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# Ultrastructure of the seminal receptacle and the dimorphic sperm in the commensal bivalve *Mysella bidentata* (Veneroida; Galeommatoidea; Montacutidae)

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**Abstract**

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The seminal receptacle and the euspermatozoa and paraspermatozoa of *Mysella bidentata* were examined at an ultrastructural level and the results were compared with earlier findings of the same and other species of the Montacutidae. The euspermatozoon has a slender 13 μm long nucleus and a 1.1 μm long bullet-shaped acrosome. The acrosome of the paraspermatozoon is almost identical in ultrastructure to that of the euspermatozoa but is longer (1.9 μm) and more slender and is bent at an angle to the diminutive nucleus (1.1 μm long). The unpaired seminal receptacle is lined by a heavily ciliated epithelium and a non-ciliated epithelium with short and broad microvilli. Euspermatozoa only are stored in the receptacle. They are densely packed and orientated with their heads towards the non-ciliated epithelium. In this position they develop numerous extremely fine microvilli from the acrosome which apparently serve to attach them to the epithelial microvillar surface. Stored sperm may presumably remain functional for at least six months. A possible function of paraspermatozoa could be to clump sperm into sperm bags to keep them in suspension.

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## Introduction

*Mysella bidentata* (Montagu) is a small montacutid bivalve which lives in association with various burrowing invertebrates, such as polychaetes, sipunculids and echinoderms (Ockelmann and Muus 1978). In the Øresund (the Sound, separating Denmark and Sweden) *M. bidentata* occurs together with the brittle star *Amphiura filiformis* (O. F. Müller). During its second year, the species functions as a male but when it reaches three years old and is 2–3 mm long, it changes its sex to become hermaphroditic (Ockelmann and Muus 1978). The ova are spawned into and brooded within the suprabranchial cavity. Sperm transfer presumably involves sperm bags and sperm are stored in a seminal receptacle within the floor of the suprabranchial chamber.

Ockelmann and Muus (1978) observed that *M. bidentata* produces two types of sperm. Sperm dimorphism occurs in a few other species of commensal bivalves as well (Ockelmann 1965) but in none of these species have the two types of sperm cells been studied at an ultrastructural level. The present paper describes the fine structure of the dimorphic sperm in *M. bidentata*, for which we have used the terms euspermatozoa and paraspermatozoa, introduced by Healy and Jamieson (1981). In addition, we have examined the ultrastructure of the seminal receptacle and the relations between the sperm and the receptacle. The results of the study are compared to those obtained for other Montacutidae.

## Materials and Methods

Many 2.0–3.0 mm long specimens were dredged on 9 December 1999 off Hellebæk, N. Øresund, at a depth of 27 m. All belonged to the m-form of Ockelmann and Muus (1978). For light microscopy, three specimens were preserved

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in Bouin's fluid, embedded in paraplast and cut into 8-µm serial sections that were stained with haematoxylin and eosin. For transmission electron microscopy (TEM) eight specimens were fixed in 2.5% glutaraldehyde in a 0.1 M cacodylate buffer and postfixed in 2% osmium tetroxide in a 0.1 M cacodylate buffer. Ultrathin sections, cut on a Leica Ultracut UCT microtome, were treated with uranyl acetate and lead citrate for contrast and examined with a JEOL-100 SX electron microscope.

## Results

### *The reproductive organs and seminal receptacle*

The reproductive organs of *Mysella bidentata* consist of left and right halves that are fused dorsally for part of their lengths. In the hermaphroditic phase the gonad develops into a true ovotestis in which the male component occupies almost all of its dorsal fused portion (Fig. 1). In the major part of the left and right separate halves, the oocytes develop exclusively along the median wall of the ovotestis while the testis is more or less restricted to the lateral walls. The ovarian parts have minor extensions into the basal and central portion of the foot at each side of the byssus gland.

