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TWO NEW SPECIES OF THE GENUS *DUPLACRORHYNCHUS* SCHOCKAERT & KARLING, 1970, WITH REMARKS ON RELATIONSHIPS WITHIN THE GENUS AND ON THE DUPLACRORHYNCHINAE (PLATYHELMINTHES, POLYCYSTIDIDAE)

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Abstract. Two new species of Polycystididae are described : *Duplacrorhynchus megalophallus* sp. nov. from the bay of Marseille (France) and *D. heyleni* sp. nov. from Zanzibar (Tanzania). The female system of both is characterized by the presence of a muscular bulb at the proximal end of the bursal stalk, with three strong teeth in *D. megalophallus*, and in *D. heyleni* the muscle bulb is highly asymmetric. In *D. megalophallus* the copulatory bulb is divided by a septum, and the cirrus has small spines and a proximal umbrella-like expansion. In *D. heyleni* only the cirrus, which has strong teeth, is enclosed in the cirrus sack : the seminal and prostate vesicles are not. Relationships with the two known species of the genus (*D. minor* Schockaert & Karling, 1970 and *D. major* Schockaert & Karling, 1970) are discussed. The subfamily Duplacrorhynchinae Schockaert & Karling, 1970 may constitue a monophyletic taxon, characterised by the 3+2 configuration of the proboscis retractor system. Duplacrorhynchinae contains all genera of Polycystididae with an interposed prostate vesicle. The taxon *Duplacrorhynchus* may be the sister taxon of all these genera.

Key words: *Duplacrorhynchus megalophallus* sp. nov., *Duplacrorhynchus heyleni* sp. nov., Eukalyptorhynchia, systematics, phylogeny, Polycystidae, Duplacrorhynchinae.

INTRODUCTION

In 1970 SCHOCKAERT & KARLING introduced the subfamily Duplacrorhynchinae to contain « three new anatomically remarkable » species of Polycystididae : *Duplacrorhynchus minor*, *D. major* and *Yaquinaia microrhynchus*. The remarkable aspect of these species is the copulatory organ of the so-called conjuncta-duplex type (terminology of KARLING, 1956), *i.e.* with an interposed prostatic vesicle and an eversible cirrus enclosed in a muscular bulb or cirrus sack. Later, SCHOCKAERT (1971, 1974) added *Djeziraia pardii* Schockaert, 1971 to the subfamily (or at least suggested a close relationship) based on the fact that the prostate vesicle of that species is also interposed, and the stylet is single walled – a so-called « papillenstilet » (terminology of KARLING, 1956) – unlike the double-walled stylet of all other Polycystididae. However, the prostate vesicle is also interposed in the genera *Phonorhynchoides* Beklemischev, 1927 and *Annalisella* Karling, 1978 (both with a single-

walled stylet), and *Koinocystella* Karling, 1952 and *Paracrorhynchus* Karling, 1956 (both with a cirrus that is not enclosed in a cirrus sack). This is also the case in *Gemelliclinus flavidus* Evdonin, 1970, but the original description of this species does not provide enough data and the material in our possession is of inferior quality ; hence we consider this species as *inquirenda* and it will not be considered further. Do these taxa all belong in the Duplacrorhynchinae? An extensive discussion on the matter is beyond the scope of this contribution, but we present some ideas in the discussion.

In this contribution we describe two new species, considered to be representatives of the genus *Duplacrorhynchus* : *D. heyleni* n.sp. from the African East Coast (Zanzibar) and *D. megalophallus* n.sp. from the Mediterranean. Both species show some characters in which they strongly deviate from each other and from the two previously described *Duplacrorhynchus* species, but we argue for their close relationship and consider that they are members of a single monophyletic taxon.

MATERIAL AND METHODS

Both species were found in marine environments and were extracted from the sediment by decantation, using an isotonic $MgCl_2$ solution (see Schockaert, 1996). Animals were studied alive and then mounted with lactophenol. Other specimens were fixed in Bouin's fluid and serially sectioned. Sections were stained with Heidehain's iron hematoxilin, using eosin as counterstain. The material of *Duplacrorhynchus megalophallus* was collected and studied alive by Dr. Brunet.

The size of organs is expressed as a percentage of the body length as is the position of the gonopore (distance from the anterior tip of the body). Figures without a scale are freehand.

