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Taxonomic revision of Protoplanellinae (Typhloplanidae, Rhabdocoela)

With notes on other limnoterrestrial Typhloplanidae

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Abstract

Although more than 60 species of rhabdocoels are known to live in limnoterrestrial habitats, they often have been ignored in taxonomic studies, despite their abundance and high diversity. Most limnoterrestrial rhabdocoels belong to Typhloplanidae, in particular Protoplanellinae. The majority of research dates back several decades and was carried out almost exclusively in Europe.

This study focused on Protoplanellinae and other limnoterrestrial Typhloplanidae. New specimens were collected in Germany, Austria and Belgium. Furthermore, already collected specimens from Belgium, Alabama (USA) and Ontario (Canada) were examined. In total, 21 species were found. Five species are new to science, one of which belonged to a new genus: ‘nov. gen. nov. sp.’, and four can be classified under an existing genus. *Adenoplea* nov. sp. 1, *Adenoplea* nov. sp. 2, *Acrochordonoposthia* nov. sp. and *Hoplopera* nov. sp.

‘nov. gen. nov. sp.’ is characterized by the anterior position of its pharynx, the presence of a seminal receptacle in the female duct and the copulatory organ with bended, sclerotized ejaculatory duct.

***Adenoplea* nov. sp. 1** has got dermal rhabdites in its epidermis, a seminal receptacle inside the female duct, testes that differ strongly in size and a copulatory organ containing a straight, armed cirrus without diverticle at its distal end.

***Adenoplea* nov. sp. 2** possesses an egg-shaped copulatory organ with long, muscular ejaculatory duct and two types of associated prostate glands and an oval bursa, surrounded by circular muscles.

***Acrochordonoposthia* nov. sp.** has a straight cirrus with spines pointing in proximal direction and a sclerotized, cone-shaped bursa, surrounded by circular muscles.

***Hoplopera* nov. sp.** has a seminal receptacle inside the female duct, strongly developed atrial glands and a bipartite bursa with a sclerotized structure, consisting of several vertical rods delineated by vertical bars, inside the stalk.

Furthermore, seven species were reported for the first time since their original description: *Adenoplea meridionalis*, *Adenocerca minima*, *Chorizogynopora italica*, *Hoplopera opaca*, *Prorhynchella minuta* and *Ventrociliella romanae*.

As descriptions of many species are incomplete and most species lack type material, neotypes were proposed when necessary and when sufficient material was available. Neotypes were proposed for *Adenoplea perigraptopera, Carcharodopharynx arcanus*, *Adenocerca minima*, *Acrochordonoposthia nemoralis*, *Hoplopera opaca*, *Krumbachia subterranea*, *Olisthanellinella rotundula* and *Protoplanella simplex*. These neotypes were only proposed for the purpose of this thesis and should not be used as such. No type material exists for *Acrochordonoposthia conica*, *A. reversa* and *Archivortex silvestris*, but the quality of the specimens in this study was inadequate to be assigned as a neotype. For *Prorhynchella minuta*, no neotype was assigned, as material from the original description might still be in existence. Because of temporal restrictions this could not be verified..

The phylogeny of Typhloplanidae has never been thoroughly investigated and it was always unclear whether Protoplanellinae are monophyletic or not. In this study, a phylogenetic analysis including 30 sequences of Typhloplanidae, of which 18 Protoplanellinae, was conducted. The complete 18S and 28S rDNA genes were analyzed with Maximum Likelihood (ML) and Bayesian Inference (BI). Furthermore, a concatenated analysis was performed. The analysis clearly shows that Protoplanellinae are polyphyletic and fall apart in several small, often strongly supported taxa. Furthermore, *Carcharodopharynx arcanus*, which has always been placed in its own family, is a highly supported taxon within Typhloplanidae in the analysis.

Samenvatting

Hoewel er meer dan 60 soorten rhabdocoelen gekend zijn uit limnoterrestrische habitats, worden ze meestal genegeerd in taxonomische studies, ondanks hun grote aantallen en hoge diversiteit. De meeste limnoterrestrische rhabdocoelen behoren tot de Typhloplanidae, en dan hoofdzakelijk de Protoplanellinae. Het meeste onderzoek dateert echter al van verschillende decennia geleden en gebeurde allemaal in Europa.

Deze studie richtte zich op Protoplanellinae en andere limnoterrestrische Typhloplanidae. Stalen werden genomen in Duitsland, Oostenrijk en België. Verder werden reeds verzamelde specimens uit België, Alabama (USA) en Ontario (Canada) onderzocht. In totaal worden er 21 soorten behandeld. Vijf soorten zijn nieuw voor de wetenschap, waarvan een tot een nieuw genus behoorde: ‘nov. gen. nov. sp.’ en vier in bestaande genera geclassificeerd kunnen worden: *Adenoplea* nov. sp. 1, *Adenoplea* nov. sp. 2, *Acrochordonoposthia* nov. sp. en *Hoplopera* nov. sp.

‘nov. gen. nov. sp.’ heeft de pharynx in de voorste lichaamshelft, heeft een receptaculum seminis in het vrouwelijk kanaal en een copulatieorgaan met gebogen, gesclerotiseerde ductus ejaculatorius.

***Adenoplea* nov. sp. 1** bezit dermal rhabdieten in de epidermis, een receptaculum seminis in het vrouwelijk kanaal, testes die onderling sterk in grootte verschillen en een copulatieorgaan met rechte, bestekelde cirrus zonder divertikel aan het distale uiteinde.

***Adenoplea* nov. sp. 2** wordt gekenmerkt door een eivormig copulatieorgaan met lange, gespierde ductus ejaculatorius en twee types prostaatklieren en een ovalen bursa omringd door circulaire spieren.

***Acrochordonoposthia* nov. sp.** heeft een rechte cirrus die volledig bezet is met stekels die in proximale richting wijzen en een gesclerotiseerde, kegelvormige bursa omringd door circulaire spieren.

***Hoplopera* nov. sp.** bezit een receptaculum seminis in het vrouwelijk kanaal, sterk ontwikkelde atriumklieren en een tweedelige bursa met een gesclerotiseerde structuur, bestaande uit meerdere verticale staven, afgelijnd door horizontale balken, in de steel.

Zeven soorten werden voor het eerst sinds hun oorspronkelijke beschrijving teruggevonden: *Adenoplea meridionalis*, *Adenocerca minima*, *Chorizogynopora italica*, *Hoplopera opaca*, *Prorhynchella minuta* en *Ventrociliella romanae*.

Aangezien de beschrijvingen van veel soorten onvolledig zijn en typemateriaal ontbreekt bij de meeste soorten, werden indien nodig en indien voldoende materiaal beschikbaar was neotypes voorgesteld. Neotypes werden voorgesteld voor *Adenoplea perigraptopera*, *Carcharodopharynx arcanus*, *Adenocerca minima*, *Acrochordonoposthia nemoralis*, *Hoplopera opaca*, *Krumbachia subterranea*, *Olisthanellinella rotundula* en *Protoplanella simplex*. Deze neotypes werden enkel voorgesteld voor deze thesis en dienen niet als zodanig gebruikt te worden.Er bestaat geen typemateriaal van *Acrochordonoposthia conica*, *A. reversa* en *Archivortex silvestris*, maar wegens de onvoldoende kwaliteit van de gevonden specimens werd besloten geen neotype aan te duiden. Van *Prorhynchella minuta* is geen neotype aangeduid, maar mogelijk bestaat hier nog materiaal van. Wegens tijdsgebrek kon dit niet worden nagegaan en wordt voorlopig geen neotype aangeduid.

De fylogenie van de Typhloplanidae is nooit uitgebreid onderzocht en de monofylie van de Protoplanellinae wordt betwijfeld. Om dit te onderzoeken werd een fylogenetische analyse uitgevoerd met 30 sequenties van Typhloplanidae, waarvan 18 sequenties Protoplanellinae. De volledige 18S en 28S rDNA genen werden gebruikt voor de analyses, waarbij zowel van Maximum Likelihood (ML) als van Bayesian Inference (BI) gebruik werd gemaakt.. Verder werd een geconcateneerde analyse uitgevoerd. De analyse toont duidelijk aan dat de Protoplanellinae polyfyletisch zijn en uiteenvallen in verschillende kleine, vaak sterk ondersteunde taxa. Verder werd *Carcharodopharynx arcanus*, die vaak in zijn eigen familie wordt ingedeeld, sterk ondersteund als taxon binnen de Typhloplanidae.

Introduction

There are about 6500 described species of turbellarians (TYLER *et al.* 2006-2012), living in marine, brackish, limnic and terrestrial environments. Despite their large abundance and diversity, turbellarians are often ignored in taxonomic studies because of their small size and the need to study the animals alive, in whole mounts and/or serial sections for a successful identification. The number of species may be at least 10 times higher than the number of described species at this moment (SCHOCKAERT *et al.* 2008). Furthermore, recent data shows that turbellarians are even more diverse and prominent than previously thought (FONSECA *et al.* 2010).

Rhabdocoela Meixner, 1925 makes up a diverse taxon of turbellaria, which consists of about 1500 described species (VAN STEENKISTE 2012). Rhabdocoels are usually simultaneous hermaphrodites with internal fertilization. The reproductive system is very complex and is the most important characteristic for identification and taxonomical classification. In the female system, large yolk glands or vitellaria are present in addition to the ovaries. Other atrial organs, such as a copulatory bursa (bursa copulatrix), a uterus or a seminal receptacle (receptaculum seminis) are also present. The male system consists of testes, seminal vesicle (vesicula seminalis), a copulatory organ which often contains an ejaculatory duct (ductus ejaculatorius) and/or a stylet and associated prostate glands, which form a prostate vesicle.

Dalytyphloplanida Willems et al., 2006 makes up a very diverse taxon within Rhabdocoela. Within this taxon, Typhloplanidae forms the largest family, consisting of about 285 described species. Most of them occur in limnic environments, but about 30 species live in marine or brackish habitats and more than 60 species are found in limnoterrerrestrial habitats (VAN STEENKISTE *et al.* 2010; VAN STEENKISTE 2012). Typhloplanidae Graff, 1905 are classified in ten subfamilies: Ascophorinae Findenegg, 1924, Cephalopharyngidae Hochberg, 2004, Mesophaenocorinae Noreña *et al.,* 2006, Mesostominae Bresslau, 1933, Olisthanellinae Bresslau, 1933, Opistominae Luther, 1963, Phaenocorinae Wahl, 1910, Protoplanellinae Reisinger, 1924, Rhynchomesostominae Bresslau, 1933 and Typhloplaninae Bresslau, 1933. Morphologically, Typhloplanidae are characterized by the combined possession of paired testes and a single ovary. The division in subfamilies is based on the relative position of the testes to the vitellaria and the position of the protonephridiopores. As these morphological characteristics delineating the subfamilies of Typhloplanidae are not very robust, the real affinities of many species are highly doubtful (VAN STEENKISTE *et al.* 2010; VAN STEENKISTE 2012).

Table 1: Known genera of Protoplanellinae. Notice that the difference between limnic and limnoterrestrial habitats is often not very clear, e.g. very moist soil or moss growing in streams.

Described genera	**# species**	**Habitat***	**Distribution**
Acrochordonoposthia Reisinger, 1924	9	Limnoterrestrial, limnic	Palearctic, Nearctic
Adenocerca Reisinger, 1924	4	Limnoterrestrial, limnic	Palearctic
Amphibolella Findenegg, 1924	2	Limnic	Palearctic, Nearctic
Bockia Reisinger, 1924	1	Limnoterrestrial	Palearctic
Bryoplana Van Steenkiste, Davison & Artois, 2010	1	Limnoterrestrial	Nearctic
Chorizogynopora Reisinger, 1924	2	Limnoterrestrial	Palearctic
Haplorhynchella Meixner, 1924	1	Limnoterrestrial	Palearctic
Hoplopera Reisinger, 1924	6	Limnoterrestrial, marine	Palearctic
Krumbachia Reisinger, 1924	11*	Limnoterrestrial, limnic	Palearctic, Nearctic
Lioniella Riedl, 1954	1	Marine	Palearctic
Microcalyptorhynchus Kepner & Ruebush, 1935	1	Limnic	Nearctic
'Nov. gen.'	1	Limnoterrestrial	Palearctic
Olisthanelliola Schwank, 1980	1	Limnoterrestrial	Palearctic
Perandropera Reisinger, 1924	1	Limnoterrestrial	Palearctic
Proamphibolella An der Lan, 1939	2	Limnic	Palearctic
Prorhynchella Ruebush, 1939	1	Limnic	Nearctic
Protopharyngiellona Schwank, 1980	1	Limnoterrestrial, limnic	Palearctic
Protoplanella Reisinger, 1924	2	Limnoterrestrial	Palearctic
Pseudobockia Kolasa, 1981c	1	Limnic	Palearctic
Rhomboplanilla Schwank, 1980	1	Limnoterrestrial	Palearctic
Tauridella Sekera, 1912	1	Marine	Palearctic, Nearctic
Ventrociliella Kolasa, 1977	1	Limnoterrestrial	Palearctic
Yagua Marcus, 1958	1	Limnic	Neotropic

***The classification of many species of *Krumbachia* is highly uncertain and several species are sometimes classified within *Olisthanella* Voigt, 1892.**
**** One new genus was described in this study, but remains unnamed to prevent the creation of a nomen nudum.**

Because most terrestrial rhabdocoels live in moist habitats, the term semi-terrestrial or limnoterrestrial is preferred above terrestrial (VAN STEENKISTE *et al.* 2010). Most limnoterrestrial species belong to Protoplanellinae. This taxon is characterized by the presence of paired nephridiopores and the ventral position of the testes relative to the vitellaria. These characteristics are, however, not unique for this group. Paired protonephridia also occur in Olisthanellinae, and in Phaenocorinae. Furthermore, *Carcharodopharynx* Reisinger, 1924, which is traditionally classified in its own family, also possesses paired nephridiopores and ventral testes. Most species of Protoplanellinae are described from Europe. In total 22 genera are known (Table 1), containing 49 species in total. 43 species and

18 genera were found in Europe. Six genera and eight species are known from the nearctic. Furthermore the monotypic genus *Yagua* Marcus, 1958 has a neotropical distribution.

Besides Protoplanellinae there are a few other described limnoterrestrial rhabdocoels, all belonging to Dalytyphloplanida. All ten species of *Adenoplea* Reisinger, 1924 and the monotypic genus *Macrophysaliophora* Reisinger, 1924, all classified within the Typhloplaninae Luther, 1904, are found in limnoterrestrial habitats. *Carcharodopharynx arcanus* (Reisinger, 1924), which always has been classified in its own family, Carcharodopharyngidae Bresslau, 1933 and *Archivortex silvestris* Reisinger, 1924, classified within Provorticidae Beklemischev, 1927, complete the list of known limnoterrestrial rhabdocoels.

Despite their abundance, limnoterrestrial rhabdocoels have received little attention by taxonomists compared to marine and limnic species. The Palearctic has been sampled relatively well (REISINGER 1924, 1933; AN DER LAN 1955; LUTHER 1963; KOLASA 1974, 1977, 1981a,d) and sporadic records from the American continent exist (SAYRE & WERGEN 1994; VAN STEENKISTE *et al.* 2010). Other regions have never been sampled for limnoterrestrial rhabdocoels. A few limnic and marine species were also classified within Protoplanellinae. Several other papers investigate limnic Protoplanellinae from Europe (FINDENEGG 1924; MEIXNER 1924; AN DER LAN 1939; SCHWANK 1979, 1980, 1981, KOLASA 1981b,c) and America (KEPNER & CARTER 1931; KEPNER & RUEBUSH 1935; RUEBUSH 1938, 1939; MARCUS 1958). All known marine Protoplanellinae are described from Europe (GRAFF 1905; RIEDL 1954; KARLING 1957; EHLERS 1974). However, an unidentified specimen of *Tauridella* was found in Virginia, USA (FERGUSON & JONES 1949). Most research on Protoplanellinae and other limnoterrestrial rhabdocoels dates back decennia. Except for one recent paper (VAN STEENKISTE *et al.* 2010), the most recent studies on Protoplanellinae were carried out more than 30 years ago (e.g. KOLASA 1977; 1981a,b,c,d; SCHWANK 1979, 1980, 1981).

As older studies often provide incomplete descriptions and type material usually does not exist, was never kept or is lost, new specimens were collected from the original type localities. Species were redescribed and – when necessary – a neotype was assigned to clear out the taxonomy of the Protoplanellinae.

Most phylogenetic research on Platyhelminthes has been carried out on parasitic taxa (e.g. OLSON *et al.* 2001; ZAMPARO *et al.* 2001; LOCKYER *et al.* 2003) or on higher taxonomic groups (e.g. EHLERS 1985; LITTLEWOOD *et al.* 1999; WILLEMS *et al.* 2006). The first extensive analysis of the rhabdocoels was carried out by WILLEMS *et al*. (2006) and included 13 sequences of Typhloplanidae, but no Protoplanellinae. Unpublished data (VAN STEENKISTE 2012) suggests that Protoplanellinae may not be monophyletic, but thorough phylogenetic studies of Typhloplanidae, including more sequences of Protoplanellinae, are lacking.

A thorough phylogenetic analysis of Typhloplanidae has never been published but it is likely that Protoplanellinae are not monophyletic (VAN STEENKISTE 2012). In this study a phylogenetic analysis is performed with 18 sequences of Protoplanellinae and 12 of other Typhloplanidae to test the monophyly of Protoplanellinae. As the phylogenetic affinities of *Carcharodopharynx* are unknown, sequences of this taxon were also included in the analysis.

The musculature of the body wall and the genital system can be visualized by using phalloidin-linked fluorophores. This technique is often used on Acoela (TYLER & RIEGER 1999; HOOGE 2001; HOOGE & TYLER 2006) and has proven its importance in phylogenetics (TEKLE *et al.* 2005). Phalloidin fluorescence of the musculature is not often used in the study of rhabdocoels (KOTIKOVA *et al.* 2002), but could prove to be an important taxonomic tool. Fluorescence staining was performed, but this did not yield sufficient data to be useful. For this reason, the data are omitted from this thesis.

Material & methods

Sampling localities

New material was collected in Europe, with a special interest in known type localities in Germany (SCHWANK 1979; 1980) and in Austria (REISINGER 1924; AN DER LAN 1955). The specimens were obtained during sampling campaigns in Germany from 9 to 10 August 2011 and in Austria from 21 to 30 August 2011, and during several small sampling trips in Belgium and Bavaria, Germany during the entire summer of 2011.

Unprocessed animals that were collected by Dr. Van Steenkiste from Alabama, USA and Ontario, Canada in May 2009 and by Kevin Thijssen in Belgium in the period 2008-2009 were also investigated.

Extraction of the animals

Animals from dry samples of moss and forest soil were extracted using the Baermann pan method (TOWNSHEND 1963). The sample is contained within a paper tissue, which is put on a coarse sieve. The sieve is set on a plate filled with a small amount of water, just enough to partly inundate the sample. After a few hours the negatively phototactic animals will move from the sample, through the paper tissue and the sieve, to the water. By pouring the water through a sieve with very small meshes (35-40µ) and rinsing the sieve on a Petri dish the worms are concentrated in a small amount of water along with other organisms (nematodes, bdelloid rotifers, ciliates, tardigrades) in the sample, where they can be picked out.

For limnic samples (muddy sediment or submersed vegetation), the oxygen depletion method was used (SCHOCKAERT 1996). The samples are weighted by stones to keep them submerged in a jar filled with water. As the oxygen depletes on the bottom, an oxygen gradient arises. Because of this gradient, animals migrate to the more oxygen-rich upper part of the water column, where they can be picked out with a glass pipette.

Microscopy & Taxonomical methods

Flatworms that were picked out of the samples were studied and drawn alive under a light microscope before they were fixed in hot (50°C) freshwater Bouin’s fixative for at least 24 hours and stored in 70% ethanol. They were embedded in paraffin, sliced with a microtome in sections of 3 or 4µm thick and stained with Heidenhain’s iron haematoxylin, with erythrosine as counterstain. When hard parts were present (e.g. *Acrochordonoposthia, Adenocerca*), specimens were whole mounted with lactophenol.

Since most species were described long ago, it is almost never mentioned whether or not material was preserved. KOLASA (1977, 1981b,d) deposited *Adenoplea meridionalis* Kolasa 1981d and *Chorizogynopora italica* Kolasa 1981b in the Department of Animal Morphology, Poznan and *Ventrociliella romanae* Kolasa, 1977 in the Department of Agrobiology, Poznan; SCHWANK (1979) mentions whole mounts of *Krumbachia paludicola* Schwank, 1979 but did not assign a holotype. KOLASA (1981b) also clearly stated that the holotype of *Adenocerca minima* is not preserved. REISINGER (1924) never mentioned stored specimens and there are strong indications that any existing material from Graz was destroyed during the Second World War (RIEGER 1974). Contacting the Institute of Zoology at Karl-Franzens-Universität in Graz, Austria, the Department of Animal Morphology in Poznan, Poland and the Max-Planck-Institut für Evolutionsbiologie in Schlitz, Germany did not yield any material.

New species and genera described in this study also remain unnamed to prevent the creation of nomina nuda. Neotypes were assigned as this species group is very hard to study and type material is necessary to clear out its taxonomy. Holotypes, paratypes and neotypes were assigned as closely as possible to the ICZN (1999). However, they do not meet the qualifying conditions since material is not deposited in a scientific or educational institution. Therefore, these designations are merely for the purpose of this thesis and cannot be considered valid and should not be considered as such.

Phylogenetic analysis

Animals used for DNA-extraction were stored in 95% ethanol at -20°C. DNA was extracted from entire specimens with the QIAamp DNA Micro Kit® with QIAamp MinElute® columns (QIAGEN) following the added tissue protocol. Extracts were stored for PCR at -20°C.

32 sequences were used in the analysis of the 18S rDNA gene (table 2). Protoplanellinae were represented in the analysis by 18 sequences from 14 different species. Furthermore, twelve other Typhloplanidae, including two sequences of *Carcharodopharynx* were used. Two species of Dalyelliidae – *Castrella truncata* (Abildgaard, 1789) and *Gieysztoria cuspidata* Schmidt, 1861 – were used as outgroups, as the Dalyelliidae are a strongly supported clade closely related to Typhloplanidae (VAN STEENKISTE 2012). The same animals were used for

analysis of the 28S rDNA gene, except for *Protoplanella simplex* Reisinger, 1924 and *Mesostoma lingua* (Abildgaard, 1789), which did not yield a useable sequence of this gene. In addition to the separate analyses, a concatenated analysis of both genes was performed.

Table 2: List of specimens used in phylogenetic analysis

Species	Code	Location
Ascophorinae		
Dochmiotrema limicola	Ext 150	Stekene, Belgium
'Carcharodopharyngidae'		
*Carcharodopharynx arcanus**	DNAWP001BEI	Bavaria, Germany
Carcharodopharynx sp.	Ext 290	Austria
Mesostominae		
Mesostoma lingua (!)	Ext 124	Doñana, Spain
Olisthanellinae		
Olisthanella truncula	Ext 123	Doñana, Spain
Phaenocorinae		
Phaenocora foliacea	Ext 167	Goa, India
Phaenocora n. sp.	219ONT	Ontario, Canada
Protoplanellinae		
Acrochordonoposthia conica	Ext 121	Voeren, Belgium
*Acrochordonoposthia n. Sp.**	DNAWP002GRZ	Graz, Austria
*Acrochordonoposthia sp.**	DNAWP005HES	Hessen, Germany
*Adenocerca minima**	DNAWP004GRZ	Graz, Austria
*Bryoplana sp.**	AL398	Alabama, USA
*Bryoplana xerophila**	Ext 179	Alabama, USA
*Chorizogynopora italica**	DNAWP007HES	Hessen, Germany
*Hoplopera n sp**	DNAWP005GRZ	Graz, Austria
*Krumbachia paludicola**	ONT72	Ontario, Canada
*Krumbachia subterranea**	DNAWP001HES	Hessen, Germany
Olisthanellinella rotundula	DNAWP009	Graz, Austria
*Prorhynchella minuta**	AL405	Alabama, USA
Protoplanella simplex(!)	Ext 623	Doñana, Spain
*Protoplanella simplex**	DNAWP001NPA	National Park 'Hoge Kempen', Belgium
*Protoplanella simplex**	DNAWP002BEI	Bavaria, Germany
*Protoplanella simplex**	DNAWP003NPA	National Park 'Hoge Kempen', Belgium
*Protoplanella sp.**	DNAWP011GRZ	Graz, Austria
Rhynchomesostominae		
Castrada sp.	DNAAH005GRZ	Graz, Austria
Rhynchomesostoma rostratum	Ext 211	Tvärminne, Finland
Typhloplaninae		
*Adenoplea meridionalis**	Ext 735	Lommel, Belgium
*Adenoplea n. sp.**	Ext 736	Liège, Belgium
Typhloplana viridata	Ext 130	Belgium
Dalyelliidae (outgroup)		
Castrella truncata	Ext 615	Ontario, Canada
Gieysztoria cuspidata	Ext 566	Ontario, Canada

(!) Were only used in analysis of the 18S rDNA gene
*** Sequences that were processed by the author**

The 18S and 28S rDNA sequences of *Acrochordonoposthia conica* Reisinger, 1924, *Bryoplana xerophila* Van Steenkiste, Davison & Artois, 2010, *Carcharodopharynx* sp., *Dochmiotrema limicola* Hofsten, 1907, *Mesostoma lingua*, *Olisthanella truncula* (Schmidt, 1858), *Phaenocora n. sp., P. foliacea* Bohmig, 1914, *Protoplanella simplex*, *Rhynchomesostoma rostratum* (Müller, 1773), *Typhloplana viridata* (Abildgaard, 1789), *Castrella truncata* and *Gieysztoria cuspidata* were kindly provided by dr. Niels Van Steenkiste and Albrecht Houben.

