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Primary Homology Assessment in the Male Atrial System of the Polycystididae (Platyhelminthes: Eukalyptorhynchia)

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Abstract. A comparative lightmicroscopical study of the male atrial system of the Polycystididae is presented. Firstly, the different glandular structures associated with the male atrial system are compared. This has lead to the recognition of eight different types of glandular vesicles, four of which are considered prostate, the other four accessory. Secondly, the different hard structures that can be found in the male atrium are discussed and compared. These hard structures can consist of numerous hard spines (armed cirrus), but mostly the hard structure is a single- or double-walled tubiform stylet, or a plate-shaped stylet. Different types of stylets can be recognised, some of which are associated with a prostate vesicle and therefore considered prostate stylets. Using the criteria of position and conjunction, conjectures of homologies are put forward. These primary homologies are compared with homology assessments found in earlier literature.

Key words. Flatworms, Turbellaria, male atrial system, morphology, evolution.

1. INTRODUCTION

The term "atrial organs" was introduced by KARLING in his seminal 1956 paper, and indicates the structures associated with the genital atrium. In fact, it indicates all the genital organs and structures, except for the gonads and their ducts. With its 143 species known – even though some may be dubious – the Polycystididae is by far the most species rich taxon within the Kalyptorhynchia (turbellarians with a proboscis), and the diversity in the atrial organs is astonishing. This complexity has lead to difficulties in homology assessment of the various parts of the atrial system. Different views on possible homologies have been put forward (see MEIXNER 1925; KARLING 1956; SCHOCKAERT 1974; KARLING & SCHOCKAERT 1977; EVDONIN 1977; ARTOIS & SCHOCKAERT 1999a, 2000), and thus also different hypotheses on the relationships within the taxon. These relationships were, however, never approached from a cladistic point of view, not within the Polycystididae nor within the Kalyptorhynchia. We have now initiated such a cladistic analysis of the Polycystididae, and this contribution is the first discussion on characters, at the light microscopic level, that can be used in the analysis. We will not discuss all characters, associated with the male atrial system, which could be useful in the cladistic analysis, but only those that raise difficulties as to homology assessment. All species for which we had good material (108) were examined. In total 17 characters, divided into two groups, are discussed in this contribution: eight characters related to glandular organs of the male system and nine characters in the hard structures of the male system. Each of these characters is a conjecture of homology (primary homology of PATTERSON 1982), based on the conjunction and position criteria commonly used in primary homology assessment (PATTERSON 1982; DE PINNA 1991; BROWER & SCHAWAROCH 1996). Whether they represent true homologies (synapomorphies) must be assessed together with other characters (other primary homologies) in a congruence analysis. Such an analysis is not proposed in this contribution, and we do not discuss phylogenetic relationships here.

2. MATERIAL AND METHODS

2.1. Microscopical material

At present, 143 species of Polycystididae are known. Of 108 species, the material we were able to examine (sections, whole mounts, and some species studied alive) allowed a thorough analysis of all characters. We used a Reichert Polyvar compound microscope equipped with Nomarski interference phasecontrast, useful for examination of hard structures

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in whole mounts. Of the 35 species listed below, material is simply non-existent, of bad quality (or at least insufficient), or was not available for loan.

Acrorhynchus baikalensis Rubtzoff, 1928, *Acrorhynchus reprobatus* (Pereyaslawsewa, 1892), *Antiboreorhynchus torquatus* Karling, 1952, *Bermudorhynchus sterreri* Karling, 1978, *Fungorhynchus pistillatus* Karling, 1952, *Gemmeliclinus flavidus* Evdonin, 1970, *Gyrator bivittatus* Uljanin, 1870, *Leuconoplana ovata* (Uljanin, 1870), *Ludmilla graciosa* Uljanin, 1870, *Macrorhynchus spiralis* Pereyaslawsewa, 1892, *Marcusia yagana* (Marcus, 1954), *Megaloascos psammophilum* Evdonin, 1970, *Opisthocystis goettei* (Bresslau, 1906), *Opisthocystis abyssalis* Timoshkin, 1986, *Opisthocystis angarensis* Timoshkin, 1986, *Opisthocystis bilobata* (Nasonov, 1935), *Opisthocystis campanulata* (Nasonov, 1935), *Opisthocystis cariottus* Timoshkin, 1986, *Opisthocystis curvistylus* Timoshkin, 1986, *Opisthocystis multifida* (Nasonov, 1935) Karling, 1956, *Opisthocystis pedistylus* Timoshkin, 1986, *Opisthocystis sabussovi* Timoshkin, 1986, *Opisthocystis trifida* (Nasonov, 1935), *Palladia nigrescens* Evdonin, 1977, *Phonorhynchella biarcuata* Karling, 1956, *Phonorhynchoides carinostylis* Ax & Armonies, 1987, *Phonorhynchoides flagellatus* Beklemischew, 1928, *Phonorhynchus bitubatus* Meixner, 1938, *Phonorhynchus pearsi* Ferguson, Stirewalt & Kepner, 1940, *Rogneda polyrhabdota* Ax, 1959, *Rogneda tripalmata* (Beklemischew, 1927), *Polycystis georgii* Graff, 1905, *Polycystis intubata* Graff, 1905, *Polycystis tenuis* Beklemischew, 1921, *Psammopolycystis forcipiens* Brunet, 1976.

The examined material is either present in the collections of the Limburgs Universitair Centrum, or was received on loan from the Naturhistorisk Riksmuseet (Stockholm, Sweden) and from the II. Zoologischen Institut der Universitaet zu Göttingen (Germany).

2.2. List of abbreviations

acg1 accessory glands type I, **acg2** accessory glands type II, **acg3** accessory glands type III, **acg4** accessory glands type IV, **ast1** accessory stylet type I, **ast2** accessory stylet type II, **ast3** accessory stylet type III, **ast4** accessory stylet type IV, **ci** armed cirrus, **de** ejaculatory duct, **ga** common genital atrium, **gp** gonopore, **ma** male atrium, **mb** male bursa, **mub** muscle bulb, **pv1** prostate vesicle type I, **pv2** prostate vesicle type II, **pv3** prostate vesicle type III, **pv4** prostate vesicle type IV, **s** septum, **st1** prostate stylet type I, **st2** prostate stylet type II, **st3** prostate stylet type III, **st4** prostate stylet type IV, **vs** seminal vesicle, **x** see text

3. RESULTS

3.1. Glandular organs of the male atrial system

Until now, the homologies of the glandular organs in the male system of the Polycystididae appeared rather simple, and the present ideas (mainly those of KARLING 1956; SCHOCKAERT 1974; KARLING & SCHOCKAERT 1977; ARTOIS & SCHOCKAERT 1999a) can be summarised as follows:

Many Polycystididae have only one glandular organ in the male atrial system. It was then called "prostate vesicle" and considered homologous, irrespective of whether it is interposed in the male duct (conjuncta-type) or separated from it (divisa-type or "free prostate vesicle"). Many other Polycystididae have two or even more glandular organs. Usually one of these organs was then considered the "prostate vesicle", homologous with that of the first group regardless of its position in the atrium or its relations to the other atrial structures, and even regardless of its structural details. The remaining glandular organs were considered accessory, and homologies between the different accessory organs were never explicitly indicated.

