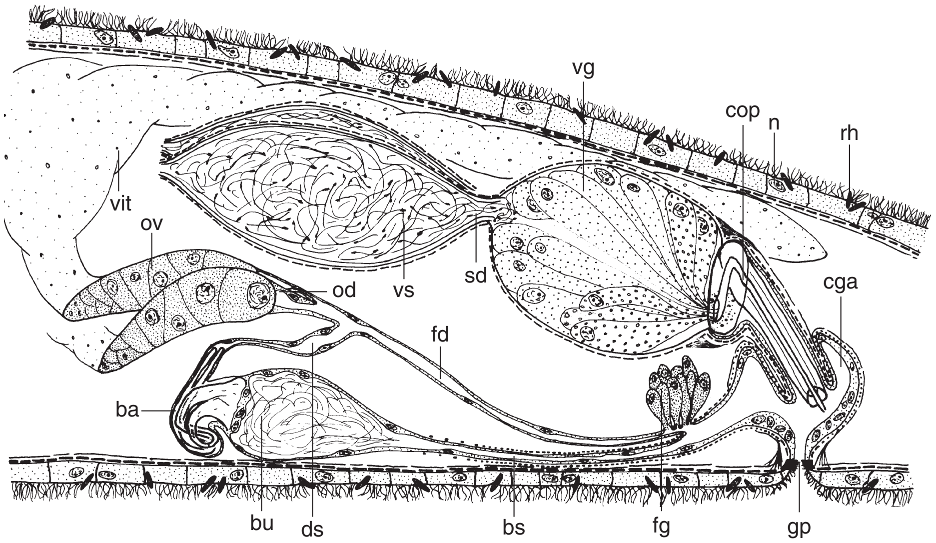

Figure 3. Reconstruction on sagittal sections of the genital system from the left hand side (***T. franki* sp. nov.**). *Abbreviations*: ba, bursal appendage; bs, bursal stalk; bu, bursa; cga, common genital atrium; cop, copulatory organ; ds, spermatic duct; fd, female duct; fg, female glands; gp, common genital pore; n, nucleus; od, oviduct; ov, ovary; rh, rhabdite; sd, seminal duct; vg, prostate vesicle; vit, vitellary; vs, seminal vesicle.

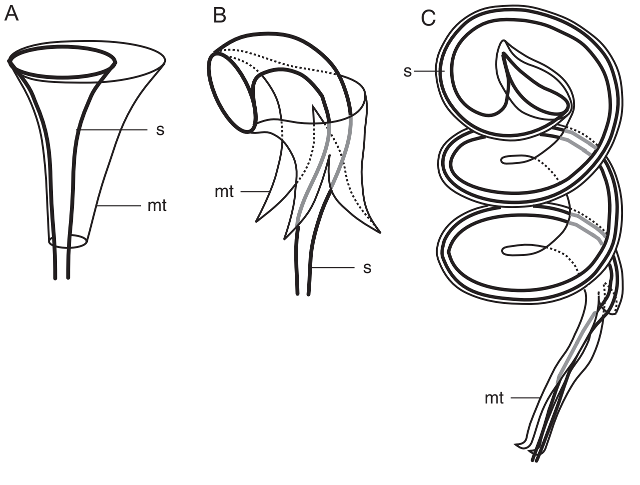

Figure 4. Schematic representation of the copulatory organ. A, theoretical diagram of a double-walled copulatory organ. B, diagram of the copulatory organ of the representatives of group 1. C, diagram of the stylet of the representatives of group 2. *Abbreviations*: mt, mantle; s, stylet.

the proximal rim of the stylet and distally divided into spiny plates (Fig. 4B). The stylet is a narrow tube, proximally curved over 90–270°, with a relatively thick wall and a broad proximal opening. In the species of the second group, the mantle follows the coils of the stylet over almost its entire length, forming two spiny plates at the very end (Fig. 4C). In sectioned material of *T. setigerum*, the way the mantle surrounds the stylet can be clearly seen. In *T. denhartogi* and in *T. spinigerum* the copulatory organ is far more complicated than in the other species, and the structure of the mantle is not yet well understood.

According to Meixner (1924b), the prostate secretion is discharged in the mantle, while sperm is released in the stylet. Observations on sectioned material of *T. franki*, *T. armatum* and *T. setigerum* revealed that both sperm and secretion are released through the (inner) stylet.

The ovaries are connected to the genital atrium by an efferent duct and an afferent system (see Luther, 1943). The oviducts are very short and lined with a nucleated epithelium. They join at the 'fecundatorium' (Luther's terminology) from where the (efferent) female duct (Fig. 3: fd) runs towards the atrium. This duct is long and narrow, lined with a low, nucleated epithelium and muscles are lacking. It enters the common genital atrium anteriorly, ventrally of the male system and dorsally of the afferent system. Eosinophilic glands (Fig. 3: fg) open into the female duct near the atrium. The afferent system consists of a bursal stalk (Fig. 3: bs), the bursa (Fig. 3: bu) and the sclerotic appendage (Fig. 3: ba) that leads into the spermatic duct (or insemination duct; Fig. 3: ds). This duct ends near the ovaries in the 'fecundatorium'. The bursal stalk is long and narrow (although short in *T. penicillatum* according to Meixner, 1924b). The whole afferent system is lined with a low nucleated epithelium. Only the bursal stalk is surrounded by (circular) muscles. There is no uterus.

Meixner (1924b) recognized two types of bursal appendage: (1) the *setigerum* type consists of two more or less coiled narrow tubes, attached to a ring- or funnel-like part at the bursa wall; (2) the *penicillatum* type consists of a bundle of many closely adhering tubules, which protrude into the bursa for some distance, where they may be held together by a casing. There are further variations, discussed below.

All *Trigonostomum* species are marine and are found all over the world, in areas ranging from tropical to polar. Most have been found in the intertidal zone, mainly on algae or seagrasses. Some species also occur in sand. Only two species, *T. messoplanoides* Artois, Vermin and Schockaert, 2000 and *T. nataschae* sp. nov., have not so far been found on algae (see further), while *T. messoplanoides* and *T. setigerum* are the only species collected at greater depths: 499–515 m and 137–150 m respectively (Westblad, 1952; Artois *et al.*, 2000).

COMPARISON OF THE SPECIES

The main differences between the species of *Trigonostomum* are to be found in the construction of the hard parts of the copulatory organ, the bursal appendage and, to a lesser extent, in the pigmentation.

As mentioned above and diagrammatically represented in Figure 4B, C, the copulatory organ consists of a proximally curved stylet surrounded by a thin-walled mantle, divided into spiniform plates. In the first group of species, the proximal rim of the mantle does not follow the curvature of the stylet, and thus only surrounds the distal (straight) part of the stylet (Fig. 4B). In two of these species (group 1A, *T. messoplanoides* and *T. venenosum*, see Fig. 6) the stylet is long and narrow and makes a proximal turn of 270°, while the mantle forms a narrow ring and bears one long, slender spine (spoon-like in *T. venenosum*). This kind of organ resembles that of species of the genus *Messoplana* Den Hartog, 1966.

The bursal appendage of *T. messoplanoides* and *T. venenosum* consists of two tubes attached to the bursa by a more or less conspicuous ring, and may be fused proximally in some individuals of *T. venenosum* (Fig. $6A_4$). In the construction of both the copulatory organ and the bursal appendage, these species demonstrate plesiomorphy, as found in several other taxa of the Trigonostominae (see Ax, 1971).

In the other species of this first group (group 1B; see Fig. 7), the stylet is relatively wider (and shorter) and makes a turn of about 90° in *T. coronatum*, *T. penicillatum* and *T. watsoni* to about 180° in the other species (*T. breitfussi*, *T. lilliei*, *T. mirabile* and *T. nataschae*). The mantle is divided into three pointed plates in the first three species, into two plates in *T. mirabile* and *T. nataschae*, and forms only one plate in *T. breitfussi* and *T. lilliei*. Some other, more subtle differences are seen: the plate is rather blunt in *T. breitfussi* and shorter than the stylet, which has a crested proximal rim, while in *T. lilliei* the plate ends in a little hook and is as long as the stylet, which in this case lacks the crested rim. In *T. nataschae*, the two plates are blunt and shorter than the stylet, while they are pointed in *T. mirabile* and at least as long as the stylet. In both these species the stylet has a wide spoon-like terminal opening, but in *T. nataschae* it has a proximal crested rim, not present in *T. mirabile*. Differences between the copulatory organs of *T. coronatum* and *T. penicillatum* are difficult to discern, while the plates in *T. watsoni* are very thin and easily folded. Moreover, in this last species, the stylet is inconspicuous and often impossible to find in some individuals. However, the bursal appendage in these three species is very different, as is the overall size of the copulatory organ, thus enabling clear distinction between the species.

The bursal appendage in a number of species of group 1B is clearly of Meixner's *penicillatum* type. It consists of many closely adhering parallel tubules, which protrude into the bursa for some distance, where they may be held together by a casing resembling a little barrel, connected to the bursa wall by a ring. This kind of appendage is found in *T. breitfussi, T. mirabile* and in *T. penicillatum*, where the tubules diverge at the very end, giving the appendage a brush-like appearance, but probably also in *T. mirabile* (see fig. 27 in Pereyaslawzewa, 1893). This brush-like appearance may be an artefact caused by squeezing the animals, especially when the tubules are long, as is the case in these two species.

In *T. nataschae* very narrow tubules protrude into the bursa beyond the proximal 'barrel' that has a rather thick wall. Just outside the bursa the tubules are still visible, but more distally they are very faint and separated into two coiled bundles, giving the impression that the bursal appendage ends in two broad tubes. The bursal appendage of *T. lilliei* has a highly coiled single part that ends in six tubules; the proximal 'barrel' seems to be missing, but there is a proximal ring at the inside of the bursa wall, beyond which the tubules protrude into the bursa (see also fig. 18 in Marcus, 1948 of *T. divae* = *T. lilliei*). The appendage of *T. coronatum*, on the other hand, has a proximal part that looks like a clover-leaf, and one, sometimes two (perhaps three: see fig. 23 in Attems, 1897) broad terminal 'tubes' with a fine striation. In *T. watsoni* the bursal appendage starts at the bursa with a broad funnel that splits into two highly coiled broad tubes, also with a fine striation, suggesting that they may consist of subtubules.

Though the bursal appendage of the species in this group exhibits variation (which needs further investigation), it clearly deviates in structure from that of all other members of the taxon *Trigonostomum* and even of all other taxa of the Trigonostominae. Perhaps this is a synapomorphy.

In a second group of species (group 2; see Fig. 10), the proximal mantle rim closely adheres to the proximal rim of the stylet, envelopes the stylet over its entire length, and diverges at the very end into two spiny plates with a terminal hook (see Fig. 4C). The narrow stylet is spirally coiled (except in *T. franki*, but see below) and the mantle follows the coils of the stylet. The stylet is at the periphery of the spire, while the mantle forms a double plate towards the centre, where it can be slightly thickened (wrongly called *Sekretrorh* by Graff, 1913) and shows radial wrinkles. At the very end, where the two plates are situated, there may be a straighter part of the stylet. The hooks of the plates are invariably directed towards the convex side of the curve. This combination of characters, especially the fact that the mantle entirely envelopes the stylet, is unique within the Trigonostominae and is without any doubt a synapomorphy for this group of species. On the other hand, the *setigerum* type of appendage (Fig. 8C) has the same construction as that of the majority of the other Trigonostominae, consisting of two tubules attached by a ring to the bursa, with both tubes coiled over 360°.

All animals with two, three or more spires in the copulatory organ have been considered representatives of *T. setigerum*, even those members of the Eastern Pacific population with five whole spires. According to Karling (1986), the number of spires depends on growth (and may thus vary in one and the same individual according to age), muscle contraction and degree of compression, while studied. We have collected data on all individuals of species/populations with a coiled stylet, including material available from many different localities and from the literature. The results are presented diagrammatically in Figure 5 and in Table 1.

The first three stylets represented are those of *T. franki* (Fig. 5A), *T. armatum* (Fig. 5B) and *T. setigerum* (Fig. 5C); individuals with the fourth type of stylet (Fig. 5D) have been collected on the East Australian coast. The stylet of the Galapagos population (Figs 5E, 9E) is from the micrograph in Ehlers &

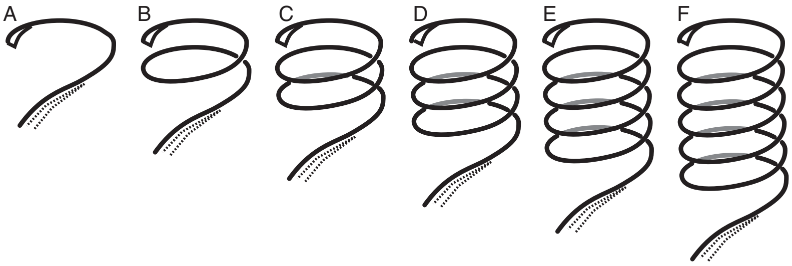

Figure 5. Schematic representation of the copulatory organ of the representatives of group 2, showing the different number of spires (dashed lines indicate the location of the mantle plates). A, ***T. franki* sp. nov.** ($^1/_2$ spire). B, T. armatum. ($1^1/_2$ spires). C, T. setigerum. ($2^1/_2$ spires). D, ***T. australis* sp. nov.** ($3^1/_2$ spires). E, ***T. galapagoensis* sp. nov.** ($4^1/_2$ spires). F, ***T. tori* sp. nov.** ($5^1/_2$ spires).

Table 1. Morphological differences between different populations of ***Trigonostomum franki*** **sp. nov.**, *T. armatum*, *T. setigerum*, ***T. australis*** **sp. nov.**, ***T. galapagoensis*** **sp. nov.** and ***T. tori*** **sp. nov.** (species group 2). *Abbreviations:* SL, stylet length (in µm); BAL, bursal appendage length (in µm); BAT, bursal appendage tubules; MSM, minimum no. of specimens measured; NM, not measurable; ? not known

Species	Distribution	Pigmentation	Coils	SL	BAL	BAT	MSM
T. franki	Caribbean, Florida	longitudinal stripe	1/2	86–101	84–100	normal	8
	East Africa	longitudinal stripe	1/2	90–101	120	normal	1
	New Caledonia	longitudinal stripe	1/2	95–111	62–166	normal constricted	4
T. armatum	northern Atlantic	none	1	158–196	NM	?	2
	southern Atlantic	rostral spot	1	434	149	normal	1
	Caribbean	rostral spot	1	274–378	100–108	normal	9
	eastern Australia	none	1	183	NM	?	1
	New Caledonia	none	1	386	127	normal	1
T. setigerum	western Mediterranean	rostral spot	2	413–492	87–113	normal	4
	eastern Mediterranean	none					
		longitudinal stripe					
		rostral spot	2	284–462	64–113	normal	21
	southern Atlantic	rostral spot	2	482	141	constricted	1
	East Africa	none	2	403	93	normal	1
	English Channel	rostral spot	2	477	NM	?	1
	Bermuda	none	2	434	NM	constricted	1
T. australis	eastern Australia	longitudinal stripe	3	414–445	34	normal	3
T. galapagoensis	Galapagos	rostral spot	4	?	?	?	0
T. tori	California	none	5	683–853	80–106	normal	3

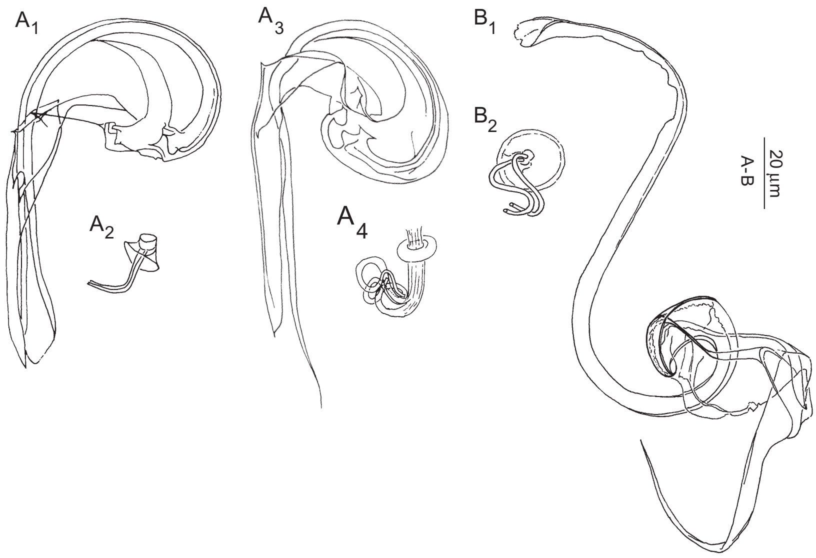

Figure 6. Copulatory organs and bursal appendages of the representatives of group 1A. A, *T. venenosum*: A$_{1,3}$ copulatory organ; A$_{2,4}$ bursal appendage. (A$_{1,2}$ specimen from Norway, neotype; A$_{3,4}$ specimen from Sardinia). B, *T. messoplanoides*: B$_1$ copulatory organ; B$_2$ bursal appendage. (from the holotype; after Artois *et al.*, 2000).

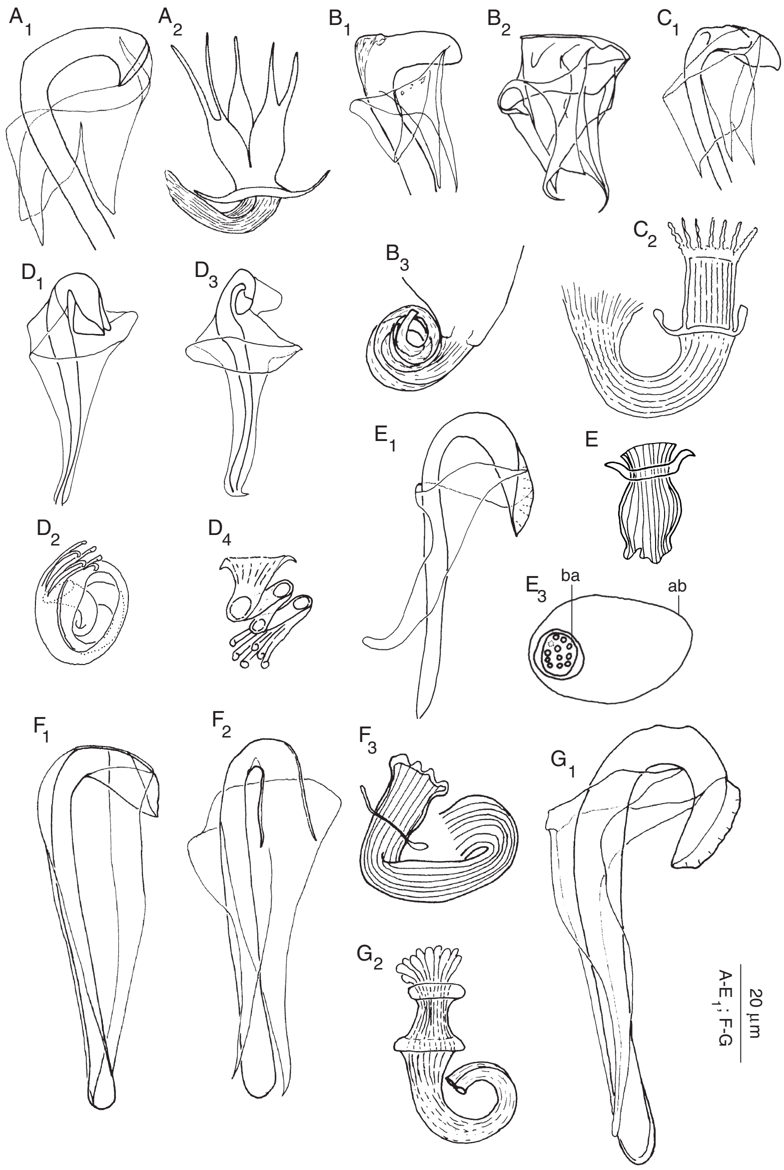

Figure 7. Copulatory organs and bursal appendages of the representatives of group 1B, A, *T. coronatum*: A_1 copulatory organ; A_2 bursal appendage. (from the neotype). B, ***T. watsoni* sp. nov.** $B_{1,2}$ copulatory organ; B_3 bursal appendage (B_1 from the holotype; $B_{2,3}$ from the paratype). C, *T. penicillatum*: C_1 copulatory organ; C_2 bursal appendage (from the neotype). D, *T. lilliei*: $D_{1,3}$ copulatory organ; $D_{2,4}$ bursal appendage. [$D_{1,2}$ specimen from Australia; $D_{3,4}$ specimen from the USA (neotype = holotype of *T. prytherchi*)]. E, *T. breitfussi*: E_1 copulatory organ; E_2 bursal appendage; E_3 section through the bursa and the bursal appendage (different specimens from Sweden; $E_{2,3}$ freehand drawings). F, *T. mirabile*: $F_{1,2}$ copulatory organ; F_3 bursal appendage ($F_{1,3}$ from the neotype; F_2 from another specimen from the Black Sea). G, ***T. nataschae* sp. nov.** G_1 Copulatory organ; G_2 Bursal appendage (from the holotype).

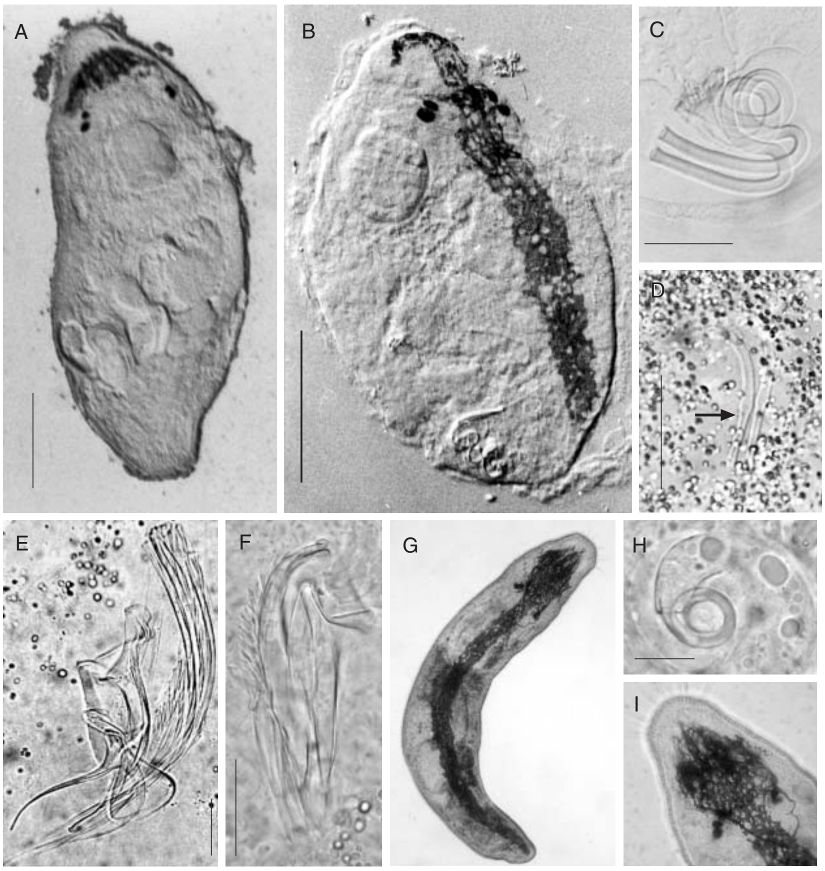

Figure 8. A, rostral spot in *T. armatum* (whole mounted specimen from Curaçao). B, dorsal stripe in *T. setigerum* (whole mounted specimen from Greece). C, bursal appendage of ***T. tori* sp. nov.** (from the paratype). D, constricted distal tubes of bursal appendage (arrow indicates constriction) in *T. setigerum* (specimen from Bermuda). E, copulatory organ of *T. denhartogi* **comb. nov.** (specimen from New Caledonia). F, copulatory organ of ***T. spinigerum* sp. nov.** (from the holotype). G, habitus of ***T. franki* sp. nov.** (live specimen from New Caledonia). H, bursal appendage of ***T. spinigerum* sp. nov.** (from the holotype). I, head region of ***T. franki* sp. nov.** showing both epidermal and subepidermal pigment (live specimen from New Caledonia). Scale bars: A, B = 200 µm. C–F = 20 µm. H = 10 µm.

Ax (1974), while the last type of stylet (Fig. 5F) is found in the population from California, as described by Karling (1986).

In *T. franki*, the stylet makes a turn of about 180° (although it shows all other characteristics of the stylet of this group of species) with an additional, more or less straightened part where the two spines of the mantle are (dashed lines in Fig. 5). This stylet thus has a half spire. In *T. armatum* there is one complete spire over 360° and an additional part ($1^1/_2$ spires), in *T. setigerum* $2^1/_2$ spires (see Figs 5C, 9C, $10C_1$), and so on. Muscle contraction and a degree of squeezing the animal causes the spires to stay close to each other or go apart (in some of our whole mounts of individuals of the Greek population, the spires had virtually disappeared). The number of coils in the spiral is constant in all studied populations, though one may count a different number of coils, depending on the side from which the stylet is viewed. In *T. setigerum*, for instance, with $2^1/_2$ spires, one counts three spires when looking at the stylet from the side where the stylet begins (including the 'half' spire), and only two when

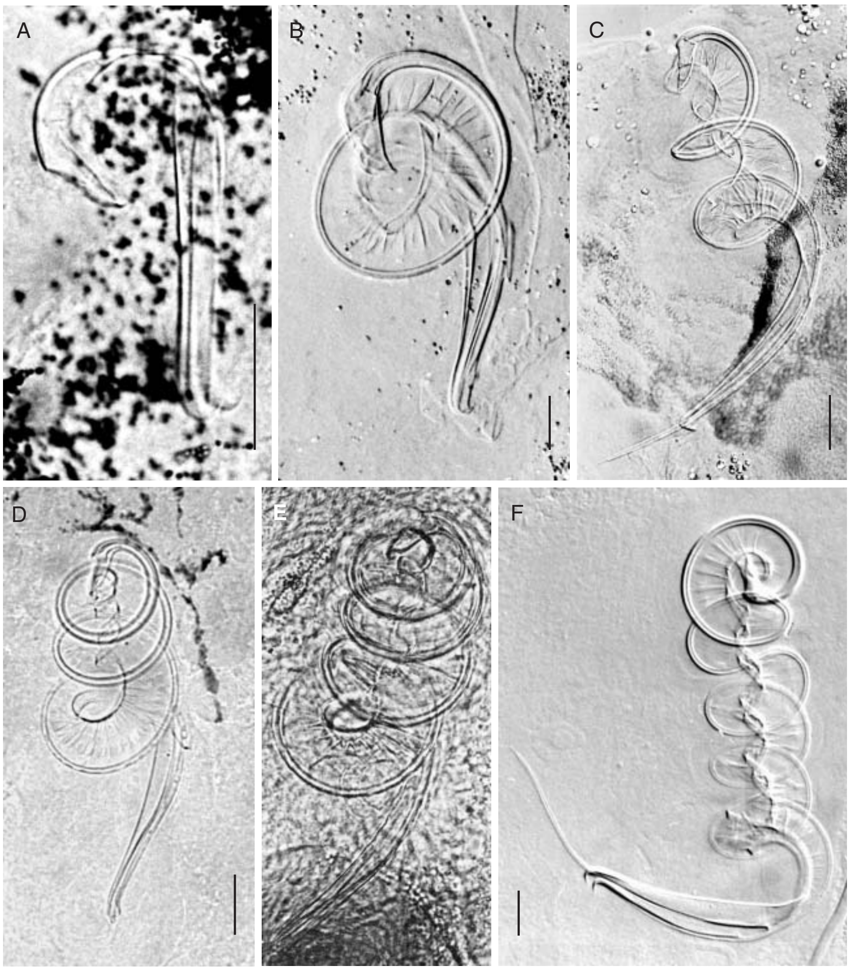

Figure 9. Copulatory organ of the representatives of group 2. A, ***T. franki* sp. nov.** (specimen from Curaçao) B, *T. armatum* (specimen from Curaçao). C, *T. setigerum* (from the neotype). D, ***T. australis* sp. nov.** (from the holotype). E, ***T. galapagoensis* sp. nov.** (from the holotype; after Ehlers and Ax, 1974: fig. 13C). F, ***T. tori* sp. nov.** (from the holotype). Scale bars: A–D, F = 20 µm.

looking from the opposite side. The spires turn clockwise (seen from the proximal end of the stylet) in all individuals; in those with a very long stylet (e.g. the Californian populations and some individuals in the Greek population), one of the spires may swing in the other direction, as often happens in spirals made of flexible material.

We believe that these stylets indicate that we are dealing with individuals from different species. Hence we give species status to the populations from Australia, the Galapagos and California, respectively, as *T. australis* sp. nov., *T. galapagoensis* sp. nov. (though no material is available, except the micrograph in Ehlers & Ax, 1974), and *T. tori* sp. nov., described as *T. setigerum* by Tor Karling in 1986. There may be some minor differences in some populations: Karling (1986) pointed to the constriction at the end of the tubes of the bursal appendage in the populations of *T. setigerum* in Bermuda, the Falkland Islands, South Georgia and in the English Channel (Fig. 8D; Table 1;

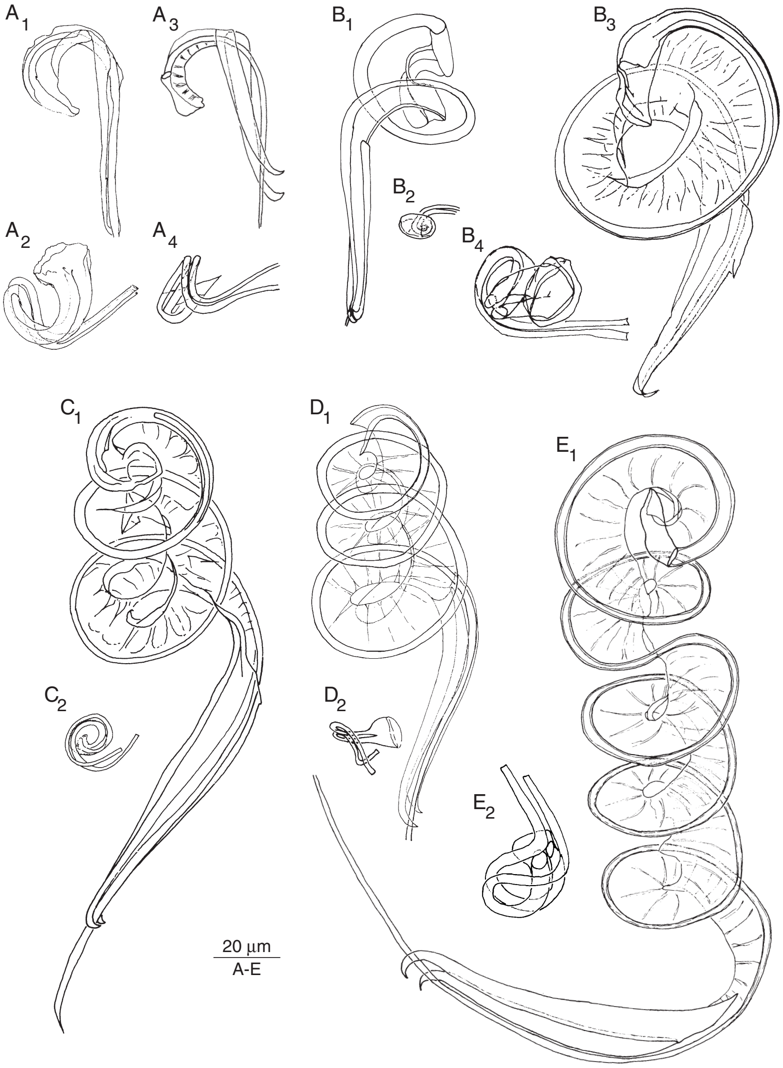

Figure 10. Copulatory organs and bursal appendages of the representatives of group 2. A, ***T. franki* sp. nov.** $A_{1,3}$ copulatory organ; $A_{2,4}$ bursal appendage ($A_{1,2}$ from the holotype; $A_{3,4}$ from a specimen from Kenya). B, ***T. armatum* sp. nov.** $B_{1,3}$ copulatory organ; $B_{2,4}$ bursal appendage ($B_{1,2}$ specimen from France; $B_{3,4}$ specimen from Curaçao). C, *T. setigerum*: C_1 copulatory organ; C_2 bursal appendage (specimen from Kenya). D, ***T. australis* sp. nov.** D_1 copulatory organ; D_2 bursal appendage (from the holotype). E, ***T. tori* sp. nov.** E_1 copulatory organ; E_2 bursal appendage (from the holotype).

Karling, 1978: fig. 28; Karling, 1986: figs 40, 48). In one specimen of *T. franki* from New Caledonia the tubes are also slightly constricted.

On the other hand, considering the distribution of these species, some doubts may arise. *T. franki* and *T. armatum* have been found together on Curaçao, *T. australis* and *T. armatum* occur sympatrically on the Australian East coast, while *T. armatum* and *T. setigerum* can be found together in both North Atlantic (Westblad's material from Plymouth; Gamble, 1893; Southern, 1912, 1936; Graff, 1913) and South Atlantic populations (Westblad's material from South Georgia, all considered *T. setigerum* by Karling, 1986). *T. setigerum* is the only species that has, so far, been found in the Mediterranean Sea, while those species with more than $2^1/_2$ spires are confined to the Pacific (the further east, the more spires). We began to wonder whether these are all populations of a single 'species complex' similar to that of the kalyptorhynch *Gyratrix hermaphroditus* Ehrenberg, 1831 (Curini-Galletti & Puccinelli, 1989, 1990, 1994, 1998, Artois *et al.*, 2000; Artois & Schockaert, 2001), albeit of a highly polymorphic species. Are we dealing with a cline in the Indo-Pacific? We prefer, with the data now available, to consider these populations as different species, until proven otherwise.

It is also in this group that a variation in pigmentation occurs (see Table 1): a mid-dorsal stripe (Fig. 8B, G), a rostral spot (Fig. 8A) or no pigmentation. Graff (1905) considered that, based on pigmentation, there were three subspecies of *T. setigerum*. However, Southern (1912) rejected this finding; we follow the latter view as these three pigmented forms are known to occur sympatrically (see Table 1 and remarks on *T. setigerum*).

Finally, two more species, *T. denhartogi* and *T. spinigerum*, deserve comment regarding the construction of the copulatory organ and the bursal appendage. With the material now available of *T. denhartogi* a first analysis of the copulatory organ can be given (Fig. 11A_3). The stylet is rather broad with a wide proximal funnel to which the proximal rim of the mantel is attached. On the concave side of the stylet the mantle forms 4–5 plates or spines, while on the convex side it bears a bundle of numerous fine folds or rods, one of which is very long and flagelliform. The bursal appendage has a straight initial part, thickened at one side, and two extremely long, highly coiled distal tubes. Both characters are clear autapomorphies for the species, and have no equivalent within the Trigonostominae. With only one individual available, the precise structure of the copulatory

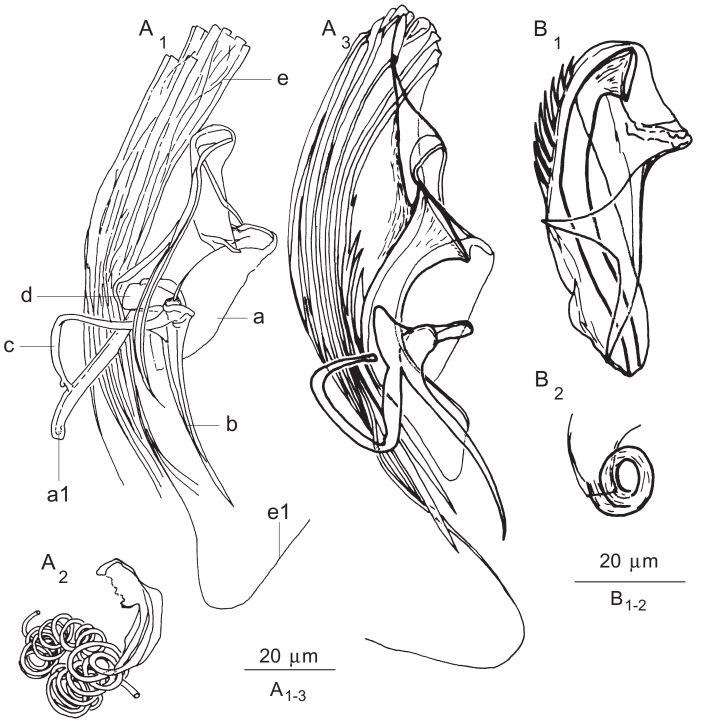

Figure 11. A, *T. denhartogi* **comb. nov.** $A_{1,3}$ copulatory organ; A_2 bursal appendage ($A_{1,2}$ specimen from Curaçao; A_3 specimen from New Caledonia). B, ***T. spinigerum*** **sp. nov.** B_1 copulatory organ; B_2 bursal appendage (from the holotype). *Abbreviations:* a–e, explained in text.

organ of *T. spinigerum* (Fig. $11B_1$) cannot yet be well understood. The mantle seems to have its rim proximal to the beginning of the stylet, and envelopes the latter completely. On the convex side and in the proximal half, the mantle bears 8–10 spines (or folds?); on the concave side and in the distal half, the mantle forms plates and spines similar to those in species of group 1B (e.g. *T. coronatum* or *T. watsoni*). The bursal appendage on the other hand is very similar to that of *T. watsoni*, constituting a proximal funnel with two broad tubes coiled over more than 360°.

To conclude, within *Trigonostomum* two common types of copulatory organ occur: (1) a proximally curved stylet surrounded by the mantle in its distal part, the latter divided into spiny plates; (2) a coiled stylet completely surrounded by a mantle that ends in two spiny plates. The first condition is plesiomorphic, the second synapomorphic. The mantle exhibits a much more complicated structure in *T. denhartogi* and *T. spinigerum*. Three types of bursal appendage occur: (1) two tubes attached to the bursa with a ring or funnel; (2) a funnel with two highly coiled tubes; (3) many tubules held together in one way or another. The first condition is plesiomorphic, the second a possible autapomorphy, and the third synapomorphic, though variations may occur. The differences discussed here, together with some other characters, are reflected in the following key.

IDENTIFICATION KEY

1. Copulatory organ 106–121 µm long and very complex, consisting of a gutter-shaped plate, enveloping a bundle of rods, of which one has a long thread-like distal tip; stylet funnel-shaped, ± 65 µm long; bursal appendage with initial straight part (22–44 µm long) and two heavily coiled distal tubes. (Figs 8E, 11A) .... *T. denhartogi* **comb. nov.**

– Copulatory organ ± 60 µm long; stylet bent over 90°, surrounded by the mantle that has 8–10 small spines at the convex side and with broad distal plates and spines distally (Fig. $11B_4$); bursal appendage with two broad, highly coiled tubes (Figs 8F, H, 11B) .... ***T. spinigerum* sp. nov.**

– Copulatory organ otherwise 2

2. Proximal part of the stylet curved over 90–270° and surrounded by the mantle only in its distal part. (Figs 4B, 6, 7) 3

– Copulatory organ spirally coiled over 360° or more (except in *T. franki*, where it is coiled over 180°); stylet surrounded over its whole length by the mantle, latter mostly wrinkled and split distally into two narrow, spiny plates with a terminal hook; bursal appendage consisting of two tubes (coiled over 270–360°), with a straight distal part; animals with dorsal stripe or rostral spot or without pigmentation (Figs 4C, 5, 8A, B, G, I, 9, 10) 7

3. Stylet long and narrow with a proximal turn of 270°; mantle forming a narrow ring and carrying a long and slender spine; bursal appendage with two tubes, proximally with a ring (Fig. 6) 4

– Stylet broad, proximally curved over 90° or 180°; mantle split into one, two or three plates (Fig. 7) 5

4. Copulatory organ consisting of a ±225 µm long slender stylet; mantle with flagelliform spine (± 75 µm); bursal appendage ± 22 µm long (Fig. 6B) .... *T. messoplanoides*

– Copulatory organ 74–130 µm long; stylet 117–194 µm long; mantle with a straight spine of 70–78 µm; bursal appendage ± 54 µm long (Fig. 6A) .... *T. venenosum*

5. Stylet bent over 90°, mantle split into three pointed plates:

* Copulatory organ 30–45 µm long; stylet 44–53 µm long; bursal appendage 60–70 µm long, with a proximal barrel-like casing and ± 12 tubules, diverging distally (Fig. 7C) .... *T. penicillatum*

* Copulatory organ 28–36 µm long; stylet 29–41 µm; bursal appendage 62–78µm long, consisting of a short funnel-shaped proximal part and two coiled (>360°) tubes, all with a faint striation (Fig. 7B) .... ***T. watsoni* sp. nov.**

* Copulatory organ ± 44 µm long; stylet ± 70 µm long; bursal appendage ± 78 µm long, with a proximal part, shaped like a clover leaf, and one to three distal bent tubes with a fine striation (Fig. 7A) .... *T. coronatum*

– Stylet bent over 180°; mantle with one or two plates 6

6. Mantle split into two plates, stylet with spoon-like terminal opening (Fig. $7F_{1,2}$, G_1) (note that the copulatory organ of *T. franki* may easily be confused with this type):

* Plates of the mantle blunt and shorter than the stylet; copulatory organ ± 78 µm long; stylet ± 104 long, proximally with a crest; bursal appendage ± 65 µm long, curved proximally over 270°, with proximal barrel-like part and a bundle of very narrow, faint tubules, which form two bundles distally (Fig. 7G) .... ***T. nataschae* sp. nov.**

* Plates of the mantle pointed and at least as long as the stylet; copulatory organ slender, 72–74 µm long; stylet 83–84 µm long; bursal appendage ±66 µm long, with a proximal barrel-like part and ±10 distal tubules (Fig. 7F) .... *T. mirabile*

– Mantle with only one plate (see Fig. 7D$_{1,\ 3}$, E$_1$):

* Plate of the mantle as long as the stylet and ending in a little hook; copulatory organ 42–46 µm long; stylet 40–57 µm long; bursal appendage 95–98 µm long consisting of a coiled tube, which distally splits into five to six tubules (Fig. 7D) .... *T. lilliei*

* Plate of the mantle blunt and shorter than the stylet; copulatory organ ± 62 µm long; stylet ± 64 µm long, proximally with a crest; bursal appendage 20–24 µm long with proximal barrel-like part and ±10 distal tubules (Fig. 7E) .... *T. breitfussi*

7. Copulatory organ 86–111 µm long, proximally bent over 180° and continuing in a straighter part, where the two spiny plates are; bursal appendage 62–166 µm long, coiled over 270°; animals mostly with dorsal pigment stripe (Figs 5A, 9A, 10A) .... ***T. franki* sp. nov.**

– Copulatory organ with 1–5 complete spire(s) of 360°, continuing in a straighter section, where the two spiny plates are situated (Fig. 5B–F):

* Copulatory organ with one spire, 158–434 µm long; bursal appendage 50–149 µm long, coiled over 270° (Figs 5B, 9B, 10B) .... *T. armatum*

* Copulatory organ with two spires, 284–498 µm long; bursal appendage 64–161 µm long, coiled over 360° (Figs 5C, 9C, 10C) .... *T. setigerum*

* Copulatory organ with three spires, 414–445 µm long; bursal appendage ± 34 µm long, coiled over 270° (Figs 5D, 9D, 10D) .... ***T. australis* sp. nov.**

* Copulatory organ with four spires (Figs 5E, 9E) .... ***T. galapagoensis* sp. nov.**

* Copulatory organ with five spires, 683–853 µm long; bursal appendage 80–106 µm long, coiled over 270° (Figs 5F, 9F, 10E) .... ***T. tori* sp. nov.**

TAXONOMY

TRIGONOSTOMUM SCHMIDT, 1852

Trigonostomum Schmidt, 1852: 500; Graff, 1905: 73, 113; 1908: 2542; 1913: 302–303; Meixner, 1924b: 91–92, 96, 103; Luther, 1948: 36, 38; Den Hartog, 1964: 373, t. 1; Ax, 1971: 146–150, fig. 1.
Vortex Schmidt, 1857: 352, 356.
Spiroclytus Schmidt, 1857: 352, 356; Claparède, 1863: 15.
Orcus Uljanin, 1870: 19.
Kylosphaera Jensen, 1878: 16, 36, 44–45.
Hyporhynchus Graff, 1882: 336.
Hyporcus Graff, 1905: 73, 1910; 1908: 2542; 1913: 299.
Woodshollia n.n. Graff, 1910: 947.
Woodsholia Graff, 1911a: 198; 1911b: 61; 1913: 312.
Woodsholia Graff, 1911b: 65.

Diagnosis: Trigonostominae with anterior integumental invagination, connected with the body wall by several muscles. Pharynx situated anteriorly, strongly inclined forwards, with 11 radial muscles lengthwise and 24 in cross section. Paired testes at 50%, caudal to the pharynx.

Type species: Trigonostomum setigerum Schmidt, 1852

TRIGONOSTOMUM ARMATUM (JENSEN, 1878) GAMBLE, 1900

(FIGS 5B, 8A, 9B, 10B; TABLE 1)

Alternative species name: trigonostomum-armatum
Kylosphaera armata Jensen, 1878: 7, 12, 14, 17, 45–47, t. 3, figs 14–22.
Hyporhynchus armatus Graff, 1882: 337; Gamble, 1893: 466–467; Attems, 1897: 228, t. 2, fig. 26.
Trigonostomum armatum Gamble, 1900: 813; Southern, 1912: 3, 9; Graff, 1913: 305–307, fig. 265; Southern, 1915: 34; Meixner, 1924b: 89, 94, 96, 99–100, 102; 1925: 256; Steinböck, 1931: 13, 23; Southern, 1936: 45, 57; Steinböck, 1938: 13, 22; Ax, 1952: 90–91, fig. 1; Westblad, 1954: 9.

Known distribution: Norway (Jensen, 1878; Graff, 1882; Westblad, 1954), English Channel (Gamble, 1893), North Sea (Attems, 1897; Meixner, 1924b, 1925), Ireland (Gamble, 1893, 1900; Southern, 1912, 1915, 1936), Faeroe Islands (Steinböck, 1931), Iceland (Steinböck, 1938), Baltic Sea (Ax, 1952).

New localities: Norway, Bergen, Karlsög, on algae, 9 July 1953, Westblad (coll. SMNH). Norway, Trondheim, Munkholmen, in sand, 45–50 m deep, 22 July 1955. Westblad (coll. SMNH). Norway, Leröy-Buröy, in fine-grained sand, 5–7 m deep, 1 August 1968, Karling (coll. SMNH; type locality).

United Kingdom, Plymouth, Salcombe, Saltstone, on algae, 11 July 1949, Westblad (coll. SMNH). Sweden, Gullmaren, Gåsövik, sheltered bay on brown algae, 19 August 2001. France, Wimereux, Langue du Chien, on algae, 20 October 1999. Curaçao (Dutch Antilles), Playa Canoa, on green algae, 10 December 1998. Curaçao (Dutch Antilles), Dam di Cabicuchi, 'Spaanse water', on *Turbinaria*-like algae, 14 and 30 December 1998 and 5 January 1999. Australia, New South Wales, Arrawarra, south of the marine station, on *Sargassum* sp., 1 November 1997. New Caledonia, Nouméa, Nouville, on algae in a lagoon, 10 August 2003. South Georgia, Cumberland Bay, May Creek, on seaweed, 9 May 1902 (coll. SMNH).

Material examined: Several individuals studied alive. Neotype (SMNH, no. 46427) from Norway. Whole mounts from France (1), Australia (1), New Caledonia (1), Curaçao (10) and South Georgia (SMNH, no. 46448). Serially sectioned specimens from Sweden (3), Curaçao (1), Norway (SMNH, no. 46425–6) and Plymouth (SMNH, nos. 46419–20).

Diagnosis: Trigonostomum species with coiled copulatory organ, with one whole spire. Stylet 158–434 μm long, enveloped by the mantle over its entire length. Mantle distally split into two spiny plates with terminal hook. Bursal appendage 50–149 μm long, with two tubules, proximally curved over 270° and with straight distal part.

***TRIGONOSTOMUM AUSTRALIS* SP. NOV.**
(FIGS 5D, 9D, 10D; TABLE 1)

Alternative species name: trigonostomum-australis sp. nov.

Holotype: Whole mount, Australia, Queensland, North Stradbroke Island, Point Lookout, algae in tidepool, 12 August 1996.

Other material: Observations on live material. Two whole mounts from Australia, New South Wales, Arrawarra, on *Sargassum* sp., 28 August 1996 and 1 November 1997.

Etymology: Reflects the species' occurrence in the southern hemisphere.

Diagnosis: Trigonostomum species with coiled copulatory organ, with three whole spires. Stylet 414–445 μm long, enveloped by the mantle over its entire length. Mantle distally split into two spiny plates with terminal hook. Bursal appendage 34 μm long, proximally curved over 270° and with straight distal section.

TRIGONOSTOMUM BREITFUSSI (GRAFF, 1905) MEIXNER 1924
(FIG. 7E)

Alternative species name: trigonostomum-breitfussi.
Hyporcus breitfussi Graff, 1905: 112, t. 3, figs 12–16; 1913: 301–302, figs 261, 262.
Hyporhynchus breitfussi Meixner, 1925: 256.
Trigonostomum breitfussi Meixner, 1924b: 89, 91–94, 96–99, 105, figs 3, 4; Steinböck, 1932: 309; Ax, 1952: 91–92, fig. 2; Westblad, 1954: 9; Armonies & Hellwig-Armonies, 1987: 104, table 5; Joffe & Kotikova, 1989: 70–72, 74–77, 79–82, figs 2, 3, 6 and 7.

Known distribution: Barents Sea (Graff, 1905), Baltic Sea (Ax, 1952), Greenland (Steinböck, 1932), North Sea (Meixner, 1924b, 1925; Ax, 1952; Armonies & Hellwig-Armonies, 1987), Norway (Westblad, 1954).

New locality: Sweden, Gullmaren, Kristineberg, 23, 26 and 27 July 1932, Westblad (coll. SMNH; type locality). Sweden, Gullmaren, on algae, 6 August 1945, Westblad (coll. SMNH). Sweden, Gullmaren, Harpo Bedar, on red algae, 20 m deep, 7 August 2001.

Material examined: Observations on live material from Sweden. Neotype (SMNH, no. 47461). Two whole mounts (SMNH, nos. 47462–3) and two serially sectioned specimens (SMNH, nos. 47469–70) from Sweden.

Diagnosis: Trigonostomum species with copulatory organ ± 62 μm long. Stylet ± 64 μm long, proximally bent over, 180° and with a crest. Mantle with one blunt plate (shorter than the stylet), surrounds only the distal part of the stylet. Bursal appendage 20–24 μm long, proximally with a barrel-like casing and nine or ten distal tubes.

Remarks: The observation of Ax (1952) that there is only one plate-like structure instead of two (Graff, 1905), surrounding the tubiform stylet, can be confirmed. This plate has a spine-like projection at its distal end, which lies close to the stylet but is displaced in highly squeezed animals (Fig. $7E_1$). The bursal appendage is short (Graff, 1913 24 μm; Ax, 1952: 20–21 μm), barrel-shaped and consists of nine, maybe ten, short tubes (Fig. $7E_3$).

TRIGONOSTOMUM CORONATUM (GRAFF, 1882) GRAFF, 1913
(FIG. 7A)

Alternative species name: trigonostomum-coronatum
Hyporhynchus coronatus Graff, 1882: 340, t. 9, fig. 21.
Hyporhynchus intermedius Attems, 1897: 228, t. 2, figs 22, 23.

Trigonostomum intermedium Graff, 1913: 308, fig. 267; Meixner, 1924b: 96, 98; Southern, 1936: 45, 57.

Trigonostomum coronatum Graff, 1913: 307–308, fig. 266; Meixner, 1924b: 96; Steinböck, 1933: 29.

Trigonostomum quadrifolium Riedl, 1954: 220–223, figs 28, 29.

Known distribution: Madeira (Graff, 1882), Irish Sea (Southern, 1936), North Sea (Attems, 1897), Mediterranean Sea (Steinböck, 1933; Riedl, 1954).

New locality: France, Banyuls, Ile Gros, on algae left of the jetty, 23 June 2000 (type locality).

Material examined: One specimen studied alive and mounted (neotype, LUC no. 228).

Diagnosis: Trigonostomum species with copulatory organ of ± 44 µm. Stylet ± 70 µm long, proximally bent over 90 °. Mantle split into three pointed plates, surrounding only the distal part of the stylet. Bursal appendage 78 µm long, with proximal crown-like part and one terminal bent, striated tube.

Remarks: According to Graff (1882, 1913) the copulatory organ of *T. coronatum* consists of an inner and an outer tube. The inner tube is proximally bent and twice as long as the outer one, which forms a broad mantle around the distal part of the inner tube. These observations can more or less be confirmed on the specimen from Banyuls, although it was squashed and the copulatory organ was rather damaged. The bursal appendage consists of a crown-like proximal part, enveloped by the bursa, and a tubular distal part. The bursal appendage of the Banyuls specimen consists of a plate proximally carrying a crown-like part, which is enveloped by the bursa, probably consisting of four plates, which are proximally split. Distally, the bursal appendage forms two bent tubes. These tubes show an inconspicuous striation, giving the impression that they consist of a number of smaller tubules.

Based on this resemblance, the Banyuls specimen is placed within *T. coronatum*. However, two other species have the same structure of the bursal appendage – *T. intermedium* Attems, 1897 and *T. quadrifolium* Riedl, 1954 – although little is known of the structure of their copulatory organs. In *T. quadrifolium* the appendage carries only one tube (Riedl, 1954), whereas the tube is apparently split into three tubules in *T. intermedium* (Attems, 1897; Graff, 1913), of which no material was available. These species also differ in the number of plates forming the crown of the appendage: four in *T. quadrifolium* (Riedl, 1954) and five in *T. intermedium* (Attems, 1897: fig. 22; Graff, 1913) and *T. coronatum* (Graff, 1882). Based on the descriptions of *T. intermedium* and *T. quadrifolium* and our experience that the mentioned differences are often difficult to assess, we synonymise both species with *T. coronatum*.

TRIGONOSTOMUM DENHARTOGI (KARLING, 1978)
COMB. NOV.
(FIGS 8E, 11A)

Alternative species name: trigonostomum-denhartogi
Proxenetes denhartogi Karling, 1978: 233, figs 35, 36.

Known distribution: Bermuda (Karling, 1978).

New localities: Kenya, Mombasa area, McKenzie Point, in shallow pool on seagrass, 30 September 1991. Curaçao (Dutch Antilles), Dam di Cabicuchi ('Spaanse water'), on *Turbinaria*-like algae from exposed rocks, 14 December 1998. Curaçao (Dutch Antilles) 'Spaanse water', mixed sample of algae, 30 December 1998. New Caledonia, Nouméa, Nouville, on algae in a lagoon south of the asylum, 3 August 2003. New Caledonia, Nouméa, Anse Vata, on algae (*Ulva* sp. and *Enteromorpha* sp.) from a little estuary, 22 August 2003.

Material examined: Holotype (SMNH, no. 2965). Live material and five whole mounts, one from each new locality.

Diagnosis: Trigonostomum species with very complex copulatory organ, 106–121 µm long. Mantle with numerous folds, rods and spines, one of which has a thread-like distal part. Stylet 61–66 µm long. Bursal appendage with a straight initial part, 22–44 µm long, and two heavily coiled tubes.

Remarks and additional data: Karling (1978) described this species from Bermuda based on one whole mounted specimen, but without observations of live animals. On the holotype, the anterior invagination ('proboscis') is not visible and Karling therefore did not observe this important feature. Based on the structure of the copulatory organ he reluctantly placed the species within *Proxenetes* Jensen, 1878. He explicitly mentioned, however, that the bursal appendage was very unlike that of any other species of *Proxenetes*, where the bursal appendage consists of a split tube, surrounded by a ring. Observations on live material clearly show that this species indeed belongs to *Trigonostomum*, as it has the typical anterior invagination.