Type material of the new species is deposited in the zoological collection of the Department SBG, Limburgs Universitair Centrum, Diepenbeek, Belgium. Type material of the species considered in the discussion are in the Swedish Museum of Natural History (Stockholm) where they were studied by T.A. during a visit in 1997. Some type material is also located in the Diepenbeek collection.

ABBREVIATIONS IN THE FIGURES

acg :	accessory glands	od :	oviduct
ag :	atrial glands	ov :	ovary
b :	bursa	p :	proboscis
bs :	bursal stalk	pb :	proboscisbulb
ci :	cirrus	ph :	pharynx
co :	copulatory organ	r1, r2, r3 :	three different retractors
dir :	dorsal integument retractor	rs :	seminal receptacle
e :	eye	t :	testis
evs :	external seminal vesicle	ut :	uterus
fd :	female duct	vg :	prostate vesicle
g :	glands	vi :	vitellarium
ga :	common genital atrium	vir :	ventral integument retractor
gp :	gonopore	vs :	seminal vesicle
ivs :	internal seminal vesicle	x :	explanation in text
mb :	muscular bulb		

DESCRIPTIONS OF THE NEW SPECIES

***Duplacrorhynchus megalophallus* sp. nov.**

Distribution. Bay of Marseilles, between the Chateau d' If and the Isle of Ratonneau, in *Amphioxus*-sand (14-16 m deep), 3/2/1966 (Type locality). Bay of Marseilles, between the Island of Jarre and the coast, on the «Plateau des Chèvres», in *Amphioxus*-sand (8-10 m deep), 3/2/1966.

Material. Drawings of living animals by Dr. Brunet. Several individuals sectioned, one of them designated as the holotype (sectioned horizontally, leg. Dr. Brunet).

Derivatio nominis. The species epithet refers to the very large copulatory organ; megalos (Gr.): big, phallos (Gr.): penis.

Description

The living animals are colourless, 0.8-1 mm long, with paired eyes. The epidermis is syncytial with flattened, lobate nuclei, 5 µm high, with cilia of 4 µm. Ovoid rhabdites are present, with a length of about 1/3 of the height of the epithelium. The pharynx is situated in the first body half and is of the same construction as in the other members of the genus (see SCHOCKAERT & KARLING, 1970).

The proboscis is 20% of the body length in living animals (160 µm according to Brunet's measurements) and about 30% in sectioned material. The proboscis cavity has an anucleated epithelium and is surrounded by internal circular and external longitudinal muscles. The apex of the conus is very small. There are no nuclei at the junction of the cone and the sheath epithelium. There are three pairs of proboscis retractors and two pairs of integument retractors.

The gonads are paired. Both testes are situated laterally and extend from about the anterior edge of the pharynx to the ovaries. The ovaries are rather small and situated ventro-laterally behind the pharynx. The vitellaria lie dorsally and extend from the level of the pharynx towards the caudal body end. The common gonopore is situated at about 85%. The genital atrium is divided into two parts by a slight constriction, one in which the copulatory organ opens while the uterus and the bursal stalk end in the other one. The atrium is lined with a nucleated epithelium and surrounded by weak longitudinal muscle fibers. In some sections the epithelium has a ruffled aspect (pseudociliation) or seems to have disappeared (pseudocuticula). Basophilic atrial glands are found where the copulatory organ connects to the atrium.

The copulatory organ has a caudal position, dorsally from the common genital pore. Its position may be variable: in living animals and in several sectioned animals its position is behind the genital pore, while in the holotype it is situated in front of it. The copulatory system consists of a large ovoid bulb that is surrounded by a spiral muscle sheath and is divided into two parts by a transverse septum. The distal part contains the cirrus, the proximal part the enlarged seminal duct (internal seminal vesicle) and the intracapsular parts of the prostate glands. The seminal duct narrows towards the septum and perforates it, to continue as the cirrus. The prostate glands enter the seminal duct here. The nucleated parts of the prostate glands are entirely extracapsular. The prostate secretion consists of coarse basophilic granules. There is also an extracapsular seminal vesicle.

