ARTHROPOD SYSTEMATICS & PHYLOGENY

75 (2): 245–260
8.9.2017

SENCKENBERG

The morphology of the male and female reproductive system in two species of spider crabs (Decapoda: Brachyura: Majoidea) and the issue of the *velum* in majoid reproduction

Katja Kienbaum *[,§,1], Gerhard Scholtz[1,2] & Carola Becker[1,2,3]

[1] Humboldt-Universität zu Berlin, Institut für Biologie, Vergleichende Zoologie, Philippstr. 13, 10115 Berlin, Germany; Katja Kienbaum * [katja.kienbaum@gmail.com]; Gerhard Scholtz [gerhard.scholtz@rz.hu-berlin.de]; Carola Becker [c.becker@qub.ac.uk] — [2] Cluster of Excellence "Image Knowledge Gestaltung", Humboldt-Universität zu Berlin, Sophienstr. 22a, 10178 Berlin, Germany — [3] Queen's University Marine Laboratory; 12–13 The Strand, Portaferry, Co. Down; BT22 1PF, UK — [§] Former name 'Katja Jaszkowiak'; * Corresponding author

Accepted 04.iv.2017.
Published online at www.senckenberg.de/arthropod-systematics on 30.viii.2017.

Editors in charge: Stefan Richter & Klaus-Dieter Klass

Abstract

The reproductive system of spider crabs (Majoidea) has raised considerable interest due to the complexity of female sperm storage organs. In several majoid species, the seminal receptacle has been described as being divided into a dorsal storage chamber and a ventral fertilization chamber separated by a muscular velum. The velum is supposed to control the amount of sperm used for fertilization and to play an important role in sperm competition. Here, we present a study on the reproductive systems of the two majoid species, *Mithraculus sculptus* (Lamarck, 1818) and *Stenorhynchus seticornis* (Herbst, 1788) using various morphological techniques such as µCT scans and 3D-reconstructions, complemented by paraffin histology. The male gonopods of the herein investigated species are similar in their general morphology and in the presence and distribution of setae. The tubular first gonopod holding the ejaculatory canal is much longer than the short and stout second gonopod, which is supposed to function as a piston in the transport of sperm into the female ducts. The female reproductive system of *M. sculptus* and *S. seticornis* conforms to that of other Eubrachyura in possessing paired ovaries, oviducts, seminal receptacles, vaginae, and vulvae. Based on our 3D-reconstructions we demonstrate that there is no division of the seminal receptacle into two chambers separated by a velum. In contrast to this, we observed a spatially restricted invagination of the seminal receptacle. A comparison of our data with those of previous studies, allows for the conclusion that the invagination of the seminal receptacle may have been misinterpreted and mistaken for a velum by other authors. Thus, the division of the seminal receptacle into two chambers separated by a velum is a character which needs to be re-evaluated.

Key words

Seminal receptacle, storage chamber, insemination chamber, gonopods, setae, 3D-reconstruction.

1. Introduction

The Majoidea or spider crabs is a very diverse brachyuran group, comprising more than 800 species, with a worldwide distribution in marine waters (De Grave et al. 2009). It has been proposed to be a basal branching lineage within the Eubrachyura based on morphological (Jamieson et al. 1995) and molecular data (Spears et al. 1992; Porter et al. 2005). However, data concerning the phylogenetic position of the Majoidea within the Eubrachyura is sparse and partially contradictory (Brösing et al. 2006; Tsang et al. 2014). Whilst their monophyly is widely accepted (Hultgren & Stachowicz 2008; Mahon & Neigel 2008; Tsang et al. 2014), relationships among

the majoid groups remain disputed. The only constant is the monophyly of the Oregoniidae and their position as sister group to the remaining Majoidea (see Hultgren et al. 2009 and Guinot et al. 2013 for review). The morphology of brachyuran reproductive systems has been discussed with respect to phylogenetic (Guinot 1977; McLay & López Greco 2011; Guinot et al. 2013; Becker & Scholtz 2017) and functional analyses (Diesel 1989; Beninger et al. 1991).

The male copulatory system is a complex arrangement of three paired parts, consisting of the first and second gonopods and the penes. The tubular first gonopod (G1) forms the ejaculatory canal with a basal and distal opening. During copulation, the second gonopod (G2) and the penis are inserted into the G1 through the basal opening. The penis extrudes the sperm into the ejaculatory canal of the G1. It is then pushed by the G2 into the female genital duct (Bauer 1986). Gonopods contain valuable phylogenetic information (Beninger et al. 1991; Beninger & Larocque 1998). This is based on their diverse and specific morphology including the patterns of setae in combination with the conservative nature of gonopods (Martin & Abele 1986; Vallina et al. 2014).

The female reproductive system of eubrachyuran crabs consists of paired ovaries, oviducts, seminal receptacles, vaginae, and vulvae. The seminal receptacles play a major role in eubrachyuran reproduction due to their ability to store sperm from one or more males that can be used to fertilize several consecutive broods (Cheung 1968).

The reproductive morphology of the Majoidea has been addressed in several studies (Diesel 1991; Beninger et al. 1993; Lanteigne et al. 1996; Sainte-Marie & Sainte-Marie 1998; Rotllant et al. 2007; González-Pisani et al. 2011; Antunes et al. 2016). In contrast to other eubrachyurans, a division of the seminal receptacle into two distinct chambers has been described in some majoids (Diesel 1989). According to this view, a muscular diaphragm, the velum, separates the seminal receptacle into a dorsal secretory "storage chamber" filled with sperm and a ventral cuticle "insemination chamber" with the oviduct junction (Diesel 1989). The putative existence of a velum has stimulated inferences on the reproduction of spider crabs in terms of the location of fertilization (Diesel 1991) and sperm competition of eubrachyurans in general (McLay & López Greco 2011). To date, it is not clear which majoid groups actually possess a velum and whether all structures referred to as a velum (Sal Moyano et al. 2010; González-Pisani et al. 2011) are really similar to its original description and definition (Diesel 1989).

In this study, we investigate the male and female reproductive morphology of the majoids *Stenorhynchus seticornis* (Herbst, 1788), belonging to the Inachoididae, and *Mithraculus sculptus* (Lamarck, 1818) of the Majidae. Our main goal is to re-evaluate characters of the female reproductive system described by Diesel (1989, 1990, 1991), in particular the division of the seminal receptacle into two chambers. By means of the latest morphological methods such as µCT-scans and 3D-reconstruction, together with established histological tools, our results reveal that seminal receptacles of both studied species are not divided into two separate chambers and shed doubt on previous interpretations of such a division and the presence of a velum (Diesel 1989; Sal Moyano et al. 2010; González-Pisani et al. 2011). The present study demonstrates the value of 3D-reconstructions to understand the spatial organization of reproductive systems and the need for further studies in order to re-evaluate the majoid reproductive systems.

2. Materials & methods

2.1. Material

The specimens of *Stenorhynchus seticornis* and *Mithraculus sculptus* were obtained from commercial vendors (www.shop-meeresaquaristik.de). Three females of each species were used for histology and the 3D-reconstruction of the reproductive organs. All investigated females were mature and two females of *M. sculptus* were ovigerous.

2.2. Histology

For the histological analyses, specimens were cold-anaesthetised in a freezer at –18°C for 15 minutes. Whole specimens were preserved either in Bouin's solution or in "Susa Heidenhain" (MORPHISTO® Evolutionsforschung und Anwendung GmbH, Frankfurt am Main, Germany) for 48–73 hours. For decalcification, specimens were treated in Ethylenediaminetetraacetic acid (EDTA) for 48–72 hours. Specimens were then dehydrated through a series of ethanol solutions and infiltrated (Shandon Hypercenter XP, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and embedded with paraffin. Sections were prepared at 6–8 µm using a rotary microtome Leica RM2255 (Leica Microsystems GmbH, Wetzlar, Germany). All histological sections were stained with the trichromatic Masson-Goldner "light green" (MORPHISTO®, Frankfurt am Main, Germany).

2.3. Scanning electron microscopy (SEM)

The dissected gonopods of two male specimens of each species were cleaned manually after an ultrasonic bath. The first and second gonopods were critical point dried (Bal-Tec CPD 030, Balzers, Liechtenstein) and sputter coated with a gold layer (Bal-Tec SCD 005, Balzers, Liechtenstein). The micrographs were taken using a LEO (Zeiss) 1430 scanning electron microscope (Carl Zeiss Nano Technology Systems GmbH, Oberkochen, Germany) and images were processed with the software Corel DRAW X6 (Corel, Ottawa).

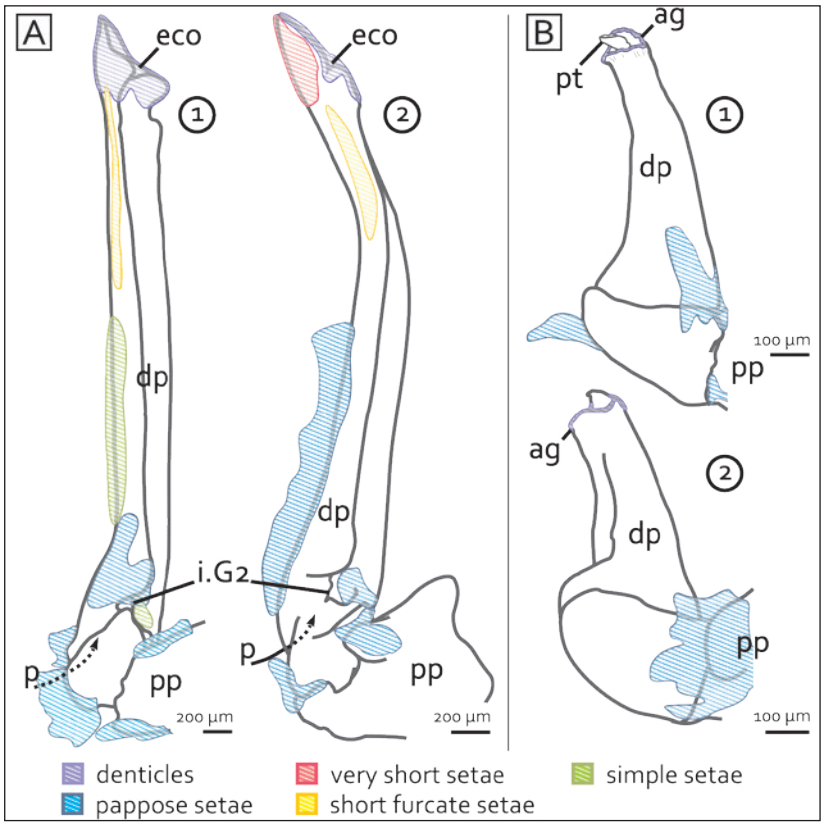

Fig. 1. Arrangement of different setae types along the gonopods. **A**: First gonopod of (**1**) *Mithraculus sculptus* and (**2**) *Stenorhynchus seticornis*. **B**: Second gonopod of (**1**) *Mithraculus sculptus* and (**2**) *Stenorhynchus seticornis*. — ***Abbreviations***: ag = apical girdle; dp = distal podomere; eco = ejaculatory canal opening; i.G2 = insertion of G2; p = penis; pp = proximal podomere; pt = protuberance.