The specimens from Curaçao and New Caledonia are ± 0.8 mm long. The copulatory organ is of exactly the same structure as in the specimen from Bermuda (Karling, 1978), consisting of an outer plate-like structure (Fig. $11A_1$: a) that forms a broad gutter enclosing several long rods (Fig. $11A_1$: e). One of these rods has a long distal thread-like point (Fig. $11A_1$: e1). A second, triangular, plate-like part (Fig. $11A_1$: d) surrounds the rods and carries three distal hooks (Fig. $11A_1$: a1, b and c). The length of the copulatory organ (excluding the thread-like tip) is 107–111 µm (Curaçao) and 109–121 µm (New Caledonia), which is almost identical to that of the holotype (115 µm: Karling, 1978). The exact number of rods could not be determined. The tubular stylet is only clearly visible in the New Caledonian specimens (Figs 8E, $11A_3$). It is 61–66 µm long (n = 2) and rather broad, with a wide proximal funnel to which the mantle is attached. The bursal appendage of the specimens from Curaçao and New Caledonia clearly consists of two heavily coiled tubes and a proximal basal piece. This proximal part is 31 µm and 44 µm in the two specimens from Curaçao, 28 µm and 36 µm in the specimens from New Caledonia, and 22 µm in the specimen from Bermuda (Karling, 1978). Karling (1978) could not determine the exact number of coiled tubes in the Bermuda individuals.

***TRIGONOSTOMUM* FRANKI SP. NOV.**
(FIGS 2, 3, 5A, 8G, I, 9A, 10A; TABLE 1)

Alternative species name: trigonostomum-franki sp. nov.

Holotype: Whole mount, Curaçao (Dutch Antilles), Dam di Cabicuchi ('Spaanse water'), on *Turbinaria*-like algae from exposed rocks at the side of 'Caracasbaai', 14 December 1998 (LUC no. 225).

Paratype: Whole mount, same data as for the holotype (LUC no. 226).

Other material: Observations on live material. Two whole mounts and one serially sectioned specimen from Curaçao (Dutch Antilles), 'Spaanse water', mixed sample of algae containing mainly *Caulerpa* sp. and *Halimedia gantia*, 30 December 1998. One whole mount from Florida (USA), Fort Pierce, in fine detritus-rich sand between rocks, 16 November 1994. Three whole mounts – Kenya, Mombasa area, McKenzie Point – on algae and seagrasses, 4 and 15 June 1987 and 27 September and 1 October 1991. One whole mount from Tanzania, Zanzibar, Pete, on seagrasses, 16 August 1995. Three whole mounts, New Caledonia, Nouméa, Nouville, on algae in a lagoon south of the asylum, 3 and 10 August 2003. One whole mount, New Caledonia, Nouméa, Baie des Citrons, on algae in a lagoon, 8 August 2003. Live observations from New Caledonia, Nouméa, Nouville, on algae covered with shells and sand on a reef in the western part of the Kuendu Bay, 16 August 2003.

Etymology: Dedicated to Mr Frank Van Belleghem, who helped to collect the material on Curaçao.

Diagnosis: Trigonostomum species with moderately coiled copulatory organ, with 1/2 spire. Stylet 86–111 µm long, enveloped by the mantle over its entire length. Mantle distally split into two spiny plates with terminal hook. Bursal appendage 61–166 µm long, with two tubules, proximally curved over 270° and with straight distal part.

***TRIGONOSTOMUM GALAPAGOENESIS* SP. NOV.**
(FIGS 5E, 9E; TABLE 1)

Alternative species name: trigonostomum-galapagoensis sp. nov.

Trigonostomum setigerum Ehlers & Ax, 1974: 664–666, 668, fig. 13A–C.

Holotype: One micrograph (fig. 13C in Ehlers & Ax, 1974), Galapagos (Ecuador), Santa Cruz, Bahía Academy (Ehlers & Ax, 1974).

Etymology: The islands where the type material was found.

Diagnosis: Trigonostomum species with coiled copulatory organ, four whole spires. Stylet enveloped by the mantle over its entire length. Mantle distally split into two spiny plates with terminal hook. Bursal appendage coiled, with two tubules.

Remarks: Pigment spot rostrally, between the eyes (see Ehlers & Ax, 1974: fig. 13A). The length of both hard parts, copulatory organ and bursal appendage, could not be measured, because this species is only known from a micrograph of a live individual (Ehlers & Ax, 1974: fig. 13), without a scale bar.

TRIGONOSTOMUM LILLIEI (GRAFF, 1911A) MEIXNER 1924B
(FIG. 7D)

Alternative species name: trigonostomum-lilliei

Woodsholia lilliei Graff, 1911b: 61–65, fig. 3, t. IV, figs 29–43; 1913: 312–314, figs 277–279; Meixner, 1924b: 91, 92.

Trigonostomum prytherchi Kepner, Ferguson & Stirewalt, 1941: 243–252, figs 1–3, pl. 3.

Trigonostomum divae Marcus, 1948: 121–125, 189, t. III, figs 13–18.

Trigonostomum lilliei Meixner, 1924b: 92, 94, 96, 99, 102.

Known distribution: North American Atlantic coast (Graff, 1911b, 1913; Kepner *et al.*, 1941), Brazil (Marcus, 1948).

New locality: Australia, New South Wales, Arrawarra, rocky tidepool at low tide, on brown algae, 29 August 1996; south of the marine station, mideulittoral, on *Sargassum* sp., 1 November 1997.

Material examined: Observations on two live specimens, two whole mounts (all from Australia), the holotype of *T. prytherchi*, which is designated neotype (whole mount; SI-NMNH, Cat. no. 20593; Kepner *et al.*, 1941) and Marcus' (1948) material of *T. divae* (sections and whole mounts; SMNH, nos. 42204–8).

Diagnosis: Trigonostomum species with copulatory organ 42–46 μm long. Stylet 40–57 μm long, proximally bent over, 180°. Mantle with one hooked plate (as long as the stylet) surrounds only the distal part of the stylet. Bursal appendage 95–98 μm long, heavily coiled, consisting of one tube, which is distally split into five or six finer tubes.

Remarks and additional data: The stylet of the examined specimens is 40–57 μm long (57 μm in the holotype of *T. prytherchi*; 40–43 μm in Marcus' material of *T. divae*, $n = 2$; 44–46 μm in the Australian specimens, $n = 2$). The bursal appendage is a 95–98 μm long ($n = 2$). The bursal appendage could not accurately be measured in the second Australian specimen, or in Marcus' material.

According to the description, including figures, of Kepner *et al.* (1941), it seems that they observed not one but three bursal appendages (including two smaller nonfunctioning appendages). These observations could be due to a misinterpretation of the serially sectioned material, because a thorough examination of the type material revealed only one bursal appendage.

According to Marcus' (1948) drawings and description, the difference between *T. divae* and *T. lilliei* is the presence of only five tubes in the bursal appendage instead of six, a feature, which is often very difficult to assess. The overall construction of the bursal appendage of *T. divae* (i.e. the number and extent of coils) is identical to that of the Australian specimens. The bursal appendage of *T. prytherchi* is also identical in structure. The difference between *T. prytherchi* and *T. lilliei* would also be the ending of the prepharyngeal cavity in the 'proboscis' cavity as observed by Graff (1911b: fig. 31). According to Meixner (1924b) this feature is the result of the degree of contraction, and he therefore rejected the taxon *Woodsholia*. Because of these doubtful differences between these 'species' and the overall similarity in the structure of both the copulatory organ and the bursal appendage, we synonymise *T. prytherchi* and *T. divae*, with *T. lilliei*.

TRIGONOSTOMUM MESSOPLANOIDES ARTOIS *ET AL.*, 2000

(FIG. 6B)

Alternative species name: trigonostomum-messoplanoides

Trigonostomum messoplanoides Artois *et al.*, 2000: 104–105, fig. 1.

Known distribution: Weddell Sea, Antarctica (Artois *et al.*, 2000).

Material examined: Holotype (LUC, no. 207).

Diagnosis: Trigonostomum species with stylet 225 μm long, proximally bent over 270°. Mantle with stylet 75 μm long, flagelliform spine surrounding only the distal part of the stylet. Bursal appendage 22 μm long, with a proximal ring and two distal tubes.

Remarks: The copulatory organ of *T. messoplanoides* resembles that of some species of *Messoplana* (Artois *et al.*, 2000), for instance *M. elegans* (Luther, 1948) Den Hartog, 1966, *M. helgolandica* Ax, 1971, *M. pacifica* Karling, 1986 and *M. rugata* Ehlers, 1974. However, the presence of a ventral invagination clearly confirms that it is a species of *Trigonostomum*.

TRIGONOSTOMUM MIRABILE (PEREYASLAWZEWA, 1893) GRAFF, 1913

(FIG. 7F)

Alternative species name: trigonostomum-mirabile

Hyporhynchus mirabilis Pereyaslawzewa, 1893: 267, t. 4, fig. 27.

Trigonostomum mirabile Graff, 1913 : 310–311, fig. 273; Meixner, 1924b: 96, 98; Ax, 1959: 98–99, figs 80, 81; Mack-Fira, 1968: 179–180, figs 2–4; 1974: 249, 265, 273, 281, 284.

Proxenetes lictor Beklemischev, 1927: 190–191, 203–204, t. I, figs 8, 9.

Known distribution: Black Sea (Pereyaslawzewa, 1893; Beklemischev, 1927; Ax, 1959; Mack-Fira, 1968, 1974); Sea of Marmara (Ax, 1959).

New locality: Romania, Black Sea, Agigea, on *Ceramium* sp., 26 September 1968, Mack-Fira (coll. SMNH; type locality).

Material examined: Two individuals from Agigea (mounted on the same slide: SMNH, no. 47474). One of them designated neotype.

Diagnosis: Trigonostomum species with copulatory organ 72–74 μm long. Stylet 83–84 μm long, proximally bent over, 180°. Mantle with two pointed plates (as long as the stylet) surrounds only the distal part of the stylet. Bursal appendage 66 μm long, proximally with a barrel-like casing and ± ten distal tubules.

Remarks: The synonymization of *Proxenetes lictor* with *T. mirabile* was made by Ax (1959), but it was already recognized by Beklemischev in a personal note on the offprints of his article (see Ax, 1959).

***TRIGONOSTOMUM NATASCHAE* SP. NOV.**
(FIG. 7G)

Alternative species name: trigonostomum-nataschae sp. nov.

Holotype: Whole mount, Kerguelen (France, subantarctic territory), Port Raymond, tidepool with fine sand and silt, mixed with shells, 23 November 1992 (LUC no. 227).

Other material: Observations on live material from Kerguelen.

Etymology: Dedicated to Mrs Natascha Steffanie, technical assistant at LUC, Diepenbeek (Belgium).

Diagnosis: Trigonostomum species with copulatory organ ± 78 µm long. Stylet ± 104 µm long, proximally bent over, 180° and with a crest. Mantle with two blunt plates (shorter than the stylet) surrounds only the distal part of the stylet. Bursal appendage ± 65 µm long, with proximal barrel-like part and very narrow tubules, forming two curved bundles of tubules distally.

TRIGONOSTOMUM PENICILLATUM (SCHMIDT, 1857) MICOLETZKY, 1910
(FIG. 7C)

Alternative species name: trigonostomum-penicillatum
Vortex penicillatus Schmidt, 1857: 352, t. 1, fig. 3.
Hyporhynchus penicillatus Diesing, 1862: 227; Graff, 1882 : 341, t. 9, figs 15–20; Gamble, 1893: 467; Fuhrmann, 1898: 459; Gamble, 1900: 813; Meixner, 1925: 256; 1926: 577.
Trigonostomum intermedium n.n. Graff 1910: 4.
Trigonostomum penicillatum Micoletzky, 1910: 174; Graff, 1913 : 308–309, figs 268–270; Southern, 1912: 3, 9; 1915: 34; Meixner, 1924a: 202–203; 1924b: 89, 92, 94, 96, 98, 99, 105; Southern, 1936: 45, 58; Westblad, 1954: 8; Den Hartog, 1964: 378; Ax, 1971: 216–217, fig. 45.
Trigonostomum marki Graff, 1911b: 60, t.4, figs 44, 45; 1913: 309–310, fig. 271; Meixner, 1924b: 96, 99.

Known distribution: North American Atlantic coast (Graff, 1911b), French Atlantic coast (Ax, 1971), English Channel (Gamble, 1893), North Sea (Graff, 1913; Meixner, 1924b), Ireland (Southern, 1912, 1936), Norway (Westblad, 1954), Mediterranean Sea (Schmidt, 1857; Graff, 1913; Meixner, 1925, 1926) and Adriatic Sea (Meixner, 1925, 1926).

New localities: Great Britain, Plymouth, 5 July 1949, Westblad (coll. SMNH). France, Corsica, Ocellutia, large sandflat with coarse sand, 10–12 m deep, 19 October 1982 and 18 September 1983. Italy, Sardinia, Porticciolo, on algae at ± 10 m deep, 14 August 1994 (type locality). France, Banyuls, Ile Gros, on green algae near the jetty behind the station, 20, 22 and 23 June 2000. Yugoslavia, Adriatic Sea, Dubrovnik, 24 June 1952, Westblad (coll. SMNH). Bulgaria, Varna (Black Sea), on algae, 21 December 1953, Valkanov (coll. SMNH).

Material examined: Observations on live material from Corsica, Sardinia and Banyuls. Neotype (LUC no. 229) from Sardinia. One whole mount from Bulgaria (SMNH, no. 47485) and serially sectioned specimens from Great Britain (SMNH, nos. 47480–3) and Yugoslavia (SMNH, nos. 47484). Seven whole mounts from Banyuls and three from Corsica.

Diagnosis: Trigonostomum species with copulatory organ 30–45 µm long. Stylet 44–53 µm long, proximally bent over 90°. Mantle with three spiny plates, surrounds only the distal part of the stylet. Bursal appendage 60–70 µm long, with a barrel-like casing and ± 12 distal tubules.

Remarks: According to Meixner (1924b), the bursal appendage of *T. penicillatum* partially consists of ± 26 fine rods. However, our observations on serially sectioned material of *T. penicillatum* (SMNH) revealed only 12 fine, slightly bent rods in the bursal appendage of a specimen from Plymouth, though more in the individuals in the whole mounts.

In 1897, *T. intermedium* was described by Attems, but the same name was used by Graff as a *nomen nudum* in 1910 for a species he later (Graff, 1911b) described as *T. marki*. The structure of the bursal appendage of *T. marki* is identical to that of *T. penicillatum* (Graff, 1911b, 1913). According to Graff (1913) the species differ in the structure of the copulatory organ. In *T. penicillatum* the organ has a stylet, enclosed by a mantle, which carries three plates; the same structure is described and drawn by Graff (1911b: 61, table 4, figs 44, 45; 1913: 309, fig. 271) for *T. marki*. *T. marki* is therefore synonymized with *T. penicillatum*.

TRIGONOSTOMUM SETIGERUM SCHMIDT, 1852
(FIGS 5C, 8B, D, 9C, 10C; TABLE 1)

Alternative species name: trigonostomum-setigerum
Trigonostomum setigerum Schmidt, 1852: 500, t. 47, fig. 13; Diesing, 1862: 229; Graff, 1905: 113–114, t. 3, figs 17–21; Micoletzky, 1910: 173; Southern, 1912: 3, 9; Graff, 1913: 303–305, figs 263, 264; Southern, 1915: 34; Meixner, 1924a: 202–203; 1924b: 89, 90, 92, 93–94, 96, 99–101, 102, figs 1, 2, 5; 1925: 256; 1926: 577; Steinböck, 1933: 10–11; Southern, 1936: 45, 57; Meixner, 1938: 25, 114, fig. 23; Westblad, 1952: 30–31; Ax, 1959: 97; Riedl, 1959: 319–322,

fig. 6; Den Hartog, 1964: 375; Karling, 1978: 231, figs 27, 28; 1986: 209–210, figs 39, 40, 47,48; Ax & Armonies, 1990: 100.

Trigonostomum setigerum setigerum Graff, 1905: 113, t. 3, figs 19–21; 1913: 305.

Trigonostomum setigerum album Graff, 1905: 114, t.3, figs 17, 18; 1913: 305.

Trigonostomum setigerum lunulatum Graff, 1905: 114; 1913: 305.

Spiroclytus nisus Schmidt, 1857: 356, 365, t. 3, fig. 8; Diesing, 1862: 225.

Spiroclytus euryalus Schmidt, 1857: 356, 365, t. 3, fig. 8.

Spiroclytus setigerus Claparède, 1863: 15.

Vortex ornatus Uljanin, 1870:, 18, t. 4, fig. 15.

Hyporhynchus setigerus Graff, 1882: 338–339, t. 9, figs 6–14, t. 11, fig. 27; Pereyaslawzewa, 1893: 267, t.4, fig. 29, t. 10, figs 60a–e, 63d; Fuhrmann, 1898: 459; Sekera, 1901: 81; Sabussow, 1905: 488.

Known distribution: Mediterranean Sea (Schmidt, 1852, , 1857; Graff, 1905; Sabussow, 1905; Micoletzky, 1910; Meixner, 1925; Steinböck, 1933; Riedl, 1959), Adriatic Sea (Meixner, 1925, 1926), Black Sea (Uljanin, 1870; Pereyaslawzewa, 1893; Graff, 1905; Ax, 1959), Sea of Marmara (Ax, 1959), northern Atlantic Ocean (Fuhrmann, 1898; Graff, 1905; Southern, 1912, 1915, 1936; Karling, 1978), North Sea (Meixner, 1924b), southern Atlantic Ocean (Westblad, 1952).

New localities: France, Corsica, Port de la station Stareso, on algae, 10 April, 9 May and 19 October 1982; Punta Reveletta, on algae, 11 May and 22 October 1982. France, Banyuls, Ile Gros, on green algae near the jetty behind the station, 20–23 June 2000. Greece, Perea, east side of the beach, coarse-grained detritus-rich sand mixed with shell gravel; on green and red algae, 22 July 2002; on green algae and seagrasses, ± 2 m deep, 31 July 2002. Greece, Nea Michaniona, flat exposed beach, on *Enteromorpha* sp. and seagrasses, 22 July 2002. Greece, Aghias Triada, flat beach, on green algae and seagrasses ± 2.5 m deep, 22 July 2002 and 6 August 2002 (type locality). Greece, Nea Fokea (Kassandra Peninsula), heavily exposed beach, on algae, 28 July 2002. United Kingdom, Plymouth, Wembury, tidepool, 21 July 1949, Westblad (coll. SMNH). Kenya, Tiwi, on algae, 6 October 1991.

Material examined: Several specimens studied alive. Neotype (LUC no. 230) from Greece. Whole mounts from Banyuls (2), Bermuda (SMNH, no. 46460), Corsica (5), Falkland (SMNH, nos. 46429, 46430, 46440), Greece (26), Kenya (1), Plymouth (SMNH, no. 46452) and South Georgia (SMNH, no. 46448). Serially sectioned specimens from Falkland (SMNH, nos. 46431–9, 46441–4), Plymouth (SMNH, nos. 46449–51), South Georgia (SMNH, nos. 46445–7) and Greece.

Diagnosis: Trigonostomum species with coiled copulatory organ, with two whole spires. Stylet 284–498 μm long, enveloped by the mantle over its entire length. Mantle distally split into two spiny plates with terminal hook. Bursal appendage 64–161 μm long, with two tubules, proximally curved over 360°.

Remarks: Graff (1905, 1913) recognized three subspecies, based on the pattern of pigmentation: *T. setigerum setigerum* (with dorsal stripe), *T. setigerum lunulatum* (with a rostral spot between the eyes) and *T. setigerum album* (without pigmentation). The validity of these subspecies was questioned by Southern (1912). Observations on a large number of live animals from one population in Greece showed that the three subspecies occurred sympatrically and that the difference between these three is not always clear. For example, specimens with a rostral spot (*T. s. lunulatum* of Graff, 1905) showed a large variation in size of the spot, making the difference with *T. s. album* very small. There is also variation in the length and width of the dorsal stripe: some individuals have a short dorsal stripe, which is broader between the eyes, while in other specimens the dorsal stripe is long and very broad over its whole length, giving it a network-like appearance. Based on the occurrence of all forms in one population (Greece, Thessaloniki), the lack of other distinctive features and the large variation of the three 'forms', the three subspecies are synonymized with *T. setigerum*.

***TRIGONOSTOMUM SPINIGERUM* SP. NOV.**

(FIGS 8F, H, 11B)

Alternative species name: trigonostomum-spinigerum sp. nov.

Holotype: Whole mount, New Caledonia, Nouméa, Nouville, on algae in a lagoon south of the asylum, 3 August 2003 (LUC no. 231).

Etymology: Refers to the spines on the mantle surrounding the stylet; *spinigerum* (L.) = prickly, spiny, thorny.

Diagnosis: Trigonostomum species with copulatory organ 61 μm long. Stylet 57 μm long, proximally bent over almost 90°. Mantle surrounds the whole stylet and carries 8–10 small spines on the convex side. Bursal appendage ± 35 μm long, proximally funnel-shaped with two heavily coiled distal tubes, very faintly striated.

Remarks: The bursal appendage is measured (and drawn) on the whole mount, in which it appeared extremely thin-walled. Therefore, the length (35 μm) has to be interpreted as the minimum length. Furthermore, only one distal tube could be observed (two in the live individual), probably because both tubes are situated exactly above each other in the mounted specimen.

TRIGONOSTOMUM TORI **SP. NOV.**

(FIGS 5F, 9F, 10E; TABLE 1)

Alternative species name: trigonostomum-tori sp. nov.
Trigonostomum setigerum Karling, 1986: 209–210, figs 45, 46.

Holotype: One whole mount, USA, California, Pacific Grove (SMNH, no. 46459; Karling, 1986).

Paratypes: Two whole mounts (SMNH, nos. 46457–8; Karling, 1986).

Etymology: Dedicated to Prof. Dr Tor G. Karling, who collected the material.

Diagnosis: Trigonostomum species with coiled copulatory organ, with five whole spires. Stylet 683–853 μm long, enveloped by the mantle over its entire length. Mantle distally split into two spiny plates with terminal hook. Bursal appendage 80–106 μm long, with two tubules, proximally curved over 270° and with straight distal part.

TRIGONOSTOMUM VENENOSUM (ULJANIN, 1870) MEIXNER, 1924B

(FIGS 1, 6A)

Alternative species name: trigonostomum-venenosum
Orcus venenosus Uljanin, 1870: 19, t. 2, fig. 5.
Hyporhynchus venenosus Graff, 1875: 419; 1882: 341; Pereyaslawzewa, 1893: 265, 266, t. 4, fig. 28; Attems, 1897: 227, t. 2, figs 24, 25.
Hyporcus venenosus Graff, 1905: 110, t. 3, figs 9–11; Southern, 1912: 3, 8–9; Graff, 1913: 299–301, fig. 260; Southern, 1915: 34; Meixner, 1924b: 91, 92; Southern, 1936: 45, 57; Meixner, 1926: 577.
Trigonostomum venenosum Meixner, 1924b: 89, 92, 94, 96, 99, 102; 1925: 256; Steinböck, 1931: 12, 23; 1938: 12–13, 22; Ax, 1959: 98, figs 78, 79; Mack-Fira & Cristea-Nastasesco, 1971: 225, 227, figs 5, 6; Mack-Fira, 1974: 249, 265, 273, 281–282, 284.

Known distribution: Iceland (Steinböck, 1938), Ireland (Southern, 1912, 1936), Faeroe Islands (Steinböck, 1931), North Sea (Attems, 1897; Meixner, 1924b, 1925), Mediterranean Sea (Graff, 1882; Meixner, 1926), Adriatic Sea (Meixner, 1926), Black Sea (Uljanin, 1870; Pereyaslawzewa, 1893; Graff, 1905; Ax, 1959; Mack-Fira & Cristea-Nastasesco, 1971; Mack-Fira, 1974).

New localities: Sweden, Gullmarsfjord, Gåsövik, among algae, August 1945, Westblad (coll. SMNH). Norway, Bergen, Bay south of Tyssöy, stones, gravel, algae and fine shell-sand, 5–8 m depth, 29 July 1968, Karling (coll. SMNH). Norway, Bergen, sound between Leröy and Buröy; sand and mud, 5 m depth; 1 August 1968, Karling (coll. SMNH). France, Corsica, Port de la station Stareso, on algae, 6–4 m deep, 9 May 1982 and 12 March 1983, Martens; Bay of Calvi, 11 April 1984 and 26 March 1985. Italy, Sardinia, Porticello, about 0.2–1.5 m deep, on *Vaucheria*-like algae on rocks, 14 August 1994. France, Banyuls, Ile Gros, on green algae near the jetty behind the station, 22–23 June 2000. Kerguelen, Port Couvreux, bay at the right, green algae, 25 November 1992.

Material examined: Several specimens studied alive. Neotype (SMNH, no. 47499). Whole mounts from Sardinia (2), Corsica (4), Banyuls (3), Kerguelen (6), Norway (SMNH, nos. 47496–8) and Romania (SMNH, no. 47500–1). Two serially sectioned specimens (SMNH, nos. 47493–4) from Sweden.

Diagnosis: Trigonostomum species with copulatory organ 74–130 μm long. Stylet 117–194 μm long, proximally bent over 270°. Mantle with one pointed plate, surrounds only the distal part of the stylet. Bursal appendage ± 54 μm long, with a proximal ring and two distal tubes.

Remarks and additional data: The length of the stylet shows large variation between the populations of Banyuls (117–124 μm; $n = 2$), Sardinia (148–156 μm; $n = 2$), Corsica (165–179 μm; $n = 2$) and Kerguelen (157–194 μm; $n = 3$). The stylet resembles that of some species of *Messoplana* (e.g. *M. elegans* Luther, 1948; *M. helgolandica* Ax, 1971; *M. pacifica* Karling, 1986; *M. rugata* Ehlers, 1974).

TRIGONOSTOMUM WATSONI **SP. NOV.**

(FIG. 7B)

Alternative species name: trigonostomum-watsoni sp. nov.

Holotype: Whole mount, Australia, New South Wales, Arrawarra, on *Pavonina*-like algae in shallow tidepool between rocks near beach, 27 August 1996.

Paratype: One whole mount.

Other material: Observations on live material. Six whole mounts and six serially-sectioned specimens, Australia, New South Wales, Lennox Head, on beach with coarse sand and on algae in tide pool, 27 October and 1 November 1997. One whole mount, Australia, New South Wales, Arrawarra, on *Sargassum* sp. in permanent pool, 27 August 1996. One whole mount, Australia, New South Wales, Arrawarra, Mullaway headland, on algae in deep rock pools, 24 July 2003.

Two whole mounts, New Caledonia, Nouméa, Magenta, permanent pool near mangroves, on large algae covered with epiphytes, 22 August 2003.

Etymology: Dedicated to Dr Nikki Watson, Armidale, Australia, who assisted with the collection of material at Arrawarra.

Diagnosis: Trigonostomum species with copulatory organ 28–36 µm long. Stylet 29–41 µm long, proximally bent over 90°. Mantle with three spine-like plates surrounds only the distal part of the stylet. Bursal appendage 62–78 µm long, with two coiled (more than 360°) striated tubes.

SPECIES INQUIRENDAE

TRIGONOSTOMUM BRUNCHORSTI GRAFF, 1905

Trigonostomum brunchorsti Graff, 1905: 115–116, t. III, figs 24, 25; 1913: 311, figs 274, 275; Meixner, 1924b: 96, 98; Steinböck, 1932: 309.

Distribution: Norway (Graff, 1905), Greenland (Steinböck, 1932).

According to Graff's (1905, 1913) description, the overall morphology of the copulatory organ resembles that of *T. penicillatum*. The two plate-like structures differ somewhat in shape, ending both proximally as well as distally in a hook-like structure (see Graff, 1905: taf. 3, fig. 24). The bursal appendage is described as resembling that of *T. venenosum*. The position of the pharynx in the middle of the body is exceptional for a member of *Trigonostomum* and new material is needed to confirm the validity and/or the taxonomy of this species.

TRIGONOSTOMUM PIRIFORME (PEREYASLAWZEWA, 1893) GRAFF, 1905

Hyporhynchus piriformis Pereyaslawzewa, 1893: 266, t. 4, fig. 30.

Trigonostomum piriforme Graff, 1905: 115, t. III, figs 22,23; 1913: 310, fig. 272; Meixner, 1924b: 96, 99.

Distribution: Black Sea (Pereyaslawzewa, 1893; Graff, 1905)

According to Graff's (1905) drawings and description, the copulatory organ consists of three parallel plate-like structures, but without a central stylet. The bursal appendage is described as being identical with that of *T. penicillatum*. This species could be identical with *T. penicillatum*, but observations on new material are needed.

MARINELLIA LINGULIFERA RIEDL, 1954

Marinellia lingulifera Riedl, 1954: 223–231, figs 30–32.

Distribution: Mediterranean Sea (Riedl, 1954).

This species was considered by Riedl (1954) to be a close relative of *Trigonostomum*, mainly based on the presence of an antero-ventral invagination forming a kind of 'proboscis'. Other features in which *M. lingulifera* resembles *Trigonostomum* species are the pharynx, which is strongly inclined forwards and shows very strong external muscles and weak radial ones (Riedl, 1954: 227), the double connection in the female system (Riedl, 1954: fig. 31), the presence of a bursa (Riedl, 1954: fig. 31: rs) with a long and narrow bursal stalk, paired ovovitellaria and a bursal appendage that consists of two coiled tubes, fused at their base (Riedl, 1954: 225). Three other features of *M. lingulifera* are not found in any *Trigonostomum* species: the presence of a muscular tongue in front of the pharynx (Riedl, 1954: 227–228, fig. 31: z), a muscular septum connecting the invagination with the pharynx (Riedl, 1954: fig. 31: ms), and an unpaired seminal vesicle (Riedl, 1954: 226). Ax (1971) considered *M. lingulifera* to be the sister group of *Trigonostomum*, based only on the presence of the antero-ventral invagination. There is, however, no material of this species available and some of the observations, such as the unpaired seminal vesicle, the detailed structure of the copulatory organ and the invagination certainly need confirmation. In addition, the copulatory organ and the bursal appendage are insufficiently described and not even figured in the original description.

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II

ZOOLOGICAL SCIENCE **21**: 333–341 (2004)

Reports on the Free-Living Platyhelminthes from Australia: Typhloplanoida, with the Description of Three New Taxa

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ABSTRACT—Five typhloplanoids from the Australian East Coast are reported, three of them new to science. Two taxa are members of Promesostomidae: *Vauclusia conica* n.g. n.sp., characterised by a cone-shaped stylet, the presence of a female bursa and a very long, partially-swollen female duct; *Brinkmanniella australiensis* n.sp. has a funnel-shaped stylet with a smooth distal tip. *Pilamonila bimascula* n.g. n.sp. is a representative of the Solenopharyngidae, characterised by a stylet within a cirrus. The known species found are *Ceratopera axi* and *Ptychopera scutulifer*.

Key words: taxonomy, 'Turbellaria', Promesostomidae, Solenopharyngidae, Trigonostomidae

INTRODUCTION

Relatively few free-living platyhelminth species from Australia, excluding Polycladida, have been reported and described. To date, most of the microturbellarians are Proseriata, with 41 species reported and described (Curini-Galletti 1997, 1998; Curini-Galletti and Cannon, 1995, 1996a, b, 1997; Martens and Curini-Galletti, 1989; Curini-Galletti *et al.*, 2002; Faubel and Rohde, 1998). Also known are four species of Macrostomida (Faubel, Blome and Cannon, 1994; Sluys, 1986), a single representative of Kalyptorhynchia (the polycystidid *Gyratrix hermaphroditus* Ehrenberg, 1831, which seems to be a complex of sibling species (Curini-Galletti and Puccinelli, 1990, 1998)), five species of Dalyellioida (of which only one, *Luriculus australiensis* Faubel *et al.*, 1994, is from a marine habitat (Faubel, Rohde and Watson, 1994; Hartenstein and Dwine, 2000; Hochberg and Cannon, 2001, 2002a; Schmarda, 1859)) and 15 species of Typhloplanoida. Among the latter, ten species are from freshwater habitats (Hochberg and Cannon, 2002a; Kolasa and Schwartz, 1988; Noreña-Janssen and Faubel, 1992; Schmarda, 1859). The five marine typhloplanoids are: *Magnetia queenslandica* Hochberg and Cannon, 2002 (see Hochberg and Cannon, 2002b), and four species of *Trigonostomum* (Willems *et al.*, in press). However, in an ecological study on tropical intertidal sediments Dittman (1991) recognised 108 different species, 16 of which were Typhloplanoida. In this contribution we report on five more typhloplanoids, of which three are new to science and two are known from localities outside Australia (*Ceratopera axi* (Riedl, 1954) Den Hartog, 1964 and *Ptychopera scutulifer* Ehlers and Ax, 1974), bringing the total number of named marine typhloplanoids to ten for Australia. They have all been found on the East Coast in areas around Townsville, Brisbane (North Stradbroke Island), Sydney and between Byron Bay and Coffs Harbour.

Next to their scientific names according to the Linnean system (and the International Code on Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999)), we also propose a converted name for each species in the phylogenetic system (Phylocode; http://www.ohio.edu/phylocode) following the system proposed by Artois (2001).

MATERIAL AND METHODS

The specimens for this study were collected during two separate expeditions: the first in August-September 1996 by Tom Artois and Ernest Schockaert (ES) and the second by ES in September-November 1997. The animals were extracted from the sediment or from algae using the $MgCl_2$-decantation method (see Schockaert, 1996), studied alive and whole mounted with lactophenol. Remaining specimens, if any, were fixed in marine Bouin's solution, embedded in paraffin, serially sectioned (4 μm sections) and stained with Heidenhain's iron haematoxylin, using eosin as a counterstain.

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Camera lucida drawings of hard parts were made, using Nomarski interference. Drawings without a scale bar are freehand. Measurements of hard parts are taken axially, unless indicated otherwise. The positions of the gonopore and organs, and the measurements of the pharynx are expressed in percentages of the total body length (distances from the anterior tip of the body).

The type material of the new species will be deposited in the collections of the Queensland Museum, Brisbane, Australia. Voucher specimens of *Ceratopera axi* (Riedl, 1954) Den Hartog, 1964 from California and the Falklands, were loaned from the Swedish Museum of Natural History in Stockholm (SMNH). A whole mount of *Ptychopera scutulifer* Ehlers and Ax, 1974 from Somalia is present in the collections of the LUC (Diepenbeek, Belgium).

Abbreviations used in the figures

b: brain; bs: bursal stalk; cg: caudal glands; cga: common genital atrium; ci: cirrus; cil: cilia; de: ejaculatory duct; e: eye; ecm: external circular muscle; elm: external longitudinal muscle; fb: female bursa; fd: female duct; fg: female glands; gg: prostate glands; gm: glands of Minot; gp: common genital pore; i: intestine; icm: internal circular muscle; id: insemination duct; ilm: internal longitudinal muscle; lm: longitudinal muscle; m: mouth; ma: male atrium; od: oviduct; ov: ovary; pc: prepharyngeal cavity; pg: pharynx glands; ph: pharynx; pl: pharynx lumen; ppt: pharynx protractors; rg: rostral glands; rh: rhabdite; rm: radial muscle; s: stylet; sph: sphincter; t: testis; v: vas deferens; vd: vitelloduct; vg: prostate vesicle; vit: vitellaria; vs: seminal vesicle; y, z: features described in respective text.

TAXONOMIC ACCOUNT

TRIGONOSTOMIDAE GRAFF, 1905 *sensu* DEN HARTOG, 1964

Ceratopera **Den Hartog, 1964**

Ceratopera axi **(Riedl, 1954) Den Hartog, 1964**

ceratopera-axi (Riedl, 1954) Den Hartog, 1964
Proxenetes axi Riedl, 1954
Ceratopera bifida Ehlers and Ax, 1974

Locality in Australia. Arrawarra (New South Wales): on small, shell-shaped brown algae (*Pedina* sp.) (29/08/1996) and on *Pavonina*-like algae (27/08/1996) in intertidal rockpools.

Known distribution. Gulf of Naples and Sicily (Riedl, 1954); Galapagos (Ehlers and Ax, 1974); Falkland Islands and California (Karling, 1986); Weddell Sea and La Réunion (Artois *et al.* 2000).

Material. Observations on live, mature specimens and two whole mounts (one from each new locality). Whole mounts from Falkland and California (collections of SMNH).

Remarks. Following Karling (1986) and Artois *et al.* (2000), we consider *C. bifida* Ehlers and Ax, 1974 a junior synonym of *C. axi* (Riedl, 1954) Den Hartog, 1964. The stylet of one of the Australian specimens is 70 µm long (measured along the axis of the stylet; 56 µm if measured from top to bottom as in Ehlers and Ax, 1974). The bursal appendage of this specimen is 94 µm long and splits distally. Neither part could be measured in the second specimen. In comparison with other populations (see Table 1), the Australian specimen has the smallest stylet.

Table 1. Measurements of the stylet and the bursal appendage of *Ceratopera axi* in different populations.

	Stylet	Bursal appendage	Reference
Australia	70 µm	94 µm	this paper
Galapagos	94–95 µm	67–87 µm	Ehlers and Ax, 1974
Falklands	120–180 µm	91 µm	Karling, 1986
California	93–117 µm	102 µm	Karling, 1986
La Réunion	105 µm	77 µm	Artois *et al.*, 2000
Weddell Sea	124 µm	?	Artois *et al.*, 2000

Ptychopera **Den Hartog, 1964**

Ptychopera scutulifer **Ehlers and Ax, 1974**

ptychopera-scutulifer Ehlers and Ax, 1974

Localities in Australia. Arrawarra (New South Wales): southern part of the beach, on *Sargassum*-like algae in a large permanent pool at the beginning of a mass of rocks (27/08/1996). North Stradbroke Island, Amity Point (Queensland): in muddy sediment from amongst mangroves (14/08/1996).

Known distribution. Galapagos (Ehlers and Ax, 1974); Somalia (Schockaert and Martens, 1985).

Material. Two mature specimens studied alive and mounted (one from each new locality). A whole mount of a specimen from Somalia (collections of LUC).

Remarks. The specimen from Stradbroke Island has a 54 µm-long stylet (measured axially). The sclerotised part of the afferent female duct (Ehlers and Ax, 1974: ductus spermaticus) between the swollen part (Ehlers and Ax, 1974: receptaculum seminis) and the atrial bursa (Ehlers and Ax, 1974: bursa copulatrix) is 49 µm long and is bent over 90°. These data correspond with the measurements on specimens from the Galapagos (38–40 µm-long stylet, axially measured; 23 µm-long sclerotised part of the afferent duct; Ehlers and Ax, 1974) and from Somalia (45–47 µm-long stylet; 20 µm sclerotised part of the afferent duct; Schockaert and Martens, 1985). On the other hand, the stylet of the Arrawarra specimen measures 98 µm, while the distal part of the afferent duct is 140 µm long. Whether these aberrant values represent a fixed difference between populations or are due to individual variability is not yet clear. It is also the only specimen found on algae. We, however, prefer to retain the Arrawarra-specimen within this species until/unless further material should suggest otherwise.

PROMESOSTOMIDAE DEN HARTOG, 1964

Brinkmanniella **Luther, 1943**

Brinkmanniella australiensis **n.sp.**

brinkmanniella-australiensis n.sp.
(Fig. 1)

Locality in Australia. Arrawarra (New South Wales): on crustaceous algae on rocks on the beach north of the headland

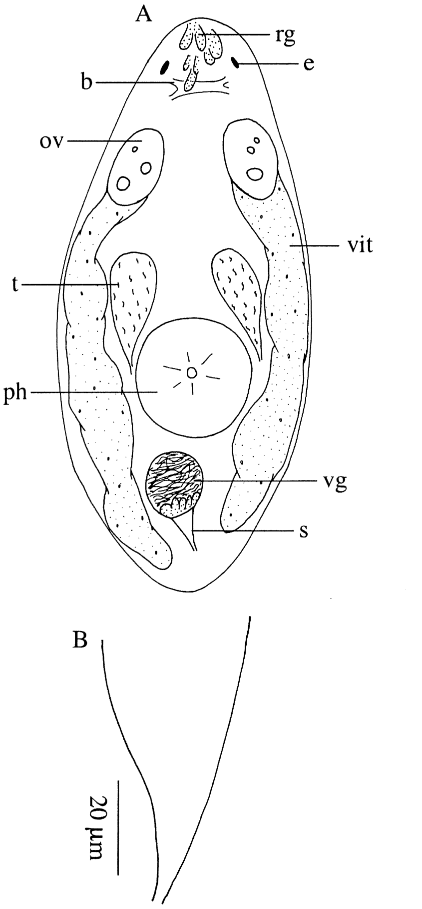

Fig. 1. *Brinkmanniella australiensis* n.sp. - A. General organisation (from a live specimen). - B. Stylet (from the holotype).

(27/08/1996) (type locality).

Material. One animal studied alive and mounted (holotype).

Etymology. The specific name refers to the species' occurrence in Australia.

Description. The animal is 0.9 mm long (measured on whole mount), pale yellow and has two eyes. The general organisation does not deviate from that of other *Brinkmanniella*-species (Fig. 1A; see also Ehlers, 1974; Karling, 1986; Luther, 1943, 1948; Marcus, 1951; Schockaert and Martens, 1985). The stylet (Fig. 1B) is thin-walled, straight and funnel-shaped. It is 57 µm long, and 32 µm wide proximally and slightly constricted about halfway along its length.

Diagnosis Brinkmanniella australiensis. Species of *Brinkmanniella* with straight funnel-shaped stylet of 57 µm long and proximally 32 µm wide, constricted halfway along its length and with a smooth distal tip.

Discussion. The representatives of the taxon *Brinkmanniella* are characterised by the rostral position of the ovaries, the absence of any special female atrial organs, apart from the female duct, and the caudally-situated pharynx. Schockaert and Martens (1985) gave a good overview of the main characters of *Brinkmanniella*-species. The stylets of *B. obtusa* Luther, 1943, *B. augusti* Marcus, 1951 and *B. palmata* Karling, 1986, show "fingers" at the distal opening (Karling, 1986), while *B. australiensis*, *B. macrostomoides* Luther, 1948, *B. procerastyla* Ehlers, 1974 and *B. microps* Schockaert and Martens, 1985 lack these "fingers". Of these latter four species, only *B. microps* and *B. australiensis* have a straight tubiform stylet, while it is curved in the other two species. The stylet of *B. microps* is only 30 µm long and 10 µm wide proximally and not constricted midway as in *B. australiensis*. The stylet of *B. australiensis* is twice as long with a width/length ratio of about 1/2.

Vauclusia conica **n.g. n.sp.**

vauclusia-conica n.sp.

(Fig. 2)

Locality in Australia. Sydney, Vaucluse beach (New South Wales): flat beach with fine sand and numerous crab holes, in eulittoral (10/10/1997) (type locality).

Material. One individual studied alive and mounted (holotype). Three serially-sectioned specimens (paratypes).

Etymology. The genus name/praenomen refers to the type locality. The specific name emphasizes the overall structure of the copulatory organ. Conicus (Lat.): cone-shaped.

Description. The slender animal is ± 1.4 mm long (measured on the whole mount), without eyes. The cellular epidermis is ± 3.5 µm thick, with cilia of 3 µm long. The basement membrane is ± 1 µm thick. At the rostral end of the body, two types of large rhabdite glands are present. The first type, situated at the periphery of the glandular mass, produces large, basophilic rhabdites of 8.5–10.5 µm long. The glands in the centre produce eosinophilic rhabdites of the same size, but less densely packed within the cell bodies. All glands end at the rostral body tip, the basophilic ones stretching to the testes, the eosinophilic ones not further than the brain. There are also glands in the caudal body region (Fig. 2E: cg), opening ventrally behind the gonopore and producing large, basophilic rhabdites.

The mouth is situated at ± 65% and can be closed by a strong sphincter. The prepharyngeal cavity is lined with a low, nucleated epithelium and surrounded by only longitudinal muscles. The distal rim of the pharynx is lined with a very low epithelium, with a thick basement membrane and

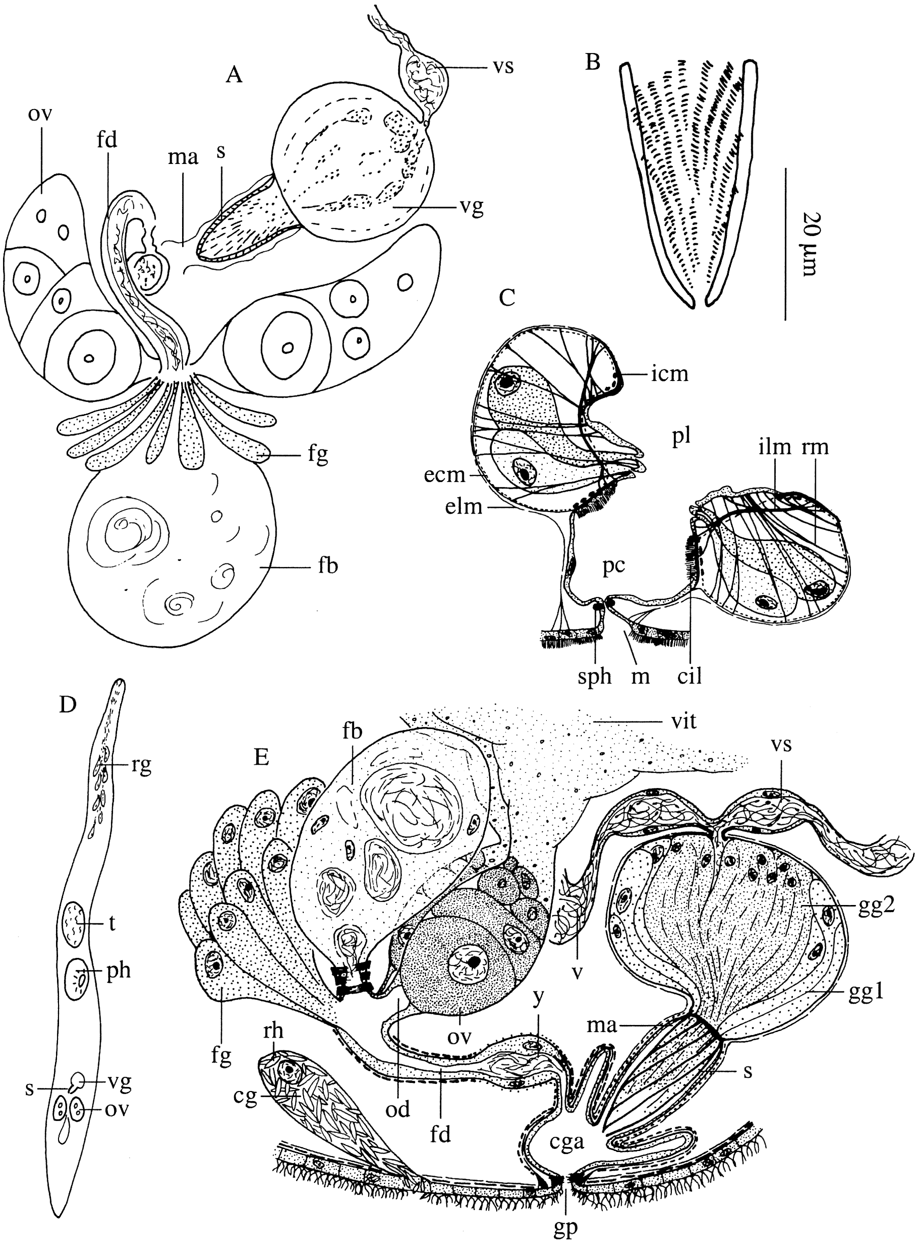

Fig. 2. *Vauclusia conica* n.g. n.sp. - A. Organisation of the genital system (from a live specimen). - B. Stylet (from the holotype). - C. Pharynx (reconstruction on sagittal sections). - D. Habitus of a live animal. - E. Reconstruction of the atrial organs from the right side.

cilia (Fig. 2C: cil). The pharynx lumen (Fig. 2C: pl) is lined with a low, anucleated epithelium. The epithelium of the proximal pharyngeal rim is degenerated, leaving only a thick pseudocuticula. The inner circular muscles are thicker near the proximal and distal ends of the bulb. The exact number of internal longitudinal muscles could not be determined.

There are two types of eosinophilic pharyngeal glands, one coarse-grained and one fine-grained. They open into the lumen somewhat proximally from the distal sphincter, with the fine-grained ones most distally. In one of the sectioned specimens a third, basophilic gland could be observed. However, the exact location and the place of discharge could not be determined. The external pharyngeal muscle layers consisting of an inner circular (Fig. 2C: ecm) and an outer longitudinal one (Fig. 2C: elm), are rather weak.

The common genital pore is situated at ± 80%. The common genital atrium is lined with a high, anucleated epithelium and surrounded by an inner longitudinal and an outer circular muscle layer. Although the live specimen (Fig. 2D) apparently only showed one testis, there are clearly two testes in the sectioned specimens. The testes are situated ventrally just in front of the pharynx, at both sides of the body. The vasa deferentia are clearly visible in the sectioned specimens.

The paired seminal vesicles (only one observed in the live specimen; Fig. 2A) are lined with a low, nucleated epithelium. They continue towards the prostate vesicle, fusing when entering the prostate vesicle. The prostate vesicle (Fig. 2A: vg) is surrounded by a layer of longitudinal muscles, and contains fine-grained (Fig. 2E: gg2) and coarse-grained eosinophilic glands (Fig. 2E: gg1), the fine-grained glands located in the centre of the bulb. All glands are entirely intracapsular. The stylet (Fig 2B), connected to the prostate vesicle, fills almost the entire male atrium. This stylet is cone-shaped, 29 μm long, 17 μm wide proximally and 5 μm wide distally. The exact structure of the stylet could not be determined, but probably consists of about eight elongated and spirally-running bars (or ridges), which show some striation. The male atrium (Fig. 2E: ma) enters the common genital atrium from the rostral side. It is lined with the same epithelium and surrounded by the same muscle layers as the common genital atrium, only the muscle layers have changed position, revealing an inner circular and an outer longitudinal muscle layer.

The ovoid ovaries are situated caudally from the gonopore. They form the distal part of the ovovitellaria. The female duct (Fig. 2E: fd) is long and enters the common genital atrium at the caudal side. It is lined with a high, anucleated epithelium and surrounded by strong circular muscles. Distally it is swollen and contains many sperm (Fig. 2E: y). This part is lined with a nucleated epithelium. Proximally the female duct ends in the female bursa (Fig. 2E: fb). It receives both oviducts somewhat distally from the bursal entrance. The part of the female duct between the bursa and the oviducts ("bursal stalk") is surrounded by circular muscles. The oviducts are rather short and lined with a low anucleated epithelium. A large bundle of coarse-grained eosinophilic glands (Fig. 2E: fg) enters the female duct ventrally at the bifurcation into the oviducts. A uterus is lacking.

Diagnoses Vauclusia. Promesostomidae with the pharynx situated in the middle of the body. Inversion of muscle layers at the transition from the common genital atrium to the male genital atrium. Paired testes and seminal vesicles. Globular prostate vesicle with two types of secretion. Cone-shaped stylet, consisting of several plate-like bars (or ridges). Paired ovovitellaria. Very long female duct. Distal part of the female duct swollen and filled with sperm. Female bursa and female glands present. Type species: V. conica.

Vauclusia conica. Provisionally with the same diagnosis as the genus. Stylet 29 μm long.

Discussion. The combination of paired, solid testes, paired ovovitellaria, lack of a second connection of the female gonads with the exterior and the presence of only one genital pore are diagnostic features of the Promesostomidae (see Den Hartog, 1964). The new species fits into this diagnosis. Its possible relationships within this taxon are less clear. The fact that the ovaries in *V. conica* are not separated from the vitellaria, and the shortness of its male genital atrium exclude it from the present taxa Adenorhynchinae Ax and Heller, 1970 and Promesostominae Luther, 1948. However it shares some characters with this last taxon such as the presence of a terminal female bursa and terminal female glands. The connection of the ovaries with the vitellaria (ovovitellaria) also suggests a relationship with members of the Brinkmanniellinae Luther, 1948. Both in the members of the Adenorhynchinae and the Brinkmanniellinae the male atrium is very short. The relative values of all these characters can only be assessed by a detailed cladistic analysis including many more 'Typhloplanoida'. Therefore, we refrain from allocating *V. conica* to any of the subtaxa within the Promesostomidae.

SOLENOPHARYNGIDAE GRAFF, 1882

***Pilamonila bimascula* n.g. n.sp.**

pilamonila-bimascula n.sp.

(Figs 3–4)

Locality in Australia. Arrawarra (New South Wales): south of the headland, on *Sargassum*-like algae in a large permanent pool and on algae in a permanent pool in front of the marine station, lower eulittoral (27/08/1996) (type locality).

Material. Three mature animals studied alive and mounted (one designated holotype, the others paratypes) and three sectioned specimens (designated paratypes).

Etymology. The genus name/praenomen refers to the chain of globular structures in the female system. Pila (Lat.): ball. Monile (Lat.): necklace. The specific name emphasizes the presence of a stylet and an armed cirrus. Bis (Lat.): twice. Masculus (Lat.): manly.

Description. The animal is 0.4–0.5 mm long (measured on whole mount), with two eyes. The body is whitish with black to yellow-brown spots when observed under incident light, pale brown, and opaque when observed with transmitted

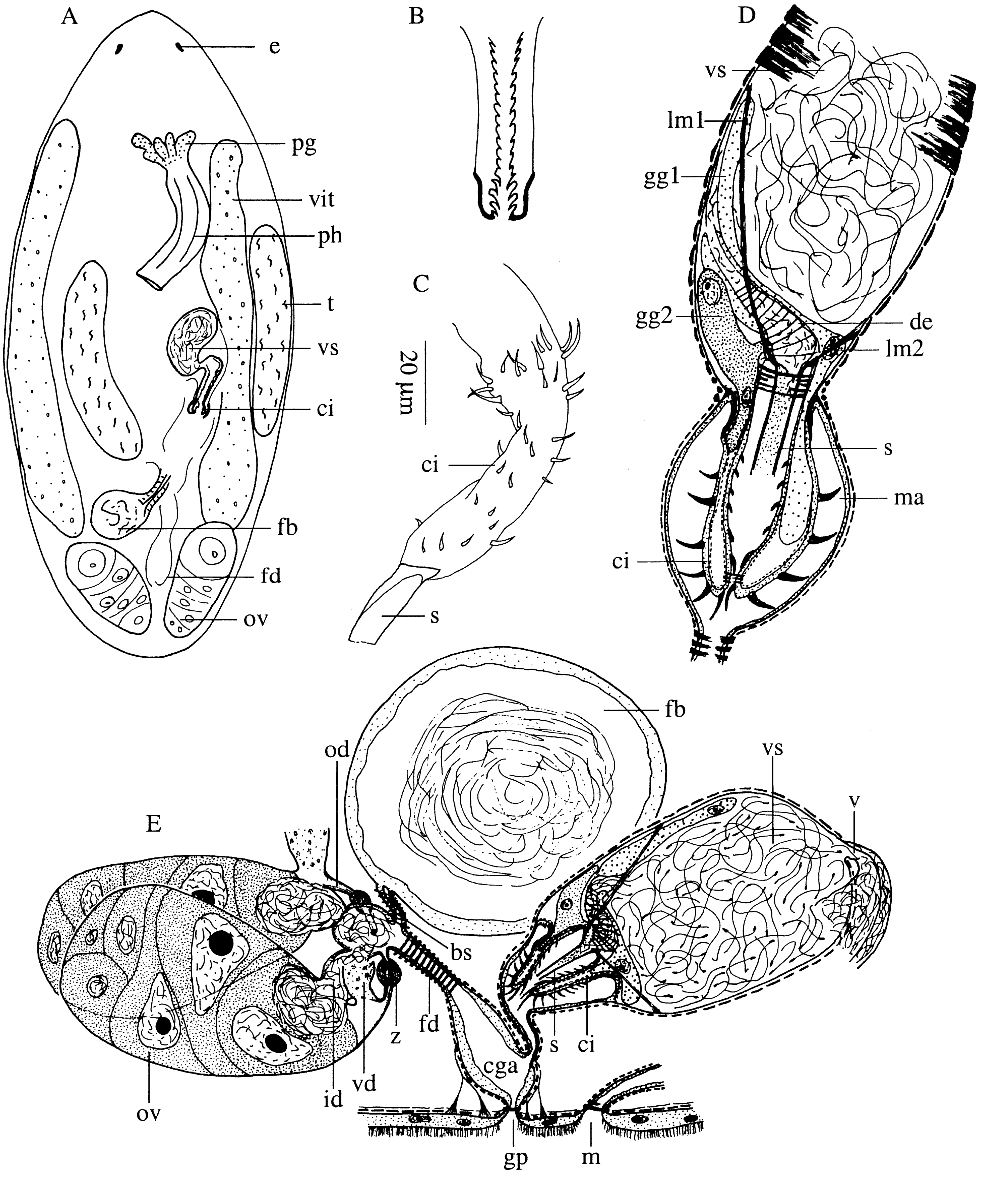

Fig. 3. *Pilamonila bimascula* n.g. n.sp. - A. General organisation (from a live specimen). - B. Inverted cirrus (from a live specimen). - C. Everted cirrus and stylet (from the holotype). - D. Male genital system (reconstruction on sagittal sections). - E. Reconstruction of the atrial organs from the right side.

light. The syncytial epidermis is 3 µm thick with cilia of 3 µm long. The basement membrane is ± 1 µm thick. Rhabdites are very small and equally distributed over the whole epidermis.