The seminal receptacle is a curved, transversely placed sac or tube, blind at both right and left tapering ends. It is placed posteriorly and superficially in the visceral mass, between the floor of the suprabrancial cavity and the two fused posterior foot retractors. Two (right and left) narrow, ciliated ducts issue from its broader middle part to open at each side into the suprabranchial cavity (Fig. 1). Under the light microscope two different epithelia are distinguishable. All over its dorso-posterior wall the receptacle is lined with a non-ciliated cuboidal epithelium of relatively large cells with large nuclei. Along its ventro-anterior wall the epithelial cells are lower, have smaller nuclei and are heavily ciliated. This same type of epithelium also lines the two ducts and the immediate area of the receptacle from which they issue. The receptacle contained only euspermatozoa, which were associated with the non-ciliated epithelium, orientated with their elongated heads pointing towards its surface. Paraspermatozoa were never found.

### *Ultrastructure of the sperm*

Both eu- and paraspermatozoa from the testis and euspermatozoa from the seminal receptacle of hermaphroditic specimens were studied (euspermatozoa only because the paraspermatozoa never enter the receptacle). Although spawning of eggs mainly occurs from June to September (Ockelmann and Muus 1978) eight of the 11 specimens taken in December had the testis well developed and/or the receptacles full of sperm.

The euspermatozoon from the testis has an almost straight and very elongate head (Figs 2A, 3A). Probably due to shrinkage caused by fixation and dehydration, the total length of the head in our preparations is 15–16 µm, compared to 20–22 µm measured from live material by Ockelmann and Muus (1978). According to the same authors, the tail filament of live sperm is 70–75 µm long. The head consists of a bullet-shaped acrosome, 1.1 µm long and 0.5 µm wide, a nucleus, ≈ 13 µm long and 0.7–0.8 µm wide, and a middlepiece, 1.5 µm long and 1.0 µm wide.

The acrosome is composed of an acrosomal vesicle and associated subacrosomal material. The acrosomal vesicle is invaginated along its base to become V- or U-shaped in longitudinal section (Figs 3B, 4A). Its blunt tip contains a relatively electron-opaque material with no apparent internal structure. Most of the vesicle is made up of a homogeneous and less electron-opaque substance. Near the middle of the acrosomal vesicle and apposed to the hollowed-out inner vesicular membrane is an electron-dense, ring-shaped zone, which is nearly oval in section (Figs 2B, 4A). Immediately anterior to this zone is another, much thinner, ring of similar diameter, which contains an electron-translucent material (Fig. 2B). The basal invagination of the acrosomal vesicle is occupied by a flocculent mass of subacrosomal substance which is most condensed basally.

A 0.2-µm gap separates the acrosome from the anterior tip of the nucleus. The chromatin is fully condensed. The diameter of the nucleus is almost uniform throughout its length (Figs 2A, 3A). There is a small apical fossa but no basal fossa. Profiles of the middlepiece show a maximum of five to seven mitochondria (Fig. 2C). There are two centrioles, of which the distal one gives rise to the flagellum (Fig. 4B,C). The proximal centriole lies transversely to the distal centriole and at the base of the nucleus.

The paraspermatozoon has almost the same structural elements as the euspermatozoon, but the dimensions differ considerably (Figs 3D, 4A). The head is not straight but is always bent at an angle of up to 90°, or even more, at the boundary between the acrosome and the nucleus (Figs 3C, 4A). In live sperm this was only observed when the sperm were stuck to a surface (Ockelmann and Muus 1978). Compared to the euspermatozoa, the acrosome is more slender (a maximum of 0.35 µm at its base) and elongated to a length of 1.8–1.9 µm. The fully condensed nucleus has the shape of a short cylinder, 1.1 µm long and 0.35 µm wide. The middlepiece is shorter (0.9 µm long), but is structurally identical to that of the euspermatozoon.

The basal invagination of the acrosomal vesicle containing subacrosomal material is almost as deep as the vesicle is long and the electron-dense material present at the tip of the vesicle in the euspermatozoa is absent. The two ring-shaped (electron-dense and electron-translucent) zones are more terminally placed (Figs 3D, 4A). The subacrosomal substance is more condensed basally than in the euspermatozoa. Ockelmann and Muus (1978) stated that the tail filament was considerably longer than in the euspermatozoa, namely 125–130 µm.