Table 3: Primers used for PCR and sequencing

Gene	Primer	Sequence (5'_3')	Function	Reference
18S	TimA	AMC TGG TTG ATC CTG CCA G	PCR/Seq	Norén & Jondelius (1999)
	600F	GGT GCC AGC AGC CGC GGT	Seq	Willems *et al.* (2006)
	600R	ACC GCG GCT GCT GGC ACC	Seq	Willems *et al.* (2006)
	1100F	CAG AGG TTC GAA GAC GAT C	Seq	Nóren & Jondelius (1999)
	1100R	GAT CGT CTT CGA ACC TCT G	Seq	Nóren & Jondelius (1999)
	18S7F	GCA ATA ACA GGT CTG TGA TGC	Seq	Nóren & Jondelius (1999)
	18S7FK	GCA TCA CAG ACC TGT TAT TGC	Seq	Nóren & Jondelius (1999)
	TimB	TGA TCC ATC TGC AGG TTC ACC T	PCR/Seq	Nóren & Jondelius (1999)
28s (part 1)	LSU5	TAG GTC CAG CCG CTG AAY TTA	PCR/Seq	Littlewood *et al.* (2000)
	L300F	CAA GTA CCG TGA GGG AAA GTT G	Seq	Littlewood *et al.* (2000)
	L300R	CAA CTT TCC CTC ACG GTA CTT G	Seq	Littlewood *et al.* (2000)
	L1600F	GCA GGA CGG TGG CCA TGG AAG	Seq	Littlewood *et al.* (2000)
	L1600R	CTT CCA TGG CCA CCG TCC TGC	Seq	Littlewood *et al.* (2000)
	LSUD6-3	GGA ACC CTT CTC CAC TTC AGT C	PCR/Seq	Littlewood *et al.* (2000)
28S (part 2)	1600F	AGC AGG ACG GTG GCC ATG GAA G	PCR/Seq	Lockyer *et al.* (2003)
	U2229	TAC CCA TAT CCG CAG CAG GTC T	Seq	Lockyer *et al.* (2003)
	L2230	AGA CCT GCT GCG GAT ATG GGT	Seq	Lockyer *et al.* (2003)
	L2450	GCT TTG TTT TAA TTA GAC AGT CGG A	Seq	Lockyer *et al.* (2003)
	U2562	AAA CGG CGG GAG TAA CTA TGA	Seq	Lockyer *et al.* (2003)
	L2987	CTG AGC TCG CCT TAG GAC ACC T	Seq	Lockyer *et al.* (2003)
	U3139	AAG TTA CCA CAG GGA TAA CTG GCT	Seq	Lockyer *et al.* (2003)
	L3449	ATT CTG ACT TAG AGG CGT TCA	PCR/Seq	Lockyer *et al.* (2003)

Both the complete 18S rDNA and the 28SrDNA, approximately 1800 and 3300 bp respectively were amplified. The PCR reaction was performed in 25µl PuReTaq Ready-to-Go PCR beads (GE Healthcare) with 3µl DNA extract, 2.5µl of each primer and 17µl purified water. Primers are represented in table 3. The thermal cycling for 18S was initiated with a denaturation of 95°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 90 seconds, with a final phase of 72°C for 5 minutes. The 28S rDNA gene was amplified in two parts. The amplification of the first 1700bp started with a 5 minutes long phase at 95°C. Subsequently, 30 cycles were performed starting at 94°C for one minute, followed by 50°C for one minute and 72°C for 90 seconds. A phase of 5 minutes at 72°C ended the protocol. Amplification of the last 2000 bp of the 28S rDNA gene started with a phase of 94°C for 2 minutes, followed by 30 replications of 30 seconds at 94°C, 30 seconds at 58°C and 2 minutes at 72°C to conclude the amplification with a 7 minute phase at 72°C. The PCR-products were verified on an agarose gel, stained with GelRed^{TM}. The sequencing was conducted by Macrogen.

The sequence reads were assembled and the primers were removed in Geneious Pro 5.3.6 (DRUMMOND *et al.* 2010). A BLAST search (ALTSCHUL *et al.* 1990) was performed on the NCBI-website (http://www.ncbi.nlm.nih.gov) to check for contaminations. MAFFT v. 6 (KATOH *et al.* 2002) was used for aligning the sequences. The Q-INS-i-option, which takes into account the structural information as a form of base-pairing probability, was used (KATOH & TOH 2008 a,b; KATOH *et al*. 2009). Ambiguous sites in the alignment were located with Aliscore v. 2.0 (MISOF & MISOF 2009; KÜCK *et al.* 2010) and cut out by Alicut v. 2.3 (KÜCK 2009). Gaps were treated as fifth characters (–N), were checked for replications (-r) and a sliding window with size 4 was chosen (w=4). The Multiple Sequence Alignments (MSA) were tested for substitution saturation by plotting the transition and transversions rate versus the divergence in DAMBE v. 5.2.30 (XIA *et al.* 2003; XIA & LEMEY 2009). The best model for the analyses was chosen with jModelTest v. 0.1.1 (POSADA 2008). For the 18S-dataset, Tim2+I+G was chosen as the most appropriate model, followed by GTR+I+G (General Time Reversible model with discrete gamma-distributed rate variation among sites and allowing for invariant sites). For both the 28S and the concatenated dataset, the GTR+I+G model was chosen as best model. As the used phylogenetic programs don't support the Tim2+I+G model, the GTR+I+G model was used for all analyses.

A maximum likelihood analysis was carried out with RAxML v. 7.2.8 (STAMATAKIS 2006) by using the graphical interface raxmlGUI v. 1.1 (SILVESTRO & MICHALAK 2011). This program was used because of its fast and robust tree search and bootstrapping algorithms. An analysis with 100 runs and an additional bootstrap with 1000 replications was performed.

Bayesian inference was performed in MrBayes v. 3.2.0 (HULSENBECK & RONQUIST 2001; RONQUIST & HULSENBECK 2003). Two independent runs were carried out, each with 10,000,000 generations and four chains (one cold and three heated chains at default temperature), sampled every 100 generations. The burn-in was set at 25000 (25% of all trees) to ensure that samples were only taken after the parameters had converged.

Taxonomic account

Typhloplanidae Graff, 1905

Typhloplaninae Luther, 1904

Adenoplea Reisinger, 1924

Adenoplea nov. sp. 1

Figure 1 A-D

Material: Three specimens studied alive, one of which sagittally-sectioned and one whole mounted.

Description: Animals measure over 1mm in length. The anterior body end is pointed whilst the posterior end is bluntly rounded. Sometimes a small tail can be observed on swimming animals. In both the anterior and the posterior end very typical glands (tg) were present. They were rod-shaped, like big rhabdites, strongly eosinophilic and lying more or less parallel to each other. Adenal rhabdites can be observed in two tracts that slightly anastomose near the front end of the body. The rhabdoid glands lie just in front of the brain and are arranged in two groups. The distal half of the epidermis is completely filled with coarse, strongly eosinophilic secretions, which are probably dermal rhabdites (dr). The rosulate pharynx is located in the middle of the body and is slightly slanted forwards. It measures 35µm across. The excretory opening was not observed with certainty on the studied specimens, although in other species of *Adenoplea* the protonephridia exit through the mouth.

The ovary is located dorsally. A seminal receptacle is also present within the oviduct. The vitelloduct was not observed. The female duct runs to an atrium that is lined with an epithelium and surrounded by an outer musculature. The gonopore is situated at ±65% of the body.

The paired, different sized testes lie in front of the pharynx and ventral of the vitellaria. One testis is relatively large, whilst the other is barely visible. The vasa deferentia enter the copulatory organ laterally. The copulatory organ measures over 50µm and is surrounded by two layers of diagonal muscles. Large, coarse grained eosinophilic glands are associated with the organ, but it is unclear where they enter. The copulatory organ consists of a proximal half, containing an oval, bulbular seminal vesicle and a distal half, which bears a cirrus (Fig 1 C-D). The more or less straight cirrus measures about 30µm in length and is covered with small spines on its distal part. On its utter distal part, larger spines pointing outward are present. The distal end of the copulatory organ is provided with a sclerotized cone, which envelops part of the cirrus.

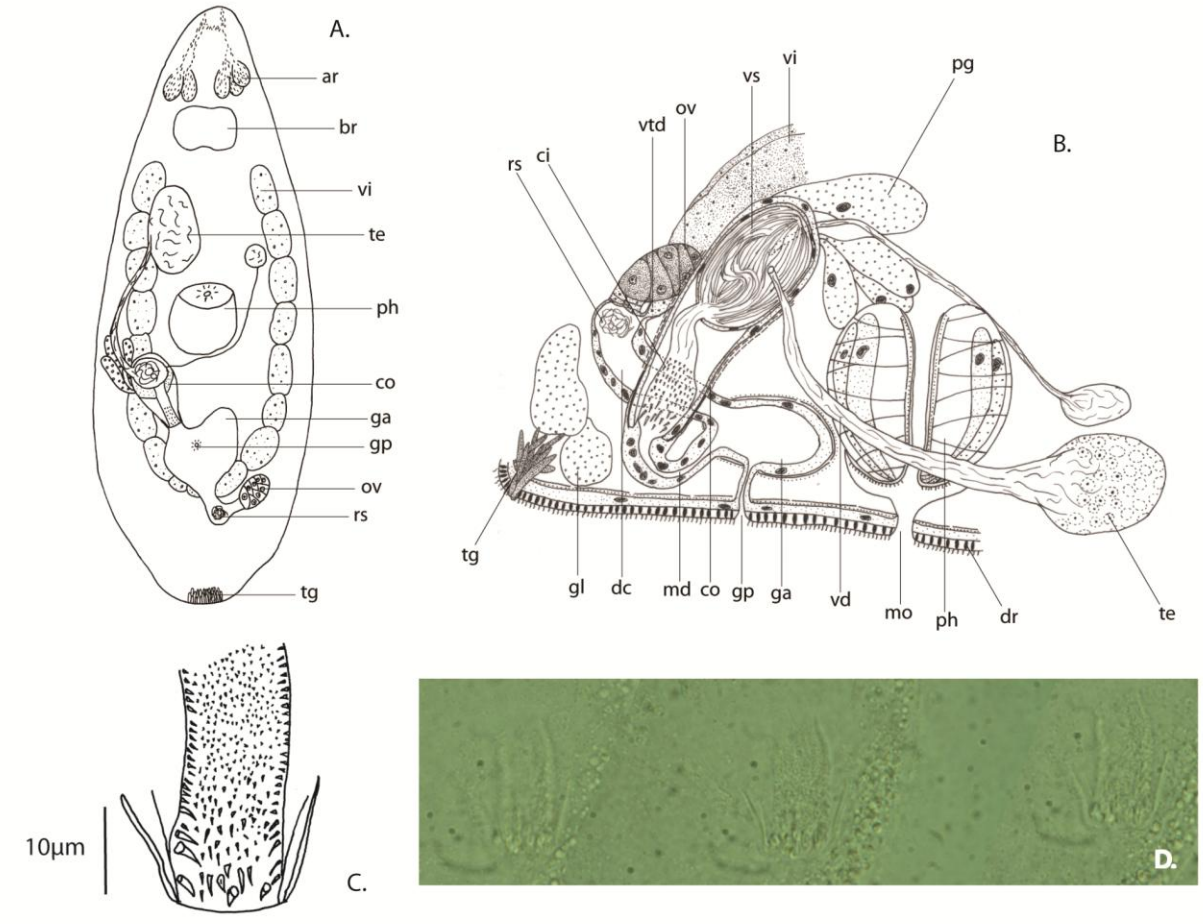


Figure 1: *Adenoplea* nov. sp.: A. habitus of living animal. B. Sagittal reconstruction of the holotype. C. Armed part of the cirrus. D. Armed part of the cirrus photographed at different focus levels.

Discussion: The genus *Adenoplea* is subdivided in two groups (REISINGER 1924). The inermis-type possesses a protrusible, sack-shaped ejaculatory duct. Sperm and secretions exit the organ together. The armata-type, on the other hand, possesses spines on the cirrus. Secretions and sperm exit the organ separately. Because of the typical armed cirrus this species should undoubtedly be classified within the armata-group. The three other species that belong to this group are *A. armata* Reisinger, 1924, *A. paraproxenetes* Reisinger, 1924 and *A. nanus* Sayre & Wergin, 1994. *A. armata* measures about 1mm in length, the copulatory organ measures 50-55µm and contains an S-shaped cirrus with 2.5-3.0µm long hooks at the distal end of the copulatory organ. *A. armata* also lacks a bursa. *A. paraproxenetes* is much larger, measuring 1.5-1.8mm in length. The vasa deferentia are swollen to form seminal vesicles before entering the copulatory organ. The copulatory organ itself measures 120-125µm. However, SCHWANK (1981) reports a size of 170µm. The cirrus is also S-shaped in this species, and covered with 8µm long, flat, lamellar spines in its distal part. On the distal side of the copulatory organ a diverticle containing a complex six-part stylet is present. A large, muscular, slightly sclerotized bursa is present. *A. nanus* is a small species, measuring 0.42-0.59mm and possesses a muscular bursa and a 25-37µm long copulatory organ with an S-shaped cirrus with spines measuring 2.5-3.0µm.

Adenoplea sp. differs from the 3 other species by having a more or less straight cirrus and by possessing dermal rhabdites. Furthermore the testes are of equal size in all other species, whilst those of *Adenoplea* nov. sp. 1. differ significantly. Furthermore, the other species all have a seminal receptacle that is a distinct diverticle, while the receptacle in *Adenoplea* nov. sp. 1 is situated inside the oviduct. In addition, it lacks the diverticle at the distal end of the copulatory organ, in contrast to *A. paraproxenetes* and *A. armata*.

The nature of the rod-shaped glands that were present at both the anterior and the posterior end of the body is unclear. Because of their position on the body they may be adhesive glands. Many glands in the posterior part of the body did not show a very clear association with the copulatory organ and may also be associated with the tail region.

The animals seem to feed on rotifers; Mastaxes were found in the gut.

Diagnosis: Animals about 1mm long. With rod-shaped glands at front and back end. Adenal and dermal rhabdites present. Elongate copulatory organ with bulbus and straight, armed cirrus lacking a diverticle. Large size difference between testes. Long, saccate bursa present. Receptaculum seminis is an accumulation of sperm in the female duct.

Holotype: A sagittally-sectioned specimen (3238), collected on 29 August 2011 at the Kreuzberg near Weyer, Austria (47°51’36”N; 14°39’09”E). Forest soil of *Pinus*, *Sambucus, Corylus* and *Cornus,* together with two other adult specimens.

Paratype: One whole-mounted specimen, same date and locality as holotype (TotoWP008GRZ).

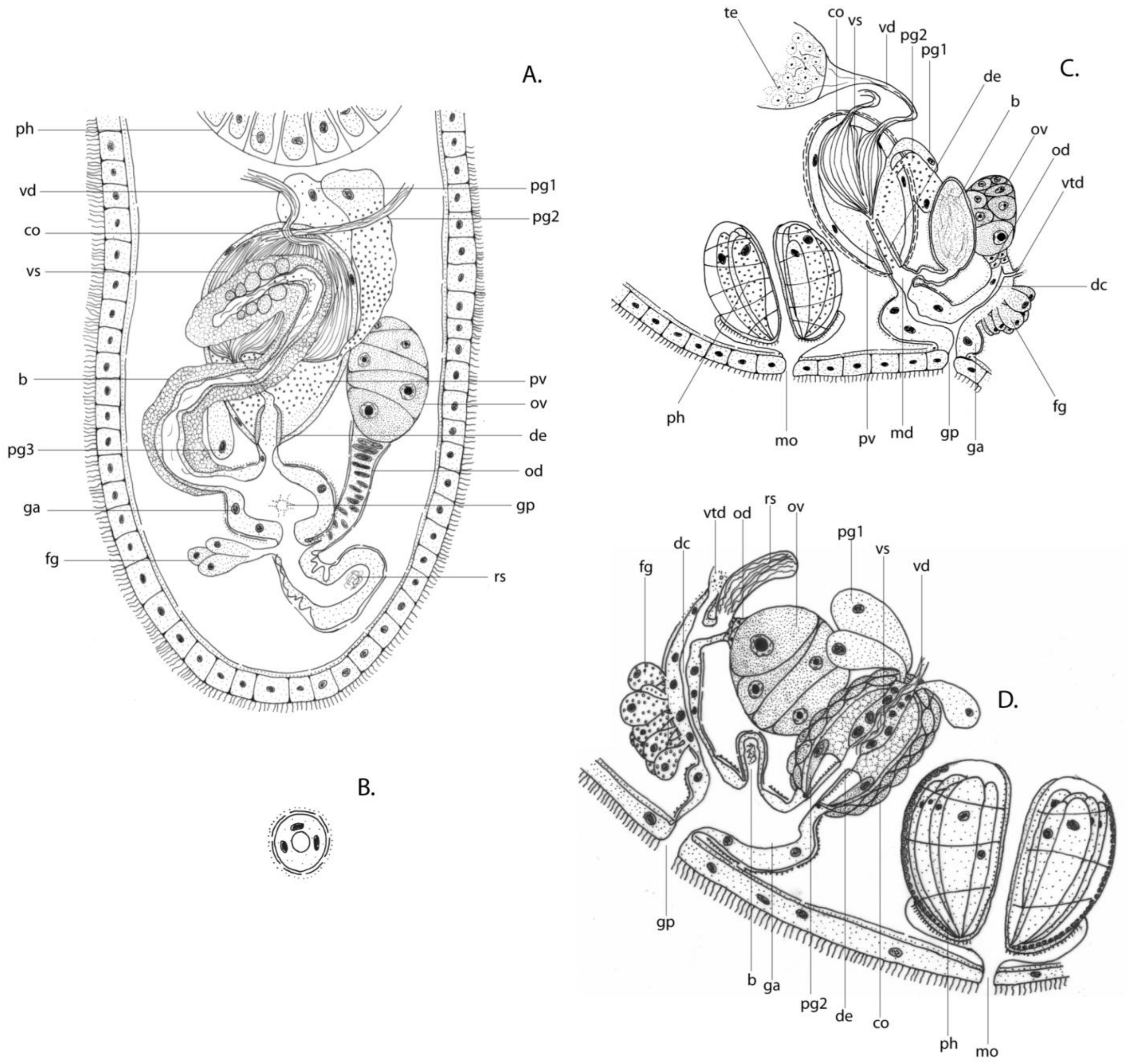


Figure 2: *Adenoplea perigraptopera*: A. Frontal reconstruction of the neotype, ventral view. B. Gonopore; *Adenoplea* nov. sp. 2: C. Sagittal reconstruction of the holotype; *Adenoplea meridionalis*: D. Sagittal reconstruction.

Adenoplea perigraptopera Reisinger, 1924

Figure 2 A-B

Material: One frontally-sectioned specimen.

Description: Animal measures about 2mm in length. The body is slender, 5-6 times as long as wide. Adenal rhabdites occur in two tracts in the front of the body. The rhabdoid glands lie in two groups at ±25% of the body, just behind the brain. A large, weakly stained gland with more or less fine secrete was present just behind the brain. Dermal rhabdites were not observed. The rosulate pharynx lies just behind the middle of the body and has a diameter of 95-100µm. The protonephridia exit through the mouth.

Vitellaria were still developing on the observed specimen and the vitelloduct was not visible. The ovary is connected to the genital atrium by a long oviduct, which is lined with a high epithelium and surrounded by longitudinal muscles. The seminal receptacle is a short, bended evagination of the oviduct with similar musculature. Distal from the receptacle, small, eosinophilic, coarse grained shell glands (fg) are present. The female duct enters the atrium from its dorsal side. The genital atrium is lined with a high epithelium and surrounded by an inner layer of longitudinal muscles and an outer layer of circular muscles and is connected to the gonopore (fig. 2 B), situated at ±80% of the body, by a short gonoduct, which possesses a similar epithelium and musculature.

The large, paired, egg-shaped testes lie in front of the pharynx. The vasa deferentia enter the copulatory organ separately at the proximal end. The egg-shaped to oval copulatory organ measures 77µm in length. Coarse grained eosinophilic (pg1, pg2) and fine grained basophilic prostate glands (pg3) enter the organ laterally. The proximal half contains a bulbular seminal vesicle, filled with sperm. The distal part holds a prostate vesicle, which is filled with large granules of basophilic secrete and a short, muscular and slightly sclerotized ejaculatory duct. The copulatory organ is connected to the atrium by a very short male duct. The bursa is an elongate, narrow muscular sac with numerous bends and is surrounded by a loose matrix. Its length clearly exceeds that of the copulatory organ. It is covered with circular muscles in its distal 90%. At its proximal end, strong spiral muscles are present. The bursa is directly connected to the genital atrium.

Discussion: The shape of the copulatory organ and the short ejaculatory duct are typical of species belonging to the group of *A. inermis* Reisinger, 1924. *A. inermis* does not possess a bursa. Furthermore the structure of the bursa of *A. pinguis* Reisinger, 1924, *A. weyeri* An der Lan, 1955 and *A. meridionalis* Kolasa, 1981d is completely different from that of this species. The structure of the bursa is the most typical characteristic of the *A. perigraptopera* and makes identification of this species straightforward.

Remark: As the type material is believed to be lost and type material is considered essential to clarify the taxonomy, a neotype was assigned in accordance to article 75 of the ICZN (1999). Intensive searches near the original type locality in Graz did not yield any specimens, but a specimen was found in Belgium. As other records (Schwank 1981) suggest that this species has a wide range and because of the high confidence in the identity of our specimen, it is assigned the status of neotype.

Known distribution: In the vicinity of Graz, Austria, common in forest soil (Reisinger 1924). Schlitz, Hessen, Germany in moss in helocrene springs (SCHWANK 1981).

Neotype: A frontally-sectioned specimen (2284) found in Vyele-et-Tharoul, Liege, Belgium (50°27’6”N; 5°16’46”E), single specimen in moss, collected by Kevin Thijssen on 18 August 2008.

Adenoplea **nov. sp. 2**

Figure 2 C

Material: Three sagittally-sectioned animals.

Description: Animals measure 1mm in length. Living animal has a white-yellowish colour. Behind the brain, a group of basophilic glands is present. Adenal rhabdites are present in the anterior part of the body. The rhabdoid glands lie in two groups. Dermal rhabdites were not observed. The rosulate pharynx has a diameter of ±55µm and is positioned at ±65% of the body. Protonephridiopores were not observed, but should exit through the mouth, like in all other species in the genus.

The vitellaria reach forward to the level of the rhabdoid glands. The vitellaria fuse to form a single vitelloduct. The ovary is connected to the atrium by a long female duct which runs to the posterior end of the animal and makes a slight bend to end in the atrium. The oviduct meets the vitelloduct just before the turn. A large group of coarse grained eosinophilic shell glands (fg) is present in the bend of the ductus communis. The atrium is lined with a high epithelium and surrounded by muscles. The gonopore is situated at ±90% of the body.

The large, oval, paired testes lie in front of or lateral to the pharynx and ventral to the vitellaria. The vasa deferentia enter the copulatory organ separately at its proximal end and swell before opening in the organ. At least two types of glands are associated with the copulatory organ. First there are strongly stained eosinophilic glands (pg2) with coarse grained secretions, which enter the organ at its proximal end. Furthermore basophilic glands (pg2) with finer grained secretes and a weakly coloured gland with coarse grained secretions are present. The oval copulatory organ measures 60-65µm and is surrounded by two layers of diagonal muscles. The proximal part of the organ holds a spindle-shaped seminal vesicle. The distal half holds a prostate vesicle containing coarse grained secretions and an ejaculatory duct surrounded by longitudinal muscles. The male duct is a short, narrow channel, surrounded by a layer of circular muscles. The oval bursa is slightly smaller than the copulatory organ and completely surrounded by circular muscles. It ends in the distal part of the male duct.

Discussion: The specimens clearly belong to the genus *Adenoplea*, and more specifically the *A. inermis*-group because of the structure of the copulatory organ, which is similar to that of *A. perigraptopera*, except for its smaller size, longer ejaculatory duct and spindle-shaped seminal vesicle. It differs from *A. inermis* Reisinger, 1924, which does not possess a bursa. The bursa of *A. meridionalis* Kolasa, 1981d is much smaller and the copulatory organ of this species is surrounded by strong spiral muscles and possesses a longer ejaculatory duct, a smaller seminal vesicle and a much smaller prostate vesicle. *A. perigraptopera* Reisinger, 1924 is much bigger and possesses a very elongate bursa with strong diagonal muscles at its proximal end. *A. weyeri* An der Lan, 1955 also differs in the structure of the bursa, which is much more elongate and possesses many crypts. Furthermore, the ejaculatory duct of the latter is much shorter. *A. pinguis* Reisinger, 1924 has a shorter ejaculatory duct, a sclerotized bursa and a large seminal receptacle. The specimens cannot be classified as an existing species and should be considered as a new species.

Diagnosis: 1mm long animal with yellowish colour. Adenal rhabdites present in two tracts. Female system with bended female duct. Seminal receptacle lacking. Copulatory organ with at least two types of prostate glands. Spindle-shaped seminal vesicle. Muscular ejaculatory duct in distal third of copulatory organ. Elongate, oval bursa completely surrounded by circular muscles, opens in male duct.

Holotype: A sagittally-sectioned specimen (2286) found in Vyele-et-Tharoul, Liege, Belgium (50°27’6”N; 5°16’46”E), together with several specimens in moss, collected by Kevin Thijssen on 18 August 2008.

Paratypes: A transverse-sectioned specimen (2285) and a sagittally-sectioned specimen (2290) same date and locality as holotype.

Adenoplea meridionalis Kolasa, 1981d

Figure 2 D

Material: One frontally-sectioned and three sagittally-sectioned animals.

Description: The animals measure 1mm in length. The outer part of the epidermis of sectioned animals is only weakly stained, while the inner two thirds are strongly basophilic. Adenal rhabdites are present in the anterior end of the body. Dermal rhabdites were not observed. The rosulate pharynx has a diameter of 60-65µm and lies behind the middle of the body. The protonephridia open in the mouth.

The vitellaria reach to the middle of the body and fuse at the posterior end to form a single vitelloduct. The ovary is more or less round. The oviduct is very short and fuses almost immediately with the vitelloduct. At the same point the seminal receptacle is connected to the female duct. It is shaped as a long, slightly crescent stalk, filled with sperm. The long ductus communis is provided with coarse grained eosinophilic shell glands. It is connected to the genital atrium, which is delineated with a high epithelium and surrounded by muscles. The gonopore is situated at ±80% of the body.

Testes were not observed with certainty. The vasa deferentia fuse before entering the organ. The egg-shaped copulatory organ measures 35µm in length and is surrounded by two layers of strong spiral muscles. Large, basophilic prostate glands (pg1) enter the copulatory organ on its proximal side. Furthermore, small basophilic glands (pg2) are present inside the distal part of the copulatory organ. The proximal part of the organ holds a small seminal vesicle. Distally a muscular, sclerotized ejaculatory duct is present. The sclerotization seems to consist of two laminae. The ejaculatory duct can be closed by a strong sphincter. The male channel is very short and connects the copulatory organ with the atrium. The 15µm long bursa possesses a thin wall and is surrounded by weak longitudinal muscles. The bursa opens directly in the genital atrium.

Discussion: The animals match the description of *A. meridionalis*. Other species of the *Adenoplea inermis*-group lack a bursa, or hava a much larger, differently shaped bursa. Furthermore, the structure of the copulatory organ, which contains a relatively long ejaculatory duct, a small seminal vesicle and a wall surrounded very strong spiral muscles, differs completely from other species in the genus. It was not feasible to get the type material in time, so further comparisons with type material will be needed to get absolute certainty about the identity of our specimens.

Known distribution: Fosso Contesora, Italy, found in mosses on the banks of a helocrene spring (KOLASA 1981d).

New locality: The ‘Lommelse Sahara’, Lommel, Belgium (51°14’48”N; 5°17’46”E), together with several other specimens in moss in a pine forest, collected by Kevin Thijssen on 23 July 2008.

“Carcharodopharyngidae” Bresslau, 1933

Carcharodopharynx Reisinger, 1928

Carcharodopharynx arcanus (Reisinger, 1924), Reisinger, 1928

Synonym: *Acanthopharynx arcanus* Reisinger, 1924
Figure 3 A-D

Material: Three adult specimens studied alive, one of which sagittally-sectioned, many juvenile individuals.