The elongated pharynx (Figs 3A: ph, 4C) is situated in the first body half with the mouth at ± 65% (on sections). It is inclined towards the rostral body end. A narrow duct connects the mouth with the deep prepharyngeal cavity (Fig.

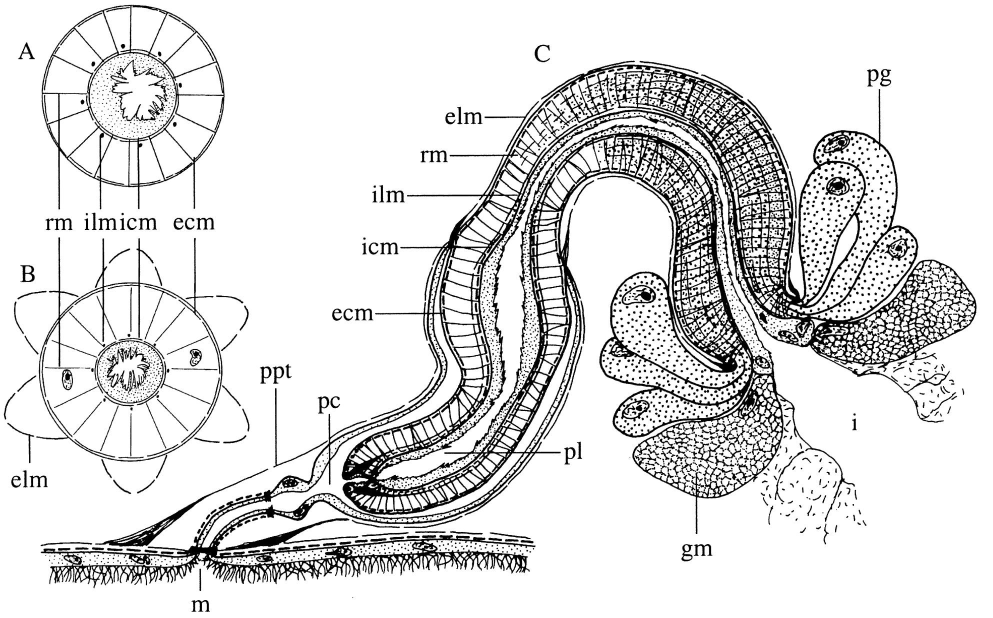

Fig. 4. *Pilamonila bimascula* n.g. n.sp. - A, B. Sections through the pharynx (A. Distally; B. Proximally). - C. Reconstruction of the pharynx from the right side.

4C: pc). This duct is lined with a low, anucleated epithelium and surrounded by strong circular muscles. At the transition of this duct with the prepharyngeal cavity the epithelium is much higher and shows some nuclei. The prepharyngeal cavity is lined with a low, nucleated epithelium and surrounded by longitudinal muscles. The distal pharyngeal rim has no cilia. The pharynx is surrounded by an outer longitudinal and an inner circular muscle layer. The former is, at least in the proximal part of the pharynx, arranged in six bundles forming a six-pointed star (Fig. 4B). Each "point of the star" contains four longitudinal fibres, showing a total of 24 external longitudinal muscles. The internal muscles consist of weak radial muscles (Fig. 4C: rm), a circular (Fig. 4C: icm) and a longitudinal layer (Fig. 4C: ilm). There are eight inner longitudinal and 16 radial muscle fibres (Fig. 4A). The epithelium of the lumen has degenerated to a pseudociliation. The pharynx contains coarse-grained eosinophilic glands and some coarse-grained basophilic glands. The latter enter the pharynx proximally, and thus have an extrapharyngeal part. The exact place where both types of glands open into the lumen could not be determined, but must be close to the distal end of the pharynx.

The common genital pore lies at 70%, just behind the mouth, and can be closed by a sphincter. The common genital atrium is lined with a high, anucleated epithelium and is surrounded by an inner circular and an outer longitudinal muscle layer.

The paired and elongated testes lie ventrally of the prostate vesicle, just behind the pharynx. The vasa deferentia are short, and connect the testes with the intracapsular seminal vesicle (Fig. 3E: vs). This vesicle fills almost the entire proximal part of the copulatory bulb, and narrows distally to the ejaculatory duct (Fig. 3D: de). The ejaculatory duct runs centrally through prostate glands. It is lined with an anucleated, membranous epithelium, surrounded by weak circular muscles and connected to a small, tubiform stylet (Fig. 3C–E: s). There are basophilic (Fig. 3D: gg1) and eosinophilic (Fig. 3D: gg2) prostate glands, situated in the most distal part of the prostate vesicle and discharging their contents through the stylet. This stylet is 12–19 μm long and 4–5 μm wide (in the holotype: 19 μm and 4 μm respectively). It was visible in only two of the three whole mounts, but in all sectioned specimens. The copulatory bulb is surrounded by a circular muscle layer. From the stylet two groups of muscle fibers run obliquely through the copulatory bulb. The dorsal group attaches to the wall of the copulatory bulb in its proximal part (Fig. 3D: lm1), the ventral group in its distal part (Fig. 3D: lm2). The stylet lies in a cirrus, which bears small spines (1–2 μm) in its proximal part and large spines (5–8 μm) distally. Both parts are separated by a sphincter, and in the sectioned individuals the distal part of the cirrus was everted, and when completely everted (as in two of the whole mounts; Fig. 3C) the stylet appears at the end of the cirrus. The cirrus is lined with a low, membraneous, anucleated epithelium, as also is the male atrium. Only at the transition between the male atrium and the spiny cirrus is the epithelium hardened, and this forms a 'cap' at the end of the cirrus when it is completely inverted (Fig. 3B).

The female duct (Fig. 3E: fd) enters the common genital atrium dorsally. It is lined with a low, anucleated epithelium and surrounded by circular muscles. Proximally the female duct widens and is filled with sperm. It bifurcates towards the ovaries and these insemination ducts (Fig. 3E: id) are swollen and also filled with sperm. Both ovaries have a second connection (Fig. 3E: od) to the swollen part of the female duct. These ducts receive the vitelloducts (Fig. 3E: vd) and are therefore to be considered the oviducts. In the middle of the oviducts a sclerotized ring appears (Fig. 3E: z). The bursa (Fig. 3E: fb) lies above the female duct. This large, globular bursa is also connected to the swollen part of the female duct by a short, very narrow canal, surrounded by circular muscles (Fig. 3E: bs). A uterus is lacking.

Diagnoses Pilamonila. Solenopharyngidae with the mouth situated at 2/3 of the body. Pharynx elongated, with well-developed basophilic glands proximally. Testes paired. Large internal seminal vesicle. Male copulatory organ consists of a spiny cirrus and a simple tubiform stylet within the cirrus. Ovaries paired. Large female bursa with muscular bursal stalk. Double connection between ovaries and female duct: swollen spermatic ducts filled with sperm, and narrow oviducts, halfway surrounded by a thickened sclerotised ring. Type species: *P. bimascula.*

Pilamonila bimascula. Provisionally with the same diagnosis as the genus. Stylet ± 15 μm long, cirrus with small proximal spines (1–2 μm) and large distal spines (5–8 μm).

Discussion. Based on the ventrocaudally-oriented, elongated pharynx, strongly-developed extrapharyngeal glands, the caudally-situated mouth, the presence of a common genital pore, paired testes, unpaired seminal vesicle, intracapsular prostate glands, the presence of a cirrus and paired vitellaria, this species is placed within the Solenopharyngidae Graff, 1882 (see Ehlers, 1972). The general structure of the male copulatory organ does not seem to deviate from that of other solenopharyngids, except for the presence of a sclerotised structure at the end of the ejaculatory duct. This hard part is a 'real' single-walled stylet in contrast with the sclerotised lining of the ejaculatory duct as in *Proceropharynx litoralis* Ehlers, 1972 and *Lenopharynx tubatus* Schockaert and Martens, 1985 (see Schockaert and Martens, 1985). The paired ovaries of *P. bimascula* suggest a placement within the subfamily Lenopharynginae Ehlers, 1972. However, the organisation of the female system with the double connection between the ovaries and the female duct is completely aberrant from that in all other representatives of the Solenopharyngidae. At the moment we refrain from speculation about the relationships of *P. bimascula* within the Solenopharyngidae and placement in any of the subfamilies recognised by Ehlers (1972).

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III

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Freshwater Rhabdocoela (Platyhelminthes) from Ephemeral Rock Pools from Botswana, with the Description of Four New Species and One New Genus

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ABSTRACT—Four new species of freshwater rhabdocoel flatworms from ephemeral rock pools in southeastern Botswana are described and discussed. Two of them, *Syringoplana kolasai* n. gen. n. sp. and *Mesostoma thamagai* n. sp. belong to the Typhloplanidae Graff, 1905. The unique construction of the excretory system is the main characteristic of *S. kolasai*. *M. thamagai* can be separated from other *Mesostoma* Ehrenberg, 1837 species by the presence of a bundle of eosinophilic glands at the transition from oviduct to seminal receptacle. The other two taxa, *Gieysztoria isoldeae* n. sp. and *G. faubeli* n. sp. belong to the Dalyelliidae Graff, 1905. *G. isoldeae* is characterised by the presence of four separate hollow spines in the male atrium, which are connected to two accessory glandular organs. *G. faubeli* can be separated from other *Gieysztoria* Ruebush and Hayes, 1939 species by the detailed construction of the stylet. Apart from these two species the occurrence of an unidentified *Microdalyellia* Gieysztor, 1938 species is mentioned.

Key words: Rhabdocoela, *Syringoplana*, *Mesostoma*, *Gieysztoria*, new taxa

INTRODUCTION

Although free-living flatworms ("Turbellaria") are of major importance in freshwater and marine ecosystems, our knowledge of this group is very scant. This is especially true for tropical and subtropical areas, such as South America, Australia and Africa. Knowledge of the African freshwater Turbellaria mostly comes from older literature. A comprehensive list of the African species of freshwater Turbellaria and of the literature concerning them is given by Young (1976). This author recognises 83 valid species. Since then not much work on African freshwater Turbellaria has been done (De Vries, 1988; Kolasa, 1976; Kolasa and Mead, 1981; Young, 1977).

In this contribution we describe four new rhabdocoel species from ephemeral rock pools in southeastern Botswana. These pools were sampled within the framework of an intercontinental comparative study on the faunal community structures of ephemeral rock pools in subtropical areas. Two of the species described here, *Mesostoma thamagai* n. sp. and *Syringoplana kolasai* n. gen. n. sp., belong to the large and widespread rhabdocoel taxon Typhloplanidae Graff, 1905. The other two, *Gieysztoria isoldeae* n. sp. and *G. faubeli* n. sp. fit into the Dalyelliidae Graff, 1905, another species-rich and widespread taxon of rhabdocoel flatworms. A third representative of the Dalyelliidae, a species of *Microdalyellia* Gieysztor, 1938, was also collected. However, the only whole mounted specimen of this species is in such bad condition that it does not allow further identification.

MATERIAL AND METHODS

All turbellarians were hatched from dry sediment samples containing the resting propagules collected from ephemeral rock pools on a granite escarpment in southeastern Botswana. The samples were taken with a spoon and a little brush during the dry phase of the pools. Sediment was transported to Belgium in plastic bags. To obtain hatchlings, about 200 g of sediment was incubated in distilled water, under 24-hour light conditions and at a temperature of 25°C. Usually the first turbellarians were visible after one or two days. They were fed with living *Daphnia* and fairy shrimps (freshwater Anostraca).

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Specimens of most species could be studied alive. If hard parts were present, whole mounts were prepared using lactophenol. Specimens intended for sectioning were fixed using hot (50°C) Bouin's fixative. They were embedded in paraffin and serially sectioned (5 μm), then stained with Heidenhain's haematoxylin, using erythrosine as counterstain.

Hard parts were measured axially. Drawings without a scale are freehand.

Type material will be deposited in the collections of the research group Biodiversity, Phylogeny and Population Studies of the Limburgs Universitair Centrum (LUC), Diepenbeek, Belgium.

TAXONOMIC ACCOUNT

TYPHLOPLANIDAE GRAFF, 1905

***Syringoplana kolasai* n. gen. n. sp.**

(Fig. 1)

Locality. Botswana, Thamaga (24°41'50"S, 25°31'00"E), ephemeral rock pools on isolated granite domes (type locality). Sediment collected 22/12/1998, inundated in the laboratory 4/10/2002.

Material. Observations on a live animal. Several sectioned specimens, a sagitally-sectioned one designated holotype (LUC nr. 232), the others paratypes (LUC nr. 233–238).

Etymology. The genus is named after Syrinx, one of the Naiads (water nymphs) of Greek mythology. Species epithet in honour of Dr. Jurek Kolasa (Hamilton, Canada).

Description. The animals are relatively small, oval-shaped, with a blunt rostral and a pointy caudal end ("tail"). Animals coloured dark brownish green, owing to the presence of a subepidermal pigment, which is less dense at the ventral side. Eyes absent.

Epidermis cellular, ciliated all over the body surface. Dermal rhabdites absent. Anteriorly there are some large rhabdite glands that produce large, adenal rhabdites.

The excretory system consists of two protonephridial ducts, situated at both sides of the body. They end in a common excretory pore, which is just caudal from the gonopore and lies in a ventro-caudal pit. Several large, coarse-grained basophilic glands surround this pit. The excretory pore can be closed by a sphincter.

The mouth is at 1/3 of the body length and is surrounded by a weak sphincter. The prepharyngeal cavity is relatively small and lined with a very low epithelium without nuclei. It is surrounded by a weak internal circular and a strong external longitudinal muscle layer. The globular pharynx is very large and oriented vertically. Its distal border is lined with a very low, membranous, anucleated epithelium, with very short cilia. The pharynx lumen is also lined with a very low, anucleated epithelium that has no cilia. The pharynx musculature consists of a weak internal circular muscle layer (around the pharynx lumen), a thick internal longitudinal muscle layer consisting of 24–26 muscles, an external circular and an external longitudinal muscle layer underneath the septum of the bulb, and a longitudinal muscle layer just outside the septum, continuous with the longitudinal muscles of the prepharyngeal cavity. Strong radial muscles connect the wall of the pharynx lumen with the septum of the bulb. There are two types of pharyngeal glands, with the eosinophilic fine-grained ones entering the pharynx lumen proximally from the basophilic coarse-grained ones.

The genital system lies just caudally from the pharynx, the gonopore situated ventrally at about 2/3 of the body length, just in between the excretory pore and the mouth. The two large testes are situated at both sides of the body. The single ovary is situated at the level of the gonopore. The vitellaria are long and narrow, extending dorsally from the testes at both sides of the body. The common genital atrium is surrounded by an inner circular and an outer longitudinal muscle layer, and lined with a high, nucleated, lightly sclerotised epithelium.

The copulatory organ enters the genital atrium dorsally. It is a large, ovoid bulb, with a septum enclosing the seminal vesicle, the prostate glands and the cirrus (conjuncta-duplex copulatory organ; terminology of Karling, 1956). Two spirally-running muscle layers surround the bulb. The vasa deferentia join each other just before entering the copulatory bulb at its proximal end. Within the bulb the seminal duct enlarges to form a seminal vesicle, which is surrounded by a weak circular muscle layer. The seminal duct enters the cirrus about midway along the bulb. The cirrus consists of a rather broad tube armed with small, sharp spines and is surrounded by an inner circular and an outer longitudinal muscle layer. The coarse-grained basophilic prostate glands enter the copulatory bulb at the same place as do the vasa deferentia, feeding into the prostate vesicle, which in turn enters the cirrus at the same place as does the seminal vesicle. The internal prostate vesicle is surrounded by a weak circular muscle layer.

The female duct enters the genital atrium caudally, while proximally it ends in the small ovary. Somewhat halfway, it makes a 180° turn and starts running anteriorly. At the turn, a large bundle of fine-grained eosinophilic glands enters the duct. Just before it reaches the ovary it widens to a seminal receptacle, containing eosinophilic glandular secretion and many sperm. The duct is lined with a high, nucleated epithelium and surrounded by an inner circular and an outer longitudinal muscle layer.

The vitelloduct could not be seen. The single uterus leaves the common genital atrium anteriorly.

Diagnosis. *Syringoplana* n. gen. Typhloplanidae with pharynx rosulatus in the first 1/3 of body. Dermal rhabdites lacking. Excretory system with two lateral nephridial ducts ending in a single ventral excretory pore, caudal from the gonopore. Excretory pore surrounded by large basophilic glands. Testes paired, lying laterally from the pharynx, ventrally from the vitellaria. Copulatory organ of the duplex-type, caudal to the pharynx. Seminal vesicle single, intracapsular. Prostate vesicle intracapsular. With a cirrus with fine spines. Ovary single. Oviduct enlarged to a seminal receptacle. Large bundle of eosinophilic glands entering the female duct

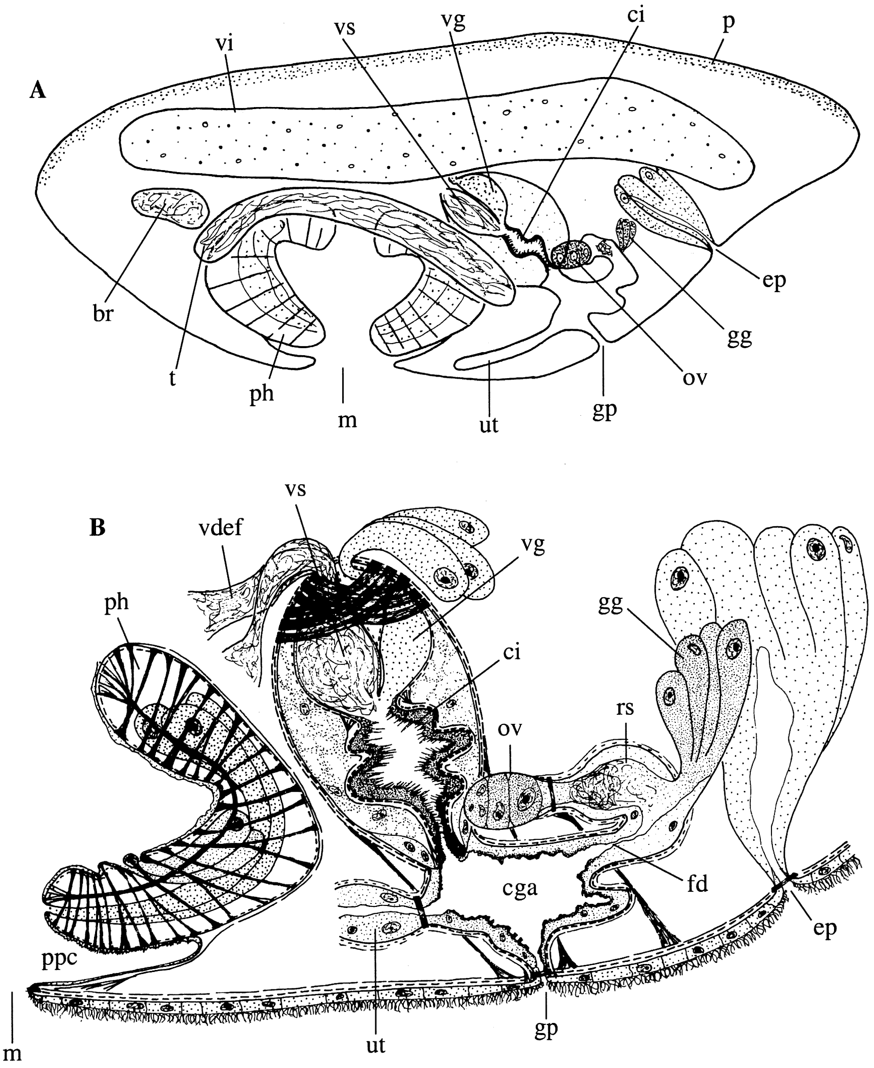

Fig. 1. *Syringoplana kolasai* n. gen. n. sp. (A) Semi-diagrammatic reconstruction of the whole animal from sagital sections. (B) Reconstruction of the genital system (holotype). br, brain; cga, common genital atrium; ci, cirrus; ep, excretory pore; fd, female duct; gg, glands; gp, gonopore; m, mouth; ov, ovary; p, pigment grains; ph, pharynx; ppc, prepharyngeal cavity; rs, seminal receptacle; t, testis; ut, uterus; vdef, vas deferens; vi, vitellarium; vg, prostate vesicle; vs, seminal vesicle.

distally from the seminal receptacle. Type species: *Syringoplana kolasai* n. sp. Provisionally with the same diagnosis as the genus.

Discussion. Syringoplana kolasai differs from all other taxa of Typhloplanidae by the presence of only one ventral excretory pore, separate from the mouth, and lying just caudally from the gonopore. In most of the other species of Typhloplanidae, the protonephridial ducts have two separate pores at the body surface (Protoplanellinae Reisinger, 1924, Olisthanellinae Luther, 1904, Ascophorinae Findenegg, 1924, Phaenocorinae Wahl, 1910). Alternatively, the protonephridial ducts join each other and enter the prepharyngeal cavity near the mouth (Mesostominae Luther, 1904, Typhloplaninae Luther, 1904) or in the common genital atrium (Rhynchomesostominae Bresslau, 1933).

Not considering the unique construction of the excretory system, the new taxon shows a combination of features characteristic of the Protoplanellinae: a typical globular, ventrally-oriented pharynx rosulatus, testes ventral to the vitellaria and the excretory ducts ending at the body surface (but separately in the Protoplanellinae). The presence of a spiny cirrus makes the copulatory organ resemble that of *Acrochordonoposthia* Reisinger, 1924. The pharynx is, however, in a more central position, in comparison with the anterior position in *Acrochordonoposthia*.

From the above it is clear that the new species cannot be put into any of the existing genera, and therefore must be placed in a genus of its own. The position of this new genus within the Typhloplanidae remains unclear.

Mesostoma thamagai **n. sp.**

(Fig. 2)

Locality. Botswana, Thamaga (24°41'50"S, 25°31'00"E), ephemeral rock pools on isolated granite domes (type locality). Sediment collected 22/12/1998, inundated in the lab 4/10/2002.
Material. Observations on live animals. Several serially-sectioned animals, a sagitally-sectioned one designated holotype (LUC nr. 239), the others paratypes (LUC nr. 240–247).
Etymology. Named after the type locality.

Description. Elongated, elliptical animals with two eyes. They are 3–4 mm long when reared in the lab. Animals collected in the field were not measured, but appear to be somewhat larger. Adult animals coloured dark brown by subepidermal pigment. Juveniles are somewhat lighter.

Epidermis cellular, consisting of large octagonal cells, ciliated and containing numerous large, rod-shaped rhabdites.

Protonephridia extend at both sides of the body and end separately in the prepharyngeal cavity.

The pharynx is of the rosulatus type and is identical to that of other *Mesostoma* Ehrenberg, 1837 species. It is situated at 1/3 of the body length. The mouth is lined with a degenerating, ruffled epithelium (pseudociliation) and can be closed by a sphincter. The prepharyngeal cavity is lined with a low epithelium with a few nuclei. Most of the nuclei of this epithelium are, however, insunk in a nuclear pouch situated at the junction between the prepharyngeal epithelium and the pharynx bulb. The prepharyngeal cavity is surrounded by longitudinal muscles, which continue around the pharynx bulb. The distal pharynx rim is lined with a low, ciliated epithelium that becomes unciliated towards the pharynx lumen. There are 32 internal longitudinal muscles. There are three types of pharyngeal glands: coarse-grained eosinophilic, coarse-grained basophilic and fine-grained eosinophilic. All three types enter the pharynx lumen near the distal end of the pharynx bulb, the fine-grained eosinophilic ones probably most proximally.

There are two very large testes, extending at both sides of the body. They are connected to each other at three places: caudally, rostrally and at 3/4 of the body length. The very small ovary is situated just behind the pharynx, at the left hand side. The vitellaria are paired and situated ventrally to the testes.

The gonopore lies just behind the mouth, both being situated within a depression of the ventral body wall. The com-

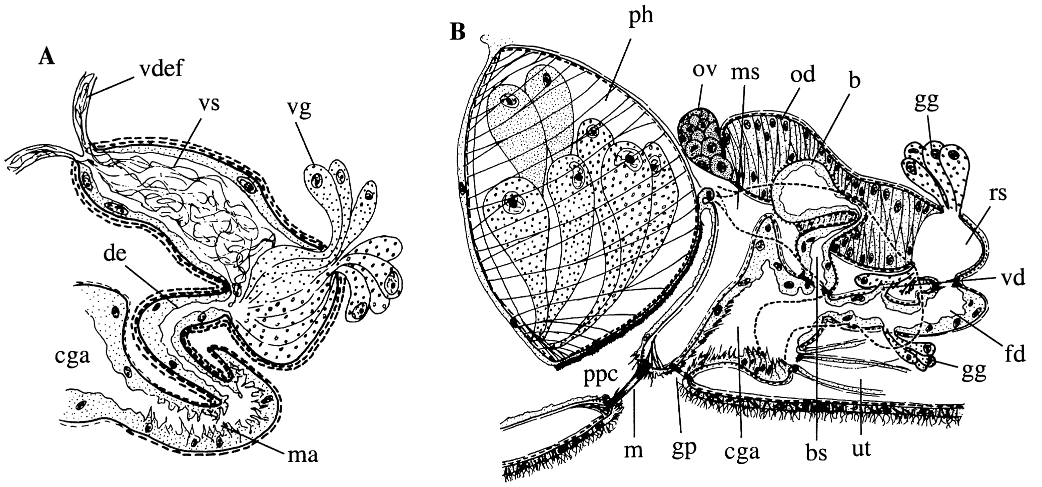

Fig. 2. *Mesostoma thamagai* n. sp. (A) Reconstruction of the male atrial system (holotype). (B) Reconstruction of the genital organs (holotype). b, bursa; bs, bursal stalk; cga, common genital atrium; de, ejaculatory duct; fd, female duct; gg, glands; gp, gonopore; m, mouth; ma, male atrium; ms, male genital system; od, oviduct; ov, ovary; ph, pharynx; ppc, prepharyngeal cavity; rs, seminal receptacle; ut, uterus; vd, vitelloduct; vdef, vas deferens; vg, prostate vesicle; vs, seminal vesicle.

mon genital atrium is very large. It is lined with a high, nucleated epithelium and surrounded by a weak inner circular and an outer longitudinal muscle layer. Towards the gonopore it narrows to a duct that is lined with a pseudociliation and surrounded by a thicker circular muscle layer.

The male atrial system (called "copulatory organ" by Noreña Janssen and Faubel, 1992) consists of a long duct that is surrounded by a pigment similar to that found beneath the epidermis. It is surrounded by two spirally-running muscle layers over its entire length. Proximally the duct is widened to form an elongated seminal vesicle, which is lined with a high, nucleated epithelium. Distally from the seminal vesicle the duct abruptly narrows to form a somewhat coiled, very muscular ejaculatory duct that is lined with a high, nucleated epithelium (the so-called "ductus ejaculatorius exterior" or "penispapilla" of Noreña Janssen and Faubel, 1992). At the transition between the seminal vesicle and the ejaculatory duct eosinophilic and basophilic prostate glands enter the male system through its caudal wall. Both types of glands are coarse-grained. Neither the glands nor the gland necks are surrounded by muscle layers ("false vesicula granulorum" of Noreña Janssen and Faubel, 1992). Distally, the ejaculatory duct merges into the male atrium, which is the most distal part of the male duct and is lined with a pseudociliation. It is surrounded by a strong circular muscle layer.

The oviduct is very long and broad and lined with a very high epithelium. A lumen could not be observed. Distally the oviduct enters a spherical seminal receptacle, which in most specimens contained many sperm. The receptacle is lined with a low epithelium without nuclei, and there is a large sphincter at its distal end. At the transition between female duct and receptacle there is a large bundle of eosinophilic glands. The most distal part of the female duct (the "ductus communis" of Noreña Janssen and Faubel, 1992) is lined with a very high, nucleated epithelium. It enters the common genital atrium dorso-caudally, ventrally from the bursal stalk. It receives the basophilic shell glands ventrally, and the vitelloduct dorsally.

The uteri are paired and enter the common genital atrium separately through its caudal wall.

The bursal stalk leaves the common genital atrium dorsally from the female duct. It is lined with a high, nucleated epithelium and is surrounded by a thick circular muscle layer. The bursa proper is spherical, and in many specimens it was inflated and filled with many sperm. It is lined with a high epithelium without nuclei.

Diagnosis. *Mesostoma thamagai* n. sp. *Mesostoma* species with dark brown, subepidermal pigment. Same pigment present around the male genital system. Gonopore very close behind the mouth. Vasa deferentia entering the copulatory bulb separately from the prostate glands. Prostate glands eosinophilic and basophilic, entering the copulatory bulb dorsally. Ejaculatory duct distally very muscular, with a high epithelium. Eosinophilic glands enter the female system at the transition from oviduct to seminal receptacle. Uteri paired.

Discussion. This species clearly belongs to the Mesostominae as defined by Noreña Janssen and Faubel (1992). The lack of a true prostate vesicle, a spermatic duct and a prepharyngeal dermal pit furthermore mark it as a *Mesostoma* species.

The most recent and comprehensive treatment of this extremely complicated taxon was made by Noreña Janssen and Faubel (1992). These authors recognise 60 species of *Mesostoma*. Twenty eight of them they consider *species dubiae*, one a *species inquirenda*. The remaining 31 species they divide into four morphological groups. Since 1992 only two species of *Mesostoma* have been described: *M. tubiseminalis* Smith, 1998 (designated to the *M. maculatum*-group; see Smith, 1998) and *M. extremiensis* Noreña *et al.*, 1999 (with a typical pincers-like end of the ejaculatory duct; see Noreña *et al.*, 1999). Based on the relative position of the prostate glands (separated from the entrance of the vasa deferentia), the fact that the male duct is directed dorso-caudally (at least the distal part) and the fact that the male system lacks the typical shape of the species of the *M. lingua*-group (dorso-ventrally orientated, globular to pyriform with a relatively short and conical ejaculatory duct or "penispapilla"), *M. thamagai* can be included in the *M. ehrenbergi*-group. Noreña Janssen and Faubel (1992) include five species within this group, of which only *M. ehrenbergi* (Focke, 1836) Örsted, 1844, *M. productum* (Schmidt, 1848) Graff, 1882 and *M. magnum* Kolasa and Schwartz, 1988 have paired uteri that enter the common atrium separately, as is the case in *M. thamagai*. *M. thamagai* most closely resembles *M. magnum*, and they share a number of features that are unique to both: the prostate glands enter the male duct dorsally, the male atrium is tube-shaped, and pigment surrounds the male atrial system. *M. thamagai* differs from *M. magnum* in that the seminal vesicle is much more elongated. A unique feature distinguishing *M. thamagai* from all other species of *Mesostoma* is the presence of a bundle of eosinophilic glands at the transition between oviduct and seminal receptacle. The combination of all these features indicates that *M. thamagai* is indeed a new species.

Whether the four species-groups recognised by Noreña Janssen and Faubel (1992) represent natural, monophyletic groups is doubtful. The subdivision is rather artificial, based on a limited number of morphological features. In addition several features occur in more than one group. For instance, paired uteri are not unique to the *M. ehrenbergi*-group, but also occur in species of other groups (e.g. *M. sibolae* Kolasa, 1976 of the *M. lingua*-group and *M. columbianum* Hyman, 1939 of the *M. maculatum*-group). Moreover, this feature was considered dubious and probably depends on the number of eggs in the uteri (see Kolasa, 1976). A thorough cladistical analysis including a re-evaluation of morphological characters complemented with molecular data is badly needed to reveal the phylogenetic relationships within *Mesostoma*.

Young (1976) mentioned seven valid *Mesostoma* species occurring on the African continent: *M. brincki* Marcus, 1970, *M. ehrenbergi, M. evelinae* Marcus, 1955, *M. ewerum* Du Bois-Reymond Marcus, 1951, *M. inversum* Beauchamps, 1954, *M. lacteum* Neppi, 1904 and *M. lingua* (Abildgaard, 1789) Schmidt 1848. Additionally, he mentioned two other South African species as *species dubiae*: *M. antarcticum* Dreyer, 1918 and *M. karrooense* Dreyer, 1914. Of these two, especially *M. karrooense* has often been considered insufficiently described (Ruebush, 1939; Du Bois-Reymond Marcus, 1951; Marcus, 1955), and therefore has not been considered in more recent faunistic or taxonomic studies. In the same year, Kolasa (1976) described *M. africanum* Kolasa, 1976 from south Algeria and gave a list of six "undoubtedly valid" African species: *M. africanum, M. brincki, M. evelinae, M. ewerum, M. lingua,* and *M. productum*. His list and that of Young (1976) differ in the fact that Kolasa (1976), at the time of his writing, was not aware of the record of *M. ehrenbergi* by Young and Young (1976) and (probably) considered *M. inversum* a junior synonym of *M. productum* as later also did Noreña Janssen and Faubel (1992), although Kolasa did not mention this explicitly. Moreover, he apparently considered *M. lacteum* to be a *species dubia*. In 1981, Kolasa and Mead described a new species from Nigeria: *M. zariae* Kolasa and Mead, 1981. In their monograph of the Mesostominae, Noreña Janssen and Faubel (1992) erected a new monospecific genus to classify the somewhat aberrant species *M. evelinae*: *Marcomesostoma* Noreña Janssen and Faubel, 1992. Moreover, they considered *M. antarcticum* a junior synonym of *M. mutabile* Böhmig, 1902, probably based on the statement by Dreyer (1918) that there was virtually no difference between the two species except for their geographical distribution and for some minute differences in the female system. From what we could retrieve from literature, this synonymisation seems justified. They also explicitly considered *M. lacteum* a *species dubia*. Following these changes and additions, nine valid *Mesostoma* species are now recorded from Africa: *M. africanum, M. brincki, M. ehrenbergi, M. ewerum, M. lingua, M. mutabile, M. productum, M. thamagai* and *M. zariae.* Three of these are cosmopolitan (*M. ehrenbergi, M. productum, M. lingua*), while *M. mutabile* also occurs in South America. The remaining five are only known from the African continent.

DALYELLIIDAE GRAFF, 1905

Gieysztoria isoldeae **n. sp.**

(Fig. 3)

Locality. Botswana, Thamaga (24°41'50"S, 25°31'00"E), ephemeral rock pools on isolated granite domes (type locality). Sediment collected 22/12/1998, inundated in the laboratory 4/10/2002.

Material. Observations on live animals. One whole mount, designated holotype (LUC nr. 248). Seven sectioned specimens, designated paratypes (LUC nr. 249–255).

Etymology. Species named after the first author's daughter Isolde.

Description. Animals ± 0.8 mm long (measured on sectioned specimens), light brown, with two eyes. The rostral end is rounded, the caudal end tapers off ("tail").

The cellular epidermis is about 6 μm thick, with cilia of about 5 μm long. The epidermis contains numerous rhabdites, which are straight rods, stretching the entire thickness of the epidermis. These rhabdites are distributed evenly over all of the epidermis. Large and conspicuous rhabdite glands are present in the tail area, and end at the body surface near the tail.

The mouth is situated slightly ventrally at the anterior body end. The prepharyngeal cavity is rather short, and lined with a very low epithelium. It is surrounded by relatively strong longitudinal muscles. The pharynx bulb measures about 20–25% of the body length and is situated anteriorly. Morphologically it is the same as in most other dalyelliids (see Luther, 1955). However, distal sensory areas ("Taster"; see Luther, 1955) and ciliated areas in the pharynx lumen ("Brusten"; see Luther, 1955) were not observed. There are 26 internal longitudinal muscles, which alternate with 26 radial muscles on each transverse section.

There are two testes, which extend ventro-laterally at both sides of the gut. Rostrally they start far behind the pharynx; their caudal ends are at the anterior end of the copulatory organ. The single ovary is rather small and oviform. It is situated dorso-caudally from the gonopore, somewhat on the left hand side. The vitellaria are situated dorsally from the testes. They are slender, and join each other caudally to form a broad single vitelloduct that enters the oviduct. The common gonopore is situated ventrally, at ± 2/3 of the body length. It can be closed by a thick sphincter, and is surrounded by a bundle of large glands, which discharge their content in the gonopore. The common genital atrium is large and globular. It is lined with a very high, nucleated epithelium and surrounded by an inner circular and an outer longitudinal muscle layer. It receives the male system anteriorly, the female system more dorso-laterally.

The male system consists of an elongated, oval copulatory organ. In its most proximal part it contains a single, large seminal vesicle that narrows distally to a seminal duct, which is surrounded by a circular muscle layer (only visible in one of the sectioned specimens). At the place where the seminal vesicle narrows, it is surrounded by prostate secretion (glands), part of which is coarse-grained eosinophilic, the other part is coarse-grained basophilic. More distally the prostate secretion disappears, but empty gland necks conspicuously surround the seminal duct. The nuclei of the prostate glands were not observed. Seminal vesicle and prostate glands are surrounded by a muscular septum, consisting of at least two muscle layers which cross each other. Between seminal vesicle/prostate glands and the septum there is a space filled with loose parenchyma. As such the copulatory organ is clearly of the conjuncta-duplex type (terminology of Karling, 1956). Distally the septum narrows and

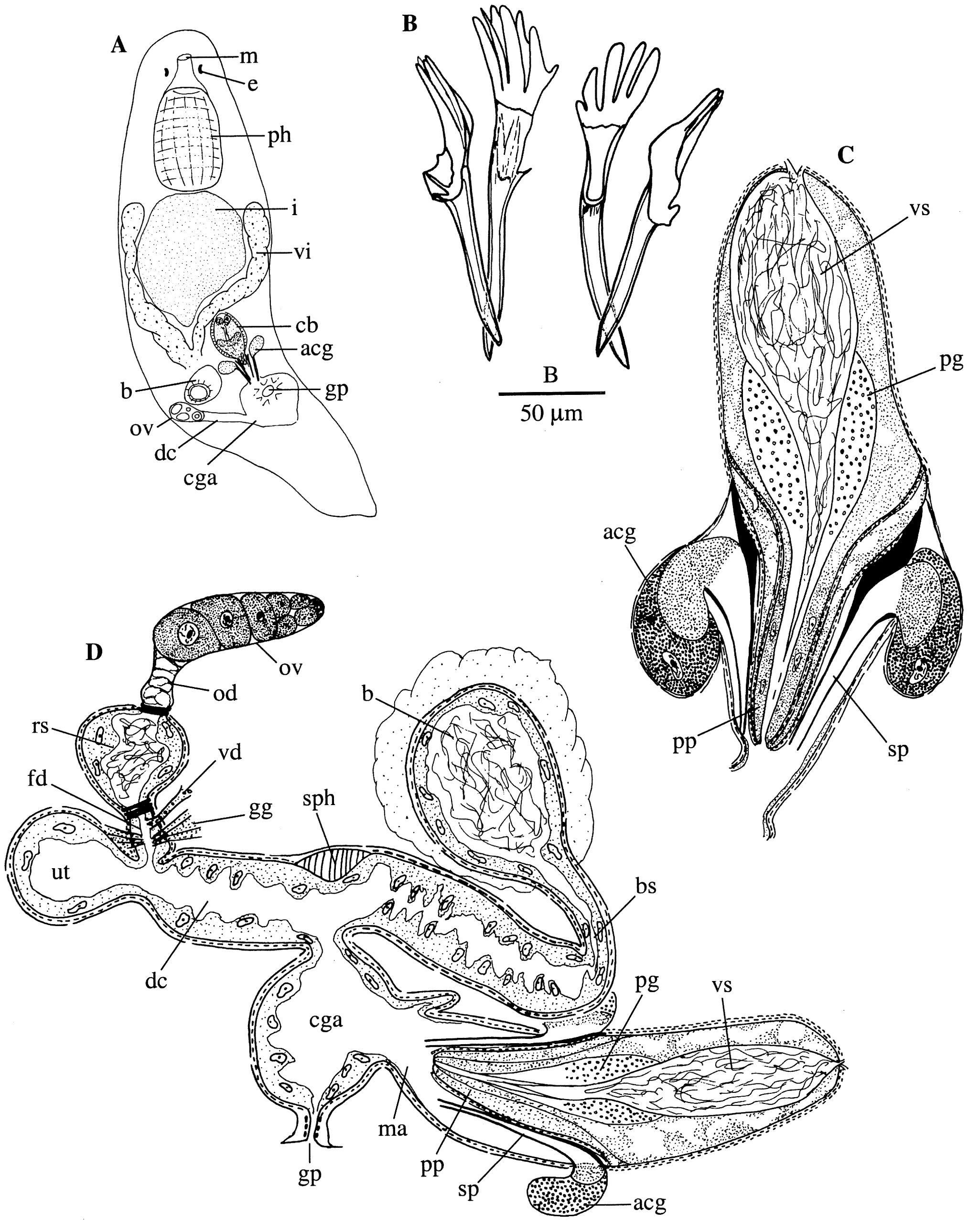

Fig. 3. *Gieysztoria isoldeae* n. sp. (A) Habitus (from a living animal). (B) Spines of the male system (holotype). (C) Reconstruction of the male genital organs (from several paratypes). (D) Reconstruction of the genital system (from several paratypes). acg, accessory glands; b, bursa; bs, bursal stalk; cb, copulatory bulb; cga, common genital atrium; dc, ductus communis; e, eye; fd, female duct; gg, glands; gp, gonopore; i, intestine; m, mouth; ma, male atrium; od, oviduct; ov, ovary; pg, prostate glands; ph, pharynx; pp, penial papilla; rs, seminal receptacle; sph, sphincter; sp, spine; ut, uterus; vd, vitelloduct; vi, vitellarium; vs, seminal vesicle.

splits into two layers, both surrounded by an inner circular and an outer longitudinal muscle layer. In this way a very muscular penial papilla is formed, which is situated centrally in the male atrium. From centre to periphery the penial papilla consists of: the narrow seminal duct, the necks of the prostate glands, parenchyma (distally denser and with many nuclei), a muscular septum, parenchyma and again a muscular septum. The distal opening of this penial papilla can be closed by a sphincter. At the proximal end of the penial papilla (where the septum narrows), four hollow and tubiform spines enter the male atrium, two of them situated ventro-laterally, the other two laterally. The ventrolateral spines (152 and 162 μm) are somewhat larger than the lateral ones (129 and 134 μm). Because of a prolongation of one of the sides, the proximal opening of each spine is large and asymmetrical. These prolonged sides consist of 4–6 digitiform proturbances. They lie next to the septum and support it. The distal part of each spine flanks the penial papilla. A bundle of coarse-grained light basophilic glands enters each lateral spine, and maybe also the ventral ones. These basophilic glands are surrounded by fine-grained eosinophilic ones, which also enter the lateral spines and probably also the ventral ones. These glandular complexes are surrounded by a common muscle layer that is continuous with the muscles forming the septum and surrounding the male atrium. The male atrium itself is lined with a very low epithelium and is surrounded by an inner circular and an outer longitudinal muscle layer.

As it leaves the common genital atrium, the female system immediately splits into the bursal stalk, running anteriorly, and the ductus communis, running caudally. The bifurcation is surrounded by a very thick circular muscle layer, which is extremely thick dorsally, forming an asymmetrical sphincter. Bursal stalk, bursa and ductus communis are lined with the same high, irregular, nucleated epithelium as is the common genital atrium, and are surrounded by an inner circular and an outer longitudinal muscle layer. The bursal stalk is rather broad, but narrows towards the bursa. At the place where it narrows it starts to run caudally. The bursa can have different appearances depending on the state of the animal: very small (underdeveloped; before copulation), or globular or oval filled with many sperm. The fully developed bursa is surrounded by an eosinophilic tissue. The ductus communis is also rather broad, and proximally splits into the uterus and the female duct. The uterus is situated terminally and is globular when no eggs are present. It is lined with the same epithelium and surrounded by the same muscle layers as is the ductus communis. The female duct runs dorsally. It is short and proximally widens to form a seminal receptacle, which often contains sperm. Female duct and seminal receptacle are lined with the same epithelium and surrounded by the same muscle layers as is the ductus communis. At the transition from female duct to seminal receptacle the circular muscle layer thickens to form a sphincter. The vitelloduct and several basophilic and eosinophilic glands ("Schalendrüsen" of Luther, 1955) enter the female duct just distally from the sphincter. The short oviduct enters the seminal receptacle opposite the female duct. It consists of very flat cells that are piled upon each other. These cells appear to be hollow.

Diagnosis. Gieysztoria species with a very well-developed penial papilla. Male system with four separate hollow spines, not connected to each other by a girdle. All spines with proximal digitiform protuberances. Ventrolateral spines larger (± 157 μm) than the lateral ones (± 132 μm). A large complex of glands enters the spines.

Discussion. See the discussion of *Gieysztoria faubeli*.

***Gieysztoria faubeli* n. sp.**

(Fig. 4)

Locality. Botswana, Thamaga (24°41'50"S, 25°31'00"E), ephemeral rock pools on isolated granite hills (type locality). Sediment collected 22/12/1998, inundated in the laboratory 4/10/2002.

Material. Observations on live animals. 10 whole mounts, 1 of them designated holotype (LUC nr. 256), the other 9 paratypes (LUC nr. 257–265).

Etymology. Species named in honour of Dr. Anno Faubel (Hamburg).

Description. Small animals, 0.5–0.7 mm long, pale brown, with two eyes. The pharynx is a typical pharynx doliiformis at the rostral end of the body.

The construction of the genital system does not differ from that of the other *Gieysztoria* species. The genital pore is in the caudal 1/3 of the body. Testes were not observed. The ovary is small and is situated at ± 2/3 of the body length. The vitellaria are small and distally join each other in a broad vitelloduct.

The pyriform prostate vesicle contains an internal seminal vesicle at its proximal end and a number of prostate glands at its distal end. Distally it ends in the stylet, which is 69–84 μm long (m=73 μm; n = 8) and 31–44 μm broad (m=38 μm; n=7). Proximally the stylet consists of a girdle (terminology of Luther, 1955), which is divided into a proximal part with fine longitudinal and transverse ridges, and a distal rectangular part with only a few longitudinal ridges. Two long S-shaped spines make up the sides of the girdle and protrude into the male atrium. Distally the girdle carries two central slender spines. At each side of these central spines, six broader and stouter spines occur. All spines are hollow (exc. the two large lateral ones?), and their openings are easily visible right up against the rim of the girdle. The longitudinal ridges of the distal part of the girdle are in line with the edges of the spines, and split up and continue as the longitudinal ridges of the proximal part of the girdle. The girdle does not show any holes.

The very muscular bursal stalk ends in the common genital atrium in between the oviduct and the copulatory organ. The uterus never contains more than one egg.

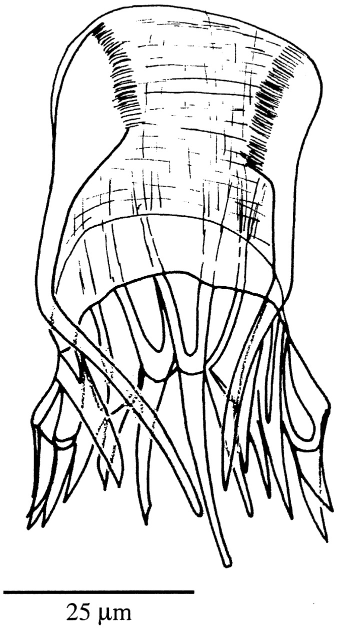

Fig. 4. *Gieysztoria faubeli* n. sp. Stylet (holotype).

Diagnosis. Gieysztoria species with a ± 73 μm long and 38 μm broad stylet. Girdle large, with two large spines forming the sides. Apart from these two spines, two long, slender central spines and 12 smaller, stouter spines connected to the distal rim of the girdle. Girdle without holes.

Discussion. Gieysztoria is a very species-rich and anatomically rather homogeneous taxon. Large differences between the species can, however, be found in the detailed construction of the stylet. Luther (1955) splits the genus into the "Aequales" with stylets with equal spines, and the "Inaequales", with heteromorph spines on the stylet. Within the Aequales, five species have separate hollow spines in the male system, without a girdle connecting the bases of these spines: *G. cuspidata* (Schmidt, 1861) Ruebush and Hayes, 1939, *G. quadridens* (Böhmig, 1897) Ruebush and Hayes, 1939, *G. inflata* (Okugawa, 1930) Ruebush and Hayes, 1939, *G. eastmanni* (Graff, 1911) Ruebush and Hayes, 1939 and *G. pseudoblodgetti* Luther, 1955. Now also *G. isoldeae* can be added to this list, although its spines are certainly not all of exactly the same length (so not really "Aequales"). *G. quadridens* was described from East Africa (Böhmig, 1897), but the only feature giving some information on its identity is the fact that it has "four simple chitinous spines " in the male system. Apart from *G. cuspidata*, the species mentioned are all badly known and mostly insufficiently described (see Luther, 1955) and we therefore consider them *species inquirendae*. Apart from the lack of a girdle in the stylet, also the presence of a very well-developed penial papilla is a feature typical of both *G. isoldeae* and *G. cuspidata*. Although it is also present in most of the other *Gieysztoria* species, it is never so well developed there. *G. isoldeae* differs from *G. cuspidata* in the form of the spines, which are more slender than in *G. cuspidata* and have the typical proximal digitiform protuberances. Moreover, the large accessory glands entering the spines are typical of *G. isoldeae*. In *G. cuspidata*, there are small cells at this place (as is the case in most *Gieysztoria* species) that form the spines, but they do not form a glandular organ. The presence of an asymmetrical sphincter at the junction of female duct and bursal stalk also is characteristic of *G. isoldeae*.

It could be argued that the observed similarities between *G. isoldeae* and *G. cuspidata* warrant the erection of a separate genus for these two species. However, here we refrain from doing so because the monophyly of such a group cannot be proven at this moment. The erection of a new taxon and the coining of a new name should fit into a much broader study of the taxonomy of the Dalyelliidae, backed up with a thorough phylogenetic analysis of the group, which is planned by the first author in the near future.

G. faubeli is a more typical representative of the taxon *Gieysztoria*, with the bases of the different spines attached to a proximal girdle. The fact that the spines are of different forms places this species within the Inaequales. Luther (1955) considers the presence of holes in the girdle ("fenestrae") a very important taxonomical feature, dividing the species of Inaequales into the "Fenestratae"(with holes in the girdle) and the "Radiatae" (without holes). *G. faubeli* clearly belongs to the latter. A typical feature of *G. faubeli* is the fact that the two lateral spines have long proximal ends that form both sides of the girdle. This is also the case in *G. kolasai* Young, 1977 and *G. saganae* Young, 1977, both species from East Africa. In these species, however, one of the lateral spines bears all of the other spines, and the girdle is basically without spines. In *G. faubeli* on the other hand, all spines are attached to the girdle. Other resembling species are *G. pseudodiademata* Noreña Janssen, 1995, *G. santafeensis* Noreña Janssen, 1995, *G. quadrata* Noreña Janssen, 1995 and *G. tigrensis* Noreña Janssen, 1995, all species described from Argentina by Noreña Janssen (1995). The girdle of the stylet of *G. faubeli* is, however, relatively larger, and it is the only species with a stylet with two central spines, flanked by six smaller spines at each side and two large ones forming the sides of the girdle. Regrettably, no whole mounts of the Argentinian species are available, which makes a direct comparison between *G. faubeli* and these species impossible (the same goes for the species described by Young, 1977). The features mentioned above, as exhibited by our specimens, however, indicate that they belong to a different species.

G. isoldeae and *G. faubeli* are, respectively, the ninth and tenth species of *Gieysztoria* to be recorded from Africa. The eight others are [see Young (1977)]: *G. quadridens, G. expedita* (Hofsten, 1907) Ruebush and Hayes, 1939, *G.*

rubra (Fuhrmann, 1894) Ruebush and Hayes, 1939, *G. kolasai, G. saganae, G. donnae* Young, 1977, *G. joannae* Young, 1977 and *G. papii* Young, 1977. *G. isoldeae* and *G. faubeli* are the first to be recorded from the southern part of the continent.

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IV

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"Typhloplanoida" (Platyhelminthes: Rhabdocoela) from the Indian Ocean, with the description of six new taxa

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Abstract

Seven typhloplanoids from the Indian Ocean are discussed; six of them are new to science. We place five of the new species within the Trigonostomidae. *Gandalfia bilunata* gen. nov. sp. nov. is characterized by a tubiform stylet and two semilunar structures in the afferent duct. *Mahurubia clava* gen. nov. sp. nov. has a gutter-shaped stylet and a club-shaped mouthpiece on the bursa. *Parapharyngiella involucrum* gen. nov. sp. nov. is characterized by a stylet in the form of a question mark and a bursal mouthpiece with a club-shaped and a barrel-shaped part. *Poseidoplanella halleti* gen. nov. sp. nov. is characterized by an unpaired ovary, a funnel-shaped stylet connected with a plate, and a small, tubular bursal mouthpiece. Furthermore, new data are given on *Ceratopera axi*. The two other new species belong to the Typhloplanidae. *Aegira annabellae* gen. nov. sp. nov., a marine species, is characterized by a box-shaped stylet and a long, pseudocuticularized ejaculatory duct. *Castrada trispina* sp., a freshwater species, has a copulatory bursa with three spines. *Mahurubia clava* gen. nov. sp. nov. and *Parapharyngiella involucrum* gen. nov. sp. nov. occur at Zanzibar, Tanzania. The other five species have been found on Kerguelen.

Keywords: *Biodiversity, taxonomy, Trigonostomidae, Turbellaria, Typhloplanidae*

Introduction

The marine turbellarian fauna of the Southern Hemisphere in general and of the Indian Ocean in particular is little known. This is especially true for the so-called microturbellaria, and the "Typhloplanoida" is no exception. There are only some scattered reports on marine typhloplanoids in the Indian Ocean, from Somalia (Schockaert and Martens 1985), Kenya (De Clerck and Schockaert 1995), the Seychelles (De Clerck 1994; De Clerck and Schockaert 1995), and La Rèunion (Artois et al. 2000). Recently, five *Trigonostomum* species, three of them new to science, were reported from Kerguelen, Zanzibar and Kenya,

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(Willems et al. 2004a). This brings the total number of known Typhloplanoida for the Indian Ocean to 14 species. In this contribution five new marine taxa (three from Kerguelen and two from Zanzibar) and one new freshwater species (from Kerguelen) are described. In addition, new data on *Ceratopera axi* (Riedl, 1954) Den Hartog, 1964 from Kerguelen are given.

In addition to the traditional Linnean binomen, for each species we also propose an alternative form of species name, which could be used in a system of phylogenetic nomenclature (De Queiroz and Gauthier 1990, 1992, 1994). This converted name (for each species) is chosen according to the system proposed by Artois (2001) and can be found directly below the Linnaean name.

Material and methods

The material was collected during two sampling expeditions. The specimens from Kerguelen were collected by Ernest Schockaert during an expedition to La Réunion, Crozet and Kerguelen in November and December 1992. Material from Zanzibar was collected by Huguette Sas, Tom Artois and Ernest Schockaert during a stay in August 1995.

The marine animals were extracted from sediment and algae using the $MgCl_2$ decantation method (see Schockaert 1996), whereas the freshwater *Castrada* species was collected by the oxygen depletion method (see Schockaert 1996); a moss sample was inundated, causing animals to move to the water surface. A few individuals of each species were studied alive and whole-mounted with lactophenol. The remaining specimens were fixed in marine Bouin's solution, embedded in paraffin, serially sectioned (4 μm sections), and stained with Heidenhain's iron haematoxylin, using eosin as a counterstain.

