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The Antarctic holothurian genus *Echinopsolus* Gutt, 1990 (Dendrochirotida, Cucumariidae): brood pouches, spermatozoa, spermatozeugmata and taxonomic implications

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Abstract

An examination of seven Antarctic brooding cucumariid and psolid holothurian species revealed a variety of characters all of them have in common: (1) All are gonochoric. (2) A genital papilla is present on the oral disc (permanent and digitiform in males). (3) Females brood their offspring in five anterior interradial brood pouches that are situated at the transition of body to introvert. (4) Multiple spermatozoa are always bundled to bunch-like spermato-zeugmata. (5) The spermatozoa have a fusiform head and a hollow cylinder-like mid-piece encircling the anterior end of the flagellum. This combination of characters so far is unique, and indicates a close relationship based on common origin. As a consequence, we unite all species sharing this set of synapomorphies in the genus *Echinopsolus* Gutt, 1990. The herewith included species are: *E. acanthocola* Gutt, 1990, *E. acutus* (Massin, 1992) comb. nov., *E. charcoti* (Vaney, 1906) comb. nov., *E. koehleri* (Vaney, 1914) comb. nov., *E. mollis* (Ludwig & Heding, 1935) comb. nov., *E. parvipes* Massin, 1992 and *E. splendidus* (Gutt, 1990) comb. nov.. Because the current assignment of *Echinopsolus* to the family Psolidae can not be retained, the genus is tranferred to the family Cucumariidae, as relationships to taxa within this family are obvious. The peculiar spermatozoa and spermato-zeugmata of all *Echinopsolus* species are described using light- and electron-microscopical techniques and the results are evaluated and discussed concerning their taxonomy and phylogeny.

Key words: Echinodermata, Antarctic radiation, sperm ultrastructure, Southern Ocean

Introduction

A considerable number of holothurian species of the dendrochirotid families Cucumariidae Ludwig, 1894 and Psolidae Burmeister, 1837 are known to protect their brood. Recent summaries list about 40 cucumariid (O'Loughlin 1994; O'Loughlin *et al.* 2009) and 11 psolid species (McEuen & Chia 1991). Various modes of brood-protection have been realized in both families (see McEuen & Chia 1991: tab. 2; O'Loughlin 1994: tab. 1): offspring may be brooded externally (e.g. held between tentacles; kept on substrate beneath ventral body, sometimes in folds or depressions), or internally (in variously shaped brood pouches, i.e. pocket-like inversions of the body wall with an opening to the exterior; in ovaries; in coelomic cavity or in coelomic brood sacs).

Interradial anterior brood pouches, situated at the transition of body to introvert, so far are only known from a variety of Antarctic Cucumariidae and Psolidae (Tab. 1). The number of brood pouches seems to be variable. The majority of species have five pouches, one in each interradius, while two cucumariid species possess two pouches and one species has three (see Tab. 1). Remarkable in this context is the common presence of a genital papilla on the oral disc, between or ventral to the mid-dorsal pair of tentacles (Tab. 1). Details for a variety of species are given by O'Loughlin (2001) and O'Loughlin *et al.* (2009). According to O'Loughlin *et al.* (2009: p. 217), these species have in common, that "males have a long genital papilla between the dorsal tentacle pair (...), and do not have marsupia; females have a short genital papilla between the dorsal tentacle pair (...), and have up to five anterior interradial marsupia with external pores.". Also the fact, that all species sharing this set of characters so far are restricted to Antarctica, was first stressed by these authors (O'Loughlin *et al.* 2009).

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TABLE 1. Literature records of Antarctic Cucumariidae and "Psolidae" with anterior interradial brood pouches and a genital papilla on oral disc complemented by data from the current investigation. *lli*—left lateral interradius; *mdi*—mid-dorsal interradius; *rli*—right lateral interradius; *rvi*—right ventral interradius +—present; *1*—"*Cucumaria georgiana*" group species sensu O'Loughlin *et al*. (2009); *2*—according to O'Loughlin *et al*. (2009: p. 217), "*Cucumaria georgiana*" group species have in common that "...males have long genital papilla.." and "...do not have marsupia...", while "...females have short genital papilla..." and "...up to five anterior interradial marsupia...", without giving further details to the included species; *3*—fide O'Loughlin *et al.* (2009).

Species	number of brood pouches	genital papilla	spermato-zeugmata	*"Echinopsolus"* spermatozoa	Source	Name used herein
Cucumariidae						
[1]*Cucumaria acuta* Massin, 1992	5	+	+	+	herein	*Echinopsolus acutus* (Massin, 1992)
		+			Massin 1992	
[1]*Cucumaria attenuata* Vaney, 1906	5	+			Ekman 1925	?
		+			Massin 2010	
Cucumaria coatsi [non Vaney, 1908]	5				Ekman 1925	?
Cucumaria ferrari (Bell, 1908)	3				McClintock *et al.* 1994	?
[1]*Semperia georgiana* Lampert, 1886		+			Lampert 1886	?
[1]*Cucumaria georgiana* (Lampert, 1886)	5				Ekman 1925	?
"*Cucumaria georgiana*" group species	5	+			O'Loughlin 1994, 2001	?
	[2]up to 5	[2]+			[2]O'Loughlin *et al.* 2009	
[1]*Cucumaria joubini* Vaney, 1914	5	+			Vaney 1914, 1925	?
[1]*Cucumaria lateralis* Vaney, 1906	2 (mdi, rli)	+			Vaney 1906a, b	?
	2 (rli, rvi)				Vaney 1908	
	2				Vaney 1925	
[1]*Cucumaria vaneyi* Cherbonnier, 1949	2 (lli, mdi)				Massin 1992	?
Microchoerus splendidus Gutt, 1990	5	+	+	+	herein	*Echinopsolus splendidus* (Gutt, 1990)

……*continued on the next page*

TABLE 1. (Continued)

Species	number of brood pouches	genital papilla	spermato-zeugmata	"Echinopsolus" spermatozoa	Source	Name used herein
		+			Gutt 1990	
	5	+			O'Loughlin 1994, 2001	
Pseudocolochirus mollis Ludwig & Heding, 1935	5	+	+	+	herein	*Echinopsolus mollis* (Ludwig & Heding, 1935)
	5	+			Ludwig & Heding 1935	
Psolidiella mollis (Ludwig & Heding, 1935)	5	+			O'Loughlin *et al.* 2009	
"Psolidae"						
Echinopsolus acanthocola Gutt, 1990	5	+	+	+	herein	*Echinopsolus acanthocola* Gutt, 1990
	?				[3]De Ridder *et al.* 2005	
	up to 5	+			O'Loughlin *et al.* 2009	
Echinopsolus parvipes Massin, 1992	5	+	+	+	herein	*Echinopsolus parvipes* Massin, 1992
		+			Massin 1992	
	up to 5	+			O'Loughlin *et al.* 2009	
Psolus charcoti Vaney, 1906	5	+	+	+	herein	*Echinopsolus charcoti* (Vaney, 1906)
	5				Ekman 1925	
	5	+			O'Loughlin 2001; O'Loughlin *et al.* 2009	
Psolus koehleri Vaney, 1914	5	+	+	+	herein	*Echinopsolus koehleri* (Vaney, 1914)
	5				Vaney 1914, 1925	
	5	+			Ekman 1925	

The "*Cucumaria georgiana*" group was first established by Gutt (1988) and further specified by Massin (1992) and O'Loughlin *et a*l. (2009). Include are 11 Antarctic *Cucumaria* species (see O'Loughlin *et al.* 2009: tab. 1), which are of a more or less dubious taxonomic status. Five of these are known to brood their offspring in interradial anterior brood pouches (Tab. 1). According to Massin (1992: p. 187) "*Cucumaria georgiana*" group species are "...small Antarctic dendrochirote holothurians, with the tube feet on 1–3 rows in each ventral radius and more randomly scattered dorsally..." their calcareous deposits "..., abundant in young specimens and often rare or absent in adults, are knobbed or spiny perforated plates, which show great variability...".

An examination of the rich holothurian samples collected in recent years by a variety of Antarctic expeditions with FS "Polarstern" in the Weddell Sea and the Antarctic Peninsula area (EASIZ I to III, BENDEX), revealed the presence of at least 11 different species, which are brooders with five anterior interradial brood pouches and which have distinct genital papillae present on the oral disc in both sexes (although smaller in females). Detailed investigations of male specimens of these species revealed a further, so far unknown feature all of them have in common: peculiarly modified spermatozoa, which are bundled to bunch-like spermatozeugmata.