Description: Animals measure 0.8-1.5mm in length. Animals are usually very dark due to gut contents. The anterior body end is rounded. The posterior end possesses a small tail. Small, eosinophilic tail glands were observed. Adenal rhabdites are lacking. Small, closely spaced dermal rhabdites were present all over the epidermis. The 75µm long, strongly modified pharynx (fig 3 C-D) is situated at ±25% and it is pointing forward. It is divided in two parts: the 48µm long posterior part (pp) is similar to that of a normal rosulate pharynx. The anterior part (ap), however, is narrowed and its lumen forms a channel that is covered with sclerotized spines (ssp). The 27µm long anterior part contains many retractor muscles (rm). On living animals it was often observed that animals retracted the anterior part (fig 3 C), so the shape of the channel could vary between a short and broad trunk and a very elongate and narrow channel. The pharynx is surrounded by two muscular layers: an inner circular layer (cm) and an outer longitudinal one (lm). The lumen of the posterior part of the pharynx is surrounded by a layer of circular muscles (cm) and a layer of longitudinal muscles (lm). No such musculature was observed in the anterior part. The spiny channel exits in the prepharyngeal cavity (ppc), which is surrounded by a layer of thin longitudinal muscles. The mouth (mo) has a subterminal position. Protonephridia exit the body through a pair of nephridiopores, which are situated at ±35% of the body.

Most organs of the genital system are situated in the middle of the body. The vitellaria extend from the anterior end to ±40% of the body and fuse at their anterior end. They are located dorsally, but reach to the ventral part of the body at their anterior end. The ovary lies ventrally. The short oviduct is very broad and possesses a high epithelium. After fusing with the unpaired vitelloduct to form the ductus communis, it quickly narrows.

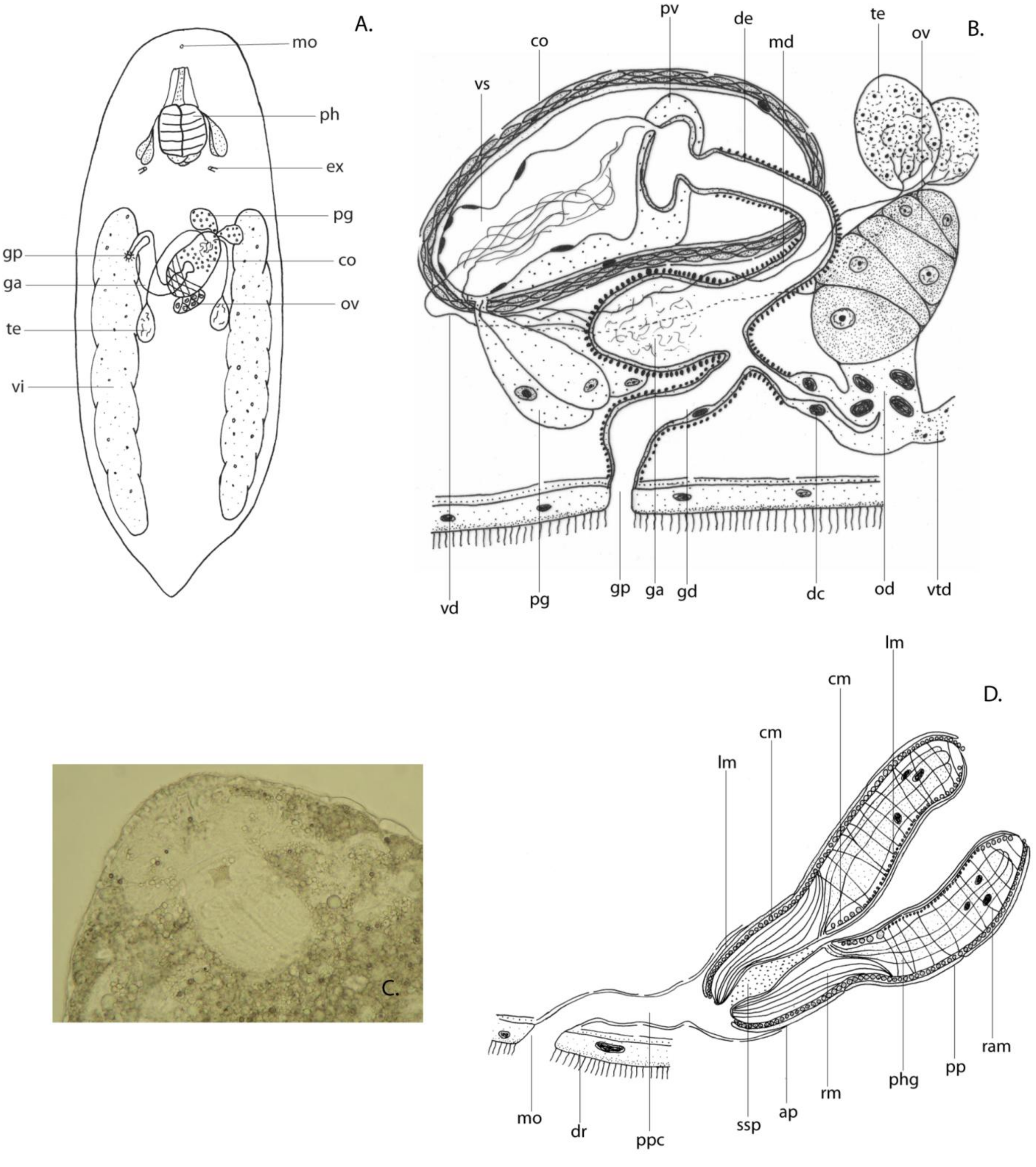


Figure 3: *Carcharodopharynx arcanus*: A. Habitus of living animal. B. Sagittal reconstruction of neotype. C. Pharynx photographed at magnificationa 10x40. D. Sagittal reconstruction of the pharynx.

The distal half of the ductus communis is very narrow and possesses strong circular muscles. The atrium (ga) is surrounded by slightly stronger circular muscles and is filled with a compact, eosinophilic mass. The female duct seems to fuse with the rest of the genital system distal from the atrium. A long gonoduct (gd), surrounded by circular muscles, is present. The gonopore is situated at ±50% of the body.

The small, round testes lie at ±60% of the body and ventral to the vitellaria. The vasa deferentia enter the organ separately at its proximal end. The 57µm long, oval shaped copulatory organ is surrounded by two strong layers of spiral muscles and a thin outer layer of longitudinal muscles. Large, eosinophilic, fine grained glands enter the copulatory organ proximally. The prostate vesicle is filled with these secretions. Furthermore, a long seminal vesicle is present. The distal 1/3 of the copulatory organ possesses a broad ejaculatory duct. It is surrounded by a layer of circular muscles. At its proximal 20% it possesses a slight constriction and at the proximal end a broad pocket, which receives secretions and sperm, is present. The male duct possesses the same circular musculature as most channels in the genital system. A bursa was not observed

Discussion: *C. arcanus* cannot be mistaken for any other species because of its typical pharynx. Because of its peculiar structure, the species is often classified in its own family. Molecular evidence, however, suggests that the species should be classified in Typhloplanidae. Our specimens were completely consistent with the animals from the original description (REISINGER 1924). Despite the fact that only one species is described within the genus, the specimens collected by SCHWANK (1981) differ in having a cirrus that is completely covered with small spines. These specimens might represent a – for now – undescribed species.

Remarks: The neotype was collected in Bavaria, Germany, as the material from our sampling campaign in Graz was of poor quality. Because of the large range of the species, the relatively small distance between the old type locality and the locality of the neotype and the certitude of the identity of the specimen, it is considered legitimate to assign a neotype from this locality.

Known distribution: Graz, Austria, widespread in forest soils (REISINGER 1924), Pallanza, Italy in forest soils (STEINBÖCK 1951), Tvärminne, Finland in mosses (LUTHER 1963), Sierra de Carzola and Finca Caleron, Spain (AN DER LAN 1963), Schlitz, Germany, in beech litter near helocrene springs (SCHWANK 1981), Carpathians, Poland (KOLASA in SCHWANK 1981).

Neotype: Sagittally-sectioned specimen (3497), collected in Oberau, Bavaria, Germany (47°33’33”N; 11°6’57”E) in forest litter, on 13 July 2011, together with one other adult specimen.

Other new localities: Kordel, Germany (49°49’24”N; 6°38’6”E) one juvenile specimen in mosses growing on a wall, on 24 July 2011.

In the vicinity of Graz, Austria, a juvenile animal in humus of a beech forest on the Hohe Rannach (47°9’53”N; 15°24’50”E) and two juvenile specimens in humus at the Rohrerberg (47°7’44”N; 15°24’53”E), on 26 August 2011.

At the Kreuzberg, Weyer, Austria, one adult specimen in humid mosses and one juvenile animal in leafy humus (47°51’36”N; 14°39’9”E), on 29 August 2011.

Hasselt University, Diepenbeek, Belgium (50°55’37”N; 5°23’9”E) one juvenile specimen in moss growing on a concrete bridge, on 22 May 2012.

Protoplanellinae Reisinger, 1924

Adenocerca Reisinger, 1924

Adenocerca minima Kolasa, 1981b

Figure 4 A-D

Material: Four specimens studied alive, one of which whole-mounted and one frontally-sectioned.

Description: The species measures 0.6mm in length. The anterior body end is slightly truncated, while the posterior end usually possesses a small tail. Adenal rhabdites are large and sharp, and are most prominently visible in two tracts. Rhabdoid glands lie in two groups behind the brain. Small dermal rhabdites were present all over the body. The rosulate pharynx measures 33µm across and is located just behind the middle of the body. It is a little slanted forward in some individuals. The protonephridiopores are situated lateral to the pharynx.

The vitellaria reach up to the anterior third of the body and fuse at their posterior end. The vitelloduct was not observed with certainty, but is probably unpaired. The large ovary lies dorsally at ±80% of the body. A seminal receptacle is present in the oviduct, right next to the ovary. The female duct opens in the atrium through its dorsal wall. The atrium is lined with a high epithelium. The gonopore is located at 80% of the body.

Two small testes are located behind the pharynx, at 65-70% of the body. The vasa deferentia enter the 28µm long copulatory organ laterally. The pear-shaped copulatory organ contains a round seminal vesicle in its proximal part and a cirrus in its distal part. The more or less straight cirrus is provided with small, basophilic prostate glands, which do not reach out of the copulatory organ. The distal half of the cirrus possesses small spines. At the distal end, a crown of 6µm long, crescent spines (sh) pointing in distal direction is present. In one specimen, six spines were counted, however could not be confirmed if this number is fixed for all individuals. The most distal part of the copulatory organ is sclerotized and forms a cone around the crown spines. The male duct is very short and the copulatory organ opens almost directly in the atrium.

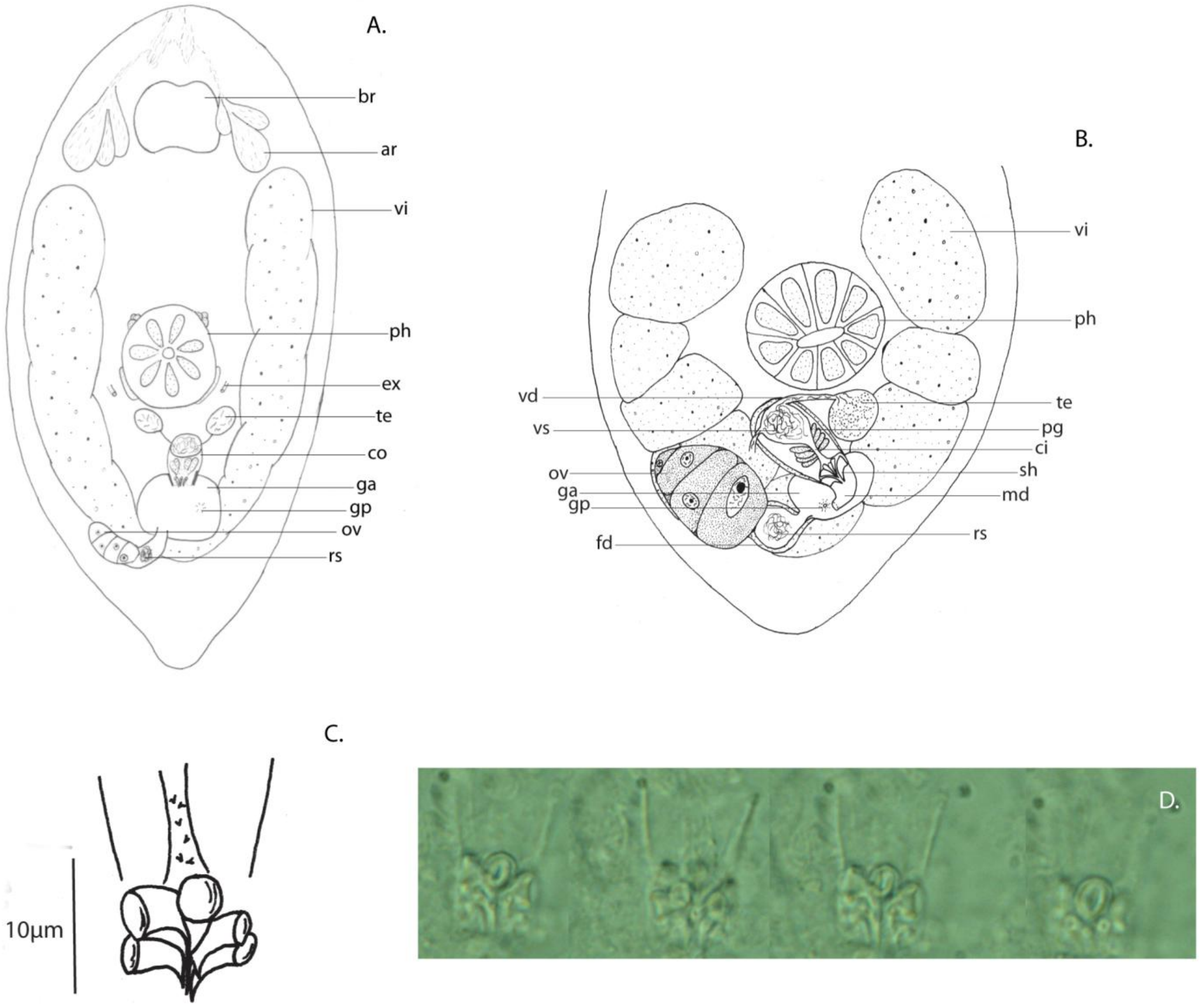


Figure 4: ***Adenocerca minima*****: A. Habitus of living animal. B. Schematic reconstruction, ventral view. C. Armed cirrus of the neotype. D. Armed cirrus of the neotype photographed at different focus levels.**

Discussion: The individuals clearly belong to the genus *Adenocerca* because of the posterior position of the pharynx, the position of the testes, the absence of a bursa and the shape of the copulatory organ. There is a clear resemblance with *A. bresslaui* Reisinger 1924. The hooks in the copulatory organ are, however, arranged in a different way: the 4-6 most proximal hooks are the biggest in *A. bresslaui*; the distal spines are much smaller. *A. clinopharynx* Reisinger 1924 and *A. teshirogii* Kolasa 1981a do not possess large spines in the copulatory organ. The specimens are considered as *A. minima* because of the conspicuous crown of hooks in the copulatory organ. The original description was based on only one specimen, which was not preserved. The specimen examined by KOLASA (1981b) possessed a crown of 7 hooks, but like in *A. bresslaui*, the number of hooks in the copulatory organ might not be fixed. Furthermore this specimen was only 300µmm long, about half the size of the individuals from this study. Except for these differences the specimens completely match the original description of *A. minima* and therefore are considered the same species.

The animal seems to feed on rotifers. Mastaxes were found in the gut of several specimens.

Remarks: KOLASA (1981b) explicitly stated that no material was preserved. The original type locality is the Fosso Contesora in Northern Italy, whilst our specimens were collected near Graz, Austria, 500km away from the original type locality. The animals were found in a very similar habitat and are morphologically identical. For this reason a neotype is assigned in accordance with the ICZN (1999).

Known distribution: Fosso Contesora, Italy, in wet mosses on the banks of the stream (KOLASA, 1981b).

Neotype: Whole-mounted specimen (TotoAH007GRZ)**,** found between Glashütten and Trahütten, south of Graz, Austria (46°49’46”N; 15°06’09”E), together with three other specimens in humid moss in a small stream in a pine forest, on 23 August 2011.

Acrochordonoposthia Reisinger, 1924

The genus *Acrochordonoposthia* consists of nine known species, all described from Europe. Animals usually measure around 1mm in length. All species possess very inconspicuous adenal rhabdites at their anterior body end. Dermal rhabdites are present over the complete body surface, but are very small and can only be observed on prepared animals. The rosulate pharynx is clearly slanted forward and is situated at 30-35% of the body. The paired protonephridiopores are located posterior to the pharynx in most species.

Vitellaria reach up to the level of the pharynx and fuse over a broad anastomosis at their posterior end to form an unpaired vitelloduct. A short oviduct departs from the ovary and fuses almost immediately with the vitelloduct. In some species a seminal receptacle is present inside the oviduct. The ductus communis opens in the genital atrium, which is lined by epithelium and surrounded by a weak muscular layer. The gonopore is usually situated at 75-80% of the body.

The round to oval testes are usually relatively small. Vasa deferentia enter the copulatory organ laterally. Prostate glands may be associated with the copulatory organ. The copulatory organ varies in size, depending on the species. It is an oval to elongated structure, surrounded by muscles of unknown orientation. It contains a round seminal vesicle in its proximal part and a cirrus in its distal part. Both compartments are separated by a septum. The cirrus can be straight or bended, depending on the species. The pattern of the sclerotized spines also differs with the species. At the distal end, the copulatory organ is sclerotized to form a cone around the distal end of the cirrus. The male duct is relatively long and opens in the genital atrium. Some species possess a bursa, the structure of which depends on the species.

Because the identification to the species level is not always straightforward, a key containing all eight described species and one new species was added.

Key to the species of the genus Acrochordonoposthia

1. a) Huge copulatory organ (over 400µmm).
A. robusta **Luther, 1963**
b) Copulatory organ much smaller (up to 70µm). 2

2. a) Animals very small (< 200µm). Copulatory organ oval, small and with a short cirrus. ***A. ramolia*** **An der Lan, 1958**
b) Animals bigger (700-1100µm). Copulatory organ more elongated, bigger and with longer cirrus. 3

3. a) Bursa present. 4
b) Bursa absent. 8

4. a) Eight cuticular crown spines, divided in two groups of four at the distal end of the cirrus.
A. diademula **Schwank, 1980**
b) Crown spines absent. 5

5. a) Cirrus bended and covered with spines, except for most distal 20-30%. Large bursa, stalk provided with strong sphincters.

A. reversa **Reisinger, 1924**

b) Distal part of the cirrus always covered with spines. 6

6. a) Cirrus straight, completely covered with spines. Distal spines larger than proximal ones. Strongly sclerotized bursa, surrounded by circular muscles.

Acrochordonoposthia **nov. sp.**

b) Cirrus with one or two bends. Only distal 2/3 covered with spines. Saccate bursa, weakly sclerotized. 7

7. a) 3 pairs of prostate glands present. No constriction present between head and rest of the body.

A. conica **Reisinger, 1924**

b) 1 pair of large prostate glands present. Constriction present between the head and the rest of the body.

A. ophiocephala **Reisinger, 1924**

8. a) Very long, strongly wound cirrus. No seminal receptacle present. Prostate glands not clearly visible.

A. apopera **Reisinger, 1924***

b) Short, straight cirrus. Seminal receptacle present. Prostate glands despersed in little groups.

A. nemoralis **Reisinger, 1924**

*SCHWANK (1980) mentions animals showing strong similarities with *A. apopera*, but possessing a large bursal cavity.

Acrochordonoposthia conica Reisinger, 1924

Figure 5 A-E

Material: Seven animals studied alive, one of which sagittally-sectioned, three of which whole mounted. Two additional sagittally-sectioned specimens.

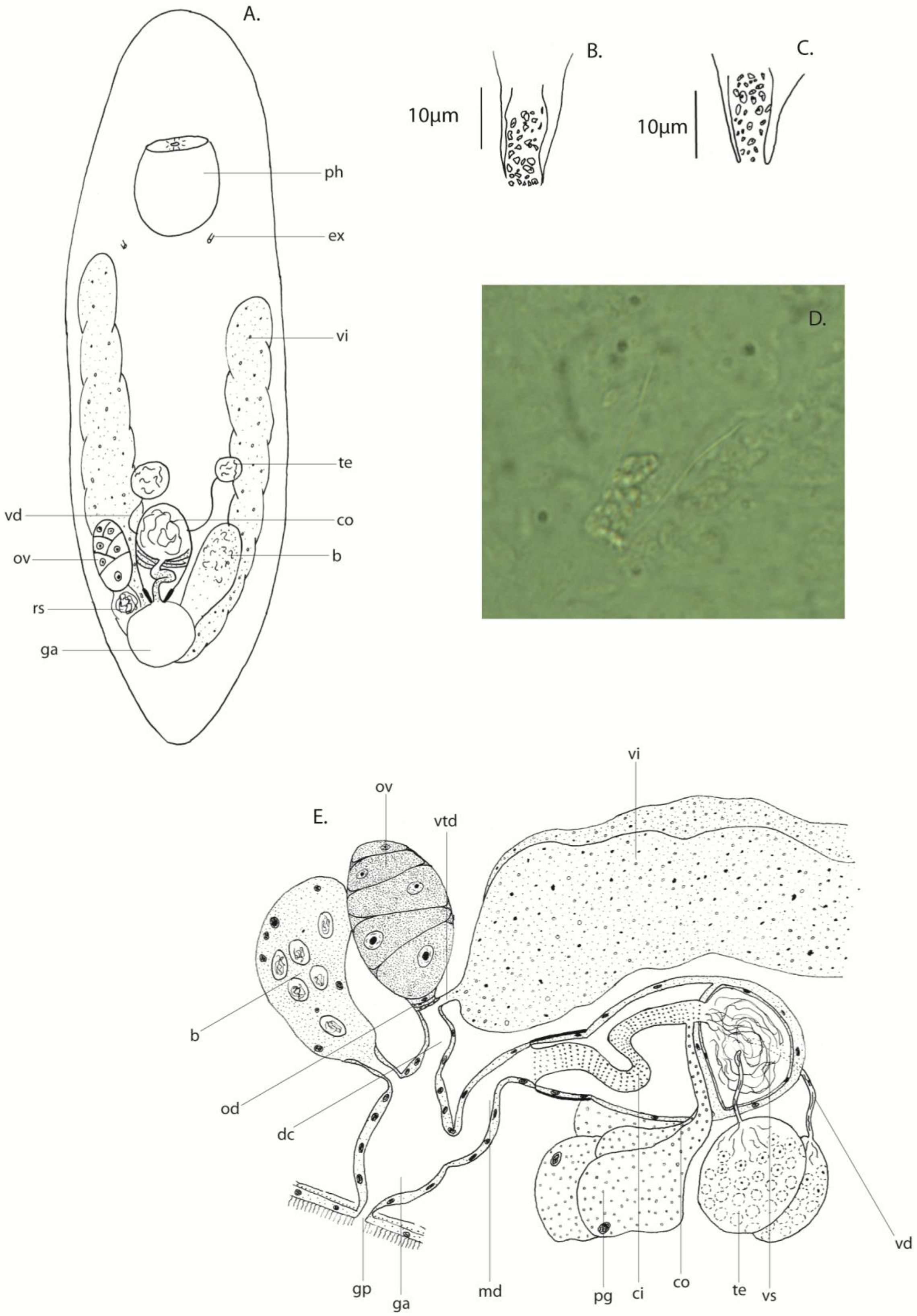


Figure 5: ***Acrochordonoposthia conica*****: A. Habitus of living animal. B. Armed cirrus of the neotype. C. Armed cirrus of another whole mounted specimen. D. Photograph of armed cirrus of neotype. E. sagittal reconstruction of genital system.**

Description: Animals measure 0.8-1.0mm in length. The anterior end is somewhat pointed. The posterior end is rounded. The length to width ratio is about 5 to 6. A seminal receptacle is present in the oviduct. The copulatory organ measures 30µm in length. Three pairs of large, coarse grained, eosinophilic prostate glands end in the proximal part of the cirrus. The 18µm long cirrus contains two bends and is covered with small spines in its distal two-thirds. The large, sac-shaped bursa contains many vacuoles in its wall and is slightly sclerotized. It is connected to the genital atrium by a short duct.

Discussion: The cirrus, sack-shaped bursa and the presence of three pairs of prostate glands are diagnostic for *A. conica*.

On the specimens from Voeren, Belgium, no seminal receptacle was observed. Furthermore, the whole cirrus of this species is covered with spines, instead of only the distal two thirds. This specimen is provisionally identified as *A. conica*, but might represent a new species.

This species seems to be the most common limnoterrestrial rhabdocoel in Europe, with a known range extending from Italy to Greenland. The species seems to feed on rotifers, as mastaxes were observed in the gut of several specimens.

Known distribution: Several localities in Austria, in mosses and moist forest soil (REISINGER 1924; AN DER LAN 1963), Godhavn, Greenland, in damp mosses (STEINBÖCK & REISINGER 1931), Långskär and Tvärminne in Finland, in moist mosses (LUTHER 1963), several localities in Germany, Italy (Papi 1952, 1959) and France (AN DER LAN 1963), Poznan, Poland, in moist leaf fall (KOLASA 1971).

New localities: Nuropstraat, Voeren, Belgium (50°45’38”N; 5°52’21”E), two specimens in moss growing on a tree, collected by Kevin Thijssen on 2 March 2009.

Oberau, Bavaria, Germany (47°N33’33”N; 11°6’57”E), three specimens in forest litter, on 13 July 2011.

Hohe Rannach, Graz, Austria (47°9’53”N; 15°24’50”E), a single specimen in humus of a beech forest.

On the Kreuzberg, Weyer, Austria (47°51’24”N; 14°39’14”E), two specimens in moss growing on a dead trunk, on 29 August 2011.

Hasselt University, Diepenbeek, Belgium (50°55’37”N; 5°23’9”E), a single specimen in moss growing on a concrete bridge, on 22 May 2012

Acrochordonoposthia nemoralis Reisinger, 1924

Figure 6 A-D

Material: Five specimens studied alive, two of which were whole mounted and two were sagittally-sectioned.

Description: Animals reach a length of ±0.7mm. The anterior end is slightly rounded; the posterior end possesses a small tail. A small seminal receptacle is present in the oviduct. The copulatory organ measures ±30µm in length. The distal part bears a 20-25µm long cirrus. It is straight, although in some specimens it contains a bend in the proximal half. The distal half is provided with small spines, which grow bigger at the distal end. Several groups of small, basophilic prostate glands enter the cirrus. The animals do not possess a bursa.