Camera lucida drawings of hard parts were made with Nomarski phase contrast microscopy. Drawings without a scale bar are freehand. Measurements of hard parts are taken axially, unless indicated otherwise. The position of the gonopore and organs, and the measurements of the pharynx, are expressed in percentages of the total body length (distance from the anterior tip of the body).

The type material of the newly described species is deposited in the zoological collection of the Limburgs Universitair Centrum (LUC), Diepenbeek, Belgium.

To enable us to compare the *Ceratopera axi* (Riedl, 1954) Den Hartog, 1964 specimens from Kerguelen with those of other populations, we borrowed voucher specimens from California and the Falklands from the collections of the Swedish Museum of Natural History in Stockholm (SMNH). Two whole mounts from Australia are also present in the collections of the LUC (Diepenbeek, Belgium).

The following abbreviations are used in the figures: ac, atrium copulatorium; ad, afferent duct; av, additional vesicle; bc, bursa copulatrix; bg, basophilic glands; bh, bursal hook; bs, bursal stalk; bu, bursa; cg, caudal glands; cga, common genital atrium; cil, cilia; de, ejaculatory duct; ds, seminal duct; e, eye; ecm, external circular muscle; elm, external longitudinal muscle; fa, female atrium; fd, female duct (=efferent duct); fg, female glands; gg, prostate glands; gp, common genital pore; gw, "Greifwulst"; icm, internal circular muscle; ilm, internal longitudinal muscle; lm, longitudinal muscle; m, mouth; ma, male atrium; mp, mouthpiece; mt, "mantle"; od, oviduct; ov, ovary; pc, prepharyngeal cavity; pg, pharynx glands; ph, pharynx; pl, pharynx lumen; rg, rostral glands; rh, rhabdite; rm, radial muscle; rs, seminal receptacle; s, stylet; so, stylet opening; t, testis; ut, uterus; v, vas deferens; vd, vitelloduct; vg, prostate vesicle; vit, vitellarium; vs, seminal vesicle; x, y, z, features described in respective text.

Taxonomic account

Systematic relationships within the "Typhloplanoida" s.s. (see Ehlers 1985) have never been assessed from a cladistical point of view. Pending a cladistical analysis of the "Typhloplanoida" by the first author, we will, for the time being, use the system proposed by Den Hartog (1964) and refined by Ax and Heller (1970). In this system the "Typhloplanoida" are divided into eight families, all of them further subdivided into several subfamilies. None of these taxa has yet been assessed for its monophyletic status. We would like to stress that the discussions in this paper must be seen as provisional, and are only intended to compare the new species with strongly similar species (as a differential diagnosis) and are not intended as primary homology assessments (*sensu* Patterson 1982).

TRIGONOSTOMIDAE Graff, 1905 *sensu* Den Hartog, 1964

PARAMESOSTOMINAE Luther, 1948

Gandalfia bilunata gen. nov. sp. nov.

gandalfia-bilunata sp. nov.

(Figure 1)

Diagnosis

Gandalfia gen. nov. Paramesostominae with the pharynx situated in the middle of the body, with a ciliated collar in the distal part of the lumen. Unpaired testis and seminal vesicle. Distal part of the seminal vesicle and the proximal part of the prostate vesicle enclosed within the same muscle layer. With a straight, single-walled stylet. Ovaries paired. Glands enter the female duct about half way. Muscular afferent duct, widened near the common genital atrium, with basophilic glands and two sclerotized semilunar structures. Type species: *Gandalfia bilunata*.

Gandalfia bilunata sp. nov. Provisionally with the same diagnosis as the genus. Stylet ±240 μm long.

Locality

Kerguelen, Port Couvreux: between *Ceramium*-like red algae and thread-like green algae, mid-eulittoral, 0.4 m deep at high tide (25 November 1992) (type locality).

Material

Four specimens studied alive, and mounted, one of them designated holotype (LUC no. 266), the others paratypes (LUC nos 267–269). Five serially sectioned specimens (paratypes; LUC nos 270–274).

Etymology

The genus name/praenomen refers to the wizard Gandalf from the books by J. R. R. Tolkien; gender: feminine. The species epithet refers to the two semilunar structures in the bursa; *bis* (Latin): twice, *luna* (Latin): moon.

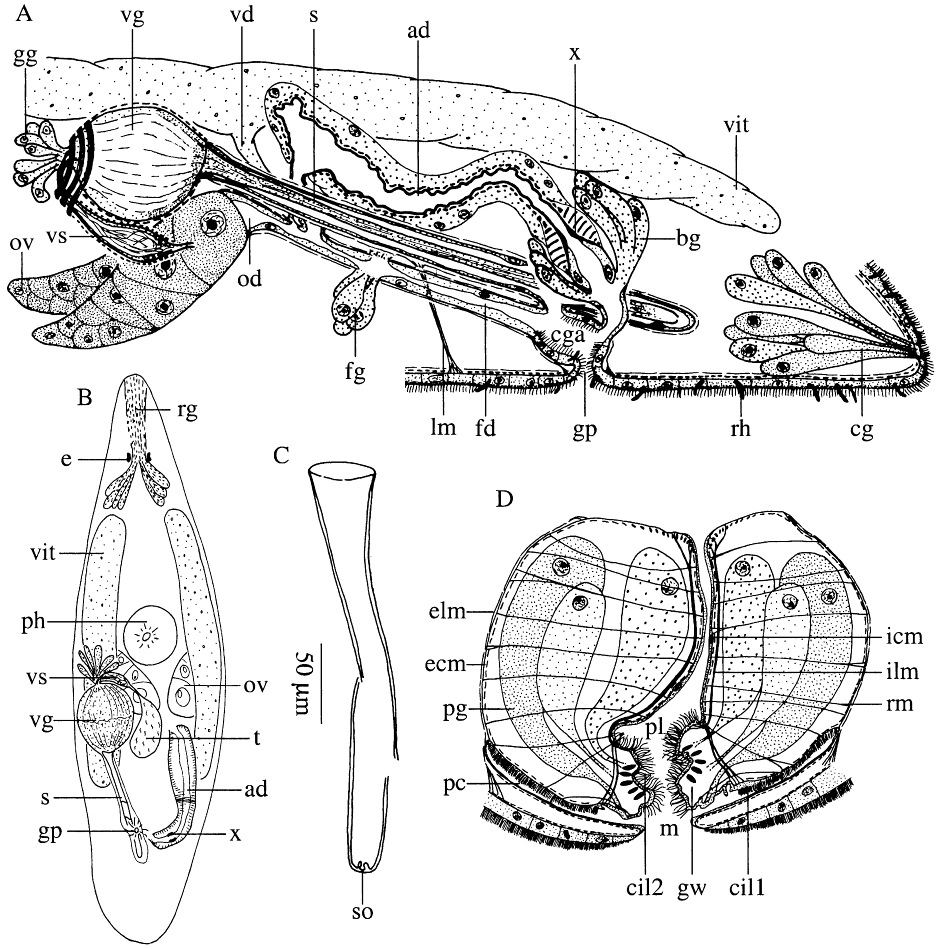

Figure 1. *Gandalfia bilunata* gen. nov. sp. nov. (A) Reconstruction of the atrial organs from the left side; (B) general organization (from a live specimen); (C) stylet (from the holotype); (D) pharynx (reconstruction on sagittal sections).

Description

Transparent, yellowish white animals, 1–1.6 mm long (measured on whole mounts), with two eyes. The cellular epidermis is about 4–5 µm thick, with cilia 2 µm long. It contains large, rod-shaped, slightly bent rhabdites, which are as long as the epithelium is high. Large eosinophilic rhabdite glands (Figure 1B: rg) converge to two long rhabdite strands towards the rostral tip of the body. The caudal glands are well developed (Figure 1A: cg), mainly eosinophilic, with a few basophilic ones.

The pharynx (Figure 1D) is situated at ±40%. The epithelium on the distal rim of the pharynx is provided with short, densely packed cilia (Figure 1D: cil1). The epithelium of the lumen is very low, with several nuclei and very high cilia in the distal part (Figure 1D: cil2). This part forms a so-called "Greifwulst" (prehensile girdle; Figure 1D: gw) with a sphincter of five thick fibres and a few weak radial muscles, running from the proximal end of the ciliation to the medial part of the rim of the pharynx bulb. There are 16 internal longitudinal muscles. The pharynx bulb is filled with large glands, one type with a basophilic and two different types with eosinophilic secretion. The basophilic glands and the most proximal, eosinophilic glands enter the pharyngeal lumen at the level of the distal sphincter, whereas the other eosinophilic glands open near the distal rim.

The common genital pore is at 80–85%. The common genital atrium is lined with a high, cellular epithelium with cilia. It is surrounded by longitudinal muscles only.

There is only one small testis (see Figure 1B), dorsally next to the prostate bulb. The vas deferens is very short, leading to the seminal vesicle (Figure 1A, B: vs). This vesicle is lined with a low, nucleated epithelium, and surrounded by spirally running muscles. These muscles are very thick distally and also surround the proximal part of the prostate vesicle. The prostate vesicle, as seen in live animals (Figure 1B: vg), is divided into proximal and distal halves, each with a different secretion. In the sectioned material, on the other hand, the prostate vesicle is completely empty, while filled gland necks can be seen in the stylet (fixation artifact?). Where the glands, which have an extracapsular nucleated part (Figure 1A: gg), enter the prostate stylet, a number of nuclei are present (not drawn in Figure 1A). The fragile, single-walled, tubiform stylet (Figure 1C) is broken in every one of the whole mounted specimens, and is 212–250 µm long (mean=237 µm; $n=3$). The proximal opening is 30–38 µm wide (mean=35 µm; $n=3$). From this opening the stylet narrows, and at about one-quarter of its length it continues as a straight tube, 17–23 µm in diameter (mean=19 µm; $n=3$). The opening is situated at the blunt end (Figure 1C: so). A weak muscle (Figure 1A: lm) extends from the male atrium to the ventral body wall.

There are two large, elongated ovaries, lying ventrally just behind the pharynx. The paired vitellaria extend dorsally at both sides of the body, from behind the eyes towards the level of the gonopore. In the sectioned specimens both vitellaria anastomose with each other, which was not observed in live animals. The two short oviducts join each other to form the female (=efferent) duct (Figure 1A: fd). The vitelloducts enter the oviducts just before this junction. The female duct is surrounded by longitudinal muscles and lined with a high, nucleated epithelium. About half way, a large bundle of basophilic glands (Figure 1A: fg) enters the female duct. A second duct, the afferent duct (Figure 1A: ad), connects the common genital atrium with the junction of both oviducts. It is lined over its whole length with basement membrane only (pseudocuticula) and is surrounded by circular muscles, except for the part near the ovaries where it has a very thin wall. The muscular part is surrounded by parenchymatic tissue (myoblasts?), containing a large number of nuclei. Near the common genital atrium, the afferent duct is slightly widened and surrounded by very thick circular muscles. The basement membrane is very thick, forming two semilunar structures (Figure 1A, B: x), especially obvious in live animals. A large bundle of basophilic glands (Figure 1A: bg) opens into the distal part of the afferent duct, at the transition to the common genital atrium. A uterus is lacking.

TRIGONOSTOMINAE Luther, 1948

Ceratopera axi (Riedl, 1954) Den Hartog, 1964

ceratopera-axi (Riedl, 1954) Den Hartog, 1964
Proxenetes axi Riedl, 1954.
Ceratopera bifida Ehlers and Ax, 1974.

Localities

New locality. Port Couvreux (Kerguelen): between filiform green algae, mid-eulittoral, 0.4 m deep at high tide (25 November 1992).

Other locality in the Indian Ocean. La Réunion (Artois et al. 2000).

Known distribution. Gulf of Naples and Sicily (Riedl 1954); Galapagos (Ehlers and Ax 1974); Falkland Islands and California (Karling 1986); Weddell Sea (Artois et al. 2000); Eastern Australia (Willems et al. 2004b).

Material

Observations on live specimens and one whole mount. Whole mounts from Falklands and California (collections of SMNH) and Australia (collections of LUC).

Remarks

The stylet of the specimen from Kerguelen measures 121 µm (88 µm if measured as in Ehlers and Ax 1974). The bursal appendage (or mouthpiece) is 117 µm long. These measurements correspond with those on the specimen from the Weddell Sea (Artois et al. 2000), they are slightly larger than those of the specimen from La Réunion (Artois et al. 2000) and much larger than those of the Australian (Willems et al. 2004b) and the Galapagos specimens (Ehlers and Ax 1974). The last-mentioned population was formerly described as a different species, *C. bifida* Ehlers and Ax, 1974. According to Karling (1986), Artois et al. (2000) and Willems et al. (2004b), *C. bifida* Ehlers and Ax, 1974 is considered a junior synonym of *C. axi* (Riedl, 1954) Den Hartog, 1964.

As the variation in stylet size within *C. axi* is rather large, it would not be surprising that this "morphological plasticity" in fact hides a complex of cryptic species, as is the case for *Gyratrix hermaphroditus* Ehrenberg, 1831 (see Curini-Galletti and Puccinelli 1989, 1990, 1994, 1998; Artois et al. 2000; Artois and Schockaert 2001) or the "*setigerum* species complex" within the taxon *Trigonostomum* Schmidt, 1852 (see Willems et al. 2004a). However, since the only known difference between the different populations of *C. axi* is this size variation of the stylet, it is at the moment highly arbitrary to split this complex into different formal species. Such an action should be backed up by a much more thorough morphological (and molecular) study of the species, which is clearly not within the scope of this study.

Mahurubia clava gen. nov. sp. nov.
mahurubia-clava sp. nov.
(Figure 2)

Diagnosis

Mahurubia gen. nov. Trigonostominae with the pharynx in the middle of the body. Testes and seminal vesicles paired, far behind the pharynx. Prostate stylet consisting of two parts, one of which is gutter-shaped and receives the prostate secretion, the other one a folded plate-like structure, which carries several spines distally. Additional vesicle (with sperm) at the proximal part of the female (efferent) duct. Afferent system consisting of tortuous duct and large, thick-walled bursa. Mouthpiece of bursa club-shaped. Type species: *Mahurubia clava*.

Mahurubia clava sp. nov. Provisionally with the same diagnosis as the genus. Stylet 87 µm long.

Locality

Zanzibar, Mahurubi Palace Ruins: open beach with fine sand in front of the mangroves, at low tide (5 August 1995) (type locality); same locality: sandflat with crab holes (17 August 1995).

Material

Three individuals studied alive and mounted: one designated holotype (LUC no. 275), another one paratype (LUC no. 276). Three serially sectioned animals (paratypes; LUC no. 277–279).

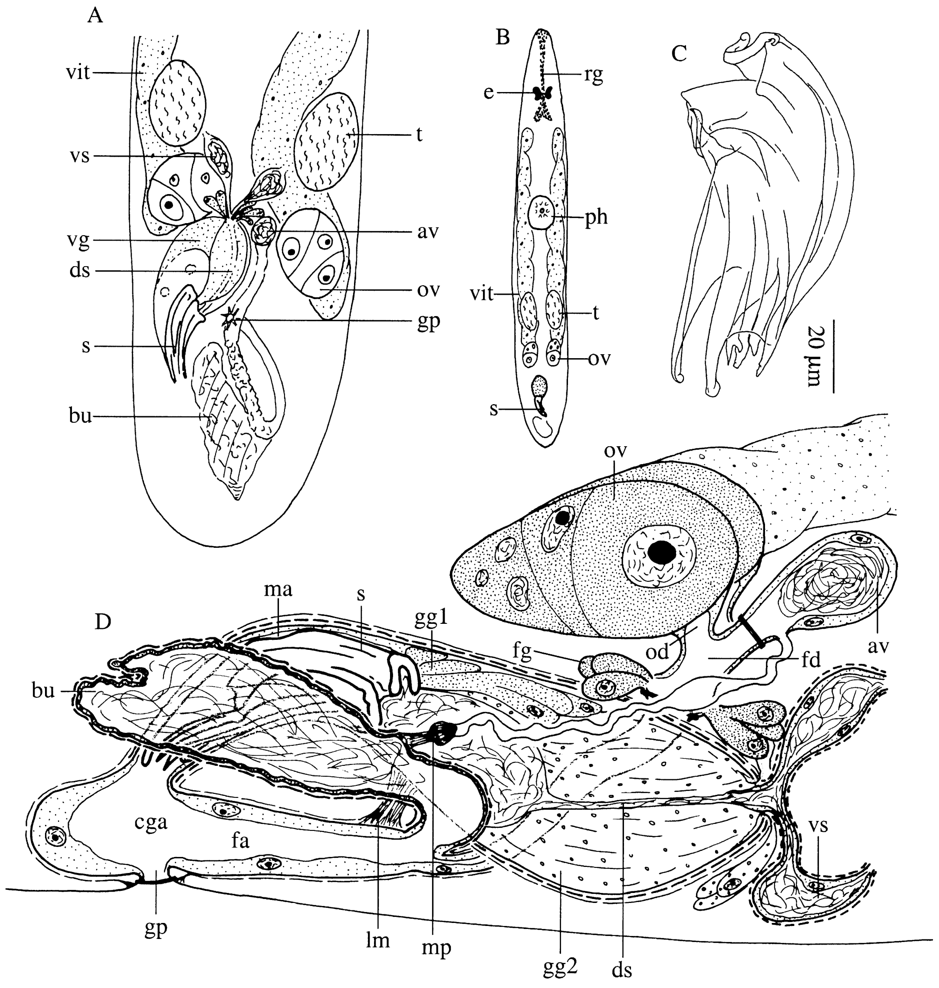

Figure 2. *Mahurubia clava* gen. nov. sp. nov. (A) General organization (from a live specimen); (B) habitus of a live animal; (C) stylet (from the holotype); (D) reconstruction of the atrial organs from the right side.

Etymology

The genus name/praenomen refers to the type locality, Mahurubi (Zanzibar, Tanzania); gender: feminine. The species epithet refers to the shape of the mouthpiece; *clava* (Latin): club.

Description

The animals are ±0.6–0.9 mm long (measured on whole mounts), with two eyes. The cellular epidermis is about 2 μm thick, with cilia 1.5 μm long. Large rhabdites occur throughout the epidermis, but are slightly more concentrated at the rostral and caudal ends. Rostrally there are two long strands of rhabdite glands (Figure 2B: rg). Caudal glands are also well developed.

The pharynx is situated at about 40%. There are basophilic and eosinophilic pharyngeal glands. The nucleated epithelium of the pharyngeal lumen is low. There are 24 internal longitudinal and 24 radial muscles in horizontal sections.

The common genital pore lies at ±90%. The common genital atrium is rather wide, lined with a high, nucleated epithelium, and surrounded by longitudinal muscles only. It extends rostrally to form the female atrium and communicates dorsally with the male atrium.

Paired testes are situated far behind the pharynx, just in front of the ovaries (Figure 2A, B). Seminal vesicles are paired, lined with low, nucleated epithelium and surrounded by spiral muscles. Both vesicles narrow and subsequently fuse to form the seminal duct (Figure 2D: ds), just before entering the prostate vesicle. The seminal duct continues through the prostate vesicle as a very narrow duct and widens just before entering the stylet. The prostate vesicle (Figure 2A: vg) is very large and elongated. It is surrounded by inner circular and outer longitudinal muscles. Two kinds of prostate glands are present: eosinophilic ones (Figure 2D: gg2) with extracapsular nucleated parts, and basophilic glands, which are completely intracapsular (Figure 2D: gg1). The very complex stylet (Figure 2C) is 81–90 μm long (mean=87 μm; n=3) and consists of two parts: a bent, gutter-shaped one (maybe a closed tube) and a folded plate-like part at the concave side, which is attached to the gutter-shaped part over most of its length. Sperm is probably discharged in between these two parts, while the gutter-shaped one receives the prostate secretion. The male genital atrium is lined with a membranous epithelium and surrounded by inner circular and outer longitudinal muscles. Distally, the circular layer ends where the male atrium enters the common genital atrium. A short, but strong bundle of longitudinal muscle fibres (Figure 2D: lm) connects the proximal part of the male atrium with the common genital atrium.

The paired, ovoid ovaries are situated on both sides of the prostate vesicle. They form the most caudal part of the elongated ovovitellaria (Figure 2B: ov+vit), which extend from just behind the eyes to the prostate vesicle. There is a large variation in size of the ovaries between different individuals, possibly due to differences in developmental stage. The female duct (=efferent duct; Figure 2D: fd) enters the female atrium (Figure 2D: fa) anteriorly. The common genital atrium, the female atrium and the female duct are all lined with a high, nucleated epithelium and surrounded by longitudinal muscles. Distally from the junction of the oviducts a large bundle of eosinophilic glands (Figure 2D: fg) opens into the female duct, which is guarded by a sphincter. Proximally from the junction, the female duct ends in a small sperm-containing vesicle (Figure 2D: av), lined with a low, nucleated epithelium and filled with motile sperm in the living animal. This additional vesicle can be closed by a weak sphincter. At the transition between the female duct and the vesicle a tortuous duct (=part of the afferent duct) connects a large bursa (Figure 2D: bu) with the ovaries. Where the duct leaves the bursa, a sclerotized, club-shaped mouthpiece (Figure 2D: mp; not visible on whole mounts) is present. The bursa proper is thick-walled, surrounded by strong spirally running, almost longitudinal muscles, and enters the female atrium dorsally, just above the female duct. A uterus is lacking.

Parapharyngiella involucrum gen. nov. sp. nov.
parapharyngiella-involucrum sp. nov.
(Figure 3)

Diagnosis

Parapharyngiella gen. nov. Trigonostominae with the pharynx situated in the first body half. Paired testes in front of the pharynx. Paired ovovitellaria with the ovaries and atrial organs situated just caudally to the pharynx. Prostate stylet a curved tube, distally surrounded by the mantle. Additional vesicle (with sperm) at the proximal part of the female (efferent) duct. Afferent system consisting of curling duct and large, thin-walled bursa (with sclerotized bursal stalk). Mouthpiece of bursa consisting of a club-shaped part surrounded by a barrel-shaped part. Type species: *Parapharyngiella involucrum*.

Parapharyngiella involucrum sp. nov. Provisionally with the same diagnosis as the genus. Stylet 48 μm long.

Locality

Zanzibar, Mahurubi Palace Ruins: beach with detritus-rich sand, churned up by crabs, at low tide (5 August 1995) (type locality).

Material

Four specimens studied alive and whole mounted, one of them designated holotype (LUC no. 280), the others paratypes (LUC nos 281–283). One serially sectioned specimen (paratype; LUC no. 284).

Etymology

The genus name/praenomen refers to the position of the genital system, just behind the pharynx; *para* (Greek): close to; gender: feminine. The species epithet refers to the partially sclerotized wall of the bursal stalk, giving it the appearance of a kind of envelope; *involucrum* (Latin): envelope.

Description

The animals are ±1 mm long (measured on whole mounts), with two eyes. The cellular epidermis is about 3 μm thick with cilia of 3–4 μm long. Numerous minute rhabdites occur throughout the epidermis. Close behind the eyes, some basophilic glands (Figure 3A: rg) are present and produce rod-shaped adenal rhabdites, which are ±6 μm long. The gland necks are organized in two long tracts, fusing at the level of the eyes and opening terminally at the rostral body end.

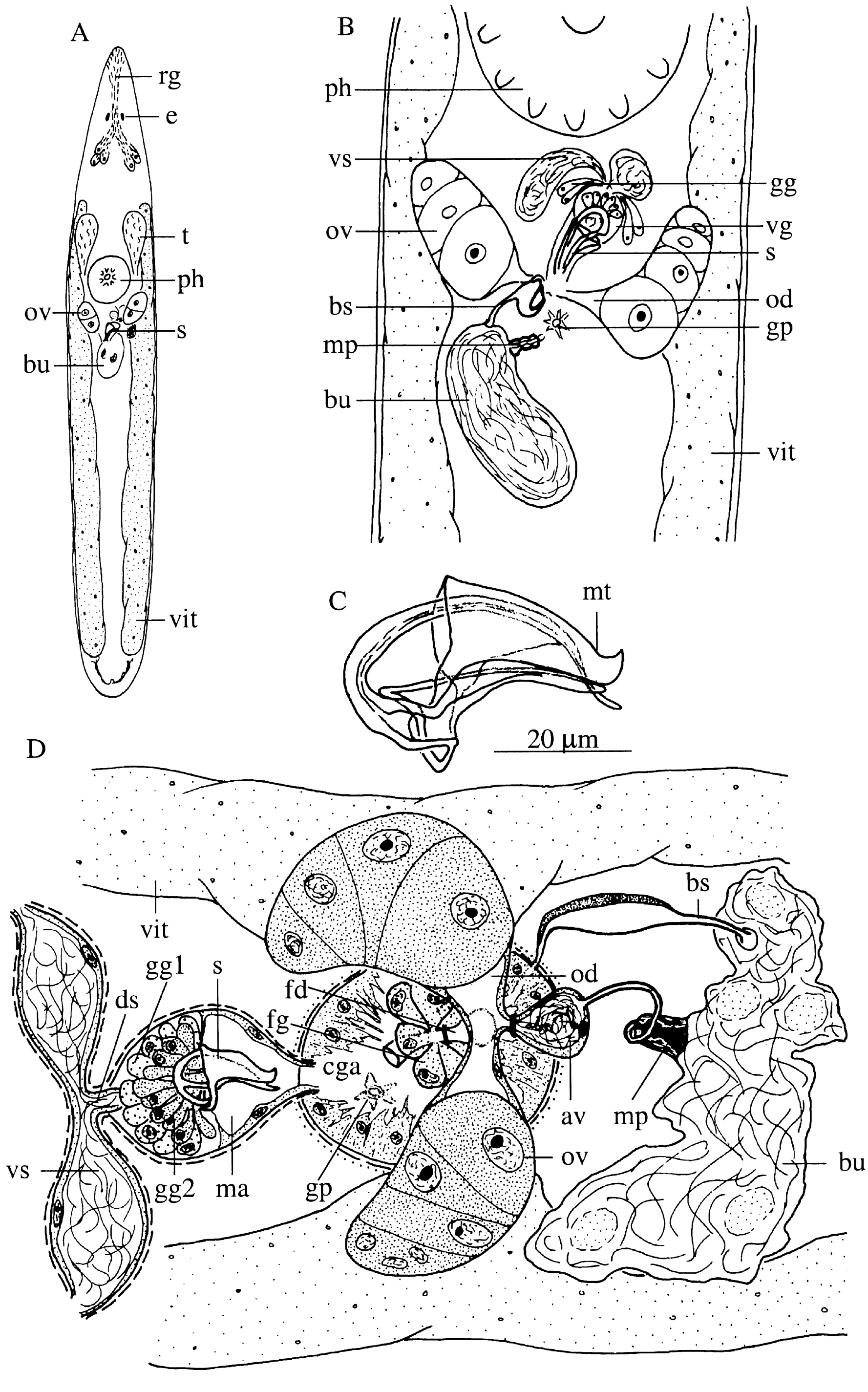

Figure 3. *Parapharyngiella involucrum* gen. nov. sp. nov. (A) Habitus of a live animal; (B) general organization (from a live specimen); (C) stylet (from the holotype); (D) horizontal reconstruction of the atrial organs.

The pharynx is situated at about 30%. It has 24 internal longitudinal muscles. The more detailed structure could not be determined, but does not seem to differ from that of most other trigonostomids.

The paired testes are rather small and globular and lie just in front of the pharynx (Figure 3A). The dorsally situated, paired ovovitellaria extend from the level of the testes to the caudal body-end, with the ovaries just behind the pharynx. Also all other reproductive structures are located immediately behind the pharynx (see Figure 3A, B). The common genital pore is situated at ±40%. The common genital atrium is surrounded by inner circular and outer longitudinal muscles, and lined with a nucleated and frayed epithelium (pseudociliation).

The paired seminal vesicles are lined with a low, nucleated epithelium and surrounded by circular muscles. Just before entering the prostate vesicle (Figure 3B: vg), both seminal vesicles join to form the seminal duct (Figure 3D: ds). It runs axially through this prostate vesicle, but is only clearly visible in the proximal part. Both prostate vesicle and male atrium, are surrounded by circular muscles. There are two types of prostate glands: eosinophilic glands situated dorsally (Figure 3D: gg2) and basophilic ones ventrally (Figure 3D: gg1). Extracapsular parts of the glands were seen only on live material (Figure 3B: gg). The complex stylet (Figure 3C) is 44–55 μm long (mean=48 μm; n=4) and consists of a very narrow, thick-walled tube, giving it the shape of a question mark when viewed laterally. The distal part (about 60%) is surrounded by a large plate ("mantle" of Ax 1971). Distally, this plate (Figure 3C: mt) ends in a broad hook.

The ovoid ovaries are situated dorsal to the prostate vesicle. The short oviducts are lined with a low, nucleated epithelium and join to form the female duct (=efferent duct; Figure 3D: fd), which enters the atrium dorsally. The female duct is surrounded by a strong sphincter at its proximal end, where a large bundle of eosinophilic glands (Figure 3D: fg) surrounds and enters the female duct. Proximally the female duct ends in a small, globular sperm-containing vesicle (Figure 3D: av), the entrance of which is guarded by a sphincter. The afferent system consists of a large bursa (Figure 3D: bu) and a sclerotized duct, which connects the bursa with the sperm-containing vesicle on the female duct. Where it leaves the bursa, this duct widens and contains a large mouthpiece (Figure 3D: mp; not visible on whole mounts), which is a complex sclerotized structure, consisting of an inner club-like part, surrounded by a barrel-shaped part. The latter part is probably the thickened wall of the surrounding duct. The bursa has a thin and extremely folded wall, is filled with sperm and eosinophilic secretion, and is connected to the genital atrium by a narrow duct (bursal stalk; Figure 3D: bs), which is lined with a thick sclerotized basement membrane. In about its middle, the bursal stalk widens and the basement membrane is clearly thicker on one side, giving it the appearance of a kind of envelope. A uterus is lacking.

MARIPLANELLINAE Ax and Heller, 1970
Poseidoplanella halleti gen. nov. sp. nov.
poseidoplanella-halleti gen. nov. sp. nov.
(Figure 4)

Diagnosis

Poseidoplanella gen. nov. Mariplanellinae with complex stylet, consisting of tubiform and plate-like parts. Unpaired seminal vesicle. Extracapsular prostate glands. Afferent system

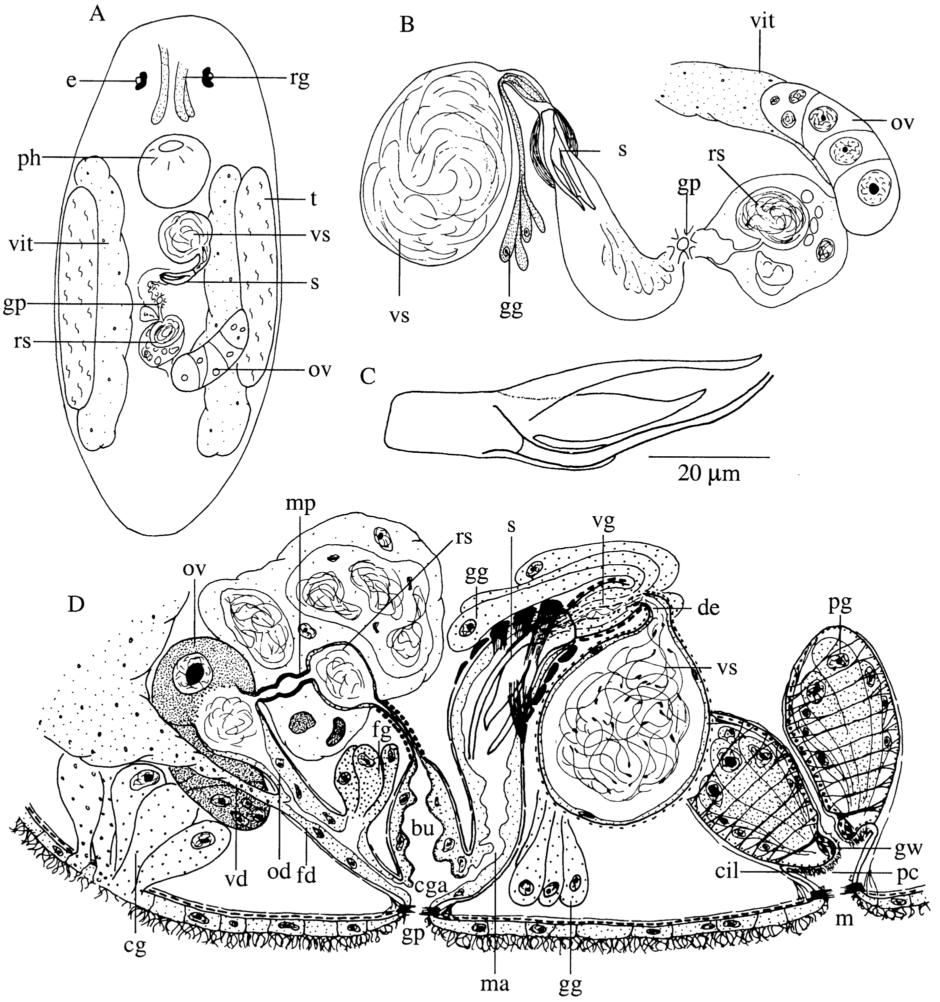

Figure 4. *Poseidoplanella halleti* gen. nov. sp. nov. (A) Habitus of a live animal; (B) general organization (from a live specimen); (C) stylet (from the holotype); (D) reconstruction of the atrial organs and the pharynx from the right side.

consisting of dome-shaped bursa, muscular duct, seminal receptacle and sclerotized mouthpiece. Type species: *Poseidoplanella halleti.*

Poseidoplanella halleti sp. nov. Provisionally with the same diagnosis as the genus. Stylet 61–67 µm long, consisting of a tube connected to a triangular plate that carries a spur.

Locality

Kerguelen, Port Couvreux: small bay, on thread-like green algae, mid-eulittoral, 0.4 m deep at high tide (25 November 1992) (type locality).

Material

Several individuals studied alive. Five whole mounts, one of them designated holotype (LUC no. 285), the others paratypes (LUC nos 286–289). Two serially sectioned specimens (paratypes; LUC nos 290–291).

Etymology

The genus name/praenomen refers to Poseidon, the Greek god of the sea. The suffix -*planella* ("little flat animal") is used in analogy with *Mariplanella* and *Lonchoplanella*, the genus names of the two other species in the subfamily (see Discussion); gender: feminine. The species is dedicated to Mr Peter Hallet, a friend of the first author.

Description

The colourless to pale yellow animals are 0.7–1.0 mm long (measured on whole mounts) and have two eyes with lenses. The cellular epidermis is about 3 µm thick (only slightly thicker at the rostral and caudal body end) with cilia ±2 µm long. Cilia are almost completely lacking at the dorsal side. The rod-shaped rhabdites are ±3 µm long and occur exclusively in the dorsal body epithelium. Two small, eosinophilic rhabdite glands (Figure 4A: rg) are present between the eyes in the rostral part of the body. Caudal eosinophilic and basophilic glands are very well developed (Figure 4D: cg).

The small, globular pharynx (Figure 4A, D: ph) is situated in the first body half at ±25%. The distal rim carries short cilia (Figure 4D: cil). The prepharyngeal cavity (Figure 4D: pc) is lined with a low, anucleated epithelium and surrounded by longitudinal muscles. The pharynx lumen is lined with a low, anucleated epithelium without cilia. The exact number of inner longitudinal and radial muscles could not be determined. Distally the inner circular muscles form a thick sphincter within the so-called "Greifwulst" (prehensile gridle; Figure 4D: gw), which lacks cilia. The pharynx is filled with probably only one type of basophilic gland.

The genital pore is situated at ±60% (Figure 4A, B, D: gp) and can be closed by a sphincter. The common genital atrium is very small, lined with a high, nucleated epithelium and surrounded by longitudinal muscles. It communicates with the male atrium rostrally, with the female duct caudally and with the bursa dorsally.

The paired, elongated testes lie caudally of the pharynx, ventrally of the vitellaria. The unpaired seminal vesicle is large and globular and lies just caudally of the pharynx. It is lined with a low, nucleated epithelium and surrounded by weak circular muscles. A short ejaculatory duct (Figure 4D: de) leads into the small barrel-shaped prostate vesicle (Figure 4D: vg), which contains the gland necks of the elongated, extracapsular prostate glands, and is surrounded by circular muscles. The stylet consists of a narrow tube (44–54 µm long; mean=49 µm; $n=5$) attached to a plate-like structure (54–58 µm long; mean=56 µm; $n=5$) that has a proximal rectangular part and a distal elongated triangular part. The stylet is attached to the plate at the transition of both parts, from where also a slender spur departs (40–47 µm long; mean=43 µm; $n=5$). The whole structure, plate plus stylet, measures 61–67 µm (mean=65 µm; $n=5$). Both sperm and prostate secretion are discharged through the tubiform part of the stylet. The male atrium is rather long, lined with a high, anucleated epithelium, and proximally surrounded by very strong spirally running muscles. In the distal part of the male atrium the muscles become weaker and circular.

The single ovary is situated caudally. The paired vitellaria lie dorsally and extend from the pharynx to the caudal body end. The vitelloducts join and, subsequently enter the female duct from behind and ventrally (Figure 4D: vd). The female or efferent duct (Figure 4D: fd) is lined with a high, nucleated epithelium (the lumen is very narrow) and surrounded by weak longitudinal muscles. Distally to the vitelloduct, basophilic glands enter the female duct from the dorsal side (Figure 4D: fg). In the proximal part of the female duct the lumen becomes wider, and close to the ovary it is swollen and filled with sperm. The afferent system begins with a dome-shaped bursa at the dorsal side of the common genital atrium. This bursa is lined with the same high, nucleated epithelium as the atrium. The bursa is connected to a globular sperm-containing vesicle or receptaculum seminis (terminology of Ax and Heller 1970 and Ehlers 1974) through a very narrow, muscular duct. The vesicle is embedded in a stromatic tissue with cavities filled with sperm in various stages of digestion, and is connected to the oviduct by a short, sclerotized duct or mouthpiece, not seen, however, in the living animal (Figure 4D: mp; terminology of Ax and Heller 1970 and Ehlers 1974). A uterus is lacking.

Discussion of the new Trigonostomidae species

The four new species described above are members of the taxon Trigonostomidae Graff, 1905 (*sensu* Den Hartog, 1964), and belong to the subtaxa Paramesostominae Luther, 1948, Trigonostominae Luther, 1948, and Mariplanellinae Ax and Heller, 1970.

In the Trigonostomidae, the ovaries are connected to the atrium by two ducts: an efferent duct ("abführend" in Luther 1948), through which the fertilized egg descends to the atrium, and an afferent system ("zuführend" in Luther 1948) through which the alien sperm (presumably) reach the ovaries after copulation. This feature has always been considered diagnostic for the Trigonostomidae (see Den Hartog 1964; Ax and Heller 1970; Ax 1971). However, Karling et al. (1972) expressed some doubts about the homology of this double connection in all trigonostomids, and indeed such a double connection can also be found in, for example, some Polycystididae Graff, 1905 (see Artois and Schockaert forthcoming) and in some Solenopharyngidae Graff, 1882 (e.g. *Proceropharynx litoralis* Ehlers, 1972 and *Adenopharynx mitrabursalis* Ehlers, 1972; see Ehlers 1972). Moreover, the construction of the whole female system may be quite different in the various Trigonostomidae species. A thorough discussion on the matter is, however, beyond the scope of this contribution. We have used the terminology of previous authors to indicate different parts of the afferent system, without implying any homology. Comparison of each new species with known species is made within the present taxonomic system, pointing only to the most conspicuous differences (as a differential diagnosis).

Gandalfia bilunata sp. nov. exhibits all characters of the Paramesostominae enumerated by Karling et al. (1972, p 261), and shows the highest resemblance with *Astrotorhynchus bifidus* (McIntosh, 1874) Graff, 1905. *Astrotorhynchus bifidus* has a retractable "proboscis", a uterus and spiral ridges on the stylet (see Luther 1950), whereas *G. bilunata* has none of these characters. Furthermore, in *G. bilunata* there is only one testis (two in *A. bifidus*; Luther 1950) and the basement membrane of the atrial bursa is thickened to form two semilunar structures, obvious in live animals, but absent in *A. bifidus*.

Mahurubia clava sp. nov. and *Parapharyngiella involucrum* sp. nov. must be considered members of the Trigonostominae (for diagnosis, see Den Hartog 1964 and Ax 1971). They have paired ovovitellaria, paired seminal vesicles, the copulatory organ is clearly of the trigonostominae construction, and the afferent female duct ends in a sclerotized mouthpiece (except in *Petaliella spiracauda* Ehlers, 1974). Contrary to the situation in all other Trigonostominae, this mouthpiece is club-shaped in the two African species, and lies within a duct, which ends in an additional sperm-containing vesicle close to the ovaries. Such a vesicle is absent in all other known species. Moreover, the copulatory organ in each of the two new species is clearly different from those of all other Trigonostominae, allowing easy recognition.

The Mariplanellinae have a single ovary, separated from the (paired) vitellaria (see Ax and Heller 1970), and *Poseidoplanella halleti* sp. nov. must therefore be considered a representative of this taxon. So far, the taxon Mariplanellinae encompassed two species, *Mariplanella frisia* Ax and Heller, 1970 and *Lonchoplanella axi* Ehlers, 1974. Both these species have the pharynx in the second half of the body and the genital organs in the caudal body part (see Ax and Heller 1970; Ehlers 1974). *Poseidoplanella halleti* has the pharynx at 25%, and the genital organs in the middle of the body, and it has a single seminal vesicle, while the other two species have only one. The hard parts of the copulatory organ are clearly different in the three species.

TYPHLOPLANIDAE Graff, 1905

RHYNCHOMESOSTOMINAE Bresslau, 1933

Castrada trispina sp. nov.

castrada-trispina sp. nov.
(Figure 5)

Diagnosis

Castrada species with three spines in the copulatory bursa, two of them of the same shape and size (40 and 46 µm long), the third one of a different shape and larger (88 µm long). Ejaculatory duct a simple, bent, pseudocuticularized tube.

Locality

Kerguelen, Baie de la Table; on mosses in a freshwater torrent near a cascade on the beach (4 December 1992) (type locality).

Material

Several individuals studied alive. One whole mount, designated holotype (LUC no. 300). Four serially sectioned specimens on seven slides (paratypes; LUC nos 301_{A-C}–304_{A-B}).

Etymology

The species epithet refers to the presence of three spiny thorns in the copulatory bursa; *spina* (Latin): thorn.

Description

Small and slender animals, colourless and without eyes. Small rhabdite glands (Figure 5: rg) with slender, elongated rhabdites present in the rostral region, which is very agile in live individuals. The syncytial epidermis is about 4 µm thick ventrally, dorsally thinner (2–3 µm), with cilia ±5 µm long. These cilia are much shorter and less densely distributed

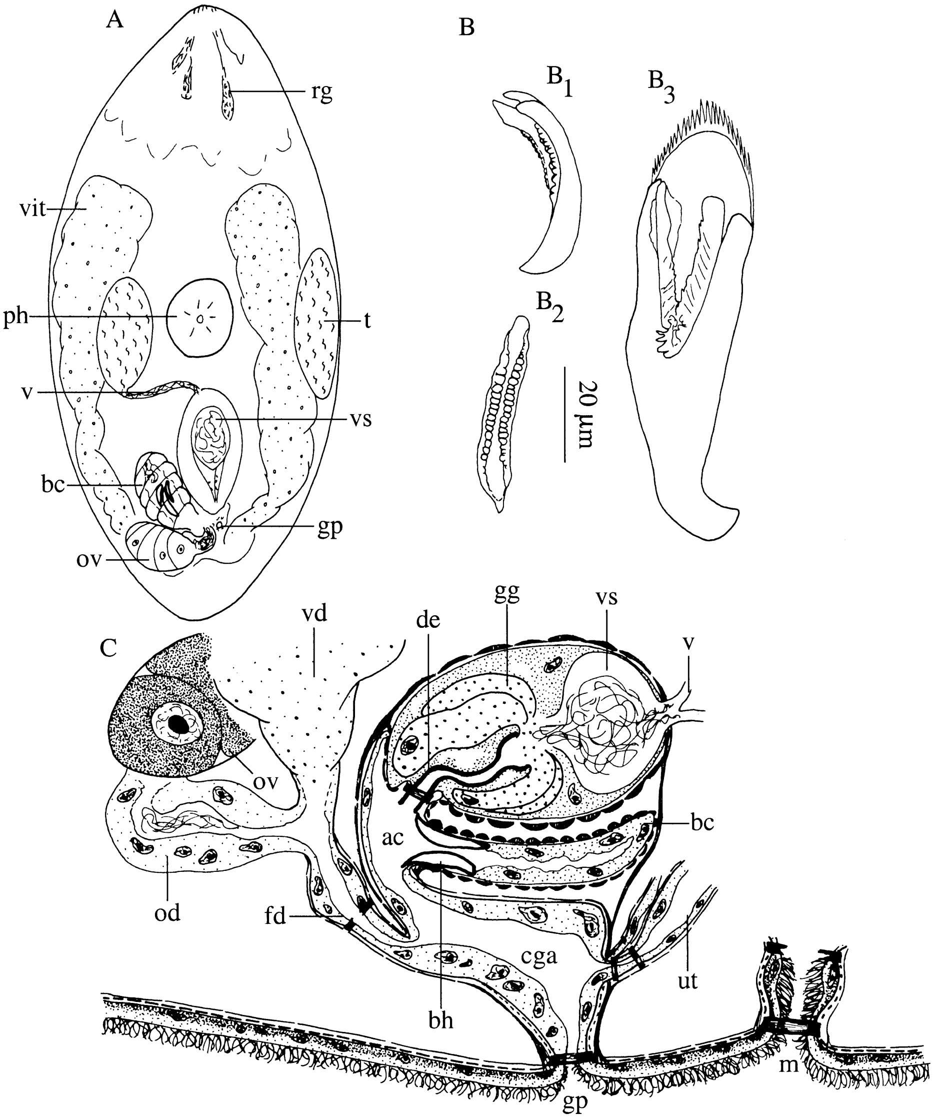

Figure 5. *Castrada trispina* sp. nov. (A) General organization (from a live specimen); (B) bursal hooks; (C) reconstruction of the atrial organs from the right side.

dorsally. Dermal rhabdites could not be discerned. In sections, the epidermis shows a basal, strongly stained part in which the nuclei lie, and an outer, weakly coloured part, as in a number of *Castrada* species (Luther 1946, p 48).

The globular pharynx is situated mid-body. The detailed structure does not deviate from that of other *Castradella* and *Castrada* species (Luther 1946, 1963; Papi 1959). The prepharyngeal cavity consists of two parts. Proximally it is wide and lined with a low, anucleated epithelium, whereas it narrows distally, forming a short tube. This tubular part can be closed by two sphincters, one proximally near the transition to the larger cavity, the second one at the mouth. The tubular part is lined with a high, nucleated epithelium, covered with cilia. This epithelium strongly resembles the body epithelium. In all *Castrada* species, a very similar tubular part of the prepharyngeal cavity is present, into which the protonephridial ducts open, and that therefore is called the excretion cup (Luther 1946; Papi 1959). However, in live individuals of *Castrada trispina* sp. nov. the protonephridia clearly did not open into the mouth, albeit that the exact place of opening could not be determined.

The paired testes are situated left and right of the pharynx, ventrally to the vitellaria. The male genital system contains two major parts: the copulatory bulb and the copulatory bursa, both in close association with each other and surrounded by the same muscle layer. The vasa deferentia are narrow and enter the seminal vesicle together. This copulatory bulb is surrounded by very strong circular muscles and contains the seminal vesicle and the basophilic prostate glands, with no extracapsular parts. Seminal vesicle and glands open into the cup-shaped proximal part of the pseudocuticularized ejaculatory duct (Figure 5C: de), which becomes a narrow tube distally. The ejaculatory duct enters the proximal part of the common genital atrium (atrium copulatorium in Papi 1959) and can protrude into it. Here, the copulatory bulb can be closed by a sphincter. The atrium copulatorium also receives the copulatory bursa (Figure 5C: bc), which is a narrow, muscular sac, ventral to the copulatory bulb. A bursal stalk is absent. The bursa itself is lined with a high, nucleated epithelium, surrounded by strong circular muscles, and contains three hooks (Figure 5C: bh). Two of them are more or less sickle-shaped (Figure 5B: B_1, B_2) and lie near the opening of the bursa into the atrium copulatorium, keeping the opening wide. They are 40 and 46 µm long and their edges carry a row of knobs, reminiscent of a cobblestone pattern when viewed from above (as in Figure 5B: B_2). The third spine (Figure 5B: B_3) is somewhat larger (88 µm long), funnel-shaped and distally bent. The rounded proximal rim carries some small, thin spines. A thick-walled V-shaped structure probably represents the rim of the opening of the funnel. The exact position of this spine in the copulatory bursa could not be determined in sections. This hook is quite similar in outline to that drawn by Luther (1946, Figure 69) in the copulatory bursa of *Castrada annebergensis* Luther, 1946 and there called "spermatophore".

The unpaired ovary is situated in the caudal part of the body. The vitellaria extend from the rostral to the caudal body end. The female duct enters the common genital atrium at the caudal side. It is rather narrow, lined with a high, nucleated epithelium and surrounded by longitudinal muscles, except for a weak sphincter near the atrium. Proximally it receives the vitelloduct and the oviduct, which is widened, has almost no lumen but contains a small amount of sperm. The paired uteri (Figure 5C: ut) enter the common genital atrium from the rostral side.

Discussion

This species clearly belongs to the Rhynchomesostominae, as it shows all diagnostic features of this taxon, as outlined by Papi (1959). Within the Rhynchomesostominae, *Rhynchomesostoma* Luther, 1904 is characterized by an anterior invagination of the epidermis and *Mesocastrada* Volz, 1898 by the presence of an accessory glandular organ connected to the common genital atrium (Papi 1959; Luther 1963). The lack of these features is typical of the taxon *Castrada* Schmidt, 1861, in which our species thus can easily be placed. In the past, the taxonomy of *Castrada* has been much debated (see for instance Luther (1948, p 117–118) and Papi (1959, p 45–47)). Originally all species were placed within the genus *Castrada*. Later on Nassonov (1926) erected the genus *Castradella*, based on the opening of the protonephridia on the body surface (in the mouth in *Castrada*), whereas Luther (1963) placed both taxa as subgenera into the taxon *Castrada*. As the taxonomic value of this diagnostic feature (location of protonephridia opening) is questionable and the division of the taxon *Castrada* into two genera is largely subjective, we prefer following the view of Luther (1963) in order to prevent establishing an unnecessary surplus of names.

Very few species within the taxon *Castrada* have large hooks in the male genital system: *C. baldii* Steinböck, 1949, *C. cristatispina* Papi, 1951, *C. gladiata* (Schwank, 1980), *C. granea* Braun, 1885, *C. neocomensis* Volz, 1898, *C. quadridentata* Hofsten, 1907, and *C. trispina*. The new species resembles *C. cristatispina* most because only in these two species are the hooks all situated in the copulatory bursa, whereas in the other species at least some of the hooks are situated in the atrium copulatorium and/or in a sac-like bulge of this atrium. However, in *C. cristatispina* there are many more hooks, although an exact number is not given (Papi, 1951). Furthermore, *C. cristatispina* has zoochlorellae and a blind sac on the atrium copulatorium, absent in *C. trispina*, and the detailed structure of the bursal hooks is different.

INCERTAE SEDIS

Aegira annabellae gen. nov. sp. nov.

aegira-annabellae sp. nov.

(Figure 6)

Diagnosis

Aegira gen. nov. Typhloplanidae with prostate vesicle surrounded by two spirally running muscle layers. Unpaired, intracapsular seminal vesicle. Pseudocuticularized ejaculatory duct. Prostate glands and ejaculatory duct open into a simple, thin-walled stylet. Simple female genital system with short female duct, proximally split into oviduct and bursa. Vitellaria connected with oviduct through single vitelloduct. Female glands and uterus absent. Type species: *Aegira annabellae*.

Aegira annabellae gen. nov. sp. nov. Provisionally with the same diagnosis as the genus. Box-shaped stylet 25–32 μm long, 14–19 μm wide with distal hook.

Locality

Kerguelen, Port Couvreux: small bay, on *Ceramium*-like red algae, mid-eulittoral, 0.4 m deep at high tide (25 November 1992) (type locality).

Material

Several individuals studied alive. Seven whole mounts, one of them designated holotype (LUC no. 292), three others paratypes (LUC nos 293–295), two of them on the same slide as the holotype. The three remaining whole mounts are of poor quality. Four serially sectioned specimens (paratypes; LUC nos 296–299).

Etymology

The genus name/praenomen refers to the Viking god of the sea, Aegir; gender: feminine. The species epithet is dedicated to Miss Annabel Schreurs, the first author's partner.

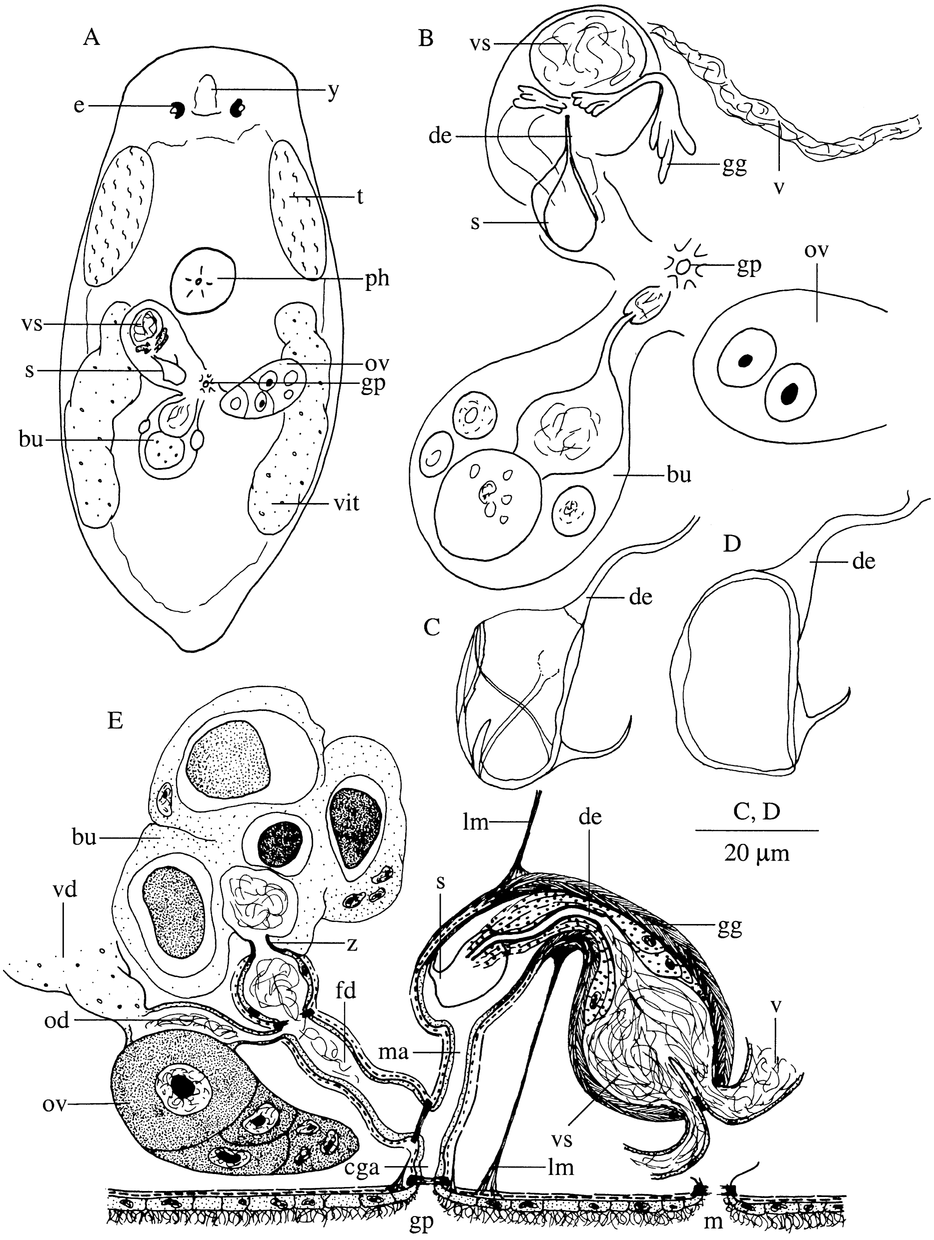

Figure 6. *Aegira annabellae* gen. nov. sp. nov. (A) Habitus of a live animal; (B) general organization (from a live specimen); (C) stylet (from the holotype); (D) stylet (from a paratype); (E) reconstruction of the atrial organs from the right side.