The vast majority of holothurian spermatozoa investigated so far are of a uniform type (Chia *et al.* 1975; Jamieson 1985; Hodgson & Bernard 1992; Tyurin & Drozdov 2003), similar to spermatozoa found in other echinoderm classes like Crinoidea, Asteroidea and Ophiuroidea (Chia *et al.* 1975; Jamieson 1985). This type has been denoted as "echinosperm" by Jamieson (1985), and Chia *et al*. (1975: p. 554) characterize it as follows: "…the head is essentially spherical with the acrosome positioned at the anterior end of the nucleus in a depression, while the mitochondrial middle piece takes the form of an annular band at the posterior end of the nucleus…".

The only holothurian species known to depart from the "echinosperm" type are *Cucumaria lubrica* H.L. Clark, 1901, *Cucumaria pseudocurata* Deichmann, 1938 (which both brood their young between the ventral surface and the substrate, e.g. Atwood & Chia 1974; Atwood 1975) and *Semperiella drozdovi* Levin & Stepanov, 1999 (see Tyurin & Drozdov 2003). *Cucumaria pseudocurata* has elongated dorso-ventrally compressed spermatozoa, that have their acrosome located on the side of the nucleus, and the majority of the mitochondrial mass on the opposite side (Atwood 1975; Chia *et al.* 1975). Sperm is released in the form of a modified spermatophore, where the spermatozoa are tightly packed into dense strands of mucus, hence quite resistant to dilution (Atwood 1975; McEuen 1988). The spermatozoa of *C. lubrica* are cylindrical or cigar-shaped (Atwood & Chia 1974; Chia *et al.* 1975) and numerous spermatozoa are bundled into bunches by interwined tails (McEuen 1988). After spawning these spermatozoan bundles remain together for an extended length of time before dispersing in the water (Atwood & Chia 1974; Engstrom 1982; McEuen 1988). In *S. drozdovi* the spermatozoa have an "echinosperm" architecture except for the conical shape of their sperm heads (see Tyurin & Drozdov 2003).

Quite recently, spermatozoan bundles comparable to those of *Cucumaria lubrica* (see previous paragraph) have been described for males of the cucumariid holothurian *Pseudrotasfer microincubator* Bohn 2007, which is also a brooder. Contrary to the aforementioned species, females of *P. microincubator* hatch their brood in the ovaries (Bohn 2007).

The aim of this study is to describe in detail the peculiar spermatozoa and spermatozeugmata of seven Antarctic Cucumariidae, using light- and electron-microscopic techniques and to evaluate and discuss the results concerning their taxonomy and phylogeny.

Material and methods

The holothurians investigated in the current study were collected in the course of various Antarctic expeditions with FS "Polarstern" to the Weddell Sea and the Antarctic Peninsula area using Agassiz and bottom trawls (Tab. 2). On board, specimens were sorted to morpho-species and afterwards either fixed in buffered formalin or conserved in 75 % ethanol. The material is deposited in the Bavarian State Collection of Zoology in Munich.

Images of holothurian specimens were generated with the extended focal imaging software CombineZM (Hadley 2008) from a series of partially focused digital images taken with a digicam on a standard stereo microscope.

To investigate spermatozoa and spermatozeugmata with a scanning electron microscope (SEM) single gonad tubules were removed from male specimens and dehydrated according to the AXA method (for details see Bohn 2007). The dry gonad tubules were placed on aluminium stubs with adhesive carbon tabs and opened up with a fine pin to spread the spermatozeugmata on the stub. Finally, the SEM specimens were sputtered with gold in a Biorad Polaron SEM coating system (2.4 kV, 120") and studied with a LEO 1430 VP scanning electron microscope at 25 kV.

TABLE 2. Locality data of *Echinopsolus* specimens pictured herein for specimen details, scanning electron microscopy, histology or fine structure.

Species	Specimen	Expedition	Station	Latitude	Longitude	Depth (m)
E. acanthocola	JMB-00090, JMB-01779	EASIZ III	PS 56/109-1	71°11.3' S	12°18.5' W	311–316
	JMB-01760	BENDEX	PS 65/090-1	70°56.1' S	10°31.7' W	274–288
E. acutus	JMB-01731	EASIZ I	PS 39/06 AGT 1	71°31.8 'S	13°34.5 'W	254–261
	JMB-01712	EASIZ II	EASIZ II	71°09.7' S	12°28.7' W	341–360
E. charcoti	JMB-01832, JMB-01837	EASIZ III	PS 56/065-1	71°17.6' S	13°48.0' W	615–648
E. koehleri	JMB-01833, JMB-01836	EASIZ III	PS 56/085-1	71°11.3' S	12°15.4' W	309–318
E. mollis	JMB-01680		PS 39/11 GSN 4	73°22.6' S	21°10.6' W	333–338
	JMB-01831	BENDEX	PS 65/336-1	70°50.7' S	10°28.3' W	276–281
E. parvipes	JMB-01825, JMB-01826	EASIZ III	PS 56/065-1	71°17.6' S	13°48.0' W	615–648
E. splendidus	JMB-01696, JMB-01698	EASIZ II	PS 48/077	71°09.7' S	12°28.7' W	341–360
	JMB-00122	EASIZ III	PS 56/085-1	71°11.3' S	12°15.4' W	309–318
	JMB-00088, JMB-01702	EASIZ III	PS 56/109-1	71°11.3' S	12°18.5' W	311–316

For histology and fine structure investigations short pieces of the tubules (preferably cut out at a swelling) were dehydrated in a graded acetone series and embedded in epoxy resin (Epon 100, Roth Company). Semithin sections (0.5–1 µm) were cut with glass knives on a RMC MT-XL 7000 ultramicrotome, stained with Richardson's reagent (Richardson *et al.* 1960) and documented with a digicam on a standard widefield light microscope. Thin sections (80 nm) were cut with a 45° diamond knife, mounted on formvar coated copper grids, stained with lead citrate and uranylacetate and inspected with a Zeiss EM10 at 80 kV and a FEI Technai Spirit transmission electron microscope at 120 kV.

Results

The rich Antarctic holothurian samples at hand, collected in recent years in the Weddell Sea and the Antarctic Peninsula area, contain about 11 discernible species, which share a variety of characters. So far only seven of these holothurians can be assigned to well-defined species and therefore are included in this investigation (*Cucumaria acuta* Massin, 1992; *Echinopsolus acanthocola* Gutt, 1990; *Echinopsolus parvipes* Massin, 1992; *Microchoerus splendidus* Gutt, 1990; *Pseudocolochirus mollis* Ludwig & Heding, 1935; *Psolus charcoti* Vaney, 1906; *Psolus koehleri* Vaney, 1914). For now omitted, but to be clarified and described later on, are species which have a dubious taxonomical status ("*Cucumaria georgiana*" group) or are new to science.

The species investigated agree in the following characters (see also Tab. 1). (1) All are gonochoric. (2) A genital papilla is present on the oral disc, between or ventrally of the dorsal pair of tentacles. In males these papillae are permanent, large (up to 4 mm in length) and digitiform (Figs 1a–b). In females, the genital papilla is sometimes absent, but usually small (up to 1 mm in length) and wide conical to digitiform (Fig. 1c). (3) Females brood their offspring in five anterior interradial brood pouches that are situated at the transition of body to introvert (Figs 1d–f) and open via a small interradial slit.

Histological and fine structural investigations of the spermatozoa of these species revealed some further features shared by all.