In a few taxa the conjuncta situation was considered a secondary situation (KARLING 1955, 1956; SCHOCKAERT 1974) since in these taxa a free prostate vesicle occurs as well (and was considered homologous with the free prostate organ of the groups above). Based on several other features, these taxa were considered related to each other. *Annulorhynchus adriaticus* Karling, 1956 and the *Gallorhynchus* Schockaert & Brunet, 1971 species share these same features and are thus also considered related to these taxa, and therefore the conjuncta situation in *Annulorhynchus* Karling, 1956 and *Gallorhynchus* was also considered derived, even though these two taxa have no free prostate vesicle. From the above it thus appears that there would be three kinds of glandular organs considered homologous: the prostate organ (primarily interposed or free), the secondary interposed glandular vesicle, and the accessory glandular structures.

KARLING & SCHOCKAERT (1977) and ARTOIS & SCHOCKAERT (1999a) expressed some doubts about the homology of the various free prostate organs, and suggested possible homologies with some of the so-called "accessory" gland vesicles. Some of these ideas have inspired us during this study.

Using criteria of structure and position, our current observations revealed eight different types of glandular elements, a homology assessment quite different from ideas in the past. These glandular structures are discussed now: three kinds of free prostate vesicles (prostate vesicles type I–III, the interposed prostate vesicle (prostate vesicle type IV), and four different accessory glandular elements. The use of the common terms "prostate vesicle" or "accessory glands" does not imply any *a priori* statement of homology.

3.1.1. The prostate vesicle of type I (Figs. 1A, 3A). Species in the taxa *Galapagorhynchus* Artois & Schockaert, 1999; *Macrorhynchus* Graff, 1882; *Paulodora* Marcus, 1948; *Phonorhynchus* Graff, 1905; *Polycystis* Kölliker, 1845.

The overall shape of this type of free prostate vesicle is globular to spindle-shaped. It contains an eosinophilic and a basophilic secretion. It is surrounded by three spiral muscle layers, each with a different slope (*Macrorhynchus*; see Fig. 15 in SCHOCKAERT & KARLING 1975; Fig. 8 in ARTOIS & SCHOCKAERT 2001), by two muscle layers (e.g. *Polycystis*, Fig. 1A) or by a single, very thick muscle layer (e.g. *Phonorhynchus*, Fig. 3A). Within this muscle sheath the gland necks form fascicules that are spirally woven around each other (*e.g. Polycystis*, Fig. 1A), or both types are clearly separated, one of them peripheral as in *Galapagorhynchus* Artois & Schockaert, 1999 and in *Phonorhynchus helgolandicus* (Metschnikow, 1865) Graff, 1905, where there are 18 peripheral strands of basophilic secretion, separated from each other by the eosinophilic secretion (KARLING 1956).

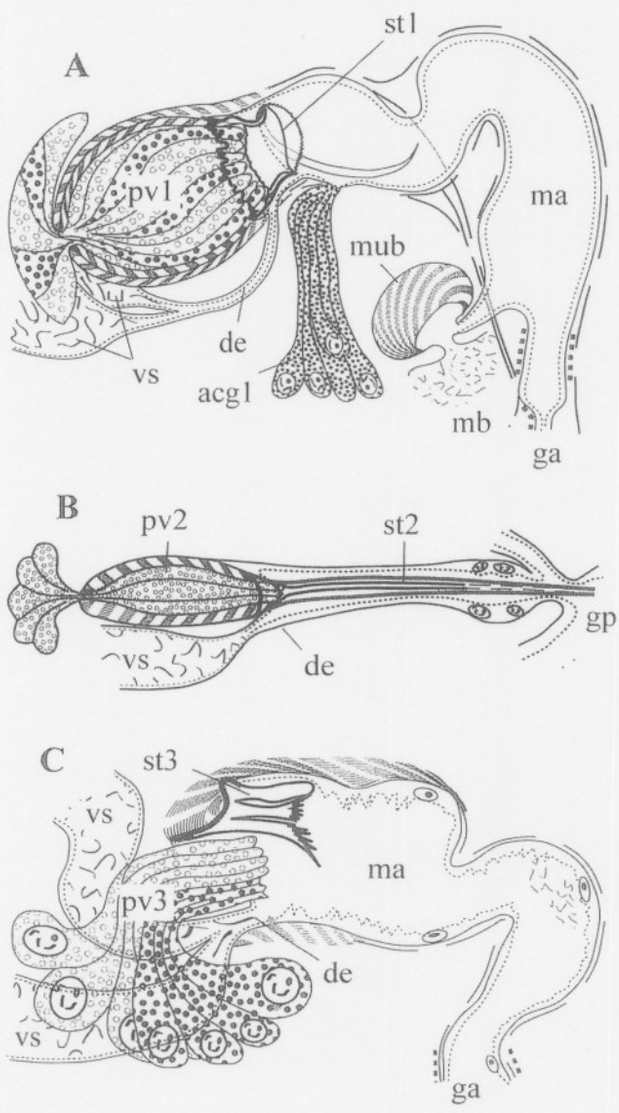

Fig. 1. Schematic representation of the male atrial organs of **A.** *Polycystis naegelii*. **B.** *Gyratricella attemsi*. **C.** *Alcha evelinae*.

This type of prostate vesicle is always connected to a double-walled stylet of which the outer stylet has a complex construction and where the gland necks enter the inner stylet (prostate stylet type I; see section 3.2.2). When there are two or three muscle layers around the vesicle, the inner layer is almost longitudinal and attaches to the inner side of the outer stylet. The outer layer(s) then continue(s) around the male atrium. When there is only one muscle layer, inner fibres attach to the stylet whereas outer fibres continue around the male atrium. The ejaculatory duct enters the atrium next to the base of the stylet, with which it is closely associated (exc. *Galapagorhynchus*) (see also section 3.2.2).