2.4. Micro-computer-tomography (µCT)

One specimen of each species was fixed in "Susa Heidenhain" (MORPHISTO®, Frankfurt am Main, Germany) for 48–73 hours and washed repeatedly in 70% ethanol. After dissecting the pleon together with the attached gonopods, samples were dehydrated through a series of ethanol solutions. For contrast improvement, samples were immersed in a 1% iodine-ethanol solution for 24 h and subsequently critical point dried (Bal-Tec CPD 030, Balzers, Liechtenstein). The samples were X-ray scanned using a Nanotom (Phoenix | x-ray, GE Sensing and Inspection Technologies) high resolution µCT system.

2.5. Image processing and 3D-reconstruction

The reconstruction of three-dimensional (3D) models was carried out with the Amira software (FEI Visualization Sciences Group, Bordeaux). Series of histological sections were photographed using a stereo microscope Axioskop 2 equipped with a camera Axio Cam HRc and processed with the Axio Vision 4.3 software (Carl Zeiss Vision GmbH). The images were turned into grey scale and aligned. After the alignment, the 3D-reconstruction was carried out by processing image stacks of these virtual sections. The contours of each reproductive structure (differentiated gray scale values) were marked on the virtual cross section with a polygon, and the polygons then used to calculate a surface model of the reproductive system. 3D-reconstruction of the µCT scans was carried out by processing image stacks of virtual sections. These sections were then edited the same way as the aligned histological sections. All images were processed in Corel DRAW X6 and Corel PHOTOPAINT X6 (Corel, Ottawa).

3. Results

3.1. Male gonopods

3.1.1. Overall morphology

The seminal duct passes through the coxa of peraeopod 5 and emerges through the sexual opening (gonopore) as a penis. The gonopore lies adjacent to the basal part of the first gonopod (G1). The small penis enters the G1 on the opposite side of the opening for the second gonopod (G2) (Fig. 1). In both investigated species the tubular first gonopod is longer than the stout second gonopod (Fig. 1).

The G1 (Figs. 2, 3) is tripartite (Fig. 2F). The elongated distal shaft is characterized by the tubular cuticle that forms the ejaculatory canal, whose suture is visible from the outside. The distal, subterminal opening of the ejaculatory canal (eco) is directed medially (Figs. 2A,B, 3A). The proximal opening for the second gonopod (G2) is formed between overlapping cuticular folds of the proximal side of the shaft (Figs. 2C, 3B). Gonopod tegumental glands or rosette glands (rg) are situated proximally with-

Table 1. Location and arrangement of types of setae on first gonopod (G1) and second gonopod (G2) of *Stenorhynchus seticornis* and *Mithraculus sculptus*; + = present; – = absent.

Location / arrangement on gonopod			setae type	*S. seticornis*	*M. scultus*
G1	proximal + middle podomere	· along the dorsal edges	pappose (Fig. 2C–D; Fig. 3D)	+	+
	distal podomere	· surrounding the basal opening for the G1 and penis · along the basal two-thirds of the dorsolateral edge · one row on basal postero-medial edge		+	+
				+	–
				+	–
		· along the middle of the dorso- and ventro-lateral area	simple	–	+
		· along the distalmost dorso- and ventro-lateral area	simple, very short (Fig. 2B)	+	+
		· on the dorso-lateral side of the tip	bifurcate, short (Fig. 3A)	+	–
		· one row along one-third of the dorso-lateral side		+	–
		· around the ejaculatory canal opening	denticles (Fig. 3A)	+	+
G2	proximal + middle podomere	· grouped along the distal edge	pappose (Fig. 3C)	+	+
	distal podomere	· grouped along the proximal edge		–	+
		· around the distal apical girdle	denticles (Fig. 2E)	+	+

in the shaft of the G1, arranged around its basal opening (Fig. 2F1–3). Several muscle strands are present in the gonopods connecting the three podomeres (Fig. 2F). Within the G1 three muscles are observed (referred to as m1–m3). While the m1 and m2 have only one origin and projection, the m3 has two proximal origins on the ventral and dorsal side of the proximal podomere.

In *Stenorhynchus seticornis* the slightly twisted shaft is bent laterally and tapers distally (Fig. 1A2). Its distal end is somewhat bulbous with a pointed tip (Fig. 3A).

In *Mithraculus sculptus* the shaft is elongated, straight, and slightly bent in dorso-lateral direction on the first quarter of the basal part. The tip is cone-shaped and elongated on the ventro-lateral side. It is thereby forming a pointed tip with a wide opening of the ejaculatory canal. A small hook like appendix is present on the dorsomedial side below the edge of the tip (Fig. 2A,B).

The G2 of both species is tripartite as well. The distal podomere has a compact shaft with a smooth surface and only few folds along its ventro-medial side (Fig. 3C). Along the distal part of the shaft the cuticle is very compact and almost completely fills out the entire lumen (Fig. 2F4). The G2 has three muscles (referred to as m*1–m*3). One of them (m*1) is running within the distalmost segment only (Fig. 2F4).

In both species an apical girdle (sensu BENINGER et al. 1991) surrounds the tip (see dashed lines in Figs. 2E, 3C). The G2 of *S. seticornis* presents a process at the tip that is pointing dorsally. In *M. sculptus* the tip of the G2 bears a central protuberance (Fig. 2E).

3.1.2. Setation

The types of setae and their distribution along the surface of the gonopods are similar in *S. seticornis* and *M. sculptus* (see Table 1 and Fig. 1A,B).

3.2. Female reproductive system

The females of *Mithraculus sculptus* and *Stenorhynchus seticornis* have very similar reproductive systems that differ only in some small details. Thus, the results presented in the following apply to both species as long as not mentioned separately.

3.2.1. Ovary and oviduct

The ovaries are paired, elongated organs located dorsally in the cephalothorax with two ovary strands running along each body half as anterior and posterior lobes. The left and right strands connect ventral to the heart. Whereas those of *M. sculptus* are restricted to the thorax, the posterior ovarian lobes of *S. seticornis* extend into the pleon and are additionally fused posteriorly.

The tissues and cell types that line the ovaries and the oviduct are continuous (Fig. 4A–F). Each strand wherein the oocytes develop is highly convoluted and internally lined by a mono-layered epithelium (Fig. 4A–C,F). The cells that form the oviduct have a cubic shape with round basally located nuclei, whereas the cells of the ovary are more elongated and have oval nuclei. Both structures are externally coated by a thin layer of connective tissue (Fig. 4D,E–F).

Different stages of oocyte development are present within the ovaries (Fig. 4B,C). The germinative zones, where oogonia proliferate, can be distinguished from the adjacent maturation zones where the oocytes develop. However, in females with large mature ovaries, the mature oocytes are intermingled with strands of germinative zones. Within the germinative zones, batches of oogonia (10–20 µm) with a low amount of cytoplasm and relatively large nuclei, originate (Fig. 4C,F). Previtellogenic oocytes with a larger proportion of cytoplasm and an irregular cell shape (20–120 µm), are also present adjacent to the germinative zone (Fig. 4C,F). The ovaries contain no oocytes in early vitellogenesis (meaning the stage of the maturing cells, in which the inclusion of yolk has started only recently) but show mature oocytes at sizes of 140–300 µm, completely filled with yolk (Fig. 4A–C,E). In several females, oogonia and previtellogenic oocytes are also present within the oviduct close to the connection to the seminal receptacle (Fig. 4D,F).