Description

The colourless to pale yellowish animals are 0.4–0.8 mm long (measured on whole mounts) and have two eyes with lenses. In the free-swimming animal, the anterior end is slightly narrowed behind the eyes, giving it the appearance of a spatula. Between the eyes a small protrusion of the intestine is present (Figure 6A: y). Rhabdite glands are absent. The cellular epidermis is about 2 μm thick (only slightly thicker at the rostral and caudal body ends) with cilia ±3 μm long. The cilia are more densely distributed on the ventral side of the body. The rod-shaped rhabdites are ±5 μm long and occur exclusively in the dorsal body epithelium. The intestine of almost all studied individuals is filled with diatoms.

The pharynx (Figure 6A: ph) is situated in the hind part of the first body half, at ±45%. The prepharyngeal cavity is lined with a very low, anucleated epithelium and surrounded by longitudinal muscles. The distal rim of the pharynx is void of cilia. The epithelium of the lumen is very low and anucleated. There are about 20 internal circular muscles, which are very strong. There are 16 internal longitudinal and 16 radial muscles. The outer circular muscles are rather weak. Basophilic glands are present, whereas eosinophilic ones could not be discerned. The exact location where the glands open into the lumen could not be determined due to the state of the sections. Furthermore, in all studied sections a large, circular cavity is present in the pharynx bulb, in between the glands.

The genital pore is situated at ±55% and can be closed by a strong sphincter. The common genital atrium is small, lined with an anucleated epithelium and surrounded by outer longitudinal and inner circular muscles. It receives the male atrium from above and the female duct from behind.

The paired testes lie in the anterior body part and extend from just behind the eyes to the pharynx, in front of the vitellaria. The vasa deferentia are swollen (Figure 6B, E: v), filled with sperm and lined with a low, nucleated epithelium. They are rather long and winding and run separately into the copulatory bulb, in which they join, thus forming a single, intracapsular seminal vesicle (6B, E: vs). The seminal vesicle tapers towards the ejaculatory duct, which is thick-walled and sclerotized. The copulatory bulb is surrounded by two thick, spirally running muscle layers and fixed to the body wall by strong muscles (Figure 6E: lm): one to the dorsal and one to the ventral side of the animal. Besides the seminal vesicle, the bulb also contains coarse-grained, eosinophilic prostate glands (Figure 6E: gg) in its distal part. The prostate secretion enters the stylet, along with the ejaculatory duct. The stylet (Figure 6C, D) is a very simple, thin-walled and cup-shaped box, which can show some wrinkles distally (Figure 6C). It is 25–32 μm long (mean=28 μm; n=4), 14–19 μm wide (mean=16; n=4) and distally carries a slender, bent spine, 10–11 μm long (n=2). The stylet lies in an asymmetrical widening of the male atrium, which is lined with a thin, sclerotized epithelium and surrounded by an inner circular and an outer longitudinal muscle layer. These are the continuations of the muscle layers surrounding the copulatory bulb. Distally of the stylet, the male atrium narrows and forms a duct, which is lined with a low, anucleated epithelium and surrounded by outer longitudinal and inner circular muscles.

The single ovary is situated in the caudal body half. The vitellaria extend from the pharynx to the caudal region and lie at the same dorsoventral level as the testes. The ovary is connected to the female duct by a funnel-shaped oviduct (Figure 6E: od), lined with a low anucleated epithelium and filled with sperm. Proximally, the oviduct receives the broad vitelloduct. Distally, the oviduct narrows and opens into the broad female duct (Figure 6E: fd), which is lined with a low, anucleated epithelium and surrounded by inner circular and outer longitudinal muscles. The female duct is constricted by a sphincter proximally from the oviduct, where it forms a globular, sperm-filled vesicle, which is lined with a low, nucleated epithelium. This vesicle opens into a very large bursa, filled with a number of large inclusions, and a few cavities filled with sperm. In between the female duct and the bursa, the epithelium becomes sclerotized, in some sections giving the appearance of a sort of mouthpiece (Figure 6E: z). A uterus is lacking.

Discussion

A striking feature of *Aegira annabellae* is the presence of only one ovary. This feature is found in the Mariplanellinae (a subtaxon of the Trigonostomidae) and the Typhloplanidae. As explained above, all Trigonostomidae are characterized by the presence of an afferent duct in the female system (see also Den Hartog 1964; Ax and Heller 1970; Ax 1971), absent in *Aegira annabellae*. Most members of the Typhloplanidae are freshwater species, whereas *A. annabellae* is marine. However, some marine representatives of the Typhloplanidae are known, and as the presence of a single ovary is diagnostic of the Typhloplanidae, *A. annabellae* can be placed within this taxon. The placement of *A. annabellae* within one of the eight subtaxa is rather difficult. The division of the Typhloplanidae into these subtaxa is based on the position of the excretory pore and the testes relative to the vitellaria (see Luther 1963). Both features are very difficult to assess in our material. As it is a marine species, the protonephridia are not very well developed or even absent. Furthermore, the testes are situated in front of the vitellaria and more or less lie at the same level (dorsoventrally). In addition, the combination of a pseudocuticularized ejaculatory duct and a stylet is unique within the Typhloplanidae. Therefore, *A. annabellae* is placed within the Typhloplanidae as a taxon incertae sedis.

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V

Typhloplanoida (Platyhelminthes, Rhabdocoela) from New Caledonia and eastern Australia, with the description of six new taxa

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FIELD: Taxonomy

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Abstract Thirteen species of Typhloplanoida from the Australian east coast and New Caledonia are reported, six of them new to science. Three of these new species are representatives of the Promesostomidae: *Coronhelmis cuypersi* new species, *Coronhelmis novaecaledoniae* new species, and *Kymocarens kanakorum* new species. *Austradenopharynx reynaertsi* new genus and species is a member of the Solenopharyngidae. Two species are placed within the Typhloplanidae: *Kaitalugia lydieae* new genus and species and *K. falcata* new species. Furthermore, new localities are given for *Messoplana minuta*, known from the Weddell Sea, *Brinkmanniella palmata*, occurring on the North American Pacific coast, the Swedish west coast and in the Black and Mediterranean Seas, *Ceratopera axi*, a cosmopolitan species, and *Vauclusia conica* and *Pilamonila bimascula*, two Australian species. For the sake of completeness, two more species of which insufficient material is available, are mentioned. A complete species list of all marine Typhloplanoida found in the region is given.

Key words systematics; taxonomy; biodiversity; 'Turbellaria'; Promesostomidae; Solenopharyngidae; Trigonostomidae; Typhloplanidae; Australia; New Caledonia

INTRODUCTION

Our knowledge of marine turbellarians, and of typhloplanoids in particular, from Australia and the adjacent island territories is very scattered. However, in an ecological study in northeastern Australia, Dittman (1991) recognised more than 100 different species of Turbellaria, of which 16 were undescribed typhloplanoids. Up to the present, 14 different species of Typhloplanoida have been described from the region (see Willems *et al.*, 2004a, 2004b; Hochberg, 2004; Hochberg & Cannon, 2003). An overview of all species occurring on the coasts of New Caledonia and eastern Australia, including those described in this contribution and two new species of *Promesostoma* Graff, 1882 (see further), is given in a species list for the region.

Six new species are described in the present contribution: three species from New Caledonia only; two species from the east coast of Australia only; and one species occurring in both regions. Furthermore, additional data are given on *Ceratopera axi* (Riedl, 1954) Den Hartog, 1964, *Brinkmanniella palmata* Karling, 1986, *Messoplana minuta* Artois *et al.*, 2000, *Vauclusia conica* Willems *et al.*, 2004 and *Pilamonila bimascula* Willems *et al.*, 2004. For the sake of completeness, two more species are added, *Pratoplana* spec. and *Ptychopera* spec.. For both, only observations on live material have been made, and one whole mount in bad condition is available, not allowing exact identification. However, both species may be recognised when found in the future.

Additionally two *Promesostoma* species were collected from New Caledonia. Because of the taxonomical complexity and species-richness of this taxon, both species are described in a monographical treatment of *Promesostoma*, which is already submitted for publication elsewhere.

MATERIAL AND METHODS

The specimens for this study were collected during three expeditions: the first in August-September 1996 by Tom Artois and Ernest Schockaert (ES), the second by ES in September-November 1997 and the third by ES in July and August 2003.

The animals were extracted from the sediment or from algae using the $MgCl_2$-decantation method (see Schockaert, 1996), studied alive and whole mounted with lactophenol. Additional specimens were fixed in marine Bouin's solution, embedded in paraffin, serially sectioned (4 µm sections) and stained with Heidenhain's iron haematoxylin, using eosin as a counterstain.

Camera lucida drawings of hard parts in the whole mounts were made with Nomarski microscopy. Drawings without a scale bar are freehand. Photographs are all from live animals and are taken with an Olympia C-5050 Zoom digital camera.

Measurements of hard parts are taken axially, unless indicated otherwise. The position of the gonopore and organs, and the measurements of the pharynx are expressed in percentages of the total body length (distance from the anterior tip of the body).

Voucher specimens of *Ceratopera axi* (Riedl, 1954) Den Hartog, 1964 from California and the Falklands were loaned from the collections of the Swedish Museum of Natural History in Stockholm (SMNH). Two whole mounts from Australia, one from Kerguelen, one from La Réunion and one from the Weddell Sea are present in the collections of the LUC (Diepenbeek, Belgium). Material of several solenopharyngid species from the SMNH collections was used for reference. The type material of *Vauclusia conica*, *Messoplana minuta* and *Pilamonila bimascula* is present in the LUC collections.

The type material of *Austradenopharynx reynaertsi* new genus and species and *Coronhelmis cuypersi* new species, both from Australia, will be deposited in the collections of the Queensland Museum, Brisbane, Australia. The type material of the New Caledonian species will be kept in the LUC collections.

Abbreviations used in the figures

b: brain; ba: bursal appendage; bc: copulatory bursa; bgg: basophilic prostate glands; bh: bursal hook; bu: bursa; cga: common genital atrium; cil_{1-2}: cilia; cm: circular muscles; cop: copulatory organ; csp: cirrus spines; de: ejaculatory duct; ds: spermatic duct; e: eye; egg: eosinophilic prostate glands; epg: extrapharyngeal glands; evs: extracapsular seminal vesicle; fd: female duct; gg: prostate glands; gl: glands; gp: common genital pore; gw: ‘Greifwulst’ (prehensile girdle); igg: intracapsular prostate glands; ipg: intrapharyngeal glands; ivs: intracapsular seminal vesicle; lm: longitudinal muscles; m: mouth; ma: male atrium; mb: muscular bulb on female duct; od: oviduct; ov: ovary; pc: prepharyngeal cavity; pg: pharynx glands; ph: pharynx; pl: pharynx lumen; rg: rostral glands; rs: seminal receptacle; s: stylet; sep: muscular septum; sph: sphincter; t: testis; ut: uterus; v: vas deferens; vd: vitelloduct; vg: prostate vesicle; vit: vitellarium; vs: seminal vesicle; x, y, z: features described in respective text.

TAXONOMIC ACCOUNT

Family Promesostomidae Den Hartog, 1964
Subfamily Brinkmaniellinae Luther, 1948

***Brinkmanniella palmata* Karling, 1986** Fig. 1

NEW LOCALITIES: Ile Nou (Nouméa, New Caledonia): on Kuendu Beach in coarse-grained sand, 3 Aug 2003 and on algae in the lagoon, 10 Aug 2003.

KNOWN DISTRIBUTION: North American Pacific coast (Karling, 1986), Black Sea (leg. V. Mack-Fira; see Karling, 1986), Mediterranean Sea (leg. P. Martens; see Karling, 1986), Swedish west coast (Karling, 1986).

MATERIAL EXAMINED: Two specimens (one from each locality) studied alive and mounted.

REMARKS: The delicate stylet of the New Caledonian specimens is 56-58 µm long (n = 2) and shows six or seven ‘fingers’, which lie closely together (see Fig. 1). The distal tip is somewhat widened. The structure of the stylet resembles that of two out of six species of *Brinkmanniella* Luther, 1943: *Brinkmanniella obtusa* Luther, 1943 and *B. palmata* Karling, 1986, the difference between the two being the number of ‘fingers’ (six in *B. palmata*, seven to eight in *B. obtusa*) and the fact that the distal tip is pointed (in *B. obtusa*) or blunt (Karling, 1986). In the specimens from New Caledonia the distal tip of the stylet is blunt, not, however forming a fist-like structure as in *B. palmata* specimens from the North American Pacific coast, the Swedish west coast and the Black and Mediterranean Sea. It is, however, never pointed as in *B. obtusa*.

***Coronhelmis cuypersi* new species** Fig. 2A-C, Tab. 1

LOCALITY: Arrawarra (New South Wales, Australia): beach south of the marine station, between rocks, fine sand, 30 Oct 1997 (Type locality).

MATERIAL EXAMINED: One specimen studied alive and mounted, designated holotype.

ETYMOLOGY: Species dedicated to Dr. Ann Cuypers (Centre for Environmental Sciences, LUC, Diepenbeek).

DESCRIPTION: The Australian specimen is ± 1.0 mm long (measured on the whole mount). The pharynx is situated in the middle of the body (Fig. 2A: ph). The prostate vesicle (Fig. 2A, B: vg) is situated in the last third of the body and shows a little cap (Fig. 2B: x) as seen in the live animal. In the whole mount it seems to be an everted (permanently?) muscular collar. From here starts a rather long ejaculatory duct (Fig. 2B: de), which widens slightly distally where it is armed with small spines (thickened basement membrane; Fig. 2B: y). The whole system, prostate vesicle and ejaculatory duct, is enclosed in a septum, forming a typical duplex-type copulatory organ (terminology of Karling, 1956). The stylet (Fig. 2C) surrounds the distal part of the ejaculatory duct, is 24 µm long, proximally 19 µm wide, with a proximal thin-walled, 11 µm-long part (‘Manschette’ in Luther, 1948) and a single ring of spines distally. The spines (more than 30) are all ± 15 µm long. A copulatory bursa was not observed.

DIAGNOSIS: *Coronhelmis* species with stylet 24 µm long and 19 µm wide, with one ring of more than 30 spines and 11 µm-long 'manschette'. Slender spines ± 15 µm long. Long ejaculatory duct with small spines.

DISCUSSION: see Discussion on the taxon *Coronhelmis*.

Coronhelmis novaecaledoniae new species Fig. 2D-F, Tab. 1

LOCALITY: Ile Nou (Nouméa, New Caledonia): on Kuendu Beach in coarse-grained sand, 3 Aug 2003 (Type locality).

MATERIAL EXAMINED: One specimen studied alive and mounted, designated holotype (LUC no. 305).

ETYMOLOGY: The species name/epithet refers to its occurrence on New Caledonia.

DESCRIPTION: The animal is ± 1.2 mm long (measured on the whole mount). The pharynx is situated in the middle of the body (Fig. 2E: ph). The overall structure of the genital system (based on observations of a live animal; Fig. 2D) does not deviate from that of other species of *Coronhelmis* Luther, 1948 (see for instance Luther, 1948; Ehlers, 1974; Ax, 1994). The stylet (Fig. 2F) is 19 µm long and 29 µm wide. It consists of a proximal thin-walled, ± 9 µm long part ('Manschette' in Luther, 1948) and one ring of spines (15 to 20) distally. The 9 µm long spines resemble a small leaf and are elongated oval in shape with a thickened ridge in the middle, which extends beyond the proximal rim. In the centre of the stylet a flower-shaped structure is present, which possibly is the thickened wall of the ejaculatory duct.

DIAGNOSIS: *Coronhelmis* species with stylet 19 µm long and 29 µm wide, with one ring of 15 to 20 spines and ± 9 µm long 'Manschette'. Spines 9 µm long, leaf-shaped with a thickened median ridge, extending beyond the proximal rim.

DISCUSSION ON THE TAXON *CORONHELMIS*: *Coronhelmis novaecaledoniae* and *C. cuypersi* possess almost all diagnostic characters of the taxon *Coronhelmis* Luther, 1948: an elongated body, a copulatory bursa and an oval-shaped copulatory organ connected to a stylet that consists of a proximal part or 'Manschette' and a distal ring of spines (diagnosis of Luther, 1948). A copulatory bursa was not observed in *C. cuypersi* (as for example also in *C. conspicuus* Ax, 1994, *C. noerrevangi* Ax, 1994 and *C. exiguus* Ax, 1994; see Ax, 1994). An overview of all known species is given in Tab. 1. *C. urna* Ax, 1954 is regarded as a junior synonym of *C. lutheri* Ax, 1951, following Luther, 1962.

The only differences between the species are to be found in the detailed structure and size of the stylet (see Tab. 1). The stylet of *C. novaecaledoniae* is

Table 1. Comparison of all *Coronhelmis* species. (?: not known)

	C. conspicuus	*C. cuypersi*	*C. exiguus*	*C. inornatus*	*C. lutheri*	*C. multispinosus*	*C. noerrevangi*	*C. novaecaledoniae*	*C. tripartitus*
Length animal (mm)	1.5-2	1	0.8-1.0	0.6-0.8	0.8-1.4	0.5-1.5	0.8-1.2	1.2	0.6-1.0
Stylet length (µm)	60-80	24	30	11-12	17-26 (45 under strong pressure)	16-23 (± 56 under strong pressure)	17-22	19	15-20
Length 'manschette' (µm)	18-25	11	± 5	absent	8-9	± 33 µm (under strong pressure)	?	9	?
Stylet width (µm)	?	19	?	± 10	12-24	18	17-20	29	11-14
No. of spiny rows (strong pressure)	4-5 (?)	?	1	1	max. 8	1	3	1 (?)	1
No. of spiny rows (no pressure)	2-3	1	1	1	1	1	1	1	1
No. of spines	>30	>30	8-10	10-15	>30	± 10	15	15-20	25-30
Length spines (µm)	20-30	15	?	max. 11-12	5-10	13 (25)	5-9	9	?
Ejaculatory duct	short	long	short	short	short	short	short	short	short
Habitat	freshwater outlet	marine, eulittoral	freshwater outlet	marine, supralittoral	freshwater outlets; brackish sandflats	brackish sandflats	marine& brackish sandflats; freshwater outlets	marine, eulittoral	marine, supralittoral
Distribution	Greenland	East Australia	Greenland	North Sea	Baltic Sea; North Atlantic; Alaska	Baltic Sea; North Sea	Greenland, Iceland, Färöer	New Caledonia	North Sea
Literature	Ax, 1994; Ax, 1995b	this paper	Ax, 1994; Ax, 1995b	Ehlers, 1974	Ax, 1951, 1954, 1994, 1995a; Luther, 1962; Karling, 1974; Ehlers, 1974; Ax & Armonies, 1987, 1990	Luther, 1948, 1962; Karling, 1974; Ehlers, 1974; Ax & Armonies, 1987; Müller & Faubel, 1993	Ax, 1994; Ax, 1995b	this paper	Ehlers, 1974

easily distinguished from that of other *Coronhelmis* species by the detailed structure of the spines. In all these species the spines are extremely simple, not leaf-shaped with a median ridge, as in *C. novaecaledoniae*.

The stylet of *C. cuypersi* closely resembles that of *C. lutheri* (see for instance Ax, 1951: Fig. 23; Ax, 1994: Figs 1A-B, 2A-B; Luther, 1962: Fig. 19H; Ehlers, 1974: Fig. 11A, C; Ax & Armonies, 1987: Fig. 20D-E). In this species the stylet consists of telescopic rings, only visible under high pressure of the cover slide (see for instance Karling, 1974: Fig. 73; Ehlers, 1974: Fig. 11B; Ax, 1994: Figs 1C, 2C). However, the long ejaculatory duct, armed with spines, is a unique feature within the taxon *Coronhelmis*; in all other *Coronhelmis* species the stylet is almost directly connected to the prostate vesicle, and the ejaculatory duct is extremely short.

Kymocarens kanakorum **new species** Fig. 3

LOCALITY: Ile Nou (Nouméa, New Caledonia): on Kuendu Beach in coarse-grained sand, 3 Aug 2003 (Type locality).

MATERIAL EXAMINED: One specimen studied alive and mounted, designated holotype (LUC no. 306).

ETYMOLOGY: The species name refers to the original inhabitants of New Caledonia, the Kanaki.

DESCRIPTION: Animal ± 0.5 mm long (measured on the whole mount), with eyes. Two large rhabdite tracts present, running from behind the eyes to the sides of the "head" (Fig. 3A: rg). The caudal end of the animal is blunt, more or less forming a tail plate.

Pharynx situated in the middle of the body (Fig. 3A: ph).

The common genital pore is situated at ± 80%. Some small glands open into the common genital atrium, together with a large vesicle or copulatory bursa (Fig. 3B: bc). The paired testes lie in front of the pharynx (Fig. 3A: t). Paired, elongated ovovitellaria (Fig. 3A: ov + vit) extend over almost the entire body length, with the ovaries in the most caudal part (Fig. 3 A-B: ov).

The paired seminal vesicles fuse and open into the copulatory bulb (Fig. 3B: vg), which is filled with prostate secretion and an internal seminal vesicle. The bulb is connected to a slightly bent tubular stylet (Fig. 3C), which is 56 µm long and 14 µm wide at the proximal opening. This stylet shows a transverse, thin ridge, situated at ± 85% of the stylet length. The terminal opening is asymmetrical in the single studied specimen.

The female genital system is relatively simple, only consisting of the two ovaries and a small sperm-containing vesicle in between both ovaries (Fig. 3B: bu).

DIAGNOSIS: *Kymocarens* species with eyes and simple, tubular stylet, 56 µm long and 14 µm wide, with a transverse ridge distally.

DISCUSSION: Although this species was only studied alive and on a single whole mount, a number of diagnostic features were observed, resulting in its placement within the taxon *Kymocarens* Ehlers & Ehlers, 1981. This taxon is characterised by the presence of large and conspicuous rostral rhabdite tracts, an adhesive tail plate, paired testes in front of the pharynx, paired seminal vesicles, a tubular stylet, paired ovovitellaria with the ovaries caudally, a bursal organ connected to the common genital atrium, and a female duct with a sperm-containing vesicle (see diagnosis in Ehlers & Ehlers, 1981). Apart from *Kymocarens kanakorum*, two additional species of *Kymocarens* are known from the Galapagos and only from a few specimens. The New Caledonian species differs from both other species in the size and detailed structure of the stylet. In *K. proxenetoides* Ehlers & Ehlers, 1981 it is a simple, 49-50 µm long, elongated funnel, without ridges, whereas it is only 30 µm long and carries two terminal hooks and a thin plate in *K. tibialis* Ehlers & Ehlers, 1981 (see Ehlers & Ehlers, 1981). Furthermore, the latter species lacks eyes, which are present in *K. proxenetoides* and *K. kanakorum*.

Incertae sedis

***Vauclusia conica* Willems *et al.*, 2004**

NEW LOCALITY: North Stradbroke Island (Queensland, Australia): Dunwich, fine sand between mangroves in front of the marine station, 12 Aug 1996.

KNOWN DISTRIBUTION: Sydney, Australia (Willems *et al.*, 2004a).

MATERIAL EXAMINED: One specimen studied alive and mounted. Holotype (whole mount) and three paratypes (sections) from Sydney (see Willems *et al.*, 2004a).

ADDITIONAL REMARKS: The live animal from Stradbroke Island clearly showed two testes, as in the sectioned material from Sydney, whereas only one was visible in the live individual from Sydney (Willems *et al.*, 2004a). The stylet of the new specimen is 30 µm long and 23 µm wide proximally. These measurements correspond with those of the holotype (29 µm long and 17 µm wide; see Willems *et al.*, 2004a).

Family Solenopharyngidae Graff, 1882
Subfamily Solenopharynginae Ehlers, 1972

***Austradenopharynx reynaertsi* new genus and species** Fig. 4

LOCALITY: Sydney (New South Wales, Australia): Vaucluse Beach, flat beach with fine sand and numerous crab holes, 10 Oct 1997 (Type locality). North Stradbroke Island (Queensland, Australia): Adams Beach, sand plate with crab holes, medium coarse sand, 16 Sept 1996.

MATERIAL EXAMINED: Several specimens studied alive, one of them mounted and designated paratype. One serially-sectioned animal, designated holotype.

ETYMOLOGY: The genus name/praenomen refers to its southern distribution and resemblance to *Adenopharynx mitrabursalis* Ehlers, 1972. Auster (Lat.): south (wind). Species dedicated to the first author's lifetime friend, Mr. David Reynaerts.

REMARK: The sectioned individual was damaged ventrally, leaving little of the ventral epidermis and muscle layers. This made the determination of the exact location of the mouth and gonopore somewhat speculative, and details on the structure of their connection to the prepharyngeal cavity and to the common genital atrium cannot be given. Due to the tiny size of the animal, some details regarding the genital system, and especially the female system, are could not be determined precisely.

DESCRIPTION: The transparent animal is extremely small, only about 0.1 mm long (measured on the whole mount) and brightens when observed with Nomarski microscopy. Eyes are lacking. The syncytial epidermis is ± 3 µm thick, slightly thinner dorsally, with cilia ± 2 µm long. Dermal rhabdites are absent. In the live animal two rostral glands are visible (Fig. 4B: rg).

The mouth is situated at ± 60% (see remark above). The prepharyngeal cavity (Fig. 4C: pc) is lined with a low, anucleated epithelium, surrounded by longitudinal muscles and a few weak circular ones around its distal part only. The pharynx (Fig. 4B: ph), short and almost globular in live animals, bulges deeply into the prepharyngeal cavity and is slightly inclined backwards. As in other solenopharyngids, there is a proximal, rather short, tubular part. The outer circular muscles are very strong, whereas the inner circular layer is much weaker. The exact number of internal longitudinal and radial muscles could not be determined. The radial muscles are thicker in the most proximal and in the tubular part of the pharynx. At least two types of pharyngeal glands are present. The large, coarse-grained basophilic glands have an extra-capsular part (Fig. 4C: epg), surrounded by longitudinal muscles. Small, eosinophilic glands (Fig. 4C: ipg) occur in between them in the distal part of the pharynx.

The common genital pore (Fig. 4B: gp) lies at ± 70% (see remark above), just behind the mouth. The common genital atrium (Fig. 4A, C: cga) is lined with a very low, anucleated (?) epithelium and surrounded by inner circular and outer longitudinal muscles. From the dorsal side, it receives the copulatory bursa (Fig. 4C: bc). It is lined with a thickened basement membrane (pseudocuticula; clearly visible on the whole mount), covered with the remains of the epithelium. The basement membrane even forms two (or more?) large blunt teeth (Fig. 4C: bh), just before the bursal stalk enters the genital atrium. The bursa is surrounded by circular muscles and distally by an outer longitudinal layer as well. The paired testes are situated in the most anterior part of the body and are connected with each other through a wide 'bridge', in some live specimens giving the impression of an unpaired testis (see Fig. 4B: t). The unpaired ovary is situated caudally. The paired vitellaria extend over almost the entire body length.

The duplex-type copulatory organ (Fig. 4A; terminology of Karling, 1956) enters the common genital atrium from the side, ventrally from the bursa, and is ± 110 µm long (measured on the whole mount). It is entirely surrounded by a strong, inner circular muscle layer (Fig. 4A: cm), forming a sphincter close to the atrium (Fig. 4A: sph), and an outer longitudinal muscle layer (Fig. 4A: lm). The proximal part of the copulatory bulb contains a globular seminal vesicle (Fig. 4A: ivs), lined with a high, nucleated epithelium and containing thick, lively sperm. A thin muscular septum (Fig. 4A: sep) separates the seminal vesicle from the distal part of the copulatory organ. Sperm are released into the ejaculatory duct (Fig. 4A: de). Sperm are much thinner here. The ejaculatory duct (± 80 µm long; ± 3 µm wide) is lined with a thick basement membrane (or pseudocuticula). Muscles surrounding the ejaculatory duct could not be observed. Prostate glands (Fig. 4A: igg) enter the ejaculatory duct just distally from the seminal vesicle. At its very end (± 3/4 of its total length), distally from the sphincter, the ejaculatory duct widens (± 9 µm); here the basement membrane is more thickened and forms many small spines (Fig. 4A: csp). This part of the ejaculatory duct bulges into the common genital atrium and resembles a pair of pincers in the live animal.

The female system is extremely complex. The single ovary is slender and elongated (in the sectioned individual) and is connected to the genital atrium via a very narrow female duct, which receives the single vitelloduct near the ovary. The most proximal part of the female duct is widened, almost funnel-shaped, and contains sperm. This part is also connected caudally to a large resorptive bursa (Fig. 4C: bu). Additionally, this bursa is connected through a globular sperm-containing vesicle (Fig. 4C: rs) to the common genital atrium, very close to the copulatory bursa. This vesicle is surrounded by circular muscles and is lined with a low, anucleated epithelium. A large sack, completely filled with large cells (Fig. 4C: ut) opens into the genital atrium from the caudal side (uterus in Ehlers, 1972).

DIAGNOSIS: *Austradenopharynx* new genus. Solenopharynginae with short, globular pharynx situated in the middle of the body. Copulatory organ of the duplex-type with pseudocuticularised ejaculatory duct. Copulatory bursa pseudocuticularised with two large, blunt teeth. Single ovary connected to common genital atrium through two ducts: a narrow female duct and a resorptive bursa that opens into the atrium near the copulatory bursa.

Austradenopharynx reynaertsi new species. Ejaculatory duct distally widened, with small spines.

DISCUSSION: This species clearly is a member of the taxon Solenopharyngidae Graff, 1882, as it shows all diagnostic features of the taxon (see Ehlers, 1972). Furthermore, it can be placed within the subtaxon Solenopharynginae Ehlers, 1972, based on the presence of a cirrus-like copulatory organ, a copulatory bursa and an unpaired ovary that is connected with the common genital atrium through two different ducts (Ehlers, 1972). Within the Solenopharynginae, the different taxa mainly differ in the detailed structure of the copulatory organ. However, a copulatory organ with a pseudocuticularised ejaculatory duct that carries spines only distally is unique within the taxon. In all other Solenopharynginae the cirrus is pseudocuticularised over its whole length or carries spines over its whole length, but never a combination of both as in *Austradenopharynx reynaertsi*. Furthermore, a copulatory bursa that is lined with a very thick and strengthened basement membrane over its whole length, and has large teeth is also unique within the Solenopharynginae. A copulatory bursa that carries spines is only present in representatives of the taxon *Trisaccopharynx* Karling, 1940, whereas its wall is lined with a thick pseudocuticula (without spines or teeth) in *Adenopharynx mitrabursalis* Ehlers, 1972, *Culleopharynx armatus* (Riedl, 1956) Ehlers, 1972 and *Solenopharynx flavidus* Graff, 1882. Therefore, we conclude that *A. reynaertsi* is best placed in its own monospecific taxon for the time being. The alternative would be to synonymise all above taxa; a discussion on this issue is beyond the scope of this contribution.

Incertae sedis

Pilamonila bimascula Willems *et al.*, 2004

NEW LOCALITIES: Ile Nou (Nouméa, New Caledonia): Nouville, on algae in the lagoon south of the asylum, 3 and 10 Aug 2003; reef in western part of Kuendu Bay, on ramified algae, together with shell gravel and sand, 16 Aug 2003. Baie des Citrons (Nouméa, New Caledonia): on algae in the lagoon, 08 Aug 2003; Baie de Magenta (Nouméa, New Caledonia): permanent pool near mangroves, on large algae covered with epiphytes, 22 Aug 2003.

KNOWN DISTRIBUTION: Arrawarra, New South Wales, Australia (Willems *et al.*, 2004a).

MATERIAL EXAMINED: Observations on live animals. Four whole mounts and seven serially-sectioned animals. Type material (see Willems *et al*., 2004a).

ADDITIONAL REMARKS: The specimens from New Caledonia are 0.3-0.6 mm long (measured on whole mounts). The observations on the serially-sectioned individuals confirm the data given by Willems *et al.* (2004a). The organisation of the genital system, even its detailed structure, is identical with that of the Australian specimens (see Willems *et al.*, 2004a). The stylet could not be discerned in the whole mounts and even in the sectioned specimens it is inconspicuous. This is largely due to the state of contraction of the cirrus. When completely inverted (as in the New Caledonian specimens) the spines lie on top of the stylet, which also is extremely thin-walled. The only observed difference with the individuals of Australia is the cellular epidermis (stated as syncytial in Willems *et al.*, 2004a due to misinterpretation of the sectioned specimens from Australia).

Family Trigonostomidae Graff, 1905 *sensu* Den Hartog, 1964
Subfamily Trigonostominae Luther, 1948 *sensu* Den Hartog, 1964

Ceratopera axi (Riedl, 1954) Den Hartog, 1964

SYNONYMIES: *Proxenetes axi* Riedl, 1954; *Ceratopera bifida* Ehlers & Ax, 1974

NEW LOCALITIES: Baie des Citrons (Nouméa, New Caledonia): on algae in the lagoon, 8 Aug 2003; Ile Nou (Nouméa, New Caledonia): Nouville, on algae in the lagoon south of the asylum, 16 Aug 2003.

KNOWN DISTRIBUTION: Gulf of Naples and Sicily (Riedl, 1954); Galapagos (Ehlers & Ax, 1974); Falkland Islands and California (Karling, 1986); Weddell Sea and La Réunion (Artois *et al.* 2000); Eastern Australia (Willems *et al.*, 2004a); Kerguelen (own data).

MATERIAL EXAMINED: Two specimens studied alive and mounted (one from each new locality). Whole mounts from Falklands and California (collections SMNH), Australia, the Weddell Sea, La Réunion and Kerguelen (collections LUC).

ADDITIONAL REMARKS: The stylet of the specimens from New Caledonia measures 78-98 µm (measured along the axis of the stylet; 56-62 µm if measured from top to bottom as in Ehlers & Ax, 1974). The bursal appendage is 80 µm long (measurable on only one specimen). These data can be placed within the known range of measurements for this species (see Willems *et al.*, 2004a: Tab. 1; Willems *et al.*, in press). However, as the variation in the size of the stylet is rather large, it could very well be that it concerns a species complex in stead of a single species, as indicated earlier (see Willems *et al.*, in press). Since this size variation is the only known difference between the populations, it is highly arbitrary to split this complex into different species. Such an act should be backed up by a more thorough morphological analysis, which is clearly without the scope of this contribution.

Messoplana minuta Artois *et al.*, 2000 Fig. 5

NEW LOCALITY: Arrawarra (New South Wales): Arrawarra Beach, in sand of rockpool north of headland, 24 July 2003.

KNOWN DISTRIBUTION: Weddell Sea (Artois *et al.*, 2000).

MATERIAL EXAMINED: One specimen studied alive and mounted. Type material (see Artois *et al.*, 2000).

ADDITIONAL REMARKS: The animal is 0.7 mm long (measured on the whole mount) and rather slender (Fig. 5A). The structure of the genital system (Fig. 5B) does not deviate from that of other species of *Messoplana* Den Hartog, 1966. The stylet of the Australian specimen (Fig. 5C) consists of a slightly bent tube, which is 34 µm long. Proximally it is attached to a hook-shaped, 22 µm-long accessory spine. The base of the copulatory organ, which carries both the stylet and the accessory spine, is 32 µm wide. The bursal appendage (Fig. 5D) is curved (>360º), 66 µm long and apparently not split distally. In the specimens of *Messoplana minuta* from the Weddell Sea the bursal appendage is split into two short tubes. This is extremely difficult to observe in our specimen, as well as in the type material of *M. minuta*. Nevertheless, the shapes and sizes of the copulatory organ and the bursal appendage of the Australian specimen are almost identical to those of the specimens from the Weddell Sea (Artois *et al.*, 2000).

Ptychopera spec. Fig. 6

LOCALITY: North Stradbroke Island (Queensland, Australia): Point Lookout, small rocky bay on the north side, on *Ulva*-like algae in a tidepool, 14 Sept 1996.

MATERIAL EXAMINED: One specimen studied alive and mounted (in bad condition).

DESCRIPTION AND REMARKS: Only one individual of this species was observed alive and the whole mount is in a bad condition. Therefore this description must be seen as provisional.

The pharynx is situated in the first half of the body (Fig. 6A: ph). Paired testes and ovovitellaria are present. Paired seminal vesicles open into a large, globular prostate vesicle, which is connected to a complex stylet. Where a large bursal organ opens into the common genital atrium, some small, hard teeth are present. A pseudocuticularised, winding tube (Fig. 6B: ds) probably forms the connection between this bursa and the ovaries, functioning as a spermatic duct. These characters allow the placement of this species into the taxon *Ptychopera* Den Hartog, 1964 (see Den Hartog, 1964). Only the detailed structure of the stylet could not be determined, because of the bad condition of the whole mount, making the identification of this species extremely difficult. We refrain from identifying this species, which is probably new to science, as no other *Ptychopera* species has similar teeth at the opening of the bursa. At the moment only one other species of this taxon is known to occur in Australia: *Ptychopera scutulifer* Ehlers & Ax, 1974 (see Willems *et al.*, 2004a). However, in this species the pharynx is located in the most caudal part of the body and the stylet is very different in shape, in comparison with the situation observed on live material of the new specimen.

Family Typhloplanidae Graff, 1905
Subfamily Typhloplaninae Luther, 1963

Pratoplana spec. Fig. 7

LOCALITY: Magnetic Island (Queensland, Australia): Cockle Bay, amongst mangroves in detritus-rich sediment with crab holes, 8 Sept 1996.

MATERIAL EXAMINED: One animal studied alive and mounted (in bad condition).

DESCRIPTION AND REMARKS: Only one individual of this species was observed alive and the whole mount is in a very bad condition. Therefore this description must be seen as provisional.

The animal is small, only about 0.3 mm (measured on the whole mount), with two eyes and two conspicuous rhabdite tracts (Fig. 7A: rg) in between them. The pharynx (Fig. 7A: ph) is situated just behind the middle of the body. The paired testes lie besides the pharynx. There is only one seminal vesicle (Fig. 7A-B: vs), which is obviously intra-capsular. The prostate vesicle also contains some coarse-grained prostate glands (Fig. 7B: gg), which have an extra-capsular part and enter the prostate vesicle from the side. The prostate vesicle itself is connected to a small, pseudocuticularised spine (Fig. 7B: s; thickened basement membrane of the male duct?). The vitellaria extend from about 1/4 of the body length caudally to just behind the pharynx. The large, elongated ovary lies immediately behind the pharynx. A large vesicle is present in the caudal body part. The exact nature of it could not be discerned, but probably it is a part of the female system.

This marine representative of the Typhloplanidae, which encompasses mostly freshwater species, is a representative of the taxon *Pratoplana* Ax, 1960. This taxon is characterised by the presence of a muscular prostate vesicle connected to a small funnel-shaped stylet, paired vitellaria, an unpaired ovary, caudally from the pharynx, a widened female duct, functioning as a ‘vesicula resorbiens’ and a seminal receptacle (Ax, 1960; Ehlers, 1974). It has three representatives: *P. ayorae* Ehlers & Ehlers, 1989, *P. galeata* Ehlers, 1974 and *P. salsa* Ax, 1960. The specimen from Australia differs from *P. galeata* by the presence of eyes, by the shape of the prostate vesicle, which is extremely elongated in *P. galeata*, and by the presence of an intra-capsular seminal vesicle, paired extra-capsular in *P. galeata* (see Ehlers, 1974). Furthermore, the stylet of *P. galeata* is 25 µm long and almost tubular, cup-shaped (Ehlers, 1974), whereas it is short, funnel-shaped in the Australian specimen. *P. salsa* and *P. ayorae* resemble each other in the structure of the stylet, which is a 6-7 µm long funnel-shaped piece, with a narrow distal tip (Ax, 1960; Ehlers & Ehlers, 1989), somewhat resembling the stylet of the Australian specimen. However, both species differ in the number of seminal vesicles: paired extra-capsular in *P. salsa* (Ax, 1960) and unpaired intra-capsular in *P. ayorae* (Ehlers & Ehlers, 1989). The Australian specimen therefore closely resembles *P. ayorae*, in the structure of the stylet and the number of seminal vesicles. However, since the detailed structure and the size of the stylet were impossible to determine because of the poor state of the whole mount, we refrain from allocating this specimen to an existing species or erecting a new species.

Incertae sedis

Kaitalugia lydieae **new genus and species** Figs 8, 9

LOCALITY: Baie des Citrons (Nouméa, New Caledonia): on algae from the lagoon, 8 Aug 2003 (Type locality).

MATERIAL EXAMINED: Observations on live animals. Three whole mounts, one of them designated holotype (LUC no. 307), the others paratypes (LUC no. 308-309). Seven serially-sectioned animals, designated paratypes (LUC nos 310-316).

ETYMOLOGY: Genus name/praenomen refers to Kaitalugi, a mythical island in Melanesia, where ships are wrecked. Species dedicated to Mrs. Lydie Grosemans, technical assistant at LUC, Diepenbeek (Belgium).

DESCRIPTION: The uncoloured animals are 0.4-0.5 mm long (measured on whole mounts) and have two eyes. Rostral rhabdite glands (Fig. 8A: rg) are well developed, filled with thick rod-shaped, ± 2 µm-long rhabdites. The cellular epidermis is ± 3-4 µm thick and carries ± 3 µm-long cilia. Dermal rhabdites are slender, rod-shaped, 3-6 µm long and scattered over the body surface, slightly more densely packed on the dorsal side.

The rosulate pharynx (Fig. 8A, C, D: ph) is situated in the second body half, at about 60%. The mouth can be closed by a strong sphincter and is connected to the narrow prepharyngeal cavity by a very short muscular tube. The prepharyngeal cavity proper is lined with a low, anucleated (?) epithelium and surrounded by longitudinal muscles. The distal rim of the pharynx carries cilia (Fig. 8B: cil_1), a feature difficult to discern because the cavity is very narrow. The pharynx lumen is lined with a low anucleated (?) epithelium. There are 24 internal longitudinal muscles. Both radial and external circular muscle layers are weak, whereas the internal circular muscles are thickened, especially near the proximal opening of the pharynx. Basophilic and eosinophilic gland ducts open into the lumen, distally from the so-called 'Greifwulst' (prehensile girdle; Fig. 8B: gw), which carries long cilia (Fig. 8B: cil_2).

The common genital pore lies at ± 85% and is guarded by a sphincter. The common genital atrium is lined with a low anucleated (?) epithelium and is surrounded by weak, inner circular and outer longitudinal muscles. The paired testes are situated slightly in front of and at both sides of the pharynx. The large, single ovary is situated caudally. The vitellaria lie dorsally of the testes and extend from ± 30% to the caudal body end.

In the live animal, two extra-capsular seminal vesicles were seen (Fig. 8A: evs), however, they were not observed in sectioned material, and may only represent temporary swellings of the vasa deferentia. The elongated copulatory bulb (Fig. 8A: vg) is directed rostrolaterally towards the pharynx (directed more caudally in Fig. 8B for clarity) and is surrounded by a strong inner circular muscle layer and a weak outer longitudinal one. The copulatory bulb contains a large elongated seminal vesicle (Fig. 8B: ivs), which is lined with a low epithelium, next to two types of glands, each with an extra-capsular part: coarse-grained basophilic (Fig. 8B: bgg) and fine-grained eosinophilic ones (Fig. 8B: egg). Both sperm and prostate secretion are discharged into the stylet (Fig. 8E), which is 27 µm long, 21 µm wide proximally and 8-9 µm wide distally (n = 2). It is a simple, short funnel-shaped piece, which narrows slightly distally, and shows a bent hook at one side. It lies in the male atrium (Fig. 8B: ma), which is lined with a low, anucleated epithelium and surrounded by inner circular and outer longitudinal muscles, and enters the common genital atrium from its caudal side.

The very large ovary is situated dorsocaudally and connected with the common genital atrium through a wide female duct. Near the ovary, the female duct is surrounded by some weak circular muscles. More distally, it is lined with a high, nucleated epithelium, surrounded by very weak longitudinal muscles, and filled with sperm. It is swollen asymmetrically, forming a kind of bursal organ in most sectioned individuals. Towards the atrium, the female duct narrows and is guarded by a muscular bulb (Fig. 8A-D: mb) that consists of strong radial muscles and is surrounded by longitudinal muscles, which are clearly stronger in this part than around the rest of the female duct. This muscular bulb is extremely obvious in live animals and contracts regularly (Fig. 9A-D). The female duct enters the common genital atrium from the rostrodorsal side, where a large bundle of basophilic glands (Fig. 8B-C: gl) opens into the genital atrium. A uterus is lacking.

DIAGNOSIS: *Kaitalugia* new genus. Typhloplanidae species with pharynx situated midbody. Distal rim and prehensile girdle ('Greifwulst') of pharynx with cilia. Elongated copulatory bulb with intracapsular seminal vesicle. Stylet simple, funnel-shaped. Female duct swollen and filled with sperm, distally with a very strong muscular part. Large bundle of glands at transition of female duct to genital atrium.

Kaitalugia lydieae new species. *Kaitalugia* species with simple stylet, 27 µm long, 21 µm wide proximally and 8-9 µm wide distally, with a bent hook at one side.

DISCUSSION: *Kaitalugia lydieae* is characterised by the presence of a single ovary, which is a diagnostic feature for both Mariplanellinae Ax & Heller, 1970 and Typhloplanidae Graff, 1905. However, Mariplanellinae is a subtaxon of Trigonostomidae Graff, 1905 (sensu Den Hartog, 1964) of which the representatives have, apart from the female duct, a second connection between the ovary and the common genital atrium (Den Hartog, 1964; Ax, 1971; Ax & Heller, 1970). Since this double connection is lacking in *K. lydieae*, this new species therefore has to be placed within the Typhloplanidae, which mainly contains freshwater species.

Within this family several taxa are recognised, almost all of which are based on doubtful or difficult to observe characters, such as the position of the testes relative to the vitellaria and the opening of the excretory system (mostly invisible in marine species). Therefore this species is not allocated to any of the subfamilies, but, for the time being, placed as a species incertae sedis within the taxon Typhloplanidae.

Within the Typhloplanidae few species are marine, and *K. lydieae* most closely resembles *Thalassoplanella collaris* Luther, 1946 and *Thalassoplanina geniculata* (Beklemischev, 1927) Ax, 1959. However, both species are easily distinguished from *K. lydieae* by the detailed structure of their stylet (see Luther, 1946; Ax, 1959), whereas the overall structure of the male system is very similar in all three species. The major differences are to be found in the female system. In *T. collaris* a separate copulatory bursa and a second rudimentary ovary are present (Luther, 1946). Both features are absent in *K. lydieae*. Furthermore, the female duct of *T. collaris* is not swollen over almost its whole length, as in *K. lydieae*, but is connected to a terminal seminal receptacle (Luther, 1946). In *T. geniculata*, on the other hand, the female system is also swollen over its whole length, but is connected to a terminal seminal receptacle (or vesicula resorbiens; Ax, 1959) and shows a bulge (bursa copulatrix in Ax, 1959) about half way along its length. Additionally, the female duct of *T. geniculata* is strongly muscular over its whole length, while in *K. lydieae* a highly muscular bulb in the distal part of the female duct occurs.

Kaitalugia falcata new species Fig. 10

LOCALITIES: Ile Nou (Nouméa, New Caledonia): Nouville, on algae from the lagoon south of the asylum, 16 Aug 2003 (Type locality). Arrawarra (New South Wales, Australia): Mullaway Headland, on red and brown algae in rock pools, 24 July 2003; Arrawarra: southern part of the beach near rocks, on *Sargassum*-like algae in a permanent pool, 27 Aug 1996.

MATERIAL EXAMINED: Observations on live animals. Four whole mounts, two of them in bad condition. One whole mount designated holotype (LUC no. 317).

ETYMOLOGY: Species name/epithet refers to the presence of a long, scythe-shaped spine on the stylet. Falcatus (Lat.): sickle- or scythe-shaped.

DESCRIPTION: The unpigmented animals are ± 0.4 mm long (measured on whole mounts). Rostrally two conspicuous rhabdite glands are present, running in between the eyes. The pharynx (Fig. 10A: ph) is situated just behind the middle of the body.

The testes lie at the same level as the pharynx, in the middle of the body. One external seminal vesicle (Fig. 10B: evs) was clearly visible in a live specimen. This seminal vesicle narrows, forming a seminal duct, which enters the copulatory bulb proximally. The bean-shaped copulatory bulb contains the intra-capsular seminal vesicle (Fig. 10B: ivs) and the prostate glands (with extra-capsular cell bodies). It is situated caudally of the pharynx and connected to the stylet (Figs 10A-B: s, 10C), which is 29-34 µm long (n = 3; m = 31 µm), 17 µm wide proximally and 7-11 µm wide distally. The stylet is funnel-shaped with three distal projections, the first straight and dagger-like (12-15 µm long), the second short and bent (10 µm long) and the third long, narrow and scythe-shaped (16-25 µm long).

The vitellaria extend from just behind the rostral glands to the level of the pharynx, whereas the single ovary is situated caudally. A large bursal organ (swollen female duct?) is present and contains a star-shaped structure (Fig 10A-B: z; not visible on whole mounts).

DIAGNOSIS: *Kaitalugia* species with 29-34 µm-long stylet carrying two additional projections distally: a straight dagger-like one (12-15 µm long) and a slender scythe-shaped one (16-25 µm long).

DISCUSSION: This species must be placed within the taxon *Kaitalugia*, based on its overall resemblance with *K. lydieae*, in both the structure of the genital system (observations of live animals) and the detailed structure of the stylet. However, since no sectioned individuals were available, the detailed structure of the genital system could not be studied. The main difference with *K. lydieae* is the presence of a more or less star-shaped structure in the female duct, evidently the equivalent of the muscular bulb as in *K. lydieae*. However, it is not clear yet if this is a hard structure or a muscular contracting bulb as in *K. lydieae*.

SPECIES LIST OF TYPHLOPLANOIDA FROM THE CORAL AND TASMAN SEA

On the Australian east coast and in New Caledonia 26 marine species of Typhloplanoida occur, of which eight species are Promesostomidae, two are Solenopharyngidae, 11 are Trigonostomidae and 5 are Typhloplanidae.

An overview of these species and their distribution, is given in Tab. 2. For *Ceratopera axi* and all species of *Trigonostomum* Schmidt, 1852 only one reference is given, where all distribution data and references can be found.

The two new *Promesostoma* species are treated in a monography of the taxon.

Table 2. Species list of all marine Typhloplanoida from the Coral and Tasman Seas, with their known distribution within (column I) and outside (column II) the region. Abbreviations: A: eastern Australia; Al: Alaska; Ber: Bermuda; Braz: Brazil; BS: Baltic Sea; cosm: cosmopolitan; Cur: Curaçao; EA: East Africa; Gal: Galapagos; MS: Mediterranean and Black Sea; NP: North American Pacific coast; NAtl: northern Atlantic Ocean; NA: North American Atlantic coast; NC: New Caledonia; SAtl: southern Atlantic; SW: Swedish west coast; WS: Weddell Sea.

	I	II	Main literature on distribution
Promesostomidae Den Hartog, 1964			
Brinkmaniellinae Luther, 1948			
Brinkmanniella australiensis Willems *et al.*, 2004	A	-	Willems *et al.*, 2004a
Brinkmanniella palmata Karling, 1986	NC	NP, MS, SW	Karling, 1986; this paper
Coronhelmis cuypersi new species	A	-	this paper
Coronhelmis novaecaledoniae new species	NC	-	this paper
Kymocarens kanakorum new species	NC	-	this paper
Promesostominae Luther, 1948			
Promesostoma new species 1	NC	-	
Promesostoma new species 2	NC	-	
Incertae sedis			
Vauclusia conica Willems *et al.*, 2004	A	-	Willems *et al.*, 2004a
Solenopharyngidae Graff, 1882			
Solenopharynginae Ehlers, 1972			
Austradenopharynx reynaertsi new genus and species	A	-	this paper
Incertae sedis			
Pilamonila bimascula Willems *et al.*, 2004	A/NC	-	Willems *et al.*, 2004a; this paper
Trigonostomidae Graff, 1905 *sensu* Den Hartog, 1964			
Trigonostominae Luther, 1948 *sensu* Den Hartog, 1964			
Ceratopera axi (Riedl, 1954) Den Hartog, 1964	A/NC	cosm	Willems *et al.*, 2004a; this paper
Messoplana minuta Artois *et al.*, 2000	A	WS	Artois *et al.*, 2000
Ptychopera scutulifer Ehlers & Ax, 1974	A	Gal, EA	Ehlers & Ax,

			1974; Schockaert & Martens, 1985
Ptychopera spec.	A	-	this paper
Trigonostomum armatum (Jensen, 1878) Gamble, 1900	A/NC	NAtl, SAtl, BS, Cur (cosm?)	Willems *et al.*, 2004b
Trigonostomum australis Willems et al., 2004	A	-	Willems *et al.*, 2004b
Trigonostomum denhartogi (Karling, 1978) Willems *et al.*, 2004	NC	Cur, Ber, EA	Willems *et al.*, 2004b
Trigonostomum franki Willems *et al.*, 2004	NC	Cur, Flor, EA	Willems *et al.*, 2004b
Trigonostomum lilliei (Graff, 1911) Meixner, 1924	A	NA, Braz	Willems *et al.*, 2004b
Trigonostomum spinigerum Willems *et al.*, 2004	NC	-	Willems *et al.*, 2004b
Trigonostomum watsoni Willems *et al.*, 2004	A/NC	-	Willems *et al.*, 2004b
Typhloplanidae Graff, 1905			
Cephalopharynginae Hochberg, 2004			
Cephalopharynx cannoni Hochberg, 2004	A	-	Hochberg, 2004
Typhloplaninae Luther, 1963			
Pratoplana spec.	A	-	this paper
Magnetia queenslandica Hochberg & Cannon, 2003	A	-	Hochberg & Cannon, 2003
Incertae sedis			
Kaitalugia lydieae new genus new species	NC		this paper
Kaitalugia falcata new species	A/NC	-	this paper

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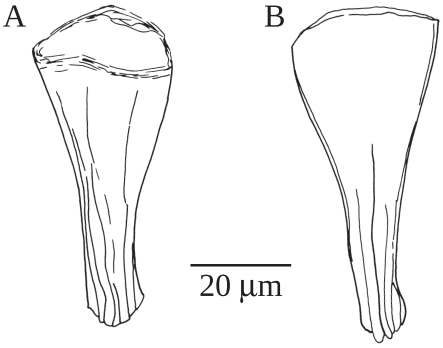

Fig. 1 *Brinkmanniella palmata*: A,B. Stylet of two different individuals.