3.1.2. The prostate vesicle of type II (Figs. 1B, 2H, 3A–D, H). Species in the taxa *Albertorhynchus* Schockaert, 1976; *Antiboreorhynchus* Karling, 1952; *Austrorhynchus* Karling, 1952; *Cincturorhynchus* Evdonin, 1970; *Danorhynchus* Karling, 1955; *Gyratricella* Karling, 1955; *Gyratrix* Ehrenberg, 1831; *Neopolycystis* Karling, 1955; *Papia* Karling, 1956;*Paraustrorhynchus* Karling & Schockaert, 1977; *Phonorhynchus*; *Porrocystis* Reisinger, 1926; *Progyrator* Sekera, 1901; *Pygmorhynchus* Artois & Schockaert, 1999; *Scanorhynchus* Karling, 1955.

A free prostate vesicle of type II is mostly short, spindle-shaped, and surrounded by one very thick, almost circular muscle sheath. It is associated with a simple, double-walled stylet, as is prostate vesicle of type I (prostate stylet type II; see section 3.2.3); however, the outer stylet does not exhibit complicated ornamentations. The inner parts of the muscle-fibres attach to the rim of the stylet, while the outer fibres continue around the male atrium. The glands produce a single, basophilic secretion and the vesicle can sometimes be very small (e.g. *Cincturorhynchus, Neopolycystis*) with only five gland necks. There is no close association with the ejaculatory duct. In a few taxa (e.g. *Gyratrix, Gyratricella, Papia*) the prostate vesicle is elongated, contains an eosinophilic secretion, and the ejaculatory duct opens next to it (but see section 3.2.3 for details). The situation in *Papia* (Fig. 3C) is unique within the Polycystididae in that the ejaculatory duct perforates the prostate organ proximally and continues through it as a narrow but thick-walled tube. This situation somewhat resembles a copulatory organ of the conjunctatype, but detailed morphology of the prostate vesicle and the fact that the ejaculatory duct is clearly demarcated over its full length indicate a free prostate vesicle type II.

3.1.3. The prostate vesicle of type III (Figs. 1C, 3D–H). Species in the taxa *Acrorhynchides* Strand, 1928; *Alcha* Marcus, 1949; *Antiboreorhynchus*; *Cincturorhynchus*; *Galapagorhynchus*; *Hawadlia* Schockaert, 1971; *Lagenopolycystis* Artois & Schockaert, 2000; *Limipolycystis* Schilke, 1970; *Myobulla* Artois & Schockaert, 2000; *Parachrorhynchus* Karling, 1956; *Paraustrorhynchus*; *Progyrator*; *Pygmorhynchus*; *Psammopolycystis* Meixner, 1938; *Sabulirhynchus* Artois & Schockaert, 2000; *Typhlopolycystis* Karling, 1956; *Rogneda* Uljanin, 1870.

A prostate vesicle of type III is found in a wide variety of taxa. It is a free prostate vesicle, which always enters the male atrium near its proximal end and bulges deeply into it. It is not enclosed by a muscle coat; at the most the circular muscles of the atrium can continue around the distal part of the vesicle. Typically there are two kinds of glands, grouped next to each other. Both groups produce a coarse-grained secretion, one eosinophilic, the other basophilic, and the gland necks are clearly arranged in strands. The basophilic secretion can be reduced or even completely absent (e.g. in *Acrorhynchides robustus* (Karling, 1931) Strand, 1928), while in other taxa it is the eosinophilic secretion that is lacking (e.g. *Acrorhynchides caledonicus* (Claparède, 1861) Strand, 1928). The ejaculatory duct