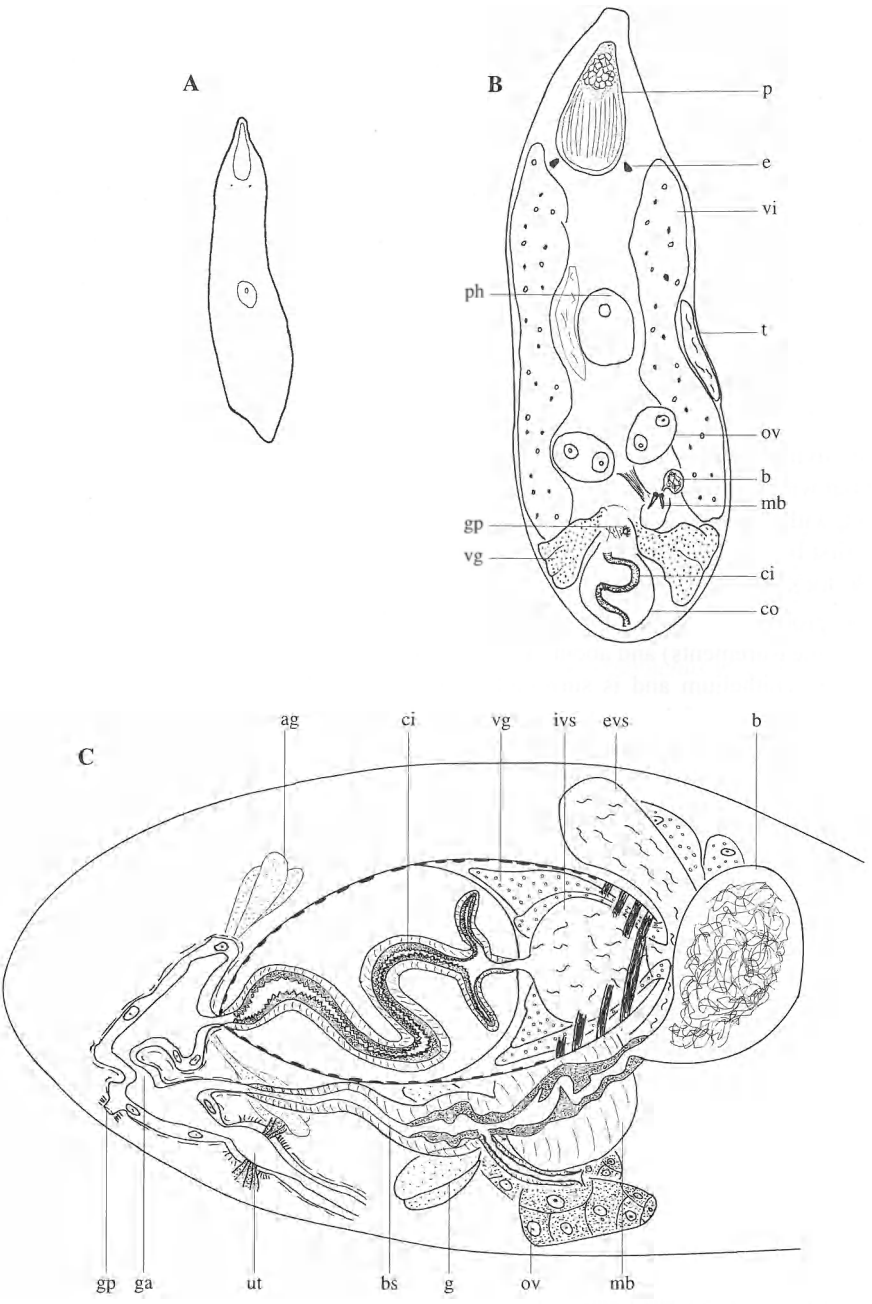

Fig. 1. – *Duplacrorhynchus megalophallus* sp. nov. – A. Habitus. – B. General organisation (from a living animal). – C. Horizontal reconstruction of the genital organs seen from the ventral aspect.

The most proximal part of the cirrus is narrow and moderately sclerotised. It widens almost immediately into an umbrella-shaped diverticulum of which the proximal wall is uniformly sclerotised; the distal wall bears tiny teeth. Such teeth occur over the whole length of the cirrus, and are all of equal size, slightly larger than those in the diverticulum. The cirrus is surrounded by a spiral muscle sheath over its whole length.