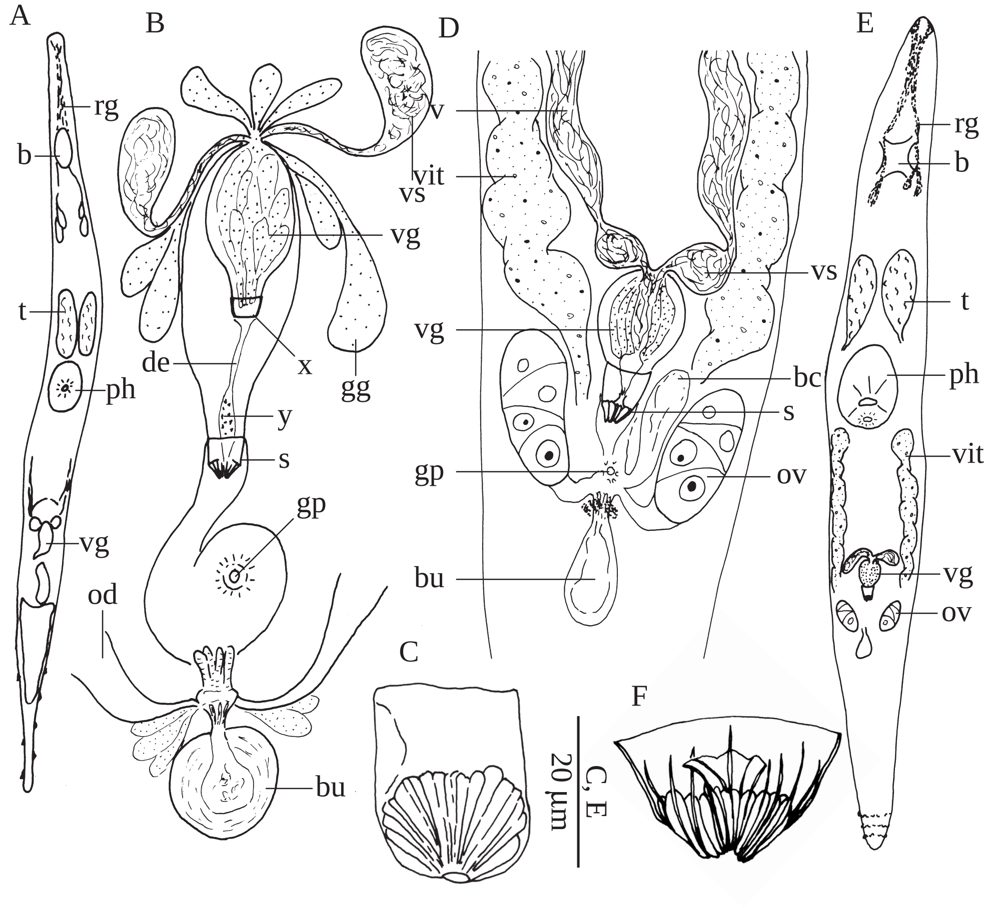

Fig. 2 *Coronhelmis cuypersi*: A. Habitus of a live animal; B. Organisation of the genital system (from a live specimen); C. Stylet (from the holotype). *Coronhelmis novaecaledoniae*: D. Organisation of the genital system (from a live specimen); E. Habitus of a live animal; F. Stylet (from the holotype).

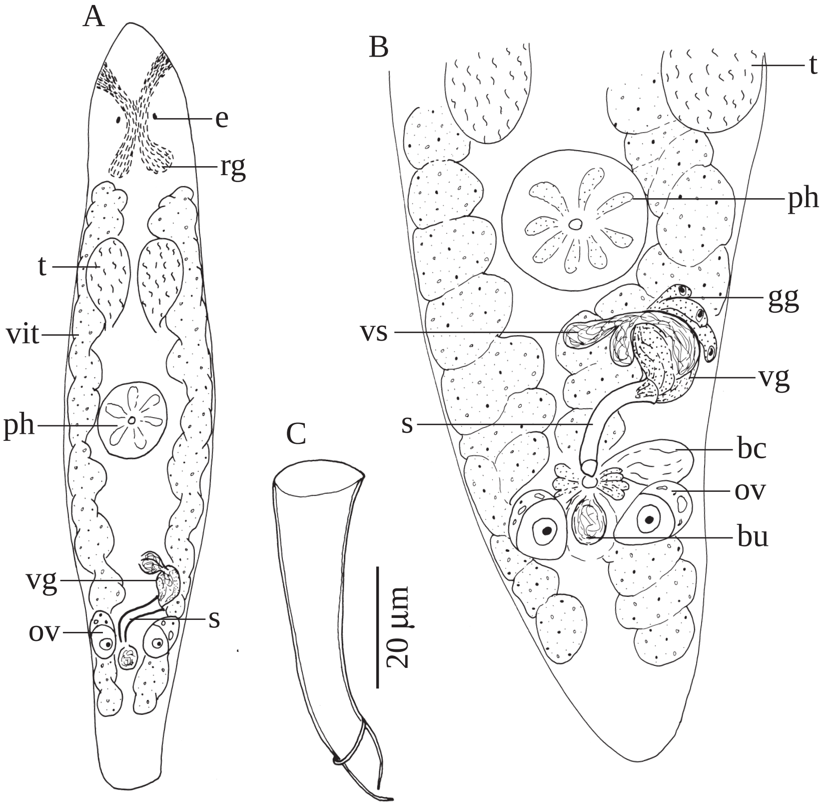

Fig. 3 *Kymocarens kanakorum*: A. Habitus of a live animal; B. Organisation of the genital system (from a live specimen); C. Stylet (from the holotype).

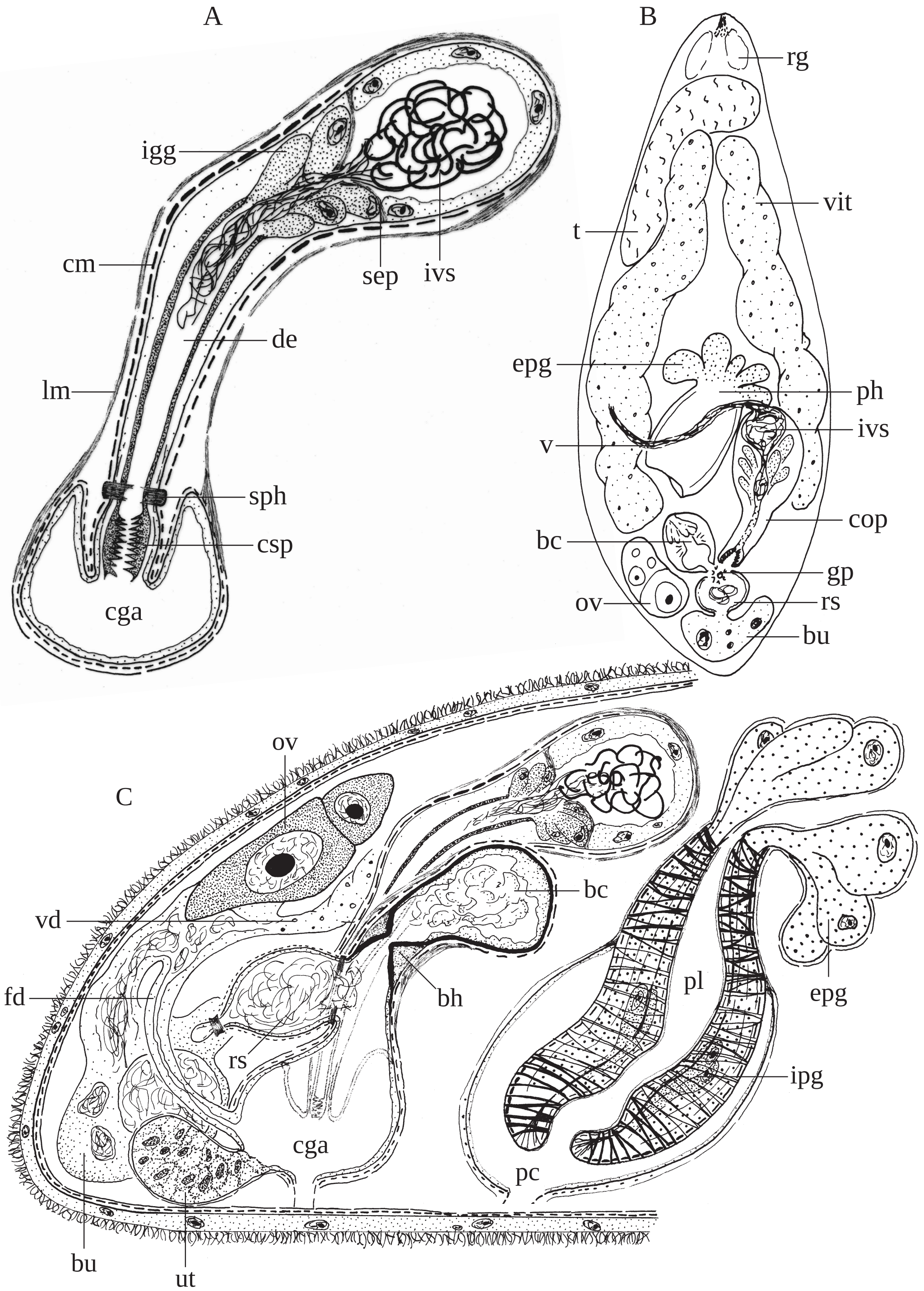

Fig. 4 *Austradenopharynx reynaertsi*: A. Male genital system (from the holotype); B. General organisation (from a live specimen); C. Reconstruction of the atrial organs from the right side (from the holotype).

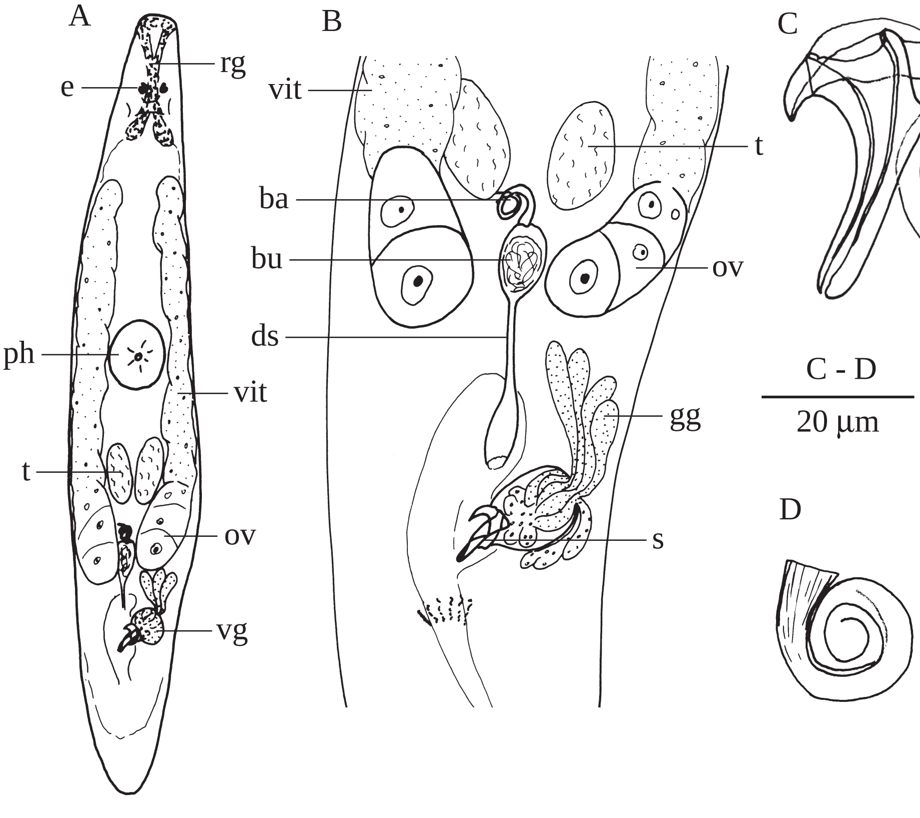

Fig. 5 *Messoplana minuta*: A. Habitus of a live animal; B. Organisation of the genital system (from a live specimen); C. Stylet (from a New Caledonian specimen); D. Bursal appendage (from a New Caledonian specimen).

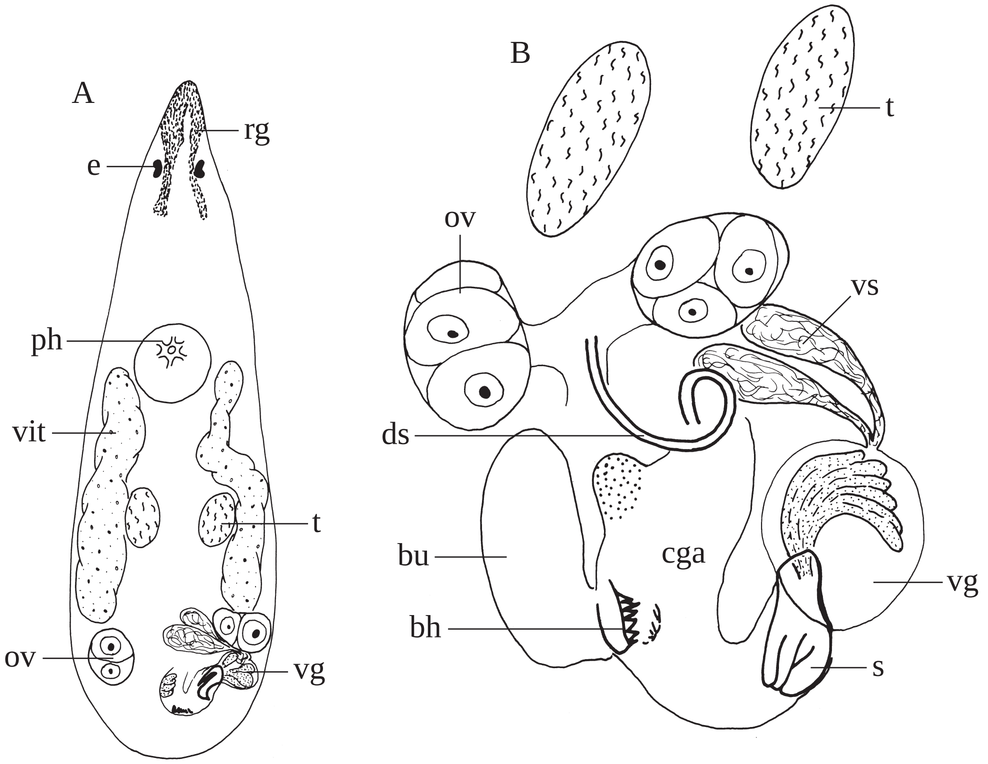

Fig. 6 *Ptychopera* spec.: A. Habitus of a live animal; B. Organisation of the genital system (from a live specimen).

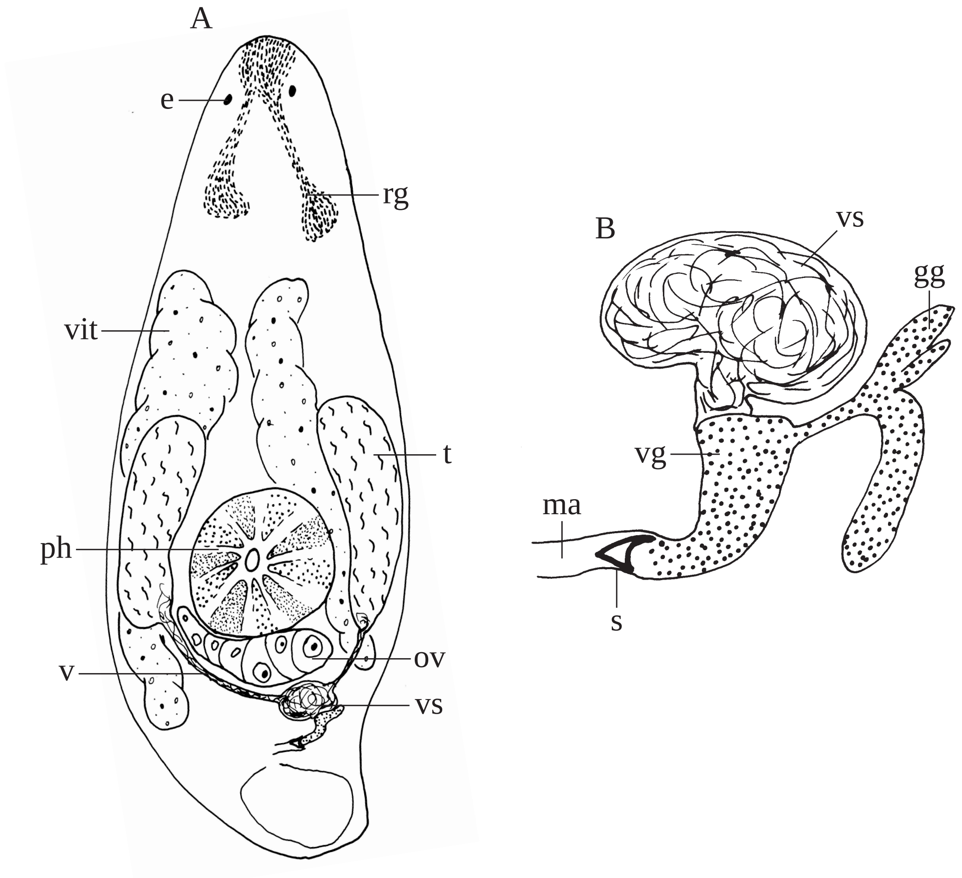

Fig. 7 *Pratoplana* spec.: A. Habitus of a live animal; B. Organisation of the male genital system (from a live specimen).

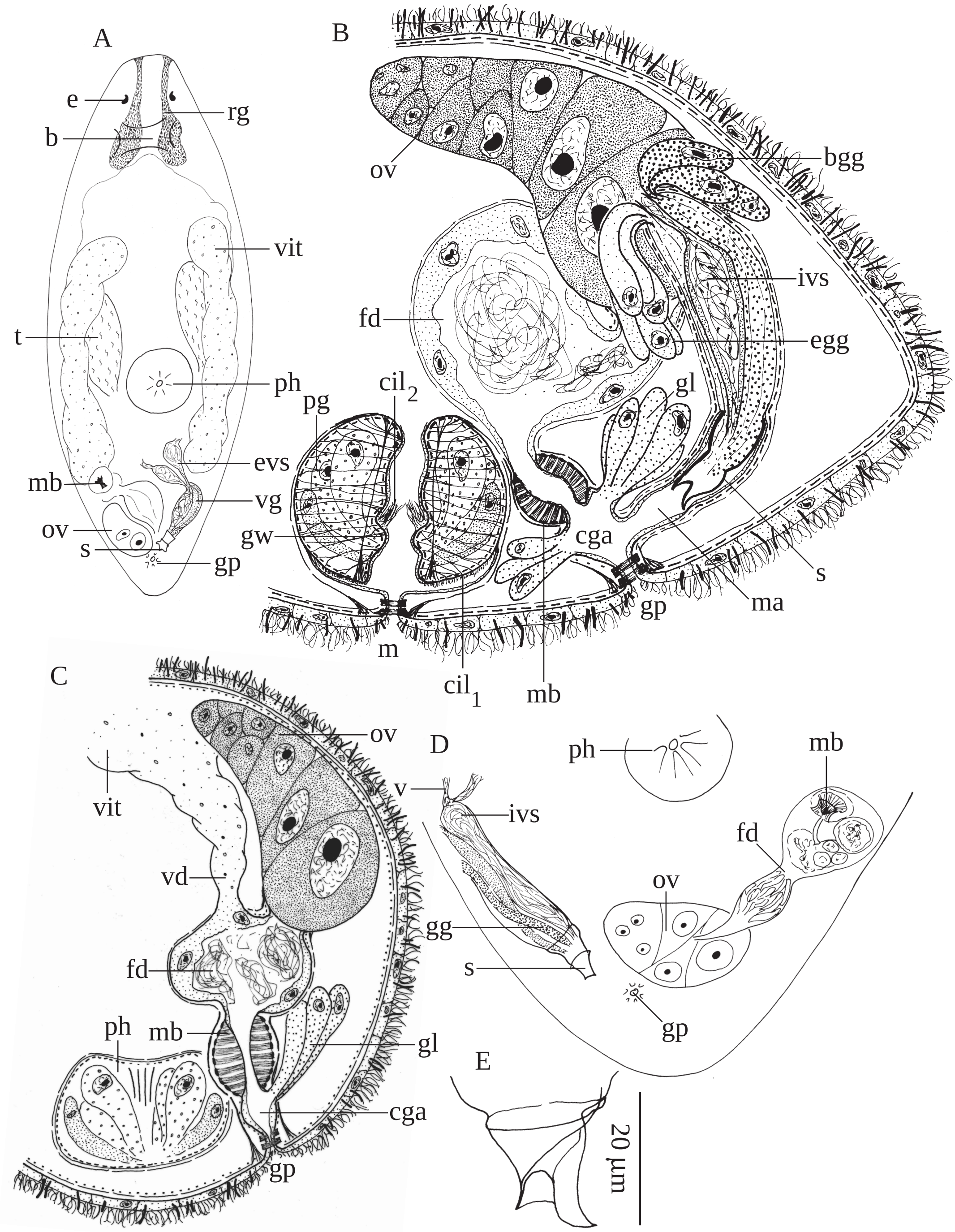

Fig. 8 *Kaitalugia lydieae*: A. Habitus of a live animal; B. Reconstruction of the atrial organs from the left side (male genital system is displaced caudally for clarity); C. Reconstruction from transverse sections of the female genital system; D. Organisation of the genital system (from a live specimen); E. Stylet (from the holotype).

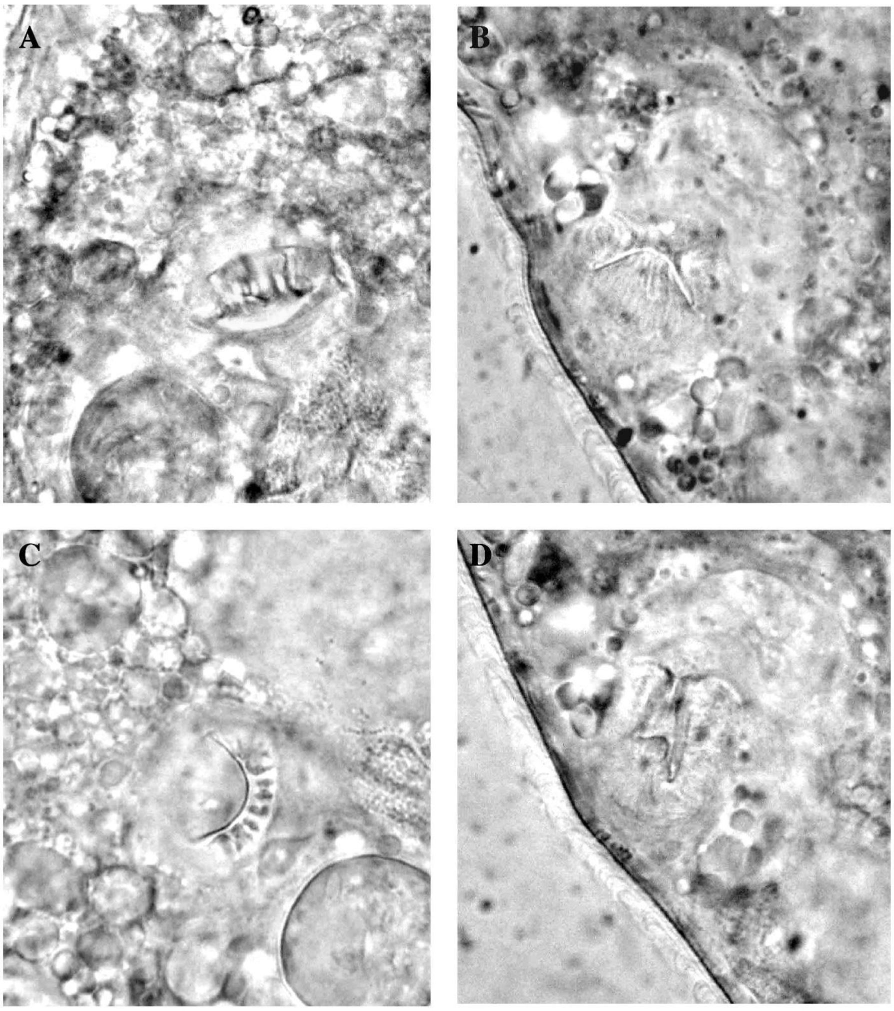

Fig. 9 *Kaitalugia lydieae*: A-D. Different stages of contraction of the muscular bulb on the female duct (from a live specimen).

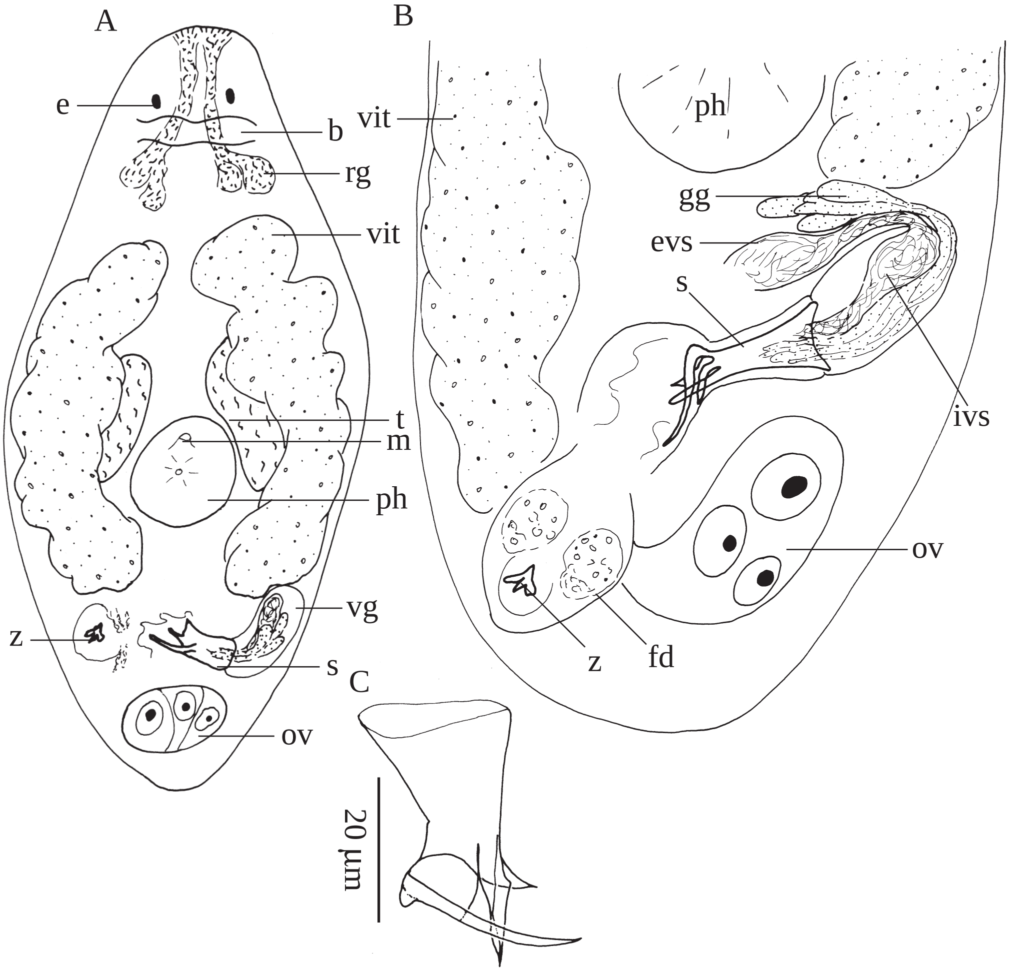

Fig. 10 *Kaitalugia falcata*: A. Habitus of a live animal; B. Organisation of the genital system (from a live specimen); C. Stylet (from the holotype).

VI

Report on the Polycystididae (Kalyptorhynchia, Rhabdocoela) from Australia, with the description of 12 new species and 6 new genera.

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SHORT TITLE: Polycystididae from Australia

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Abstract Twelve new species of Polycystididae are described from the Australian east coast. Nine of them could not be placed in any of the existing genera, and therefore 6 new genera are erected. *Alchoides* n. gen. differs from all other polycystidid genera by the presence of a bundle of glands which opens in the distal part of the male atrium (accessory vesicle type V). Two species are included in the genus, *A. alchoides* n. sp. and *A. dittmanni* n. sp., which differ from each other in form of the stylet. *Ametochus gehrkei* n. gen n. sp. can be distinguished from other polycystidids by the presence of a prostate vesicle type III associated with a prostate stylet type III, combined with the presence of an accessory stylet type III, while a prostate stylet type II is lacking. Typical for *Arrawarria inexpectata* n. gen n. sp. is the combined presence of an armed cirrus with a prostate vesicle type II connected to a prostate stylet type II. Two species are assigned to the new genus *Duplexostylus* n. gen.: *D. rowei* n. sp. and *D. winsori* n. sp. These two species have two unique features: a prostate vesicle type IV directly connected to a prostate stylet type III and an asymetrical septum which surrounds the proximal part of the male atrium. They can be distinguished from each other by differences in the detailed construction of their stylet. Two new species are placed in the new genus *Stradorhynchus* n. gen.: S. caecus n. sp. and S. terminalis n. sp. They have unpaired gonads and a prostate vesicle type IV connected to a prostate stylet type IV, the combination of both making them unique within the Polycystididae. Both species differ from each other in the form and dimensions of the stylet. *Triaustrorhynchus armatus* n. gen. n. sp. is the first species of Polycystididae to have three types of stylet in the male atrium: a prostate stylet type II, a prostate stylet type III and an accessory stylet type III. Three new species could be placed in existing genera. *Cincturorhynchus monaculeus* n. sp., *Paraustrorhynchus caligatus* n. sp. and *Polycystis australis* n. sp. All three differ from their respective relatives in the form and dimensions of their stylets. The possible relationships of all these new taxa is discussed. *Austrorhynchus hawaiiensis* Karling, 1977 is reported for the first time in Australia and the Australian population is compared with populations from other regions. The presence of *Gyratrix hermaphroditus* Ehrenberg, 1831, one new species of *Paulodora* Marcus, 1948 and a new genus of Typhlopolycystidinae Evdonin, 1977 is mentioned.

Key words systematics; taxonomy; biodiversity; 'Turbellaria'; Kalyptorhynchia; Polycystididae; Australia

INTRODUCTION

The only kalyptorhynch species ever reported from Australia is the cosmopolitan species *Gyratrix hermaphroditus* Ehrenberg, 1831 (see Curini-Galetti and Puccinelli 1990, 1998). This species however is now considered a complex of numerous species, and also in Australia several cryptic species were recognised by Curini-Galetti and Puccinelli (1990, 1998). 108 (unidentified or undescribed) species of 'Turbellaria' were reported from northeastern Australia (North Queensland) in an ecological study by Dittmann (1991), with the Kalyptorhynchia being the most species-rich taxon with 39 species. This fact shows how poorly known the marine microturbellarian fauna of this large continent actually is (for an overview, see Willems et al., 2004, in press), although it is a very important group from an ecological point of view.

In this contribution we report on 12 species of polycystidid kalyptorhynchs of which sufficient material was collected during several stays on the east Australian coast: around Townsville, on North Stradbroke Island, around Sydney and between Byron Bay and Coffs Harbour. Only one species (*Austrorhynchus hawaiiensis* Karling, 1977) is already known to science. The other 11 species are new to science, and some of them have such an aberrant anatomy that they could not be placed in any existing genus. This necessitated the erection of six new genera, the possible relationships of which will be discussed.

Apart from the species discussed we also found *Gyratrix hermaphroditus* Ehrenberg, 1831 in almost every visited location. We will not further discuss this well-known species. Two more species, one of them belonging to *Paulodora* Marcus, 1948, the other one to the taxon Typhlopolycystidinae Evdonin, 1977, will be treated in future monographic contributions on the respective taxa.

MATERIAL AND METHODS

Specimens for this study were collected during three expeditions: the first in August-September 1996 by Tom Artois and Ernest Schockaert (ES), the second by ES in September-November 1997 and the third by ES in July and August 2003. Additional material and drawings were kindly provided by Dr. Sabine Dittmann (Wilhelmshaven, Germany).

The animals were extracted from the sediment or from algae using the $MgCl_2$-decantation method (see Schockaert, 1996), studied alive and whole mounted with lactophenol. Additional specimens were fixed in marine Bouin's solution, embedded in paraffin, serially sectioned (4 µm sections) and stained with Heidenhain's iron haematoxylin, using eosin as a counterstain.

Camera lucida drawings and photographs of hard parts were made using Nomarski microscopy. Drawings without a scale are freehand. Measurements of hard parts are taken axially, unless indicated otherwise. The position of the gonopore and organs, and the measurements of the proboscis and the pharynx are expressed in percentages of the total body length (distance from the anterior tip of the body).

Terminology of the male and female system is based on Artois & Schockaert (2003, In press).

Voucher specimens of *Austrorhynchus hawaiiensis* from Hawaii were loaned from the collections of the Swedish Museum of Natural History in Stockholm (SMNH).

The type material will be deposited in the collections of the Queensland Museum, Brisbane, Australia.

Abbreviations used in the figures

acg1, accessory glands type I; acg3, accessory vesicle type III; acg5, accessory glands type V; ast3, accessory stylet type III; b, female bursa; br, brain; bs, bursal stalk; cg, caudal glands; de, ejaculatory duct; e, eye; fd1, female duct type I; fg, female glands; ga, common genital atrium; gg, glands; gp, gonopore; m, mouth; ma, male atrium; mb, muscle bulb; mbu, male bursa; od, oviduct; ov, ovary; p, proboscis; ph, pharynx; pv1, prostate vesicle type I; pv2, prostate vesicle type II; pv3, prostate vesicle type III; pv4, prostate vesicle type IV; rs, seminal receptacle; s, septum; st2, prostate stylet type II; st3, prostate stylet type III; st4, prostate stylet type IV; t, testis; ut, uterus; vd, vitelloduct; vi, vitellarium; vs, seminal vesicle.

TAXONOMICAL ACCOUNT

As the taxonomy of the Polycystididae is rather outdated (most recent review is by Evdonin, 1977) and badly in need of revision, we decided not to organise the species following any taxonomical order. Instead, the genera are arranged alphabetically.

Alchoides alchoides n. gen. n. sp.

(Figs 1A, C, E, G; 5A)

Alcha sp. in Watson (2001)

DISTRIBUTION: North Stradbroke Island (Queensland), Adams Beach, coarse sand from a sand flat with crab-holes (16/09/96) (type locality); same locality, Dunwich, on sand flat with crab-holes in the mid-eulittoral, in front of the marine station (12/08/96).

MATERIAL: Several specimens studied alive. Three whole mounts (one designated holotype, the others paratypes). One sagitally sectioned specimen (paratype).

ETYMOLOGY: The name refers to the overall resemblance of this species with *Alcha evelinae* Marcus, 1949.

DESCRIPTION: The animals are colourless, 1 mm long (measured on whole mounts) and have two eyes. The epidermis is syncytial, with several lobate nuclei. It is ± 1 µm thick, with cilia of 2 µm long and a 0,5 µm thick basal membrane. The rhabdites are mainly situated in the caudal part of the body and are ± 1/3 of the epithelium height long.

The proboscis is about 20% of the body length long. Because of the rather poor quality of the sectioned animal, its construction could not be examined in detail.

The pharynx is rather small, with a diameter of only 1/10 of the total body length. There are three types of pharyngeal glands: two basophilic ones and an eosinophilic one. The prepharyngeal cavity is lined with a membranous, anucleated epithelium, which forms a ring of pseudociliation halfway the cavity. It is surrounded by an external longitudinal and an internal circular muscle layer. The circular layer forms one sphincter at the level of the ring of pseudociliation and another one around the mouth. It is absent in the most proximal third of the cavity.

The gonads are paired. The testes are situated between the second and the last third of the body. The ovaries are ovoid with the oocytes in a row. They are situated in the caudal body end. The vitellaria are dorsally and extend at both sides of the body. The common genital pore is at ± 80% and can be closed by a sphincter. The short common genital atrium is lined with a high epithelium and surrounded by longitudinal muscles.

The long male atrium is narrow and lined with a high, nucleated epithelium. It is surrounded by a weak longitudinal muscle layer. It leaves the common atrium dorsally and bends caudally in about its half. Proximally it widens to a broad space in which a prostate stylet type III is situated. This space is lined with a low nucleated epithelium and surrounded by circular muscles. The stylet is a very complex, plate-like structure. This plate is lengthways folded, with the two halves lying beside each other. One of the halves is 34-38 µm long (m = 36; n = 3) and 28 µm broad (difficult to measure in some individuals); the other half is 21-29 µm long (m = 26; n = 3) and 12 – 15 µm broad (m = 13; n = 3). In between both plates there is a ± 20 µm long arm, which in some specimens is distally double-folded. Its distal rim is provided of large teeth. The stylet is associated with a prostate vesicle type III, which contains a coarse-grained basophilic secretion. It is surrounded by a circular muscle layer. In addition to the prostate vesicle, there are two accessory glandular organs. One consists of some diffuse eosinophilic glands that enter the male atrium dorsally near to the stylet. The second (accessory glands type V, terminology discussed in the "Discussion" on *Alchoides*; see further) produces a fine-grained basophilic secretion and opens in the distal part of the male atrium. The paired seminal vesicles are surrounded by a spirally running, almost circular muscle coat. The vasa deferentia join each other ventrally from the prostate vesicle to form the ejaculatory duct. The ejaculatory duct is surrounded by circular muscles and enters the male atrium next to the glandular vesicle.

The female duct type I is rather short and lined with a low nucleated epithelium. It is surrounded by a very weak circular muscle layer, which forms a weak sphincter distally. The vitelloducts enter the oviducts dorsally. Female glands as in *Alchoides dittmanni* (see further) could not be seen, probably because of the poor quality of the sections.

The uterus is of the normal polycystidid type.

Alchoides dittmanni n. sp.

(Figs 1B, D, F, H; 5B)

DISTRIBUTION: Broken Head (New South Wales), coarse sand between rocks, south of the abutment pier (27/10/97) (type locality).

MATERIAL: Several specimens studied alive. Four whole mounts (one designated holotype, the others paratype). Two animals sagitally sectioned (paratypes).

ETYMOLOGY: Species dedicated to Dr. Sabine Dittmann (Wilhelmshaven, Germany).

DESCRIPTION: The animals are 0,5-0,8 mm long (measured on whole mounts), colourless and have two eyes. The epidermis is syncytial and contains lobate nuclei. It is 1 μm thick with cilia of 2 μm long. The basal membrane is 1 μm thick. Rhabdites are scarcely distributed over the whole body surface and are about half the epithelium height long.

The proboscis is ± 15% of the total body length long. The epithelium of the proboscis sheath contains four nuclei. There are no nuclei in the contact zone between cone and sheath epithelium. There is one pair of ventral integument retractors. The number of proboscis retractors could not be determined with certainty, but there are probably four pairs.

The construction of the pharynx is identical to this of *Alchoides alchoides*. It is however somewhat smaller, with a diameter of only 1/15 of the body length.

The construction of the genital system resembles this of *Alchoides alchoides*. The main differences with this species are found in the shape of the stylet. It also is a double-folded plate, but it is less complex. It lacks the toothed arm, and distally ends in two blunt points. In total it is 32-36 μm long ($m = 34$; $n = 3$) and 9-11 μm broad ($m = 10$; $n = 3$). At each side, the plate has two small linguiform projections that are orientated towards each other. The accessory glands type V are much larger than in *A. alchoides* and very obvious even in live animals. A proximal bundle of accessory glands as in *A. alchoides* is lacking. The paired seminal vesicles, easily distinguishable in the live animals, could not be seen in the sectioned material. As a consequence, the exact location where the ejaculatory duct enters the male atrium could not be localised. It is probably situated at the distal tip of the stylet (observations on live material).

The female system is identical to that of *A. alchoides*, except for the presence of a large bundle of accessory glands, which opens into the female system at the bifurcation of the female duct.

DISCUSSION: The presence of only a prostate vesicle type III associated with a prostate stylet type III, without any other glandular organ and stylet in the male system, is rather uncommon within the Polycystididae. Apart from the species of *Alchoides* it is only found in *Alcha evelinae* Marcus, 1949, *Rogneda minuta* Uljanin, 1870 and *Sabulirhynchus axi* Artois & Schockaert, 2000. The species of *Alchoides* clearly differ from these other taxa, in that they have a large bundle of glands opening in the distal part of the male atrium, a feature unique within the Polycystididae. Following the same terminology as Artois & Schockaert (2003) we will call this glandular bundle of glands the accessory vesicle type V. Moreover, in both species of *Alchoides*, the prostate vesicle type III is surrounded by a weak but clearly visible circular muscle layer, a feature only found in one other Polycystidid taxon: *Galapagorhynchus* Artois & Schockaert, 1999, which is clearly not related. An important difference between *Alcha* and the three other taxa mentioned is the presence of insemination ducts (terminology of Artois & Schockaert, in press) in this taxon, which are clearly lacking in the species of *Alchoides*.

The organisation of the female system is extremely simple in the two species of *Alchoides* and is identical to the situation in many other polycystidid taxa e.g. *Rogneda* Uljanin, 1870 and *Ametochus* nov. gen (see further). Whether terminal female glands are really lacking in *A. alchoides* is doubtfull, as it is also possible that we did not find them because of the rather poor quality of the sections. Their presence/absence in this species should be checked on new material.

Both species of *Alchoides* can easily be identified by the construction and dimensions of the stylet. Moreover, the bundle of glands which enters the proximal part of the male atrium in *Alchoides alchoides*, is absent in *A. dittmanni*. Because this bundle is not found in any of the other Polycystididae it is probably an autapomorphy of *A. alchoides*.

***Ametochus gehrkei* n. gen. n. sp.**

(Figs 2; 5C-D)

DISTRIBUTION: North Stradbroke Island (Queensland), Adams Beach, coarse sand from a eulittoral sand flat with crab-holes (16/09/96) (type locality); same locality, Dunwich, sand flats with coarse or fine crab-holes in mid-eulittoral, in front of the marine station (12/08/96, 13/08/1996 & 20/08/1996). Sydney (New South Wales), Vaucluse Bay & Rose Bay, beach with fine sand and crab-holes, mid-eulittoral (10/10/97).

MATERIAL: Several specimens studied alive. Thirteen whole mounts, ten of which are from the type locality (one designated holotype). Several animals serially sectioned.

ETYMOLOGY: Ametochos (Gr.): neutral, as the genus has no real eye-catching features. The species is dedicated to John Gehrke, the janitor of the marine station on Stradbroke Island.

DESCRIPTION: The animals are colourless to pale yellow, 1 mm long (measured on whole mounts), with two eyes. The epidermis is syncytial. It is 4 µm thick with cilia 2 µm long. The basal membrane is ± 1 µm thick. The rhabdites are less than half the epithelium height long, and are absent at the level of the proboscis. Caudal glands are well developed.

The proboscis is small, about 1/8 of the body length, and is situated in the first body half. The epithelium of the proboscis sheath is high, without nuclei. Nuclei are also lacking at the junction of the sheath and cone epithelia. The sheath is surrounded by an inner circular and an outer longitudinal muscle coat. There are four pairs of proboscis retractors and a single pair of ventral integument retractors.

The pharynx is situated in the first body half and slightly inclined forwards. Four teeth are present around the proximal pharyngeal opening. The prepharyngeal cavity is lined with a low anucleated epithelium. About in the middle of the prepharyngeal cavity, the epithelium forms a ring of pseudociliation. The cavity is surrounded by an internal circular and an external longitudinal muscle layer. The pharyngeal lumen has a relatively high epithelium, which contains four nuclei arranged in pairs at different heights. There are 24 internal longitudinal muscles. There are three types of pharyngeal glands, which open in the distal part of the pharyngeal lumen.

The gonads are paired. The testes are small and lie at both sides of the body a bit behind the pharynx. The ovoid ovaries are situated caudally. The long vitellaria extend dorsally at both sides of the body. The common genital pore lies at 75% of the body length and can be closed by a sphincter. The common genital atrium is lined with a high epithelium, containing a few nuclei. The atrium is surrounded by external circular and internal longitudinal muscles. Both layers continue along the male genital atrium, which is lined with a pseudociliated epithelium.

The prostate stylet type III is situated in the proximal part of the male atrium. It consists of a ring, which is 26-40 µm long (m = 33 µm, n = 12) and 8-17 µm wide (m = 10 µm, n = 12). Part of this ring carries a large and thin plate, which is folded at one side. The edge of the plate that is most near to the fold is clearly toothed; the proximal edge is thin and smooth. The fold itself is thickened. Also the opposite edge is much thicker and ends in a 57-68 µm long serrate spine (m = 59; n = 12). At about 1/3 of its length the spine makes a 90° twist. Where the plate attaches to the ring, it shows a hole with sturdy edges ("window", arrow in Fig. 2B), which is about 9-14 µm (m = 12; n = 10) diameter. The plate is about 9-19 µm high (m = 14; n = 10) and 33-41 µm broad (m = 38; n = 10) at one side of the fold, 6-11 µm broad (m = 8; n = 10) at the other. A prostate vesicle type III enters the male atrium proximally. The glands bulge out deeply into the male atrium through the proximal ring of the stylet. Two sorts of secretion are present: a coarse-grained eosinophilic one and a fine-grained basophilic one. Only the distal part of the vesicle is surrounded by a layer of circular muscles. These muscles are continuous with the circular muscles of the male atrium. The elongated and fusiform seminal vesicles are situated ventrally from the glandular vesicle. Distally they fuse to form the ejaculatory duct. It was not possible to determine the exact place where the ejaculatory duct opens into the male system, but it is probably situated near the proximal ring of the stylet. More distally in the male atrium there is an accessory stylet type III. This accessory stylet is a hollow spine of 34-55 µm long (m = 44 µm, n = 12) with a hook-shaped distal end. It is associated with some diffuse accessory glands, which produce a basophilic secretion. (accessory vesicle type III).

The female duct type I enters the common atrium caudally. It is lined with a low, anucleated epithelium and surrounded by a strong circular muscle layer. The oviducts are swollen, filled with sperm, and distally surrounded by circular muscles. The ventral wall of each oviduct has a large bulge, which is filled with sperm (seminal receptacle). The vitelloducts enter the oviduct dorsally. At the bifurcation of the female duct into the oviducts, there is a large bundle of glands.

The uterus is of the normal polycystidid type.

DISCUSSION: A prostate vesicle type III in close association with a prostate stylet type III is a rather common combination within the Polycystididae, being found in a wide variety of taxa. However, the occurrence of both these structures together with an accessory stylet type III is almost unique within the Polycystididae, and is only found in *Triaustrorhynchus armatus* nov. gen. nov. sp. (described in this paper, see below). *T. armatus* differs however clearly from *A. gehrkei* in that it also has a prostate stylet type II connected to a prostate vesicle type II, which is lacking in *A. gehrkei*. The occurrence of an accessory stylet type III is rather uncommon within the Polycystididae anyhow. It is only found in *Porrocystis* Reisinger, 1924 and *Austrorhynchus* Karling, 1952, where it occurs in a combination with a prostate stylet type II attached to a prostate vesicle type II. In none of these species it is combined with an accessory vesicle type III, as is the case in *Ametochus*. The unique combination of all features mentioned marks *Ametochus* as a new genus.

The female system is very much like this of *Alchoides*. The seminal receptacles that are connected to the oviducts are, however, almost unique within the Polycystididae. A comparable situation is only found in *Paulodora subcontorta* (Schockaert, 1982) Artois & Schockaert, 1998 and some yet undescribed related species of *Paulodora*. This is however most probably a paralellism, as both genera do not share any other typical characteristics.

Arrawarria inexpectata n. gen. n. sp.

(Figs 3; 5E)

Arrawarra n. gen. in Littlewood et al. (1999)
Arrawaria in Joffe & Kornakova (2001)
Arrawaria sp. in Littlewood & Olson (2001)

DISTRIBUTION: Arrawarra (New South Wales), small shell-shaped brown algae from a tide pool in the mid-eulittoral (28/08/1996) (type locality); same locality, various algae from several tide pools near the marine station (27 & 28/08/1996; 01/11/1997). Sydney (New South Wales, Australia), various algae from Bondi Beach (06/10/1996).

MATERIAL: Several specimens studied alive. Ten whole mounts, one of them designated holotype, two others paratype. A total of ten animals serially sectioned.

ETYMOLOGY: Genus name derived from the type locality (Arrawarra). The species epithet refers to the unexpected combination of an armed cirrus with a prostate vesicle type II connected to a prostate stylet type II.

DESCRIPTION: Colourless animals of about 0,8 – 1,2 mm long (measured on whole mounts), with two eyes.

The epidermis is syncytial, ± 4 µm thick, with cilia of 3 µm long and a thick basal membrane. Rhabdites are few, spindle-shaped and about half the epithelium height long. Caudal glands are well developed.

The proboscis is about 1/5 of the body length long. The prepharyngeal cavity is lined with a high, nucleated epithelium and is surrounded by an inner circular and an outer longitudinal muscle layer. The circular layer is missing in the distal 1/3 of the cavity. There are no nuclei at the contact zone between sheath and cone epithelia. There are six bundles of fixators, one ventral pair of integument retractors and four pairs of proboscis retractors.

The pharynx is in the first body half and inclined forwards. The prepharyngeal cavity is lined with a very low, anucleated epithelium, which forms a ring of pseudociliation in the middle of the cavity. The cavity is surrounded by an inner circular and an outer longitudinal muscle layer. The circular layer forms a sphincter at the mouth and at the ring of pseudociliation, but is lacking in the proximal third of the cavity. There are four hard teeth around the proximal pharyngeal opening. The number of internal longitudinal muscles in the pharynx bulb amounts to 24. There are three kinds of pharyngeal glands that open into the distal part of the pharyngeal lumen.

The gonads are paired. The testes are rather small and situated behind the pharynx at both sides of the body. The ovoid ovaries are situated caudally, just behind the gonopore. The vitellaria extend dorsally at both sides of the body. The gonopore is at about 70%. The common genital atrium is small and lined with pseudociliation. It is surrounded by an inner circular and an outer longitudinal muscle layer. The circular layer forms a thick sphincter around the gonopore.

The male atrium enters the common atrium dorsally. It is very long and bends caudally. The distal 1/4 of the male atrium is lined with a high, nucleated epithelium and surrounded by a hardly visible inner circular and an outer longitudinal muscle layer. The rest of the male atrium is lined with a pseudocuticula, forming small spines (armed cirrus). The inner muscle layer surrounding this part is very thick. Proximally a prostate vesicle of type II enters the male atrium and discharges its secretion through a prostate stylet type II. The prostate vesicle consists of about four gland necks, with a coarse-grained basophilic secretion. It is surrounded by a very thick circular muscle layer, which distally becomes more spiral, even longitudinal. The outer fibres are continuous with the muscles surrounding the male atrium; the inner fibres attach to the proximal rim of the stylet. The prostate stylet type II is double-walled, slightly curved and tapers towards its distal tip. It is 50-54 µm long (m = 53; n = 9) and 24-29 µm broad proximally (m = 26; n = 9). The proximal rim of the outer stylet is thickened. The inner stylet is in the distal 2/3 of the stylet. Both vasa deferentia are very swollen and have a glandular epithelium (false seminal vesicles). They join each other ventrally from the prostate vesicle type II. The seminal duct is also very swollen (seminal vesicle) and lined by a high, glandular epithelium. It enters the male atrium proximally, near the base of the stylet. Dorsally from the prostate vesicle type II, there is a prostate vesicle type III, which only consists of eosinophilic glands and is not surrounded by muscles. It enters the male atrium dorsally from, and very near to, the prostate vesicle type II.

The female duct type I enters the common genital atrium caudally. It is lined with a high, anucleated epithelium. It is very short and almost immediately splits into the two oviducts. These oviducts are swollen and filled with sperm, functioning as seminal receptacles. The oviducts and the common female duct are surrounded by circular muscles only. At the junction of the oviducts there is a large bundle of basophilic glands. The vitelloducts enter the oviducts dorsally.

The uterus is of the normal polycystidid construction. It leaves the common genital atrium out of its frontal wall.

DISCUSSION: The male system of *A. inexpectata* is somewhat resembling this of species of the taxon *Acrorhynchides* Strand, 1928, especially this of *A. robustus* (Karling, 1931) Strand, 1928. All species of *Acrorhynchides* and *A. inexpectata* have a prostate vesicle type III entering an armed cirrus, all show a combination of two false seminal vesicles and a single “real” seminal vesicle and in all the false seminal vesicles have a high and glandular epithelium. Only in *Acrorhynchides robustus* and *Arrarwaria inexpectata* the seminal vesicle proper also has a high and glandular epithelium. *A. inexpectata* differs from the species of *Acrorhynchides* in that a prostate vesicle type II connected to a prostate stylet type II is also present. The combination of such a vesicle and stylet with a cirrus is unique within the Polycystididae, and warrants the placement of *Arrawarria* in a genus of its own.

The female system is rather simple, and is similar to that of many other Polycystididae. The ovaries are ovoid, which also distinguishes *A. inexpectata* from the species of *Acrorhynchides*, which have globular ovaries.

Austrorhynchus hawaiiensis **Karling, 1977**
(Figs 4; 5F)

Austrorhynchus pectatus in Karling et al., 1972

DISTRIBUTION IN AUSTRALIA: North Stradbroke Island (Queensland), Dunwich, in an exposed sea grass field in front of the marine station (12 & 13/08/1996); same locality, Amity Point, in a sea grass field with some mud in the tidal zone (13/08/1996).

FURTHER DISTRIBUTION: Hawaii, Oahu, (Karling et al., 1972). Zanzibar Island (Tanzania), Mbweni, beach behind the Mbweni Ruins Hotel, north of the creek, in a little pool with sea grass (*Thalassia* spec?) (11/08/1995); same locality, from a beach situated a bit higher, relative coarse sand with pebbles and silt from between sea grass (*Halodule*), rich in detritus (11/08/1995); same locality, in a tide pool with broad-leafed sea grass (17/08/1995).

MATERIAL. Two whole mounts and one sectioned specimen from Hawaii (SMNH). Several animals studied alive in Zanzibar and Australia. Three whole mounts from Zanzibar (coll. LUC). Three whole mounts and three serially sectioned specimens from Australia (coll. LUC).

ADDITIONAL INFORMATION. As to general shape of the hard parts of the male system, the specimens from Zanzibar, Australia and Hawaii are indistinguishable from each other. The prostate stylet type III is very much like this of *Austrorhynchus bruneti* Karling, 1977, with a pronounced style and foot connected to each other by a narrow clasp (indicated by an arrow in Figs 4 & 5F). The double-walled prostate stylet type II of the specimens from Zanzibar is 27-31 µm long (m = 29; n = 3). The prostate stylet type III is 62-68 µm long (m = 65; n = 3) with a plate of 24-28 µm broad and 6-7 µm high (n = 2). The Hawaiian and Australian populations seem to have larger hard parts. Only one specimen from Australia could be measured properly. It has a 41 µm long prostate stylet type II and a very long prostate stylet type III (106 µm). The plate of the latter stylet is 36 µm broad and 13 µm high. The prostate stylet type II of the Hawaiian population is 35-38 µm long. The prostate stylet type III of this population is 80 µm long (n = 2), with a plate of 30-33 µm broad and 7 µm high (n = 2).

***Cincturorhynchus monaculeus* n. sp.**

(Figs 6; 9A-B)

DISTRIBUTION: Arrawarra (New South Wales), large tide pool on the southern part of the beach at the beginning of a rocky area (27/08/1996 & 27/10/1997) (type locality); same locality, large algae from a tide pool (27/10/1997); same locality, Mullaway headland, on algae in deep rock pools (24/07/2003). North Stradbroke Island (Queensland), in sea grass from tide pools in the mid-eulittoral in front of the marine station (13/08/1996 & 20/10/1997).

MATERIAL: Several animals studied alive and mounted, one of them designated holotype. One sagitally sectioned animal from North Stradbroke Island.

ETYMOLOGY: The stylet has one spine only. Mono (Gr.): single; aculeus (Lat.): sting.

DESCRIPTION: Animals 1-1,5 mm long, yellowish under incident light, with two eyes.

The internal organisation is comparable to this of *Cincturorhynchus karlingi* Schockaert, 1982. The hook-shaped prostate stylet type II is a double-walled tube, with a very broad, annular proximal base of 31-46 µm (m = 40; n = 7) diameter. It is 42-57 µm long (m = 49; n = 7). A 36-46 µm long (m = 42; n = 7), hollow spine is attached to the proximal base of this stylet and runs more or less parallel to it, but it is less curved. The prostate stylet type III consists of a proximal semicircular to horseshoe-shaped ring, which carries long, curved spines. The diameter of the base is difficult to measure in the different specimens, as its size is largely dependent on the degree of compression. It fluctuates between 55 and 118 µm (m = 84; n = 7). The spines are implanted into two different groups, one consisting of long spines (± 34 µm), the other of shorter ones (± 16 µm).

The sectioned specimen lacks a female bursa, although it was clearly visible in live animals. Probably the sectioned animal has not reached full female maturity yet.