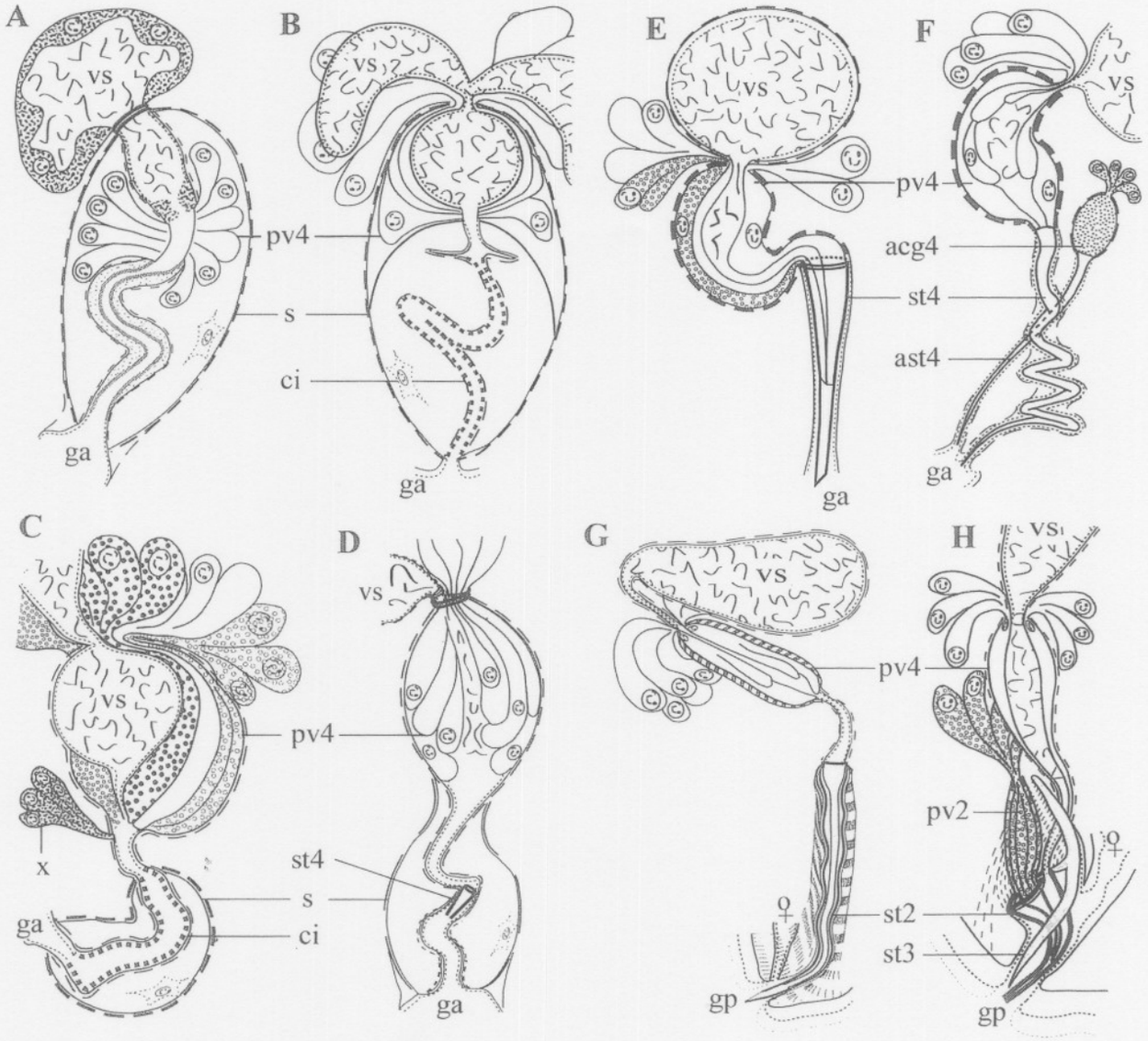

Fig. 2. Schematic representation of the male atrial organs of **A.** *Duplacrorhynchus minor*. **B.** *Duplacrorhynchus megalophallus*. **C.** *Duplacrorhynchus heyleni*. **D.** *Yaquinaia microrhynchus*. **E.** *Djeziraia pardii*. **F.** *Phonorhynchoides somaliensis*. **G.** *Gallorhynchus simplex*. **H.** *Scanorhynchus forcipatus*.

enters the male atrium close to these glands. In the majority of the taxa listed, the type III prostate vesicle is associated with a plate-like stylet (prostate stylet type III, see section 3.2.4).

3.1.4. Prostate vesicle type IV (Fig. 2). Species in the taxa *Annalisella* Karling, 1978; *Annulorhynchus*; *Danorhynchus gosoeensis* Karling, 1955; *Djeziraia* Schockaert, 1971; *Duplacrorhynchus* Schockaert & Karling, 1970; *Gallorhynchus* Schockaert & Brunet, 1971; *Koinocystella* Karling, 1952; *Neopolycystis; Phonorhynchoides* Beklemischew, 1928; *Psammopolycystis*; *Scanorhynchus* Karling, 1955; *Syltorhynchus* Noldt, 1989; *Yaquinaia* Schockaert & Karling, 1970.
Prostate vesicle type IV is the only vesicle that is interposed. It consists of glands surrounding the seminal duct, and the copulatory organ is of the so-called conjuncta-type copulatory-organ (terminology of KARLING 1956). Two subtypes can be recognised: the conjuncta-duplex where a (muscular) septum encloses part of the sperm-conducting system distally (Fig. 2A–D) and the conjuncta-simplex type, where such a septum is absent (Fig. 2E–H).
In a duplex-type organ, the copulatory organ proper is mostly a cirrus, i.e. a tube-like structure that can be everted (for details see section 3.2.1). Only in *Yaquinaia* a small, delicate, single-walled stylet is present (Fig. 2D). The prostate vesicle is mostly inside the septum, but in *Duplacrorhynchus heyleni* Artois & Schockaert, 1999 and in *Yaquinaia* the duplex bulb encompasses only that part of the cirrus distally from the prostate vesicle (Fig. 2C–D). In *D. heyleni* the glandular part is asymmetrical and very large, with four types of glands (Fig. 2C). In this species there is also a separate bundle of glands (x in Fig. 2C), which was considered accessory by ARTOIS & SCHOCKAERT (1999b). These glands are surely unique within the Polycystididae (part of the interposed ones?), and are not further discussed here.
In the copulatory organ of the conjuncta-simplex type the prostate vesicle can be connected to a single-walled stylet (Fig. 2E–F) or a double-walled stylet (Fig. 2G–H). In taxa where it is connected to a single-walled stylet, the prostate vesicle is mostly spindle-shaped, with a coarse-grained basophilic secretion and surrounded by a strong muscle layer with spirally arranged fibres. In the taxa with a double-walled stylet, the interposed prostate vesicle is mostly ovoid, contains a rather coarse-grained eosinophilic secretion and is surrounded by a weak muscle layer. Only in *Psammopolycystis falcata* Karling, 1956 is the secretion basophilic.

3.1.5. The accessory glands of type I (Fig. 1A). Species in the taxa *Paraustrorhynchus; Polycystis.*
This first category of accessory glands enters the male atrium ventrally in its proximal half. The glands form a relatively compact bundle, are not surrounded by muscles, and produce a fine-grained secretion that is mostly basophilic.

3.1.6. The accessory vesicle of type II (Fig. 3F–G). Species in the taxa *Lagenopolycystis; Limipolycystis; Myobulla; Typhlopolycystis.*
The basophilic secretion of these type II accessory glands enters a spindle-shaped vesicle that is surrounded by a spiral, almost circular muscle coat. Towards the male atrium it narrows to a duct that ends freely in the male atrium in *Myobulla* or in an accessory stylet type II in the other taxa (see section 3.2.7). The gland necks do not form fascicles inside the vesicle.

3.1.7. The accessory glands of type III (Fig. 3B). Species in the taxa *Austrorhynchus; Galapagorhynchus; Macrorhynchus* (exc. *M. croceus* (Fabricius, 1826) Graff, 1882); *Porrocystis; Progyrator; Pygmorhynchus.*
Accessory glands of type III include all cases where the epithelium of the male atrium is locally glandular. In most cases they appear as diffuse glands that are basophilic. Only in *Macrorhynchus groenlandicus* (Levinsen, 1879) Graff, 1882 and *Macrorhynchus manusferrea* Artois & Schockaert, 2001 do they form a more compact bundle that enters the male atrium dorsally above the distal tip of the stylet.

3.1.8. The accessory vesicle of type IV (Fig. 2F). Species in the taxa *Annalisella; Phonorhynchoides.*
An accessory glandular vesicle of type IV is small, spindle-shaped to globular, surrounded by a spiral, almost circular muscle layer and is filled with an eosinophilic secretion arranged in fascicles. It is always connected to a single-walled, needle-like accessory stylet.