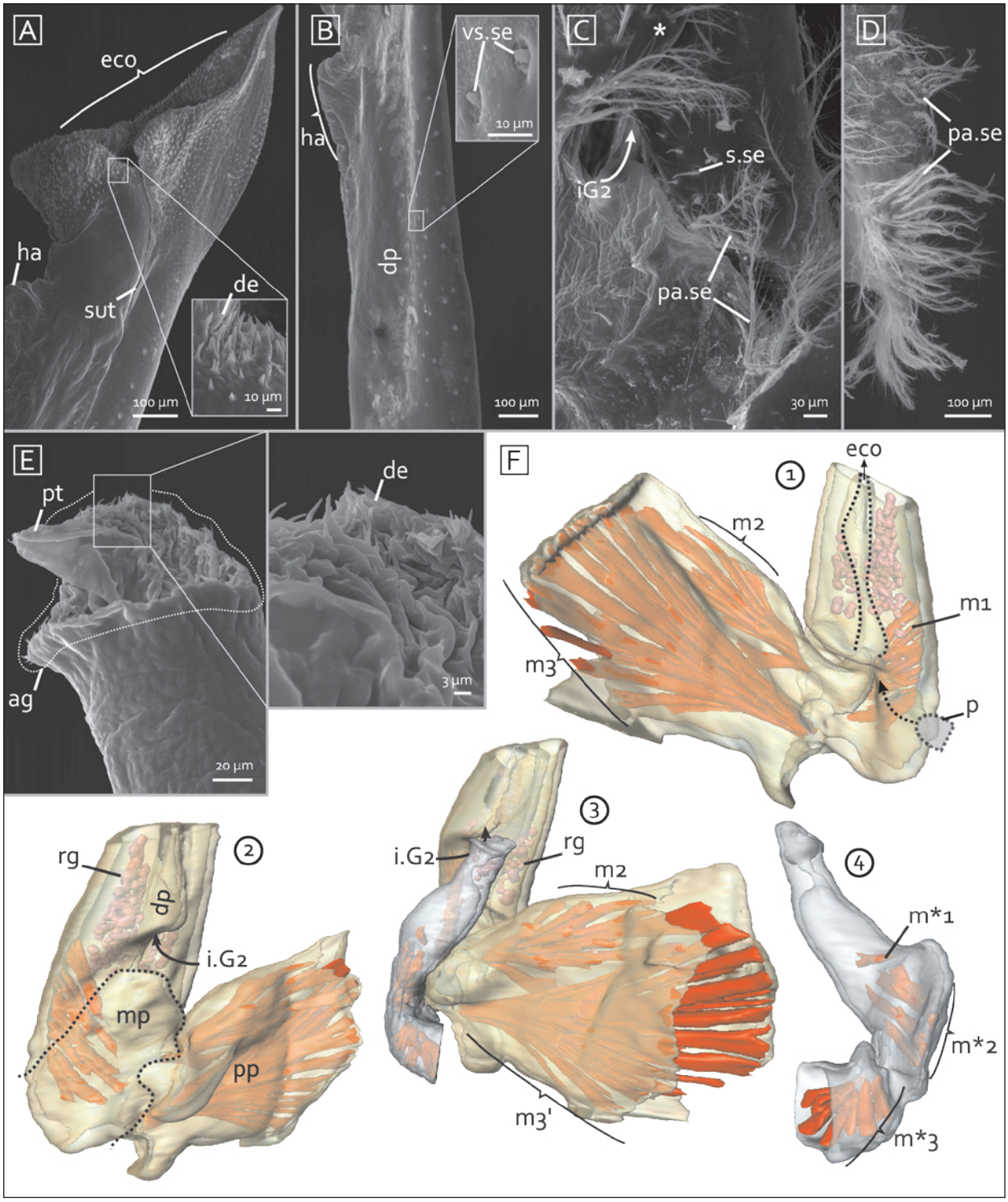

Fig. 2. First and second gonopods (G1, G2) of *Mithraculus sculptus*, SEM pictures (A–E) and 3D-reconstruction based on µCT (F). **A**: Tip of G1 with detail of denticles surrounding the ejaculatory canal opening. **B**: Part of G1 with the hook-like appendix. **C**: Proximal part of the folded cuticle (see *) forms the opening for the G2. **D**: Pappose setae along basal two thirds of dorsolateral edge. **E**: Tip of G2 with the apical girdle (including an enlargement of the denticles) and the protuberance. **F**: 3D-reconstruction of proximal part of G1 (1–3) and G2 (3, 4). Cuticle is presented semi-transparent to allow visibility of the muscle strands and rosette glands within the gonopods. (**1**) Course of ejaculatory canal indicated by black dashed lines. The position of the penis and its insertion into the G1 is indicated by a semi-transparent grey area and arrow. (**2**) All podomeres of G1 can additionally be distinguished through the attachment sites of the muscle strands. Form of middle podomere indicated by dashed line. (**3**) Position of both gonopods. G2 not inserted into G1. (**4**) Instead of all other muscle strands both attachment sides of the m*1 are situated within the distal podomere. — ***Abbreviations***: ag = apical girdle; de = denticle; dp = distal podomere; eco = ejaculatory canal opening; ha = hook-like appendix; i.G2 = insertion of G2; m1 = ventral muscle bundle within G1 running within the distal podomeres; m2, m3 = two dorsal muscle bundles within G1 that run from the distal to the proximal podomere; m2', m3' = two ventral muscle bundles within G1 that run from the distal to the proximal podomere; m*1 = muscle bundle running within distal podomere of G2; m*2, m*3 = muscle bundles within G2 running within the distal podomeres and the middle podomere to the proximal podomere, respectively; mp = middle podomere; p = penis; pa.se = pappose setae; pp = proximal podomere; pt = protuberance; rg = rosette glands; s.se = simple setae; sut = suture; vs.se = very short setae; * = cuticle folding that forms part of insertion site of G2.

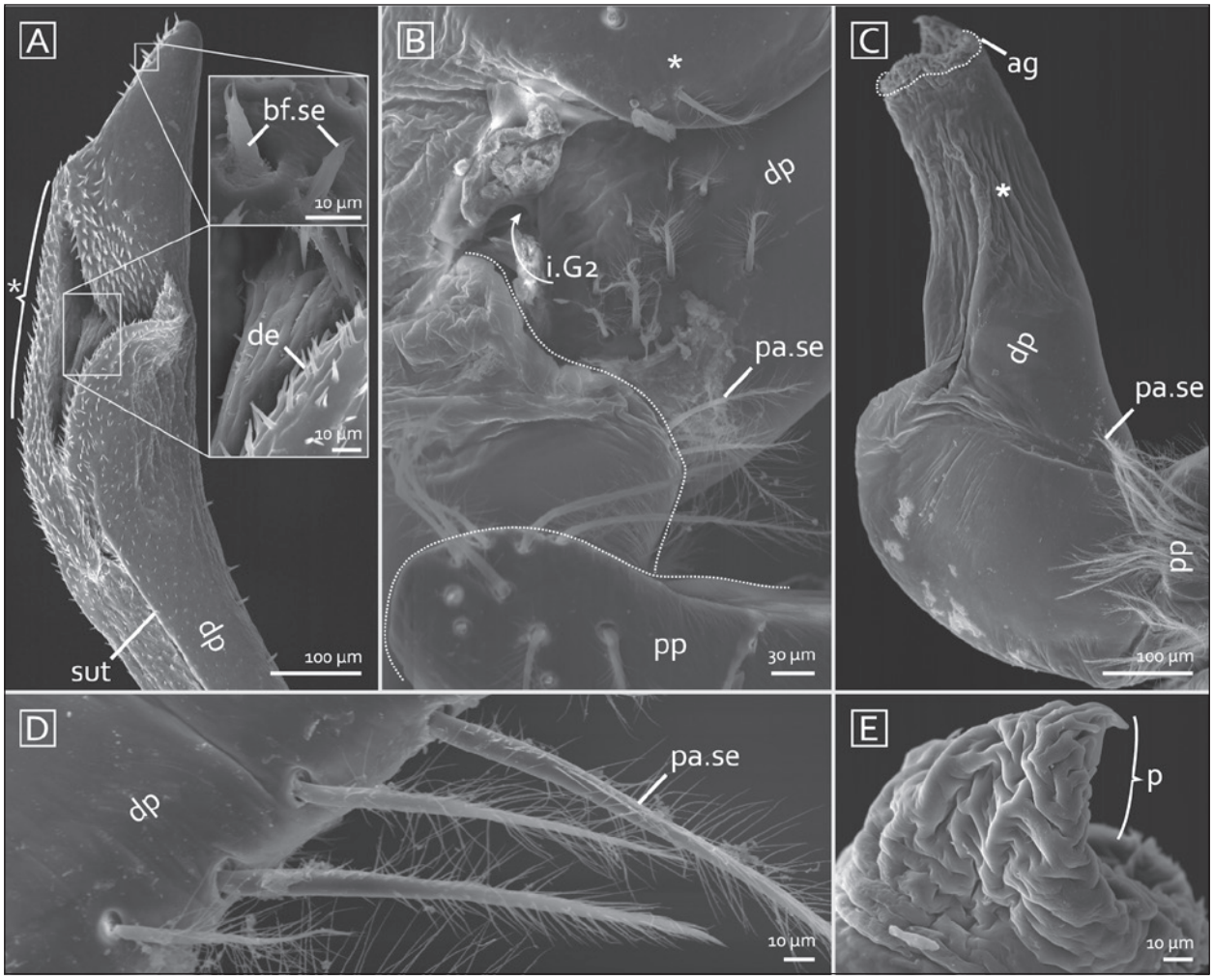

Fig. 3. First and second gonopods (G1, G2) of *Stenorhynchus seticornis*, SEM pictures. **A**: Tip of G1 with ejaculatory canal opening (see *) surrounded by denticles (lower enlargement) and bifurcate setae (upper enlargement), suture of folded cuticle clearly visible. **B**: Proximal part of folded cuticle (see *) that forms the opening for G2. Dashed lines mark middle and proximal podomeres. **C**: Overview of G2, apical girdle indicated by dashed line; * indicates area of longitudinal folds along distal podomere. **D**: Pappose setae along edge of G1 distal podomere. **E**: Process at apex of G2. — ***Abbreviations***: ag = apical girdle; am = appendix masculine; bf.se = bifurcate setae; de = denticle; dp = distal podomere; i.G2 = insertion of G2; sut = suture; pa.se = pappose setae; pp = proximal podomere.

3.2.2. Seminal receptacle

The seminal receptacle (SR) is externally coated by connective tissue. Internally, a dorsal secretory area and a ventral cuticle area can be discriminated (Figs. 5A1–4,B, 6A1,2,B–C). The tissue of the dorsal area is stratified. Due to an irregular surface, the cells that form the outer cell layer, appear loosely arranged. They are associated with the surrounding connective tissue. The adjoining proliferative cells form the middle section and an increasing degeneration of cells towards the lumen of the SR results in the release of secretions (Fig. 5D). The lumen is filled with sperm masses without any apparent layering or divisions of the sperm masses by sperm gel (Figs. 5E,G, 6D).

The oviduct runs into a thickened portion of the secretory tissue (Fig. 6C,G), close to the transition into the cuticle epithelium (Fig. 4D) and to the adjoining vagina (Fig. 5A3). Cuticle folds are present at the transition between the secretory tissue and the cuticle area of the SR (Figs. 5F, 6C,E). The cuticle that lines the SR ventrally and parts of the dorsal area is formed by a columnar epithelium.

In *M. sculptus*, the ventral cuticle-lined part of the SR forms several prominent bulges which protrude into the lumen and partly divide it into different areas (Fig. 5A3,4,B). The cuticle occurs also in the dorsal area, where it covers the secretory tissue towards the lumen (Fig. 5E).

In some sections the SR of *S. seticornis* shows a cuticle structure that transforms into a cuticle bulge that protrudes towards the lumen of the ventral area (Fig. 6D,F).

