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Functional Morphology of the Reproductive System of *Galathea intermedia* (Decapoda: Anomura)

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ABSTRACT Spermatophore formation in *Galathea intermedia* begins in the proximal part of the vas deferens. The contents subsequently form a spermatophoric ribbon, the so-called "secondary spermatophore," in its distal part. A strongly muscular ductus ejaculatorius is present in the coxa of the fifth pereiopod which builds up pressure for the extrusion of the spermatophoric ribbon. After extrusion, the ribbon is caught by the first gonopod, while the second gonopod dissolves the matrix of the ribbon. During copulation the spermatophores are randomly placed on the sternum of the female, near the genital opening, by the fifth pereiopods of the male. Subsequent ovulation of the female via the genital opening, an active process accomplished through muscular activity, results in fertilization of the eggs by the exploding spermatophores. External intersexes are characterized by both male and female external sexual characters, but in all individuals only male gonads are present. No trace of a female reproductive system could be detected. Thus, these external intersexes are exclusively functional males. J. Morphol. 262: 500–516, 2004.

KEY WORDS: sperm transfer; gonopods; spermatophores; spermatozoa; ultrastructure; Crustacea

Details concerning reproductive morphology, mating procedures, sperm transfer and storage, and fertilization are still poorly known in the Galatheidae. Ultrastructural analyses of the vas deferens of Decapoda have been limited to lobsters (Kooda-Cisco and Talbot, 1986), crayfish (Talbot and Beach, 1989), and crabs (Hinsch and Walker, 1974). More recently the morphology and structure of decapod spermatozoa and spermatophores have been increasingly studied and used for phylogenetic analysis (Kooda-Cisco and Talbot, 1982; Dudenhausen and Talbot, 1983; Subramoniam, 1984, 1993; Bauer, 1991, 1996; Bauer and Cash, 1991; Felgenhauer and Abele, 1991; Hinsch, 1991a,b; Lohrmann and Raineri, 1995; Tudge, 1991, 1992, 1995, 1996, 1997, 1999a,b; Jamieson and Tudge, 2000; Hess and Bauer, 2002). The formation and the morphology of reptant spermatophores have been described at the light microscope level by many authors (reviewed in Dudenhausen and Talbot, 1983; see Hess and Bauer, 2002, on *Clibanarius* [Anomura]).

In addition to these morphological aspects, sperm transfer and fertilization mechanisms in decapods were also of interest for phylogenetic analysis during recent decades (Bauer, 1991, on Penaeids). Bauer (1986) used data on sperm transfer for a general phylogenetic analysis and gave an overview on sperm transfer strategies in decapod crustaceans. According to that investigation, as well as to many morphological works on the copulatory systems (Grobben, 1878; Balss, 1944; Pike, 1947; Hartnoll, 1968; Greenwood, 1972; Brandis et al., 1999; Bauer, 1991, 1996; Bauer and Cash, 1991), it is apparent that despite some variability between decapod taxa, modes of sperm transfer are generally based on a relatively consistent scheme. This varies mainly in the specificity of transfer and in the mode of storage. All decapods condense the spermatozoa into spermatophores, which exhibit specific characters with taxonomic relevance in certain groups (Tudge, 1991, 1996, 1997, 1999a,b; Tudge and Jamieson, 1996a,b; Jamieson and Tudge, 2000). The spermatophores are transferred to the female by the first two pairs of male pleopods, the gonopods. These gonopods either attach spermatophores to the ventral surface of the female sternum or into a spermatheca, as, for example, is the case in eubrachyuran crabs. In pagurids the extreme modification of the abdomen, and the life in shells, make the usual sperm transfer mechanisms problematic. Thus, in this and some other groups secondary organs undertake the sperm transfer, usually accompanied by reduction of the gonopods (see Hess and Bauer, 2002).

However, in the galatheids gonopods are present in spite of sperm transfer by the fifth pereiopods (Brandes, 1897). Sperm transfer and the morphology of participating structures were first described for *Galathea strigosa* by Brandes (1897), but details

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of morphology and function are still poorly known and need reinvestigation. The present article aims to clarify spermatophore formation and the function of the transfer organs in *Galathea intermedia* on the basis of ultrastructural and histological analysis of male and female reproductive organs. *Galathea intermedia* is a small species, very common in the deeper North Sea. *Galathea intermedia* in the Helgoland Trench was surveyed from 1982–1992 by the Senckenberg Institute. A detailed study of the composition of these populations in the period from 1985 to 1992 showed that there was regular local reproduction and recruitment (Kronenberger and Türkay, 2003). Furthermore, morphological intersexes were detected, exhibiting male and female external sexual characters such as gonopods and female genital openings at the same time. The intersex proportion increased during the study, but the reasons for this were not clear. The need to explain this observation inspired the present study, the aim of which was to gather basic information on the reproductive system of the species, including the organization of the intersexes.

MATERIALS AND METHODS

Galathea intermedia Lilljeborg, 1851, was obtained by the first author in the Helgoland Trench, south of Helgoland Island in the German Bight, during a cruise aboard R. V. *Senckenberg* in June 2001.

For light microscopy (LM) animals were fixed in SUSA (Sublimat/$HgCl_2$), embedded in paraplast, cross-sectioned at 12 μm, and stained with Masson-Trichrome stain (Romeis, 1989). For electron microscopy (TEM, Zeiss EM 10) reproductive tissue was processed. Complete animals were fixed in glutaraldehyde seawater. Reproductive tissue was removed, postfixed in OsO_4, dehydrated in a graded acetone series, and embedded in low-viscosity medium (Spurr, 1969). Sections were mounted on copper grids, stained with uranyl acetate and lead citrate, and analyzed with a transmission electron microscope. Semithin sections were stained based on Richardson et al. (1960) for light microscopy. The tissue was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.6) for 2 h, rinsed with cacodylate buffer, and postfixed with 2% osmium tetroxide (2 h). Following rinsing with cacodylate buffer, the tissues were stained "en bloc" overnight with 1% uranyl acetate in 0.05 M maleate buffer (pH 5.2) and rinsed again in maleate buffer. Tissues were dehydrated with successive washings of ascending concentrations of ethanol (50–100%). Tissues were embedded in Spurr-100% ethanol (1:1) for 1 h (rotator) and overnight embedded in Spurr-100% ethanol (3:1), followed by embedding in Spurr's plastic (100%) for 6 h and another 16 h in a heater (65°C). Ultrathin (<0.2 μm) and semithin sections (0.2–1 μm) were cut. Ultrathin sections were placed on copper grids, stained with lead citrate, and rinsed with distilled water. Cells from the stained sections were photographed with TEM. Semithin sections were cut and stained (Richardson et al., 1960) for light microscopy. For SEM, samples were dehydrated through an ethanol series up to 100% ethanol and critical point-dried. Then samples were sputter-coated with gold using argon gas and an electric field.

For the analysis of intersexes, a collection of about 4,386 specimens from the Helgoland trench and other areas of the German Bight, taken during all seasons over a period of 7 years, were examined. This collection was separated into male (only with male gonopores), female (only with female gonopores), and intersex (with male and female gonopores) samples. Thirty intersex specimens, collected during all seasons, were dissected to analyze gonads. For toxicological analysis, in particular for the detection of tributyltin (TBT), intersex specimens were sent to the International Graduate School, Laboratory for Environmental Analytical Study (Zittau, Germany). TBT in many organisms causes imposex effects (Oehlmann et al., 1995).

RESULTS

Male Reproductive Structures

General overview (Fig. 1). The male reproductive system of *Galathea intermedia* consists of symmetric testes with vasa deferentia connecting to paired gonopores located on the ventral side of the coxae of the fifth pereiopods (Fig. 1). Each of the testes is situated in the cephalothoracic region, dorsolateral to the gut and ventral to the heart, surrounded by lobes of the hepatopancreas. The posterior part of each of the testes merges into the vas deferens. The latter is divisible into 1) a proximal part of narrow, closely packed coils with small diameter, extending posteriorly for a short distance, gradually increasing in diameter and leading into 2) an intermediate, irregularly, and strongly twisted tube with a larger diameter than that of the proximal part, forming irregular descending and ascending coils or spirals; 3) the distal part of the vas deferens forming a long straight tube merging into 4) a muscular terminal ampulla, the ejaculatory duct, which commences shortly in front of the coxa of the fifth pereiopod. In contrast to that of the vas deferens, the lumen of the ejaculatory duct is surrounded by a thick muscular layer composed of circular striated muscle cells, very similar to the situation in the diogenid anomuran *Clibanarius vittatus* (Hess and Bauer, 2002).

Histology and Ultrastructure of the Male Reproductive Duct (Fig. 2)

Testes. The testes are located in the anterior cephalothoracic region. They form wide tubules, surrounded by a basilar membrane. Different compartments of the testes enclose early stages of undifferentiated sperm cells. As the testes merge into the proximal vas deferens, the initially amorphous sperm mass becomes surrounded, and thereby subdivided and fractionated by the dense primary spermatophore layer.