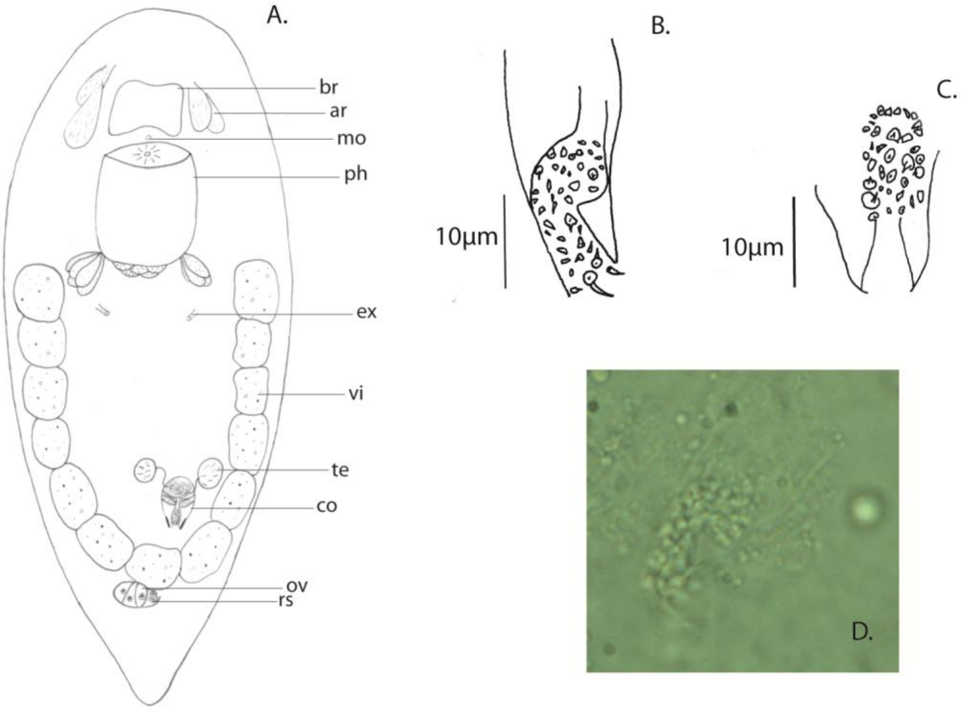


Figure 6: ***Acrochordonoposthia nemoralis*: A. Habitus of living animal. B. Armed cirrus of the neotype. C. Armed cirrus of other whole mounted specimen. D. Photograph of armed cirrus of the holotype.**

Discussion: The species is easily recognizable because of the absence of a bursa and the small, straight cirrus. In some specimens the cirrus seemed to possess a small bend at its proximal end, in contrast to the animals of Reisinger (1924) and Luther (1963), which had a more or less straight cirrus, but this bend was much less pronounced than in other species, like *A. conica*.

The animals seem to feed on rotifers.

Known distribution: In the vicinity of Graz, in moss (REISINGER, 1924).
Near Hanko, Finland, in moss (LUTHER, 1963).

Neotype: A whole-mounted specimen (TotoWP005GRZ) on the Platteberg, Graz, Austria (47°6’49”N; 15°28’9”E), together with four other specimens in moss, on 23 August 2011.

Acrochordonoposthia reversa Reisinger, 1924

Material: Two animals studied alive, both whole-mounted

Description: Animals reach a length of 0.8mm. The anterior end is bluntly rounded; the posterior end is provided with a small tail. In contrast to other species of this genus, the protonephridiopores are located anterior to the pharynx. A seminal receptacle was not observed. The copulatory organ measures 60-70µm in length. The cirrus (Fig 7 A-E) is long and contains three to four clear bends in its proximal part, but straightens on the whole-mounted specimens. It is almost completely covered with spines (ss) pointing to the distal end. Only the most distal 20% of the cirrus does not possess any spines. The most proximal spines are a little smaller than those on the distal part of the cirrus. At the distal 65% of the cirrus a few larger hooks (sh) are present. The cirrus is at its widest at 33% of its length. At 65% it suddenly narrows. A group of prostate glands (pg) enter the cirrus at its proximal end. A sac-shaped organ situated next to the copulatory organ probably serves as the bursa (b?), but its precise structure could not be observed.

Discussion: The shape of the cirrus and the organization of the spines are typical for *A. reversa*. The large hooks at 65% of the cirrus and the lack of spines on the most distal part are unique for this species. In its original description a large bursa, shaped like a long-stalked pear and provided with 5-6 strong sphincters around its stalk is mentioned. This structure was not observed with certainty on our specimens but might be present, as a bursa-like structure was observed. Despite this, the specimens could probably be classified as *A. reversa* because of the very typical structure of the cirrus, but the designation of a neotype is renounced because of the lack of information on the bursa.

The animals seem to feed on rotifers.

Known distribution: Graz, Austria, in mosses (REISINGER, 1924), doubtful record from Hessen, Germany in damp mosses (SCHWANK, 1981).

New locality: Kreuzberg, Weyer, Austria (47°51’36”N; 14°39’9”E), two specimens in moss growing on a dead trunk, on 29 August 2012.

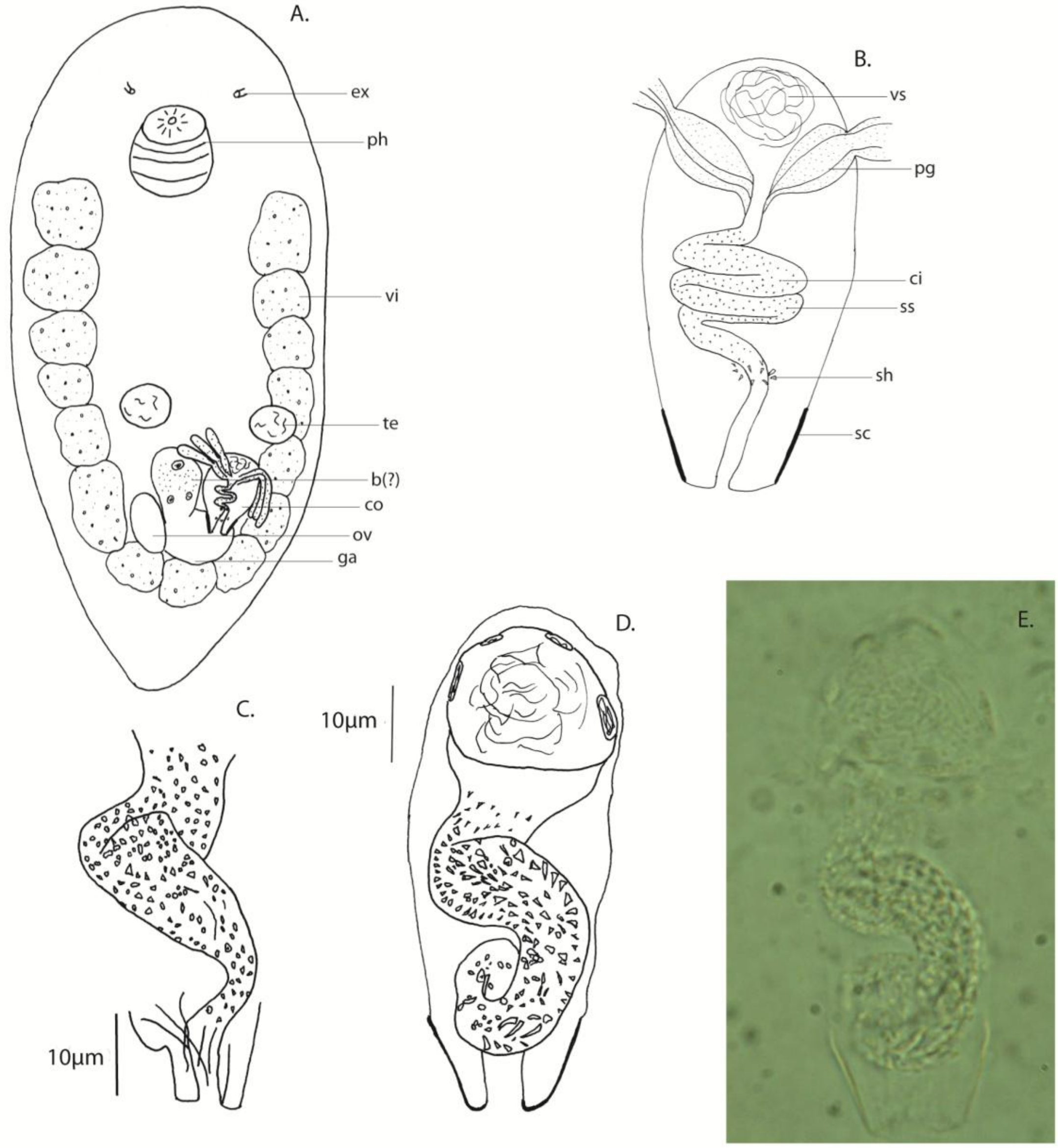


Figure 7: ***Acrochordonoposthia reversa*****: A. Habitus of living animal. B. Copulatory organ on living animal. C. Armed cirrus of whole mounted specimen. D. Copulatory organ of other whole mounted specimen. E. Photograph of copulatory organ of whole mounted specimen.**

Acrochordonoposthia nov. sp.

Figure 8 A-D

Material: Seven specimens studied alive, one of which was sagittally-sectioned , one frontally-sectioned and one whole-mounted.

Description: The animals reaches a length of almost 1mm. The anterior end is pointed, whilst the posterior end is more rounded. A seminal receptacle is lacking. The copulatory organ measures 63-72µm in length. The distal part, which holds the cirrus, is surrounded by circular muscles.. The 25-35µm long cirrus (Fig 8 C-D) is more or less straight and is covered with spines that are increasingly large at the distal end and point towards the proximal end. A large group of eosinophilic prostate glands containing coarse grained secretions enter the copulatory organ from the sides and open in the proximal part of the cirrus. A 50-55µm long, sclerotized, more or less cone shaped bursa lies ventral to the copulatory organ. It is surrounded by strong circular muscles all over its length.

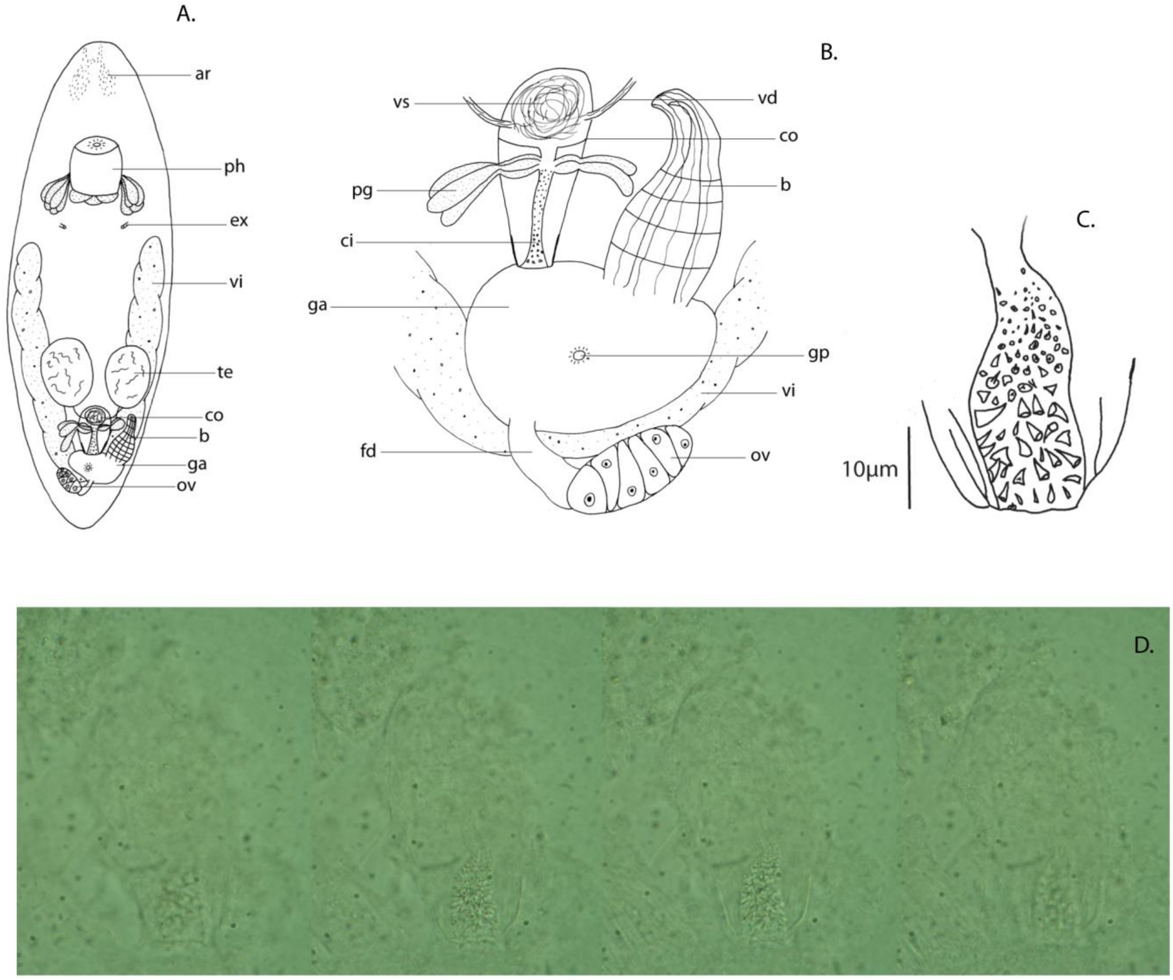


Figure 8: ***Acrochordonoposthia*** **nov. sp.: A. Habitus of living animal. B. Genital system on living animal. C. Armed cirrus of holotype. D. Armed cirrus of holotype photographed at different foci.**

Discussion: A sclerotized bursa with strong musculature has never been described in the genus *Acrochordonoposthia*. The bursae of *A. conica* and *A. ophiocephala* can be slightly sclerotized, but lack a strong musculature and don't have this typical shape. The bursa of *A. reversa* only contains strong sphincters in its distal end and has the shape of a long-stalked pear. A broad, straight cirrus, provided with spines over its complete length also occurs in *A. robusta*, but the copulatory organ of this species is much larger, measuring over 400µm.

Diagnosis: Animals measuring 1mm. Both adenal and dermal rhabdites present. Seminal receptacle lacking. 63-72µm long copulatory organ with straight cirrus, completely covered with spines growing increasingly large toward distal end. Sclerotized bursa surrounded by circular muscles.

Holotype: A whole-mounted specimen (TotoWP002GRZ), collected together with four other specimens in dry moss on the Schloβberg in the center of Graz (47°4'27"N; 15°26'10"E), on 21 August 2011.

Paratypes: One whole-mounted specimen (TotoWP001GRZ), one sagittally-sectioned specimen (3227) and one frontally-sectioned specimen (3426). Same location and date as holotype.

Chorizogynopora Reisinger, 1924

Chorizogynopora italica Kolasa, 1981b

Figure 9 A-F

Material: Nine specimens studied alive, three of which were sagittally-sectioned.

Description: The animals measure 0.7-0.8mm in length and are very translucent. The anterior end is bluntly rounded; the posterior end is usually rounded during swimming, but when not stretched, a small tail could be observed. The body is at its widest in the middle. The length-to-width ratio is about 3, giving the animals a rather plump appearance. Adenal rhabdites lie in two tracts, which partly anastomose at their distal end. The rhabdoid glands are also arranged in two groups at ±25% of the body. Dermal rhabdites were not observed. The rosulate pharynx lies just behind the middle of the body and measures 40µm across. The paired protonephridiopores lie posterior to the pharynx.

The vitellaria reach from the posterior end of the body to the level of the adenal rhabdites. At their posterior end they fuse over a small anastomosis to form an unpaired vitelloduct. A short oviduct departs from the egg-shaped ovary. Eosinophilic shell glands (fg) open in the oviduct. The seminal receptacle is a very elongate, slightly crescent structure connected to the female duct and filled with sperm cells, which are arranged parallel in longitudinal direction. Distal to the seminal receptacle, the oviduct fuses with the oviduct. The female duct is relatively broad and runs to the posterior side of the body, makes a 180° bend and fuses with the small genital atrium. The atrium is lined by a high epithelium and two muscle layers: an outer layer of circular muscles and an inner one of longitudinal muscles. Basophilic atrial glands (ag) open in the atrium. The gonopore, which was strongly muscular in some animals, is situated at 4/5 of the body. A small, muscular evagination of the atrium, which might serve as a uterus (u) was present. Some animals were carrying a strongly refracting egg. It was unclear whether it was carried in the atrium or in

The testes are very large and lie just anterior to the pharynx and ventral to the vitellaria. They are connected to the copulatory organ by very broad vasa deferentia, which narrow slightly after leaving the testes and fuse just before entering the bulbus. The 52µm long, oval copulatory organ is surrounded by two layers of diagonal muscles. Its inside is primarily occupied by a bulbus filled with sperm, which serves as seminal vesicle. The sperm cells are oriented in a longitudinal fashion in the bulbus, which is bended at its proximal end. Furthermore a slightly sclerotized ejaculatory duct is present at the side of the organ. It runs from the proximal 1/5 to the distal end and possesses circular muscles. Large eosinophilic prostate glands enter at the proximal end and form a thin strand of secretions inside the copulatory organ. The walls of the organ itself are composed of a double layer of spiral muscles. The male channel is short and possesses strong circular muscles. The bursa is a small structure with a sclerotized wall and surrounded by strong circular muscles. Its structure varies greatly between individuals. In some animals the bursa was very narrow and elongate, whilst in other animals it was more egg-shaped. On some living specimens groups of small spines were observed at the proximal and the distal end of the bursa. The spines at the distal end were a little bigger. The bursa is held in place by a strong retractor muscle, which is anchored to the dorsal epidermis. Its inside seems to contain granules of secrete and sperm. Granules of secrete and sperm were also observed in the bursa of some living specimens. The bursa ends directly in the genital atrium.

Discussion: The specimens are identified as *C. italica* because of the typical body shape, the structure of the copulatory organ and the bursa and the presence of an elongate receptaculum seminis. The muscular evagination of the atrium in our animals is probably the same structure that was assigned as the uterus in the original description. The specimens described by KOLASA (1981b) are very similar to the animals from this study, but the testes were much bigger in the specimens from our study. KOLASA (1981b) also observed tail glands, which were not observed in the specimens from this investigation.

The species was classified as a species of *Chorizogynopora* because of the armed and sclerotized bursa copulatrix. However, the bursa of the other species, *C. paradoxa* Reisinger, 1924 is very different in that it only possesses spines in its muscular stalk, while they only occur in the terminal sac of *C. italica*. Furthermore, in *C. paradoxa* the bursa is very closely associated with the copulatory organ and both are enveloped by a common muscular wall. For these reasons it might be that the bursa, or at least the cuticular spines on the bursa are not homologous between both species and that *C. italica* should be classified in another genus. Furthermore, *C. paradoxa* possesses a ductus genito-intestinalis, which was never observed on *C. italica*. However, *C. italica* shows some remarkable similarities with *Krumbachia appendiculata* Schwank, 1980 and *K. microbursalis* Kolasa, 1981a. Both species do not possess spines in their bursa, but except for this, the structure of the bursa and the copulatory organ, certainly in the case of *K. appendiculata* is very similar. It is, however, difficult to judge whether *C. italica* should be transferred to *Krumbachia*, put in a new genus, probably together with these *Krumbachia*-species, or still be classified within *Chorizogynopora,* because *C. paradoxa*, the only other species in the genus is only known from one single specimen and no drawings exist of this peculiar species.

Some animals were carrying strongly refracting eggs. It is unclear whether they were carried in the atrium or in the structure denoted as the uterus.

The animals feed on nematodes. Whole animals could be observed in the gut of some specimens.

Known distribution: Fosso Contesora creek in Tuscany, Italy, in heavily moistened mosses growing in the stream KOLASA (1981b).

New localities: Various localities near Schlitz, Hessen, Germany in moss growing in streams: one specimen in the Breitenbach (50°39’31”N; 9°37’46”E) on 9 August 2011; four in a source of the Fulda (50°29’27”N; 9°56’52”E and 50°29’20”N; 9°56’57”E, two individuals on each locality) and four specimens in the Graswiesenbach, Vogelsberg (50°32’8”N; 9°12’0”E), on 10 August 2011.

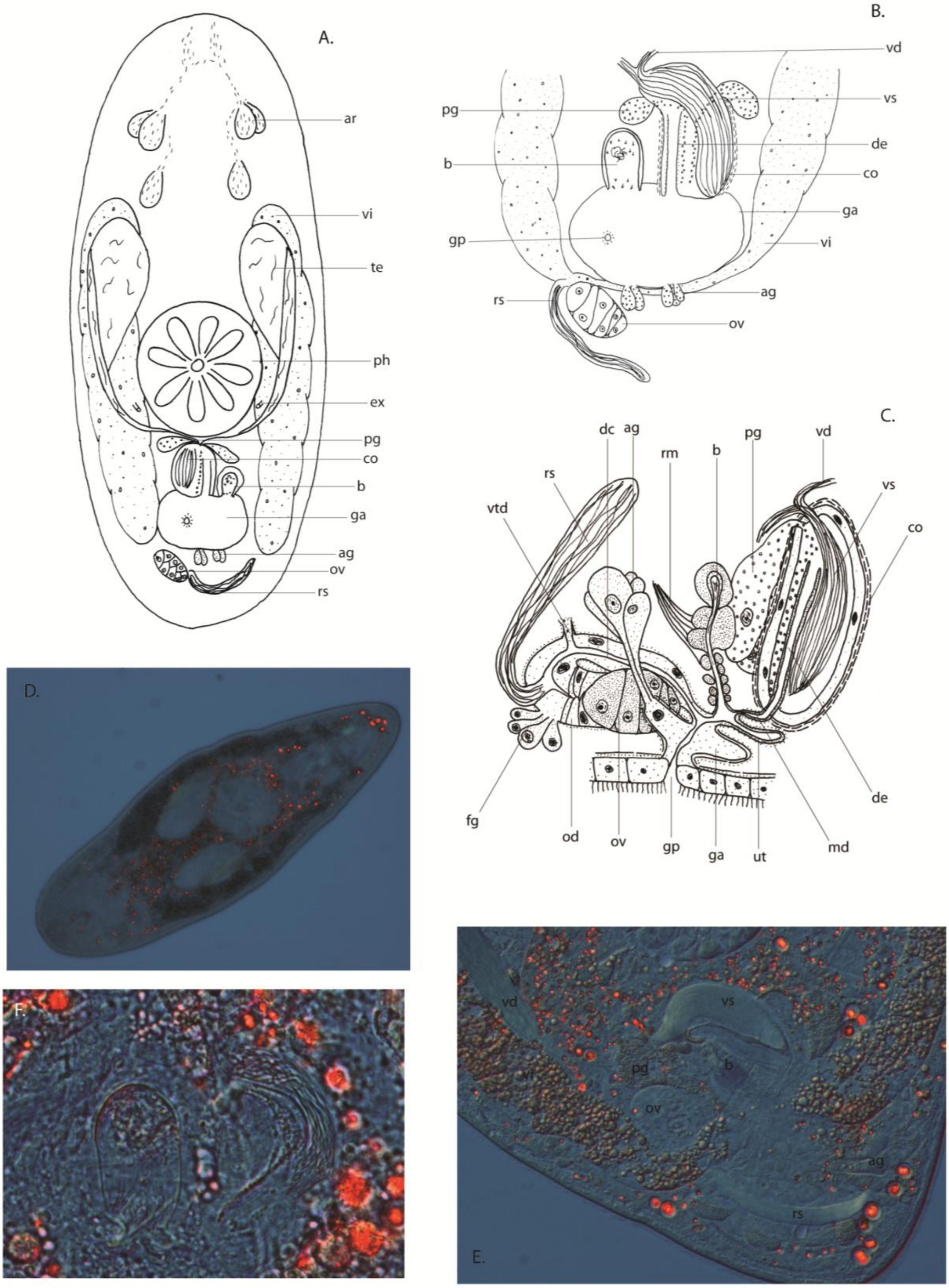


Figure 9: ***Chorizogynopora italica*****: A. Habitus of living animal. B. Genital system in living animal. C. Sagittal reconstruction of the genital system. D. Living animal, photographed at magnification of 10x10. E. Genital system photographed at magnification of 25x10. F. Bursa and copulatory organ of other specimen, photographed at magnification of 40x10**

Hoplopera Reisinger, 1924

The main characteristic of this genus is the presence of a bursa containing a typical sclerotized structure. Furthermore the species possess both adenal and dermal rhabdites. The pharynx is situated behind the middle of the body and testes lie in front of the pharynx. Tail glands are present in every species.

There are six described species of *Hoplopera,* all described from Europe. Four species live in limnoterrestrial environments: *H. opaca* Reisinger, 1924, *H. maculata* Reisinger, 1924, *H. macropharynx* Reisinger, 1924 and *Hopolopera* nov. sp. The two other species, *H. pusilla* and *H. littoralis* are known from marine or brackish habitats. The ecology of these two species seems very different from the limnoterrestrial species. However, KARLING (1957) found *H. littoralis* in coastal groundwater and mentions that the marine origin of this species is not certain. AX (2008) suggests that it might actually be a limnoterrestrial species. *H. pusilla* was found in the supralittoral zone, 8m inland. ARMONIES (1987) found this species in salty meadows with salinity below 10‰ and concludes that *H. pusilla* is a species of brackish water.

Except for these ecological differences, there are some clear morphological differences between the 'marine' and the limnoterrestrial taxa. The bursa always contains a cuticular structure, but in marine animals the bursa consists of just a single part (KARLING 1957; EHLERS 1974). All limnoterrestrial animals possess a bipartite bursa, with the cuticular structure located in the distal part (REISINGER 1924), which could be considered as a bursal stalk. Furthermore the vasa deferentia enter the copulatory organ separately from the sides in the limnoterrestrial species (REISINGER 1924), while they fuse right before entering the organ in the marine animals (KARLING 1957; EHLERS 1974). Based on morphology, it is possible that the limnoterrestrial species and the marine species probably form two distinct clades. The relationship between these clades is unknown and probably the genus should be split in two. However, further research is needed, both on the morphological and on the molecular level to clear out the taxonomy of this genus.

Hoplopera opaca Reisinger, 1924

Figure 10 A-C

Material: A single animal studied alive and sagittally-sectioned.

Description: Living animal measures about 1mm in length and is very dark. The anterior end is rounded. The posterior end possesses a very obvious tail. The body is elongated, with a length:width ratio of 7. In the tail, very large, eosinophilic, coarse grained tail glands (tg) were present. Adenal rhabdites were present, but were rather inconspicuous and only visible on the sectioned animal. Small dermal rhabdites occur all over the body. The large rosulate pharynx lies at ±75% of the body and points slightly backwards. It measures 60µm across. Protonephridia open separately, posterior to the mouth.

Vitellaria reach forward to ±33% of the body, but were still developing in this specimen. They fuse to form an unpaired vitelloduct. A broad oviduct, lined with a low epithelium, departs from the small, dorsal ovary. The vitelloduct opens in the proximal section of the female duct. The genital atrium is lined with a high epithelium. The gonopore is situated at ±80% of the body.