3.2. The hard structures of the male atrial system

The male atrium is mostly provided with some sort of device, supposed to play a role in copulation (KARLING 1969; KARLING & SCHOCKAERT 1977). It can be an eversible tube with or without numerous small teeth (armed or unarmed cirrus); or it can consist of large hard structures, then called stylets. In Polycystididae, often more than one stylet may be found in the male atrium. The stylet associated with a prostate vesicle was called the "prostate stylet", the other one was considered the "accessory stylet", even when that one is also connected to a glandular vesicle. A first attempt to assess homologies of stylets was made by KARLING & SCHOCKAERT (1977), who supposed the "accessory" stylets of some species to be homologous with the

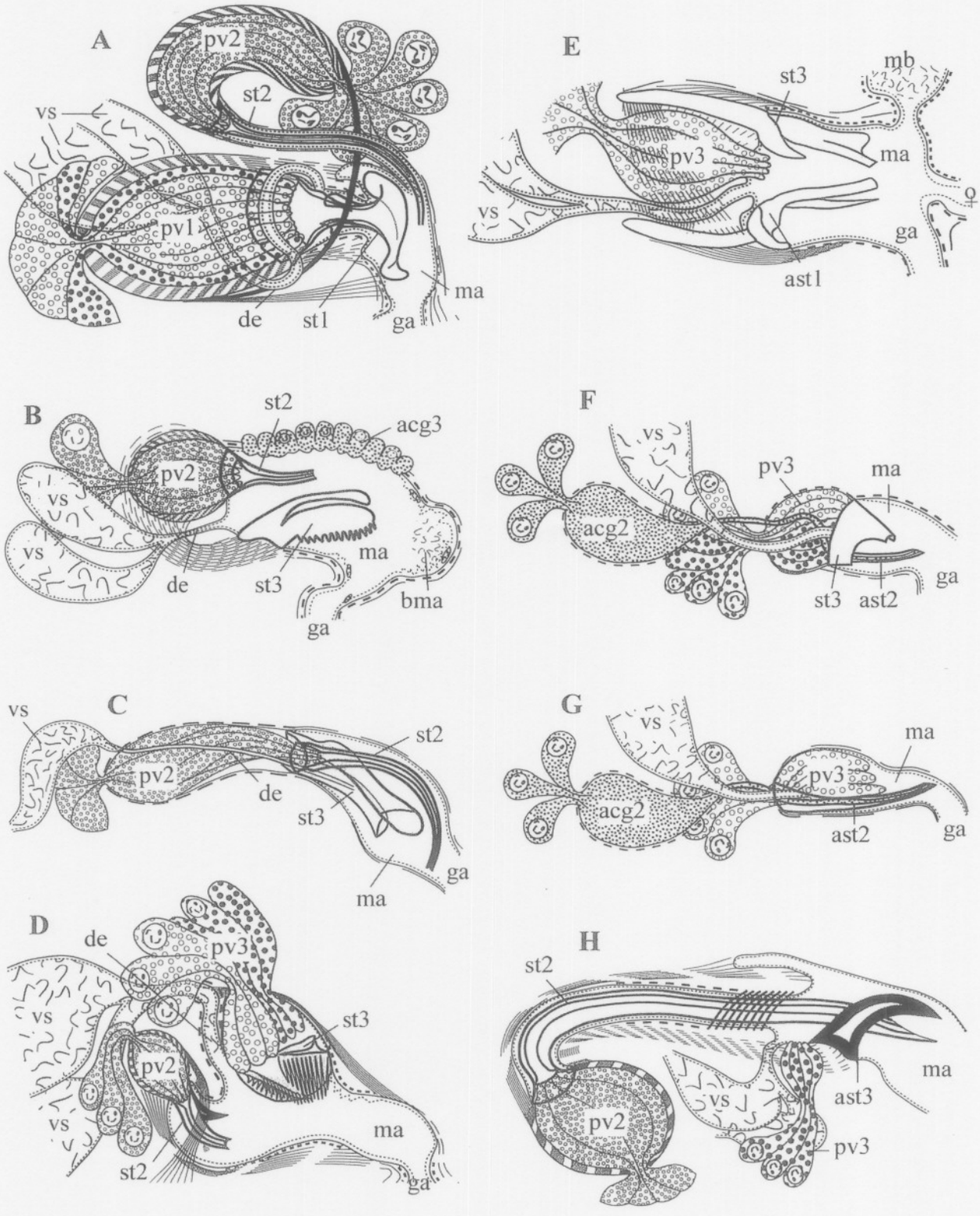

Fig. 3. Schematic representation of the male atrial organs of **A.** *Phonorhynchus helgolandicus*. **B.** *Austrorhynchus pectatus*. **C.** *Papia bifida*. **D.** *Cincturorhynchus karlingi*. **E.** *Rogneda hibernica*. **F.** *Typhlopolycystis coeca*. **G.** *Limipolycystis curvitubo*. **H.** *Antiboreorhynchus novzelae*.

"prostate" stylets of others. Indeed, as with the glandular organs, the situation is much more complex than was supposed in the past.

We recognise seven different types of stylets. Three of them can be called "prostate stylet", as each is associated with one of the types of prostate vesicle discussed above. The other four types are considered "accessory stylets". As it will appear from the discussion below, some stylets considered "accessory" in the past, are now considered "prostate stylets" and vice versa.

3.2.1. The armed cirrus (Fig. 2B–C). Species in the taxa *Acrorhynchides; Duplacrorhynchus* (exc. *D. minor* Schockaert & Karling, 1970); *Galapagorhynchus*.

In some species, the wall of the male atrium is partially or completely covered with hard teeth. It is then mostly tubiform and eversible, and is called an armed cirrus. In the *Duplacrorhynchus* species (with conjunctaduplex copulatory organ, see section 3.1.1 above) the cirrus is enclosed in the copulatory bulb. It is locally provided with small teeth in *D. major* Schockaert & Karling, 1970, or over its whole length in *D. heyleni* (Fig. 2C) and *D. megalophallus* Artois & Schockaert, 1998 (Fig. 2B). In the *Acrorhynchides* species the cirrus is not enclosed by a septum and consists of many small teeth. The situation in *Galapagorhynchus hoxholdi* Artois & Schockaert, 1999 is somewhat peculiar in that the spines in the atrium are few and irregularly distributed, and that this species also possesses a double-walled prostate stylet of type I (see section 3.2.2). In some taxa that lack a stylet (*Duplacrorhynchus minor*, *Koinocystella*, *Parachrorhynchus*), the wall of the male atrium does not bear teeth. It is however (probably) eversible and therefore also often referred to as (unarmed) cirrus (e.g. SCHOCKAERT & KARLING 1970; ARTOIS & SCHOCKAERT 2001).

3.2.2. The prostate stylet of type I (Figs. 1A, 3A, 4A). Species of the taxa *Galapagorhynchus*; *Macrorhynchus*; *Paulodora*; *Phonorhynchus*; *Polycystis*.

A prostate stylet of type I is always connected to a prostate vesicle of type I (see section 3.1.1 above). It is a double-walled stylet of which the inner stylet is a simple tube, whereas the outer stylet is very complex, often with several flap-like projections and folds. The proximal end is funnel-shaped and the inner stylet begins at some distance from the rim of the outer stylet. The ejaculatory duct opens in the atrium next to the base of the stylet, and the folds and flaps of the outer stylet evidently help in the evacuation of sperm.