3.2.3. The vagina

The cuticle of the SR is continuous with the cuticle of the vagina. In cross sections the vagina is crescent shaped, resembling the "concave type" vagina (sensu HARTNOLL 1968) with the inner wall invaginated into the outer wall occluding the vagina lumen. Two different cuticle layers

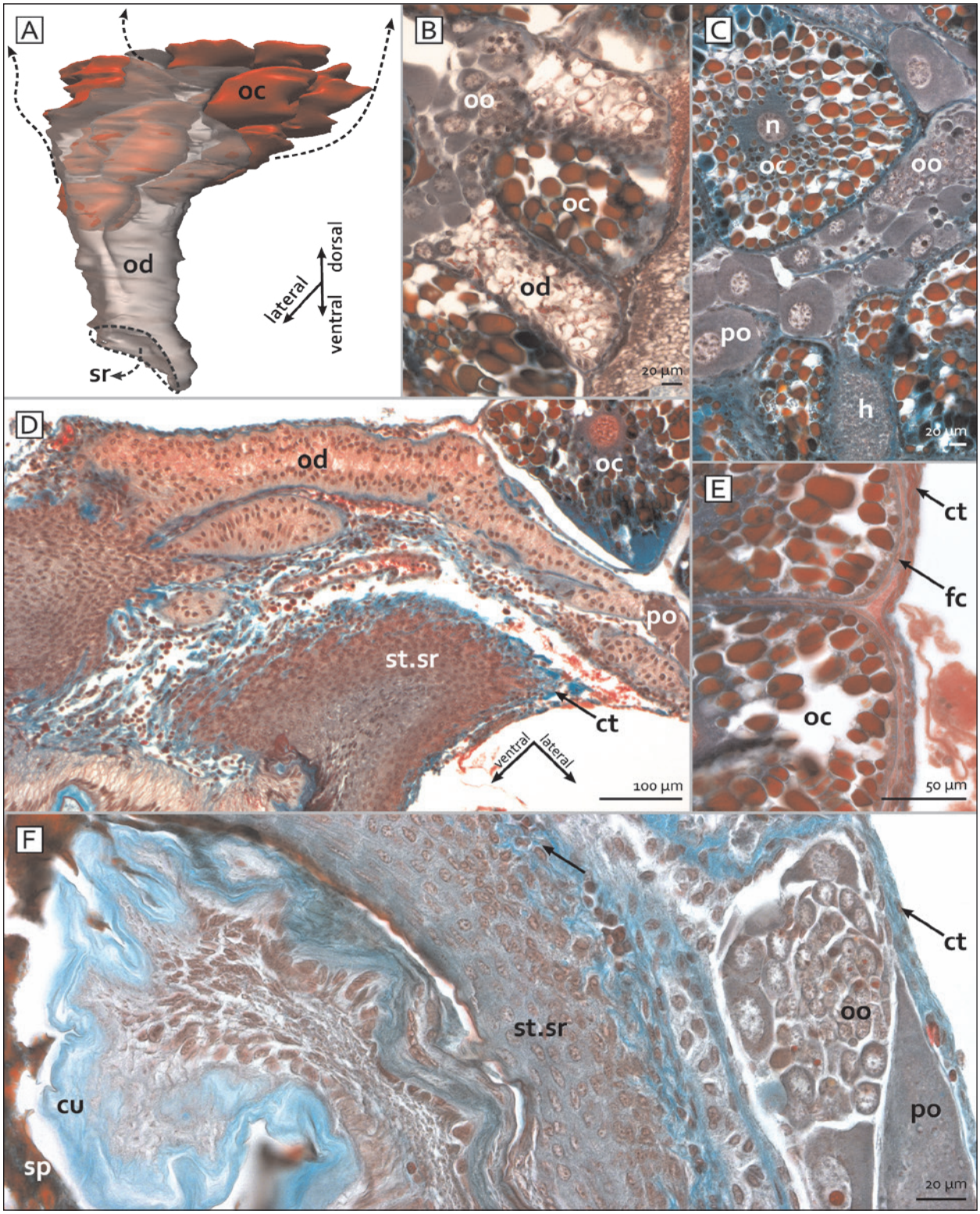

Fig. 4. Ovary and oviduct of *Stenorhynchus seticornis* (A,B,E) and *Mithraculus sculptus* (C,D,F). **A**: 3D-reconstruction of oviduct and parts of ovary based on histological sections showing mature oocytes within oviduct. Black dashed arrows = continuation of tissue; dark dashed line and arrow = orifice to seminal receptacle. **B**: Histological section through oviduct within ovary. Oogonia enclosed within oviduct, the mature oocytes arranged adjacent to it. **C**: Cellular organisation within ovary and oviduct. Germinative zones arranged in strands in between the mature oocytes. Some haemal vessels can be found. Notice the central nucleus in the mature oocytes. **D**: Histological longitudinal section of oviduct connecting ovary to seminal receptacle. **E**: Magnification of two adjacent mature oocytes within ovary. Notice the "follicle cells" that lie between the oocyte cell membrane and a very fine membrane that is present adjacent to the oocytes. **F**: From right to left: Oogonia and previtellogenic oocytes within oviduct in very close proximity to orifice connecting oviduct and seminal receptacle. The oviduct lies adjacent to the secretory tissue of the seminal receptacle. Towards the seminal receptacle lumen a cuticle bulge covers the secretory tissue. The lumen of the seminal receptacle is filled with sperm mass. Arrows = connective tissue around both structures. — ***Abbreviations***: ct = connective tissue; cu = cuticle; h = haemal vessel; st.sr = secretory tissue of the seminal receptacle; fc = follicle cell; oc = mature oocyte; n = nucleus; oo = oogonia; od = oviduct; po = previtellogenic oocyte; sp = sperm mass; sr = seminal receptacle.

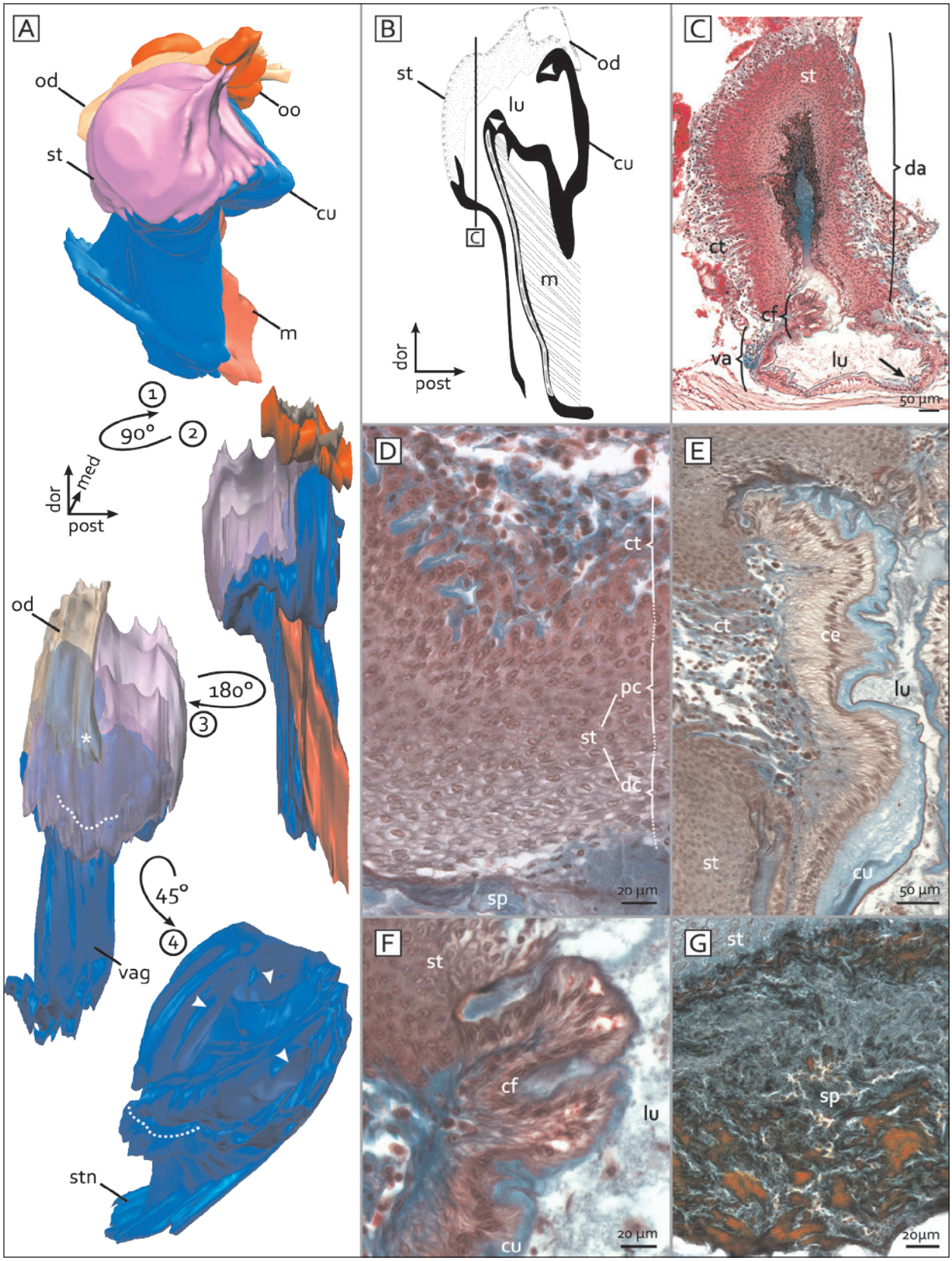

Fig. 5. Seminal receptacle and associated structures of *Mithraculus sculptus*. **A**: Different perspectives of a 3D-reconstruction of seminal receptacle and parts of oviduct based on histological sections. (**1**) all parts in shaded outlines; (**2**) secretory tissue transparent; (**3**) secretory tissue and oviduct transparent; (**4**) only cuticular parts of reproductive system visible, view from dorso-medial and cuticle slightly tilted in anterior direction. Cuticle of ventral area shows prominent bulges that protrude into the lumen (see arrowheads part 4) and divides it into different compartments. In some areas these bulges cover the secretory tissue towards the lumen (transparent in part 3). The oviduct connection to the seminal receptacle is medio-ventrally (see * in part 3) in very close proximity to the vagina opening (dashed lines in part 3 + 4). **B**: Idealized schematic drawing of seminal receptacle. Notice that the oviduct lies adjacent to the secretory tissue. Flexible parts of inner vagina wall indicated by grey area within the cuticle (arrowheads indicate cuticle bulges). **C**: Histological cross section through

can be distinguished (see Locke 2001; Fig. 7C). The epicuticle faces the vagina lumen and stains red in Masson's trichrome while the procuticle stains blue. The flexible parts of the vagina wall are equipped with musculature and the cuticle herein appears structurally different from the remaining procuticle and stain red in Massons trichrome. In both species investigated, the muscle attachment correlates with the flexible parts of the vagina.

In *M. sculptus* only the inner vagina wall is flexible and connected to the sternum by muscles running diagonally to ventro-lateral (Fig. 7A,B,D,E). In *S. seticornis*, also the outer vagina wall appears flexible towards the SR – indicated by red-staining horizontal bands of the procuticle and a muscle attachment (Fig. 7E). Those muscles run diagonally ventro-medial to the sternum. In one specimen a sperm plug was found within the vagina lumen (Fig. 7F).