The small testes lie behind the pharynx, but only one could be clearly seen on the sectioned specimen. The vasa deferentia enter the copulatory organ separately. The copulatory organ is a 20µm long, oval structure, surrounded by muscles of unknown orientation. It contains sperm in its proximal part, which probably serves as seminal vesicle. An ejaculatory duct was not observed. Prostate glands are present inside, but never exit the organ. The short male channel is surrounded by circular muscles. The bursa is divided in two parts: a bulbous proximal part containing sperm (b) and delineated with a thin wall, and a stalk (bst). The stalk is surrounded by circular muscles and contains the sclerotized structure (ss). It seems to consist of several vertical bars, which are fused in their distal part. On the sectioned specimen, however, this structure was barely visible. The distal part is thin-walled, bulbous and contains sperm. The bursa ends in the genital atrium through a wide, slightly muscular duct, which fuses with the male channel.

Discussion: This specimen clearly belongs to the genus *Hoplopera* because of the sclerotized structure in the bursa, the presence of dermal rhabdites and the tail glands. Within this genus, there are four known limnoterrestrial species. This species is considered as *H. opaca* because of its shape, the great size of its tail glands, the absence of a seminal receptacle and the shape of the sclerotized structure in its bursal cavity. *H. maculata* Reisinger 1924 is larger, reaching lengths up to 1.5mm, possesses dark spots on parts of its epidermis and in most of its organs, has a copulatory organ five times bigger than *H. opaca*, possesses a seminal receptacle and a more complex sclerotized structure. *H. macropharynx* Reisinger 1924 is smaller, the tail glands are much smaller and less striking and the sclerotized structure is different. *Hoplopera* nov. sp. differs from this species in having less conspicuous tail glands, a large group of atrial glands and a different sclerotized structure in the bursa.

Known distribution: Near Graz, Austria, in moist forest soils (REISINGER, 1924).

Neotype: A sagittally-sectioned specimen (3242) found on the Hohe Rannach near Graz (47°9’53”N; 15°24’50”E), single specimen in leafy humus of a beech forest on 26 August 2011.

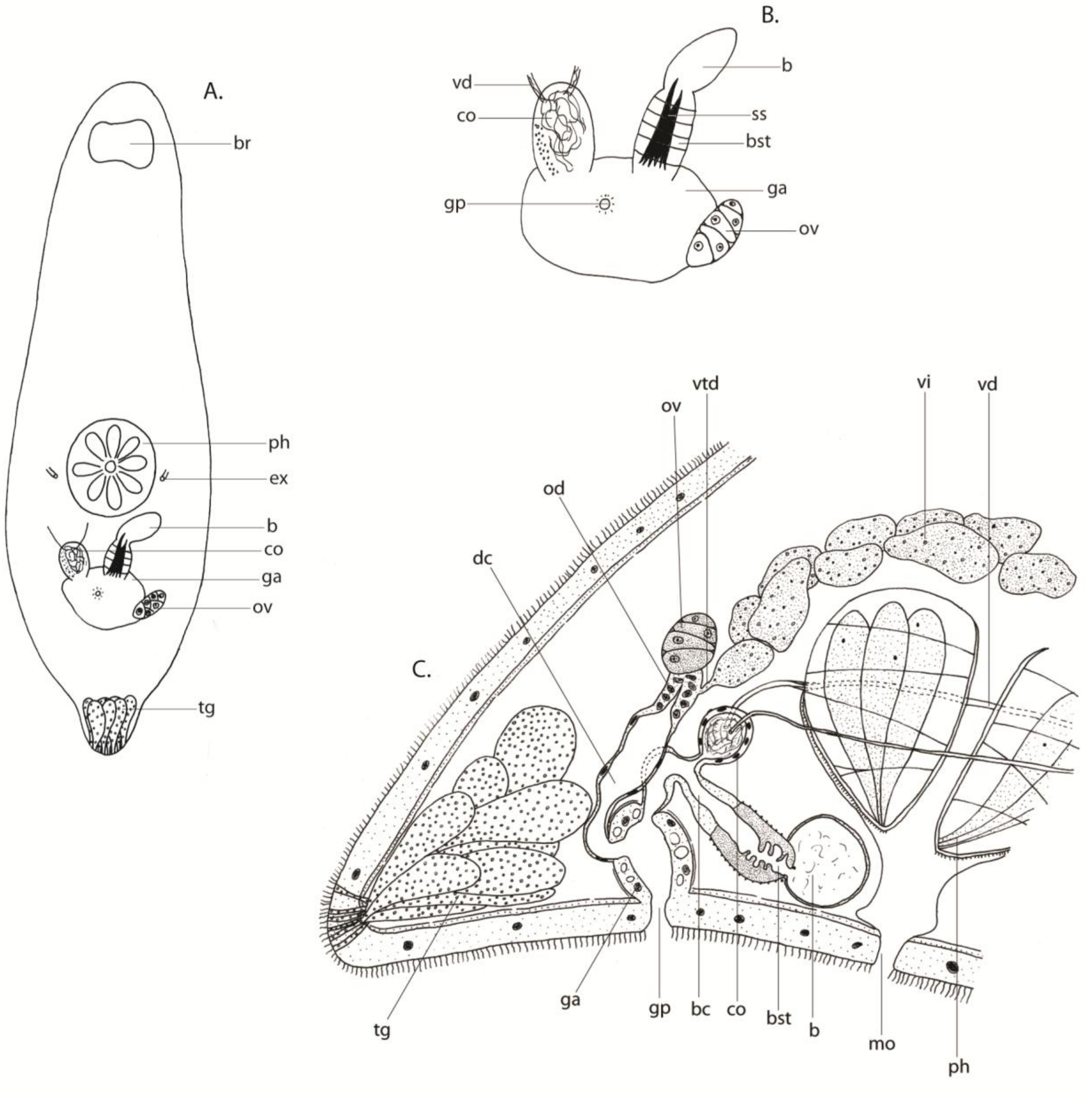


Figure 10: *Hoplopera opaca*: A. Habitus of living animal. B. Genital system on living animal. C. Sagittal reconstruction of the neotype.

***Hoplopera* nov. sp.**

Figure 11 A-D

Material: Four specimens studied alive, one of which was frontally-sectioned and one whole mounted.

Description: Living animals measure 0.7-0.9mm. The anterior end is rounded. In the posterior end, a small tail is present. In the tail region, some small, inconspicuous tail glands are present. Sharply pointed adenal rhabdites are present in 2 tracts and are produced by two groups of rhabdoid glands. Very small dermal rhabdites occur all over the body. The rosulate pharynx measures 52µm across and is located just behind the middle of the body. The paired protonephridiopores lie posterior and lateral to the mouth.

The vitellaria reach up to the anterior third of the body and fuse over a broad anastomosis at their posterior end. They probably enter the oviduct through a single vitelloduct, but this could not be observed with certainty. The long female duct departs from the large oviduct. It is lined with a high epithelium and contains several bends. A seminal receptacle is present in its proximal part. The genital atrium is lined with a high epithelium and surrounded by muscular layers. At its posterior side, a large group of eosinophilic atrial glands is present. The gonopore is present at ±80%.

Two small, spindle-shaped testes lie in front of the pharynx and ventral to the vitellaria. In some animals one testis seemed to be a little bigger than the other one. The thin vasa deferentia enter the copulatory organ from the sides. The copulatory organ is an egg-shaped, 24µm long structure,

surrounded by two layers of diagonal muscles. A small bulbus on the proximal side serves as a seminal vesicle. A short ejaculatory duct exits the copulatory organ. It is surrounded by circular muscles. The copulatory organ possesses numerous basophilic prostate glands on its inside (pg1). Furthermore, a pair of external, fine grained, basophilic prostate glands (pg2) was present. The relatively long male duct opens in atrium after fusing with the bursal channel. The bursa is divided in two parts: an 18µm long proximal part with a thin wall (b), containing sperm, and a 22µm long distal part (bst). The distal part, which could be considered as the bursal stalk, is surrounded by circular muscles. Inside its matrix a channel, which is also surrounded by circular muscles and contains a sclerotized structure (ss), is present. This structure consists of two groups of vertical rods, the one positioned above the other and separated by a single horizontal bar. The proximal and distal end of this structure are also bounded by a horizontal bar. As a whole, the structure has a funnel-like shape. The bursa opens in a bursal channel, which fuses with the male duct.

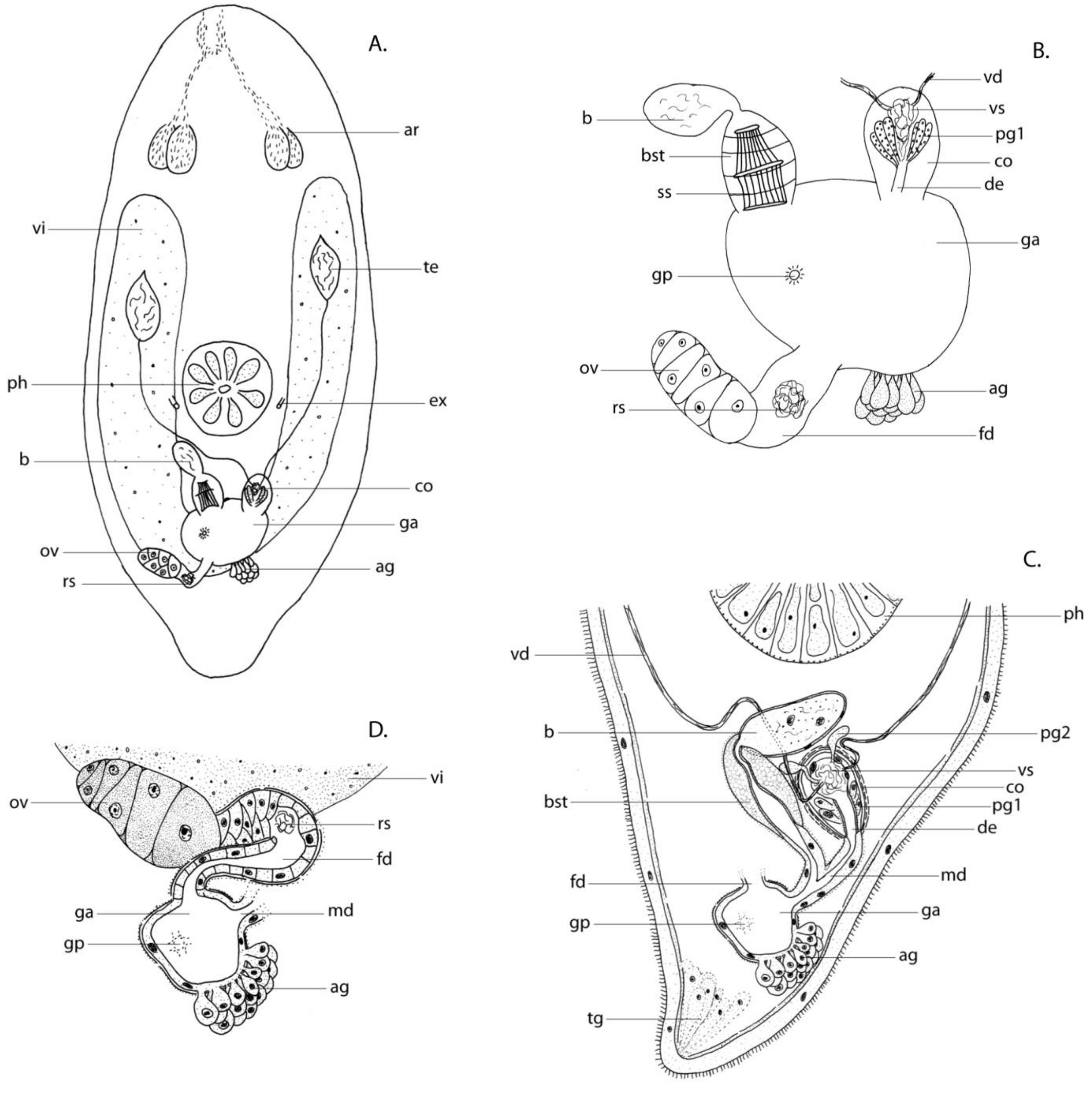


Figure 11: *Hoplopera* nov. sp.: A. Habitus of living animal. B. Genital system on living animal. C. Frontal reconstruction of male genital system of the holotype, ventral view. D. Frontal reconstruction of female system of the holotype, ventral view.

Discussion: The individuals clearly belong to the genus *Hoplopera* because of the presence of adenal and dermal rhabdites, the presence of tail glands and the bipartite bursa with a cuticular structure in the proximal part. Three limnoterrestrial species of *Hoplopera* were already described. The characteristics of those species are summarized in table 4. The animals differ from *H. opaca* in their smaller size, the tail glands which are much smaller and less conspicuous and differences in the sclerotized structure in the bursa. *H. maculata* is much bigger, possesses refracting spots over all of its body, and has a very complex cuticular structure in its bursa and a larger copulatory organ and bursa. The animals found in this study probably share the most similarities with *H. macropharynx*. However, the specimens all possess a seminal receptacle, the structure in the bursa is more complex than that of *H. macropharynx* and the pharynx is relatively small and round instead of oval. Furthermore, the very conspicuous atrial glands were not observed in any other species within the genus *Hoplopera*. Differences between the limnoterrestrial species of *Hoplopera* are summarized in table 4.

Table 4: Morphological differences between limnoterrestrial species of the genus *Hoplopera*

	H. opaca	***H. maculata***	***H. macropharynx***	***H. spec. 1***
Size	1-1.2mm	1.5mm	0.7-0.9mm	0.7-0.9mm
Colour	Dark	Refracting spots on body	Hyaline	Relatively dark
Tail glands	Very large	?	Inconspicuous	Inconspicuous
Receptacle	Absent	Present	Absent	Present
Cuticular structure	6-10 vertical bars, fused at distal end	Very complex with spoon-shaped plates which fuse proximally	6-8 thin bars, fused to a ring on the proximal side	2 groups of vertical rods, delimited by horizontal bars
Copulatory organ	18-22µm	100µm	Like H. Opaca	24µm
Pharynx	84-87µm (Reis.) 60µm	Like H. Opaca	Large, oval	52µm
Bursa	24-30µm	120µm	35µm	40µm

Diagnosis: Dark, 0.8 mm long animals with inconspicuous tail glands. Dermal rhabdites small, adenal rhabdites in two groups at front end. Spindle-shaped testes enter copulatory organ from the sides. 24µm long copulatory organ with muscular ejaculatory duct. Bursa with sclerotized structure consisting of two groups of parallel, vertical rods, separated from each other by horizontal bars. Seminal receptacle present inside the female duct.

Holotype: A frontally-sectioned specimen (3235), collected on 23 August 2011 in a spruce forest between Glashütten and Trahütten, south of Graz, Austria (46°49’46”N; 15°6’9”E), together with three other specimens in moss growing in a small stream.

Paratype: A whole-mounted specimen (TotoWP004GRZ), same locality and date.

Krumbachia Reisinger, 1924

The genus *Krumbachia* contains 11 known species, making it the largest genus of Protoplanellinae with species described from both the palearctic and the nearctic area. The species are characterized by their copulatory organ, which possesses a sclerotized ejaculatory duct, the presence of adenal rhabdites and absence of dermal rhabdites and median to posterior position of the pharynx. Most species also possess a bursa. The genus is morphologically very diverse. Species vary in size from 0.3mm in the smallest species (*K. minuta* Ruebush, 1938) to over 3mm in the largest species (*K. paludicola* Schwank, 1979). Furthermore the size and shape of the copulatory organ, the bursa and the female system are very diverse.

The genus is without any doubt polyphyletic. It contains species like *K. guttulata* Schwank, 1980, which possesses large dermal rhabdites and a poorly described and very aberrant genital system, and poorly described species like *K. exigua* (Dörner, 1902) and *K. hungarica* (Gelei, 1904). Furthermore, many species are sometimes classified in other genera, in particular *Olisthanella*.

Because of the problems that might exist for identification of species within this genus a key to the species of *Krumbachia* is provided. Problematic species of *Olisthanella* are not included in the key. As no species of *Olisthanella* are known to live in limnoterrestrial habitats, the key can be used to identify limnoterrestrial species. In limnic habitats, however, a more extended key would be needed.

1. a) Eyes present. 2
b) Eyes lacking. 4

2. a) Length 2mm, eyes anastomosing, palearctic species.
***K. hungarica* (Gelei, 1904)**
b) Eyes separate, nearctic species. 3

3. a) Length: 0.3-0.6mm. Two large eyes. Eight granular prostate glands.
***K. minuta* Ruebush, 1938**
b) Length: 2mm. Two minute eyes. Three granulate prostate glands.
***K. virginiana* (Kepner & Carter, 1931)**

4. a) Length: 1.7-2.8mm. Dermal rhabdites present. Bursa: 70µm, muscular, 7-8 paired rows of spines. Extremely proterandric.

K. guttulata **Schwank, 1980**

b) Dermal rhabdites lacking, bursa always without spines. Usually no extreme proterandry visible. 5

5. a) 0.6mm, a pair of conspicuous refracting bodies in the anterior part of the body, vasa deferentia fusing before entering the copulatory organ. Testes lying posterior to the pharynx.

K. exigua **(Dörner, 1902)**

b) No refracting bodies present. Testes lie in front of the pharynx. 6

6. a) Small species (0.5-0.9mm). Copulatory organ: 50-65µm 7

b) Larger species (1.7-3.4mm). Copulatory organ: 120-320µm 8

7. a) Length: 0.5-0.8mm. Copulatory organ with very wide (30µm), blind ending ductus ejaculatorius. Prostate glands associated with copulatory organ present. Male duct ends in bursal wall. Bursa proximally narrowing with 12-14 circular muscles, contains cuticular rings on the inside.

K. appendiculata **Schwank, 1980**

b) Length: 0.6-0.9mm. Ejaculatory duct muscular. Usually no prostate glands present. Very small bursa, attached to wall of the copulatory organ.

K. microbursalis **Kolasa, 1981a**

8. a) Length: up to 2.1mm. Very asymmetric copulatory organ. Ejaculatory duct with 2-layered cuticula. Bursa is evagination of atrium without strong muscles.

K. hiemalis **Schwank, 1979**

b) Copulatory organ less asymmetric. Ductus is not lined with double layer of sclerotization. Bursa always muscular. 9

9. a) Length: 1.5-3.2mm. Copulatory organ about 295µm long. Ejaculatory duct possesses a lateral evagination and is surrounded by a matrix ring in its distal end.

Atrium divided in two by strong sphincter. Bursa with very strong sphincters in distal part.*

K. paludicola Schwank 1979

b) Length 1.8-2.4µm. Copulatory organ 170-185µm long. Ejaculatory duct with a bend of 80°, without lateral evagination or matrix ring. Atrium not divided in two. 10

10. a) Bursa long, with multiple bends, contains strong circular muscles. Ductus communis with sharp bends. Stalk containing the seminal receptacle very long. Vitellaria smooth.

K. styriaca Reisinger, 1924

b) Bursa a transversally flattened broad pocket. Ductus communis straight. Stalk of the seminal receptacle less distinct. Vitellaria strongly ramified, possessing side branches.

K. subterranea Reisinger, 1933

*Specimens from Ontario, Canada, identified as *K. paludicola* were found in this study. They lacked a bursal sphincter, however.

Krumbachia paludicola Schwank, 1979

Figure 12 A-C

Material: One frontally and five sagittally-sectioned specimens, drawings and pictures of living animals.

Description: Very dark animals that reach a length of more than 3mm. The body has a small constriction at ±30% of its length. Both the anterior and the posterior extremity are rounded. The body is at its widest at ±65% of its length. Adenal rhabdites are present in two tracts, dermal rhabdites are lacking. The pharynx is situated just anterior to the middle of the body, at ±45%. It measures 165-235µm across. The protonephridiopores are situated just posterior to the pharynx.

The vitellaria don’t fuse and reach up to the rhabdoid glands, and back to almost the posterior end of the body. They are connected with the rest of the female system by paired vitelloducts, which fuse just before connecting to the female duct. The ovary is large and elongate. The oviduct is relatively narrow and at the point where it meets the vitelloduct, a seminal receptacle is present. It is an elongate structure lined with a very thin epithelium and filled with sperm. At this level the female duct also receives coarse grained secretions from a large, paired group of basophilic female glands that lie close to the vitellaria. The oviduct and the vitelloduct are surrounded by weak, circular muscles. The ductus communis opens in the large, elongate atrium. The atrium and the ductus communis are lined with a high epithelium and surrounded by two muscular layers: an inner layer of longitudinal muscles and an outer layer of circular ones. Two groups of atrial glands (ag) are connected to the atrium. The glands are located ventral to the seminal receptacle and run forward, to the anterior part of the atrium. The glands produce a fine grained, eosinophilic secretion and are connected to the atrium by a compact clew of channels. The gonopore is situated at the middle of the body.

The large, elongate, paired testes lie anterior to the pharynx. The broad vasa deferentia fuse just before entering the copulatory organ. The copulatory organ is a 240-320µm long, slightly bended structure, surrounded by three layers of muscles: two very strong inner diagonal muscle layers and a weaker outer longitudinal layer. The copulatory organ possesses a bulbus in its proximal two third. It is lined with a very thin epithelium. In its distal part the organ possesses a slightly bended, sclerotized ejaculatory duct. It measures 215-250µm in length. It possesses a 5-6µm high sclerotized layer. It does not have real ventricles, but is widened at half its length. At its distal end the duct possesses a very clear constriction. At its proximal end, the duct reaches back to the proximal region of the bulbus. The duct is at its broadest at its distal end, being 30-40µm wide. At the constriction, it is only 20µm wide. Probably the ejaculatory duct arose from the same epithelium as the bulbus. A paired group of large, external prostate glands are associated with the copulatory organ. They enter together with the vas deferens. The glands produce a coarse grained, basophilic secretion. The secretions are stored inside the organ in a prostate vesicle. This structure lies next to the distal end of the bulbus. The distal part of the copulatory organ, which holds the ejaculatory duct, is filled with a loose matrix, which has a conspicuous orange colour on the living animal under transmitted light and a blue colour under reflected light. On sectioned specimens this matrix (mx) was stained eosinophilic. At the constriction of the ejaculatory duct, the matrix seems to form a ring around the ejaculatory duct. The distal end of the ejaculatory duct is connected to the male duct, which possesses a musculature similar to the atrium. The 200-260µm long bursa is a very muscular, elongated sack with a clear constriction at 1/5 of its length. The proximal end is rounded to pointy. The proximal 20% is surrounded by two weak layers of diagonal muscles, whilst the distal 80% is surrounded by an outer layer of circular muscles and an inner layer of longitudinal muscles. The longitudinal muscles are relatively thin. The circular muscles are extremely well developed in the distal half of the organ. In the proximal part, they are much smaller, but still relatively strong. Outside these muscular layers a thick eosinophilic matrix is present. Outside this matrix, a strong layer of longitudinal muscles is present. The bursa is filled with a compact mass, mainly consisting of secretions. The bursa ends in the proximal part of the male channel.

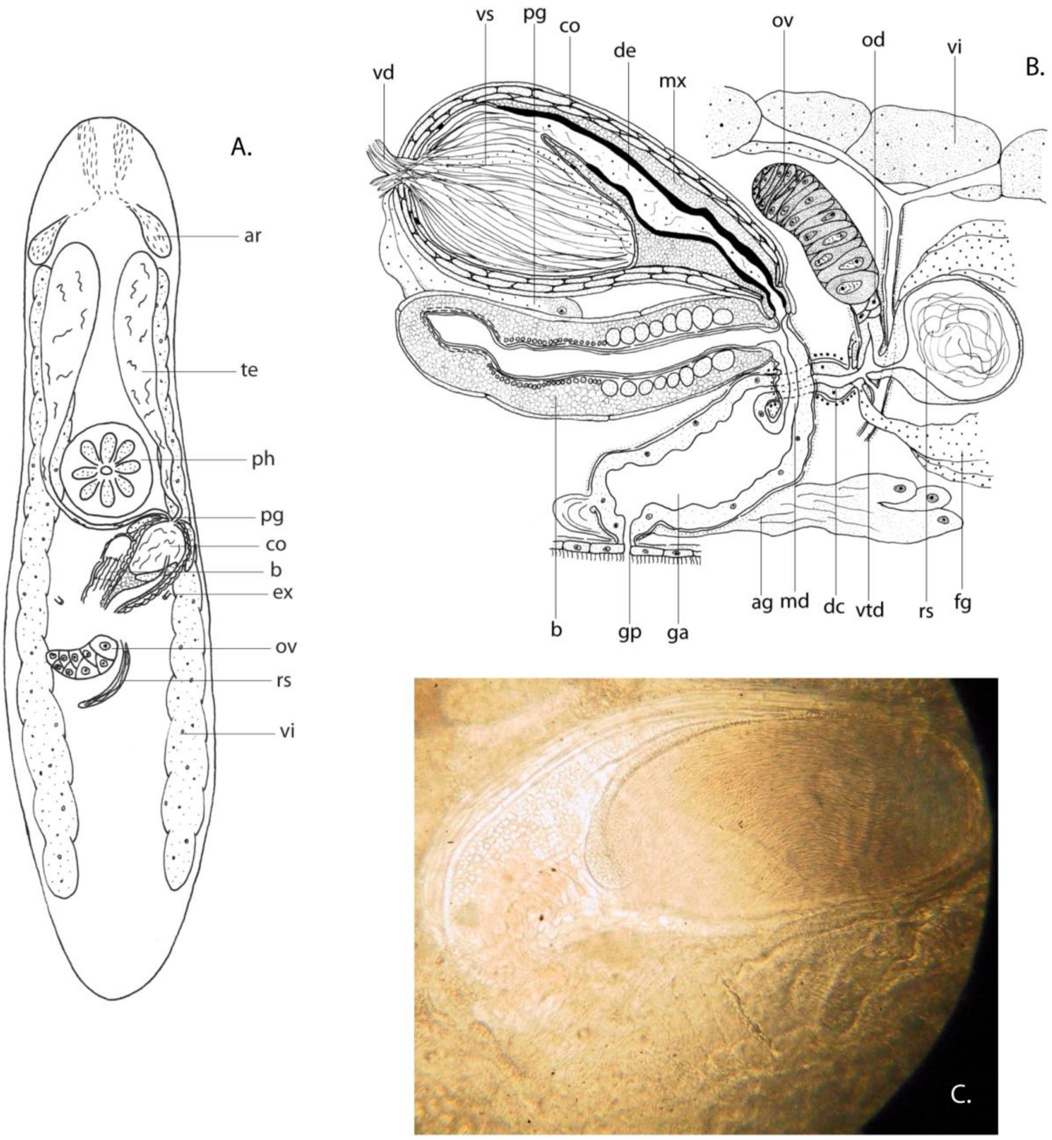


Figure 12: *Krumbachia paludicola*. A. Habitus of living animal. B. Sagittal reconstruction of the genital system. C. Photograph of copulatory organ and bursa.

Discussion: The specimens show a striking similarity to *K. paludicola*. The body shape and the reproductive system are very similar. However, the animals described by SCHWANK (1979) have an ejaculatory duct with a large side-branch, which is much less developed in the specimens from this study. A big atrial sphincter dividing the atrium in two parts, considered typical for *K paludicola* was never observed on these animals. Except for these differences, the specimens match the original description of *K. paludicola*. For this reason, the section series is preliminarily identified as *K. paludicola*. However, to be absolutely sure, it will be needed to find animals at the type locality of this species, as the only existing material of *K. paludicola* is a series of whole mounts, which probably won't suffice to provide a certain identification.