Bursal stalk and uterus (with the same structure as in most Polycystididae) end in the same diverticulum of the atrium. The bursal stalk runs straight in the cephalic direction, above the uterus, and ends in the large spherical to ovoid bursa. One sectioned individual had a nucleated epithelium in the bursa, while in all other specimens the bursa was surrounded by a thin membrane and was filled with sperm. The bursal stalk is long, surrounded by a thick circular muscle coat that becomes weaker towards the atrium. Its epithelium is anucleate distally, reduced to a pseudocuticula proximally. Close to the bursa the bursal stalk is surrounded by a very thick muscular bulb, and inside this bulb the pseudocuticula forms a ring of three strong teeth.

The female duct opens in the bursal stalk just distally of the muscular bulb. Some basophilous glands open here. From there on it also runs in a cephalic direction. It is lined by a thin pseudocuticula. The female duct then bifurcates into the two oviducts, which are very short and have anucleated epithelia. Both the oviducts and the female duct are surrounded by a thin circular muscle layer that disappears towards the ovaries.

***Duplacrorhynchus heyleni* sp.nov.**

Duplacrorhynchus spec. in WATSON, 1999

Distribution. Widely distributed in mangrove sand flats on Zanzibar Island (Tanzania): off Mahurubi Palace ruins, sand flat in the high mid-littoral with relatively clean coarse sand disturbed by crabs (5/8/1995) (type locality); same locality, in the sand of an exposed sea grass field, very rich in detritus (5/8/1995); beach behind the Mbweni Ruins Hotel, north of the creek, in a little pool with seagrass (*Thallasia* spec?) (11/8/1995), and in a tide pool with a broad leafed seagrass (17/8/1995); south of the creek, in a higher part ot the sand flat, with relatively coarse sand (11/8/1995); mangrove forest near Pete, in a tide pool with relatively fine sand and some algae (16/8/1995).

Material. Many animals studied alive, 7 whole mounts, 5 sectioned specimens, one of them designated as holotype (horizontal sections).

Derivatio nominis. Named after Wim Heylen, a friend of Artois'.

Description

Colourless and opaque animals of about 1 mm long, with paired eyes. The epidermis is syncytial with numerous optically empty vacuoles. The rhabdites are more or less oval, less numerous in the first body quarter. The position and construction of the pharynx is the same as in the other members of the genus.

The proboscis is about 20% of the body length, with a distinct apex. The epithelium of the proboscis cavity is relatively high with a ring of nuclei approximately in the middle of the cavity. The cavity is surrounded by an outer longitudinal and an inner circular muscle layer. The circular one is lacking in the distal 1/4 of the cavity. There are no nuclei at the