3.2.3. The prostate stylet of type II (Figs. 1B, 2G–H, 3A–D, H, 4B). Species of the taxa *Albertorhynchus*; *Annulorhynchus*; *Antiboreorhynchus*; *Austrorhynchus*, *Cincturorhynchus*; *Danorhynchus*; *Gallorhynchus*; *Gyratricella*; *Gyratrix*; *Neopolycystis*; *Paraustrorhynchus*; *Phonorhynchus; Porrocystis*; *Progyrator*; *Psammopolycystis*; *Pygmorhynchus*; *Scanorhynchus; Syltorhynchus*.

A prostate stylet of type II is also double-walled, rather similar to the prostate stylet type I, but with a much simpler construction. It may be straight or curved or even highly coiled as in *Albertorhynchus*, but the outer stylet never has the complicated folds or flaps as in the stylet of type I; at the most it can bear one or some spines (*Austrorhynchus* (Fig. 4B), *Cincturorhynchus*). In the majority of the taxa listed above it is connected to a prostate vesicle of type II, and only in a few cases is it connected to an interposed prostate vesicle (*Annulorhynchus, Gallorhynchus* (Fig. 2G), *Syltorhynchus*). The ejaculatory duct opens in the atrium some distance away from the stylet or close to the base of the stylet, (and through the prostate vesicle where it is interposed). However the ejaculatory duct is never as intimately associated with the stylet, as is the case with the prostate stylet of type I.

3.2.4. The prostate stylet of type III (Figs. 1C, 2H, 3B–F, 4C, F). Species of the taxa *Albertorhynchus; Alcha*; *Annulorhynchus*; *Austrorhynchus*; *Cincturorhynchus*; *Danorhynchus*; *Gallorhynchus* (exc. *Gallorhynchus simplex* Schockaert & Brunet, 1971), *Gyratrix*, *Lagenopolycystis*; *Myobulla*; *Neopolycystis*; *Paraustrorhynchus*; *Psammopolycystis*; *Rogneda; Sabulirhynchus*; *Scanorhynchus*; *Syltorhynchus*; *Typhlopolycystis*.

A prostate stylet of type III is always found in the proximal part of the male atrium. Its appearance and its association with other atrial organs are very variable. Typically it is plate-like and may be very complex, forming two or more plates, often with comb-like rims. In several species, this plate-like stylet occurs along with a prostate vesicle and -stylet of type II (e.g. *Albertorhynchus, Austrorhynchus* (Fig. 3B)), and was then considered the "accessory stylet" (or A-organ: KARLING 1977). In some species the plate may be connected to the type II stylet in such a way that it can move (e.g. *Scanorhynchus* (Fig. 2H), *Psammopolycystis, Annulorhynchus*), or it forms a sheet in which the type II stylet can glide (*Gyratrix*, *Syltorhynchus*).

In a number of species, this prostate stylet of type III is located close to a prostate vesicle of type III. When a prostate vesicle type II and a prostate stylet of type II are also present, both the prostate vesicle type III and prostate stylet type III were considered accessory (*Cincturorhynchus* (Fig. 3D), *Paraustrorhynchus*).

In *Myobulla, Lagenopolycystis* (Fig. 4F) and *Typhlopolycystis* (Fig. 3F) the stylet is relatively simple. It is gutter-like with a tubiform distal part in *Myobulla*.

In these taxa the prostate glands type III were referred to as "prostate glands" and the stylet as "prostate stylet", implying homology with any stylet connected to any prostate vesicle of other Polycystididae.

3.2.5. The prostate stylet of type IV (Figs. 2D–F, 4D). Species of the taxa *Annalisella; Djeziraia*; *Phonorhynchoides*; *Yaquinaia*.

This type of prostate stylet always is a single-walled tube and is invariably connected to an interposed prostate vesicle. It is thus part of the sperm-conducting system. It can be very long and sometimes corkscrew-like. In *Yaquinaia* on the contrary it is very small (Fig. 2D).