DISCUSSION: The new species can easily be placed within the taxon *Cincturorhynchus* Evdonin, 1970, as it shows all the diagnostic characters of this taxon: paired seminal vesicles, a small prostate vesicle type II connected to a prostate stylet type II, a prostate stylet type III consisting of a horshoe-shaped base carrying large teeth, a prostate vesicle type III asoociated with the prostate stylet type III and a terminal female bursa.

Apart from *C. monaculeus*, two other species are known of this taxon: *C. karlingi* Schockaert, 1982 from the African east coast (see Schockaert, 1982) and *C. ruber* from the Possjet Bay (Japanese Sea, Russia) (see Evonin, 1970, 1977). The three species can easily be distinguished based on differences in the construction of the prostate stylets. In *C. ruber*, the prostate stylet type II is a simple, double walled tube. In the two other species, the base of this tube is enlarged and carries a number of spines, one in *C. monaculeus*, four to seven in *C.*

karlingi. The single spine present on the prostate stylet type II of *C. monaculeus* is also much larger than each of the spines in *C. karlingi*. The prostate stylet type III in all three species consists of a horse shoe-shaped base carrying a number spines. Differences between the species can be found in the number and form of the spines. Judging from the drawings and the description of *C. ruber* by Evdonin (1970), it carries a limited number of relatively small spines. In *C. karlingi* and *C. monaculeus*, the spines are more numeruous and larger. In *C. monaculeus* they are longer and more slender than in *C. karlingi*. In *C. karlingi* they have more triangular, having the form of broad blades.

Duplexostylus winsori **n. gen. n. sp.**

(Figs 7C-E; 9C)

DISTRIBUTION: Hinchinbrook Channel (Townsville area, Queensland), sand from a tidal flat (20/11/1988 & October 1991) (type locality).

MATERIAL: Drawings of live animals by Dr. Dittmann. Three whole mounts, one of them designated holotype, the other two paratype. One sagitally sectioned animal.

ETYMOLOGY: The genus name refers to the fact that the species has a copulatory organ resembling a duplex type (terminology of Karling, 1956), combined with a stylet. The species epithet is in honour of Mr. Leigh Winsor (Townsville, Australia).

DESCRIPTION: The animals are 0,6-0,8 mm long (measured on the whole mounts), colourless and without eyes. The epidermis is syncytial, ± 5 µm high with cilia 3 µm long. The basal membrane is ± 1/4 of the epithelium height thick. The presence of rhabdites could not be determined with certainty. Caudal glands are well developed.

The proboscis is about 20 % of the body length long. The proboscis sheath is lined with a nucleated epithelium and is surrounded by an inner circular and an outer longitudinal muscle layer. There are no nuclei at the junction of sheath and cone epithelia. The organisation of the retractor system could not be determined. There are six bundles of fixators.

The pharynx is in the first body half and slightly inclined forwards. The distal part of the prepharyngeal cavity is lined with a pseudociliation, the remaining part by a membranous epithelium. It is surrounded by an inner circular and an outer longitudinal muscle layer. The circular layer is lacking in the proximal 1/3 of the cavity. The epithelium of the pharyngeal lumen lacks nuclei. Three types of pharyngeal glands enter the distal part of the pharyngeal lumen, two eosinophilic ones with a basophilic one in between. The proximal pharynx opening is surrounded by four hard teeth.

The gonads are unpaired. The small testis lies just behind the pharynx at the right hand side of the body. The ovary lies caudally from the gonopore. It is ovoid and slightly curved, with the oocytes arranged in row. The vitellarium extends dorsally at the right hand side of the body. The gonopore is at 80 % and can be closed by a strong sphincter. The common genital atrium is lined with a high, nucleated epithelium and surrounded by longitudinal muscles.

The sperm conducting system consists of a single seminal vesicle, a prostate vesicle type IV and a prostate stylet type III. The seminal vesicle is lined with a membranous epithelium and surrounded by a circular muscle layer. It narrows somewhat towards the prostate vesicle and enters it proximally (conjuncta-type copulatory organ). Within the prostate vesicle it is no longer visible, except for a small part in the distal end of the vesicle. The prostate vesicle is very large and globular. It is surrounded by a circular muscle layer, distally supplemented with thicker longitudinal muscles, which connect the vesicle with the proximal part of the male atrium (protractors of the vesicle). It contains a coarse-grained and a fine-grained basophilic secretion. The nucleated parts of the interposed prostate glands are outside the vesicle. The prostate vesicle is connected to the complex prostate stylet type III that consists of a plate, which proximally is folded in the shape of a funnel and distally continues as a gutter-like to tubular spine. The transition from funnel to spine is rather abrupt, and the proximal part of the curved spine runs perpendicularly to the funnel. At this transition, a complex fold of the funnel forms a second very broad spine, which curves in the opposite direction of the first spine. The stylet (funnel+first spine) is 146-183 µm long (m = 162; n = 3) and 71-86 µm broad proximally (m = 77; n = 3). The second spine is 46-76 µm long (m = 57; n = 3). The male atrium leaves the common atrium dorsally. It is lined with a pseudocuticula and surrounded by circular muscles. A large muscular septum encloses the larger part of the male atrium asymmetrically, only dorsally (copulatory organ of the duplex-type; terminology of Karling, 1956). It does not enclose the prostate vesicle.

The female system is very simple. The female duct type I leaves the common genital atrium caudally. It is lined with a high epithelium and surrounded by circular muscles. It broadens towards the ovarium. A large bundle of eosinophilic glands enters through the ventral wall of this broader space, the vitelloduct through its dorsal wall.

Duplexostylus rowei **n. sp.**

(Figs 7A-B; 9C)

DISTRIBUTION: Townsville (Queensland), muddy sand with much detritus from a sea grass field along “The Strand” opposite Oxley Street (25/08/1996) (type locality).

MATERIAL: One animal studied alive and mounted (holotype).

ETYMOLOGY: Dedicated to Prof. Dr. Richard Rowe (Townsville, Australia).

DESCRIPTION. This species is almost identical to *D. winsori*. As far as could be seen on the live specimen, the only difference with that species is the form of the second spine of the prostate stylet. This spine is much narrower and more elegant compared to that of the former species. The stylet is a bit smaller: 126 µm long and 52 µm broad proximally. The second spine is 47 µm long.

DISCUSSION: Unpaired gonads are only found in a restricted number of taxa within the Polycystididae and only a few of them have a prostate vesicle type IV: *Annulorhynchus adriaticus* Karling, 1956, species of *Danorhynchus* Karling, 1955 (only ovary unpaired), species of *Gallorhynchus* Schockaert, 1971, *Koinocystella inermis* Karling, 1952, *Neopolycystis tridentata* Karling, 1955 (only ovary unpaired), the species of *Psammopolycystis* Meixner, 1938, the species of *Scanorhynchus* Karling, 1955, *Stradorhynchus* nov. gen (this contribution) and *Syltorhynchus schockaerti* Noldt, 1989 (only ovary unpaired). Most of these also have a prostate stylet type III, only in *Koinocystella inermis Gallorhynchus simplex* Schockaert & Brunet, 1971 and *Stradorhynchus* it is lacking. In none of these species, however, the prostate stylet type III is directly connected to the prostate vesicle type IV, as is the case in *Duplexostylus* (see Artois & Schockaert, 2003).

Another typical feature of *Duplexostylus* is the asymetrical septum which surrounds the dorsal side of the male atrium, which indicates that the male system is of the conjuncta-duplex type. The presence of such a septum is rare in polycystidids, it only occurs in the four species of *Duplacrorhynchus* and in *Yaquinaia microrhynchus* Schockaert & Karling, 1970 (see Artois & Schockaert, 2003). In these taxa, the septum is placed symetrically around the male atrium and also encloses at least a part of the prostate vesicle (exc. in *Duplacrorhynchus heyleni* Artois & Schockaert, 1999).

The combination of unpaired gonads, the presence of a prostate vesicle type IV directly conencted to a prostate stylet type III, and the presence of a asymmetrical septum are unique within the Polycystididae, and clearly warrants the erection of a new genus. The relationships with the other taxa of Polycystididae are unclear.

***Paraustrorhynchus caligatus* n. sp.**

(Figs 8; 9E-F)

DISTRIBUTION: North Stradbroke Island (Queensland), in a sea grass field in the tidal zone (13/08/1996) (type locality); same locality, Adams Beach, in sea grasses (Zostera) from a tide pool (16/09/1996).

MATERIAL: Observations on two living animals, one from each locality, which were afterwards mounted (one designated holotype).

ETYMOLOGY: The prostate stylet type II is boot-shaped. Caliga (Lat.): boot.

DESCRIPTION: Animals about 1 mm long, yellowish to dark green pigmented. They have two eyes. The internal organisation corresponds to this of *Paraustrorhynchus pacificus* Karling & Schockaert, 1977, as far as it could be seen in the living specimens.

The male atrium is proximally very broad, and contains the prostate stylet type II and a plate-like prostate stylet type III. The prostate stylet type II is a double-walled tube, 40-58 µm long (n = 2). Its distal end makes a 45° turn, giving the stylet the overall appearance of a boot. It is connected to a prostate vesicle type II. The internal stylet can be followed almost throughout the whole length of the external one. The complex prostate stylet type III consists of a proximal plate, which serves as a base from which two arms depart. These arms protrude distally and both broaden towards their distal end. This prostate stylet could only be measured on one specimen. One of the arms is 23 µm long and 19 µm broad at its distal rim, which is completely serrated. At the base of this arm there is a triangular-shaped fold. The other arm is 37 µm long and 33 µm broad at its distal end. The distal end of this plate is only partly serrate. The distal ends of both arms are at the same level. The prostate stylet type III is associated with a prostate vesicle type III. The ejaculatory duct enters the male atrium close to this organ. More distally the male atrium is filled with many sperm.

DISCUSSION: The taxon *Paraustrorhynchus* Karling & Schockaert, 1977 is characterised by a uinique combination of features (see Karling & Schockaert, 1977): paired gonads, a large male atrium without a real male bursa, with a prostate vesicle type II connected to a prostate stylet type II, with a complex prostate stylet type III associated with a prostate vesicle type III, the ejaculatory duct ending in the male atrium very near to the prostate stylet type III, with an accessory glanduclar vesicle of type I, proximal part of male atrium with a large muscle bulb, with a female duct type I with terminal female glands but without female bursa (exc. *P. elixus* (Marcus, 1954) Karling & Schockaert, 1977 which has a female bursa) and without insemination ducts. All these characters are present in *P. caligatus*. Only the absence of the insemination ducts should be checked on sectioned material.

The hard parts of the male system of *P. caligatus* are very much like these of *P. pacificus* Karling & Schockaert, 1977, but some clear and consistent differences can be found between the two species. The prostate stylet type II of both is a rather simple tube, evenly curved in P. pacificus, with the typical boot shape in *P. caligatus*. Moreover, this stylet is much longer in *P. pacificus* than in *P. caligatus* (± 49 µm). The two arms of prostate stylet type III of P. caligatus has both arms broadening evenly towards their distal end, giving them the shape of a broad plate. One arm has a completly serrated distal rim, the other one has only the middle part of the distal rim serrated. In *P. pacificus*, both arms are much narower proximally, abruptly widening at their distal end. One of the arms has a completely serrated distal rim, while the other one is somewhat bifid distally, with a (very small) part serrated, and the other part not serrated. Moreover, the prostate stylet type III of *P. pacificus* is much larger (140 µm) than this of *P. caligatus* (37 µm).

Apart from the two species a third species of *Paraustrorhynchus* has been described from Brasil: *P. elixus* (Marcus, 1954) Karling & Schockaert, 1977. This species is however very different from the other two. It's hard parts have a totally different shape (prostate stylet type II a very simple small hook, prostate stylet type III with two articulating parts) and it has a female bursa. For a thorough discussion of this species and a comparison with *P. pacificus* we refer to Karling & Schockaert (1977).

Polycystis australis **n. sp.**

(Figs 10; 13A)

DISTRIBUTION: Arrawarra (New South Wales), brown algae from a tide pool in the mid-eulittoral (28/08/1996) (type locality). Coffs Harbour (New South Wales), Mullaway headland, brown and red algae from rock pools (24/07/2003).

MATERIAL: Several animals studied alive. Three whole mounts, one of which is designated holotype, another one paratype. One serially sectioned specimen (horizontally sectioned; designated paratype).

ETYMOLOGY: Australis (Lat.): southern.

DESCRIPTION: The animals are colourless, 1,5-2,2 mm long and have two eyes. Construction of epidermis, proboscis, pharynx and genital system as in *Polycystis naegelii* Kölliker, 1845.

The prostate stylet type I is a double-walled funnel, showing a constriction about in its middle and broadening again distally. It is 48-58 µm long and 30-53 µm broad proximally, 23-38 µm distally (n = 3). At the constriction it is 15-25 µm broad (n = 3). The distal rim of the outer stylet is partly toothed. The internal stylet is only visible in the proximal half of the outer stylet. The stylet is connected to a prostate vesicle type I, which contains two types of secretion and is surrounded by two spirally running muscle layers. The inner muscle layer attaches to the inner side of the outer stylet, the outer layer is continuous with the muscles of the male atrium. The bursal stalk leaves the male atrium just before this itself enters the common genital atrium. It is surrounded by a thick circular muscle layer and lined with a pseudocuticula. At the opening of the bursal stalk into the male atrium, the circular muscle layer is asymmetrically thickened. A real asymmetrical muscle bulb, as is found on the bursal stalk in *Polycystis naegelii* and *Polycystis ali* Schockaert, 1982 is however lacking.

At the bifurcation of the two oviducts, a very small muscular female bursa is present. It contains many sperm and nuclei.

DISCUSSION: This species undoubtedly belongs to the taxon *Polycystis* Kölliker, 1845 as diagnosed by Artois & Schockaert (1998). At the moment five species are recognised. For one of these species, *P. ali* Schockaert, 1982, Karling (1986) described three different forma's: 'Somali', 'California', and 'Galapagos'.

The species of Polycystis all have a large male bursa, ending in the distal part of the male atrium. In most species, the bursal stalk is distally provided of a very asymmetrical muscle bulb (see Artois & Schockaert, 1998). In *P. gabriellae* (Marcus, 1948) Karling, 1952 and in *P. australis*, this bulb is, however, lacking. The bursal stalk of both these species is also much longer and very muscular, with an asymmetric sphincter distally, at the place where in the other species the muscle bulb is present.

The prostate stylets of all species of *Polycystis* are rather short and broad, except for the stylet of *P. gabriellae*, which is somewhat longer and has a rather long distal tube. The stylet of *P. australis* is similar to this of *P. ali*, especially this of *P. ali* forma 'California. Typical of these two taxa is the the fact that the stylet is symmetrical, lacking the distal asymmetrical outgrow of *P. orientalis* Evdonin, 1968. It also lacks the distal hook typical of *P. hamata* Karling, 1986 and lacks the spur typical of *P. naegelii* Kölliker, 1845. *P. ali* forma 'Somali' and P. ali forma 'Galapagos' are more similar to this of *P. australis* and *P. ali* forma 'California', but differ in that the distal end is circular and broad, with the edges folded back, so that they form a sort of gutter that surrounds the stylet. The stylet of *P. australis* from this of *P. ali* forma 'California' in two important ways. It lacks the distal split, that is very prominent in *P. ali* forma 'California', and the distal rim of the stylet is not serrated over whole of its length, whereas in *P. ali* forma 'California' it is completely serrated with big and sturdy teeth.

Another typical feature of *P. australis* is the lack of any pigmentation. Within *Polycystis*, only *P. hamata* and some specimens of *P. ali* forma 'California' lack pigmentation. In the latter taxon unpigmented and weakly pigmented specimens are known (see Karling, 1986).

Stradorhynchus caecus **n. gen. n. sp.**

(Figs 11A-C; 13C)

DISTRIBUTION: North Stradbroke Island (Queensland): Myora, on a beach behind mangroves, near to a creek (14/08/96); same locality, Dunwich, in a sea grass-bed in the eulittoral (21/09/96). Coffs Harbour (New South Wales), Coffs creek, about 150 m west of the bridge, in fine-grained sand in between oysters (23/07/2003)

MATERIAL: Two specimens studied alive. One mounted (holotype) and one sagitally sectioned animal.

ETYMOLOGY: The genus name refers to Stradbroke Island, whereas the species epithet refers to the absence of eyes. Caecus (Lat.): blind.

DESCRIPTION: The animals are colourless, 0,5 mm long (measured on the mounted specimen) and have no eyes. The epidermis is syncytial, 4 µm high, with cilia 2 µm long. The basal membrane is ± 1 µm thick. The rhabdites, which are lacking around the proboscis pore, are less than half the epithelium height long. Caudal glands are well developed.

The proboscis is about 20% of the body length long. In the living animals a small apex was observed. The proboscis sheath is surrounded by internal circular and external longitudinal muscles, and is lined with an anucleated epithelium. The circular muscles are absent around the distal third of the cavity. There are no nuclei at the junction between sheath and cone epithelia. There are two pairs of integument retractors, one dorsal and one ventral pair. The exact number of proboscis retractors could not be determined with certainty, but there are probably three pairs of them. There are six bundles of fixators. A large glandular complex is situated near the brain, with the gland necks extending beside the proboscis bulb.

The pharynx is situated in the anterior body half and slightly inclined forwards. It is approximately 15% of the body length in diameter. Four teeth are present around the proximal pharyngeal opening. The prepharyngeal cavity is lined with a membranous, anucleated epithelium. About in the middle of the cavity, the epithelium forms a ring of pseudociliation. The cavity is surrounded by an internal circular and an external longitudinal muscle layer. The circular layer is absent around the most proximal third of the cavity. The pharyngeal lumen is lined with a low, anucleated epithelium. There are three types of pharyngeal glands, one basophilic one and two eosinophilic ones.

The gonads are unpaired. The testis is very long and lies at the right hand-side of the pharynx, from where it stretches out backwards. The ovoid ovary lies at the caudal body end. The vitellaria are paired and caudally connected to each other by means of a narrow "bridge" from which the single vitelloduct departs. The common genital pore lies at 80% of the body length and can be closed by a strong sphincter. The common atrium is lined with a low anucleated epithelium and surrounded by internal longitudinal and external circular muscles.

The male genital atrium leaves the common genital atrium dorsally. It is surrounded by a circular muscle layer, but is without a visible epithelium. The prostate stylet type IV fills the male atrium completely. It is 101 µm long and 26 µm wide proximally, 21 µm distally. It is ornamented with spirally running ridges over whole of its length. The distal tip of the stylet is very complex, showing several ridges and two flap-like projections. The stylet is connected to a long and pyriform prostate vesicle type IV, which contains only one type of eosinophilic secretion. The prostate secretion continues very far in the stylet, almost to its distal tip, which protrudes in the common genital atrium. The prostate vesicle is surrounded by circular muscles, which are continuous with the circular muscles surrounding the male atrium. The ejaculatory duct is surrounded by circular muscles and lined with a low, anucleated epithelium, as is the unpaired seminal vesicle. Distally, it enters the prostate vesicle through a muscular pore.

The female duct type I is lined with a low anucleated epithelium and surrounded by a thick external circular muscle layer and an internal longitudinal layer. It leaves the common genital atrium caudally. Proximally it widens to form an ovoid space that is lined with a very thick pseudocuticula. The narrow proximal part of the female duct leaves out of the frontal wall of this space. It is surrounded by a weak circular muscle layer. It almost immediately starts running dorsally. At the place where the it bends dorsally, the oviduct opens into it. This oviduct is surrounded by circular muscles and lined with a low anucleated epithelium. Its most proximal part is widened and contains sperm.

The uterus opens through the frontal wall of the common genital atrium and is of the normal polycystidid construction.

Stradorhynchus terminalis **n. gen. n.sp.**

(Figs 11D; 13D)

DISTRIBUTION: Coffs Harbour (New South Wales), Coffs creek, about 75 m west of the bridge, in fine-grained sand and algae (15 & 23/07/2003) (type locality).

MATERIAL. Several specimens studied alive. Two whole mounts, one of them designated holotype. Three serially sectioned specimens.

ETYMOLOGY. The species name refers to the fact that the spirally running ridges are restricted to the distal tip of the stylet.

DESCRIPTION. Habitus and internal organisation is identical to that of *S. caecus*, except the fact that *S. terminalis* has a somewhat reddish coloration owing to parenchymal pigment. The only difference with *S. caecus* is the morphology and dimensions of the prostate stylet type IV, which only could be measured in the holotype because of the poor quality of the other whole mounts. The stylet is 185 µm long and 40 µm wide proximally, 11 µm distally. It is ornamented with many spirally running ridges, which are are restricted to the distal third of the stylet, giving the stylet the resemblance of a “stripped-off sock”. The stylet tapers towards its complex distal tip.

DISCUSSION: The presence of only one testis and one ovary in combination with a prostate vesicle type IV connected to a prostate stylet type IV is unique within the Polycystididae. The most similar situation is found in *Koinocystella inermis* Karling, 1952, which has unpaired gonads and a prostate vesicle type IV. Moreover, both species of *Stradorhynchus* and *K. inermis* have a proximally widening of the oviduct, which functions as a seminal receptacle. Both taxa however clearly differ in two respects, the species of *Stradorhynchus* have a terminal female bursa and have a prostate stylet type IV. Both are lacking in *Koinocystella*, which has an unarmed cirrus.

Another similarity between *Koinocystella* and *Stradorhynchus* is the fact that the proboscis retractor system consists of three pairs of proboscis retractors and two pairs of integument retractors, whereas in most Polycystididae there are four pairs of proboscis retractors and one pair of (ventral) integument retractors (see Artois & Schockaert, 1999). This ‘3+2’ situation is also found in some other species which have a prostate vesicle type IV connected to aprostate stylet type IV: species of *Djeziraia* Schockaert, 1971, *Phonorhynchoides* Beklemishev 1927, *Yaquinaia*

Schockaert & Karling 1970 and *Annalisella* Karling 1978. All these species, however, have paired gonads.

The prostate stylet type IV of *Annalisella bermudensis* Karling, 1978 is remarkably similar to this of the species of *Stradorhynchus*, especially to this of *S. caecus*. In both species the stylet has spirally running ridges, 4-5 in *A. bermudensis*, ±10 in *S. caecus*. The distal tip of the stylet is hook-shaped in both species, but it is much more complex in *S. caecus*. In *S. terminalis*, the ridges are many and "packed together" at the distal tip of the stylet. *A. bermudensis* clearly differs from the species of *Stradorhynchus* as it has paired gonads, an accessory glandular vesicle type IV connected to an accessory stylet type IV and a separate male and female gonopore. Whether the similarities in shape of the stylet between *A. bermudensis* and both species of *Stradorhynchus* reflect a common origin is therefore doubtful. A very similar stylet is also found in *Astrotorhynchus bifidus* (McIntosh, 1874) Graff, 1905 (Trigonostomidae), a taxon which is clearly not related.

***Triaustrorhynchus armatus* n. gen. n. sp.**

(Figs 12; 13B)

DISTRIBUTION: Lady Bay (Sydney, New South Wales), north side of the beach, coarse shell gravel from between rocks (06/10/1996) (type locality).

MATERIAL: Observations on one living animal, which was mounted afterward (holotype).

ETYMOLOGY: The genus name reflects the similarity to *Austrorhynchus*, but indicates that the new taxon has three stylets. The epithet also refers to the presence of three stylets. Armatus (Lat.): armed.

DESCRIPTION: The only specimen available is 0,5 mm long (measured on the whole mount), and has two eyes. It is colourless. The proboscis is about 20% of the body length. The pharynx is in the first body half.

The gonads are paired. The testes lie at both sides of the body, just behind the pharynx. The ovoid ovaries are situated caudally. Vitellaria were not visible. The gonopore is at ± 75%.

There are three hard parts in the male atrium: a prostate stylet type II, a prostate stylet type III and an accessory stylet type III. The double-walled prostate stylet type II is 50 µm long. It is a simple tube distally, with the proximal rim of the outer stylet very slanting, with one side ending just proximally from the proximal rim of the inner stylet, and the other side extending much further proximally. It is connected to a glandular vesicle (prostate organ type II?). The prostate stylet type III can easily be described using the terminology of Karling (1977), as it is similar to the prostate stylet type III of some species of *Austrorhynchus* Karling, 1952. It is 91 µm long, with a proximal style and foot. The foot distally runs out in a toothed flagellum, which ends in a sharp point. The style is proximally split. This prostate stylet is associated with a large glandular organ (prostate organ type III?). Where it is connected to the wall of the male atrium, the muscles of the atrium form a thick bulb. The accessory stylet type III is found more distally in the male atrium. It is a hollow, curved tube, 27 µm long. It is associated with a small glandular organ. There are two seminal vesicles. The exact course of the ejaculatory duct could not be determined.

At the junction of both oviducts, there is a large bundle of glands.

DISCUSSION: Although sectioned material is lacking, and details of the construction of the anatomy thus cannot be given, the details seen on the live animals combined whith the whole mounts enable to describe this species. Because it is the first species of polycystidid with three hard parts in the male system, each of them associated with a glandular organ, we cannot but place it in a genus of its own.

The prostate stylet type II is a short, double walled tube, as in many other polycystidids, especially *Paraustrorhynchus pacificus*. Most similar is probably the prostate stylet type II of *P. pacificus*, as it also is an evenly curved hook, but it is much longer than in *T. armatus*. Many species of *Austrorhynchus* also have similar stylets, but in these species it typically has a proximal funnel-shaped part and and a tubular distal part, with a abrupt transition between both. Typical for *T. armatus* is the asymmetrical proximal end, which is not found in any other polycystidid with a prostate stylet type II.

The prostate stylet type III is also very similar to this of some *Austrorhynchus* species and to a lesse extent to this of the *Paraustrorhynchus* species. Moreover, both in *T. armatus*, the species of *Austrorhynchus* and the species of *Paraustrorhynchus* a large muscle bulb is associated with this stylet. In *T. armatus* a bundle of glands ends at the base of the prostate stylet type III, at the same place where in species of *Paraustrorhynchus* a prostate vesicle type III is present. Whether the glandular vesicle observed at this place in *T. armatus* is a prostate vesicle type III is most probable, but should be checked on sections. It could also be an accesory glandular vesicle of type I, which is also present in the species of *Paraustrorhynchus*, but is not associated with the prostate stylet type III. Both these vesicles are not present in the species *of Austrorhynchus*.

An accessory stylet of type III is very uncommon in polycystidids, and only occurs in *Porrocystis assimilis* (Levinsen, 1879) Karling, 1952 and species of *Antiboreorhynchus* Karling, 1952. Only in the species of *Antiboreorhynchus* it is associated with a compact bundle of accessory glands type III. These glands also occur in *P. assimilis*, but here they are more diffuse and occur over the whole length of the male atrium. The glands observed in *T. armatus* are therefore most probably comparable to these found in the species of *Antiboreorhynchus*, but again this should be checked on sections.

The female system of *T. armatus* is one of the most simple within the Polycystididae, and is similar to this of the species of *Paraustrorhynchus*. It lacks a female bursa, which is present in species of *Austrorhynchus*, *Antiboreorhynhus* and *Porrocystis*.

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We wish to thank Mrs. Natascha Steffanie for serial sectioning and staining of the animals and Dr. Sabine Dittmann for kindly providing additional material and drawings of several species, collected during her ecological research. Dr. Sven Boström is thanked for lending the material of *Austrorhynchus hawaiiensis*, present in the collection of the Swedish Museum of Natural History (Stockholm). Dr. Nikki Watson is greatly acknowledged for her help during the sampling in Australia. The first author is supported by a specialization grant of the Flemish Institute for the Benefit of Scientific and Technological Research in the Industry (IWT). The sampling expeditions were supported by grants of the Fund for Scientific Research-Flanders (Belgium).

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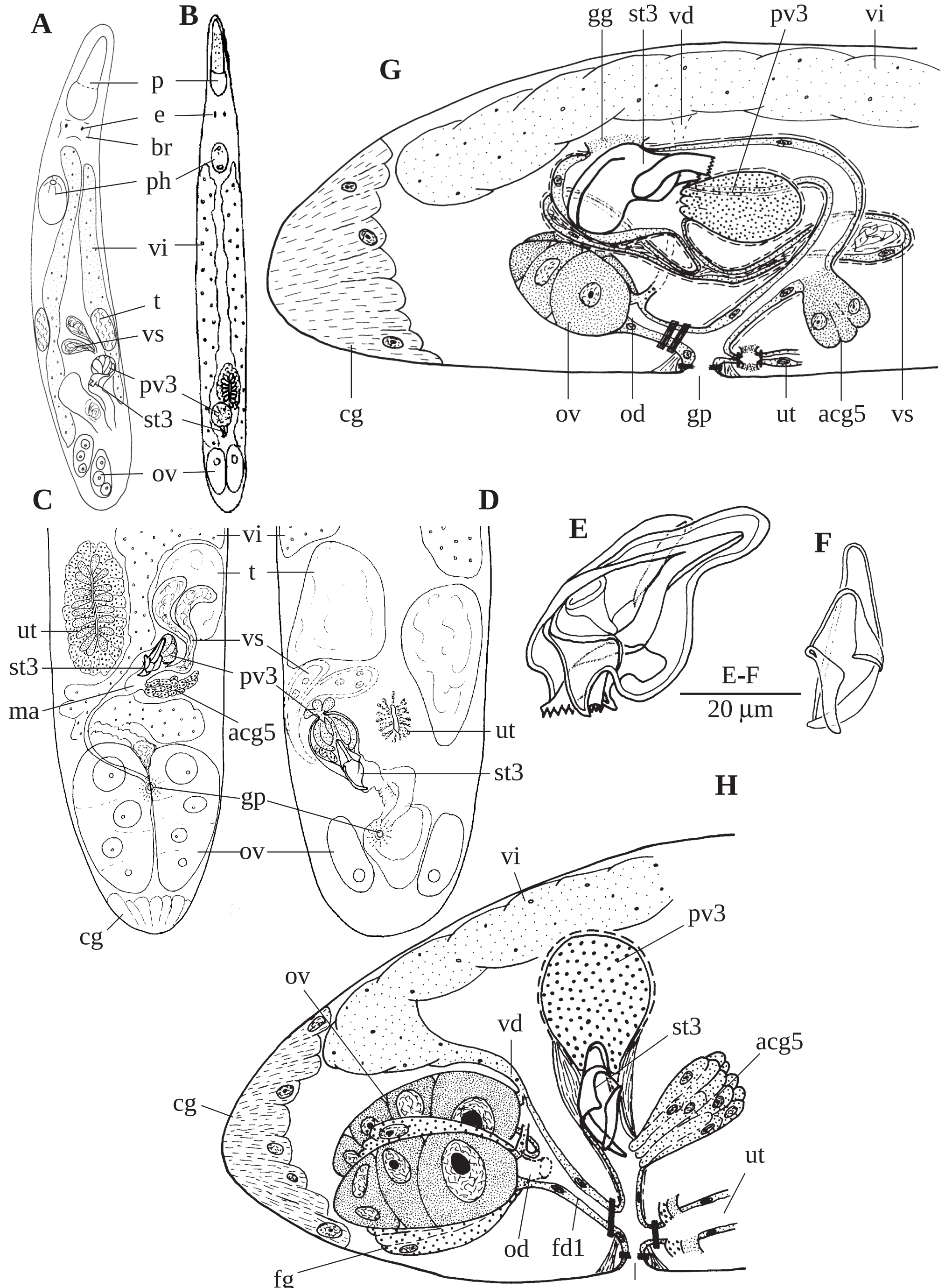

Fig. 1 *Alchoides alchoides:* A. Habitus of a live animal; C. Caudal body end with the atrial organs (from a live specimen); E. Prostate stylet type III (from the holotype); G. Reconstruction of the atrial organs from the right side.
Alchoides dittmanni: B. Habitus of a live animal; D. Caudal body end with the atrial organs (from a live specimen); F. Prostate stylet type III (from the holotype); H. Reconstruction of the atrial organs from the right side.

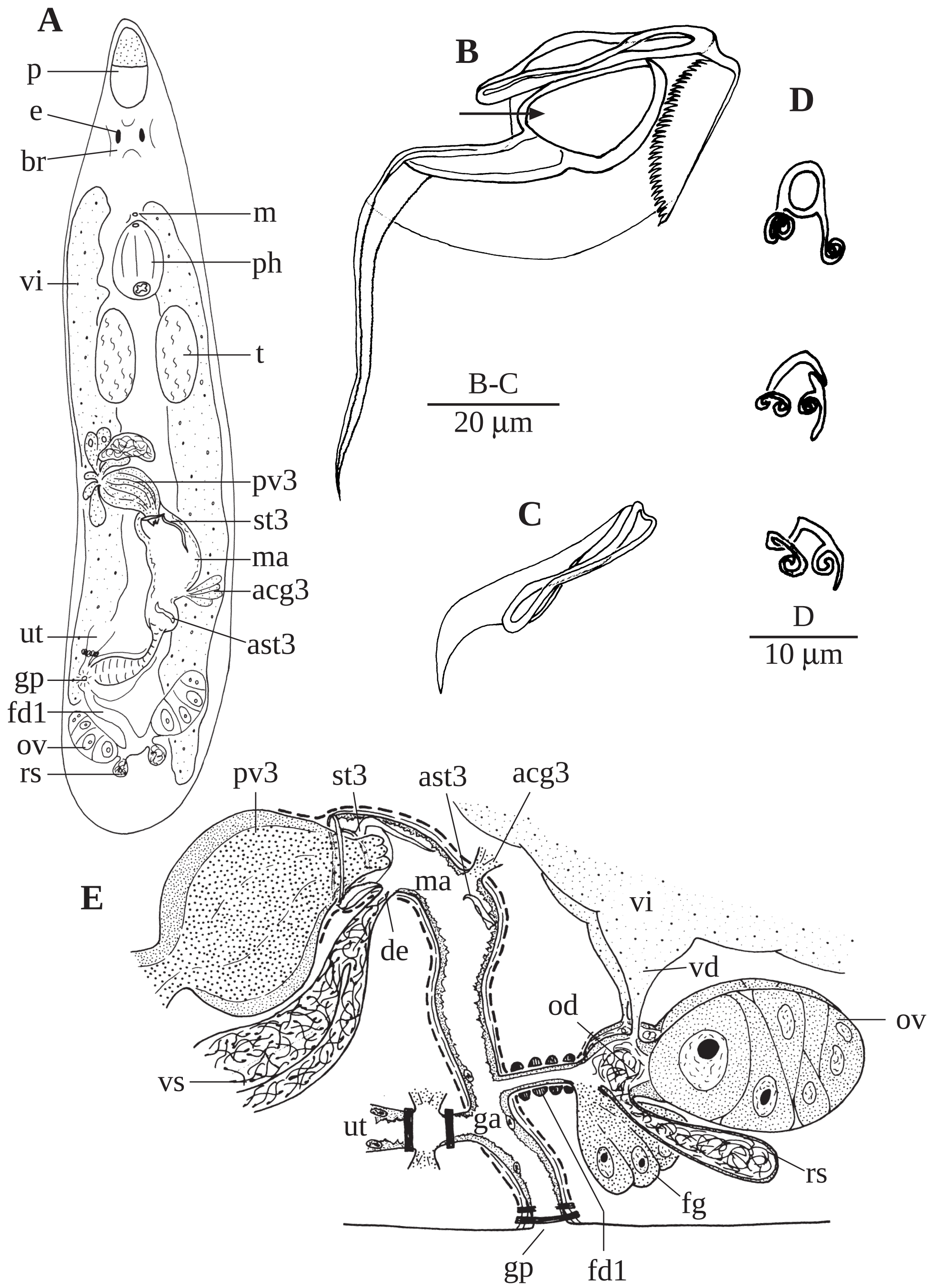

Fig. 2 *Ametochus gehrkei:* A. General organisation (from a live specimen); B. Prostate stylet type III (arrow indicates the 'window'; from the holotype); C. Accessory stylet type III (from the holotype); D. Three transverse sections through the accessory stylet type III; E. Reconstruction of the atrial organs from the left side.

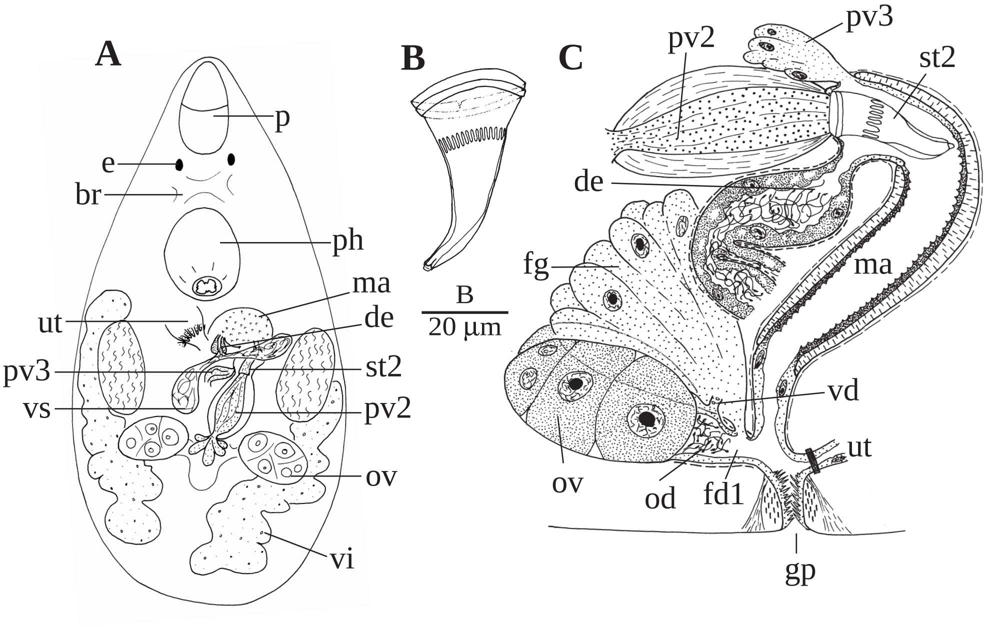

Fig. 3 *Arrawarria inexpectata:* A. General organisation (from a live specimen); B. Prostate stylet type II (from the holotype); C. Reconstruction of the atrial organs from the right side.

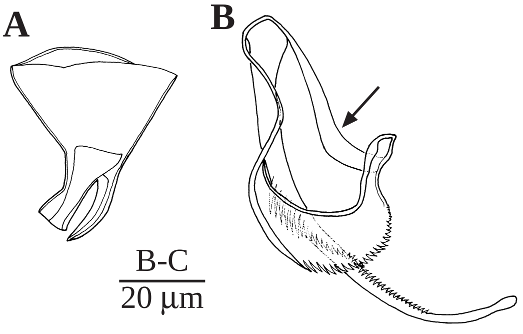

Fig. 4 *Austrorhynchus hawaiiensis:* A. Prostate stylet type II; B. Prostate stylet type III.

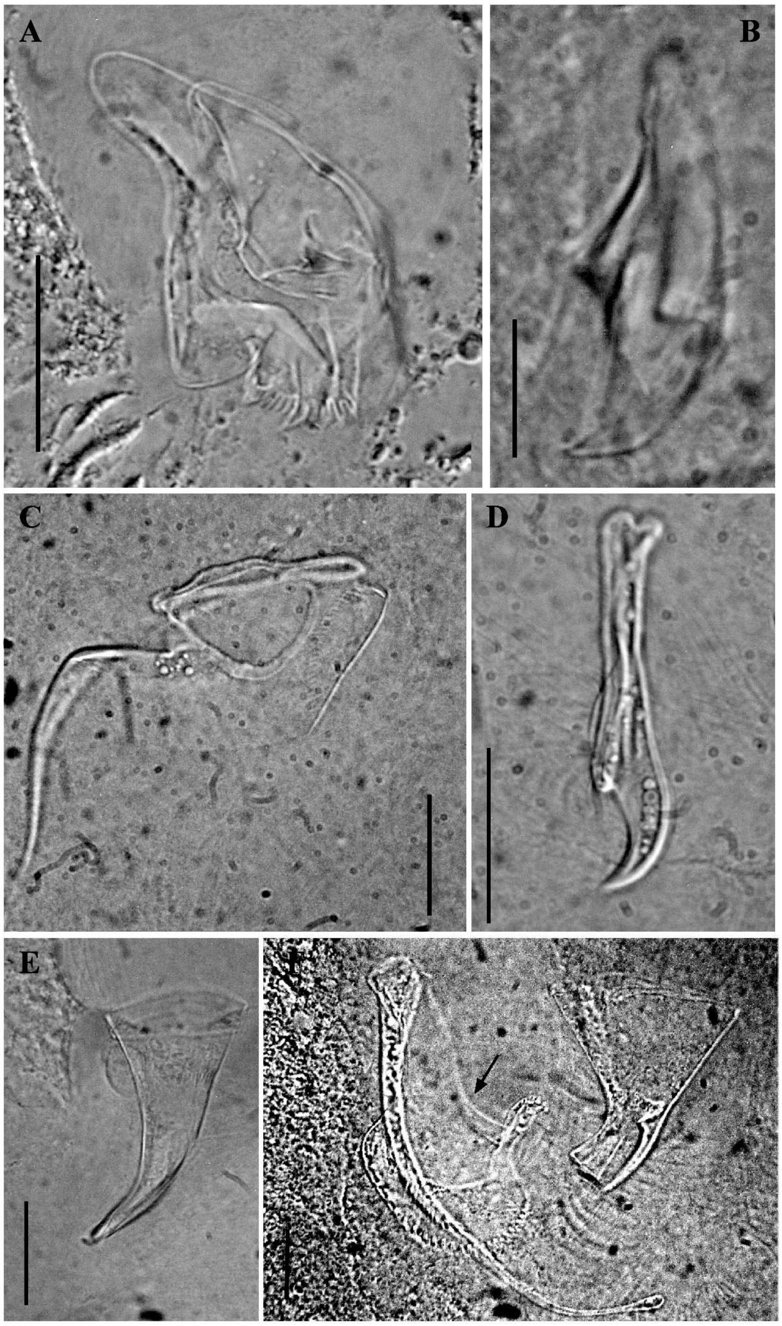

Fig. 5 *Alchoides alchoides:* A. Prostate stylet type III (from the holotype). *Alchoides dittmanni:* B. Prostate stylet type III (from the holotype). *Ametochus gehrkei:* C. Prostate stylet type III (from the holotype); D. Accessory stylet type III (from the holotype). *Arrawarria inexpectata:* E. Prostate stylet type II (from the holotype). *Austrorhynchus hawaiiensis:* F. Prostate stylet type III (left; arrow indicates the clasp) and prostate stylet type II (right). Scale bars: 20 µm.

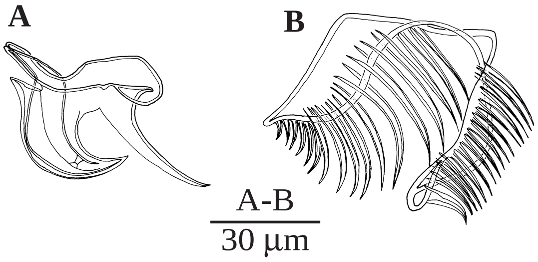

Fig. 6 *Cincturorhynchus monaculeus:* A. Prostate stylet type II (from the holotype); B. Prostate stylet type III (from the holotype).

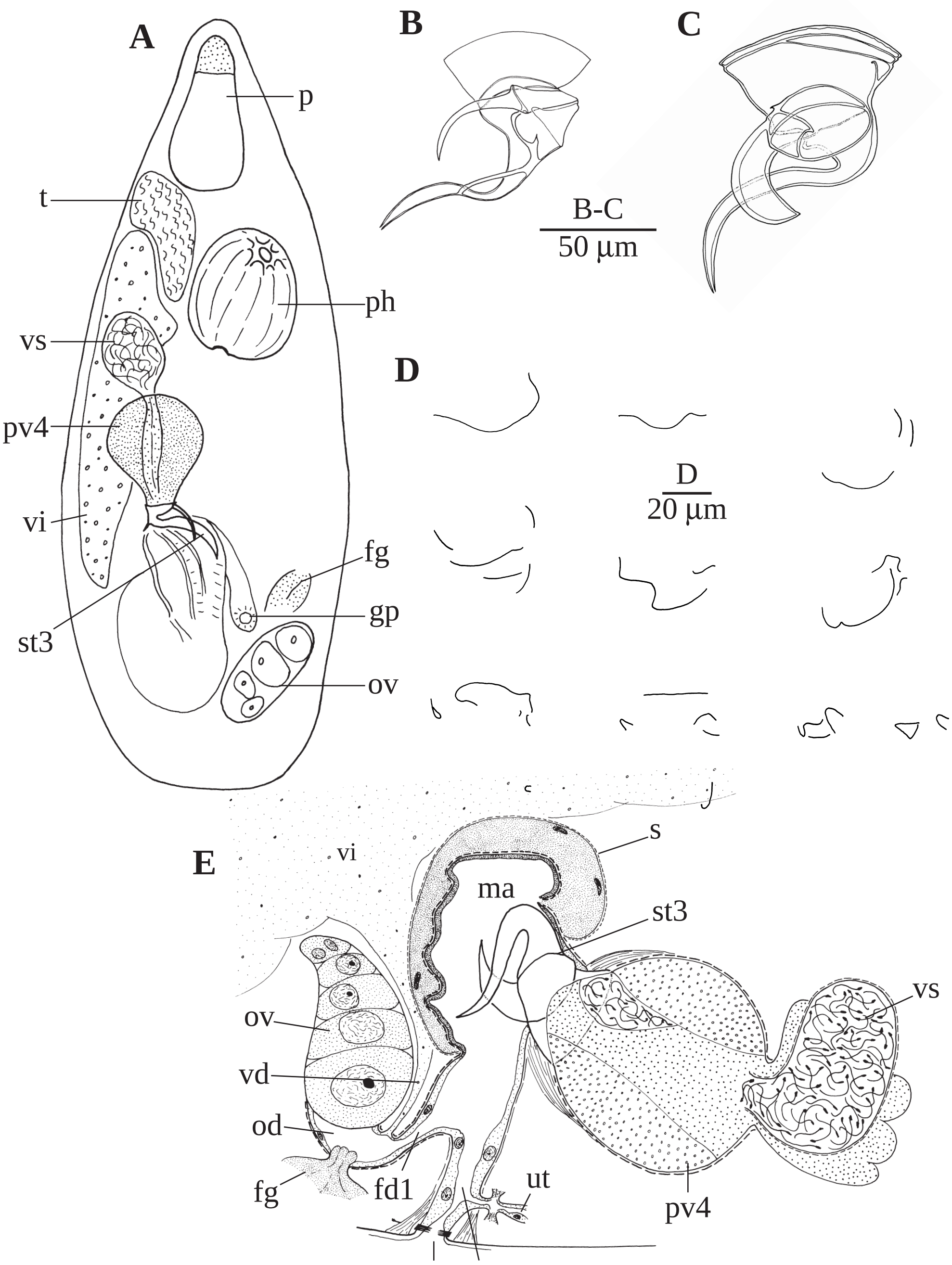

Fig. 7 *Duplexostylus rowei:* A. General organisation (from a live specimen); B. Prostate stylet type III (from the holotype); *Duplexostylus winsori:* C. Prostate stylet type III (from the holotype); D. Consecutive sections through the prostate stylet type III; E. Reconstruction of the atrial organs from the left side.

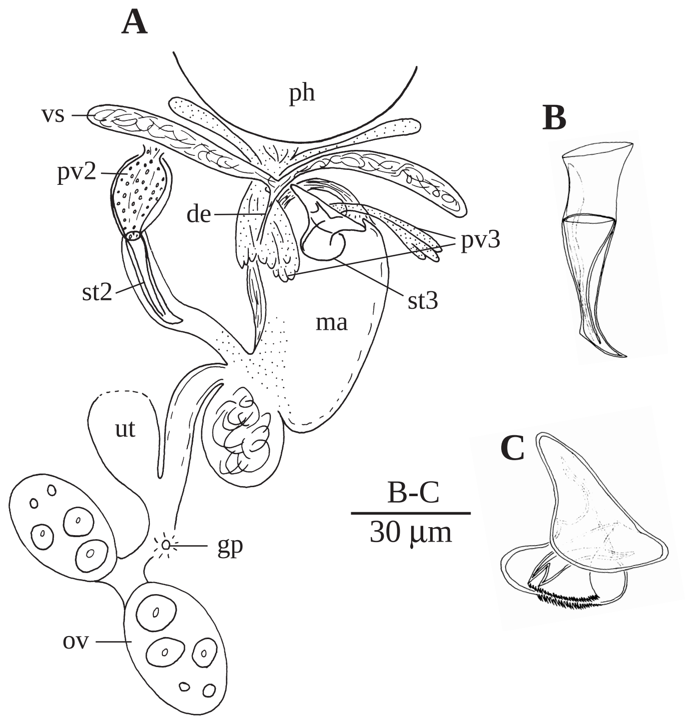

Fig. 8 *Paraustrorhynchus caligatus:* A. Atrial organs (from a live specimen); B. Prostate stylet type II (from the holotype); C. Prostate stylet type III (from the holotype).

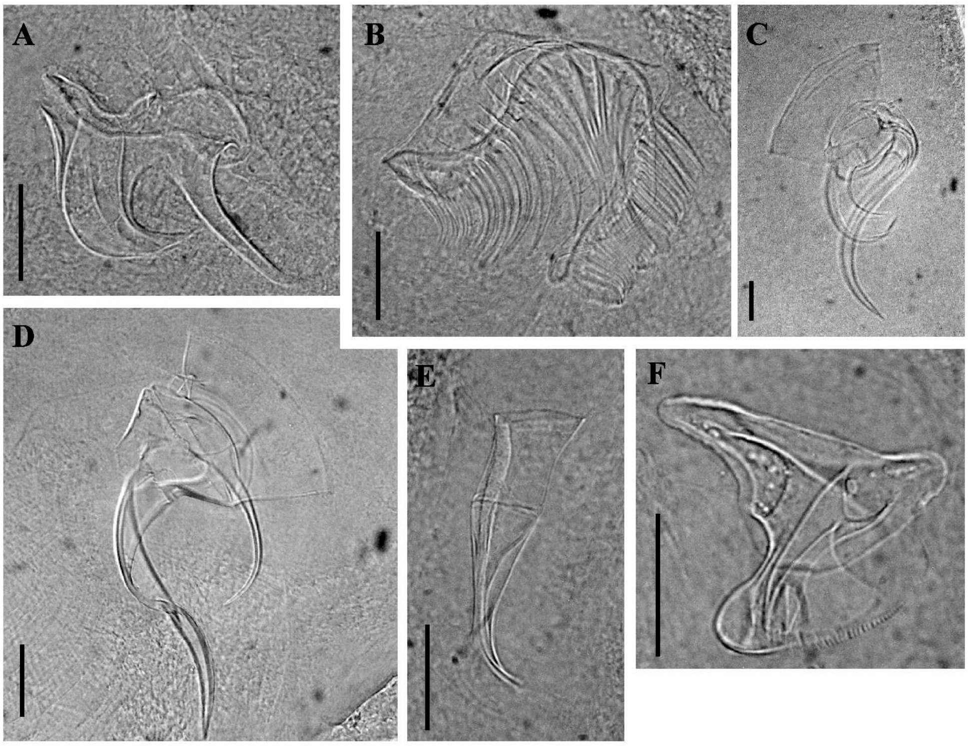

Fig. 9 *Cincturorhynchus monaculeus:* A. Prostate stylet type II (from the holotype); B. Prostate stylet type III (from the holotype). *Duplexostylus rowei:* C. Prostate stylet type III (from the holotype). *Duplexostylus winsori:* D. Prostate stylet type III (from the holotype). *Paraustrorhynchus caligatus:* E. Prostate stylet type II (from the holotype); F. Prostate stylet type III (from the holotype). Scale bars: 20 µm.

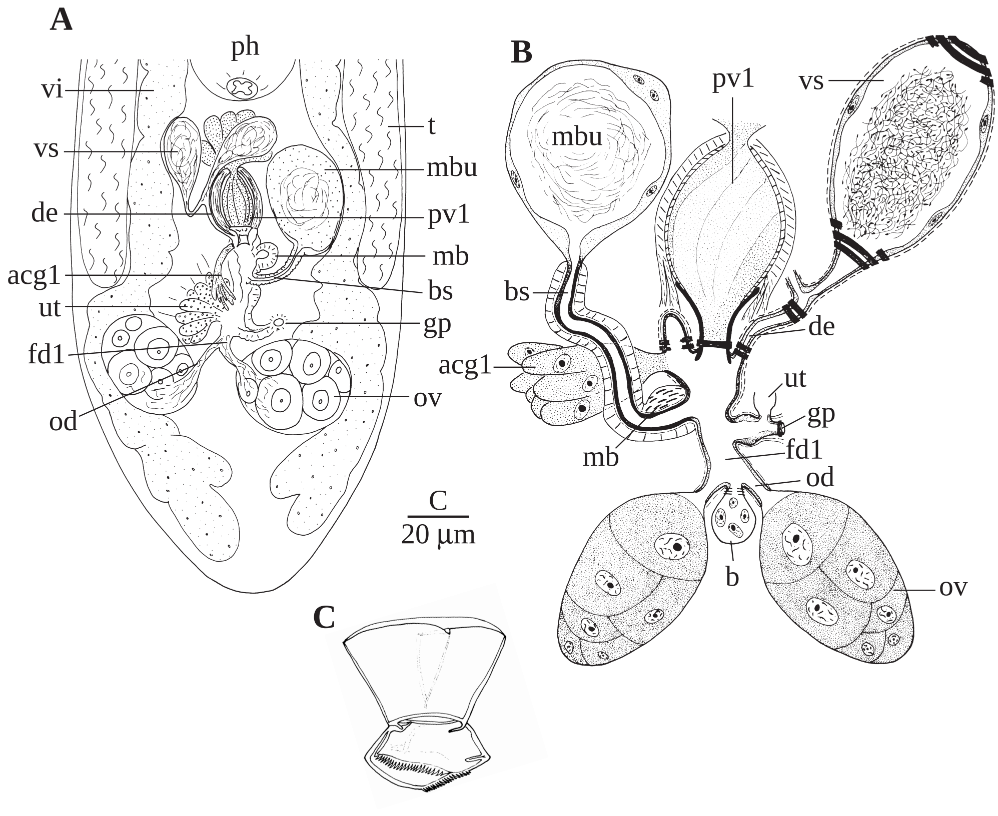

Fig. 10 *Polycystis australis:* A. Caudal body end (from a live specimen); B. Horizontal reconstruction of the atrial organs from above; C. Prostate stylet type I (from the holotype).