Vas deferens (Fig. 2). Throughout its length the vas deferens consists of two main layers: an inner secretory epithelium and an outer muscular layer (Fig. 2B–D). The epithelium is generally composed of densely packed columnar cells. These contain cytoplasmic organelles, such as cisternae of the rough endoplasmic reticulum filled with secretory products (Fig. 2D) occupying a large fraction of the cell, some less frequent Golgi bodies, and many large vesicles and nuclei. Within the vas deferens different degrees of spermatophore formation can be distinguished:

1) In the proximal vas deferens spermatozoa are embedded in seminal fluids of testicular origin

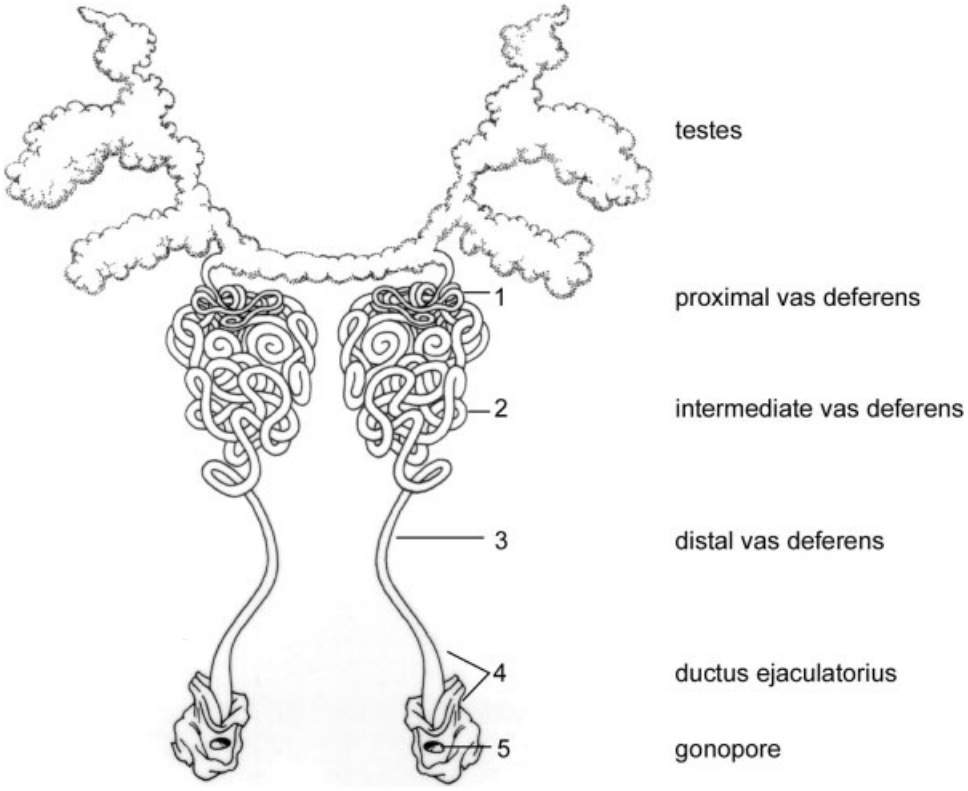

Fig. 1. Diagram of the male reproductive system of *Galathea intermedia*.

creating unformed, undifferentiated clusters of sperm.

2) These originally amorphous sperm masses become surrounded by a coat of secretions originating in the vas deferens, and thereby become subdivided or fragmented into small sections (Fig. 2A,B). The secretion appears green in Masson-trichrome stain, which indicates an acidic composition and a predominance of mucopolysaccharidic acellular material. This secretion generates the spermatophore layer, which also forms the stalk-like peduncle of the spermatophore (Figs. 2B, 3B). Thus, the formation of spermatophores commences in the very proximal vas deferens, at this stage incorporating mature as well as immature sperm cells (Fig. 3B).
3) Further down the vas deferens, the sections of agglutinated spermatozoa are reshaped by a coat composed of the above-mentioned secretory product to form definite and discrete morphologically differentiated spermatophores (Fig. 3D,E). Each spermatophore consists of an elongate ampulla containing the sperm mass and a stalk-like peduncle. Several sperm masses are contained within one spermatophore. At least one supplementary acellular layer of secretory product is being added.
4) In the distal vas deferens the spermatophores become arranged at 90° to the tube axis, with the peduncles glued together and connected ventrally by filaments. At this stage the ampullae of each discrete spermatophore are being coated by other seminal secretions, which appear red in Masson-Trichrome stain, indicating a basic composition. The complete row of spermatophores is embedded in these gelatinous seminal secretions, binding the still discrete spermatophores tightly together (Figs. 2E,F, 6C,D). In the ejaculatory duct, spermatophores are filled exclusively with mature spermatozoa (Fig. 3E).
5) The ductus ejaculatorii, or the terminal ampullae of the vas deferens, are located within the coxae of the fifth pereopods and open to the exterior via the gonopores (Fig. 6C). The openings are similar to those of the female, being guarded by flap-like opercula (Fig. 12A). The gonopores are not borne on external extensions of the ducts, as is characteristic for many decapods, e.g., Brachyura (Ryan, 1967; Brandis et al., 1999) or cambarid crayfishes (Andrews, 1911). The tissue of the ejaculatory duct consists of an extremely thick outer muscular coat of at least four layers, subtended by an inner, single-layered secretory epithelium composed of large vesicles filled with secretion product, Golgi-bodies, many nuclei, cisternae of rough endoplasmic reticulum (less than in the vas deferens), and mitochondria (Fig. 2E–G). The secretory cells bear a dense microvillous border apically, which projects into the lumen of the ductus ejaculatorius (Fig. 2H).

Spermatophore morphology (Fig. 3). The spermatophores are pedunculate, with an oblong, capacious, sperm-filled ampulla elevated on a short

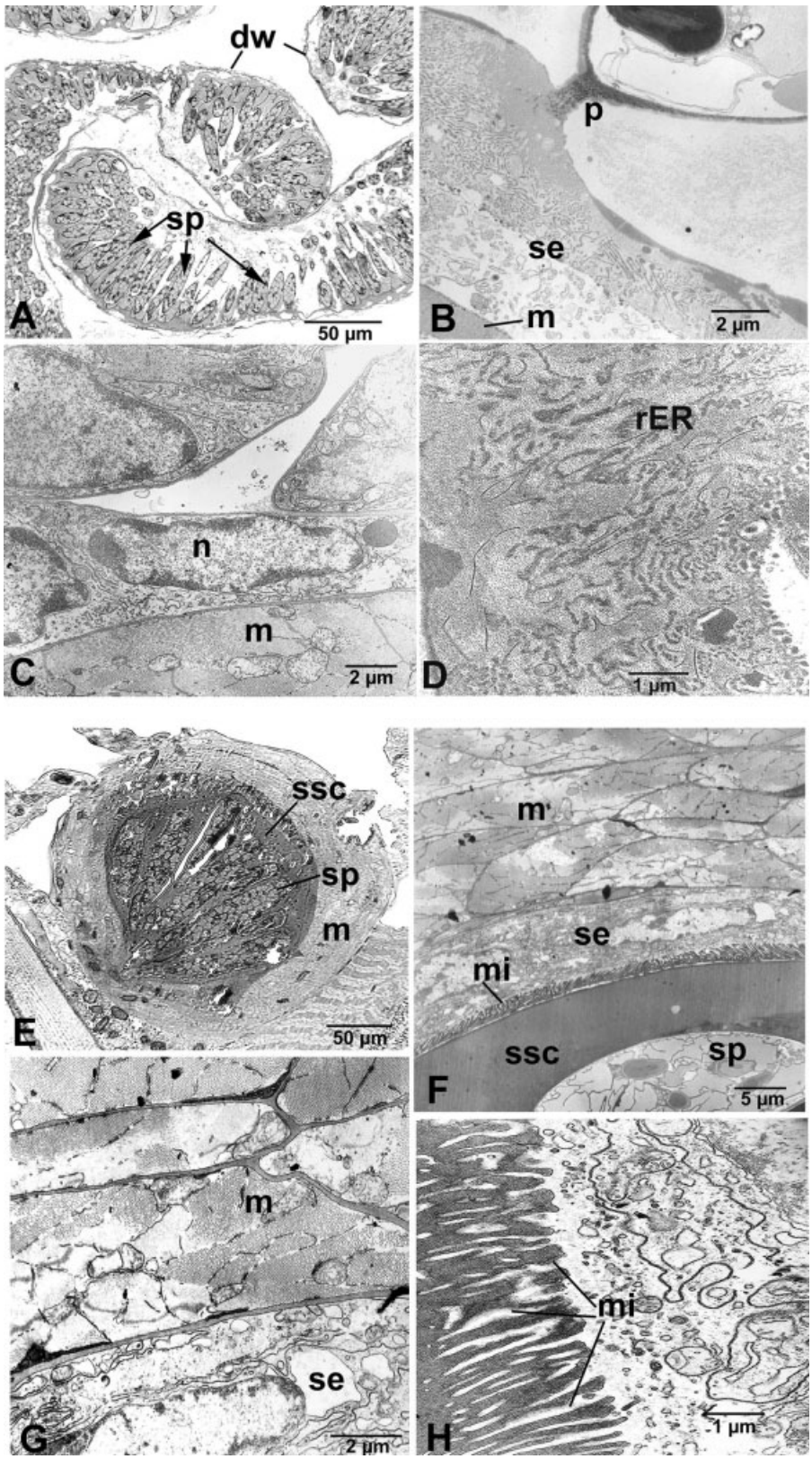

Fig. 2. Vas deferens and ductus ejaculatorius of *Galathea intermedia*. **A–D:** Vas deferens. **E–H:** Ductus ejaculatorius. **A:** The distal vas deferens with forming spermatophores. LM. **B:** Vas deferens, spermatophore formation: duct wall and a forming spermatophore. A secretory epithelium secretes acellular material that becomes the primary spermatophore layer. A muscular layer underlies the epithelium. TEM. **C:** Secretory cells and striated musculature of the vas deferens. TEM. **D:** Vas deferens, wall: details of a secretory cell. Note extensive rough endoplasmatic reticulum filled with electron-dense material. TEM. **E:** Ductus ejaculatorius: A cross-section of the most distal part of the ductus ejaculatorius in the coxa. The ductus ejaculatorius is filled with the spermatophore ribbon, which consists of the spermatophores coated by seminal secretions. LM. **F:** Wall of the ductus ejaculatorius. A thick striated muscular layer underlies a glandular epithelium with microvilli. The epithelium secretes an acellular matrix that surrounds spermatophores to form the ribbon. TEM. **G:** Details of the margin between musculature and secretory epithelium. Thick packages of striated musculature underlie the glandular epithelium. TEM. **H:** Ductus ejaculatorius: long microvilli of the glandular epithelium. Dw, duct wall; mi, microvilli; n, nucleus; p, peduncle; se, secretory epithelium; sp, spermatophore; ssc, seminal secretions; m, musculature.