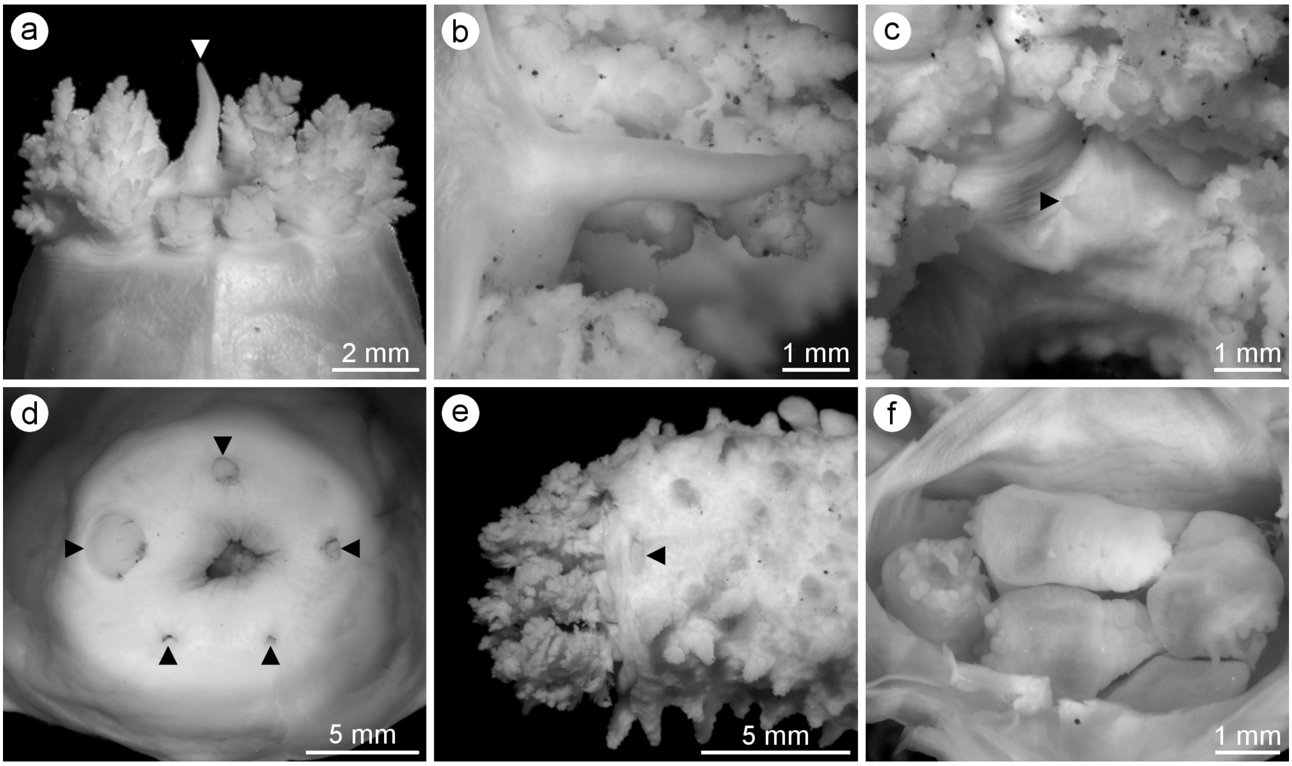

FIGURE 1. Genital papillae and brood pouches of *Echinopsolus* spp. **a.** Male genital papilla (*arrowhead*) of *E. acutus*. **b–d.** *E. splendidus*. **b.** Male genital papilla. **c.** Female genital papilla (*arrowhead*). **d.** Brood pouch openings (*arrowheads*) of a female with retracted tentacles. **e.** *E. acanthocola*, brood pouch opening of a female (*arrowhead*). **f.** *E. splendidus*, brood pouch (opened up) with developing juveniles.

Morphology of spermatozoa and spermatozeugmata

The seminiferous gonad tubules of the inspected male specimens vary widely in length and diameter between species. The tubules have a circular to oval smooth outer cross section profile (Fig. 3a) and marked longitudinal tissue-folds defining the inner surface (Figs 2d, 3a). The lumina are filled with densely packed sperm cell bundles (i.e. spermatozeugmata, Figs 2a, 3a). Each spermatozeugma is composed of several hundred, prevailingly mature sperm cells (e.g. approximately 1100 in *E. acutus*) aligned in the same orientation, united into a bunch with the sperm heads forming a compact knob or “bobble” and the flagella, which stick together by some amorphous material, forming a rotund shaft tapering towards the end. With few exceptions, spermatozeugmata are found in a paired constellation with their heads in tight contact and the flagella pointing in the opposite direction (Figs 2b, d, e).

Mature and almost mature sperm cells of the seven species investigated in this study all have the same general structure, subdivided into a head, a mid-piece and a tail (flagellum), with some species-specific variation in detail (Figs 2–4, Tab. 3).

The head always has a circular cross section and an elongate spindle-shaped (fusiform) profile in any axial cutting plane (Figs 2f–g, 3c, e, 4a–b, e–f). With a maximum diameter in the basal quarter, it tapers to a rounded tip containing the globular acrosome (Figs 4c, f, g). The overall shape of the head is similar in *E. acanthocola*, *E. acutus*, *E. mollis*, *E. parvipes* and *E. splendidus*, the latter especially pointed, the first one extra slender (length-to-max. diameter ratios see Tab. 3). The sperm head in *E. koehleri* is less pointed (torpedo-shaped, Figs 2g, 4f), that of *E. charcoti* comparatively stocky (acorn-shaped, Fig. 2c). At the basis, i.e. the anchoring site of the ciliary tail, the head shows a small, somewhat folded invagination, the centriolar fossa (Fig. 5a). After standard TEM preparation the head has a very electron dense appearance with several bright plaques (nuclear “vacuoles”) of up to 70 nm (Figs 4b, f, 5a, d). The cell membrane envelopes the nuclear material tightly, small residues of the cytoplasm are found only between the basal head curvature and the mitochondrial ring of the mid-piece.

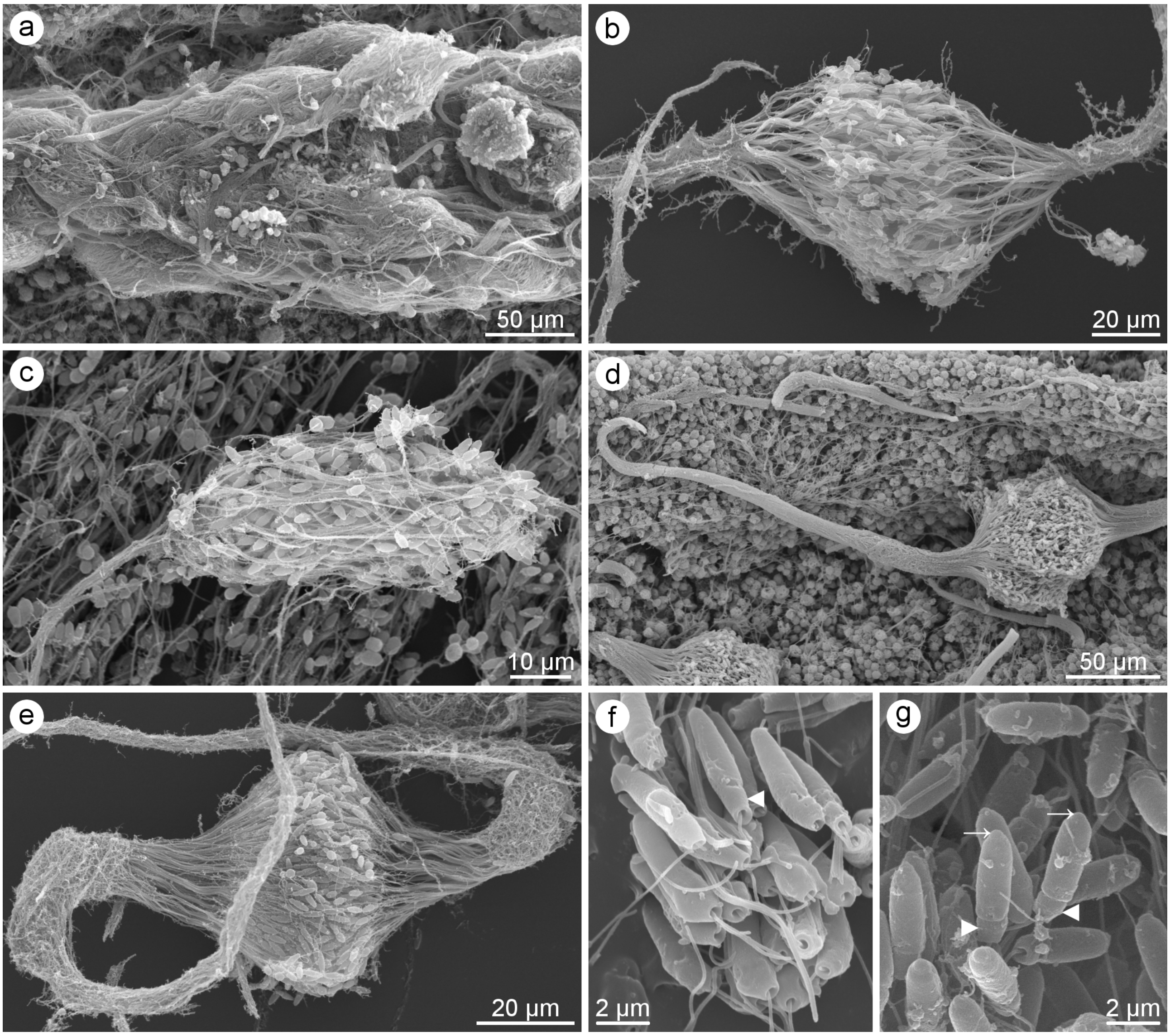

FIGURE 2. Ultrastructure (SEM) of spermatozeugmata and sperm cells of *Echinopsolus* spp. **a.** Mass of sperm bundles in gonad tubule (opened up) of *E. koehleri*. **b–e.** Spermatozeugmata. **b.** *E. acanthocola*. **c.** *E. charcoti*. **d.** *E. mollis*. **e.** *E. parvipes*. **f–g.** Spermatozoa. **f.** *E. acanthocola*. **g.** *E. koehleri*. *Arrows*—acrosomes; *arrowheads*—cylinder shaped mid-pieces.