4. Discussion

4.1. The male gonopods

4.1.1. Overall shape and functions in sperm transfer

The distal segment of the first gonopod (G1) of brachyuran males is folded longitudinally, forming the ejaculatory canal with a basal and a distal opening (Rorandelli et al. 2008; Sal Moyano et al. 2011; Vallina et al. 2014). During copulation, the second gonopod (G2) and the penis are both inserted into the G1 through a basal opening. Together, they form a complex copulatory system to transport the sperm masses into the female seminal receptacles through the vagina during copulation. The relative length of the G1 and G2 is variable among the Brachyura (see McLay & Becker 2015). A short G2 is characteristic for the Majoidea (Beninger et al. 1991; Diesel 1991; Neumann 1996; Rorandelli et al. 2008; Sal Moyano et al. 2011; this investigation) and also present in other eubrachyuran groups (e.g. Ocypodidae: Lautenschlager et al. 2010; Pinnotheridae: Becker et al. 2012). The characters present in the investigated G2 of *Mithraculus sculptus* and *Stenorhynchus seticornis* resemble those of *Chionoecetes opilio* (O. Fabricius, 1788) (Beninger et al. 1991). Even though observations of copulations are rare and data concerning the actual movement of gonopods are lacking, certain hypotheses about the transport of sperm have been developed (Beninger et al. 1991; Becker et al. 2012). In a system with a short G2, the G1 is the actual sperm conduit that interacts with the vagina while the G2 is supposed to have an accessory function by moving the sperm distally within the ejaculatory canal (Beninger et al. 1991). The narrow ejaculatory canal allows only minor movements of the G2 within the G1. With respect to the compact cuticle at the distal part of the G2, it seems unlikely that the G2 can be stretched significantly but it may however act like a seal, as has also been suggested by Beninger et al. (1991). The cuticle folds on its surface (Fig. 3C) might allow the seal to be broken by muscular contractions.

4.1.2. Setation and dentation

The arrangement of the various setal types along the G1 and G2 of the species investigated is widely consistent with data from other majoid studies (see Table 1). The presence of pappose setae (Figs. 2C,D, 3B–D) at the proximal parts of the gonopods has also been described for *C. opilio* (Beninger et al. 1991) and for *Libinia spinosa* Guérin, 1832 (Sal Moyano et al. 2011). Furthermore, the denticles located on the gonopod tips are present in many other majoid males (Beninger et al. 1991; Diesel 1991; Neumann 1996; Sal Moyano et al. 2011) and have been regarded as homologous structures (Beninger et al. 1991). The denticles surround the distal opening of the ejaculatory canal of the G1 (Fig. 2A,E) and are supposed to rupture spermatophores during sperm transfer (Beninger et al. 1991; Neumann 1996; Rorandelli et al. 2008). The observation of intact spermatophores in *S. seticornis* by Antunes et al. (2016) and *C. opilio* by Sainte-Marie et al. (2000) however contradicts this assumption.

4.2. The female reproductive system

The morphology of the reproductive system in the investigated species follows the general pattern of heterotreme eubrachyurans including other Majoidea. The oviducts connect the paired ovaries to the likewise paired seminal receptacles, which in turn open to the vulvae through the vaginae. Furthermore, the ovaries, the oviducts, and the seminal receptacles are enclosed by connective tissue (Figs. 4D,F,G, 5D,E).

Whereas some studies focused only on some aspects of the majoid reproductive system such as the development of the ovaries (Hinsch & Cone 1969; Rotllant et al. 2007), the whole reproductive system has been comprehensively described in the species *Chionoecetes opilio* (Beninger et al. 1988; Beninger et al. 1993; Lan-

seminal receptacle. A dorsal secretory area can be distinguished from a ventral cuticle area. Prominent folds at the transition between the two parts. Arrow = thin cuticle. **D**: Stratified tissue of secretory area of the seminal receptacle. **E**: Cuticle lining of seminal receptacle in dorsal area with stretched columnar epithelium. The cuticle bulges partly cover the secretory tissue that lines the dorsal area. **F**: Prominent cuticle folds at transition between secretory and cuticle area. **G**: The sperm mass within the seminal receptacle is not arranged in layers. — ***Abbreviations***: cb = cuticle bulge; ce = columnar epithelium; ct = connective tissue; cu = cuticle; da = dorsal area; dc = disintegrating cells; dor = dorsal; cf = cuticle folds; st = secretory tissue; lu = lumen; m = muscle; med = medial; oc = oocyte; od = oviduct; pc = proliferating cells; post = posterior; sp = sperm mass; stn = sternum; va = ventral area; vag = vagina.

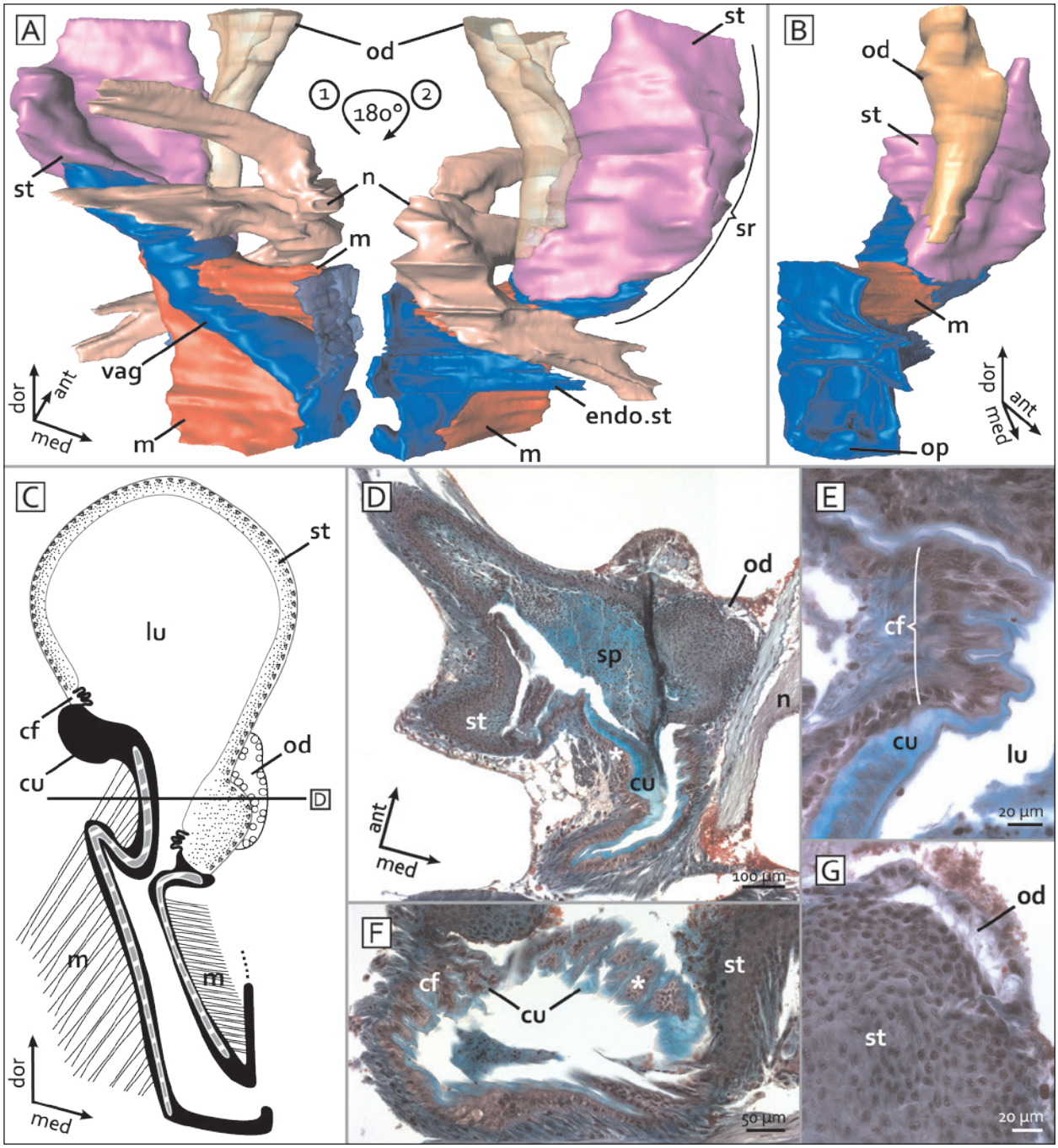

Fig. 6. Seminal receptacle and associated structures of *Stenorhynchus seticornis*. **A**: Different perspectives of a 3D-reconstruction of the seminal receptacle and parts of the oviduct based on histological sections. Oviduct and sternum shown transparent. Additionally three nerves coming from ventral nerve cord run along the seminal receptacle in very close proximity. **B**: 3D-reconstruction of seminal receptacle and parts of oviduct based on histological sections. **C**: Idealized schematic drawing of the seminal receptacle in longitudinal section. Secretory tissue forms the dorsal area and is followed ventrally by cuticle. Flexible parts of vagina indicated by grey dashed line within the cuticle. Muscles attached to both sides of vagina. The line indicates approximate position of cross section presented in Fig. 6D. **D**: Cross section through seminal receptacle. The cuticle bulge (see *) transforms into a cuticle structure that can be misinterpreted as a velum (see * in Fig. 6F). At opening of oviduct into seminal receptacle the secretory tissue clusters into a much thicker tissue than anywhere else. **E**: Histological section of folds appearing on transition between secretory and cuticle areas. **F**: Cuticle structure that could be misinterpreted as a velum (see *). **G**: Transition of oviduct into secretory tissue of seminal receptacle. — ***Abbreviations***: ant = anterior; cf = cuticle folds; cu = cuticle; dor = dorsal; endo.st = endo sternit; st = secretory tissue; lu = lumen; med = medial; m = muscle; n = nerve; op = operculum; od = oviduct; sp = sperm mass; vag = vagina; * = cuticle strap that dents into the lumen (compare Fig. 6F and D).