thick stalk, which is attached to a gelatinous base. A lateral ridge, the so-called spermatophore operculum, is the junction of the two halves of the ampulla, running around the elongate spermatophore (Fig. 3A,C–E). At this ridge, the two halves of the ampulla will split to release the spermatozoa (Fig. 3C). In the spermatophore ampulla, spermatozoa are randomly orientated and embedded in an extracellular matrix

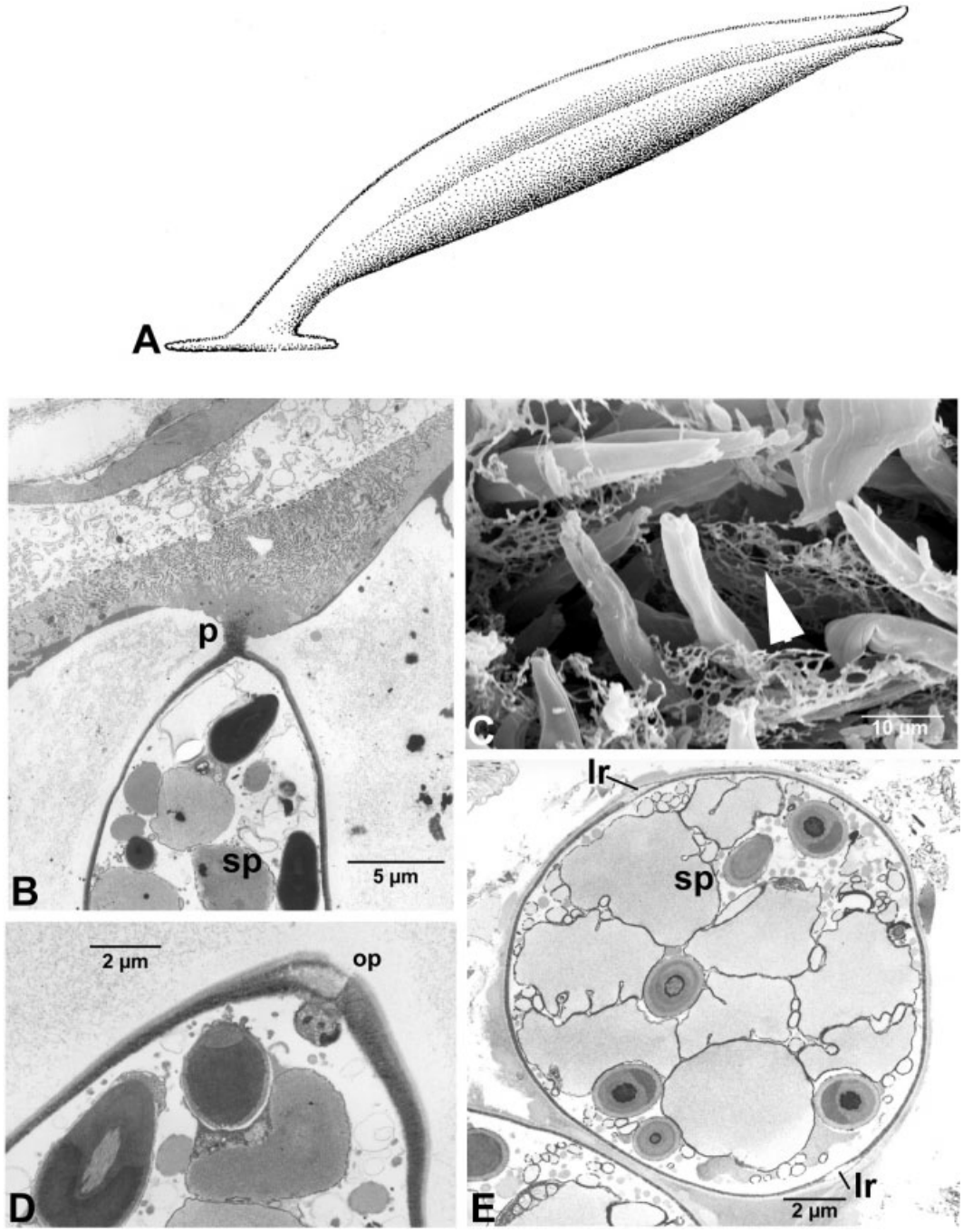

Fig. 3. *Galathea intermedia*. Spermatophore morphology. **A:** Schematic diagram of a mature spermatophore, representing the pedunculate type. The elongated ampulla is formed by two halves that are joined at the lateral or spermatophore ridge. **B:** Proximal section of a spermatophore attached to the wall of the vas deferens and filled with developing spermatozoa. Note the secretory epithelium forming the spermatophore peduncle by its secretions. TEM. **C:** Free spermatophores after extrusion from the male gonopore. Note the seminal fluid matrix as net-like fibrils between spermatophores (arrowhead). SEM. **D:** Distal part of a mature spermatophore. The wall consists at least of two different layers. The inner one is more electron dense. TEM. **E:** Cross-section of a mature spermatophore densely packed with spermatozoa. Note the details of the lateral ridges (lr) , the joining site of the two halves of the ampulla. TEM. lr, lateral ridge; op, operculum; p, peduncle; sp, spermatozoa.

(Fig. 3B,E). The spermatophore wall is at least double-layered, consisting of densely packed granular material (Fig. 3D), and is generated by additional layers of secretion product at an early stage of spermatophore formation in the proximal vas deferens (Figs. 2B, 3B).

This process of spermatophore formation results in the peduncles of the spermatophores being con-