TABLE 3. Morphometric data of *Echinopsolus* sperm cells. Diameters measured on cross sections or on oblique sections (smallest diameter). Longimetry was performed on cutting profiles that show the distal centriole and the acrosomal vesicle simultaneously. The bad structural conservation of sperm mid-pieces in *E. mollis* (no aldehyde fixation) did not allow any reliable measurement (*).

Species	***Echinopsolus***						
	acutus	***mollis***	***acanthocola***	***parvipes***	***splendidus***	***charcoti***	***koehleri***
Head length (µm)	4.1	4.1	5.0	4.1	4.2	2.9	4.6
Head diameter (µm)	1.5	1.4	1.3	1.3	1.5	1.4	1.6
Head length / diameter	2.7	2.9	3.85	3.15	2.8	2.1	2.9
Mid-piece length (µm)	1.4	*	1.8	1.6	1.6	1.4	1.6
Mid-piece diameter (µm)	1.2	*	1.3	1.2	1.3	1.4	1.5
Thickness of mid-piece wall (µm)	0.3	*	0.25	0.25	0.3	0.25–0.3	0.4

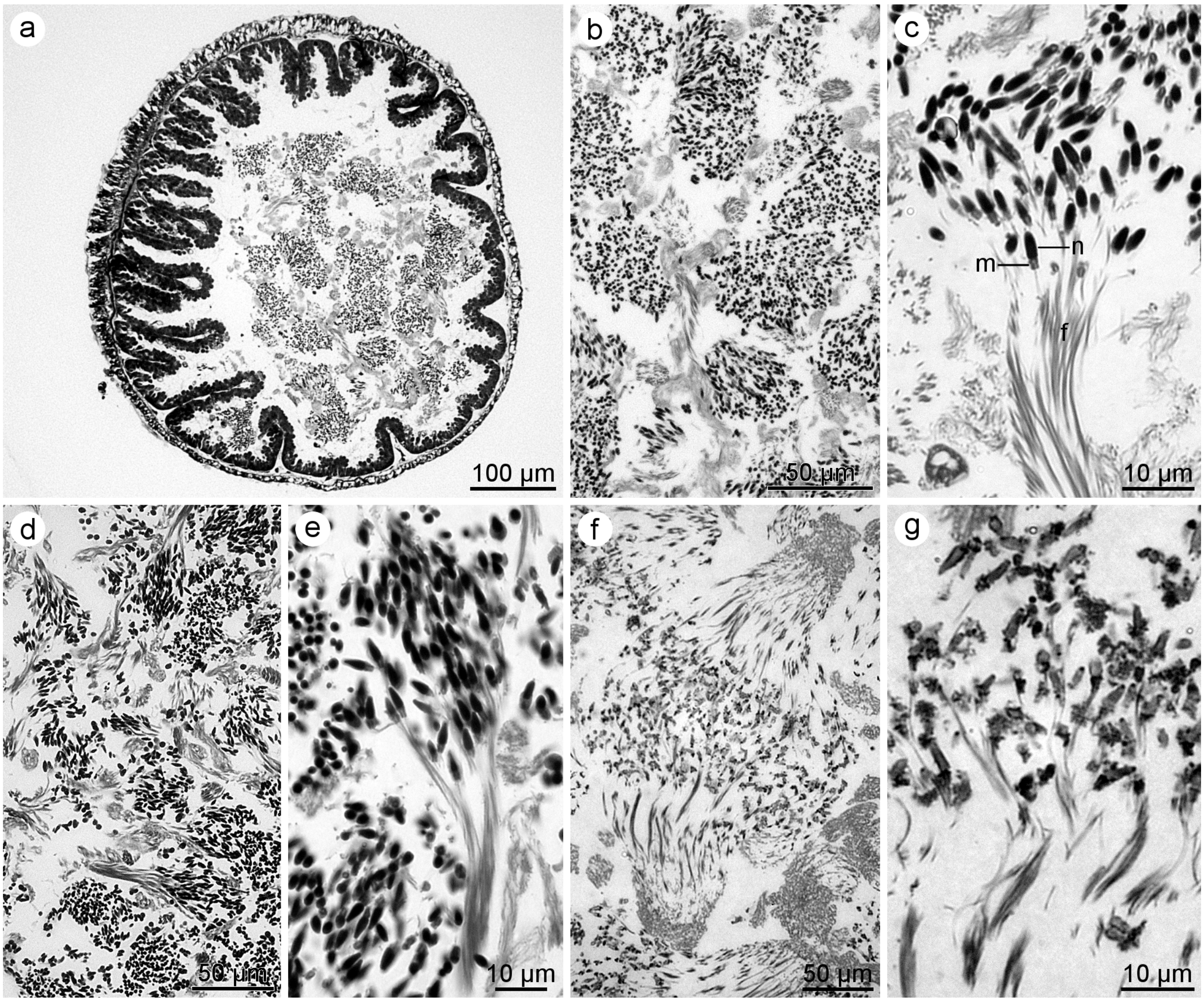

FIGURE 3. Histology of spermatozeugmata and sperm cells of *Echinopsolus* spp. **a–c.** *E. acutus*. **a.** Semithin cross section in centre of seminiferous tubule. **b.** Sperm bundles fill central space of seminiferous tubule. **c.** Longitudinal section through spermatozeugma showing sperm heads, mid-pieces and flagella. **d–e.** *E. splendidus*. **d.** Mass of sperm bundles. **e.** Longitudinal section through spermatozeugma. **f–g.** *E. mollis*. **f.** Mass of sperm bundles. **g.** Longitudinal section through spermatozeugma with poor structure preservation (alcohol conservation, no aldehyde fixation). *f*—flagellum; *m*—mid-piece; *n*—nucleus.

The membrane-bound acrosomal vesicle is positioned at the tip of the sperm head, emerging more (e.g. in *E. charcoti* and *E. koehleri*, Figs 2g, 4f) or less over the head-tip curvature. It is embedded and anchored in an apical cup-shaped recess (Figs 4g, 5a), filled with periacrosomal material of medium electron density. The vesicle has a diameter of about 400 nm (*E. acutus*, *E. acanthocola*), 500 nm (*E. charcoti*, *E. splendidus*, *E. koehleri*, *E. mollis*), or even 600 nm (*E. parvipes*) and contains a heterogeneous matrix of intermediate electron density with darker granules in the centre (Fig. 4g). In addition, a small circumscribed globular structure (diameter ca. 200 nm) of decreased electron density is found in the centre or shifted apically (Figs 5a, b).

The mid-piece has the shape of a hollow cylinder, tightly connected with the head basis and centred around the first 1.5 µm of the flagellum. With a diameter of a little less than the maximum head diameter, it is set off against the head only by a slight ring-shaped contraction. This is best seen in *E. parvipes* (Fig. 4h) and *E. koehleri* (Figs 2g, 4f). In *E. acanthocola* the transition between mid-piece and head appears rather smooth (Figs 2f, 4e), due to some cytoplasmatic residues (premature state?). As a rule, the diameter of the "cylinder" slightly decreases towards its caudal end. The thickness of this structure amounts to 220–300 nm (in *E. koehleri* up to 400 nm) and slightly tapers caudally. Ultrastructurally it turns out to be a combination of a ring-shaped mitochondrial derivative in front, tightly connected to a roughly hollow cylinder-shaped mitochondrial derivative behind (Fig. 5a). The inner wall of the latter is somehow reinforced, indicated by a membrane of increased electron density. In cross sections this mitochondrial derivative shows stacks of parallel membranes at an angle with respect to the outer and inner walls (good structural preservation provided, Figs 4i, k).