TEIGNE et al. 1996; SAINTE-MARIE & SAINTE-MARIE 1998; SAINTE-MARIE et al. 2000; BENHALIMA & MORIYASU 2001), *Hyas coarctatus* Leach, 1816 (HARTNOLL 1968; LANTEIGNE et al. 1996), *Hyas araneus* (Linnaeus, 1758) (HARTNOLL 1968), *Inachus phalangium* (Fabricius, 1775) (DIESEL 1989, 1991), *Stenorhynchus seticornis* (ANTUNES et al. 2016); *Maja brachydactyla* Balss, 1922 (ROTLLANT et al. 2007), *Libinia spinosa* (SAL MOYANO et al. 2010; GON-

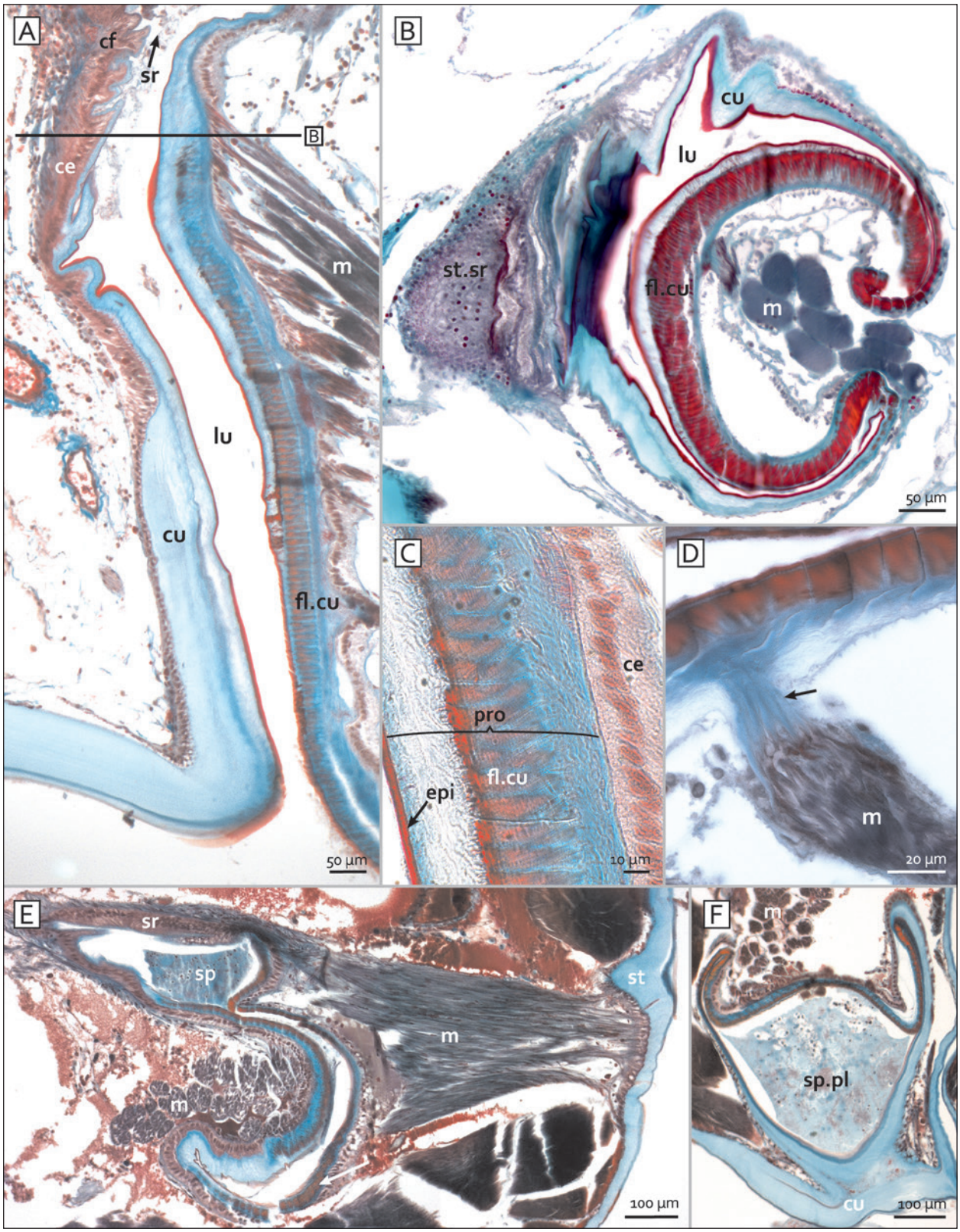

Fig. 7. Histological sections of vagina and associated structures of *Mithraculus sculptus* (A–D) and *Stenorhynchus seticornis* (E–F). **A**: Longitudinal section of vagina with attached muscles. The cuticle epithelium forms prominent folds at the transition to the seminal receptacle. The line indicates approximate position of cross section shown in B. **B**: Cross section of crescent shaped vagina at transition to seminal receptacle. The flexible inner wall is invaginated into the outer wall. **C**: Detail of the flexible vagina cuticle. Columnar epithelium lined by cuticle. Two cuticle areas can be distinguished: procuticle and epicuticle (the latter facing the lumen). **D**: Muscle attachment to the flexible cuticle of inner vagina wall. Arrow = fibrous tissue that connects the muscle to the cuticle. **E**: Cross section of crescent shaped vagina of S. *seticornis*. Muscles attached on both sides of vagina walls. Seminal receptacle filled with sperm mass. **F**: Cross section through vagina, with the sperm plug clearly visible in lumen. — ***Abbreviations***: ce = columnar epithelium; cu = cuticle; epi = epicuticle; pro = procuticle; cf = cuticle folds; fl.cu = flexible part of cuticle; st.sr = secretory tissue of the seminal receptacle; m = muscle; sp.pl = sperm plug; sp = sperm mass; sr = seminal receptacle; st = sternum; lu = lumen (of vagina).

ZÁLEZ-PISANI et al. 2011; SAL MOYANO et al. 2011), *Leurocyclus tuberculosus* (H. Milne Edwards & Lucas, 1842) (GONZÁLEZ-PISANI et al. 2011). This broad knowledge offers the possibility to identify shared characters of the majoid reproductive system (for a summary see Table 2).

4.2.1. The shape of the ovaries

The ovarian lobes of *Mithraculus sculptus* correspond to the organization of other Brachyura (MCLAY & BECKER 2015) and are consistent with the H-shape pattern, with the ovaries restricted to the cephalothorax (see KROL et al. 1992). The posterior fusion of the ovaries in *S. seticornis* (referred to as O-shape herein) is linked with an extension into the pleon. Interestingly, a similar extension of the ovaries has been described for species of three other majoid species (ROTLLANT et al. 2007; GONZÁLEZ-PISANI et al. 2011). Additionally, an extension of ovarian lobes into the pleon has previously been described for thoracotremes of the groups Grapsoidea (DE SOUZA & SILVA 2009), Pinnotheridae (BECKER et al. 2011) and Cryptochiridae (VEHOF et al. 2016).

Although the macroscopic organization is quite similar, the cell arrangement of the developing oocytes in the ovaries differs from any known description. So far, the germinative zones of heterotreme ovaries were always described as situated centrally with oocytes wandering to the periphery during their maturation progress (HINSCH & CONE 1969; JOHNSON 1980; ROTLLANT et al. 2007). In all females investigated in the present study, this usual arrangement is expanded in a more complex manner with germinative zones and adjacent previtellogenic oocytes stretching through areas of mature oocytes (Fig. 4A–C). The very small ovaries of a freshly spawned female resemble the usual arrangement to some extent but this seems to be due to the stage of the reproductive cycle. This implies that changes, not only in general size, but also in the histology within the ovaries, depend on the female reproductive cycle. The absence of vitellogenic oocytes within the ovaries may indicate a seasonal reproduction or a rapid vitellogenesis.

4.2.2. The oviduct origin or where do the "follicle cells" fit in?

At first sight, "accessory-" or "follicle cells" seem to be distributed irregularly in between the developing oocytes (Fig. 4E). In some studies they have been interpreted as the cells that surround the developing oocytes (HINSCH & CONE 1969) and form the chorionic membrane (JOHNSON 1980; DE SOUZA & SILVA 2009). Due to their distribution and arrangement within the ovary and oviduct it might be possible that in fact they are not randomly distributed, but the continuous epithelial cells of the convoluting oviduct and ovary strands (Fig. 4F).

Given that germinative zones and premature oocytes are also found within the oviduct in very close proximity to the seminal receptacle, the oviduct can be regarded as

Table 2. A comparison of the female reproductive system of investigated Majoidea. — ***Abbreviations***: cgz = central germinative zone; H = H or X shape; O = posteriorly fused ovary lobes; sph = spermatophore; st = secretory tissue; cu = cuticle; + = yes; – = no; * = with muscle within the folds; ° = plus an additional muscle; *italics* = interpretation / disputable; **bold** = consistently in all investigated species; ? = no information available.

Group	Species	References	ovary		oviduct	seminal receptacle			vagina		
			morphology/ dimension	cgz	orifice close to vagina	structure at transition st – cu	sperm distribution	sperm condition	general / oblique muscle	sperm plug	vulva closure
Oregoniidae	*Chionoecetes opilio*	BENINGER et al. 1988, 1993; LANTEIGNE et al. 1996; SAINTE-MARIE & SAINTE-MARIE 1998	H / carapace	+	+	**folds***	dorsal / ventral	sph	**concave/ +**	–	**inner vagina wall**
	Hyas araneus	HARTNOLL 1968	H / carapace	?	+	**folds**	?	?	**concave/ +**	+	**inner vagina wall**
	Hyas coarctatus	HARTNOLL 1968; LANTEIGNE et al. 1996	H / carapace	+	+	**folds***	?	?	**concave/ +**	?	**inner vagina wall**
Inachidae	*Inachus phalangium*	DIESEL 1989, 1991	H / carapace	?	+ / *ventral chamber*	***velum***	dorsal	packages	**concave/ +**	+	**inner vagina wall**
Inachoididae	*Stenorhynchus seticornis*	this study; ANTUNES et al. 2016	O / pleon	–	+	**folds**	dorsal / ventral	free mass / sph	**concave/ +°**	+	**inner vagina wall**
	Leurocyclus tuberculosus	GONZÁLEZ-PISANI et al. 2011	O / pleon	?	*? intermediate*	**folds**	dorsal / ventral	free mass	**concave/ +**	–	**inner vagina wall**
Majidae	*Maja brachydactyla*	ROTLLANT et al. 2007	O / pleon	+	+	–	dorsal / ventral	free mass	**concave/ +**	?	?
	Mithraculus sculptus	this study	H / carapace	–	+	**folds**	dorsal / ventral	free mass	**concave/ +**	–	**inner vagina wall**
Epialtidae	*Libinia spinosa*	SAL MOYANO et al. 2010, 2011; GONZÁLEZ-PISANI et al. 2011	O / pleon	?	+ / intermediate	***velum* / folds**	dorsal / ventral	packages	**concave/ +**	–	**inner vagina wall**

part of the ovary that forms the connection to the seminal receptacle (Fig. 4D,F). The structural similarity of oviduct and ovary and the view that they should not be treated as separate structures have been previously discussed by several authors (Hard 1942; Spalding 1942; Hartnoll 1968; Becker et al. 2011).

The oviduct does not form an open tube where it connects to the SR of *M. sculptus* and *S. seticornis*. Therefore, it seems likely that the tissues of the seminal receptacle and oviduct undergo cyclic changes and only form a tube when the female ovulates. This temporary orifice has been reported for the majoid *C. opilio* (Sainte-Marie & Sainte-Marie 1998) and the grapsoid *Eriocheir sinensis* H. Milne Edwards, 1853 (Lee & Yamazaki 1990).