Known distribution: *K. paludicola* is known from creeks near Schlitz, Germany, where it is reported to be common, and from interstitial sand in the Fosso Contesora in Lucca, Italy (SCHWANK, 1979).

New locality: Dundas Valley Conservation Area, Ontario, Canada (43°14'20"N; 79°59'17"W), several specimens in submersed roots in a puddle of mud in a forest, collected by Dr. Van Steenkiste on 6 May 2009.

Krumbachia subterranea Reisinger, 1933

Figure 13 A-C

Material: Two specimens studied alive, one of which was sagittally-sectioned.

Description: The animals measure over 2.5mm in length. The body is slender, with a length-to-width ratio of ±7. The anterior end is somewhat pointy, while the back end is more or less rounded. Adenal rhabdites are present in two anastomosing tracts, produced by two groups of rhabdoid glands just behind the brain. Dermal rhabdites are absent. The rosulate pharynx lies behind the middle of the body. It measures 220µm across. The paired nephridiopores lie at the same level as the mouth and very near to it.

The paired vitellaria reach just behind the rhabdite glands. They never fuse, but form two separate vitelloducts that fuse just before opening in the female duct. The ovary is more or less elongate and oval-shaped. A narrow oviduct departs from here, makes a 90° bend and meets with the paired vitelloducts. At the bend the seminal receptacle opens in the oviduct. The seminal receptacle is a large, elongate structure, filled with sperm. Where the channel to the receptaculum fuses with the oviduct, small, eosinophilic structures are present. Possibly these are the shell glands. The short ductus communis, which is surrounded by a layer of circular muscles receives the vitelloduct and opens in the atrium. The genital atrium is relatively large, is lined with a high epithelium and surrounded by an inner layer of longitudinal and an outer layer of circular muscles. A large group of eosinophilic glands (ag), containing coarse grained secretions, is present at the back end of the atrium. At the anterior end a smaller group of similar glands is also present. At both the anterior and the posterior end of the genital atrium, a cellular atrial appendage (aa) with unknown function was observed. The atrium opens in the gonopore through a gonoduct (gd) with similar epitherlium and musculature. The gonopore lies at ±75% of the body.

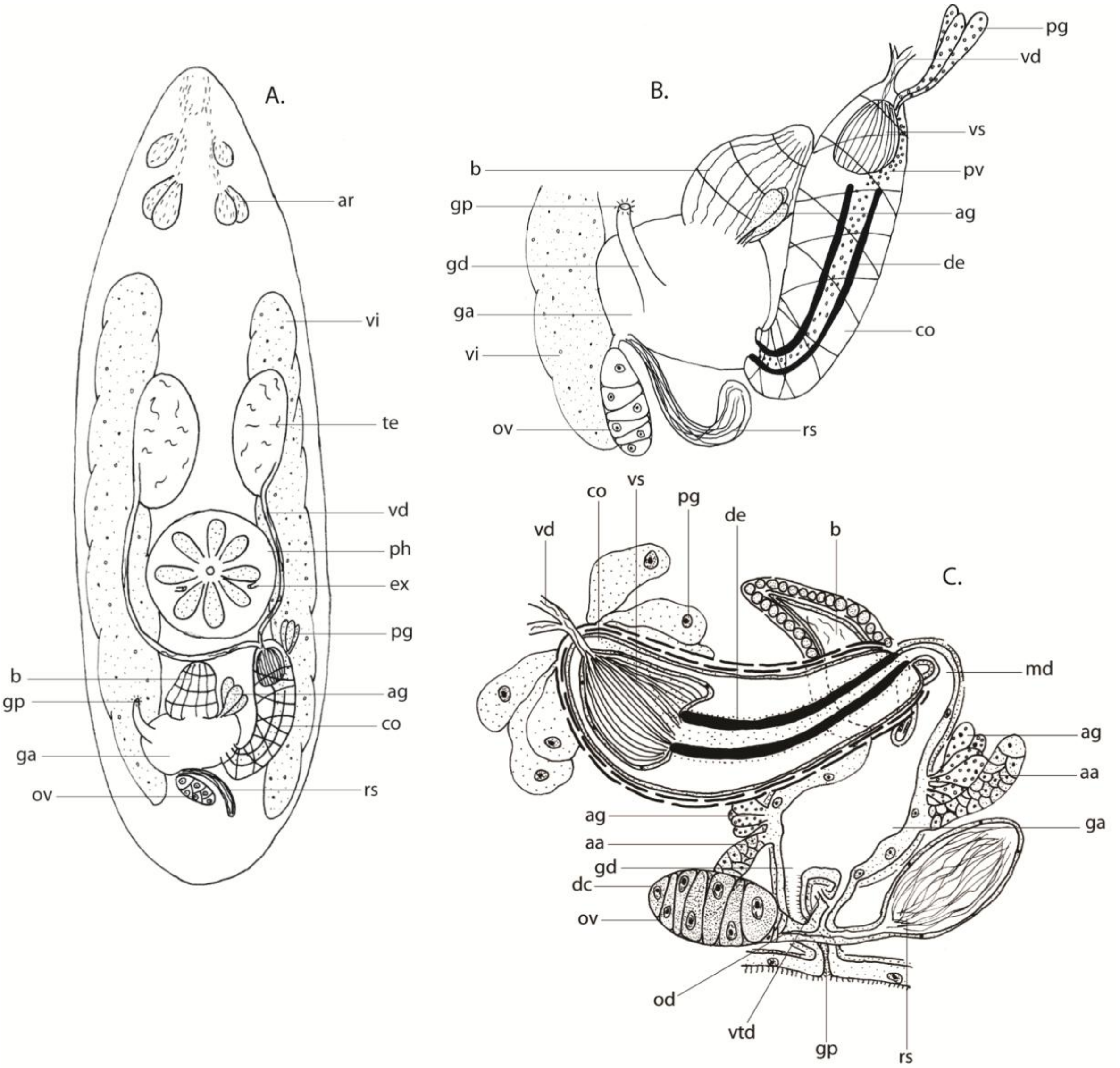


Figure 13: *Krumbachia subterranea*: A. Habitus of living animal. B. Genital system in living animal. C. sagittal reconstruction of genital system.

The large, egg-shaped testes lie anterior to the pharynx. The vasa deferentia fuse just before entering the copulatory organ. The copulatory organ is curved over its whole length, especially in its distal part, where it makes a bend of 80°. The walls are surrounded by two layers of very strong, diagonal muscles. Its proximal part has a more or less pointy end through which the fused vasa deferentia enter to form the vesicula seminalis. Several eosinophilic, coarse grained prostate glands also enter at this point. The seminal vesicle occupies the proximal quarter of the copulatory organ. The distal three quarters holds the ductus ejaculatorius. This is a curved, strongly sclerotized duct with circular musculature all over its walls. It starts as a rather broad channel at its proximal side, but gets very narrow at its distal end. The ejaculatory duct is filled with secrete from the prostate glands. At its distal end it is closed by a sphincter. The copulatory organ ends in what could be considered as an evagination of the atrium, rather than a real male duct. The bursa is a flat, strongly muscular and somewhat cone-shaped structure. It is surrounded by a strong inner layer of circular muscles and a strong outer layer of longitudinal muscles. The bursa is curved around the copulatory organ and opens directly in the atrium. Its entry is sealed by very strong sphincters.

Discussion: The specimens from Hessen clearly belong to *K. subterranea*. *K. styriaca* shows a strong resemblance with this species, but possesses an elongate and kinked bursa and a more strongly bended ductus communis.

Known distribution: *K. subterranea* is known from a cave in the German Ruhr area (REISINGER 1933), and from an inundated meadow near Schlitz SCHWANK (1981b). LANFRANCHI & PAPI (1978) mention the species to be common in Europe.

Neotype: A sagittally-sectioned specimen (3438), found in an inundated meadow at the banks of the Breitenbach creek together with another specimen, Hessen (50°46’30”N; 9°30’18”E) on 9 August 2011.

Nov. gen. nov. sp.

Fig 14 A-E

Material: A single specimen studied alive and sagittally-sectioned.

Description: The animal measures about 0.8mm in length. The anterior end is bluntly rounded, while the posterior end possesses a small tail. The epidermis is 4.5µm high on the ventral side and 3.5µm on the dorsal side. The ventral surface is entirely covered with locomotory cilia, but on the dorsal surface, ciliation is almost completely lacking. Adenal rhabdites were observed in the anterior part of the body. Rhabdoid glands are organized in two groups, located at ±25% of the body. Dermal rhabdites were not observed. The rosulate pharynx is 50µm long and 35µm wide. It is located in the middle of the body and is slanted forward. The protonephridiopores were not observed with certainty, but probably they are paired, like in all other Protoplanellinae.

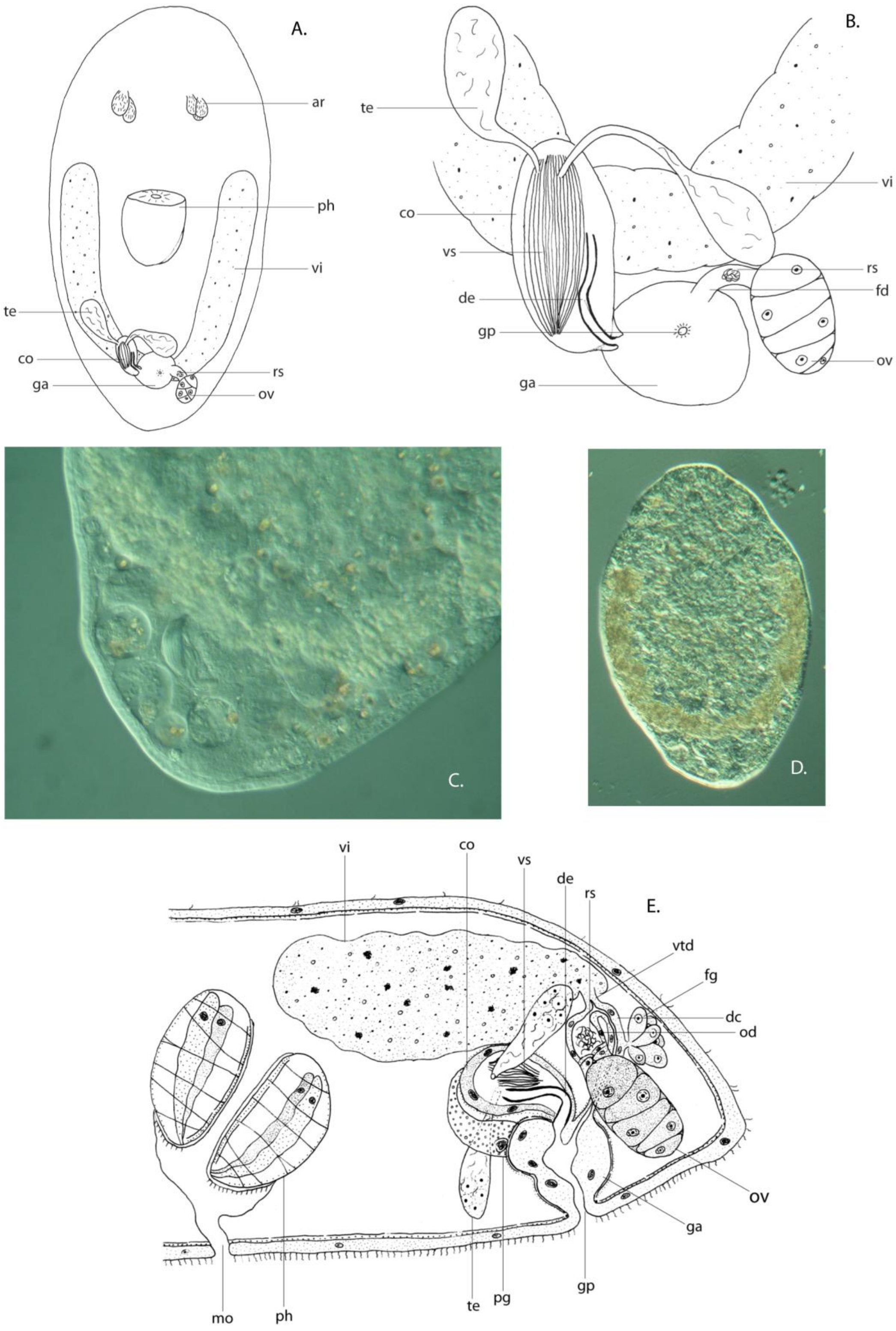


Figure 14: 'Nov. gen. nov. sp.' A. Habitus of the living animal. B. Genital system in living animal. C. Photograph of genital system in living animal at magnification 40x10. D. Photograph of living animal at magnification 10x10. E. Sagittal reconstruction of the genital system.

Vitellaria reach up to the pharynx. A short oviduct departs from the ovary. In its most proximal part the oviduct forms a seminal receptacle. More distal, the vitelloduct and the oviduct fuse to form the ductus communis. Eosinophilic female glands open in the ductus communis, which is lined by a high epithelium and surrounded by circular muscles. The atrium is lined by a high epithelium and surrounded by an inner layer of longitudinal and an outer layer of circular muscles. The gonopore lies at ±80% of the body.

The egg-shaped to elongate testes lie at ±70% of the body and lie ventral to the vitellaria. The vasa deferentia are very wide and don't have a discrete starting point: the transition between testes and vasa deferentia is very smooth. The vasa deferentia enter the copulatory organ laterally. The copulatory organ measures 34µm. Its wall is surrounded by circular muscles. The organ contains a seminal vesicle. It contains unusually thick strands of sperm, lying more or less parallel to each other. A 22µm long, strongly sclerotized ejaculatory duct is present. The duct is more or less straight at its proximal end. At its distal end, a nick of approximately 100° is present. It ends directly in the genital atrium. Eosinophilic prostate glands with coarse grained secretions enter the copulatory organ from its ventral side.

Discussion: This species shares many similarities with *Protopharyngiellona buchonina* Schwank 1980 and *Ventrociliella romanae* Kolasa 1977. It shares a similar body shape, the anterior position of the pharynx, the seminal receptacle in the female duct and the posterior position of the testes with both species.

A very typical characteristic, shared with *V. romanae* is the reduction of cilia on the dorsal side of its epidermis, which could serve as an adaptation to limnoterrestrial habitats (KOLASA 1977). However, the testes of *V. romanae* are smaller and round. The copulatory organ does not contain a seminal vesicle and the ejaculatory duct is not sclerotized. Furthermore *V. romanae* possesses a thin walled bursa, which is lacking in this species.

The structure of the copulatory organ is very similar to the one of *Protopharyngiellona buchonina*: both contain a seminal vesicle and a bended, sclerotized ejaculatory duct. Furthermore, the shape of the testes corresponds between both species. In *P. buchonina*, however, the ejaculatory duct is closed on its distal end, while it is open in '*Nov. gen. 2'*. Furthermore the duct is bended over its entire length in *P. buchonina*, whereas in the species from this study only the distal part is bended. Furthermore the vasa deferentia fuse before entering the copulatory organ in *P. buchonina*, which also possesses a very typical muscular bursa, in contrast to this specimen.

Bockia also possesses a copulatory organ with sclerotized, bended ejaculatory duct, and a pharynx in the anterior end of the body. However, this genus possesses a real pharynx doliiformis and lacks adenal rhabdites.

The specimen from this study shows similarities with several genera, but could not be classified within an existing genus. For this reason, a new genus is erected.

Diagnosis: Hyaline, 0.8mm long animals. Adenal rhabdites in two tracts in anterior end, dermal rhabdites lacking. Pharynx in anterior body half. Copulatory organ with bulbus and bended, sclerotized ejaculatory duct. Elongate testes lie in posterior part of the body and enter copulatory organ from the sides. Seminal receptacle in female duct.

Holotype: A sagittally-sectioned specimen (3437) found in nature reserve 'De Zeebermduinen' in Oostduinkerke (Koksijde) (51°8'22"N; 2°41'26"E), a single specimen in dry moss growing on dunes on 30 July 2011.

Olisthanellinella Reisinger, 1924

Olisthanellinella rotundula Reisinger, 1924

Figure 15 A-D

Material: Four specimens studied alive, one of which was sagittally-sectioned and one frontally-sectioned.

Description: Living specimens measure about 0.8 mm. The anterior end of the animals is truncated. The posterior end is rounded, but on swimming animals, a tail can be present. Large adenal rhabdites are present in two groups of rhabdoid glands, which reach back to ±40% of the body. Dermal rhabdites were not observed. The rosulate pharynx is situated just behind the middle of the body, at 55-60%. It measures 50µm across. The paired protonephridiopores are situated lateral to posterior to the pharynx.

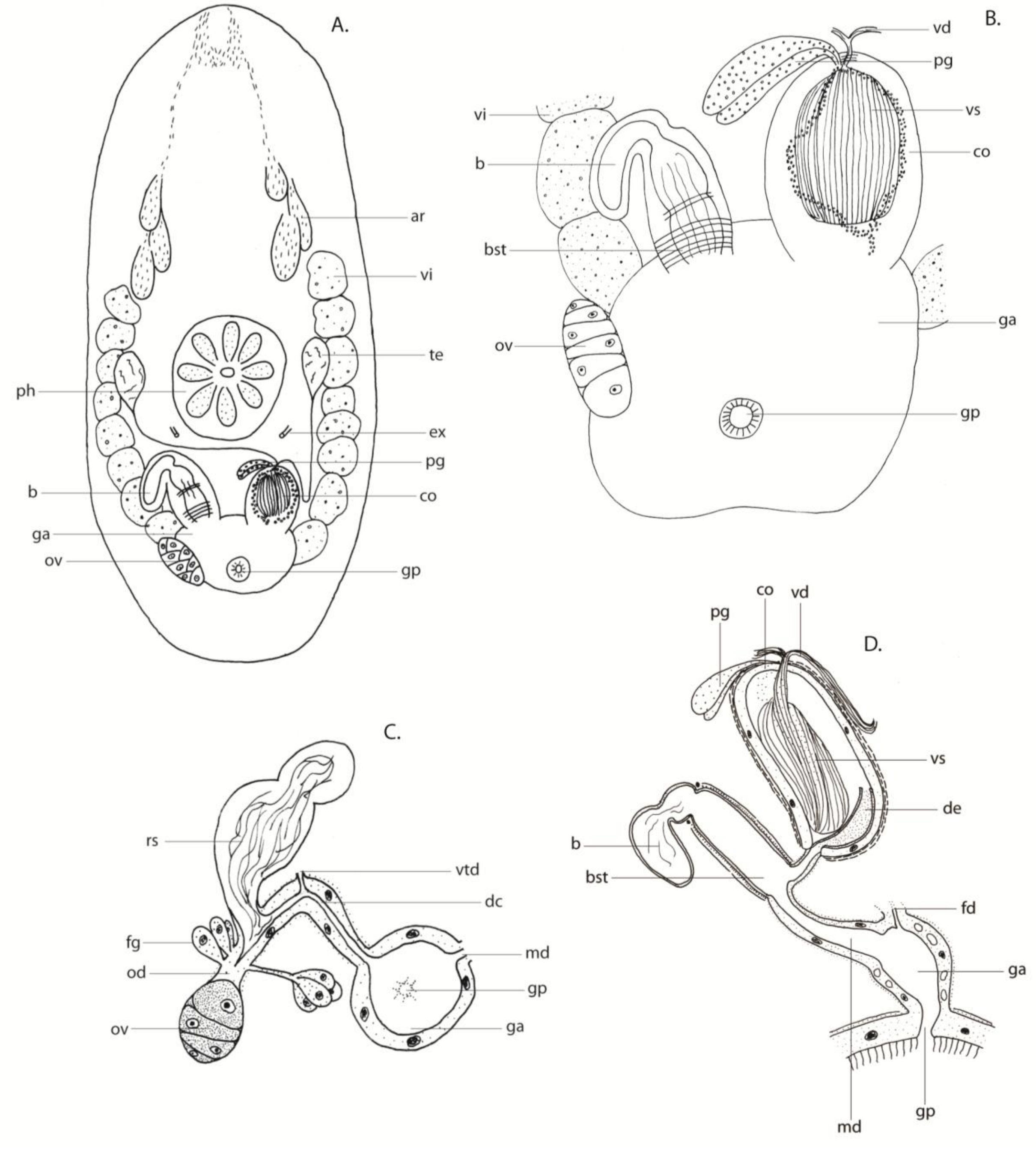


Figure 15: *Olisthanellinella rotundula*: A. Habitus of living animal. B. Genital system on living animal. C. Frontal reconstruction of the female genital system. D. Sagittal reconstruction of the male genital system of the neotype.

Vitellaria reach up to ±40% of the body. They fuse in their posterior part to form an unpaired vitelloduct. The elongate, unpaired ovary is very translucent on living animals. The oviduct receives a group of fine grained shell glands (fg). The relatively narrow ductus communis opens in the genital atrium. It contains a very large seminal receptacle that seems to evaginate from the oviduct at its proximal end. Despite its size, this structure was not observed on living specimens. The atrium is lined with a high epithelium that contains large vacuoles, and surrounded by a layer of circular muscles. The gonopore is situated at ±80%. In some animals, its sphincter is exceptionally well developed.

The paired testes are located lateral or anterior to the pharynx. The vasa deferentia enter the copulatory organ together at its proximal end. In some individuals they swell a little before entering. The copulatory organ is 45µm long and 20µm wide and is surrounded by two layers of diagonal muscles. It contains a big, bulbular seminal vesicle that is filled with sperm and occupies most of the organ. In the distal part of the organ a short but relatively wide, muscular ejaculatory duct is present. Prostate glands containing relatively coarse grained secretions enter the copulatory organ on its proximal side. Inside the organ, finer grains of secretions were also observed. In living animals, the secretions seem to run to the end of the copulatory organ in two strands, one on each side of the bulbus. The male channel is large, bended and surrounded by circular muscles all over its length. The bursa is opens in this channel at its proximal end. It is divided in a proximal, thin-walled part, filled with sperm, and a distal, muscular stalk. The stalk possesses an inner longitudinal and an outer circular layer of muscles and is weakly sclerotized on its inside. Both parts of the bursa are separated from each other by a strong sphincter.

Discussion: The most typical characteristics of this species are the bipartite bursa with muscular, sclerotized stalk and thin-walled proximal part, the copulatory organ with a muscular ejaculatory duct and the large seminal receptacle. *Olisthanellinella* is a poorly described species, but the morphology of our specimens completely matches the original description.

Known distribution: In the vicinity of Graz, where it is mentioned to be common in moist forest soil (REISINGER 1924); North-East Alps, Austria in moist forest soil (AN DER LAN & FRANZ 1954); Oldenburg, Germany (REISINGER in LUTHER 1963); Helsingfors, Finland in moss (LUTHER 1963), in salt meadows on the island Sylt, Germany (ARMONIES 1987).

Remark: The identity of the animals found by LUTHER (1963) is highly doubtful, as a large seminal receptacle was not observed and the egg-shaped, thin-walled bursa has a completely different structure from the animals described by REISINGER (1924). The specimens collected by ARMONIES (1987) were identified based on the description by LUTHER (1963).

Neotype: A sagittally-sectioned specimen (3245) found on the Kanzelkügel, Graz, Austria (47°6’49”N; 15°23’11”E) together with three other specimens in forest soil, on 26 August 2011.

Prorhynchella Ruebush, 1939

Prorhynchella minuta Ruebush, 1939

Figure 16 A-F

Material: One sagittaly sectioned specimen, two frontally-sectioned specimens. Drawings and pictures of living animals.

Description: The animal measures about 1mm in length and is very translucent. The anterior end is more or less pointy, while the posterior end is more rounded. The body is at its widest at two thirds of its length. Adenal rhabdites are present in two tracts, which reach to the level of the brain. The epidermis contained a thin, strongly basophilic layer, which may be small, very closely spaced dermal rhabdites. The animals possess a pair of ciliated pits (fig 16 C, cp) at the anterior end of the body. These are lateral invaginations of the epidermis, densely covered with relatively long cilia. Furthermore, a proboscis-like structure is present anterior to the brain. It consists of a weakly muscular sac (fig 16 C, pr) with associated basophilic glands (fig 16C, prg) that are located dorsolateral to the pharynx. The proboscis is hollow and is completely filled with these coarse grained secretions. The rosulate pharynx, situated at 25-35% of the body, measures 88µm across and is slanted forward. The paired protonephridiopores lie just behind the middle of the body.

The vitellaria occupy the posterior half of the body and don’t fuse. They are connected with the short oviduct by paired vitelloducts. An unpaired ovary is present. A receptaculum seminis is not present, but in one specimen a small accumulation of sperm was observed in the female duct. The vitelloducts open in the female duct at its proximal side. The ductus communis runs to a slightly wider part of the gonoduct, which could arguably be coined as a genital atrium. The gonoduct exits through a gonopore just before the middle of the body. One specimen was observed carrying a single, large egg.

The testes lie lateral to or just posterior to the pharynx. They are large and vary in shape, ranging from oval to pear-shaped or elongate. The vasa deferentia are relatively wide and fuse just before entering the copulatory organ. The egg-shaped, 75µm long copulatory organ is at its widest at one third of its length. It is surrounded by three muscular layers: two strong inner layers of diagonal muscles and a relatively weak outer layer of longitudinal muscles.