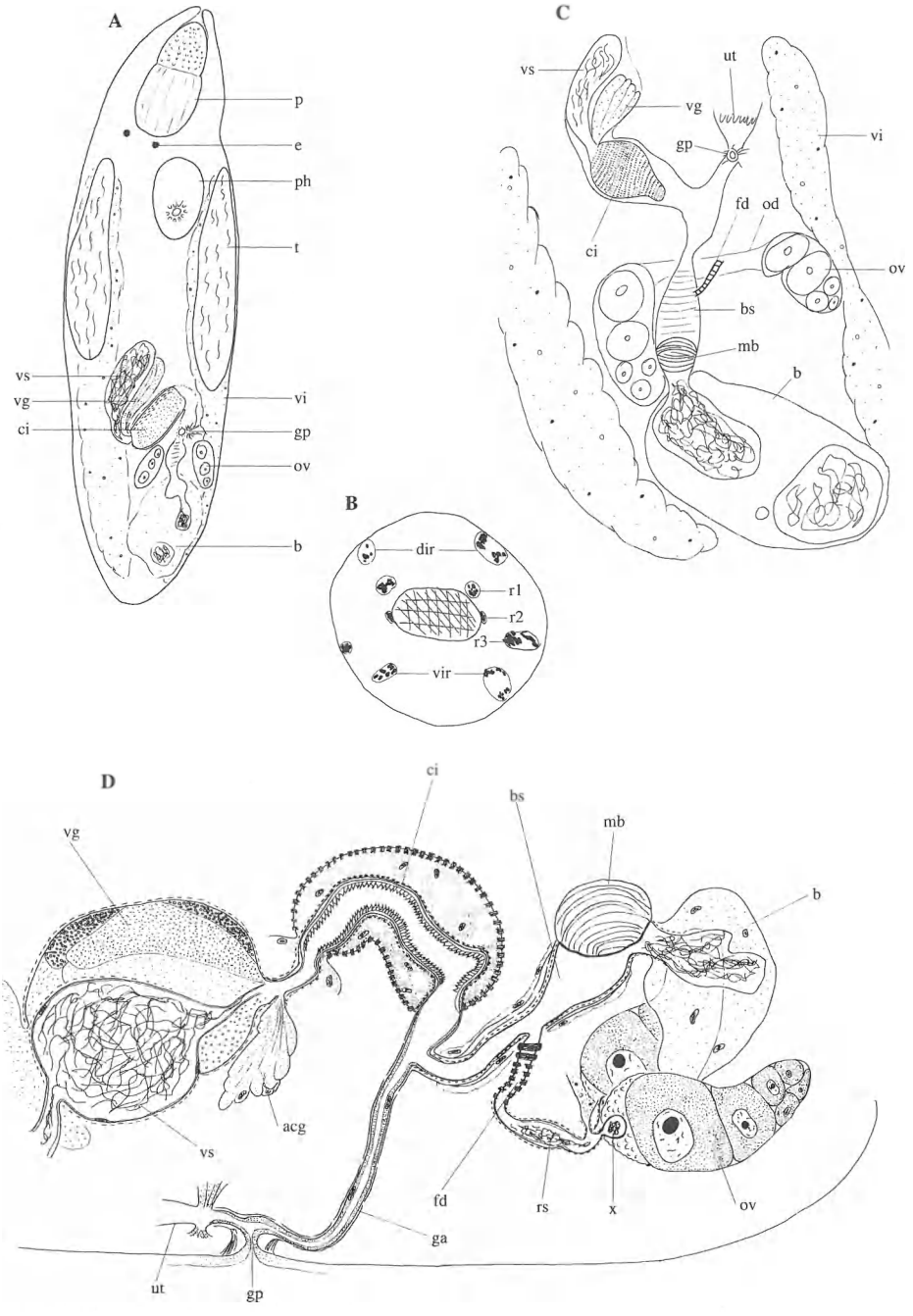

Fig. 2. – *Duplacrorhynchus heyleni* sp. nov. – A. General organisation (from a living animal). – B. Transverse section at the level of the distal end of the proboscis bulb. – C. Genital organs in a living animal. – D. Sagittal reconstruction of the genital organs seen from the left.

junction of the cavity and the cone epithelium. The basal cone epithelium is very glandular, with a strongly eosinophilic secretion. There are three pairs of proboscis retractors and two pairs of integument retractors.

The gonads are paired. The testes are situated dorsolaterally, and extend from the pharynx to the copulatory organ. The ovaries lie laterally, behind the copulatory organ. They are rather long, with the oocytes more or less arranged in rows. The vitellaria lie dorsally to the testes and extend from the level of the pharynx to the caudal body end. The gonopore is situated at about 70% and surrounded by a strong sphincter. The common genital atrium is unusually long, is lined by a nucleated epithelium and surrounded by a longitudinal muscle layer. Proximally it has a narrow diverticulum into which the copulatory organ intrudes.

The male copulatory system consists of a single seminal vesicle (lined with a low nucleated epithelium), narrowing distally into the seminal duct, which continues as a broader muscular ejaculatory duct towards the armed cirrus. The distal part of the seminal vesicle is asymmetrically surrounded by the large prostate vesicle. The seminal vesicle and the prostate vesicle are both enveloped in a common spiral (mainly circular) muscle layer. The prostate glands enter the seminal duct close to its connection with the ejaculatory duct. There are five different types of prostate glands: two with eosinophilic granules (light coarse granules, dark and very coarse granules) and three with basophilic granules (dark coarse granules, light and small granules, very light small granules). All nucleated parts of the prostate glands are outside the muscle coat. The ejaculatory duct and the cirrus are surrounded by an inner longitudinal and a strong outer circular muscle layer (both actually spiralling). The muscle layer surrounding the prostate vesicle is continuous with the circular layer. The cirrus is a broad duct, armed with numerous uniform, triangular teeth, with their sharp ends pointing proximally. It is enclosed by a highly muscular septum of which the muscles are mainly circular, and continuous with the circular muscle layer of the ejaculatory duct. A bundle of light basophilic accessory glands enters the ejaculatory duct at its initial part.