4.2.3. The seminal receptacle and the issue of the velum

In both investigated species the dorsal area of the SR is formed by a secretory tissue whose cells release secretions and degenerate towards the lumen (Fig. 5D). Secretory tissues have been described in numerous eubrachyuran species showing different dimensions within the SR (Johnson 1980; Zara et al. 2014; Ewers-Saucedo et al. 2015; Hayer et al. 2015; de Souza et al. 2017). Interestingly, the arrangement of the secretory tissue cells at the proximity to the oviduct connection of the females of *M. sculptus* and *S. seticornis* follows a similar pattern as that described as the "holocrine transfer tissue" in Pinnotheridae described by Becker et al. (2011) (see also Antunes et al. 2016) (Fig. 6C,G). If this pattern is homologous, this would undoubtedly serve as a useful character but it needs further investigations into this subject to verify this.

Diesel (1989, 1991) described a division of the SR into two discrete chambers in females of a number of majoid species. The dorsal, secretory "storage chamber" and the ventral, cuticular "insemination chamber" were interpreted as a key aspect of majoid reproduction and discussed in terms of sperm competition (Diesel 1989, 1991). According to this view, the velum that separates both chambers could allow the female to control the amount of sperm stored in the dorsal "storage chamber" and of that released into the ventral "insemination chamber" during ovulation. The concept of the velum has been adopted by some authors for other majoids and a number of eubrachyuran species (e.g., *L. spinosa*: Sal Moyano et al. 2010; González-Pisani et al. 2011; *Ucides cordatus* (Linnaeus, 1763): Sant'Anna et al. 2007). In the present study however, none of the females possesses a velum, which challenges previous observations. In some sections of *S. seticornis* a structure similar to a velum appears but the 3D-reconstruction reveals it to be an invagination of a cuticle-lined area of the SR wall (see * in Fig. 6D,F), which does not separate it into two chambers. In *M. sculptus* a structure resembling the velum in *L. spinosa* (González-Pisani et al. 2011) is present, but is just a prominent cuticle bulge that protrudes into the ventral area-lumen of the SR and stretches towards the opposite wall (Fig. 5A4).

Thus, it might be necessary to differentiate between a velum in the sense of Diesel (1989) and cuticle invaginations that incompletely divide the ventral area of the SR.

Instead of a velum, in most of the investigated majoid species some cuticle folds are present at the transition between the dorsal and ventral area of the SR (see Table 2 for a summary of the hitherto investigated majoid species). These folds can be structurally different. In *C. opilio* and *H. coarctatus* musculature inserts into the cuticle folds (Beninger et al. 1993; Lanteigne et al. 1996), whereas in all other species muscles are absent. Antunes et al. (2016) detected musculature within the folds in *S. seticornis*. However, this finding has not been confirmed in our study. The presence of cuticle folds seems to be a widely distributed eubrachyuran character (Becker et al. 2011; González-Pisani et al. 2011; de Souza et al. 2013). Nevertheless, with the sperm mass being present in the entire lumen of the SR, the cuticle folds seem not to limit its dispersion. Thus, a division in a sperm "storage- and insemination chamber" as described by Diesel (1989) is unlikely. Due to the lack of a velum or other structures such as a bursa (Vehof et al. 2017), an active participation of the female during copulation regarding the amount of sperm used for fertilization and control over specific male sperm seems improbable.

Antunes et al. (2016) observed spermatophores and free spermatozoa in the ventral region of the SR of *S. seticornis*. In contrast to this, we observed only free spermatozoa in *M. sculptus* and *S. seticornis*, which might be due to differences in the elapsed time since mating. The absence of spermatophores and sperm layering in the seminal receptacle of both species could also be due to this. Similar conclusions have been drawn for hymenosomatids as sperm masses from multiple copulations slowly mix after some time (van den Brink & McLay 2009; Klaus et al. 2014).

Concerning the oviduct orifice of eubrachyurans, Diesel (1991) differentiated between a SR of a *dorsal-type* and a *ventral-type*, with the oviduct orifice being located opposite to or adjoining the vagina, respectively. This differentiation has been widely accepted and further hypotheses concerning sperm competition were built upon it (Diesel 1991; McLay & López Greco 2011). In the herein studied species, the oviduct connection with the secretory tissue of the seminal receptacle is situated somewhat intermediate. Nevertheless, it is situated close to the cuticle area and the vagina and therefore of the ventral type (Figs. 5, 6). With regard to sperm competition, this would indicate a last male precedence. Yet, since no layering is obvious, it remains unclear if the stored sperm belongs to more than one male.

4.2.4. The vagina

The continuity of the cuticle in the SR, the vagina, and the integument suggests an ectodermal origin of all

these structures. The structures of the vagina have been elaborated in detail by HARTNOLL (1968). In his study on brachyuran female genital ducts he recognized four types of vaginae, namely (1) simple, (2) concave and concave with operculum (3) mobile, and (4) immobile.

All hitherto investigated majoid species have vaginae of the concave type and the vulva is enclosed by the deflated inner wall and opens only by contraction of the attached muscle. HARTNOLL (1968) refers to this type of closure of the genital ducts as operculum.

5. Conclusions

Several characters are shared by the species investigated in the present study (see also Tables 1 and 2):

Male gonopods. (1) The G1 is long, slender, tapers distally and forms a bulbous tip. **(2)** The opening of the ejaculatory canal is subterminal and **(3)** surrounded by denticles. **(4)** The gonopod tegumental glands (= rosette glands) are present in the proximal part of the G1 where the G2 is inserted. **(5)** The G2 is short and stout and **(6)** has longitudinal folds on its distal surface and **(7)** an apical girdle is present around its distal tip. **(8)** The penis emerges from the gonopore of the fifth coxa and enters the G1 opposite the G2.

Female reproductive system. Within the SR **(1)** a (mostly) dorsal secretory area can be distinguished from **(2)** a (mostly) ventral area lined by cuticle. **(3)** Both areas are separated by cuticle folds with or without muscle attachment. **(4)** The vagina is always of the concave pattern (sensu HARTNOLL 1968) and **(5)** the vulva is enclosed by the inner flexible wall of the deflated vaginal tube.

In contrast to earlier descriptions of majoid reproductive systems, the species investigated in the present study lack a division of the SR into a dorsal sperm "storage chamber" and a ventral "insemination chamber" separated by a muscular velum. Instead, we observed invaginations of the cuticle receptacle wall in histological sections and 3D-reconstructions which represent no anatomical or functional division of the SR. In histological sections however, those invaginations resemble the data published by DIESEL (1989, 1991) and could by mistake be interpreted as a velum. At the present stage, it remains unclear whether a divided seminal receptacle is a character which is only present in part of the Majoidea or whether histological observations of earlier studies have been misinterpreted. Our findings clearly show the benefit of 3D-reconstruction to understand the spatial organisation of reproductive structures and suggest a reconsideration of the velum as a majoid character.

6. Acknowledgements

We wish to thank the members of the group Vergleichende Zoologie at the Humboldt-Universität zu Berlin, especially Juliane Vehof for helpful conversations throughout the project and Katrin Braun for teaching the use of the Amira software to us. We thank Jutta Zeller and Kristin Mahlow (Museum für Naturkunde, Berlin), Gabriele Drescher and PD Dr. Thomas Stach (Molekulare Parasitologie) for valuable technical assistance. The helpful comments by Colin McLay and an anonymous reviewer are thankfully appreciated. ***Funding***: Katja Kienbaum (former name: K. Jaszkowiak) was funded by a doctoral fellowship of the Heinrich-Böll-Stiftung. The project was funded by the cluster of excellence "Image Knowledge Gestaltung" – an interdisciplinary laboratory, base project "Attention and Form" at Humboldt-Universität zu Berlin.

7. References

ANTUNES M., ZARA F.J., LÓPEZ-GRECO L.S., NEGREIROS-FRANSOZO M.L. 2016. Morphological analysis of the female reproductive system of *Stenorhynchus seticornis* (Brachyura: Inachoididae) and comparisons with other Majoidea. – Invertebrate Biology **135**: 75–86.

BAUER R.T. 1986. Phylogenetic trends in sperm transfer and storage complexity in decapod crustaceans. – Journal of Crustacean Biology **6**: 313–325.

BECKER C., BRANDIS D., STORCH V. 2011. Morphology of the female reproductive system of European pea crabs (Crustacea, Decapoda, Brachyura, Pinnotheridae). – Journal of Morphology **272**: 12–26.

BECKER C., TÜRKAY M., BRANDIS D. 2012. The male copulatory system of European pea crabs (Crustacea, Brachyura, Pinnotheridae). – Journal of Morphology **273**: 1306–1318.

BECKER C., SCHOLTZ G. 2017. Phylogenetic implications of sperm storage in Podotremata: Histology and 3D-reconstructions of spermathecae and gonopores in female carrier crabs (Decapoda: Brachyura: Homoloidea). – Journal of Morphology **278**: 89–105.

BENHALIMA K., MORIYASU M. 2001. Prevalence of bacteria in the spermathecae of female snow crab, *Chionoecetes opilio* (Brachyura: Majidae). – Hydrobiologia **449**: 261–266.

BENINGER P.G., ELNER R.W., FOYLE T.P., ODENSE P.H. 1988. Functional anatomy of the male reproductive system and the female spermatheca in the snow crab *Chionoecetes opilio* (O. Fabricius) (Decapoda: Majidae) and a hypothesis for fertilization. – Journal of Crustacean Biology **8**: 322–332.

BENINGER P.G., ELNER R.W., POUSSART Y. 1991. Gonopods of the majid crab *Chionoecetes opilio* (O. Fabricius). – Journal of Crustacean Biology **11**: 217–228.

BENINGER P.G., LANTEIGNE C., ELNER R.W. 1993. Reproductive processes revealed by spermatophore dehiscence experiments and by histology, ultrastructure, and histochemistry of the female reproductive system in the snow crab *Chionoecetes opilio* (O. Fabricius). – Journal of Crustacean Biology **13**: 1–16.

BENINGER P.G., LAROCQUE R. 1998. Gonopod tegumental glands: a new accessory sex gland in the Brachyura. – Marine Biology **132**: 435–444.

BRÖSING A., RICHTER S., SCHOLTZ G. 2006. Phylogenetic analysis of the Brachyura (Crustacea, Decapoda) based on characters of the foregut with establishment of a new taxon. – Journal of Zoological Systematics and Evolutionary Research **45**: 20–32.

CHEUNG T.S. 1968. Trans-molt retention of sperm in the female stone crab, *Menippe mercenaria* (Say). – Crustaceana **15**: 117–120.