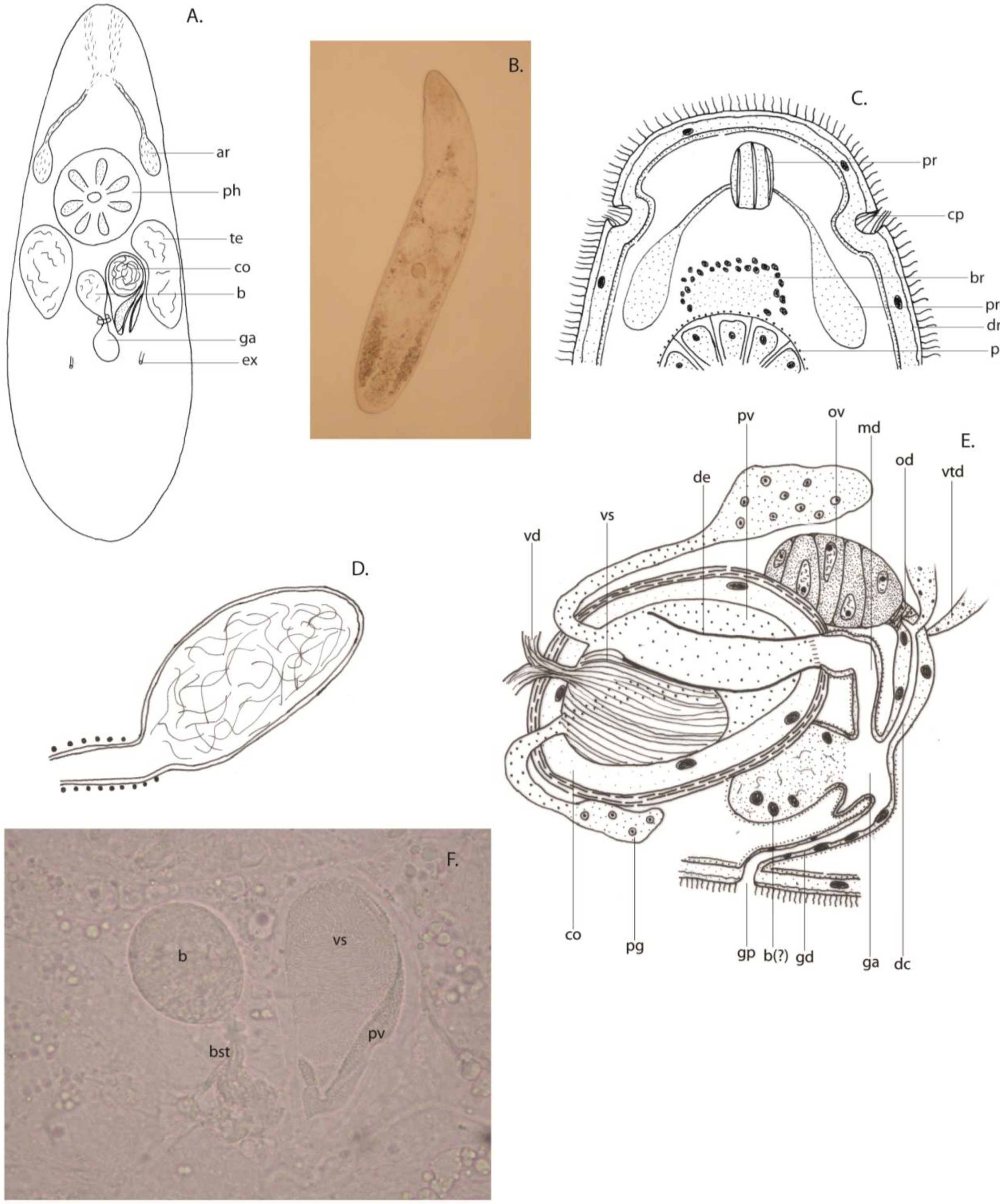


Figure 16: *Prorhynchella minuta*: A. Habitus of the living animal. B. Picture of living animal. C. Frontal reconstruction of anterior body end. D. Fully developed bursa. E. Sagittal reconstruction of genital system. F. Picture of bursa and copulatory organ.

The organ contains a seminal vesicle in its proximal third, a large prostate vesicle, filled with very coarse, basophilic secretions and a strongly sclerotized ejaculatory duct. The ejaculatory duct is slightly bended and gets narrower at its distal end. It reaches back to the most proximal third of the organ and is filled with secretions. A pair of large prostate glands is associated with the copulatory organ. They enter the copulatory organ through paired ducts. A 37µm long bursa was observed in at least one specimen (fig 16 D, F). It is an oval, thin walled structure (b), filled with sperm. It is connected to the male duct by a 16µm long bursal stalk (bst), which is surrounded by very strong sphincters, of which only two were visible on the living animal, but more on the sectioned specimen. In another specimen, an evagination of the gonoduct was observed (fig 16 E, b?). It is completely covered with circular muscles and possesses a dense mass on its inside. It is unclear whether this structure is the same organ during a different phase in development as the bursa in the other specimen.

Discussion: The specimens clearly belong to the monotypic genus *Prorhynchella* because of the presence of ciliated pits, the frontal proboscis, the position of the pharynx and the genital system and the structure of the copulatory organ. The excretory openings have a slightly different position than the animals in the original description, which had openings between the mouth and the gonopore (RUEBUSH, 1939). Furthermore their pharynx pointed backwards instead of forwards. These differences are, however, considered insufficient to classify the specimens from this study as a new species.

The ciliated pits probably serve as some kind of sensory organ. Another important characteristic of *Prorhynchella* is the presence of a proboscis-like structure at the frontal end. This characteristic is shared with *Microcalyptorhynchus* (Kepner & Ruebush, 1935) and *Haplorhynchella* Meixner, 1924, all classified as Protoplanellinae. This structure might be homologous to that of these species. However, *Microcalyptorhynchus* and *Haplorhynchella* are poorly known species. RIEGER (1974) suggests that this organ might be homologous to that of Kytorhynchidae Rieger, 1974 and doubts the taxonomy of these species. Protoplanellinae with a proboscis differ from the marine Kytorhynchidae in the unpaired ovary and the position of the pharynx (position unknown in *Haplorhynchella*). However, a molecular analysis in this study shows that *Prorhynchella* falls within Typhloplanidae, while the marine Kytorhynchidae are shown to fall within Thalassotyphloplanidae (VAN STEENKISTE 2012). This proves that probosces probably originated multiple times within the Dalytyphloplanida.

Known distribution: Freshwater ponds near Pine Orchard, Connecticut, USA (RUEBUSH, 1939).

New locality: Submersed vegetation in the southeast corner of Ponderosa Lake, Alabama, USA (32°54’53”N; 87°19’28”W), several specimens collected by dr. Niels Van Steenkiste on 27 May 2009.

***Protoplanella* Reisinger, 1924**

***Protoplanella simplex* Reisinger, 1924**

Figure 17 A-C

Material: 14 animals studied alive, three of which were frontally-sectioned and five were sagittally-sectioned.

Description: Animals measure about 0.8mm. The anterior and posterior end of the body are rounded. Adenal rhabdites were present in two anastomosing tracts; rhabdoid glands were arranged in two groups behind the brain. Dermal rhabdites were not observed. The rosulate pharynx is situated just behind the middle of the body and measures 45µm across. The protonephridiopores are situated laterally to the pharynx.

The vitellaria reach up to the anterior 25% of the body. At their posterior end they fuse over a broad anastomosis. The ovary is closely associated with one vitellarium to form an ovovitellary. The female duct is surrounded by a layer of weak, circular muscles and provided with fine grained, eosinophilic shell glands over large parts of its length. A club-shaped seminal receptacle ends in the proximal part of the female duct. The atrium is lined by a high epithelium and surrounded by an inner layer of longitudinal muscles and an outer layer of circular muscles. The gonopore is located at ±80% of the body.

The small, round testes lie posterior to the pharynx. The vasa deferentia enter the copulatory organ laterally. The 22µm long, oval copulatory organ is surrounded by a double layer of diagonal muscles. Eosinophilic prostate glands open laterally in the copulatory organ. Its proximal half contains a small seminal vesicle, which is lined by a clear epithelium. Its distal part holds the ejaculatory duct, which is surrounded by two layers of spiral muscles. The male duct possesses the same musculature as the atrium. A thin-walled structure (b?), which may serve as a bursa was observed on several living animals but was not visible on sectioned specimens.

Discussion: *P. simplex* has a very simple morphology and few diagnostic characteristics are present. The association of the ovary and one vitellarium to an ovovitellary, the structure of the copulatory organ and the position of the testes are the most important characteristics to identify this species. Due to this simple morphology, some records of this species might be based on false identifications, like LUTHER (1963) found an animal with a paired vitellarium. Specimens from VAN STEENKISTE *et al.* (2011) possessed two bursae. These small differences may be enough to consider these specimens as other, probably yet undescribed species.

The only other species in the genus is *P. macrorhabdiphora* An der Lan, 1955, which is known from only one very poorly described specimen, of which the genital system was not described, with only the huge adenal rhabdites as a diagnostic characteristic.

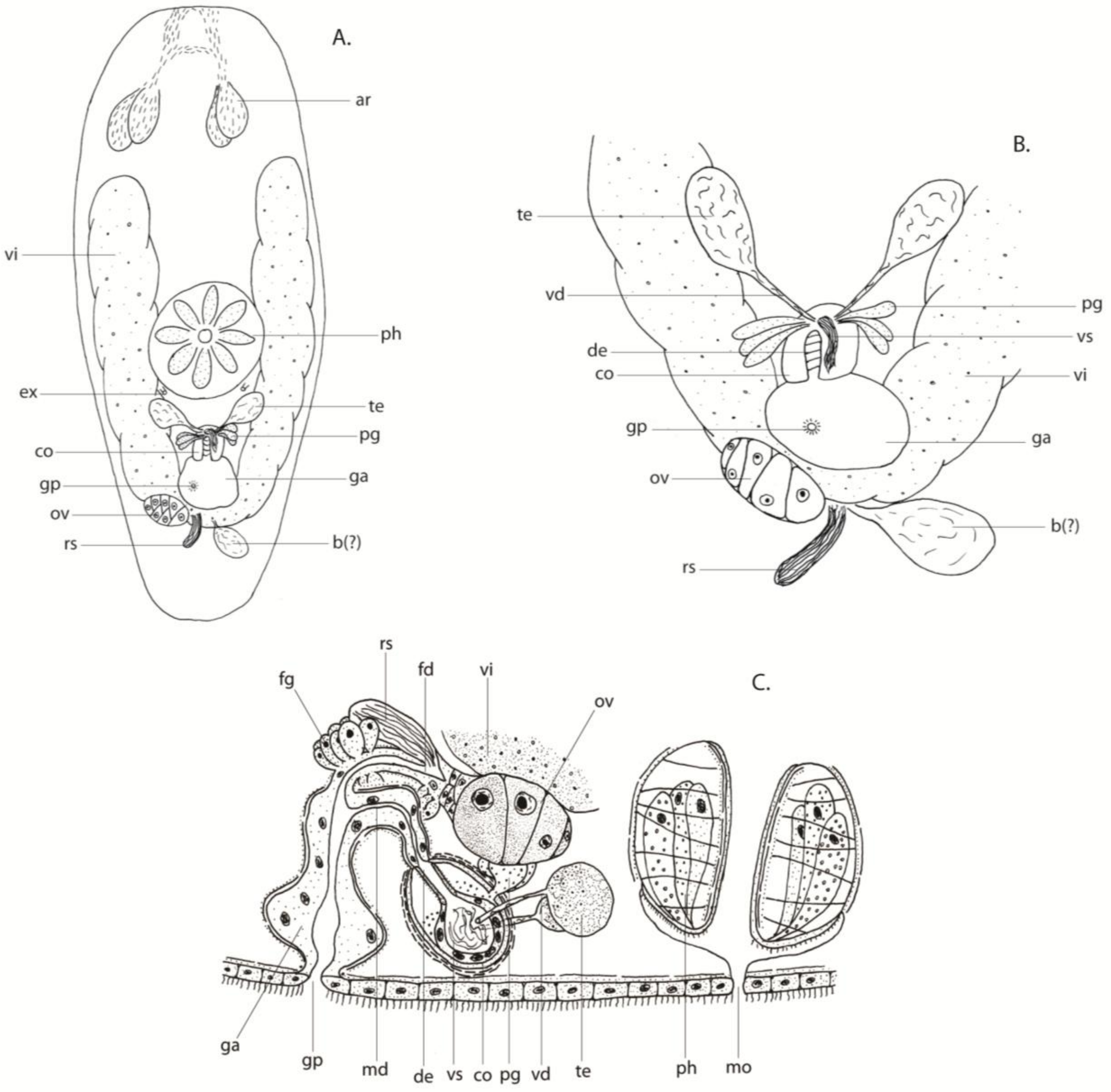


Figure 17: *Protoplanella simplex*: A. Habitus of living animal. B. Genital system of living animal. C. Sagittal reconstruction of genital system.

Remarks: Because of the few diagnostic characteristics it is considered necessary to designate a neotype for this species. Intensive surveys at the type locality failed to locate specimens. The specimens from Oberau, Bavaria, Germany, which is relatively close to the original type locality in Graz, Austria, were, however, of insufficient quality. For this reason, a specimen from Kordel, Germany was designated as the neotype, as its identification as *P. simplex* is sure. Because of the wide distribution of the species, the distance between the original type locality and the locality of the neotype was not considered problematic.

Known distribution: In the vicinity of Graz, in forest humus (REISINGER 1924; AN DER LAN 1963), on the Faroe Islands (STEINBÖCK 1932), in Tvärminne, Finland, in mosses (LUTHER 1963) near Poznan, Poland in moss and litter (KOLASA 1974) in dry moss in La Puebla del Rio, Spain (VAN STEENKISTE *et al.* 2011).

Neotype: A sagittally-sectioned specimen (3501) from Kordel, Germany (49°49’24”N; 6°38’6”E), together with one other specimen, in mosses growing on a wall, on 24 July 2011.

Other new localities: Oberau, Bavaria, Germany (47°33’33”N; 11°6’57”E), two specimens in forest litter, on 13 July 2011.
National Park ‘De Hoge Kempen’, Belgium, five specimens in mosses at a forest edge (50°56’3”N; 5°39’36”E) and five specimens in moist forest soil (50°56’2”N; 5°39’38”), on 27 July 2011.

Ventrociliella Kolasa, 1977

Ventrociliella romanae Kolasa, 1977

Figure 18 A

Material: Nine animals studied alive, one of which was sagittally-sectioned and three frontally-sectioned.

Description: The animals measure about 0.8mm in length and are very dark due to their gut contents. The anterior end is bluntly rounded to truncated, but can be protruded a little, like a small snout. The posterior end possesses a clear tail, which is much more translucent than the rest of the body. Both sensory and locomotory cilia are present, but only the ventral side of the body and a small ring at the same level as the brain are covered with cilia. Except for this ring the dorsal surface is bald. The epidermis possesses a high epithelium with vacuoles in its outer part, which show resemblance with small, very closely spaced rhabdites on the living animal. Adenal rhabdites are present in two separate tracts. On some animals they are almost absent and hardly visible. Dermal rhabdites are absent. The rosulate pharynx measures 37-42µm across. It is situated at ±35% (in some cases at ±50%) of the body and points forward. On the living animal it shows similarities with a short pharynx doliiformis, but on sectioned specimens it was clearly recognizable as a rosulate pharynx. The intestine reaches backwards to the beginning of the tail and is clearly delineated. In most animals, the paired nephridiopores are located at ±50% of the body, but in one animal the nephridiopores were situated anterior to the pharynx.

The vitellaria reach up to the pharynx and are slightly ramified. A vitelloduct was not observed, but at their posterior end, the vitellaria are fused. A short female duct runs from the small, oval ovary. At the proximal end of the female duct, a seminal receptacle is present as an accumulation of sperm inside the duct. The small atrium is lined by a high epithelium and is surrounded by a weak muscular layer. The gonopore lies at ±85% of the body.

Two small, round testes lie close to the copulatory organ. The vasa deferentia were not observed with certainty. The 20µm long, elongate copulatory organ is slightly bended and contains a small amount of sperm in its proximal part, which probably serves as a seminal vesicle. On some living animals a very thin ductus ejaculatorius was visible. It was not clear whether it was sclerotized. On sectioned animals, eosinophilic glands associated with the copulatory organ were observed. The copulatory organ ends in the genital atrium through a short male duct. A bursa was not observed.

Discussion: KOLASA (1977) classifies this species within Protoplanellinae because the protonephridia open separately and the testes lie ventral to the vitellaria. TYLER *et al.* (2006-2012) and VAN STEENKISTE *et al.* (2010) consider this species as an incertae sedis taxon within the Dalytyphloplanida. Reasons for this have, however, never been stated. Because of this, *Ventrociliella* is considered as a taxon within Protoplanellinae in this study. In the original description, the genus is said to be very closely related to *Bockia* Reisinger, 1924 because of the habit of the body, the position of the male reproductive organ, the similar structure of the pharynx and the terrestrial lifestyle. It was stated that these taxa differ because of the lack of adenal rhabdites, the presence of a real pharynx doliiformis, the occurrence of complete ciliation, the cuticular ductus ejaculatorius, the lack of a seminal receptacle and the absence of a bursa in *Bockia*. During this survey, however, specimens of *Ventrociliella* were found on the type locality of *Bockia*. The latter species is only known from one specimen, so questions its identity may be questionable.

In some of our specimens, adenal rhabdites were also lacking almost completely and were barely visible. Furthermore, the only drawing ever published of *Bockia* shows a pharynx that looks identical to the one of *Ventrociliella* (REISINGER, 1954). The peculiar structure of the pharynx of *V. romanae* makes it possible to see it as a pharynx doliiformis. The bursa described by KOLASA (1977) was not visible on the animals in this study, but the genital atrium was much larger than on the drawings from the original description, a characteristic also mentioned in the description of *Bockia* (REISINGER, 1924). In the description of *Ventrociliella* the atrium seems to go over in the bursa rather smoothly. Possibly, the bursa in the description of *Ventrociliella* is just part of the atrium. The lack of complete ciliation is also considered as a difference between *Bockia* and *Ventrociliella*, but in the description of *Bockia* no remarks on the ciliation were made, and as this is a rather subtle characteristic and because *Bockia* is described based on only one specimen, it is probable that this was overlooked by the author. As the seminal receptacle is just an accumulation of sperm in the oviduct, it would not be visible if it is not filled, which could explain the absence of a receptaculum in the only specimen of *Bockia*. The only clear difference between the two species might be that the ductus ejaculatorius of *Bockia* is slightly cuticularised, whilst it is muscular in *Ventrociliella*, but because of the small size of both animals, the reliability of this characteristic is highly doubtful. The typical, conspicuous vacuolated epidermis of *Ventrociliella* was never mentioned in the description of *Bockia*. Despite this, it is highly likely that *Ventrociliella romanae* and *Bockia deses* should be considered as synonyms, in which case *Ventrociliella romanae* Kolasa, 1977 becomes the junior synonym of *Bockia deses* Reisinger, 1924. This could, however not be proved with absolute certainty. For this reason, *Ventrociliella* and *Bockia* are still considered as distinct taxa until more research provides further information.

Remarks: KOLASA (1977) suggested that the peculiar ciliary pattern could be an adaptation to a semiterrestrial lifestyle.
The animals seem to feed on rotifers.

The only known specimen of *Bockia* was found on the Hohe Rannach, which might be an extra indication that *Ventrociliella* and *Bockia* are synonyms, as specimens of *Ventrociliella* were found on this locality.

Known distribution: Forest soil near the Fosso Contesora in Tuscany, Italy (Kolasa, 1977).

New localities: On the Hohe Rannach, near Graz, Austria (47°9’53”N; 15°24’50”E), one specimen in leafy humus of a beech forest, on 26 August 2011.

On the Rohrerberg, Graz, Austria (47°7’44”N; 15°24’53”E), one specimen between dead leaves, on 26 August 2011.

On the Kanzelkügel, Graz, Austria (47°6’49”N; 15°23’11”E), two specimens in leafy humus, on 26 August 2011.

On the Kreuzberg, Weyer, Austria, one specimen in humus of a mixed forest (47°51’36”N; 14°39’9”E) and four specimens in moss growing on a dead trunk (47°51’29”N; 14°39’14”E), on 29 August 2011.

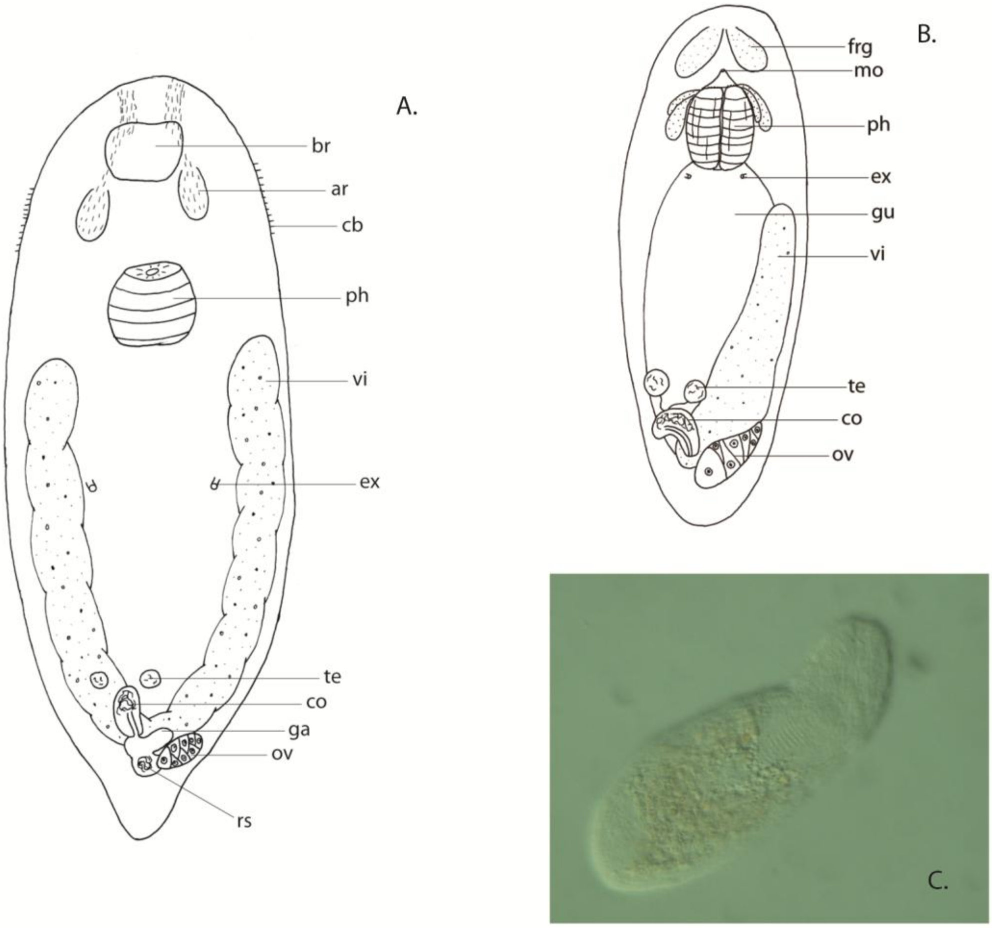


Figure 18: ***Ventrociliella romanae*****: A. Habitus of living animal.** ***Archivortex silvestris*****: B. Habitus of living animal. C. Picutre of living animal at magnification 25x10.**

Provorticidae Beklemischev, 1927

Archivortex Reisinger, 1924

Archivortex silvestris Reisinger, 1924

Material: Ten specimens studied alive, three of which were sagittally-sectioned..

Description: Animals measure 0,2mm in length. The length to width ratio is about 4-5 to 1. Both the anterior and the posterior body end are rounded. Rhabdites are lacking, but the outer part of the epidermis is completely covered in small structures that were originally described as pseudorhabdites (REISINGER 1924). The pharynx doliiformis, which is situated in the anterior 20% of the body, measures about 20x30µm. Glands (frg), lying in front of the pharynx were visible on living specimens. The protonephridia exit the body through two separate pores which are situated right behind the pharynx.

A single ovovitellary consists of an anterior vitellarium and a posterior ovary. The oviduct leads to a small genital atrium, which ends in a gonopore, located at ±75% of the body. Other female structures were not observed.

Testes are paired, round and are situated at ±65% of the body. The copulatory organ measures 13µm and is surrounded by an inner circular outer longitudinal muscle layer. The prostate vesicle is filled with eosinophilic secretions of which the glands were not observed. The ejaculatory duct is slightly bended and seems somewhat sclerotized. The male duct is very short and ends in the common genital atrium.

Discussion: *A. silvestris* is the only known species within the Provorticidae which lives in limnoterrestrial environments (REISINGER 1924; VAN STEENKISTE *et al.* 2010). Its body shape, small size, presence of a large ovovitellary and small copulatory organ make the species very recognizable.

Known distribution: In the vicinity of Graz, Austria. Common and abundant in humus of forest soils (REISINGER 1924) and in Pendling bei Kufstein, Austria (AN DER LAN 1963).

New localities: Kanzelkügel, Graz, Austria (47°6’49”N; 15°23’11”E), nine specimens in moist forest soil on 26 August 2011.
Kreuzberg, Weyer, Austria (47°51’36”N; 14°39’9”E), one specimen in humus of mixed forest on 29 August 2011.

Table 5: Abbreviations used on drawings

Abbreviation	Meaning	Abbreviation	Meaning
aa	Atrial appendage	md	Male duct
ag	Atrial gland	mo	Mouth
ap	Anterior part of pharynx	mx	Matrix
ar	Adenal rhabdites	od	Oviduct
b	Bursa	ov	Ovary
br	Brain	pg	Prostate gland
bst	Bursal stalk	ph	Pharynx
cb	Ciliary band	phg	Pharyngeal gland
Ci	Cirrus	pp	Posterior part of pharynx
cm	Circular muscle layer	ppc	Prepharyngeal cavity
co	Copulatory organ	pr	Proboscis
cp	Ciliary pit	prg	Proboscis gland
dc	Ductus Communis	pv	Prostate vesicle
de	Ejaculatory duct	ram	Radial muscle
dr	Dermal rhabdite	rm	Retractor muscle
ex	Protonephridiopore	rs	Seminal Receptacle
fd	Female duct	sc	Sclerotized cone
fg	Female glands, shell glands	sh	Sclerotized hooks
frg	Frontal gland	ss	Sclerotized structure
ga	Genital atrium	te	Testis
ga	Genital atrium	tg	Tail Gland
gd	Gonoduct	u	Uterus
gl	Gland	vd	Vas deferens
gp	Gonopore	vi	Vitellarium
gp	Gonopore	vs	Seminal vesicle
lm	Longitudinal muscle layer	vtd	Vitelloduct

Phylogenetic analysis

Results

The 18S-alignment consisted of 1813 positions. After removing ambiguous sites, 1784 positions were left, 1334 of which were constant and 450 were variable. The 28S-alignment consisted of 3771 positions, 3298 of which were left after removing ambiguous sites. 2263 sites were constant, 1035 were variable.

Saturation tests never found any evidence for substitution saturation. No complete 28S sequence of *Mesostoma lingua* and *Protoplanella simplex* Doñana were available and including them in a concatenated analysis with the 28S-sequence filled in as missing data gave unrealistic results. For this reason, these sequences were omitted from the final concatenated analysis. All runs in MrBayes converged well before the burn-in at 2,500,000 generations. The 28S tree and the concatenated tree were completely congruent, and showed relatively high support values. Trees generated by ML-analysis were mostly similar, although with weaker support values.

All trees strongly supported the existence of a clade containing the sequences of three *Acrochordonoposthia* together with *Adenocerca minima* (taxon A). This taxon seems to form a sister group relation with all other Typhloplanidae. *Carcharodopharynx* is strongly supported as a taxon within Typhloplanidae and forms the sister taxon of the least inclusive clade containing *Ventrociliella romanae* and *Bryoplana xerophila*. The taxon containing *Ventrociliella romanae, Hoplopera* nov. sp. and *Olisthanellinella rotundula* (taxon B), where *V. romanae* is the sister taxon of the other two species, is strongly supported in all analyses. Taxon B forms a sister group relation with the least inclusive taxon containing both *Chorizogynopora italica* and *Bryoplana xerophila*. Taxon C is the least inclusive clade containing both *Prorhynchella minuta* and *Bryoplana xerophila*. It is strongly supported in the 28S and the concatenated analysis, but not in the 18S analysis. The strongly supported Taxon D consisted of *Castrada* sp., *Rhynchomesostoma rostratum* and *Typhloplana viridata*. The relation between Taxon C, Taxon D and both sequences of *Krumbachia* remains unresolved. The 28S analysis suggests a weakly-supported sister group relation between Taxon C and Taxon D, but this could not be verified in the other analyses. Both sequences of *Krumbachia* cluster together in both the 28S and the concatenated analysis, but this clade has only very weak support. The polytomy consisting of *Chorizogynopora italica*, *Protoplanella sp.*, *Adenoplea meridionalis* and all sequences of *Protoplanella simplex*, except the specimen from Doñana (taxon E), is also strongly supported, but its internal resolution is only weakly supported and the different analyses disagree on it. The 28S and concatenated analysis showed a strongly supported sister group relation between Taxon E and the taxon including Taxon C, Taxon D and *Krumbachia*.