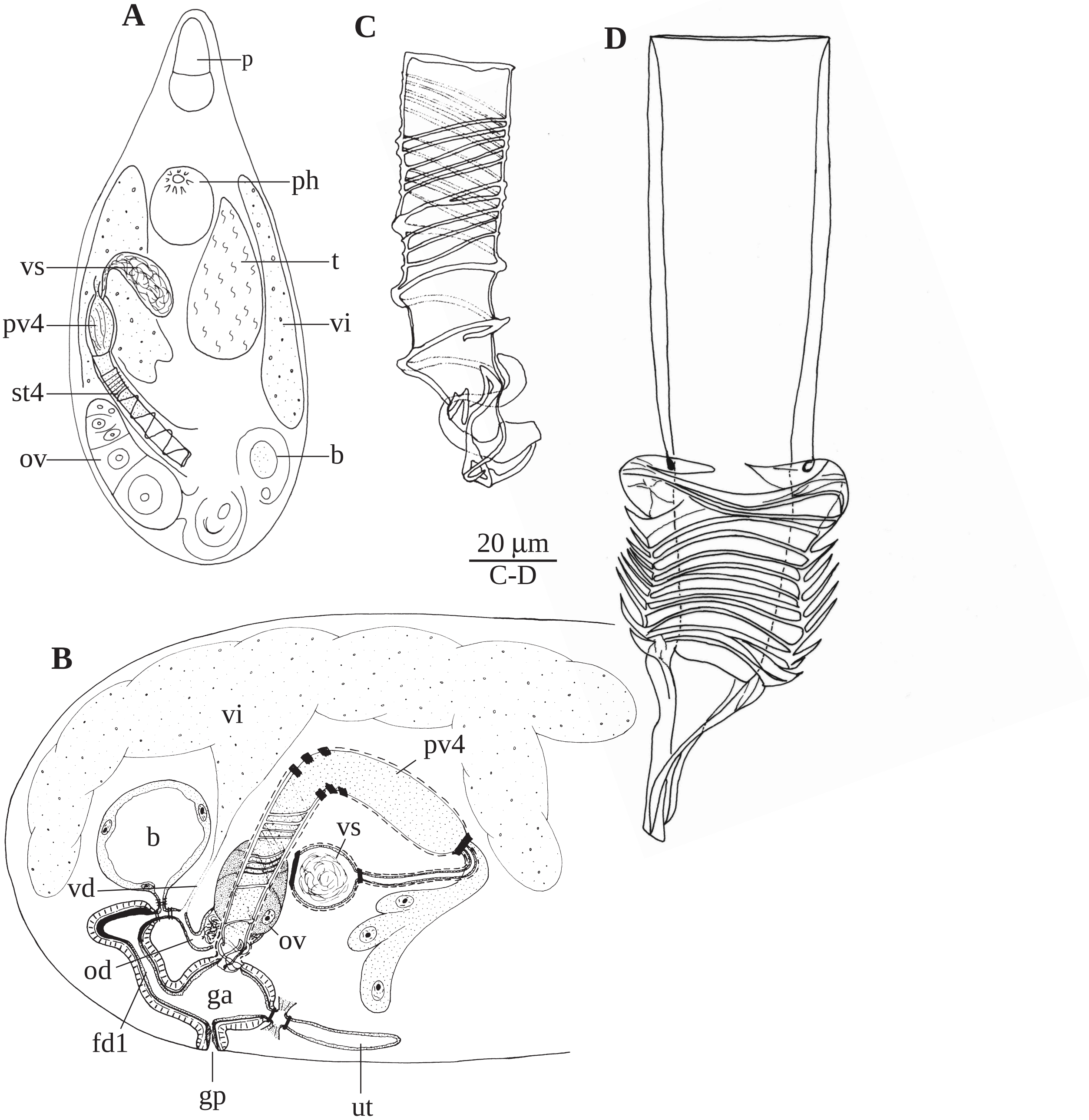

Fig. 11 *Stradorhynchus caecus:* A. General organisation (from a live specimen); B. Reconstruction of the atrial organs from the right side; C. Single-walled prostate stylet (from the holotype). *Stradorhynchus terminalis:*D. Single-walled prostate stylet (from the holotype).

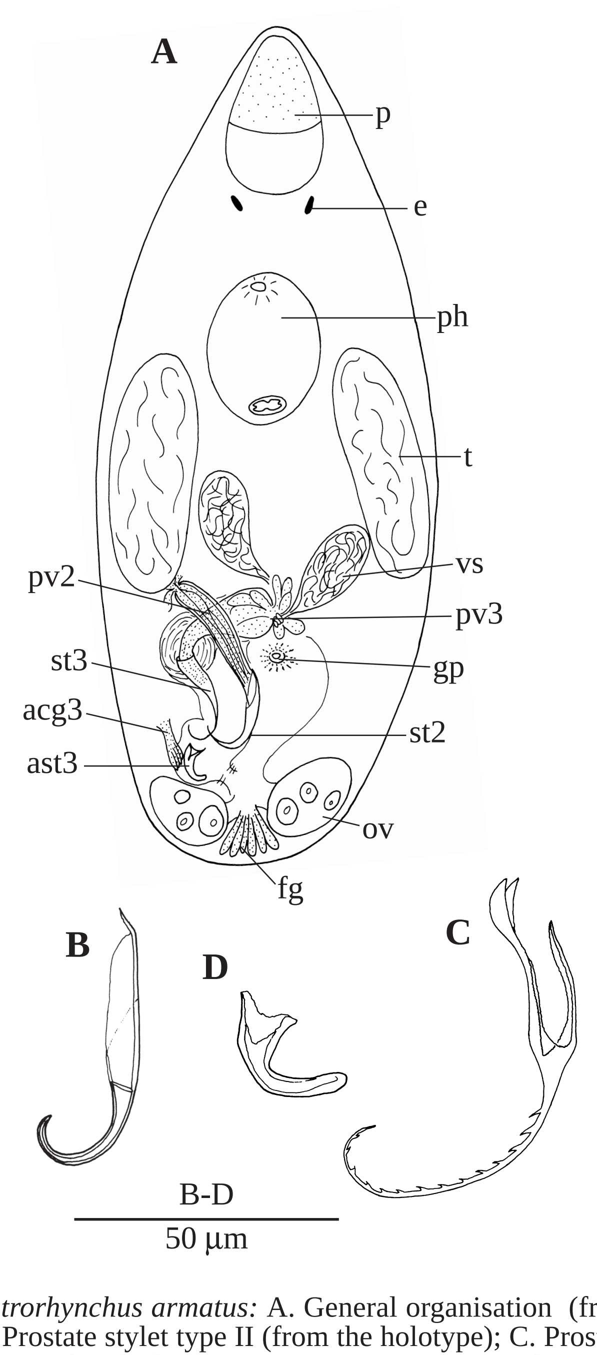

Fig. 12 *Triaustrorhynchus armatus:* A. General organisation (from a live specimen); B. Prostate stylet type II (from the holotype); C. Prostate stylet type III (from the holotype); D. Accessory stylet type III.

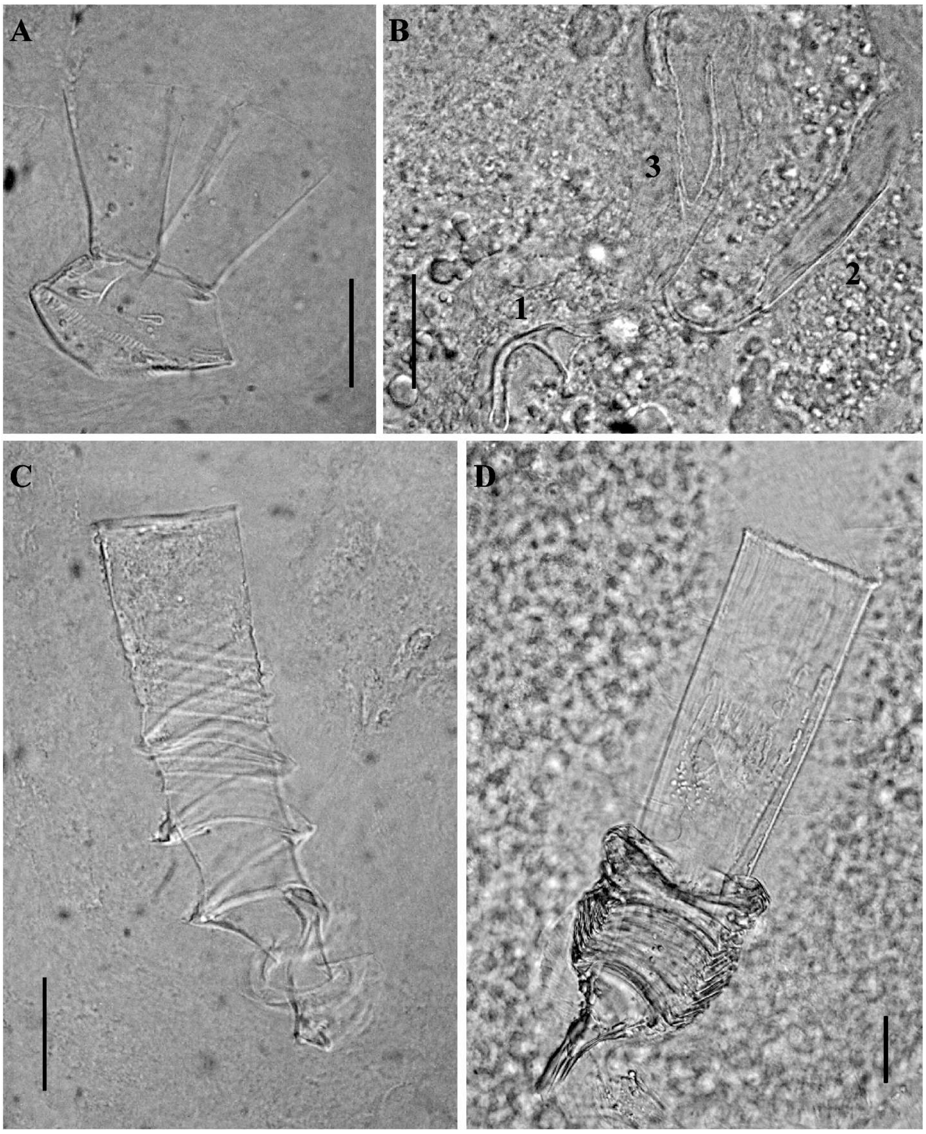

Fig. 13 *Polycystis australis:* A. Prostate stylet type I (from the holotype); *Triaustrorhynchus armatus:* B. 1. Accessory stylet type III, 2. Prostate stylet type II, 3. Prostate stylet type III (all from the holotype). *Stradorhynchus caecus:* C. Single-walled prostate stylet (from the holotype). *Stradorhynchus terminalis:* D. Single-walled prostate stylet (from the holotype). Scale bars: 20 µm.

VII

Five new species of *Promesostoma* Graff, 1882 (Platyhelminthes, Rhabdocoela, Promesostomidae), with a general discussion on this species-rich taxon

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RUNNING TITLE: The taxon *Promesostoma*

CATEGORY: Taxonomy

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Abstract The taxon *Promesostoma* is very species-rich, with more than 30 representatives from all over the world, occuring in all types of marine and brackish water habitats. In this contribution, another five new species of *Promesostoma* are described, bringing the total number on 38 species, which are all morphologically very similar, but are clearly recognisable by the detailed structure of their stylet. Two of the new species, *Promesostoma calcareum* n.sp. and *P. justinei* come from New Caledonia, whereas *P. alexanderi* n.sp. and *P. corsicum* occur in the Mediterranean Sea. *P. lincolni* n.sp. is a species from Florida, USA. Furthermore, new distribution data are given for *P.* ensifer, *P. hymanae*, *P. kergroixense* and *P. maculosum*. The new species are discussed within a larger framework, trying to identify species groups within this large taxon, mainly based on the organisation of the male genital system.

Key words: ‘Turbellaria’, Typhloplanoida, taxonomy, biodiversity, systematics

INTRODUCTION

The taxon *Promesostoma* Graff, 1882 is a very species-rich taxon of typhloplanoid Rhabdocoela. At the moment, 33 species are known and most of them occur in the northern Atlantic and the Baltic Sea. An overview of all species is given in Table 1, summarising their distribution and the main literature.

Table 1. Overview of all valid *Promesostoma* species with their distribution. The main literature that is consulted, is given (not a full reference list for each species). Species for which material was available are indicated with ‘x’.

	Distribution	**Main literature**
P. alaskanum **Ax & Armonies, 1990**	Alaska	Ax & Armonies, 1990
P. alexanderi **n.sp.**	Mediterranean Sea	this paper
P. balticum **Luther, 1918**	Baltic Sea	Luther, 1918, 1943, 1962; Karling, 1974
P. bilineatum **Pereyaslawzewa, 1892**	Black Sea, Baltic Sea	Pereyaslawzewa, 1892; Beklemischev, 1927; Ax, 1952, 1959; Mack-Fira, 1968, 1974; Karling, 1974
P. bilobatum **Ax & Armonies, 1987**	Canadian Atlantic coast	Ax & Armonies, 1987
P. bipartitum **Ax, 1956**	French Atlantic coast, North Sea	Ax, 1956b; Ehlers, 1974
P. calcareum **n.sp.**	New Caledonia	this paper
P. caligulatum **Ax, 1952**	North Sea, Baltic Sea, French Atlantic coast	Ax, 1952, 1956b; Luther, 1962; Karling, 1974; Ehlers, 1974; Faubel, 1980
P. cochleare **Karling, 1935**	North Sea, Baltic Sea, Mediterranean Sea, Canadian Atlantic coast	Karling, 1935; Ax, 1952, 1956a; Luther, 1962; Karling, 1974; Ehlers, 1974; Ax & Armonies, 1987
P. corsicum **n.sp.**	Mediterranean Sea	this paper
P. dennisalleni **Ax, 1995**	Atlantic coast USA (SC)	Ax, 1995c
P. digitosum **Ax, 1995**	Atlantic coast USA (SC)	Ax, 1995c
P. dipterostylum **Karling, 1967**	Pacific coast USA (Cal)	Karling, 1967
P. ensifer **(Uljanin, 1870) Pereyaslawzewa, 1892**	Black Sea; Mediterranean Sea	Uljanin, 1870; Pereyaslawzewa, 1892;

		Ax, 1959; this paper
P. fibulatum **Ax & Armonies, 1987**	Canadian Atlantic coast	Ax & Armonies, 1987
P. gallicum **Ax, 1956**	Mediterranean Sea	Ax, 1956a
P. gracile **Ax, 1951**	Baltic Sea, French Atlantic coast, North Sea	Ax, 1951, 1956b; Ehlers, 1974
P. hymanae **Ax, 1968**	Pacific coast USA (W), Indian Ocean (Indonesia, Kenya, Seychelles)	Ax, 1968; this paper
P. infundibulum **Ax, 1968**	Pacific coast USA (W)	Ax, 1968
P. justinei **n.sp.**	New Caledonia	this paper
P. karlingi **Ehlers, 1974**	Canadian Atlantic coast, North Sea	Ehlers, 1974; Ax & Armonies, 1987
P. kergroixense **Ehlers & Sopott-Ehlers, 1989**	French Atlantic coast, Mediterranean Sea	Ehlers & Sopott-Ehlers, 1989; this paper
P. kristenseni **Ax, 1993**	Greenland	Ax, 1993, 1995b
P. lincolni **n.sp.**	Florida	this paper
P. maculosum **Ax, 1956**	Black Sea, French Atlantic coast, Mediterranean Sea	Ax, 1956b, 1959; this paper
P. marmoratum **(Schultze, 1851) Graff, 1882**	widespread in northern Atlantic and Arctic waters; Mediterranean Sea	Schultze, 1851; Jensen, 1878; Levinsen, 1879; Graff, 1882, 1905, 1913; Gamble, 1893; Attems, 1897; Sabussow, 1905; Southern, 1912, 1936; Steinböck, 1931, 1932, 1933, 1938; Meixner, 1938; Luther, 1943, 1948, 1962; Westblad, 1954; Ax, 1954, 1956b, 1960, 1995a; Riedl, 1959; Karling, 1974; Ehlers, 1974; Ax & Armonies, 1987
P. meixneri **Ax, 1951** (with ssp. r*oscoffense*)	North Sea, Baltic Sea, French and Canadian Atlantic	Ax, 1951, 1956b; Karling, 1967; Ehlers, 1974, 1980; Ax &

	coast	Armonies, 1987
***P. minutum* Ax, 1956**	French Atlantic coast	Ax, 1956b
***P. norvegicum* Karling, 1967**	Norway	Karling, 1967
***P. nynaesiense* Karling, 1957**	Baltic Sea	Karling, 1957, 1974; Luther, 1962
***P. paracochleare* Ax, 1952**	Baltic Sea	Ax, 1952
***P. rostratum* Ax, 1951**	Northern Atlantic, North Sea, Baltic Sea	Ax, 1951, 1956b, 1993, 1995b; Ehlers, 1974; Ax & Armonies, 1987;
***P. sartagine* Ax & Ehlers, 1973**	Galapagos	Ax & Ehlers, 1973
***P. scylax* Marcus, 1952**	Brazil	Marcus, 1952
***P. serpentistylum* Ax, 1952**	North Sea	Ax, 1952; Ehlers, 1974; Jouk & Schockaert, 2002
***P. spiriferum* Karling, 1967**	Norway	Karling, 1967
***P. tenebrosum* Ax & Ehlers, 1973**	Galapagos	Ax & Ehlers, 1973
***P. teshirogii* Ax, 1992**	Japan	Ax, 1992

In this contribution, five new species are added. Two of them originate from New Caledonia, *Promesostoma calcareum* n.sp. and *P. justinei* n.sp., and are the first species of *Promesostoma*, described from the Southern Hemisphere. Two other species, *P. corsicum* n. sp. and *P. alexanderi* n.sp. occur in the Mediterranean Sea, whereas *P. lincolni* occurs on the coast of Florida (USA).

Furthermore new distribution data are given for *P. ensifer*, *P.* hymanae, *P. kergroixense* and *P. maculosum*.

In the general discussion we try to divide the species of *Promesostoma* in different groups based on the construction of the male genital system, as was also done by Karling (1967). However, the large number of species described since then and the placement of the five new species justify a revision of the system proposed by Karling (1967).

Material and methods

In the zoological collection of Limburgs Universitair Centrum (LUC), Diepenbeek, Belgium, a large number of known and new *Promesostoma* species from all over the world is present. The material is collected by the authors (New Caledonia, Belgian coast, Greece, Florida, Sylt) and earlier collaborators of our research group: Corsica and Indonesia (Dr. P. Martens), Kenya (Dr. P. Jouk and Mr. G. De Clerck) and the Seychelles (Mr. G. De Clerck). Furthermore, for this study, all *Promesostoma* material, present in the collections of the Swedish Museum of Natural History (SMNH) was also at our disposal (*P. bilineatum*

Pereyaslawzewa, 1892; ***P. cochleare*** Karling, 1935; *P. dipterostylum* Karling, 1967; *P. neglectum* Karling, 1967; ***P. norvegicum*** Karling, 1967; ***P.** nynaesiensis* Karling, 1957, *P.* ***scylax*** Marcus, 1952). The species for which material was available are indicated in Table 1.

The animals, collected by the authors, were extracted form the sediment or from algae using the $MgCl_2$ decantation method (see for instance Schockaert, 1996). They were studied alive and sorted with a dissection microscope and drawn using a microscope. Afterwards some individuals were mounted in lactophenol, while others (if any) were fixed in hot Bouin's solution, embedded in paraffin and serially sectioned. These 4 µm thick sections were stained with Heidenhain's iron haematoxylin using eosin as a counterstain. Drawings of the hard parts were made with Nomarski phase contrast microscopy on whole mounted animals. Drawings without a scale bar are freehand and made on live individuals. Measurements of the stylet were taken axially, unless indicated otherwise in the text.

All material (including types), except that belonging to the SMNH, is deposited in the collections of the Research Group Biodiversity, Phylogeny and Population Studies of the Limburgs Universitair Centrum.

Abbreviations used in the figures

ag: accessory glands; b: brain; de: ejaculatory duct; e: eye; evs: extracapsular seminal vesicle; gp: common genital pore; ivs: intracapsular seminal vesicle; ma: male atrium; ov: ovary; ph: pharynx; rg: rostral glands; rs: seminal receptacle; s: stylet; sph: sphincter; t: testis; v: vas deferens; vg: prostate vesicle; vit: vitellarium.

TAXONOMIC ACCOUNT

In this section five new species are described and some aditional data, mostly concerning the size of the stylet, for four known species is given.

Promesostoma alexanderi new species Fig. 1B

LOCALITIES: Perea (Thessaloniki Gulf, Macedonia, Greece): western part of the beach, fine sand 0.5 m deep, 6 August 2002.

MATERIAL EXAMINED: One whole mounted specimen, designated holotype.

ETYMOLOGY: Species named after Alexander the Great, who spend his youth in Pella, the ancient capital of the Macedonian kingdom.

DESCRIPTION: This species is only examined on mounted material. Therefore the description is limited to the structure of the stylet.
The stylet is 182 µm long and 5 µm wide proximally. It is slightly undulating over its whole length (due to the pressure of the cover slide?). The proximal end is cup-

shaped, ± 8 µm wide. Distally the stylet tapers and is flanked by two wing-like projections. The distal tip proper is cut-off straight.

DIAGNOSIS: *Promesostoma*-species with 182 µm long, slightly undulating stylet. Distal tip cut-off straight with two lateral wing-like projections.

Promesostoma calcareum **new species** Fig. 1A

LOCALITY: Récif de Toombo (reef off Nouméa, New Caledonia): in coarse-grained sand from the lagoon, 13 Aug 2003 (Type locality).

MATERIAL EXAMINED: One whole mount, designated holotype.

ETYMOLOGY: The species epithet refers to the presence of a sharp spur at the stylet flexure. Calcar (Lat.): spur.

DESCRIPTION: The animal is 0.6 mm long (measured on the whole mount), colourless, with two eyes. The stylet (Fig. 4D) is a slender tube (± 3 µm wide), 197 µm long. It is proximally bent (at 13% of its total length), forming an angle of almost 60º. At the flexure, a small, slender spur is present, which is approximately 10 µm long. Proximally, the stylet has a cup-shaped opening, while the distal end is blunt, cut-off oblique. No additional projections are present on the distal tip, which is cut-off oblique. Near the stylet flexure, the male genital duct shows a long and slender, sac-like protrusion, filled with sperm (seminal receptacle). The prostate vesicle is directed rostrolaterally.

DIAGNOSIS: *Promesostoma*-species with 197 µm long stylet, proximally bent over 60º and with a slender spur, ± 10 µm long. Distal tip cut-off oblique. Sac-like sperm-containing vesicle (seminal receptacle) on the male duct large.

Promesostoma corsicum **new species** Fig. 1C-D

LOCALITIES: Calvi Bay (Corsica): Port de la station Stareso: sublitoral sand, 15 m deep, 14 May, 17 September, 22 November 1983 and 24 January 1984 (Type locality); Ocellutia, large sandflat, sublitoral 10-12 m deep, 19 October 1982; Pointe Revellata: sublitoral sand, 18 and 35 m deep, 11 April and 30 June 1983; Pointe Caldano: sublitoral sand, 15 m deep, 1 July 1983.

MATERIAL EXAMINED: Several specimens studied alive. Five whole mounts, one of them designated holotype, another one paratype. The three remaining whole mounts are in bad condition, not or only partly showing the stylet.

ETYMOLOGY: Species named after the island, where the material was collected. Corsicus (Lat.): Corsican

DESCRIPTION: The animals have two eyes and are rather small, 0.4-0.7 mm long (measured on whole mounted specimens). Between the eyes a black pigment spot (white when viewed under incident light) occurs, which is divided into several parts in some individuals. Additionally, a reddish hue lies in a small strip in front of the eyes. The pharynx is situated in the middle of the body. The prostate vesicle slightly lies in front of the pharynx and is directed towards the anterior end. There is a common seminal duct, swollen to form a false seminal vesicle, which enters the prostate vesicle together with the prostate glands. The long ejaculatory duct leaves the prostate vesicle rostrally and bends back to enter the stylet. The proximal part of the stylet lies in front of the pharynx. The stylet is a very slender (4 µm wide proximally), 586-857 µm long (326-365 µm, when measured from top to bottom; n = 2) tube, which is spiralised in its distal part, showing 20 to 35 whirls. The proximal part of the stylet that is not spiralised takes 11 to 25 % of the total length (27 to 46 % compared to the top-bottom length). The distal tip of the stylet is simple, cut-off straight. The male genital duct is symmetrically widened proximally (copulatory bursa). An accessory glandular organ opens into the common genital atrium near the male genital duct.

DIAGNOSIS: *Promesostoma*-species with 586-857 µm long, very slender, tubiform stylet, distally spiralised (20-35 whirls) with a straight proximal part and a simple distal tip. Stylet partly situated in front of the pharynx. Male genital duct symmetrically widened proximally (copulatory bursa). Common seminal duct present. Prostate vesicle directed anteriorly. Accessory glandular organ present.

***Promesostoma ensifer* (Uljanin, 1870) Pereyaslawzewa, 1892** Fig. 2A-C

NEW LOCALITIES: Calvi Bay (Corsica): Port de la station Stareso: sublitoral sand, 9 May and 12 June 1982; same locality: on algae, 3-6 m deep, 10 April 1982, 26 September 1983 and 26 March 1985.

MATERIAL EXAMINED: Several individuals studied alive. Two whole mounts, one of them in bad condition, only partly showing the stylet.

ADDITIONAL REMARKS: The Corsican individuals lack any form of pigmentation, as do those from the Black Sea (see Pereyaslawzewa, 1892; Ax, 1959). The stylet of the Corsican specimen is 222 µm long and about 2 µm wide over its whole length, with the proximal opening 5 µm wide (no measurements available for Black Sea specimens). Proximally the stylet is curved over 180º. The distal tip of the stylet is curled back over 180º forming a sort of fishhook, as in the individuals from the Black Sea (see Pereyaslawzewa, 1892; Ax, 1959). Near the proximal opening of the stylet, a vesicle could be observed, which is enclosed within the widened male genital duct (= copulatory bursa), as in the specimens from the Black Sea. In several specimens it looks like a kind of funnel, which surrounds the proximal part of the stylet (see Fig. 2B-C), as in *P. meixneri roscoffense* Ehlers, 1980 (see Ehlers, 1980: fig. 2B). An accessory glandular organ is present (not observed by Ax, 1959).

This species was placed within *P. marmoratum* by Graff (1882, 1913) and Meixner (1938) based on the idea that the differences in the stylet structure could be explained by interspecific variability. However, Ax (1959) has already shown this to be untrue.

DIAGNOSIS: *Promesostoma*-species with ± 222 µm long, tubiform stylet, proximally curved over 180º, distally cut off straight with fishhook-like ending. Asymmetrically widened male duct enclosing vesicle, which surrounds stylet. Unpaired, extracapsular seminal vesicle. Accessory glandular organ present.

Promesostoma hymanae **Ax, 1968** Fig. 2D-E

NEW LOCALITIES: Ujung Pandang (Sulawesi, Indonesia): Kudingarengkeke Island, sublitoral coral sand, 0.4 m deep, 17 October 1984. Mahé (Seychelles): L'Islette, right of a small dike, coarse-grained sand mixed with detritus, 17 December 1992; Desroches (Seychelles): south-west side of the island, detritus-rich, fine-grained sublitoral sand, 2 m deep, 7 January 1993. McKenzie Point (Mombasa area, Kenya): in front of the Four Seasons Restaurant, on seagrasses and in coarse-grained sand from small rockpools, 25 and 27 September 1991; Diani Beach (Mombasa area, Kenya): sublitoral, coarse-grained sand mixed with broken sea urchin skeletons, 23 September 1985.

MATERIAL EXAMINED: Several specimens studied alive. Eight whole mounts (one from Indonesia, two from the Seychelles and five from Kenya).

ADDITIONAL REMARKS: The Indonesian specimen is 0.8 mm long (measured on the whole mount), the Kenyan specimens are slightly smaller, measuring 0.3-0.5 mm. The stylet is 217 µm long in the Indonesian specimen, 188-194 µm in the specimens from the Seychelles and ranges between 134 and 214 µm in the Kenyan animals (210 µm in the American specimen; see Ax, 1968). Most striking, the animals collected in Kenya in 1985 had a significantly smaller stylet (134-146 µm; n = 2) than those collected in 1991 (203-214 µm; n = 2). The stylet is ± 3 µm wide with a cup-shaped opening 8 µm wide. Proximally it is bent over 180°. The distal tip is cut-off straight. The whole mounted specimen from Indonesia clearly shows a strengthened, funnel-shaped structure near the stylet. This funnel is the distal part of the large seminal receptacle and is connected to the stylet by a short 'bridge' (Zapfen des Stilettrohres in Ax, 1968). The whole mounted specimens from Kenya and the Seychelles are in such a state that these details could not be observed. An accessory glandular organ is clearly present.

DIAGNOSIS: *Promesostoma*-species with 210-217 µm long, tubiform stylet, proximally curved over 180º, distally cut off straight. Sac-like sperm-containing vesicle (seminal receptacle) on the male duct large, with distal funnel-shaped part strengthened and connected to the stylet. Unpaired, extracapsular seminal vesicle. Accessory glandular organ present.

Promesostoma justinei new species Fig. 3A-C

LOCALITY: Ile Nou (Nouméa, New Caledonia): on Kuendu Beach in coarse-grained sand, 3 and 16 Aug 2003 (Type locality).

MATERIAL EXAMINED: Seven specimens studied alive and mounted, one of them designated holotype, three others paratypes. Five serially-sectioned specimens, all in bad condition.

ETYMOLOGY: Species dedicated to Prof. Dr. Jean-Lou Justine (Muséum National d'Histoire Naturelle, Paris, France).

DESCRIPTION: The animals have two eyes, are 0.5-1.2 mm long (measured on whole mounted specimens) and are rather plump. They are yellowish with a small black pigment spot (white when viewed under incident light) in front of the eyes. The stylet (Fig. 4C) is a 324-379 µm long (m = 353 µm; n = 6) slender tube, proximally curved over 360º. Proximally, the stylet has a cup-shaped opening (± 6 µm wide), while the distal tip is cut-off oblique and has no additional projections. The male genital duct is asymmetrically enlarged, forming a large sperm-containing vesicle (seminal receptacle; Fig. 4B: rs). The prostate vesicle (Fig. 4B: vg) is directed towards the rostral body end, fusiform, discharges its secretion into the stylet and contains an intracapsular seminal vesicle (Fig. 4B: ivs). Additionally, there is an unpaired, large and elongated extracapsular seminal vesicle (Fig. 4B: evs), which enters the prostate vesicle from behind, together with the large prostate glands. Some smaller glands are present near the proximal funnel of the stylet. An accessory glandular organ on the common genital atrium is absent.

DIAGNOSIS: *Promesostoma*-species with 324-379 µm long, tubiform stylet, proximally curved over 360º, distally cut off oblique. Sperm-containing vesicle (seminal receptacle) on the male duct large. Intracapsular and unpaired, extracapsular seminal vesicle. Accessory glandular organ absent.

Promesostoma kergroixense Ehlers & Sopott-Ehlers, 1989 Fig. 3D-E

NEW LOCALITIES: Calvi Bay (Corsica): Ocellutia, sublitoral sand, 10-12 m deep, 9 June 1982, 8 April and 23 November 1983 and 5 April 1984; Pointe Revellata:

sublitoral sand, 35 m deep, 30 June 1983 and 12 April 1984; Plateau de Mezzo Golfo: sublitoral sand, 64 m deep, 25 November 1983.

MATERIAL EXAMINED: Several individuals studied alive. Six whole mounts, two of them in bad condition, not showing the stylet.

DESCRIPTION AND REMARKS: The Corsican specimens are rather slender, 0.6-0.7 mm long, have two eyes (with lenses) and a large bundle of rhabdite tracts in between the eyes.

In comparison with the French Atlantic specimens (see Ehlers & Sopott-Ehlers, 1989), the genital system is very obvious and could be studied in detail on live specimens.

Both vasa deferentia join and form a common seminal duct, which can be swollen. It enters the prostate vesicle together with coarse- and fine-grained glands. The prostate vesicle proper is situated just in front of the pharynx and directed posteriorly. It is connected with the stylet through a long ejaculatory duct. The stylet is a straight (or slightly bent) tube and is entirely situated behind the pharynx. It is 137-157 µm long (m = 145 µm; n = 4) and 5 µm wide. The Atlantic specimens have a slightly shorter stylet (95-100 µm; Ehlers & Sopott-Ehlers, 1989). Distally it is asymmetrically widened with a washboard-like part at the convex side and a very thin-walled wing-like projection on the concave side. The latter projection is absent (or could not be observed) in the Atlantic specimens (Ehlers & Sopott-Ehlers, 1989). The male genital duct is asymmetrically enlarged near the cup-shaped opening of the stylet, forming a copulatory bursa.

Remarkably, only one ovary could be observed in the Corsican specimens.

A large accessory glandular organ is present on the common genital atrium.

DIAGNOSIS: *Promesostoma*-species with 137-157 µm long, straight stylet, distally with a washboard-like and a thin-walled wing-like projection. Stylet situated behind the pharynx. Male genital duct symmetrically widened proximally (copulatory bursa). Common seminal duct present. Prostate vesicle directed posteriorly. Accessory glandular organ present.

***Promesostoma lincolni* new species** Fig. 3F-G

LOCALITY: Hutchinson Island (Florida, USA): Blind Creek, flat beach with medium-grained sand, 22 November 1994 (Type locality).

MATERIAL EXAMINED: One whole mount, designated holotype.

ETYMOLOGY: Species in honour of the sixteenth president of the United States of America, Abraham Lincoln (1809-1865).

DESCRIPTION: This species is only examined on mounted material. Therefore the description is limited to the structure of the stylet and a few details regarding the male system, visible on the whole mount.

The stylet is 204 µm long, 2 µm wide with a proximal cup-shaped opening 5 µm wide. It shows a sharp angle of about 60º proximally. Distally a small, bent 6 µm long-hook is present. Opposite this hook, a straight projection, 10 µm long, occurs. This part makes the distal tip of the stylet much broader, so that the male genital duct also is clearly wider than in its proximal part. Near the angle in the stylet, a seminal receptacle is present, of which the distal part looks strengthened (pseudocuticularised wall?).

DIAGNOSIS: *Promesostoma*-species with ± 204 µm long, tubiform stylet, proximally bent with a sharp angle (± 60º), distally cut-off straight with two projections (a small, bent hook and a straight lamellar one). Seminal receptacle with distal part strengthened.

Promesostoma maculosum **Ax, 1956**

NEW LOCALITIES: Calvi Bay (Corsica): Ocellutia, sublitoral sand, 10-15 m deep, 29 June, 18 September and 23 November 1983 and 5 April 1984; Pointe Revellata: sublitoral sand, 2 and 35 m deep, 7 May 1982, 30 June and 23 September 1983; Base of Pointe Revelatta: sheltered bay, sublitoral sand, 6 m deep, 5 April, 1984; Port de la Station Stareso: sublitoral sand, 7-15 m deep, 12 March and 14 May 1983, 3 and 4 April, 1984; Calvi Plage: beach, 2 m deep, 5 May 1982.

MATERIAL EXAMINED: Several individuals studied alive. Seven whole mounts.

REMARKS: The Corsican individuals measure 0.3-0.8 mm (measured on whole mounts), a lot smaller than the Atlantic coast specimens (1.5-2 mm; Ax, 1956). The stylet is 233-339 µm long (m = 293 µm; n = 7), ± 250 µm in the specimens from the French Atlantic coast (Ax, 1956b). Approximately in the middle of the stylet, it is curled over 360º, however never visible in mounted specimens, due to the pressure of the cover slide. A wing connected to the distal tip of the stylet as the French Atlantic specimens, could not be observed (see Ax, 1968), probably due to its extreme thin-walled nature and the strong pressure of the cover slide.

DIAGNOSIS: *Promesostoma*-species with 233-339 µm long, tubiform stylet, curved over 360º in its middle, distally cut-off oblique with a thin-walled wing on one side. Male genital duct asymmetrically widened, forming a copulatory bursa. Accessory glandular organ present.

GENERAL DISCUSSION

The taxon *Promesostoma* comprises 38 species, including the five newly descibed species. Ax (1956a) discerned 3 groups of species, while Karling discussed his ideas in 1967, on the evolutionay trends in the structure of the male colupatory organ. According to Karling (1967), the underlaying cause of the different types of copulatory organs of the *Promesostoma* species is the differential (or allometric) lengthening of the stylet. Today, with twice as many species known, we may reconsider some of the variation within the taxon. In what follows, we basically follow Karling's ideas, however without attributing any taxonomic significance to the groups we present, nor implying any evolutionary or phylogenetic conclusions. Unfortunately, not all descriptions found in literature allow to decide unambiguously on the state of affairs. Moreover, a number of species have been described exclusively on the structure of the stylet. Nevertheless, we hope that this overview will clarify the situation to some extent and form the basis of a more thorough revision. The five new species are herewith also compared with the other *Promesostoma* species.

Because of the homogeneity in the overall structure and that of the female system, which is only studied for *P. marmoratum* (see Luther, 1943), the emphasis of this discussion and the resulting groupings lies on the male genital system.

In the species of Group I (Table 2a and 2b), the stylet is rather short and fits between the pharynx and the genital pore.

Table 2a - Species of Group Ia: Stylet short, behind the pharynx, Prostate vesicle in front of the pharynx, directed backwards and with a long ejaculatory duct. stylet is a simple tube or with 2-3 long spines.

	Copulatory bursa	**Seminal receptacle**	**Accessory glands**	**Stylet spines**	**Differentiations of stylet**
P. balticum	yes (?)	at the base of the stylet	?	1 long spine	distal end of stylet funnel-like
P. bipartitum	yes	no	yes	1 long spine	stylet and spine with terminal differentiations
P. cochleare	yes	no	yes	1 long spine	none
P. kergroixense	yes	no	yes	none	stylet asymmetrically widened terminally
P. paracochleare	yes	no	yes	2 long spines	none
P. tenebrosum	yes (symm.)	midway male atrium	yes	none	distal end of stylet funnel-like

The prostate vesicle lies in front of the pharynx, it is directed backwards and connected to the stylet by a long and slender ejaculatory duct. There are no external seminal vesicles and the long and sinuous vasa deferentia directly lead to the intracapsular seminal vesicle.This group is divided into two subgroups based on the structure and length of the stylet. In Group Ia (Table 2a) the stylet is a short and almost straight tube, as is most obvious in *P. tenebrosum* and in *P. kergroixense* (beit with an asymmetric terminal thickening in the latter species). This represents the (hypothetical) stage A of Karling (1967). The proximal part of the male atrium is enlarged, functioning as a copulatory bursa, indeed symmetrical in *P. tenebrosum*, as predicted by Karling, 1967. In the other species of this group the bursa is an asymmetrical bulge at one side of the male duct. The stylet of these four species may be very slightly curved and bear one or two long spines emerging from about the middle of the stilet (stage D of Karling).

Table 2b – Species of Group Ib . Stylet longer than in group Ia, slender and slightly curved, but still behind the pharynx. Prostate vesicle in front of the pharynx and with long ejaculatory duct, directed backwards or slightly sidewards.

	Copulatory bursa	**Seminal receptacle**	**Accessory glands**	**Differentiations of stylet**
P. alexanderi	?	?	?	with two wings distally
P. bilineatum	yes (symm.)	midway male duct	?	with one wing distally
P. corsicum	yes	no	yes	stylet spiralised
P. gallicum	yes (symm.)	midway male duct	no (?)	none
P. maculosum	yes	no	yes	stylet with 360° turn midway
P. norvegicum	yes	no	yes	club-shaped distally
P. serpentistylum	no	at base of stylet	yes	none

In the species of Group I b (Table 2b), the situation is basically the same, albeit that the stylet is more slender, a little bit longer and (therefor?) slightly curved. The prostate vesicle is still in front of the pharynx, sometimes slightly inclined sidewards (stage B1 of Karling, 1967). In all species, except in *P. serpentistylum*, a copulatory bursa is present. In five out of the 13 species of this Group I, there is a thinwalled vesicle attached to the male atrium, additional to the copulatory bursa: the seminal receptacle. Since both, copulatory bursa and seminal receptacle may be present in the same species (as for instance in *P. caligulatum*, *P. ensifer* and *P. gracile*), these structures cannot be homologous (contra Karling, 1967: p. 264). Moreover, the seminal receptacle may be connected to the male atrium at different locations.

Promesostoma alexanderi n.sp. can be placed within Group Ib, because of its rather short stylet, which is slightly curved and is situated behind the pharynx, with the prostate vesicle in front of the pharynx. Within this group it is the only species of which the distal tip of the stylet is flanked by two wing-like projections, giving it the impression of an arrowhead.

In all other *Promesostoma* species, the stylet is long to very long, and does not fit between the pharynx and the genital pore. In those species it is found next to the pharynx, with its anterior end anteriorly to the pharynx. The elongated prostate vesicle is now directed forewards, receiving the vasa deferentia from behind, where an unpaired (paired in *P. spiriferum*), thin walled extracapsular seminal vesicle develops. To "cope" with this "new" situation, the proximal opening of the stylet must be directed backwards, the ejaculatory duct being shorter and sometimes much shorter than in the species of Group I.

A first group of species with an elongated stylet, of which the proximal part is situated in front of the pharynx is group II (representing stage C of Karling, 1967), containing only *P. corsicum* n.sp. and *P. spiriferum*, both with a spiralised stylet. These species have no seminal receptacle, a clearly symmetrical copulatory bursa and extracapsular seminal vesicles (paired in *P. spiriferum*, unpaired in *P. corsicum*). Both species also possess a large bundle of accessory glands, opening into the common genital atrium. The main differences between both species are given in Table 3.

Table 3 – Species of Group II: stylet spiralised; prostate vesicle more or less directed sidewards.

	Eyes	**Pigmentation**	**Stylet length (in µm)**	**Seminal vesicle**
P. corsicum	present	rostral spot	586-857 (top-bottom: 326-365)	unpaired
P. spiriferum	absent	none	?	paired

In the species of Group III (Table 4a and 4b), the stylet is proximally bent over 90° (Group IIIa; Table 4a) or even less than 90° (Group IIIb; Table 4b).

Table 4a – Species of Group III a: stylet bends over about 90 degrees; prostate vesicle more or less directed sidewards.

	Copulatory bursa	**Seminal receptacle**	**Accessory glands**	**Differentiations of stylet**
P. bilobatum	no	large	yes	spoon-like with oval plates distally
P. dipterostylum	no	large (with basal funnel)	no (?)	"wings" at proximal end
P. scylax	no	large	yes	none

The prostate vesicle is next to the pharynx, directed sidewards or forewards and the ejaculatory duct mostly is of moderate length, longer than in the species of Group I and shorter than in the species of Group IV (to be considered hereafter). The stylet is slightly curved as that of the species in Group Ib, representing stage B2 of

Karling (1967). In none of these species, the male atrium is swollen to form a copulatory bursa, but they all have a very large seminal receptacle, leaving the male duct at the bent of the stylet, and directed forewards. Its wall has no muscles but the epithelium clearly shows a resorbing character. In some of the species (see Table 4a and 4b) the distal part of the seminal receptacle is strengthened (pseudocuticularised), even forming a sort of hard funnel-shaped piece.

Table 4b – Species of Group III b: proximal end of the pharynx forma a sharp bent of less than 90 degrees; prostate vesicle directed forewards.

	Copulatory bursa	**Seminal receptacle**	**Accessory glands**	**Differentiations of stylet**
P. calcareum	no	large	?	none
P. dennisalleni	no	large (with basal funnel)	?	none
P. digitosum	?	?	?	"hand with five digits" distally
P. fibulatum	no	large (with basal funnel)	no (?)	with bent lamella and lateral clasp distally
P. lincolni	no	large (with basal funnel)	?	hook-like and straight projection distally
P. nynaesiense	no (?)	?	?	spoon-like distally
P. teshigorii	no	large (with basal funnel)	no ?	none

P. calcareum n.sp. and *P. lincolni* n.sp. are easily placed in Group IIIb, based on their slightly curved stylet with aproximal angle of less than 90°. However, the former species is recognisable by the presence of a sharp spur where the stylet is bent and the absence of differentiations on the distal tip of the stylet, a combination of features, which is unique within this group. *P. lincolni* n.sp. is recognisable by the presence of two projections at the distal tip of the stylet: a hook-shaped one and a straight one.

The stylet of the species in Group IV (Table 5a and 5b) has a proximal turn of at least 180°, up to three coils of 360°.

Table 5a – Species of Group IVa: there is no copulatory bursa and the seminal vesicle is very large, directed forwards.

	Turn of stylet	**Copulatory bursa**	**Seminal receptacle**	**Acc. glands**	**Differentiations of stylet**
P. alaskana	90° +360°	no	large	?	none
P. hymanae	90°+180°	no	large (with basal funnel)	yes	none
P. infundibulum	90°+2x360°	no	large (with basal funnel)	no	none
P. justinei	90°+360°	no	large	no ?	none

In several of those species the stylet begins with a short, straight part with a clear angle of about 90° on the following section (indicated by +90° in the tables). Group IV is divided into two subgroups, based on the sperm receiving parts, associated with the male duct. In Group IVa (Table 7 a) there is no copulatory bursa, but there is a large seminal vesicle with a resorbing wall, directed forwards, exactly as in the species of Group III. In the species of Group IVb (Table 7b) there is an asymmetric enlargement of the proximal part of the male duct (the copulatory bursa), and a thin walled vesicle next to it, in some species incorporated into the copulatory bursa. This seminal receptacle is never as large, nor directed forwards as it is in the former groups.

Table 5b – Species of Group IVb: There is a well developed copulatory bursa, the seminal receptacle is relatively small incorporated into the bursa or lies next to the bursa.

	Turn of stylet	**Copulatory bursa**	**Seminal receptacle**	**Acc. glands**	**Differentiations of stylet**
P. caligulatum	90°+2x360°	yes	next to bursa	?	with hook-like projection distally
P. ensifer	90°+180°	yes	in bursa	yes	fish-hook like bent distally
P. gracile	180°	yes (?)	in bursa	no (?)	with small hook distally
P. karlingi	180°	yes	in bursa (?)	yes	with several small projections distally
P. kristenseni	360°	yes	none (?)	?	none
P. marmoratum	1x360°	yes	next to bursa	?	with small spur distally
P. meixneri	3x360°	yes	in bursa	?	with small spur distally
P. minutum	90°+2x360°	yes	next to bursa	no (?)	with lamella and thread distally
P. rostratum	180°	yes	next to bursa	no	horseshoe-shaped distally
P. sartagine	90°+180°	yes	none (?)	yes	with one wing distally

The last new species, *P. justinei* n.sp., is placed within Group Iva, based on the structure of the stylet and the absence of a copulatory bursa. Within this group, *P. hymanae* and *P. infundibulum* have a seminal receptacle with a strengthened basal funnel, which is lacking in *P. justinei* and *P. alaskana*. The main difference between both species is the stylet length: 250 µm in *P. alaskana* and 324-379 µm in *P. justinei* n.sp. Furthermore, *P. alaskana* lacks the small pigment spot between the eyes, present in *P. justinei* n.sp.

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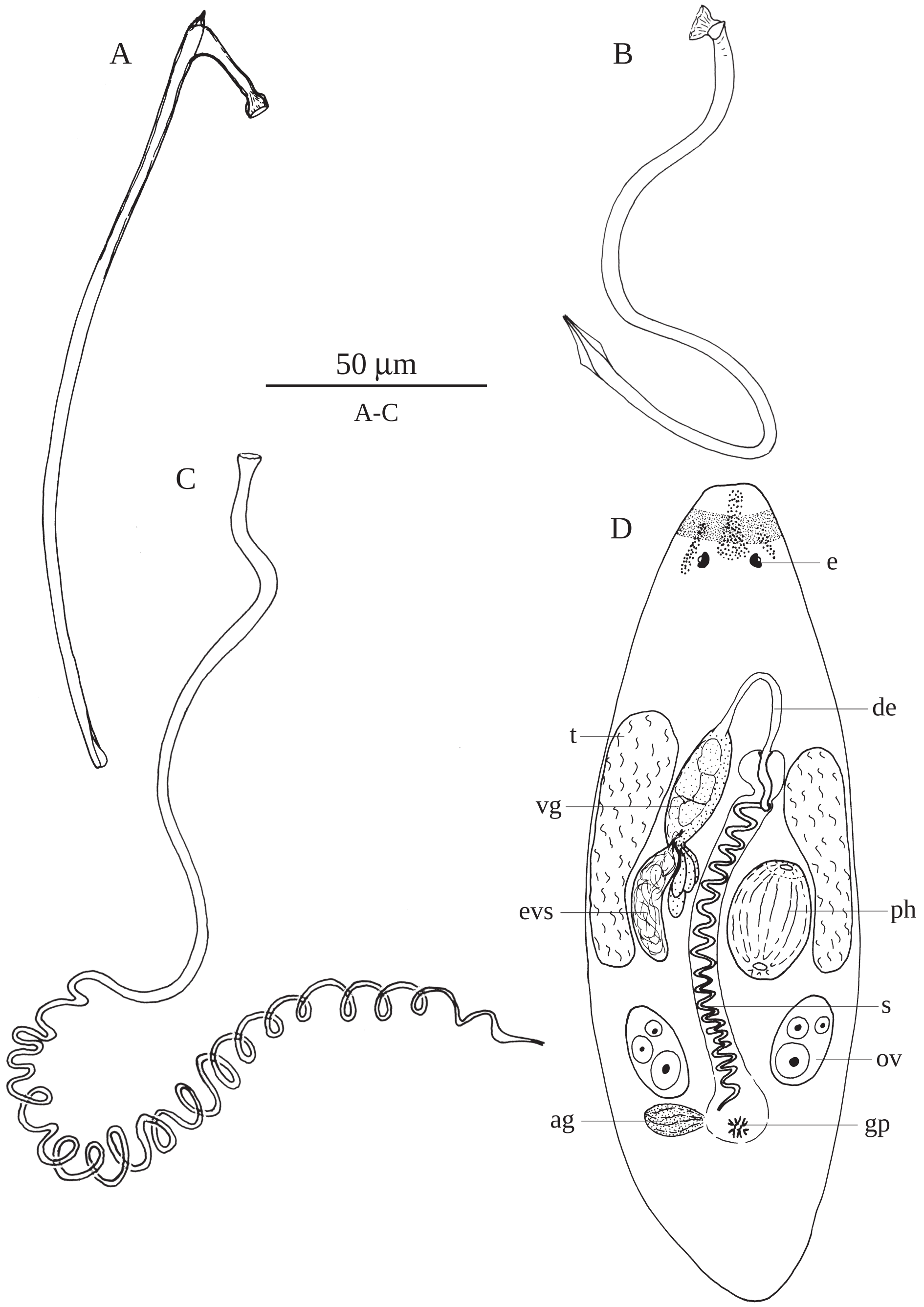

Fig. 1 *Promesostoma calcareum*: A. Stylet (from the holotype). *Promesostoma alexanderi:* B. Stylet (from the holotype). *Promesostoma corsicum:* C. Stylet (from the holotype); D. General organisation (from a live specimen).

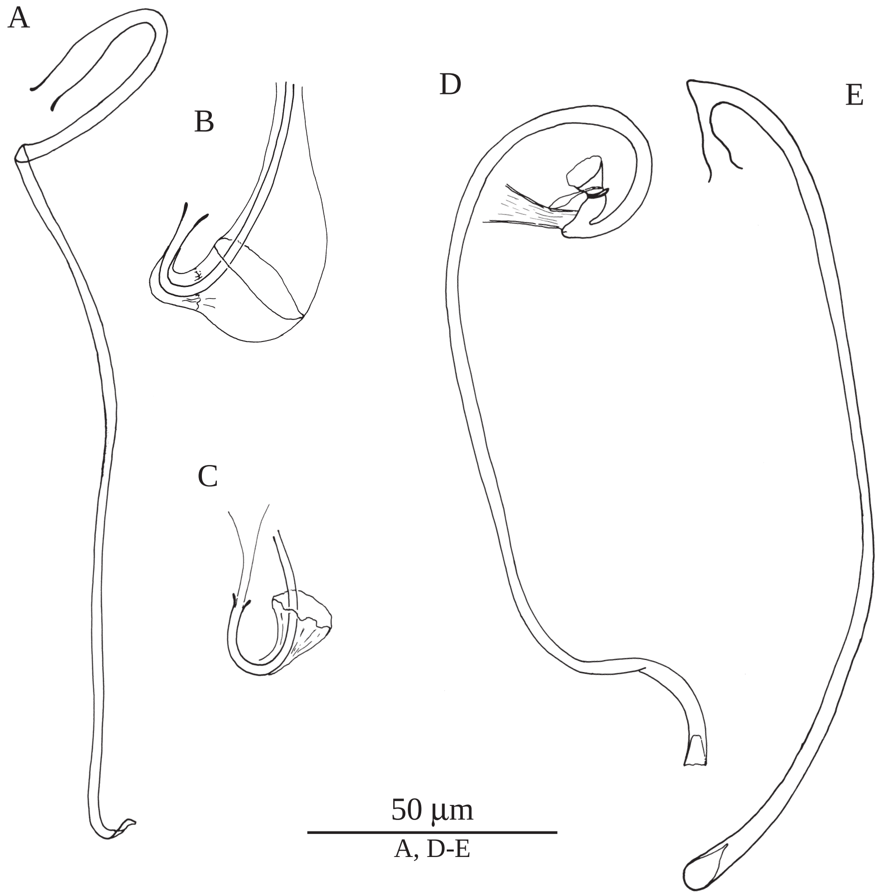

Fig. 2 *Promesostoma ensifer*: A. Stylet; B,C. Detail of the seminal receptacle, enclosed by the copulatory bursa (from two live specimens). *Promesostoma hymanae:* D,E. Stylet (from two different individuals).

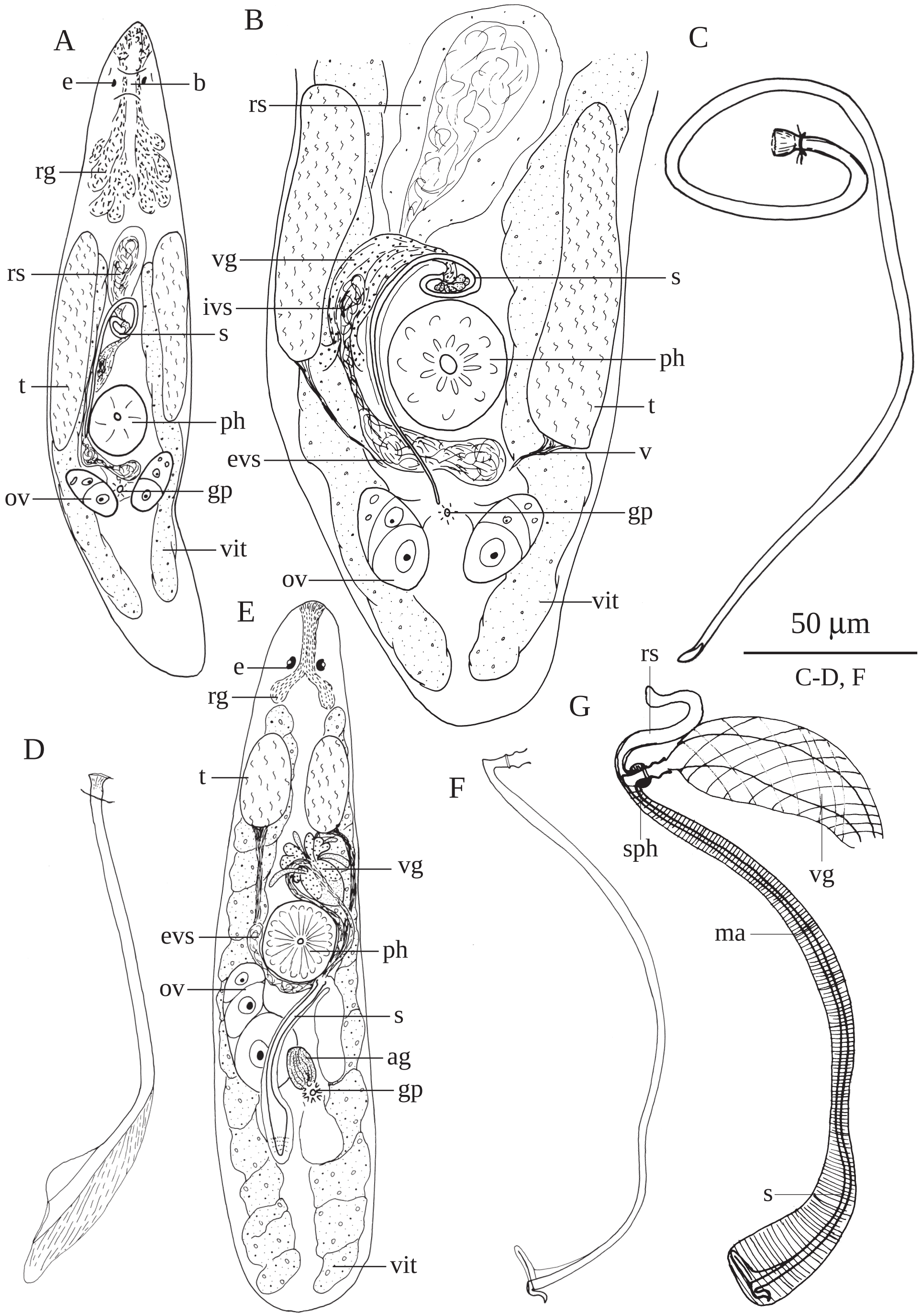

Fig. 3 *Promesostoma justinei*: A. General organisation (from a live specimen); B. Caudal body end with atrial organs (from a live specimen); C. Stylet (from the holotype). *Promesostoma kergroixense:*D. Stylet; E. General organisation (from a live specimen). *Promesostoma lincolni:* F. Stylet (from the holotype); G. Male system (from a live specimen).