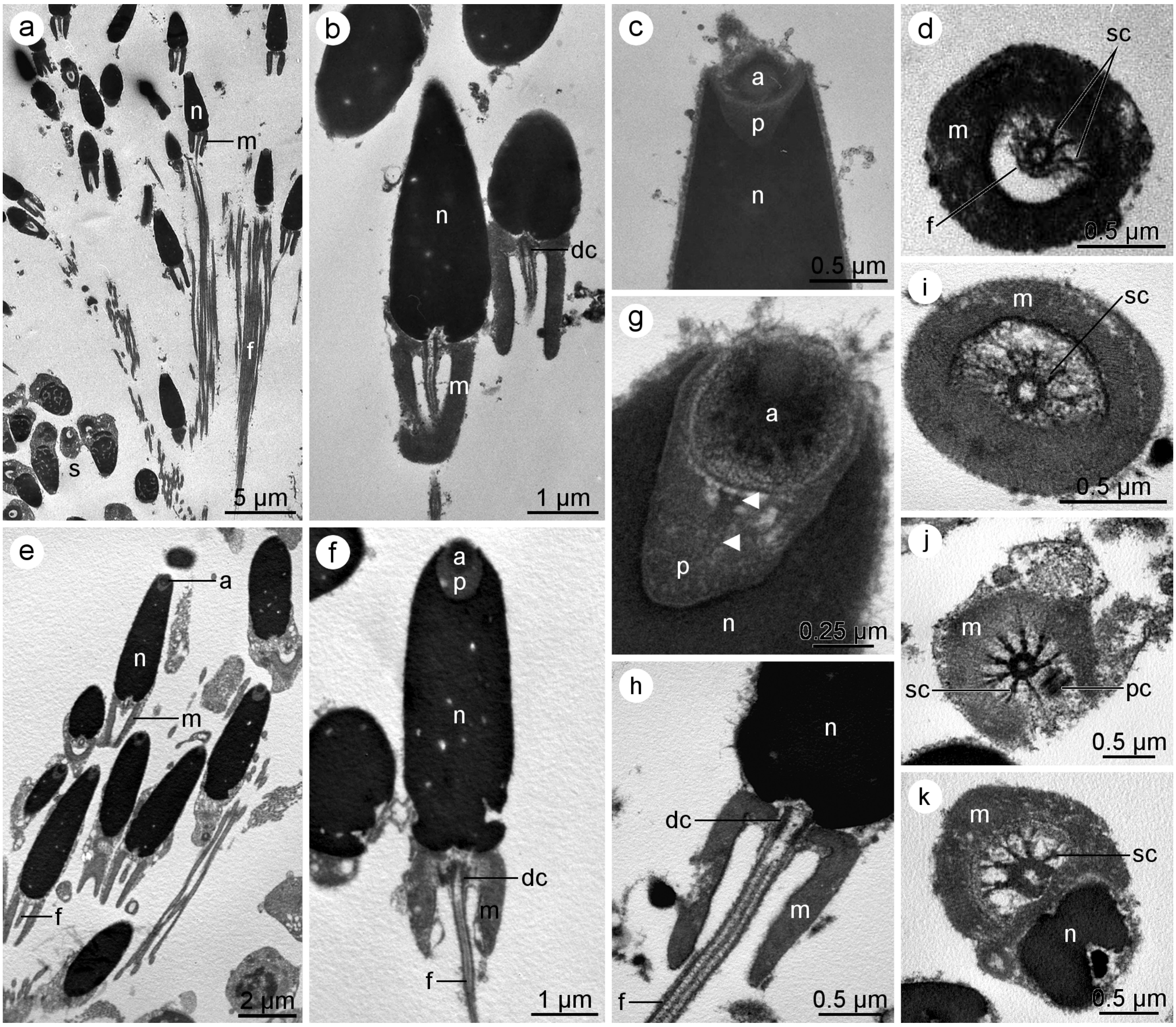

FIGURE 4. Ultrastructure (TEM) of spermatozeugmata and sperm cells of *Echinopsolus* spp. **a–d.** E. *splendidus*. **a.** Overview of ripe sperm cells and spermiogenesis-stages. **b.** Different regions of sperm cells in oblique cutting planes. **c.** Nuclear apex with cup-shaped indentation containing acrosomal complex. **d.** Cross section through mid-piece at level of centriolar satellite complex. **e.** *E. acanthocola*, longitudinal sections through sperm-cell head and mid-piece. **f.** *E. koehleri* longitudinal section through sperm-cell head and mid-piece. **g–i.** *E. parvipes*. **g.** Close up of nuclear apex with acrosomal vesicle anchored by actomere and subacrosomal plate (*arrowheads*) in periachrosomal material. **h.** Close up of the mid-piece region in axial section plane. **i–k.** Cross sections through mid-pieces at level of centriolar satellite complex. **i.** *E. parvipes.* **j.** *E. charcoti.* **k.** *E. koehleri* (premature). *a*—acrosomal vesicle; *dc*—distal centriole; *f*—flagellum; *m*—mid-piece; *n*—nucleus; *p*—periacrosomal material; *pc*—proximal centriole; *s*—spermiogenesis-stages; *sc*—centriolar satellite complex.

The flagellum has the typical 9 x 2 + 2 arrangement of microtubuli, a length of up to 150 µm (e.g. *E. parvipes*, Fig. 2e) or even 250 µm (*E. mollis*, Fig. 2d) at an average diameter between 170 and 185 nm. The first 250 nm, situated within the mid-piece cylinder, has a slightly enlarged diameter (230–280 nm) with a 9 x 2 + 0 microtubuli structure. Directly inside the insertion point and reaching a few nm into the centriolar fossa of the head, the ciliary body, with its 9 x 3 + 0 microtubuli structure, is found in axial alignment with the flagellum. The second component of the diplosome, the proximal centriole, is located in proximate neighbourhood, but paraxial and turned about 90° (Figs 4j, 5a). A rather complex ultrastructural feature of the ciliary root is the so called "satellite complex": nine electron dense branches radially emerge from the caudal half of the distal centriole in a plane normal to the sperm cell's long axis (Figs 4d, i–k, 5e) leading to a ring-shaped structure in the notch, between the mitochondrial ring and the mitochondrial cylinder. Half the way, the branches dilate to bifurcations, resulting in altogether 18 tiny spokes.