The 18S-tree was less resolved and showed lower support values. Taxon C was not supported at all. Furthermore, the relations between Taxon B, Taxon D and Taxon E were unclear in this analysis. These taxa themselves were, however, strongly supported.

Discussion

Few phylogenetic analyses have focused on rhabdocoels. Only WILLEMS *et al.* (2006) and VAN STEENKISTE (2012) performed thorough analyses of rhabdocoel phylogeny. VAN STEENKISTE (2012) included several Typhloplanidae in the analysis and provided first clues about the polyphyly of Protoplanellinae, which is also resulting from our analysis.

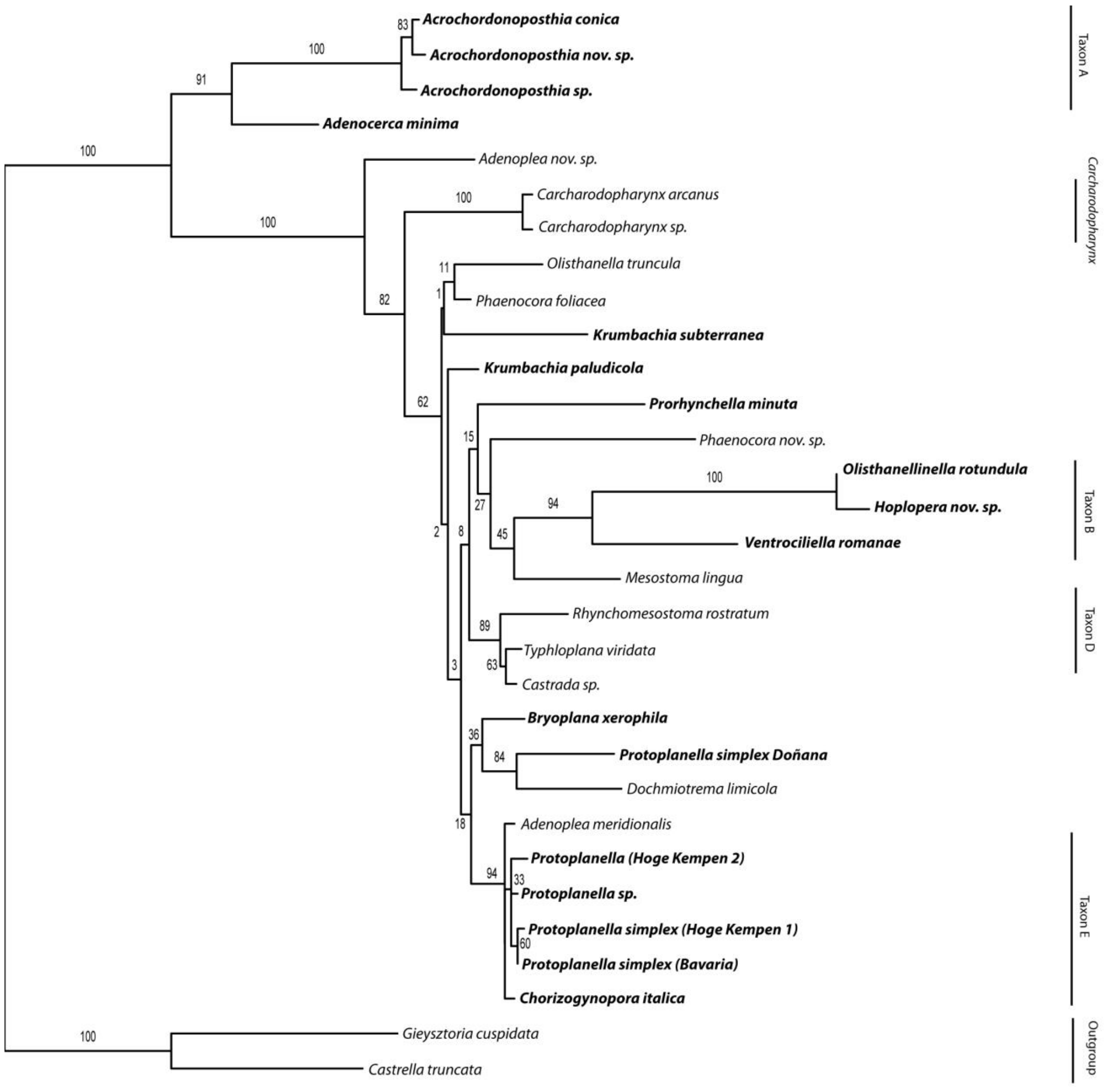
Especially the 28S and the concatenated analysis yielded very robust trees. The 18S tree shows lower support values, but was largely congruent with the other trees. ML-trees were generally less strongly supported than trees from Bayesian Inference. Some deeper branches, such as Taxon C and Taxon E were only weakly supported. To solve the relationships between these taxa, rDNA is probably too conservative and the use of a faster evolving gene might yield a better supported tree.

All analyses agree that Protoplanellinae are clearly polyphyletic. Several smaller clades consisting of Protoplanellinae, such as Taxon A, Taxon B and Taxon E are strongly supported. The taxon Protoplanellinae is based on the ventral position of the testes in relation to the vitellaria and the separate protonephridiopores. Both are highly questionable as apomorphies for this group (VAN STEENKISTE *et al.* 2010; VAN STEENKISTE 2012). Paired protonephridiopores are probably an ancestral trait (Albrecht Houben, pers. comm.) and should not be used as an apomorphy at this level. Marine Protoplanellinae were not included in this analysis and their phylogenetic position remains unclear.

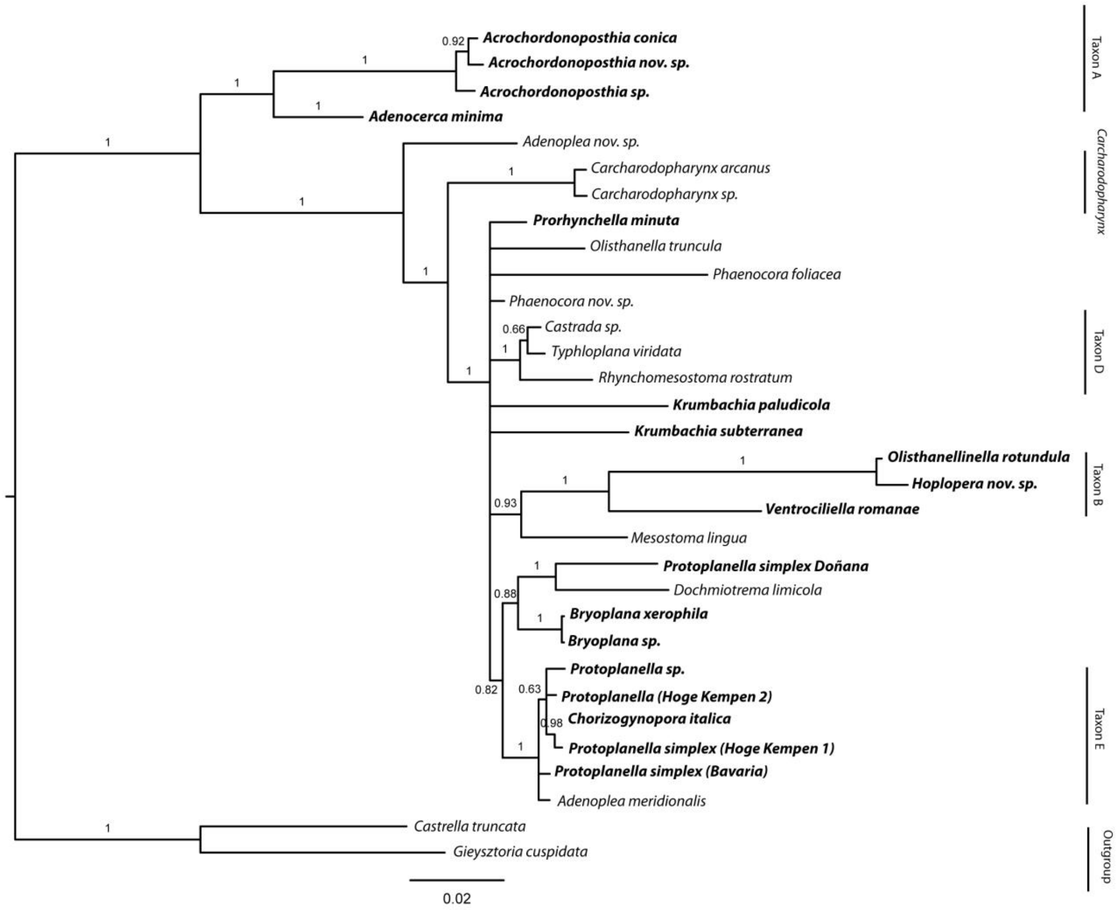
Carcharodopharynx has always been placed in its own family because of its aberrant morphology. An earlier analysis (VAN STEENKISTE 2012) indicated that *Carcharodopharynx* might fall within Typhloplanidae. Our analysis confirmed this position, with both sequences being strongly supported within Typhloplanidae. For this reason, *Carcharodopharynx* should be included within Typhloplanidae, instead of being classified within a distinct family.

Several limnoterrestrial species were included in the analysis to see whether this limnoterrestrial lifestyle originated multiple times or only once within Typhloplanidae. The trees clearly indicate that colonization of limnoterrestrial habitats happened several times. This is not surprising, as most limnoterrestrial species live in moist habitats, which are not very different from temporary limnic habitats in witnessing temporary periods of desiccation. The only known limnoterrestrial rhabdocoel that does not belong to Typhloplanidae, *Archivortex silvestris*, also colonized this habitat independently.

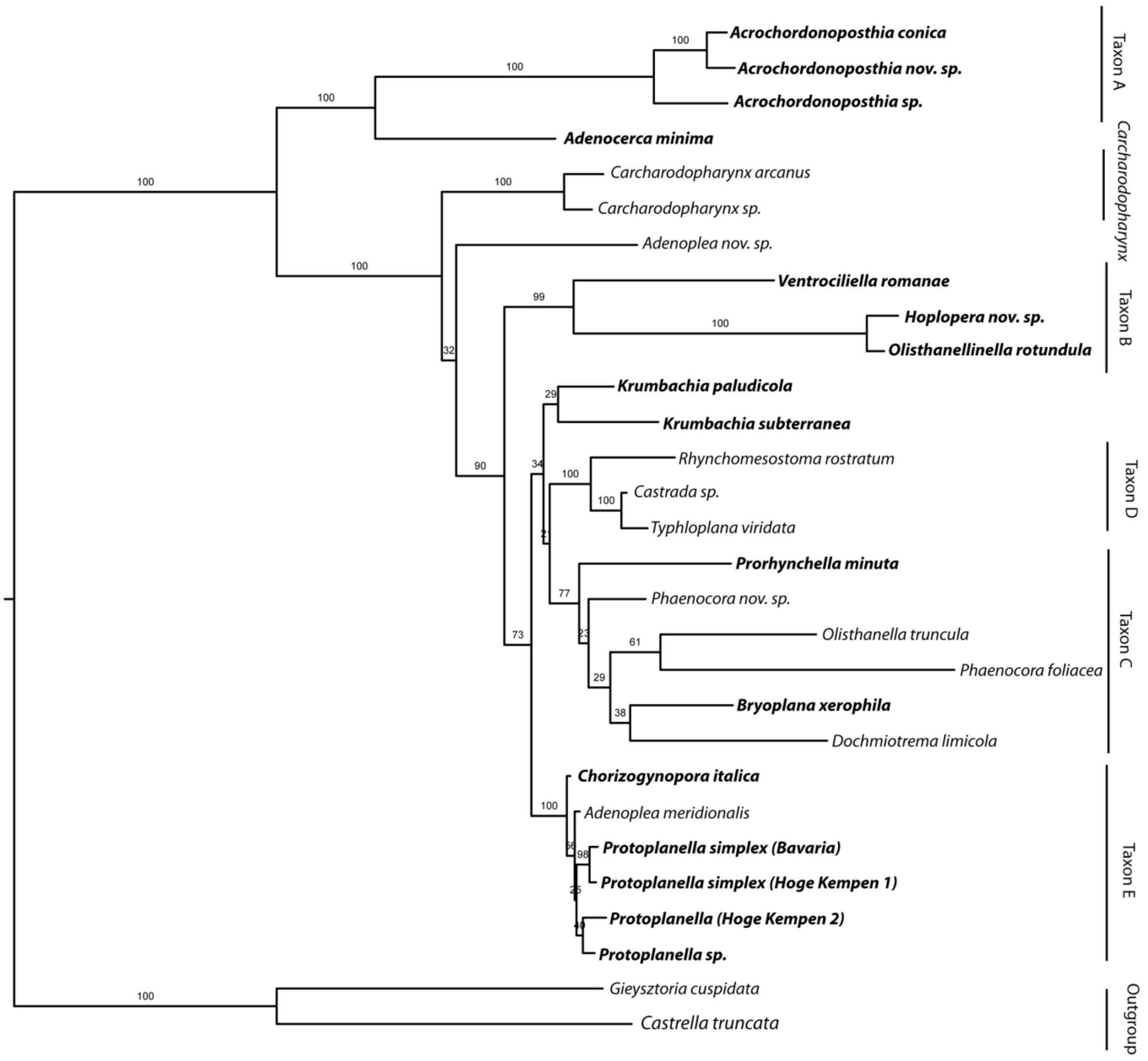
The analysis includes relatively many sequences of Protoplanellinae, but the number of other Typhloplanidae is limited, as providing a full phylogeny of Typhloplanidae was outside the scope of this study. The monophyly of some other subfamilies of Typhloplanidae, such as the Typhloplaninae, might also be questionable. The Typhloplaninae were represented by three sequences: *Adenoplea* nov. sp., *Adenoplea meridionalis* and *Typhloplana viridata*, which all fell on different positions on the tree. As all traditional subfamilies within Typhloplanidae, such as Protoplanellinae, are based on the position of the protonephridiopores and the relative position of testes and vitellaria, the taxa have only weak morphological support. A more thorough analysis with more sequences of Typhloplanidae included will be needed to clear out the phylogeny of this taxon.



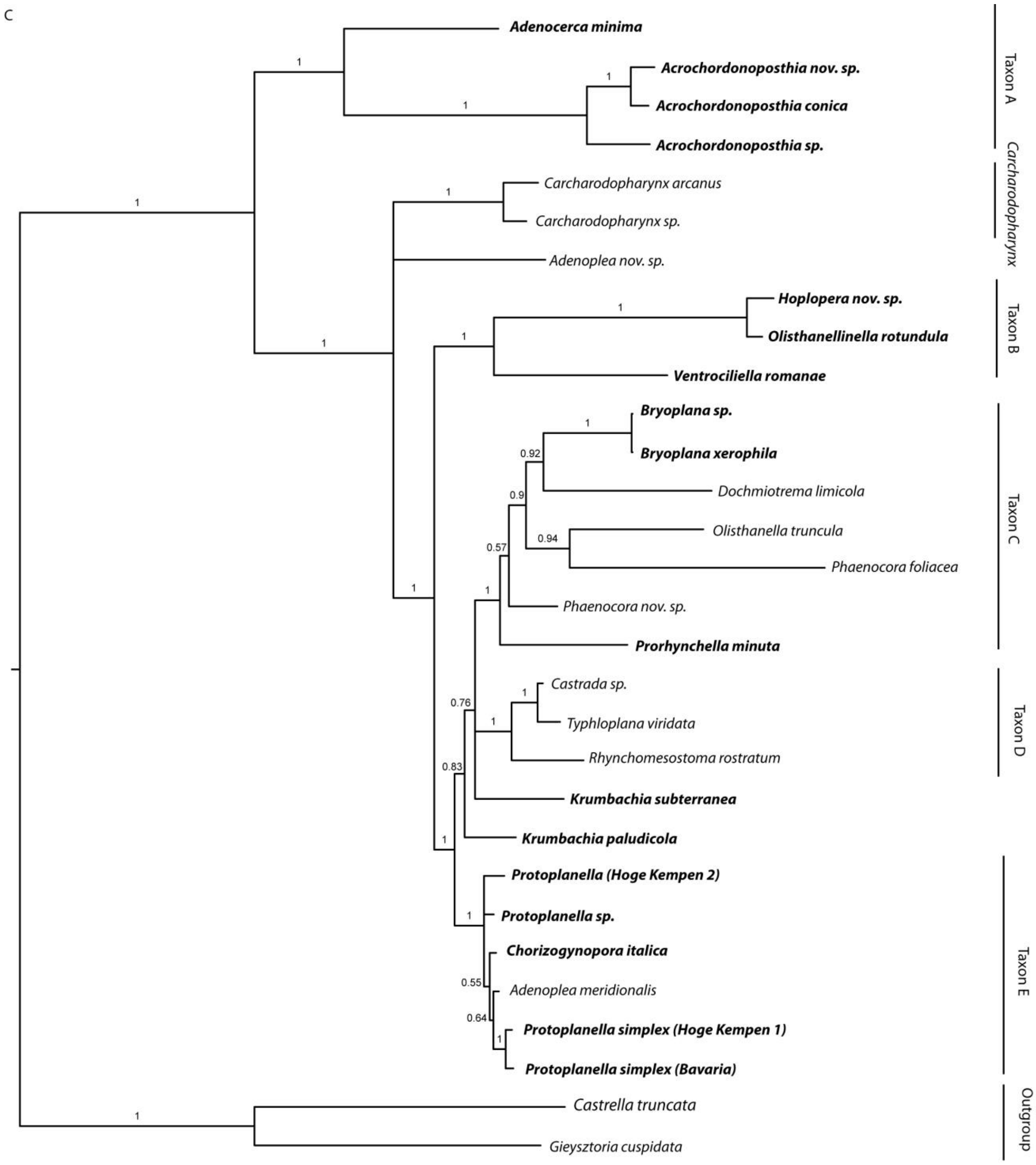
Tree 1: Maximum likelihood tree of the 18S gene. Branch labels indicate bootstrap values. Protoplanellinae are indicated in bold.



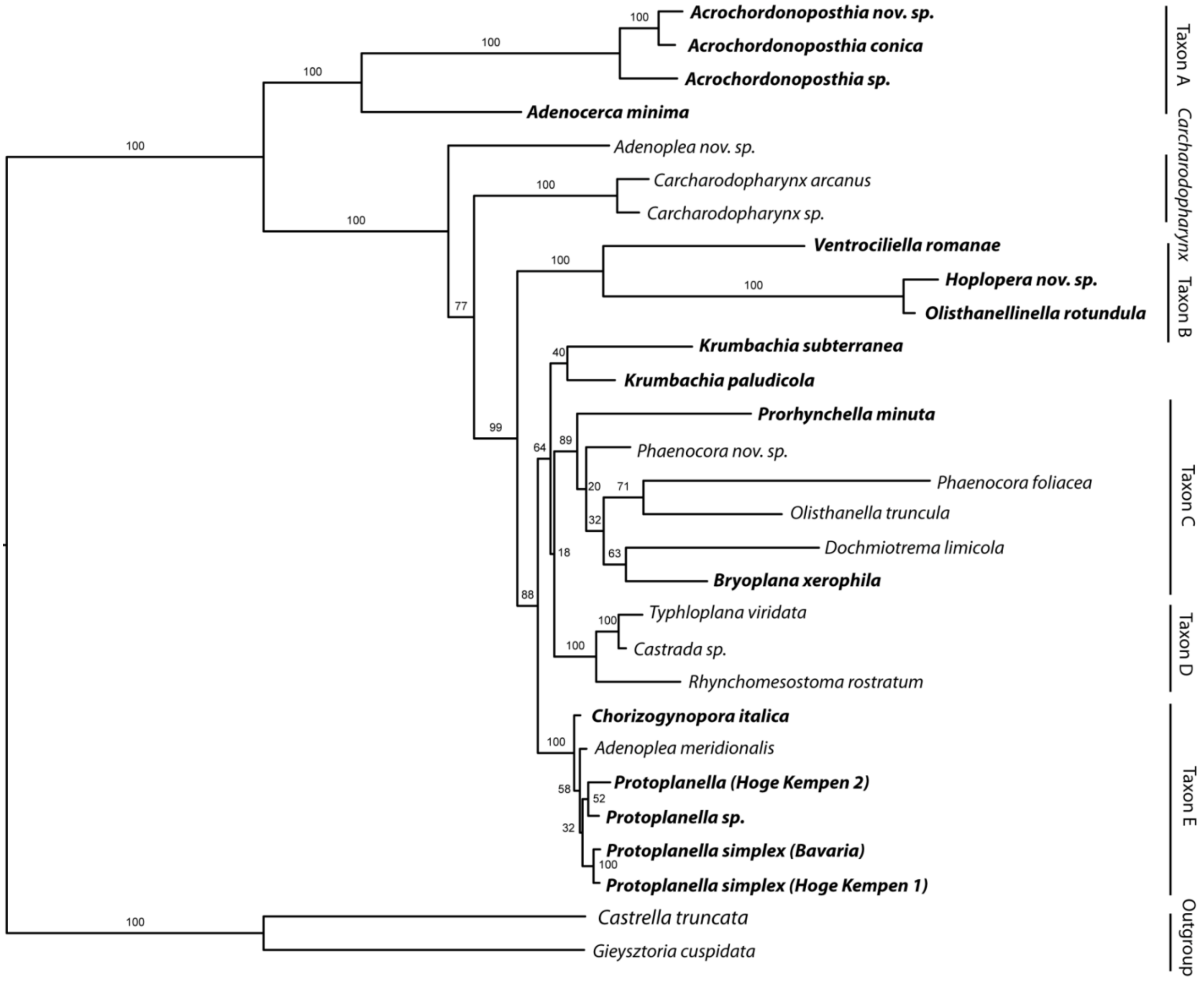
Tree 2: Bayesian inference tree of the 18S gene. Branch labels indicate posterior probabilities. Protoplanellinae are indicated in bold.



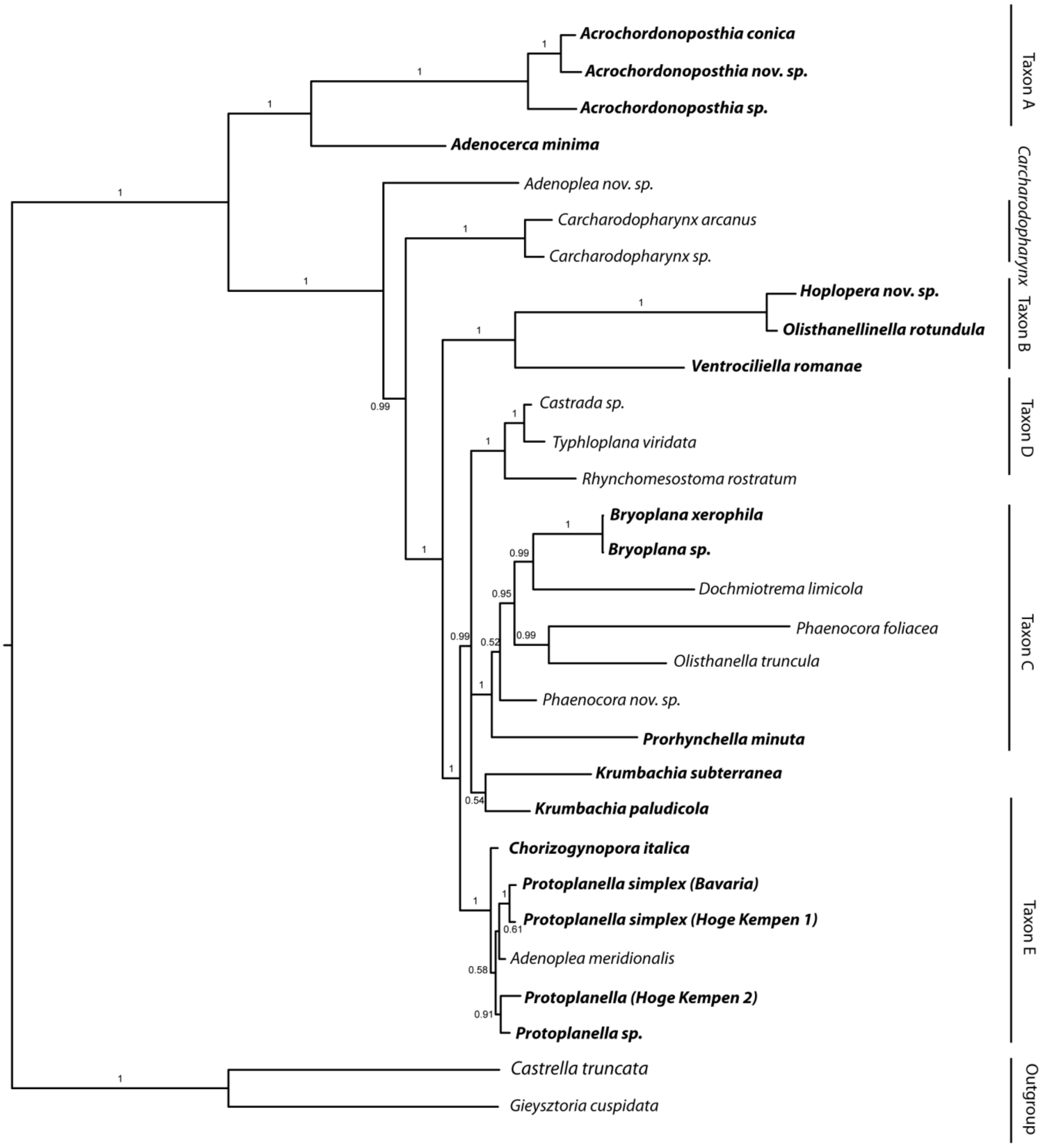
Tree 4: Bayesian inference tree of the 28S gene. Branch labels indicate bootstrap values. Protoplanellinae are indicated in bold.



Tree 4: Bayesian inference tree of the 28S gene. Branch labels indicate posterior probabilities. Protoplanellinae are indicated in bold.



Tree 5: Maximum Likelihood analysis of the concatenated dataset. Branch labels indicate bootstrap values. Protoplanellinae are indicated in bold.



Tree 6: Bayesian inference analysis of the concatenated dataset. Branch labels indicate posterior probabilities. Protoplanellinae are indicated in bold.

Conclusion

Protoplanellinae have always been ignored because of the need to study them both alive and sectioned. Most taxonomic work dates back several decennia, descriptions are often incomplete and type material often does not exist. This study partially cleared out the taxonomy of Protoplanellinae and gave a thorough account on limnoterrestrial rhabdocoels in general. However, many species remain poorly known and most parts of the world still have not been sampled yet.

The phylogenetic analysis confirms earlier suspicions that Protoplanellinae are polyphyletic. This indicates that moist terrestrial habitats were colonized several times independently. Few sequences of other subfamilies were included in this study, as a thorough analysis of Typhloplanidae fell outside the scope of this study. Future research will be needed to investigate whether the other traditional subfamilies are effectively monophyletic, or fall apart in several smaller taxa, like Protoplanellinae.

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