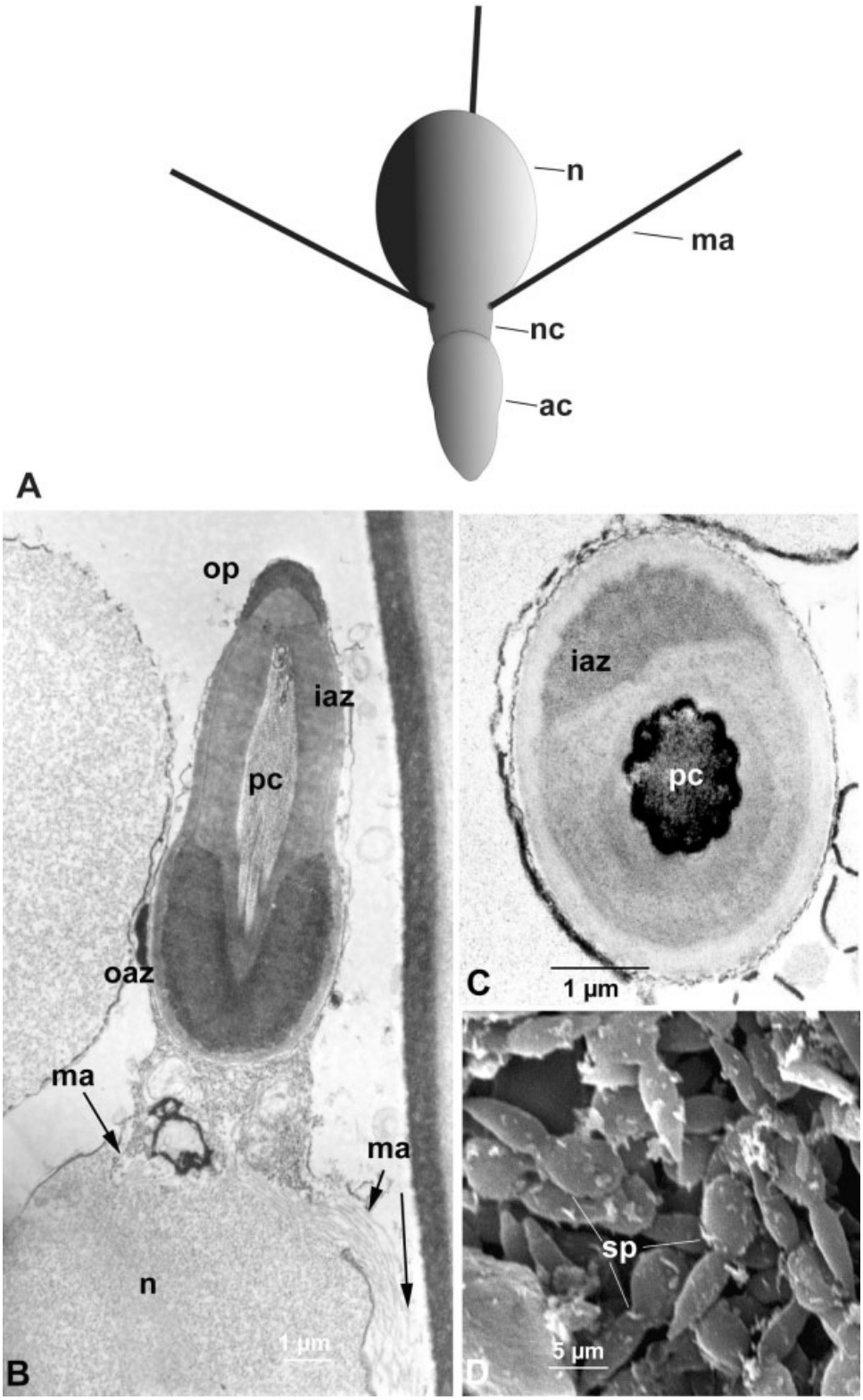

Fig. 4. *Galathea intermedia*. Aspects of spermatozoan morphology. **A:** Model of spermatozoan morphology. The spermatozoan consists of three parts, the acrosomal complex (ac), the neck (nc), and the nucleus. At the margin between neck and nucleus originate three arms formed by microtubules. **B:** Longitudinal section of acrosome of spermatozoan. The acrosomal complex forms a special perforatorial apparatus. Microtubules in the perforatorial chamber are arranged in parallel pattern. Note the microtubule arms (ma). TEM. **C:** Cross section of acrosome of spermatozoan. **D:** A broken spermatophore with spermatozoan mass (sp). SEM. ac, acrosomal complex; iaz, inner acrosome zone; ma, microtubule arm; mt, microtubules; n, nucleus; nc, neck; oaz, outer acrosome zone; op, operculum; pc, perforatorial chamber.

nected ventrally by filaments, consisting of the same material as the spermatophores, and being further ensheathed by seminal secretions, forming a long continuous cord or ribbon. The ribbon consists of substances distinctly different from those forming the spermatophores (see above). It is extruded through the gonopores at the base of the fifth pereiopod by contractions of the thick muscle layer of the ductus ejaculatorius (see Figs. 2E, 6C).

Spermatozoon morphology (Fig. 4). The acrosomal vesicle of mature sperm is large, oblong, and fusiform (Fig. 4A). The acrosome is capped by an apical electron-dense operculum and consists of an inner acrosome zone surrounding the perforatorial chamber (Fig. 4B). External to the inner acrosome zone is the outer acrosome zone. The latter is less electron dense, posteriorly filling the remainder of the vesicle. The perforatorial chamber contains microtubules. The acrosomal vesicle is followed by a long, posteriorly projecting tail composed of cytoplasm, nucleus, microtubules, and mitochondria. At least three arms emanate from the cytoplasm of the sperm cell. Motile flagella are absent.

Morphology of the male gonopods (Figs. 5–10). The first and second gonopods consist of three segments (Fig. 5). The terminal segment of the first gonopod proximally forms a stem whose margins diverge distally forming a thin, triangularly shaped plate. The ventral surface of this plate is slightly invaginated and the lateral margin strongly overlaps the ventral surface and is lined with long setae (Figs. 5, 6A,E,F). The mesial margin is slightly incurved and the distal mesial edge forms a dorsally invaginated spoon-like structure in opposition to the ventrally invaginated plate surface (Fig. 6A,E [arrows], F). The bulging margins of this structure are lined with long pappose setae (Fig. 6A,E,F). In many specimens this structure is attached to the extruding spermatophore ribbon (Fig. 6E,F). In other specimens the dorsal part is covered with layers of free spermatophores (Fig. 6G). No specialized tissues, such as glandular epithelia, were detected within this first gonopod.

The second gonopod is distinctly larger than the first one and the terminal segment forms a broad, dorsoventrally compressed plate (Figs. 5, 6B,E). The margins of the distal part of the plate are swollen and form a bulge; the mesial bulge especially is very thick, covered with long simple setae (Figs. 6B,E, 7). In cross-section this zone forms a "butterfly-like structure" with two swollen lateral margins, both converging to a narrow median connection (Fig. 7A,B). While the dorsal cuticle has a normal architecture, the ventral cuticle is reduced to a thin, membranous-like layer (Fig. 7A). The original orientation of the distal plate surface is changed by torsion such that the former dorsal margin faces the lateral side and the former ventral margin faces the mesial side (Fig. 6E). Proximally the plate margins converge strongly to a more or less rounded stem (Figs. 6, 7). The surface of the distal part of the plate is concave and the dorsomesial part is covered with a thick pad of dense, simple setae (Fig. 6E). The dorsal surface of the terminal plate is crossed by a deeply invaginated groove, which is partly overlapped by a median bulge (Fig. 7A). The groove originates on the ventral surface of the stem (Fig. 7E,F) and more distally it turns to the ventral side (see arrow in the sequence from Fig. 7D to Fig. 7A). In the

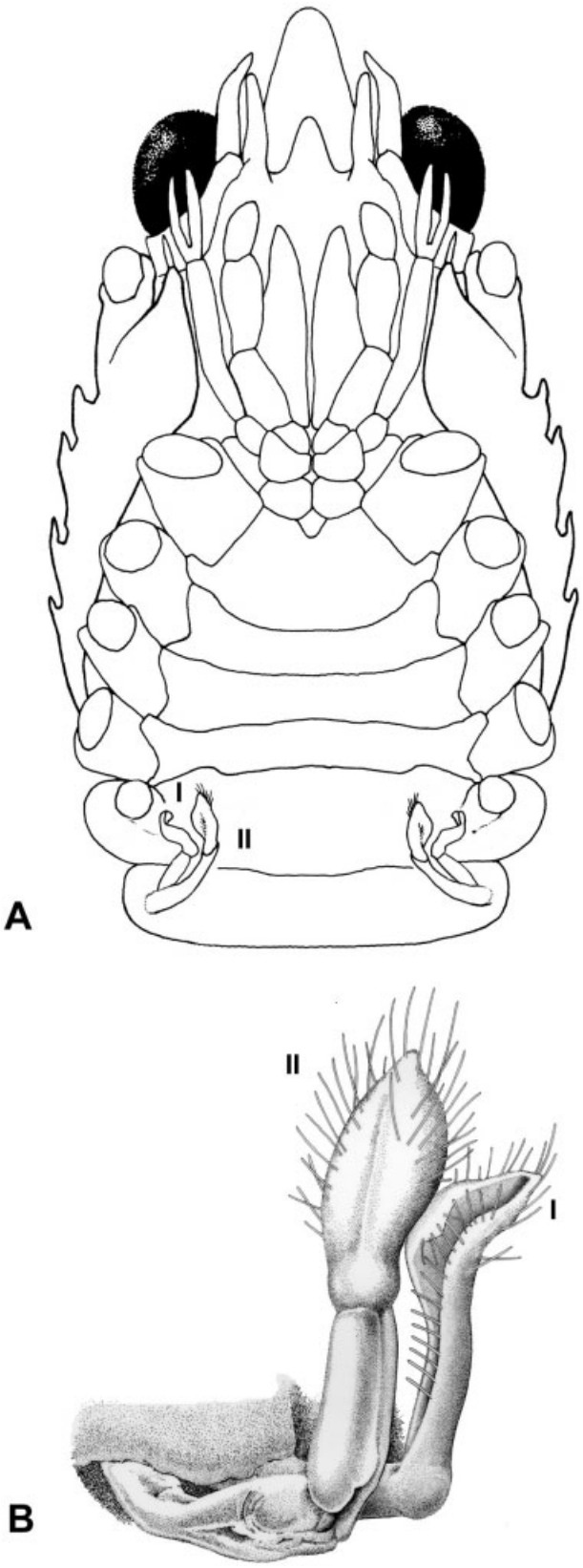

Fig. 5. Model of male gonopods of *Galathea intermedia*. **A:** Overview of the ventral side of a male of *G. intermedia.* Gonopods are in the natural position. **B:** Details of the right first and second gonopod. Terminal joint of second gonopod is turned to show the ventral surface. I, II, first and second gonopods.