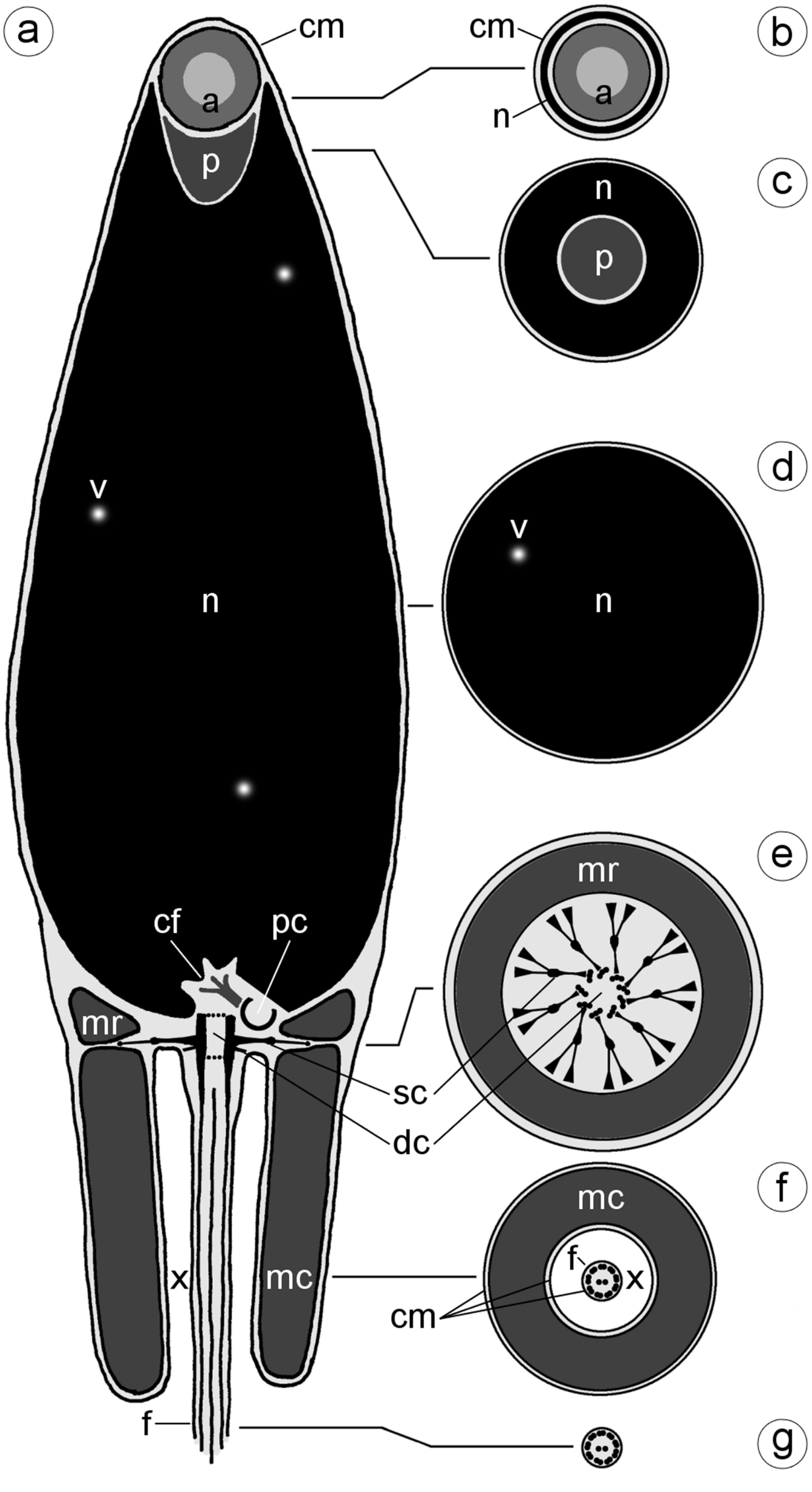

FIGURE 5. Schematic reconstruction of sperm ultrastructure of *Echinopsolus splendidus*. **a.** Longitudinal section of sperm head with acrosomal complex, diplosome with satellite complex and cylindrical mid-piece with mitochondrial ring and mitochondrial cylinder wraping basis of flagellar sperm-tail. **b–g.** Cross sections. **b.** Rim of the anterior nuclear depression with embedded acrosomal vesicle. **c.** Periacrosomal mass. **d.** Electron dense nucleus with small electron light "vacuole". **e.** Distal centriole (9 x 3 + 0 tubular arrangement) with satellite complex and subnuclear mitochondrial ring. **f.** Jacket-shaped mitochondrial derivative encircling flagellar tail-base. **g.** Flagellar tail. *a*—acrosomal vesicle; *cf*—centriolar fossa; *cm*—cell membrane; *dc*—distal centriole; *f*—flagellum; *mc*—mitochondrial cylinder; *mr*—mitochondrial ring; *n*—nucleus; *p*—periacrosomal material; *pc*—proximal centriole with fibrous arm; *sc*—satellite complex; *v*—nuclear vacuole; *x*—extracellular space within the mitochondrial cylinder.

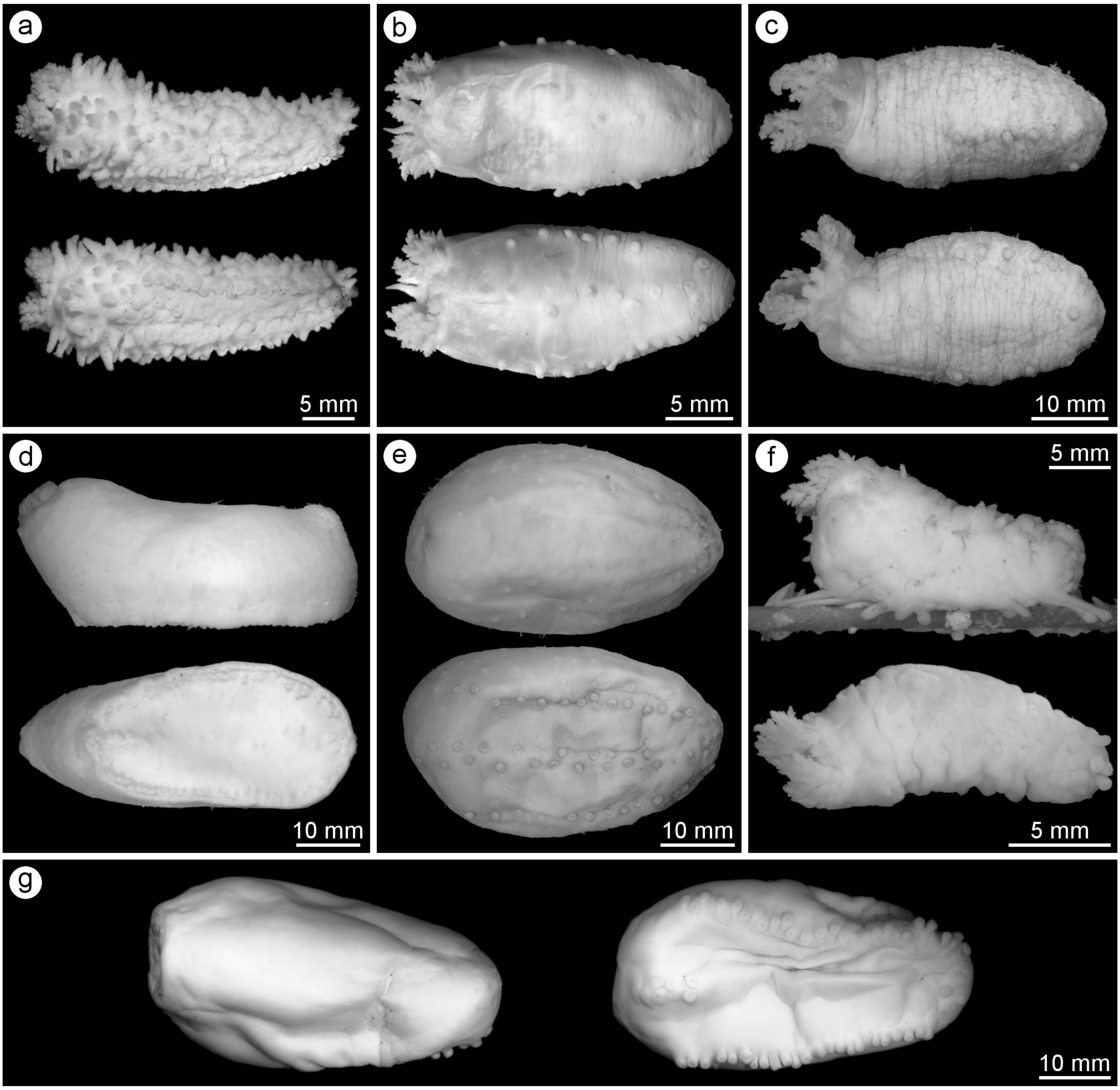

FIGURE 6. Variability of tube feet arrangement of *Echinopsolus* spp. **a.** *E. acanthocola*, lateral and ventral view. **b.** *E. acutus*, dorsal and ventral view. **c.** *E. charcoti*, lateral and ventral view. **d.** *E. koehleri*, lateral and ventral view. **e.** *E. mollis*, dorsal and ventral view (tentacles retracted, mouth to the left). **f.** *E. parvipes*, lateral and ventral view. **g.** *E. splendidus*, lateral and ventral view (tentacles retracted, mouth to the left).

Taxonomy

To the characters shared by these species, which have already been mentioned (gonochorism; sexual dimorphism, large digitiform genital papilla in males, usually inconspicuous in females; brood protection in five anterior interradial brood pouches), two further have to be added:

(1) Multiple spermatozoa are bundled by somehow agglutinating tails to bunch-like spermatozeugmata. (2) The spermatozoa are unique in having a fusiform head and a hollow cylinder-like mid-piece encircling the anterior end of the flagellum.

This combination of characters so far is unique, and in our opinion indicates a close relationship based on common origin, which should also be reflected in terms of taxonomy. As a consequence, we propose to unite all species sharing this set of synapomorphies in the cucumariid genus *Echinopsolus* Gutt, 1990 (see also Tab. 1).

Order Dendrochirotida Grube, 1840

Family Cucumariidae Ludwig, 1894

Genus *Echinopsolus* Gutt, 1990

Echinopsolus Gutt, 1990: 112. Type species: *E. acanthocola* Gutt, 1990, by original designation.
Microchoerus Gutt, 1990: 105 [non Wood 1844]. Type species: *M. splendidus* Gutt, 1990, by original designation. [*Microchoerus* Gutt, 1990 is a junior homonym of *Microchoerus* Wood, 1844]

Diagnosis [emended]: Cucumariidae with 10 tentacles, the two ventral considerably smaller. Sexes separate and dimorphic, genital papilla large digitiform in males, usually inconspicuous in females, five interradial anterior brood pouches in females (not present in males). Spermatozoa with fusiform head and hollow cylinder-like mid-piece encircling anterior end of flagellum; multiple spermatozoa bundled by agglutinating tails to bunch-like spermatozeugmata. Calcareous deposits derived from simple multi-perforate plates.