The bursal stalk enters the long genital atrium caudally, distally from the cirrus, and ends in the terminal bursa. It receives the female duct about mid-way. Close to the bursa there is a large, highly asymmetrical muscular bulb. Proximally from this bulb the bursal stalk has a relatively strong circular muscle layer, distally it has an inner circular layer and a double outer layer of longitudinal muscles that becomes single towards the muscular bulb, once the female duct has joined the stalk. The stalk is surrounded by a layer of cells, presumably muscle cells. The epithelium lining the bursal stalk and the female duct is membraneous; that covering the muscular bulb appears sclerotised («pseudocuticula»). The female duct is surrounded by circular muscles, quite strong close to the entrance in the bursal stalk, becoming weaker proximally. The short oviducts, which enter the female duct at its proximal end, also have circular muscles. These oviducts each show a small globular widening that is embedded in the stromatic tissue at each ovary (see Fig. 2D, labelled x). Sperm is found in these widenings, both evidently functioning as seminal receptacles. In its proximal part the unpaired female duct is also widened, and some sperm can be found here as well. The uterus enters the common genital atrium through its anterior wall, close to the gonopore. It has a relatively low epithelium, but does not deviate from the usual polycystidid construction.

DISCUSSION

All four *Duplacrorhynchus* species have an interposed prostate vesicle and a cirrus enclosed in a bulb: the conjucta-duplex type. However, this type of copulatory organ can be found in many Platyhelminthes, in virtually all major taxa. So this is clearly a plesiomorphy and thus invalid to define the taxon, even though it is this characteristic that brought SCHOCKAERT & KARLING (1970) to define the subfamily Duplacrorhynchinae (but see below).

The female system of the four species, however, exhibits a number of features which can be considered unique for Polycystididae: a long and highly muscular bursal stalk, a single, long, muscular female duct that joins the bursal stalk mid-way, the oviducts being very short and entering the female duct at its proximal end. The combination of these characters can thus be put forward as a synapomorphy for the four species considered. (A rather similar situation is seen in the Koinocystididae, though differences can be found and there is probably no homology). The four species are, however, very different from each other in other characteristics, as well in the female as in the male system, even to the extent that erecting a new genus for each of them might be appropriate. We have chosen not to do so to avoid an «inflation» of names, and to keep the taxon *Duplacrorhynchus* on the genus «level».

The most striking distinction in the male system of *D. heyleni* is the very large prostate vesicle and the seminal vesicle both not enclosed in the bulb. Only the cirrus is enclosed. Moreover, the seminal vesicle enters the prostate vesicle eccentrically and the prostate vesicle contains the necks of five different glands. In *D. minor* the prostate vesicle appears «reduced» and the cirrus is devoid of spines. In *D. major* the cirrus has small spines only in its middle part, while in *D. megalophallus* the bulb is divided by a muscular septum in a proximal part (containing the prostate vesicle and the internal seminal vesicle) and a distal part with the cirrus that has an umbrella-like expansion). These are all autapomorphies for each of the species. Other autapomorphies are the elongated common genital atrium and the accessory glands at the beginning of the cirrus in *D. heyleni*, and the accessory glands in the male atrium where the cirrus enters it in *D. megalophallus*. No obvious synapomorphies can be found in the male system.

Both *D. major* and *D. minor* have a voluminous eccentric seminal receptacle attached to the female duct. Furthermore, the female duct is swollen into a spherical enlargment in which a «morula-shaped» appendage can be found (SCHOCKAERT & KARLING, 1970, p. 242). At least this peculiar structure can be considered a synapomorphy for both species. Also in *D. heyleni* the female canal is enlarged. It obviously functions as a seminal receptacle and the morula-like appendage is lacking. In *D. minor* the terminal bursa is lacking, an autapomorphy of this species.

In *D. heyleni* and in *D. megalophallus* the bursal stalk is enlarged and highly muscular close to the bursa: a synapomorphy for these two species. In *D. heyleni* the muscles form a unilaterally very thick mass, while in *D. megalophallus* it contains three highly sclerotised teeth.

Within the taxon (genus) *Duplacrorhynchus, D. minor* and *D. major* appear to be closely related and a close relationship of *D. heyleni* with *D. megallophallus* can be supported as well.