3.2.6. The accessory stylet of type I (Fig. 3E). Species of the taxon *Rogneda* (exc. *R. minuta* Uljanin, 1870).

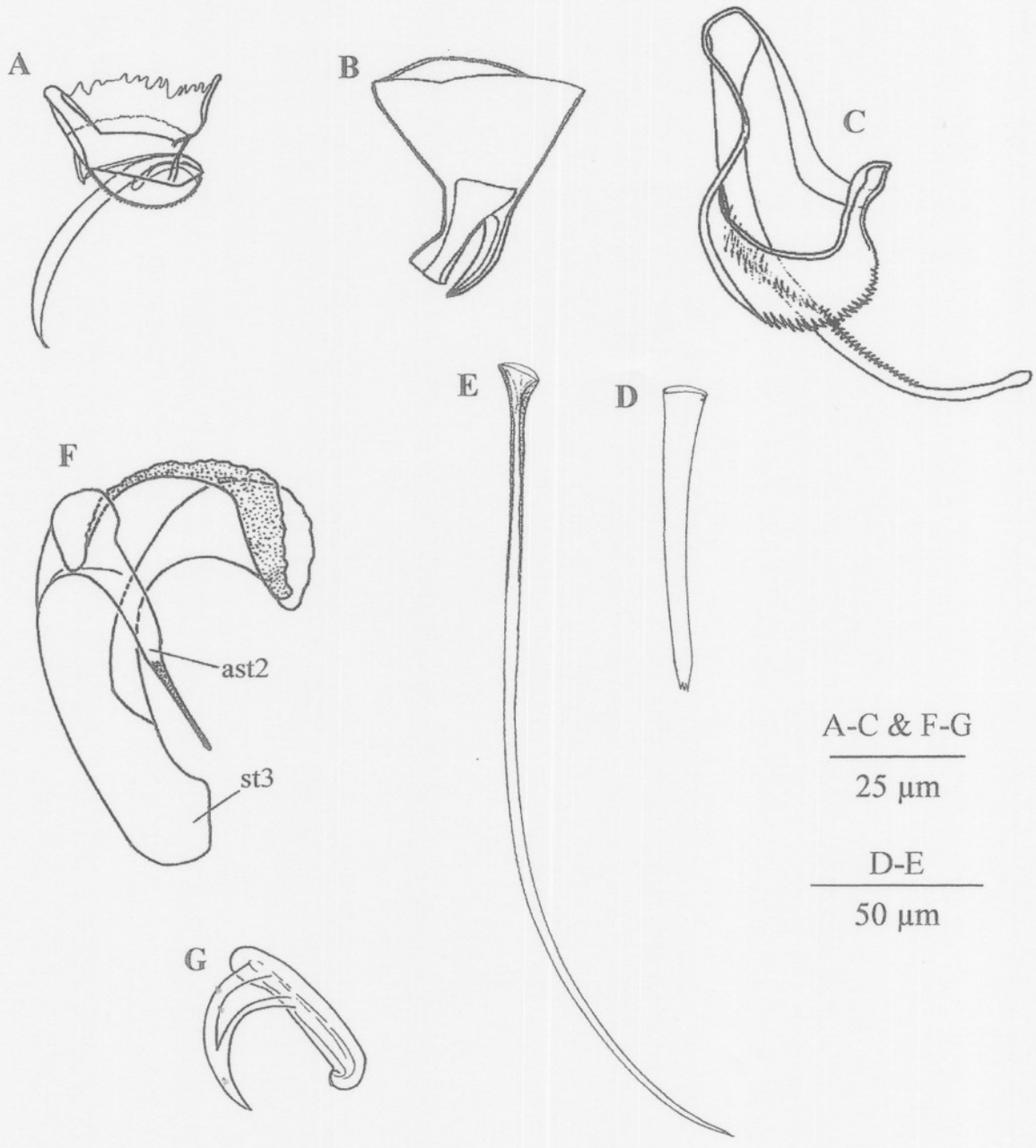

Fig. 4. Hard parts of the male atrial system. **A.** Prostate stylet type I (*Polycystis naegelii*). **B.** Prostate stylet type II (*Austrorhynchus hawaiiensis*). **C.** Prostate stylet type III (*Austrorhynchus hawaiiensis*). **D.** Prostate stylet type IV (*Phonorhynchoides haegheni*). **E.** Accessory stylet type IV (*Phonorhynchoides haegheni*). **F.** Prostate stylet type III and accessory stylet type II (*Lagenopolycystis peresi*). **G.** Accessory stylet type III (*Porrocystis assimilis*). D & E after ARTOIS & SCHOCKAERT 2001.

Apart from the prostate stylet type III, a second, almost identical stylet is present in the species of the genus *Rogneda*. We call this an accessory stylet type I. In fact it is impossible to say which is the original prostate stylet type III and which the accessory stylet type I. The choice must therefore be made arbitrarily. The prostate glands of type III and the ejaculatory duct both

3.2.7. The accessory stylet of type II (Figs. 3F–G, 4F). Species of the taxa *Lagenopolycystis; Limipolycystis; Typhlopolycystis*.

An accessory stylet of type II occurs in *Lagenopolycystis* (Fig. 4F) and in *Typhlopolycystis* (Fig. 3F) where the prostate stylet of type III bears a tubiform stylet at its proximal rim. The duct of the accessory glandular vesicle of type II ends in this accessory stylet, while prostate glands of type III open up against the prostate stylet, next to the ejaculatory duct. In *Limipolycystis* (Fig. 3G) there is only one stylet in the atrium. It receives the duct of the accessory gland reservoir (type II), while prostate glands type III and the ejaculatory duct lie aside the stylet. We therefore consider the stylet in *Limipolycystis* as homologous with the accessory stylet type II of the two other taxa. The opposite situation occurs in *Myobulla*: there is no accessory stylet type II and the single stylet is clearly the gutter-like prostate stylet type III, receiving the prostate glands and the ejaculatory duct, while the duct of the accessory gland vesicle opens freely in the atrium.

3.2.8. The accessory stylet of type III (Figs. 3H, 4G). Species of the taxa *Antiboreorhynchus; Porrocystis*.

The accessory stylet of type III is a simple hook and is always situated in the distal part of the male atrium. The ejaculatory duct opens in its neighbourhood. In *Antiboreorhynchus novzelae* a prostate vesicle type III opens next to it as well (Fig. 3H), leading KARLING & SCHOCKAERT (1977) and ARTOIS & SCHOCKAERT (1999a) to consider this spine possibly as homologous with the prostate stylet type III of some other Polycystididae. However, in a yet undescribed polycystidid from Australia, a prostate stylet type II, type III and this spine occur together, indicating that such a homology assessment may be wrong. We therefore consider this stylet an accessory stylet of a type of its own.

3.2.9. The accessory stylet of type IV (Figs. 2F, 4E). Species of the taxa *Annalisella; Phonorhynchoides*.

This single-walled accessory stylet is a long, needle-like tube that is always connected to an accessory vesicle type IV (see section 3.1.8). It occurs only in two taxa, both of which also have a single-walled prostate stylet.

4. DISCUSSION

KARLING (1956) considered the "free prostate vesicle" with the ejaculatory duct opening in the male atrium next to it (the divisa-type copulatory organ) to be characteristic for the Polycystididae. Almost all other Kalyptorhynchia have the prostate glands interposed organ proper (the conjuncta-type copulatory organ). The situation as found in the polycystidid taxon *Parachrorhynchus* with the glands entering the male duct at one side, was given as an example of how a divisa-type could have evolved from a conjuncta-type. The underlying assumption was that the free prostate organ of the Polycystididae is homologous with the interposed prostate glands of the other Kalyptorhynchia, and that interposed glands are the primary condition. However, a copulatory organ with interposed glands is also found in some polycystidid taxa (*Neopolycystis*, *Scanorhynchus, Danorhynchus gosoeensis*) that also have a free prostate vesicle; therefore the interposed glandular vesicle of these taxa was considered a secondary derivative (KARLING 1955). Based on several other features, these taxa were considered related to each other. *Annulorhynchus adriaticus* and the *Gallorhynchus* species also share these features, and therefore the conjuncta situation in *Annulorhynchus* and *Gallorhynchus* was also considered derived (KARLING 1956; SCHOCKAERT & BRUNET 1971; SCHOCKAERT 1974), even though no free prostate vesicle is present. In the early seventies, a number of other polycystidid species were described (SCHOCKAERT & KARLING 1970; SCHOCKAERT 1971) with interposed prostate glands now, however, considered the primary situation because of the similarities with the copulatory organ in other Eukalyptorhynchia. Summarising the situation in the Polycystididae, SCHOCKAERT (1974) recognised four types of copulatory organs within the family: the divisa-type, the primary interposed prostate vesicle, the secondary interposed prostate vesicle and the *Phonorhynchoides*-type (with a primary interposed prostate vesicle plus an accessory glandular organ).