Echinopsolus acanthocola Gutt, 1990
(Figs 1e, 2b, f, 4e, 6a)

Echinopsolus acanthocola Gutt, 1990: p. 113, figs 17–20.

Echinopsolus acutus (Massin, 1992) comb. nov.
(Figs 1a, 3a–c, 6b)

Cucumaria acuta Massin, 1992: 184, figs 6–8.

Echinopsolus charcoti (Vaney, 1906) comb. nov.
(Figs 2c, 4j, 6c)

Psolus charcoti Vaney, 1906a: 406.

Echinopsolus koehleri (Vaney, 1914) comb. nov.
(Figs 2a, g, 4f, k, 6d)

Psolus koehleri Vaney, 1914: p. 24, pls 1 (figs 2, 5–6), 5 (figs 1–10).

Echinopsolus mollis (Ludwig & Heding, 1935) comb. nov.
(Figs 2d, 3f–g, 6e)

Pseudocolochirus mollis Ludwig & Heding, 1935: 204, figs 62–65, pl. 2 (figs 21–38).

Echinopsolus parvipes Massin, 1992
(Figs 2e, 4g–i, 6f)

Echinopsolus parvipes Massin, 1992: 179, figs 1–5.

Echinopsolus splendidus **(Gutt, 1990)** comb. nov.
(Figs 1b–d, f, 3d–e, 4a–d, 6g)

Microchoerus splendidus Gutt, 1990: 107, figs 9–11.

? ***Echinopsolus excretiospinosus*** **Massin, 2010**

Echinopsolus excretiospinosus Massin, 2010: 271, figs 8A–D, 9A–B, pl. 1 (figs H–J).

Remarks. Judging from the morphological details given by Massin (2010), for instance position and arrangement of tube feet and morphology and arrangement of calcareous plates, a placement of this species within *Echinopsolus* in the current sense seems probable. But there are also some differences: *E. excretiospinosus* has 10 tentacles, all of about the same size (Massin 2010), while in all other species of the genus, the two ventral tentacles are considerably smaller than the remaining. Furthermore, there are no hints for the presence of brood pouches, a genital papilla or any kind of modified spermatozoa in this species (see Massin 2010).

Albeit the striking similarities shared by these species, there are also considerable differences separating them. This applies first of all to the arrangement and shape of the tube feet (for details see Figs 6a–g and Tab. 4). Dorsal tube feet are either restricted to radial rows, or are irregularly scattered or are lacking almost entirely. Also the shape of the dorsal tube feet is variable, in some species they are cylindrical and have a well developed terminal sucking disc, while in others they are conical and lack a terminal disc. The ventral tube feet usually are restricted to distinct radial rows. A distinct tendency to form a ventral sole is noticeable, which may be delimited from the remaining body either by the arrangement of the tube feet or by a distinct bulge. A concomitant phenomenon is the reduction of the number of the mid-ventral tube feet to only a few at the anterior and posterior end of the ventral sole.

TABLE 4. Morphological variability of tube feet arrangement of *Echinopsolus* species, own observations supplemented by data from literature.

Species	***Echinopsolus***						
	acutus	***mollis***	***acanthocola***	***parvipes***	***splendidus***	***charcoti***	***koehleri***
Dorsal tube feet							
Shape	cylindrical	cylindrical to conical	--------large conical-------		------------small cylindrical------------		
Arrangement	single radial rows	-------------------scattered------------------			single tube feet close to anterior end and around anus		
Ventral tube feet							
Sole	-----------indistinct----------		----------------delimited by tube feet-----------------				delimited by bulge of body wall
Lateral radii	single row	single to double row	---------------------single row--------------------------				double row
Mid-ventral radius	---------double row----------		double row restricted to anterior and posterior end of sole				double row, often scattered in middle part of sole
Sources	Massin 1992	Ludwig & Heding 1935	Gutt 1990	Massin 1992	Gutt 1990	Ekman 1925	Ekman 1925

TABLE 5. Morphological variability of calcareous deposits of the body wall of *Echinopsolus* species, own observations supplemented by data from literature. *—ventral plates similar to dorsal plates.

Species	***Echinopsolus***						
	acutus	***mollis***	***acanthocola***	***parvipes***	***splendidus***	***charcoti***	***koehleri***
Calcareous deposits	only plates					outer layer of basket-like plates, deeper layer of plates	
Variation in deposit number and size with increasing body size	decrease in number	decrease in number and size	no changes	decrease in size	decrease in number and size	no changes	
Dorsal plate arrangement	scattered	scattered	partly overlapping	partly overlapping	dense to scattered	dense to scattered	multiple layeres
Outline	irregular	rounded to elongated, one end often spiny	irregular to rounded	rounded	rounded	rounded	rounded
Surface	spiny	smooth	knobbed	knobbed	knobbed	smooth	smooth
Structure	simple	simple	multi-layered, tower-like central bump	multi-layered	simple to thickened	multi-layered	multi-layered, often tower-like bump
Maximum size (µm)	400	700	1500	600	500	1220	3300
Ventral plate arrangement	*	*	partly overlapping	partly overlapping	*	partly overlapping	multiple layers
Outline			rounded	irregular to rounded		rounded to elongated	rounded
Surface			knobbed	spiny or knobbed		smooth	smooth
Structure			often multi-layered	simple		thickened to multi-layered	simple to multi-layered
Maximum size (µm)			800	375		700	530
Sources	Massin 1992	Ludwig & Heding 1935	Gutt 1990	Massin 1992	Gutt 1990	Ekman 1925	Ekman 1925

A great diversity exists also in the shape, size and arrangement of the calcareous deposits of the body wall of the species involved (see Tab. 5), although all of these deposits can be derived from simple multi-perforate plates. These perforated plates are very variable in size, are of an irregular, elongated or rounded outline and have a smooth, spiny or knobbed surface. In some species, these plates are strengthened, either by secondary thickening of the plate (*E. splendidus*), or by adding a three-dimensional calcareous network resulting in multi-layered plates (*E. acanthocola*, *E. charcoti*, *E. koehleri*, *E. parvipes*). Two types of plates in two layers, an outer layer of small flat to deep basket-like plates and a deeper layer of very large plates, is found in two species only (*E. charcoti*, *E. koehleri*), all others have plates of one type present in the body wall, although there may be considerable differences between plates from different parts of the body. Also the arrangement of the calcareous deposits in the body wall is variable and ranges from very scattered to a test composed of multiple layers of plates. In four species, the arrangement of the plates is size-dependent, an increasing body size is accompanied by a decrease in number or size of plates, or both.

Discussion

At first glance, it may not seem convincing, that species so diverse in their external morphology, that they even have been classified in two different families, are so closely related, to justify a unification in one genus. But in our opinion, the morphological features shared by these species are so striking, that a common origin and close relationship can hardly be negated. It is true, that all morphological similarities presented, are somehow linked to brooding, and that the one or the other character has also been realized by other brooding holothurians, but the combination of characters so far is unique.

Out of 23 holothurian species known to possess genital papillae, only seven display sexual dimorphism, where males have large papillae, while papillae in females are smaller or even are lacking (O'Loughlin 2001). Significantly, two of these species, *Microchoerus splendidus* and *Psolus charcoti*, are covered herein and a third, determined as "*Cucumaria georgiana*" group species by O'Loughlin (2001), most probably also should be transferred to *Echinopsolus*. The remaining species differ from *Echinopsolus* species in the mode of brooding—*Pseudocnus laevigatus* (Verrill, 1876) has two ventral mid-body brood pouches, *Psolidocnus sacculus* (Pawson, 1983) has three dorsal internal coelomic brood sacs, *Cucumaria pseudocurata* is an external brooder and *Gephyrothuria alcocki* Kœhler & Vaney 1905 is not known to brood at all (O'Loughlin 2001).

Brood pouches, pocket-like inversions of the body wall with an opening to the exterior, are regularly found in brood protecting cucumariid holothurians, and they vary in number and arrangement in different species (see O'Loughlin 1994). So far, five anterior interradial brood pouches are only known from species inhabiting Antarctic waters (O'Loughlin 1994; O'Loughlin *et al.* 2009; herein). Three Antarctic species, *Cucumaria lateralis*, *C. vaneyi* and *C. ferrari*, which also have anterior interradial brood pouches, differ from the remaining species in having only two or three pouches (Vaney 1906a, b, 1908; Cherbonnier 1949; McClintock *et al.* 1994) instead of five. Based on the descriptions and specimens at hand, it is very likely, that all three also belong to *Echinpsolus* and reports on deviating numbers of brood pouches are erroneous. This view is also supported by the fact, that brood pouches usually are arranged in a way, that the bilateral symmetry of the body is retained, but this is not the case in *C. lateralis* and *C. vaneyi* (see Vaney 1906a, b, 1908; Cherbonnier 1949; Tab. 1).