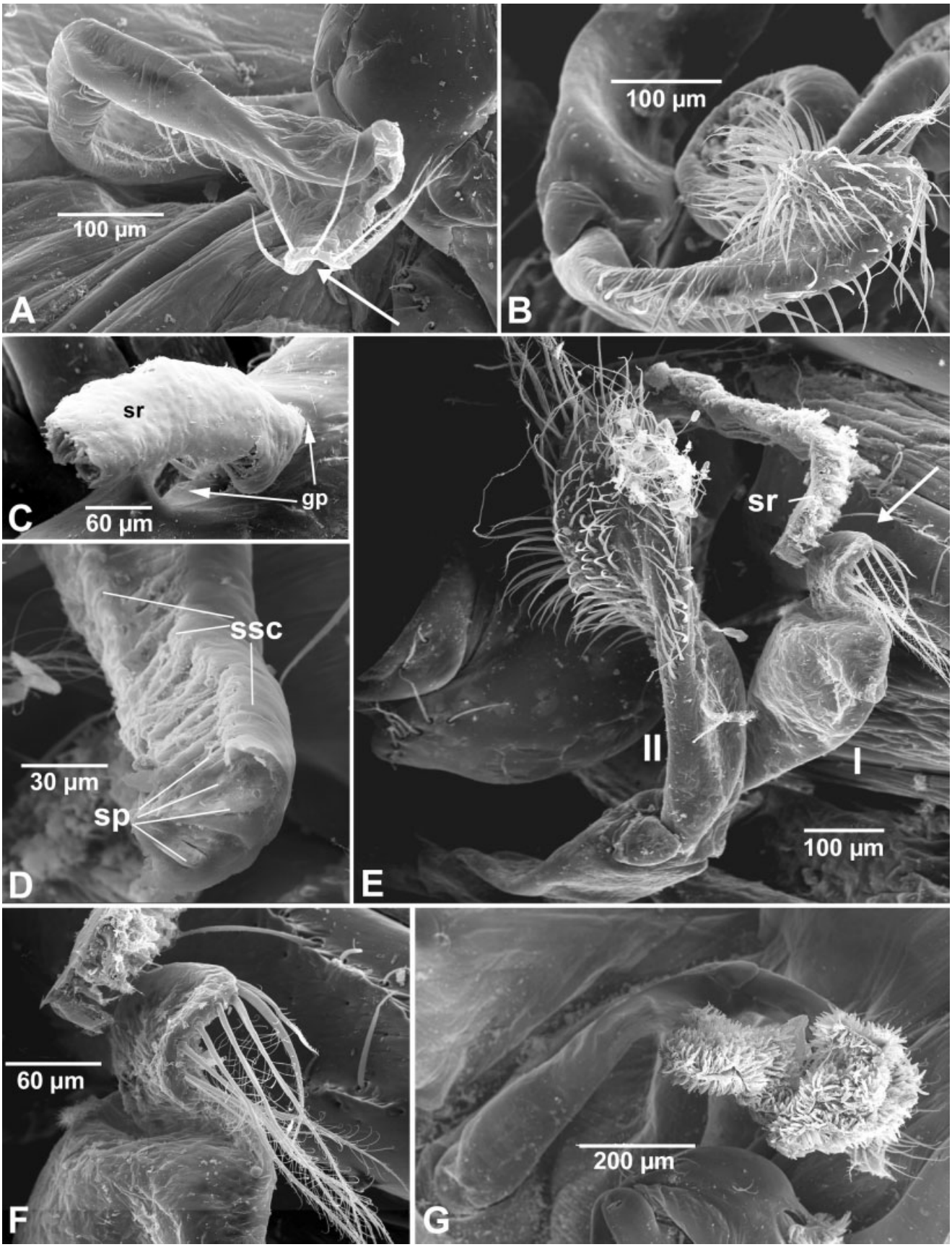

Fig. 6. *Galathea intermedia*. Male gonopods and spermatophore ribbon. SEM. **A:** Ventral view of the first gonopod. Arrow indicates the dorsal spoon-like invagination. **B:** Dorsolateral view of second gonopod. **C:** Spermatophore ribbon (sr) attached to gonopore (gp, arrows) after extrusion. **D:** Detail of spermatophore ribbon. Spermatophores are densely packed in parallel arrangement in second spermatophore material. **E:** Dorsolateral view of first and second gonopod with spermatophore ribbon attached to spoon-like invagination of first gonopod. Arrow indicates dorsal spoon-like invagination attached to spermatophore ribbon. **F:** Details of ribbon attachment. **G:** First gonopod with apically attached free spermatophores ready for transfer. I, II, first and second gonopods; gp, male gonopore; sp, spermatophores; sr, spermatophore ribbon; ssc, seminal secretions ensheathing the spermatophores.

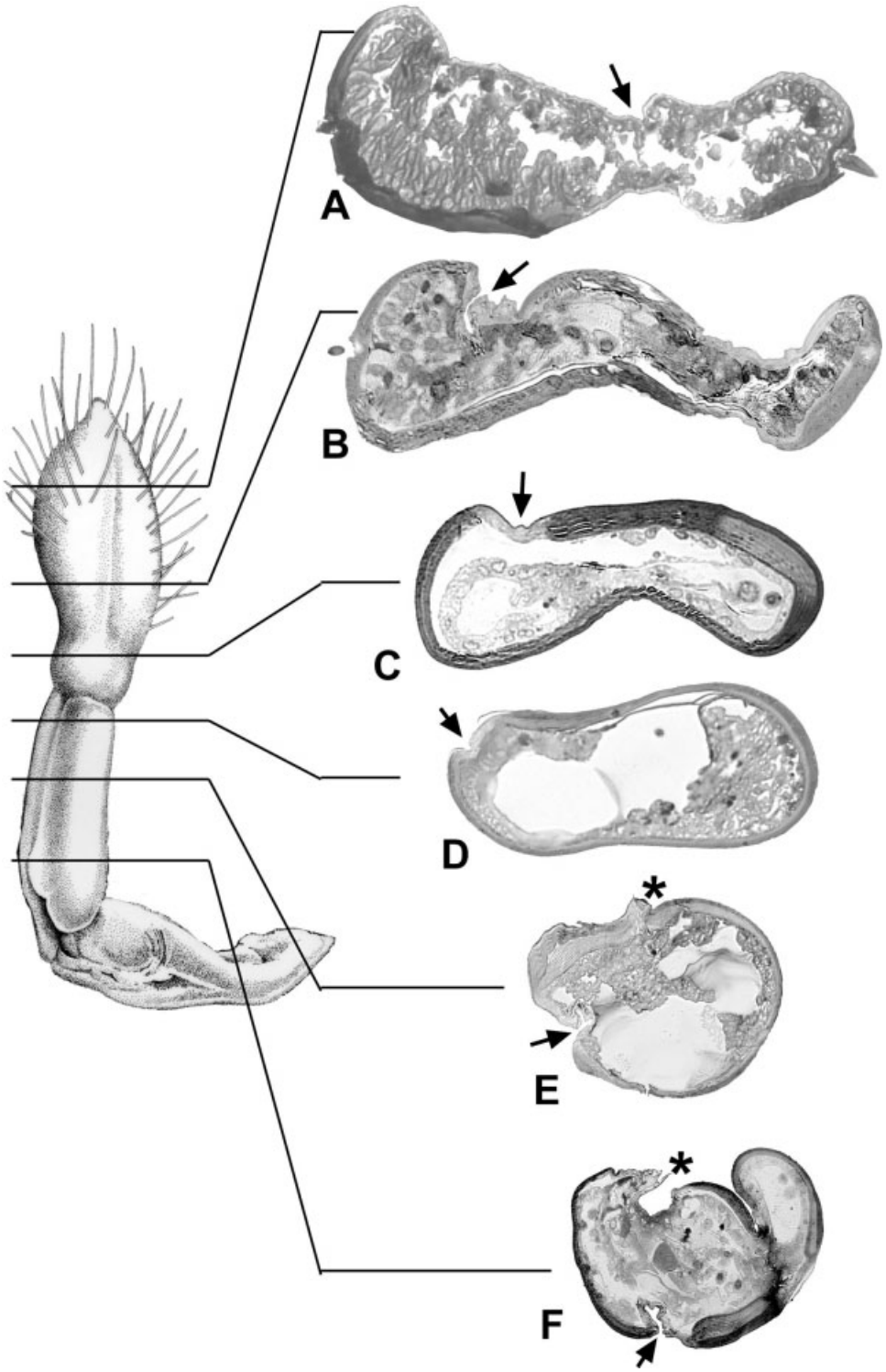

Fig. 7. *Galathea intermedia*. Cross-section series of second gonopod. Arrow indicates the course of the long groove turning from the ventral side in the proximal part to the dorsal side in the distal part. Asterisks indicate a second short groove in the proximal stem area. **A,B:** Terminal segment. **C,D:** Transition zone to the terminal segment. **E,F:** Stem area with a second dorsal groove (asterisks).

proximal part of this groove one or two gaps exist in the cuticle. These connect the groove with collecting ducts, formed by the fusion of secretory tubes (Fig. 8A–C). These tubes connect to secretory cells, filled with numerous secretory vesicles and large nuclei: the vesicles and tubes are filled with secretions (Fig. 8D–F), and fill the region beneath and lateral to the groove. The dorsal surface of the stem region is crossed by a second groove in apposition to the former one (asterisk in Fig. 7). Strong musculature is attached to the cuticle of this V-shaped groove (Fig. 9A,D). The marginal cuticle of this groove is marked by tooth-like structures (Fig. 9B,C), while the inner region is smooth. In several specimens both gonopods are attached to each other in such a way that the second gonopod