Until recently, we have followed the views of KARLING (1956), SCHOCKAERT (1974) and KARLING & SCHOCKAERT (1977) regarding the possible homologies of the different glandular structures (ARTOIS & SCHOCKAERT 1999a, 2000, 2001). However, the interposed prostate vesicles of the conjuncta-type organs always have a different position than any of the vesicles found in the divisa-type organs. On the other hand, the interposed vesicles that were formerly considered secondary do not differ in position and morphology from the interposed prostate vesicles that were considered primary. Therefore, we conclude that all interposed prostate vesicles are homologous with each other, but not

homologous with any of the glandular organs of the divisa-types. Furthermore, many of the vesicles formerly considered accessory are in fact identical in position and morphology to vesicles that were considered prostate in other species. Therefore, we decided to consider these vesicles prostate organs (prostate vesicle type II). Our prostate vesicle type III has, in the past, mostly been considered accessory, except in some species where it was the only vesicle present in the male system (e.g. *Alcha*). There it was called the prostate vesicle.

The occurrence of the different types of glandular organs within the Polycystididae is summarised in Appendix 1.

Starting from the situation as found in *Phonorhynchus*, KARLING & SCHOCKAERT (1977) distinguished two types of stylets: one closely associated with the seminal duct ("prostate"), the other not ("prostatoid" or "accessory"). They also discussed the possibility that the prostatoid stylet of *Phonorhynchus* could be homologous with the so-called prostate stylet of *Paraustrorhynchus*, *Antiboreorhynchus*, *Austrorhynchus* and *Porrocystis*. These authors based this view on the fact that in all these cases the prostatoid vesicle and stylet are not related with the ejaculatory duct, and that the prostatoid stylet is always a very simple tube. Our study confirms this view, which is further endorsed by the fact that the so-called "prostatoid" stylet is clearly double-walled as it is in the other taxa mentioned above. This feature of the "prostatoid" stylet was never mentioned in literature, and was probably never noticed, as even KARLING did not mention it in his 1982 paper, which specifically deals with the taxon *Phonorhynchus*. The conjunction of prostate and prostatoid stylets in *Phonorhynchus* clearly indicates that they have to be considered separate features in a cladistic analysis. To avoid further confusion we call them prostate stylet type I and type II, respectively.

The plate-shaped stylets that often occur in the proximal part of the male atrium were considered homologous with a prostate stylet type I by KARLING & SCHOCKAERT (1977). However, they have completely different morphology, and if they are associated with a glandular vesicle this vesicle is always a prostate vesicle type III. These observations brought us to the conclusion that the plate-like structures are best considered a separate character: prostate stylet type III.

Apart from these stylets, a single-walled prostate stylet can also be present, but only if the copulatory organ is of the conjuncta-type. All the other types of hard structures that can be found associated with the male atrium we consider accessory. A special case in this is accessory stylet type III, which was considered homologous to our prostate stylet type III by KARLING & SCHOCKAERT (1977). However, this type of stylet has a different position in the male atrium and furthermore was found in conjunction with a prostate stylet type III in a yet undescribed species from Australia, and therefore must be considered a separate character.

The occurrence of the different types of stylets within the Polycystididae is summarised in Appendix 2.

The characters and terminology presented above can also easily be used when studying other Kalyptorhynchia (or even Typhloplanoida) taxa, and could serve as a basis for comparison of features between these taxa. This can only lead to a much better view on possible homologies between the different taxa of Kalyptorhynchia, and facilitate the production of useful data matrices for analysis.

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Appendix 1 (Continued).

	pv1	pv2	pv3	pv4	acg1	acg2	acg3	acg4
Psammopolycystis	–	–	×	×	–	–	–	–
Pygmorhynchus	–	×	×	–	–	–	×	–
Rogneda	–	–	×	–	–	–	–	–
Rogneda minuta	–	–	×	–	–	–	–	–
Sabulirhynchus	–	–	×	–	–	–	–	–
Scanorhynchus	–	×	–	×	–	–	–	–
Syltorhynchus	–	–	–	×	–	–	–	–
Typhlopolycystis	–	–	×	–	–	×	–	–
Yaquinaia	–	–	–	×	–	–	–	–

APPENDIX 2

Matrix summarising the occurence of the armed cirrus and the different types of stylets within the Polycystididae (for abbreviations see Materials and methods).

	ci	st1	st2	st3	st4	ast1	ast2	ast3	ast4
Acrorhynchides	×	–	–	–	–	–	–	–	–
Albertorhynchus	–	–	×	×	–	–	–	–	–
Alcha	–	–	–	×	–	–	–	–	–
Annalisella	–	–	–	–	×	–	–	–	×
Annulorhynchus	–	–	×	×	–	–	–	–	–
Antiboreorhynchus	–	–	×	–	–	–	–	×	–
Austrorhynchus	–	–	×	×	–	–	–	–	–
Cincturorhynchus	–	–	×	×	–	–	–	–	–
Danorhynchus	–	–	×	×	–	–	–	–	–
Djeziraia	–	–	–	–	×	–	–	–	–
Duplacrorhynchus	×	–	–	–	–	–	–	–	–
Galapagorhynchus	×	×	–	–	–	–	–	–	–
Gallorhynchus simplex	–	–	×	–	–	–	–	–	–
Gallorhynchus mediterr.	-	-	×	×	–	–	–	–	–
Gyratricella	–	–	×	–	–	–	–	–	–
Gyratrix	–	–	×	×	–	–	–	–	–
Hawadlia	–	–	–	–	–	–	–	–	–
Koinocystella	–	–	–	–	–	–	–	–	–
Lagenopolycystis	–	–	–	×	–	–	×	–	–
Limipolycystis	–	–	–	–	–	–	×	–	–
Macrorhynchus	–	×	–	–	–	–	–	–	–
Myobulla	–	–	–	×	–	–	–	–	–
Neopolycystis	–	–	×	×	–	–	–	–	–
Parachrorhynchus	–	–	–	–	–	–	–	–	–
Paraustrorhynchus	–	–	×	×	–	–	–	–	–
Paulodora	–	×	–	–	–	–	–	–	–
Phonorhynchoides	–	–	–	–	×	–	–	–	×
Phonorhynchus	–	×	×	–	–	–	–	–	–
Polycystis	–	×	–	–	–	–	–	–	–
Porrocystis	–	–	×	–	–	–	–	×	–
Progyrator	–	–	×	–	–	–	–	–	–
Psammopolycystis	–	–	–	×	–	–	–	–	–
Pygmorhynchus	–	–	×	–	–	–	–	–	–
Rogneda	–	–	–	×	–	×	–	–	–
Rogneda minuta	–	–	–	×	–	–	–	–	–
Sabulirhynchus	–	–	–	×	–	–	–	–	–
Scanorhynchus	–	–	×	×	–	–	–	–	–
Syltorhynchus	–	–	×	×	–	–	–	–	–
Typhlopolycystis	–	–	–	×	–	–	×	–	–
Yaquinaia	–	–	–	–	×	–	–	–	–