Comparable holothurian spermatozeugmata, composed of various bunch-like bundled spermatozoa, so far are only known from two other Cucumariidae, namely *Cucumaria lubrica* (see McEuen 1988: fig. 4D) and *Pseudrotasfer microincubator* (see Bohn 2007: fig. 2D). Both are brooders, but neither of them has five anterior interradial brood pouches. *Cucumaria lubrica* broods the young between the substrate and the ventral surface (e.g. Atwood & Chia 1974; Engstrom 1982; McEuen 1988), while *P. microincubator* is an ovarian brooder (Bohn 2007). Both are probably not closely related to the species treated here.

The spermatozoa of the seven *Echinopsolus* species investigated are of an unique type and deviate from all other holothurian sperm cells known so far. Nevertheless, there is also morphological variation within *Echinopsolus* spermatozoa, which may turn out to be species specific. Conspicuous unifying features of *Echinopsolus* spermatozoa are the fusiform elongated head and the hollow cylinder-like mid-piece containing the mitochondrial derivative, ensheathing the anterior end of the flagellum.

In contrast, sperm cells of the "echinosperm" type, the usual type of spermatozoa found in holothurians, differ from the former by their subspherical to ellipsoid head and the shallow bowl-shaped mid-piece with the mitochondrion forming a postnuclear annular band surrounding the two centriols (e.g. Chia *et al.* 1975; Jamieson 1985; Hodgson & Bernard 1992), but not a prominent cylinder around the proximal end of the flagellum. In *Semperiella drozdovi* the head has a "conical shape … not typical for holothurians" (Tyurin & Drozdov 2003, ratio of head length to diameter only 2, compare Tab. 3) similar to the heads of the *Echinopsolus* species investigated here, but the midpiece does not form a hollow-cylinder projecting proximally. Two other holothurian species, namely *Cucumaria lubrica* and *C. pseudocurata*, are also known to possess sperm cells, deviating from the "echinosperm" type. Apart form the elongated heads, there are conspicuous differences, separating the spermatozoa found in *Echinopsolus* from the latter. Sperm cells of *C. pseudocurata* are unique, they are elongated and dorso-ventrally compressed, have the acrosome located on one side of the nucleus and the majority of the mitochondrial derivative at the base of the nucleus on the opposite side (Atwood 1975). Somewhat similar in external appearance to spermatozoa of *Echinopsolus* are the cylindrical or cigar-shaped sperm cells of *C. lubrica*, which differ from all other holothurian spermatozoa in the arrangement of the mid-piece—the mitochondrial derivative surrounds the posterior quarter of the elongated nucleus (Atwood & Chia 1974).

In situ observations of spawning *C. lubrica* specimens (Engstrom 1982; McEuen 1988) indicate, that bundled spermatozoa may have an advantage for brooding species, which do not release their eggs freely into the water column nor have any kind of copulatory behaviour: to ease fertilization of eggs, extruded sperm sinks to the bottom, preferably in the immediate vicinity of a female, and remains clumped for a longer time, rather than rapidly dispersing into the water (Atwood & Chia 1974; Engstrom 1982; McEuen 1988). The elongate genital papilla of the males is thought to be of help in forming sperm strands or strings (McEuen 1988). According to Atwood & Chia (1974), elongated sperm heads may reflect a specific adaptation to facilitate the packaging of spermatozoa to spermatozeugmata.

This scenario somewhat contradicts the idea of O'Loughlin (2001), at least in the case of *E. splendidus* and species of the "*Cucumaria georgiana*" group. He supposed copulatory behaviour for species in which the male genital papilla is permanently digitiform and the female shows externally opening brood pouches harbouring juveniles all in the same stage of development.

Albeit not observed so far, as a modification of external fertilization and brooding it would seem natural that eggs, from the bottom or directly from the genital opening, are transferred into the brood pouches with the (female's) tentacles and subsequently fertilized directly by injection of spermatozeugmata via the male genital papilla. In addition there is ultrastructural indication for poor swimming endurance of single spermatozoa: the small mid-piece volume argues for limited energy reserves and the restricted area of contact between the mitochondrial derivatives and the flagellum for a limited flow of metabolites (contrary e.g. to sperm cells, that have to drill actively through the female body wall as in the acochlidian gastropods; Neusser *et al.* 2007; Jörger *et al.* 2009). The big and prominent acrosomal vesicle on the other hand should mediate a fast penetration of the egg integument.

The limited distribution of *Echinopsolus* is noteworthy—all species investigated so far are restricted to waters south of the Polar Front, thus endemic to the Southern Ocean (in a biogeographic sense, e.g. Clarke & Johnston 2003; Clarke *et al.* 2007). This is also true for all "potential" *Echinopsolus* species, which are not included in the current investigation (see Tab. 1 and sources therein). Such a limited distribution of a presumably speciose monophyletic and morphologically diverse group implies a radiation and diversification in place.

The Southern Ocean shelf fauna has evolved for a long time in isolation; surrounding deep-sea basins and the Polar Front, which constitutes an effective natural boundary (Crame 1999), make faunal exchange with adjacent faunas difficult. Thus, a high degree of endemism is thought to be characteristic for the Southern Ocean fauna, and values for different groups at species level range from about 35 % to 90 % (Clarke & Johnston 2003). This could also be demonstrated for Weddell Sea holothurians (Gutt 1991): 45 % of the holothurian species found in the Weddell Sea area are restricted to the Southern Ocean, and if typical deep-sea species are omitted, the percentage of endemism even is much higher (92 %). But concerning higher taxonomic levels like genus or family, there is no indication for a high degree of radiation in the Southern Ocean (Gutt 1991). The investigations by O'Loughlin *et al.* (2011) as well as our own investigations clearly refute this view, for there are several groups of holothurians that experienced a remarkable radiation within this area.

Based on their external morphology and their calcareous deposits, the species treated herein, up to now have been classified in a variety of different genera in the families Cucumariidae and Psolidae. None of these genera would allow the inclusion of this monophyletic Antarctic clade without getting para- or even polyphyletic, except for *Microchoerus* and *Echinopsolus*. Both were established by Gutt (1990) for new Antarctic dendrochirote holothurians which are included in the current study. Due to the fact, that *Microchoerus* Gutt, 1990 is an unavailable name—it is a junior homonym of *Microchoerus* Wood, 1844, which was established by Wood (1844) for the extinct primate *Microchoerus erinaceus* Wood, 1844—all species dealt with are assigned to the genus *Echinopsolus*.

The morphological diversity in mind, it is obvious, that the current assignment of *Echinopsolus* to the family Psolidae can not be retained. This family is characterised by a test of imbricating and often macroscopic dorsal plates, the (usual) presence of a well defined ventral sole and a dorsally turned mouth and anus (e.g. Pawson & Fell 1965; Pawson 1982). On the other hand, relationships to taxa within the Cucumariidae are very likely and therefore *Echinopsolus* is transferred to this family. Characteristic for Cucumariidae are a simple calcareous ring without posterior processes, small inconspicuous calcareous deposits and tube feet often restricted to the radii, either scattered or arranged in regular rows (e.g. Pawson & Fell 1965; Pawson 1982). *Echinopsolus* species like *E. acutus* and *E. mollis* agree very well with this diagnosis and display at least some of the characters, which may be plesiomorphic for this genus. Most probably, plesiomorphic features are cylindrical tube feet restricted to the radii, in few radial rows on the ventral side and more irregularly scattered on the dorsal side and scattered simple perforated plates in the body wall. Conical tube feet, a loss of dorsal tube feet and a restriction of tube feet to a ventral sole along with a reduction of the mid-ventral tube feet to the anterior and posterior end of the sole are probably derived characters. This is also true for calcareous deposits of the body wall which are thickened or multi-layered and test-like arranged.

So far, these ideas have not been tested in any phylogenetic analysis and thus are somewhat speculative. Recently, O'Loughlin *et al.* (2011) were able to demonstrate in their molecular study on antarctic holothurian diversity a close relationship of *Echinopsolus koehleri* and *E. charcoti,* an obvious radiation of species belonging to the "*Cucumaria georgiana*" group, and possible cryptic species closely related to *E. mollis.* These results do not contradict our conclusions about a probable radiation of the species investigated here, subsumed within the genus *Echinopsolus*. Hopefully further investigations will provide sufficient data, preferably on morphological as well as on molecular level, to allow a clarification of the phylogenetic history of this highly interesting case of an Antarctic radiation of a rather diverse and speciose holothurian group.

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