The main diagnostic feature given by SCHOCKAERT & KARLING (1970) for the Duplacrorhynchinae is the copulatory organ of the conjuncta-duplex type, however this is a plesiomorphy (see above). The copulatory organ as in *Djeziraia* (conjucta with a «papillen

stilet») can also be found in many taxa within and without the Kalyptorhynchia. The Duplacrorhynchinae thus seems to be paraphyletic. We have reconsidered all Polycystididae with a copulatory organ where the prostate vesicle is interposed (see enumeration in the introduction). It now appears that in all these species the proboscis can be retracted by a system of three pairs of retractors (one subdorsal, one lateral, one subventral) and that the body wall around the the proboscis opening can be retracted by two pairs of integument retractors. In all other Polycystididae and in most other Eukalyptorhynchia the retractor system consists of four pairs of proboscis retractors and, in the other Polycystididae, only one pair of integument retractors (except in the polycystidids *Acrorhynchides* Strand, 1928, *Macrorhynchus* Graff, 1882, *Opisthocystis* Sekera, 1912, *Papia* Karling, 1956, and except in the Cytocystididae Karling, 1964, where the 3+2 retractor system is most probably a convergence). Consequently, this 3+2 retractor system might be considered a synapomorphy for all Polycistididae with an interposed prostate vesicle (conjuncta type = plesiomorphy) which can now be included in the taxon Duplacrorhynchinae. The bulk of the other Polycystididae constitute the sister taxon with a copulatory organ of the divisa type, *i.e.* with the prostate vesicle entering the male atrium next to the sperm conducting system. Though a thourough discussion on the relationships of the genera within the Duplacrorhynchinae is beyond the scope of this contribution, we have reasons to believe that *Duplacrorhynchus* is the sister taxon of all other Duplacrorhynchinae. Other Duplacrorhynchinae do not have the combination of characters in the female system that is considered to be the apomorphy of the taxon *Duplacrorhynchus*, and they all have a copulatory organ that deviates from the typical duplex system.

AMENDED DIAGNOSES AND DIAGNOSES OF THE NEW TAXA

Duplacrorhynchinae Schockaert & Karling, 1970

Polycystididae with paired gonads, a copulatory organ with an unpaired seminal vesicle and an interposed prostate vesicle (conjuncta type). Three pairs of proboscis retractors and two pairs of integument retractors (apomorphy).

Type-genus : *Duplacrorhynchus* Schockaert & Karling, 1970

Genus *Duplacrohrynchus* Schockaert & Karling, 1970

Duplacrorhynchinae with a cirrus enclosed in a septum. Genital pore ventral, not terminal. Female system with a highly muscular bursal stalk, the unpaired muscular female duct entering the bursal stalk in its middle part (apomorphy).

Type species : *D. minor* Schockaert & Karling, 1970

D. minor + *D. major*

Duplacrorhynchus species with a compound copulatory organ, with seminal vesicle and prostate vesicle enclosed in the bulb. Female system with an eccentric seminal receptacle and the female duct with an enlargement containing a « morula-shaped » appendage.

***D. minor* Schockaert & Karling 1970** : *Duplacrorhynchus* species without a terminal bursa, small and entirely intracapsular prostate glands ; cirrus unarmed.

***D. major* Schockaert & Karling 1970** : *Duplacrorhynchus* species with a terminal bursa, a large prostate vesicle with the nucleated parts of the prostate glands outside the septum ; small spines in the middle of the cirrus.

D. megalophallus + *D. heyleni*

Duplacrorhynchus species with a muscular enlargement in the proximal part of the bursal stalk. Cirrus with spines over its entire length.

***Duplacrorhynchus megalophallus* sp.nov. :** *Duplacrorhynchus*-species with seminal vesicle and large parts of the prostate glands within the bulb. Copulatory organ very large, divided by a muscular septum in a proximal part (containing the seminal vesicle and prostate glands) and a distal part (with the cirrus). Cirrus with an umbrella-like expansion. Enlargement of the bursal stalk with three strong teeth. Genital atrium with a male and a female part.

***Duplacrorhynchus heyleni* sp.nov. :** *Duplacrorhynchus*-species with only the cirrus enclosed in the bulb. Seminal vesicle enters the prostate vesicle eccentrically. Strong triangular teeth in the cirrus. Common genital atrium very long. Muscular enlargement of the bursal stalk that is highly asymmetrical.

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