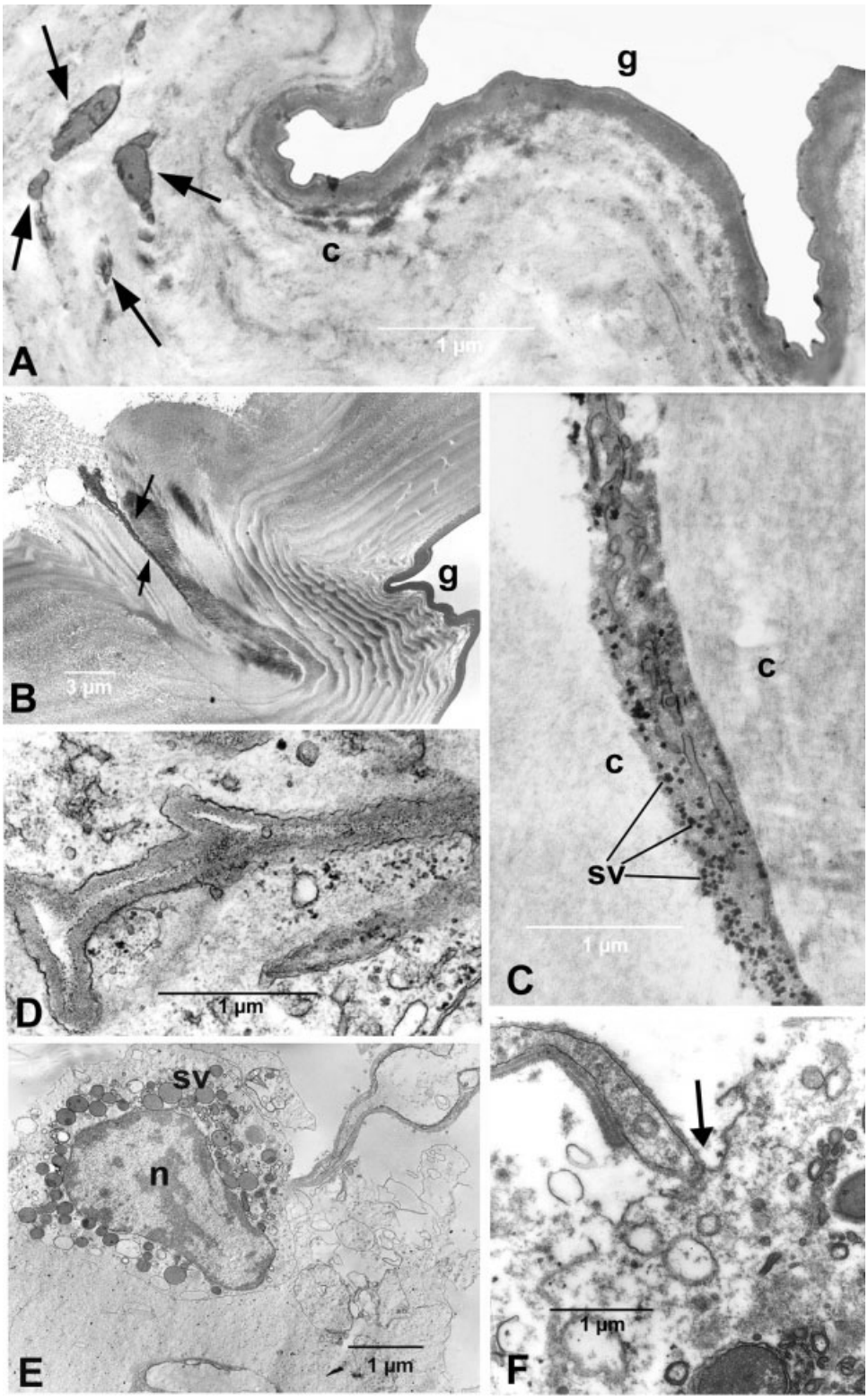

Fig. 8. *Galathea intermedia*. Details of the proximal part of the long groove of second gonopod. TEM. **A:** Groove lumen and underlying cuticle with tubes (arrows). **B:** Longitudinal section of cuticle tube (arrows). **C:** Tube details. Note the vesicles in the tube lumen. **D:** Aspects of a tube system underlying the cuticle of the groove. Several branches connect to the main tube. Tube lumen filled with secretory products. This tube system presumably connects cuticle tubes and secretory cells. **E:** Secretory cell connected to a tube. **F:** Details of cell-tube connection. Arrow indicates the connection point. g, groove; c, cuticle; n, nucleus; sv, secretory vesicles.

groove is encompassed by the ventral spoon-like structure of the first gonopod (Fig. 10).

Female Reproductive Tract (Figs. 11–13)

The ovary of female specimens of *Galathea intermedia* consists of two longitudinal branches, which lie dorsolateral on either side of the gut (Fig. 11). Both branches connect with each other by a crosspiece in the region of the heart, thus forming an H-shaped structure. The oocytes do not differ from those of other decapods (Fig. 13); however, it is notable that the accumulation of yolk appears to con-

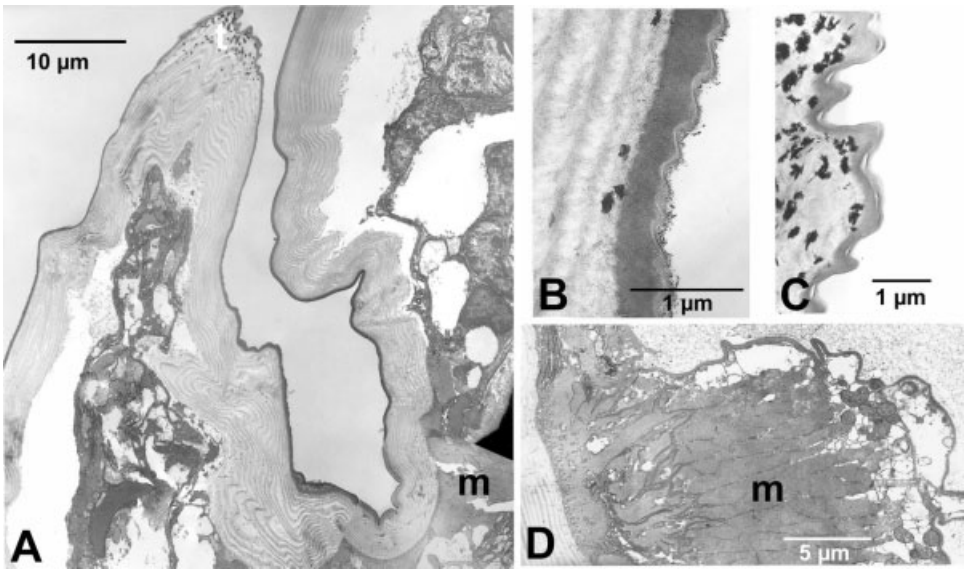

Fig. 9. *Galathea intermedia*. Details of the short groove in the stem area of the second gonopod. TEM. **A:** Overview. The outer parts are covered with teeth. **B,C:** Details of teeth. **D:** Details of the musculature attached to the cuticle lining the groove. m, musculature; t, teeth.

centrate at first in the peripheral cytoplasm (Fig. 13B). The oviducts, tubular structures that extend laterally and connect the ovary with the genital openings, the gonopores (Fig. 11), originate close to the posterior end of the branches. The paired oviducts open on the ventral side of the coxae of the third pereiopods. Each genital opening is spherical, and faces towards the sternum (Fig. 12A). Characteristic long, simple setae are situated at a constant distance near the mesio-posterior border of the genital opening (Fig. 12A). The margin of the opening is outlined by rigid integument, while the lumen is occluded by a bulge of flexible integument. In cross-section, this bulge is formed by the integument of one margin, while the opposite side in cross section is formed by a very rigid and thick cuticle (Fig. 12B). The bulge formed by the flexible integument contains musculature, which runs from its dorsal margin diagonally to the sternum (Fig. 12B,C). Above the genital opening the oviduct narrows increasingly and is lined on both sides with thin cuticle (Fig. 12D). The lateral epithelia of the oviduct contain both contractile elements (Fig. 12D–F) and glandular tissue (Fig. 12E,F).

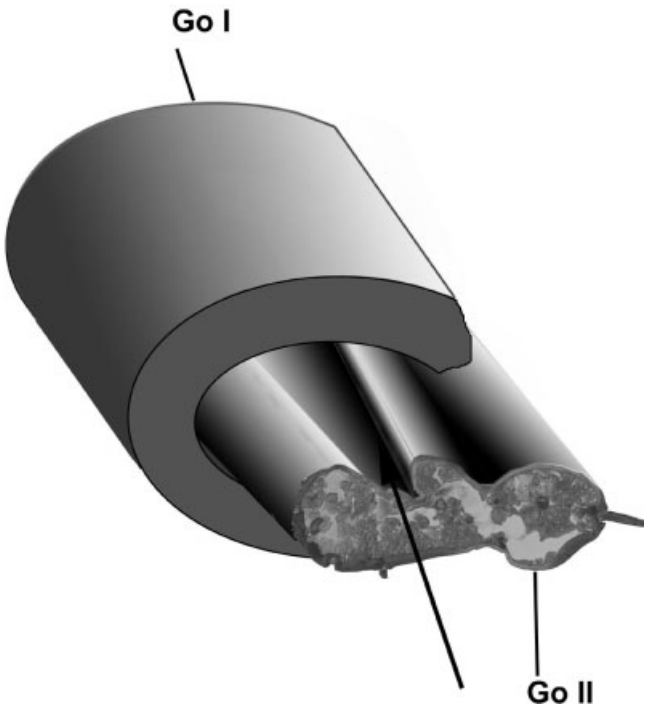

Fig. 10. *Galathea intermedia*. Model of interaction of first and second gonopod. The second gonopod groove faces the first gonopod wall and the spermatophore ribbon will be inserted in the gap between wall and groove (arrow). Go I, gonopod 1; Go II, gonopod 2.