De Grave S., Pentcheff D., Ahyong S.T. 2009. A classification of living and fossil genera of decapod crustaceans. – Raffles Bulletin of Zoology **21**: 1–109.

de Souza L.P., Silva J.R.F. 2009. Morphology of the female reproductive system of the red-clawed mangrove tree crab (*Goniopsis cruentata* Latreille, 1803). – Scientia Marina **73**: 527–539.

de Souza L.P., Silva J.R.F., Araujo A.M., Camargo-Mathias M.I. 2013. Morphology of the female genital ducts of the blue land crab *Cardisoma guanhumi* (Crustacea: Brachyura: Gecarcinidae). – Acta Zoologica **94**: 300–307.

de Souza L.P., Ogawa C.Y., Silva J.R.F., Camargo-Mathias M.I. 2017. Comparative morphology of the female genital ducts of seven eubrachyuran crabs (Saint Laurent, 1980). – Acta Zoologica **98**: 125–135.

Diesel R. 1989. Structure and function of the reproductive system of the symbiotic spider crab *Inachus phalangium* (Decapoda: Majidae): observations on sperm transfer, sperm storage, and spawning. – Journal of Crustacean Biology **9**: 266–277.

Diesel R. 1991. Sperm competition and the evolution of mating behavior in Brachyura, with special reference to spider crabs (Decapoda, Majidae). Pp. 145–163 in: Bauer R.T., Martin J.W. (eds), Crustacean Sexual Biology. – Columbia University Press, New York.

Ewers-Saucedo C., Hayer S., Brandis D. 2015. Functional morphology of the copulatory system of box crabs with long second gonopods (Calappidae, Eubrachyura, Decapoda, Crustacea). – Journal of Morphology **276**: 77–89.

González-Pisani X., Barón P., López Greco L.S. 2011. Functional anatomy of the female reproductive systems of two spider crabs (Decapoda, Majoidea). – Invertebrate Biology **131**: 61–74.

Guinot D. 1977. Propositions pour une nouvelle classification des Crustacés Décapodes Brachyoures. – Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, Serie D **285**: 1049–1052.

Guinot D., Tavares M., Castro P. 2013. Significance of the sexual openings and supplementary structures on the phylogeny of brachyuran crabs (Crustacea, Decapoda, Brachyura), with new nomina for higher-ranked podotreme taxa. – Zootaxa **3665**: 1–414.

Hard W.L. 1942. Ovarian growth and ovulation in the mature blue crab, *Callinectes sapidus* Rathbun. – Chesapeake Biological Laboratory **46**: 1–17.

Hartnoll R.G. 1968. Morphology of the genital ducts in female crabs. – Zoological Journal of the Linnean Society of London **47**: 279–300.

Hayer S., Schubart C.D., Brandis D. 2015. Morphology and function of the female reproductive system of *Ebalia tumefacta* (Decapoda, Brachyura, Leucosiidae). – Journal of Morphology **276**: 517–525.

Hinsch G.W., Cone M.V. 1969. Ultrastructural observations of vitellogenesis in the spider crab, *Libinia emarginata* L. – The Journal of Cell Biology **40**: 336–342.

Hultgren K.M., Stachowicz J.J. 2008. Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology. – Molecular Phylogenetics and Evolution **48**: 986–996.

Hultgren K.M., Guerao G., Marques F.P.L., Palero F.P. 2009. Assessing the contribution of molecular and larval morphological characters in a combined phylogenetic analysis of the superfamily Majoidea. Pp. 437–455 in: Martin J.W., Crandall K.A., Felder D.L. (eds), Crustacean Issues 18: Decapod Crustacean Phylogenetics. – Taylor & Francis / CRC Press, Boca Raton, Florida.

Jamieson B.G.M., Guinot D., Richer de Forges B. 1995. Phylogeny of the Brachyura (Crustacea, Decapoda): evidence from spermatozoal ultrastructure. – Mémoires du Muséum National d'Histoire Naturelle **166**: 265–283.

Johnson P.T. 1980. Histology of the blue crab *Callinectes sapidus* – a model for the Decapoda. – Praeger Publishers, New York. 440 pp.

Klaus S., Goh G.H., Malkowsky Y., Becker C., Plath M. 2014. Seminal receptacle of the pill box crab *Limnopilos naiyanetri* Chuang and Ng, 1991 (Brachyura: Hymenosomatidae). – Journal of Crustacean Biology **34**: 407–411.

Krol R.M., Hawkins W.E., Overstreet R.M. 1992. Reproductive components. Pp. 295–343 in: Harrison F.W., Humes A.G. (eds), Microscopic Anatomy of Invertebrates. Decapod Crustacea. – Wiley-Liss, Inc., New York.

Lanteigne C., Beninger P.G., Gionet C. 1996. Ontogeny of female primary sexual characters in the majid crabs *Chionoecetes opilio* and *Hyas coarctatus*. – Journal of Crustacean Biology **16**: 501–514.

Lautenschlager A.D., Brandis D., Storch V. 2010. Morphology and function of the reproductive system of representatives of the genus *Uca*. – Journal of Morphology **271**: 1281–1299.

Lee T.-H., Yamazaki F. 1990. Structure and function of a special tissue in the female genital ducts of the Chinese freshwater crab *Eriocheir sinensis*. – The Biological Bulletin **178**: 94–100.

Locke M. 2001. The Wigglesworth Lecture: Insects for studying fundamental problems in biology. – Journal of Insect Physiology **47**: 495–507.

Mahon B.C., Neigel J.E. 2008. Utility of arginine kinase for resolution of phylogenetic relationships among brachyuran genera and families. – Molecular Phylogenetics and Evolution **48**: 718–727.

Martin J.W., Abele L.G. 1986. Notes on male pleopod morphology in the brachyuran crab family Panopeidae Ortmann, 1893, sensu Guinot (1978) (Decapoda). – Crustaceana **50**: 182–198.

McLay C.L., López Greco L.S. 2011. A hypothesis about the origin of sperm storage in the Eubrachyura, the effects of seminal receptacle structure on mating strategies and the evolution of crab diversity: How did a race to be first become a race to be last? – Zoologischer Anzeiger - A Journal of Comparative Zoology **250**: 378–406.

McLay C.L., Becker C. 2015. Reproduction in Brachyura. Pp. 185–243 in: Castro P., Davie P.J.F., Guinot D., Schram F.R., von Vaupel Klein J.C. (eds), Treatise on Zoology – Anatomy, Taxonomy, Biology - The Crustacea. – Koninklijke Brill NV, Leiden / Boston.

Neumann V. 1996. Comparative gonopod morphology of the European spider crabs of the genus *Maja* Lamarck 1801 (Crustacea: Decapoda: Brachyura: Majidae). – Senckenbergiana Biologica **75**: 143–158.

Porter M.L., Pérez-Losada M., Crandall K.A. 2005. Model-based multi-locus estimation of decapod phylogeny and divergence times. – Molecular Phylogenetics and Evolution **37**: 355–369.

Rorandelli R., Paoli F., Cannicci S., Mercati D., Giusti F. 2008. Characteristics and fate of the spermatozoa of *Inachus phalangium* (Decapoda, Majidae): description of novel sperm structures and evidence for an additional mechanism of sperm competition in Brachyura. – Journal of Morphology **269**: 259–271.

Rotllant G., González-Gurriarán E., Fernández L., Benhalima K., Ribes E. 2007. Ovarian maturation of the multi-spawning spider crab *Maja brachydactyla* (Decapoda: Majidae) with special reference to yolk formation. – Marine Biology **152**: 383–394.

Sainte-Marie G., Sainte-Marie B. 1998. Morphology of the spermatheca, oviduct, intermediate chamber, and vagina of the adult snow crab (*Chionoecetes opilio*). – Canadian Journal of Zoology **76**: 1589–1604.

Sainte-Marie G., Sainte-Marie B., Sévigny J.-M. 2000. Ejaculate-storage patterns and the site of fertilization in female snow crabs (*Chionoecetes opilio*; Brachyura, Majidae). – Canadian Journal of Zoology **78**: 1902–1917.

Sal Moyano M.P., Gavio M.A., Cuartas E.I. 2010. Morphology and function of the reproductive tract of the spider crab *Libinia spinosa* (Crustacea, Brachyura, Majoidea): pattern of sperm storage. – Helgoland Marine Research **64**: 213–221.

SAL MOYANO M.P., GAVIO M.A., CUARTAS E.I. 2011. Copulatory system of the spider crab *Libinia spinosa* (Crustacea: Brachyura: Majoidea). – Journal of the Marine Biological Association of the United Kingdom **91**: 1617–1625.

SANT'ANNA B.S., PINHEIRO M.A.A., MATAQUEIRO M., ZARA F.J. 2007. Spermathecae of the mangrove crab *Ucides cordatus*: a histological and histochemical view. – Journal of the Marine Biological Association of the United Kingdom **87**: 903–912.

SPALDING J.F. 1942. The nature and formation of the spermatophore and sperm plug in *Carcinus maenas*. – Quarterly Journal of Microscopical Science **2**: 399–422.

SPEARS T., ABELE L.G., KIM W. 1992. The monophyly of brachyuran crabs: A phylogenetic study based on 18s rRNA. – Systematic Biology **41**: 446–461.

TSANG L.M., SCHUBART C.D., AHYONG S.T., LAI J.C.Y., AU E.Y.C., CHAN T.-Y., NG P.K.L., CHU K.H. 2014. Evolutionary history of true crabs (Crustacea: Decapoda: Brachyura) and the origin of freshwater crabs. – Molecular Biology and Evolution **31**: 1173–1187.

VALLINA M., SAL MOYANO M.P., CUARTAS E.I., GAVIO M.A. 2014. Reproductive system and size maturity of the paddle crab *Ovalipes trimaculatus* (Brachyura: Portunidae) along the Argentine coast. – Journal of Crustacean Biology **34**: 357–366.

VAN DEN BRINK A.M., MCLAY C.L. 2009. Use of the sterile male technique to investigate sperm competition, storage, and use in a pill box crab, *Halicarcinus cookii* (Brachyura: Hymenosomatidae). – Journal of Crustacean Biology **29**: 62–69.

VEHOF J., MEIJ S.E.T., TÜRKAY M., BECKER C. 2016. Female reproductive morphology of coral-inhabiting gall crabs (Crustacea: Decapoda: Brachyura: Cryptochiridae). – Acta Zoologica **97**: 117–126.

VEHOF J., SCHOLTZ G., BECKER C. 2017. Morphology of the female reproductive system of three dorippid crabs (Crustacea; Decapoda; Brachyura; Dorippidae) and the role of accessory cuticle structures associated with seminal receptacles. – Invertebrate Biology **x**(x): 1–19. DOI:10.1111/ivb.12181

ZARA F.J., PEREIRA G.R.R., SANT'ANNA B.S. 2014. Morphological changes in the seminal receptacle during ovarian development in the speckled swimming crab *Arenaeus cribrarius*. – The Biological Bulletin **227**: 19–32.