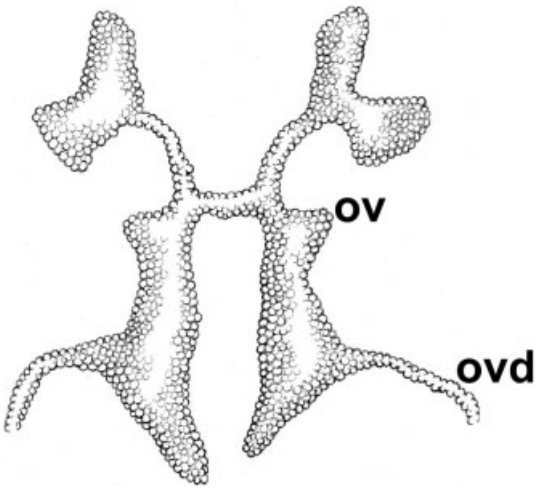

Fig. 11. *Galathea intermedia*. Model of the female reproductive system. ov, ovary; ovd, oviduct.

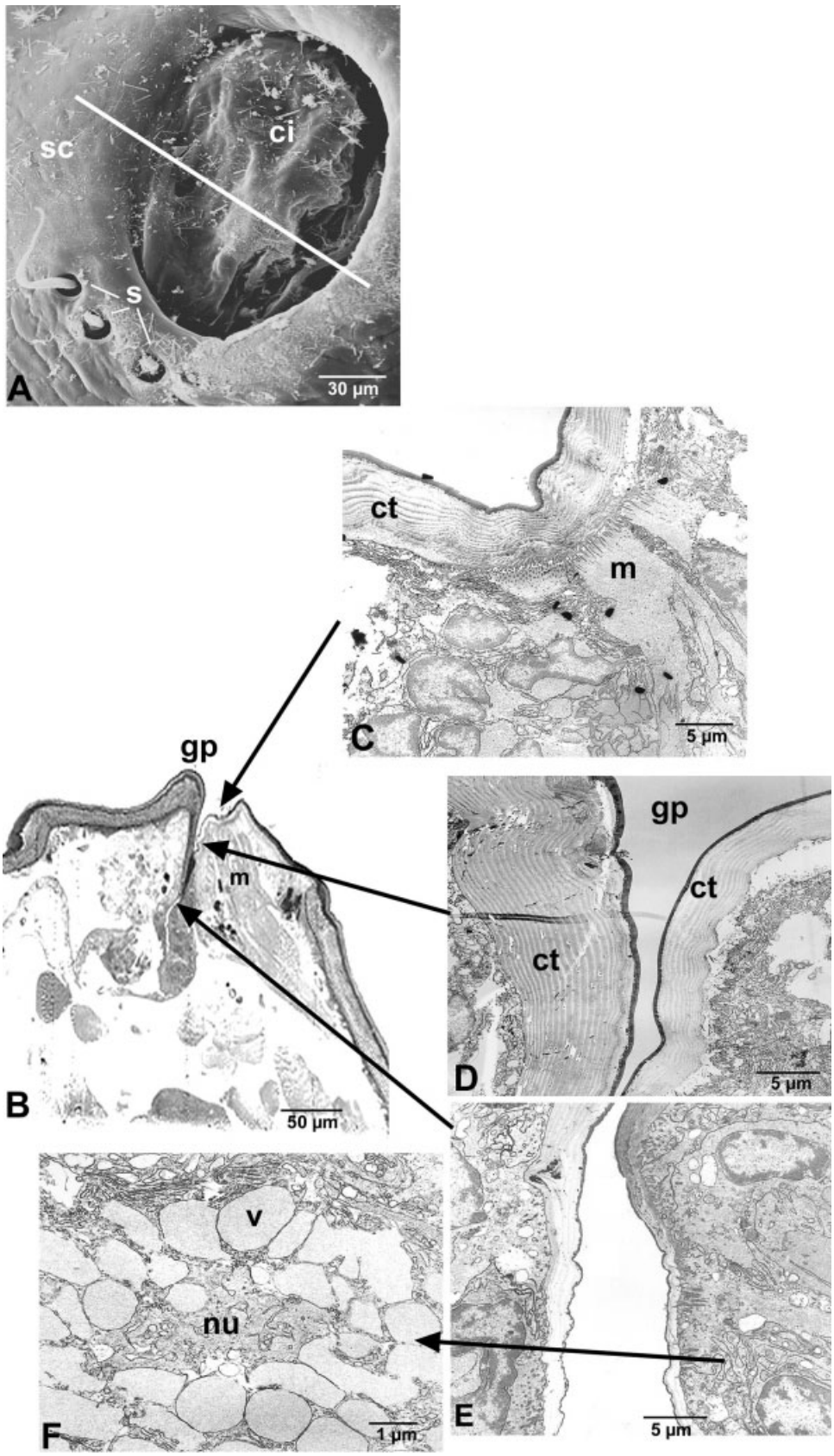

Fig. 12. *Galathea intermedia*. Morphology of the female genital pore and gonoduct. **A:** Outer morphology of the genital opening. The line indicates the cross-section shown in **B**. SEM. **B:** Cross-section of the female gonopore and terminal duct. **C:** Details of the closing integument of the gonopore. Musculature (m) allows an active opening of the gonopore. TEM. **D:** End of the gonoduct. TEM. **E:** Gonoduct and secretory cell. **F:** Proximal part of the oviduct. Note contractile elements in the epithelium. ci, closing integument; ct, cuticle; gp, gonopore; m, musculature; sc, surrounding cuticle, the cuticle lining the gonopore; v, secretory vesicle.

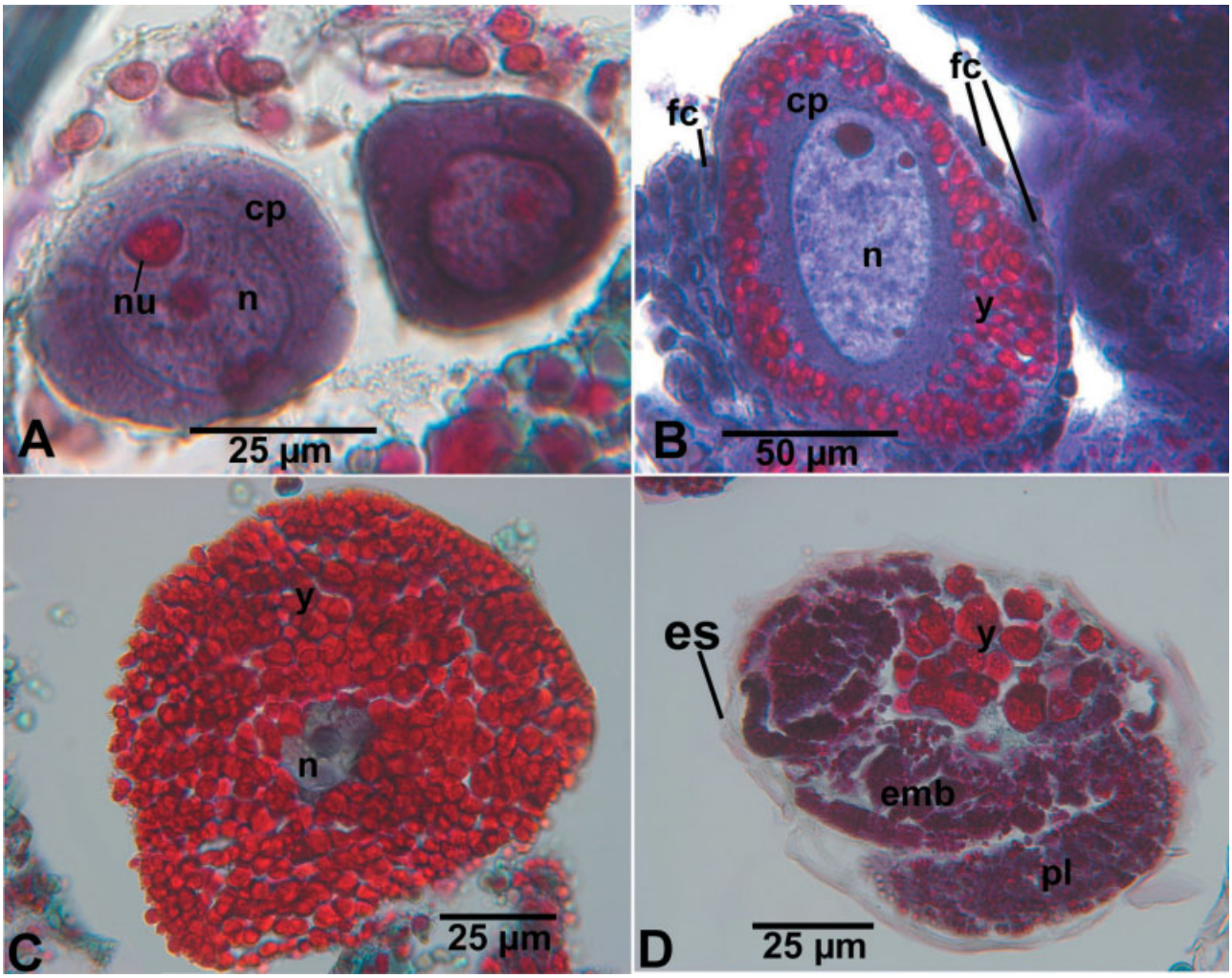

Fig. 13. *Galathea intermedia*. Oocytes. All sections stained in Masson-Trichrome stain. LM. **A:** Previtellogenic oocyte with large nucleus and only a small cytoplasmic region. Note the large nucleolus (nu). **B:** Developing vitellogenic oocyte with beginning yolk accumulation. Yolk appears to concentrate at first in the peripheral cytoplasm. The cell is surrounded by a single layer of follicle cells (fc). **C:** Vitellogenic oocyte completely filled with yolk. **D:** Developing embryo with incorporated yolk. cp, cytoplasm; es, egg shell; fc, follicle cells; n, nucleus; nu, nucleolus; pl, pleon; emb, embryo; y, yolk.

Reproductive Tract of External Intersexes

The North Sea collection of 4,368 specimens collection included 1,227 males, 2,207 females, of which 197 were ovigerous, 243 intersexes, and 383 juveniles (for a detailed analysis, see Kronenberger and Türkay, 2003). Males and females could be characterized by the exclusive occurrence of male or female gonopores. The intersex specimens, however, possess male and female gonopores. Histological analysis of 30 of these specimens showed that, irrespective of the season, in all 30 dissected intersexes only the complete male genital apparatus is present. They contain neither ovaries nor any traces of oviducts. The ectodermal integument of the external female gonopore is similar to the genital opening of a regular female. However, the characteristic long, simple setae located adjacent to the opening are missing. In some of the intersexes gonopods are very small and appear not to be functional, but these specimens also possess fully developed male gonads.

There was one exception to the above-described morphology among the 4,368 specimens examined. That specimen had gonopods on one side of the body and female genital ducts on the other, and was carrying eggs. Due to poor fixation we could not examine its inner reproductive organs, but we suspect that this might be the only functional hermaphrodite.

A toxicological analysis by the International Graduate School, Laboratory for Environmental Analytical Study (Zittau, Germany) revealed that no traces of tributyltin (TBT) could be found in *Galathea intermedia*.

DISCUSSION

Spermatophores of *Galathea intermedia* belong to the predominant spermatophore type observed in

anomuran crabs, which was described by Mouchet (1930, 1931), Balss (1944), Greenwood (1972), Tudge (1991, 1995, 1999a,b), Tudge and Jamieson (1996a,b), and Subramoniam (1993) as pedunculate or stalked. Spermatophores of this type consist of a stem and a large sperm-filled ampulla, which is composed of two halves joined along their lateral ridges (Tudge, 1999a). This suture line or spermatophore ridge is the point of weakness, where the ampulla breaks to release the spermatozoa prior to fertilization (Tudge, 1991, 1995, 1999a,b).

As opposed to aspects of spermatophore formation and spermatozoan release, few data exist on the mechanisms by which the spermatophores are transmitted from the male gonopore to the female sternum. The only detailed description was given by Hess and Bauer (2002) for *Clibanarius vittatus*. Their investigation indicated that in *C. vittatus* the spermatophoric mass is applied onto the sternal surface directly from the male gonopores without using gonopods or other intromittent structures. According to Hess and Bauer (2002), males of *C. vittatus* do not have first pleopods and the single second pleopod shows no distinct modifications. During copulation, the male gonopores are closely attached to the female sternum and spermatophores are fixed onto the sternum with adhesive seminal secretions.

The situation in *Galathea* differs from that model. As opposed to the situation in *Clibanarius,* first and second pleopods are well developed and are strongly modified into gonopods. Brandes (1897) first described the transfer and deposition of the spermatophoric mass onto the sternum of the female by the fifth pereiopods in *Galathea strigosa*. According to his account, the gonopods also do not play any discernible role in this process. Due to the striking similarities of the morphology of the sexual organs between both species, we assume the same situation exists in *G. intermedia*. However, the present study suggests that the well-developed gonopods in males very likely must play some role in spermatophore transfer or in related activities.

As opposed to the situation in *Clibanarius vittatus*, the spermatophoric mass in *Galathea intermedia* is transmitted to the female without any additional seminal secretion and is fixed to the female sternum with the basally connected peduncles (Brandes, 1897). However, before extrusion of the male gonopores the spermatophoric mass is ensheathed with seminal secretions, forming a long ribbon. Such a configuration of the spermatophore mass has been described by Brandes (1897) for *G. strigosa* and by Bott (1940) for *Pagurus prideaux*. The contrast between the ribbon totally sheathed by seminal secretions within the distal vas deferens and ductus ejaculatorius, and the free spermatophores on the female sternum connected only at their bases, suggests that the seminal secretions must be dissolved between extrusion from the male gonopore and deposition on the sternum of the female by the male fifth pereiopods.

We assume that transformation of the spermatophoric ribbon prior to deposition is performed by the gonopods: the glandular tissues in the second gonopod open into a groove, which presumably faces the spermatophore ribbon, when attached to the first gonopod cavity. The length of this groove allows impregnation of a long piece of spermatophore ribbon with secretions. This is possible because of the torsion of the second gonopod, so that both cavities have the same orientation. The marginal teeth of this second cavity on the dorsal side of the second gonopod stem, connected by strong musculature, probably hold the ribbon during treatment with secretions. We therefore conclude that the gonopods pick up the ribbon extruded from the genital opening and hold it between their terminal segments prior to treatment by these secretions. This "pick up" is done with a small dorsal spoon-like invagination of the first gonopod, as shown in Figure 6E,F. The gonopods probably also fragment the ribbon by bending it before or after the preparation process. The subsequent deposition of the prepared ribbon fragments on the sternum of the female occurs close to the latter's genital openings (Brandes, 1897).

Fertilization in *Galathea intermedia* thus is external and most presumably occurs after oocytes are actively extruded from the oviducts, on their way to the pleon. Active transport of the oocytes through the female duct in female *G. intermedia* is indicated by the presence of contractile elements attached to the oviduct and the musculature attached to the bulge.

According to our observations, oogenesis in *Galathea intermedia* corresponds to the common pattern reported in other decapod crustaceans (see Krol et al., 1992). Thus, at the time of oviposition, mature oocytes are transported through the terminal oviduct by muscle action in order to be released. After their release they pass over the spermatophores, thereby stimulating the bursting of the ampullae through the unzipping of the lateral ridge, as described for several Paguroidea (Tudge, 1991, 1995, 1999a,b), and more specifically for *Clibanarius vittatus* (Hess and Bauer, 2002). This procedure results in the release of the spermatozoa.

Bott (1940) investigated copulation and oviposition in *Pagurus prideaux*. He described similar spermatophores to those of *Galathea intermedia* and discussed the mechanism of dehiscence as an apical opening of the spermatophore. The mechanism of sperm release from the spermatophores in anomuran crabs has long been controversial. Hamon (1937) studied this process in *P. prideaux,* and considered factors such as mechanical and enzymatic digestion of the spermatophoric envelopes. Despite numerous histochemical studies (see Hinsch, 1991a,b; Subramoniam, 1991, for reviews), the dehiscence mechanisms of spermatophores remain enigmatic. Adiyodi and Subramoniam (1983) studied the bursting of spermatophores in *Emerita* sp. They report no spermatheca in this species, but there is a highly glan-

dular oviduct, which lubricates oocyte passage and facilitates spermatophore opening on the sternum. We assume a similar causal connection in *G. intermedia* between oviduct secretions and dehiscence of spermatophores. Most probably the oocytes are being impregnated with these secretions during transport through the oviduct.

The cause of the observed external intersexes in a small number of *Galathea intermedia* is still obscure. Toxicological effects caused by TBT, as documented for molluscs (Oehlmann et al., 1995, 1998; Bettin et al., 1996), Cladocera (Oberdörster et al., 1998), Copepods (Moore and Stevenson, 1994), or mysids (Davidson et al., 1986) can be excluded, as our toxicological analysis revealed no traces of TBT in specimens from our study area. However, nearly all external intersexes are functional males. In consequence, castration effects caused by parasites, as described by Reverberi (1949) or Giard (1887), can also be excluded.

Presently, the best explanation for these functional males is based on unusual changes in the activity of the androgenic gland, as described by Sagi et al. (1996a,b) for the crayfish *Cherax quadricarinatus*. During embryonic development of Malacostraca, all animals possess primordial gonads, shared by both sexes, including primordial androgenic glands (Charniaux-Cotton, 1959; Juchault, 1967). In the genetic male, the androgenic gland develops and androgenic gland hormone (AGH) is synthesized. On the other hand, in the genetic female the primordial androgenic gland does not develop and AGH is not synthesized. Male and female differentiation is directly determined by the respective action or inaction of AGH on the primordia (Charniaux-Cotton, 1965; Legrand and Juchault, 1972; Katakura, 1984; Charniaux-Cotton and Payen, 1985).

In this context, the findings on investigations of intersexes of *Cherax quadricarinatus* by Sagi et al. (1996a,b) are highly interesting. As in *Galathea*, these *Cherax* intersexes are always functional males with male and female gonopores. The explanation for this phenomenon was that the intersexes are genetic females with active androgenic glands (Sagi et al., 1996a,b, 1997, 1999, 2002; Khalaila et al., 1999, 2001; Abdu et al., 2002). The intersexes of *C. quadricarinatus* can be induced by the implantation of androgenic glands in females (Khalaila and Sagi, 1997; Sagi et al., 2002).

These results could also be an explanation for the intersex phenomena in *Galathea intermedia*, but this is presently speculation, as no experimental approach has been taken by us. Therefore, for the causal interpretation of the intersexes observed here, physiological examinations of the hormone system and their effect on the development of sexual characters would be needed, which are beyond the scope of our study.

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