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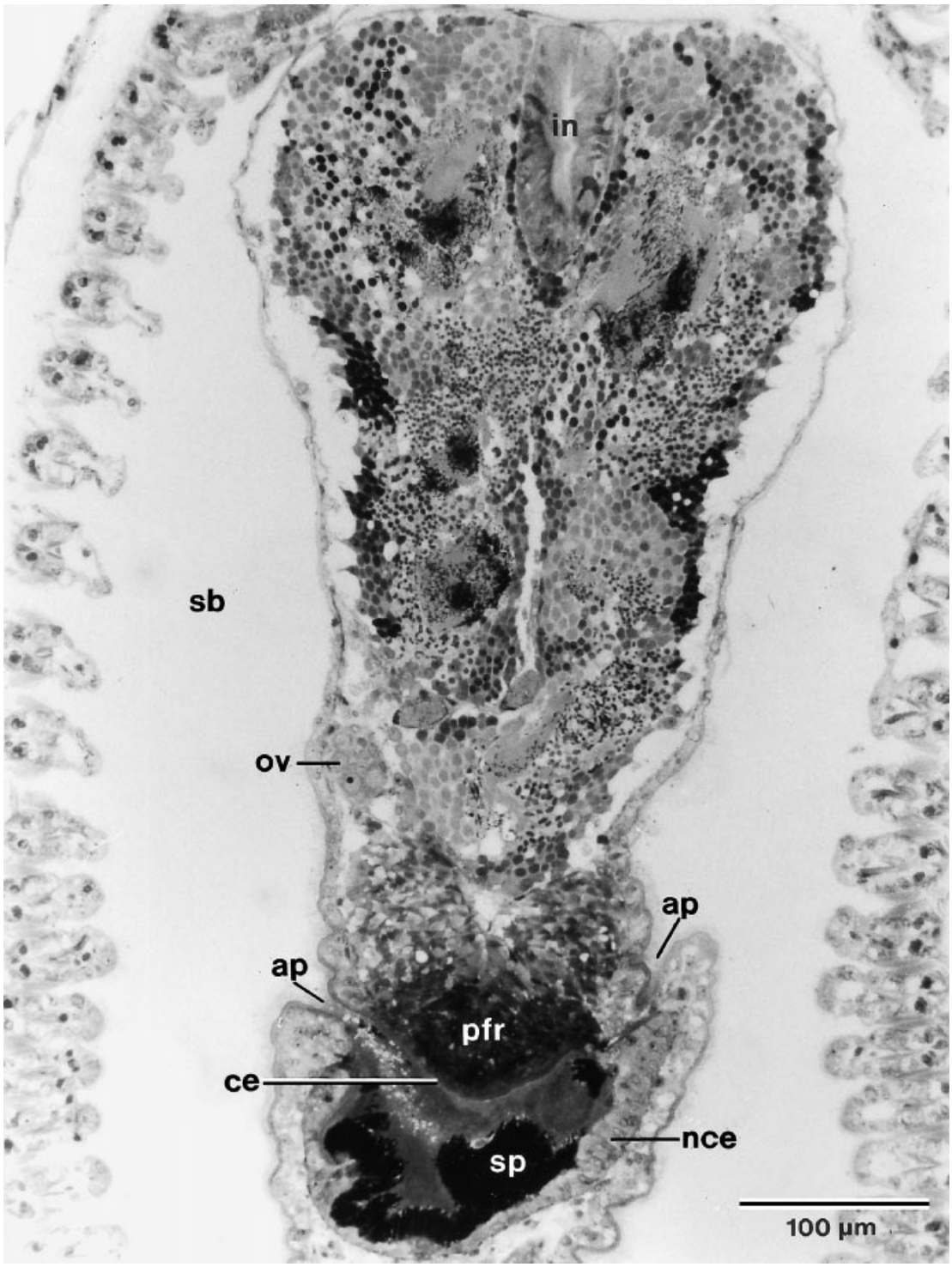


**Fig. 1**—Transverse section through testis, small part of the ovary, and the seminal receptacle of a hermaphroditic *Mysella bidentata*. ap, apertures of seminal receptacle; ce, ciliated epithelium of the receptacle; in, intestine; nce, non-ciliated epithelium of the receptacle; ov, ovary; pfr, posterior foot retractor; sb, suprabranchial cavity; sp, sperm in the receptacle; 2-µm araldite section, stained with toluidine-blue.

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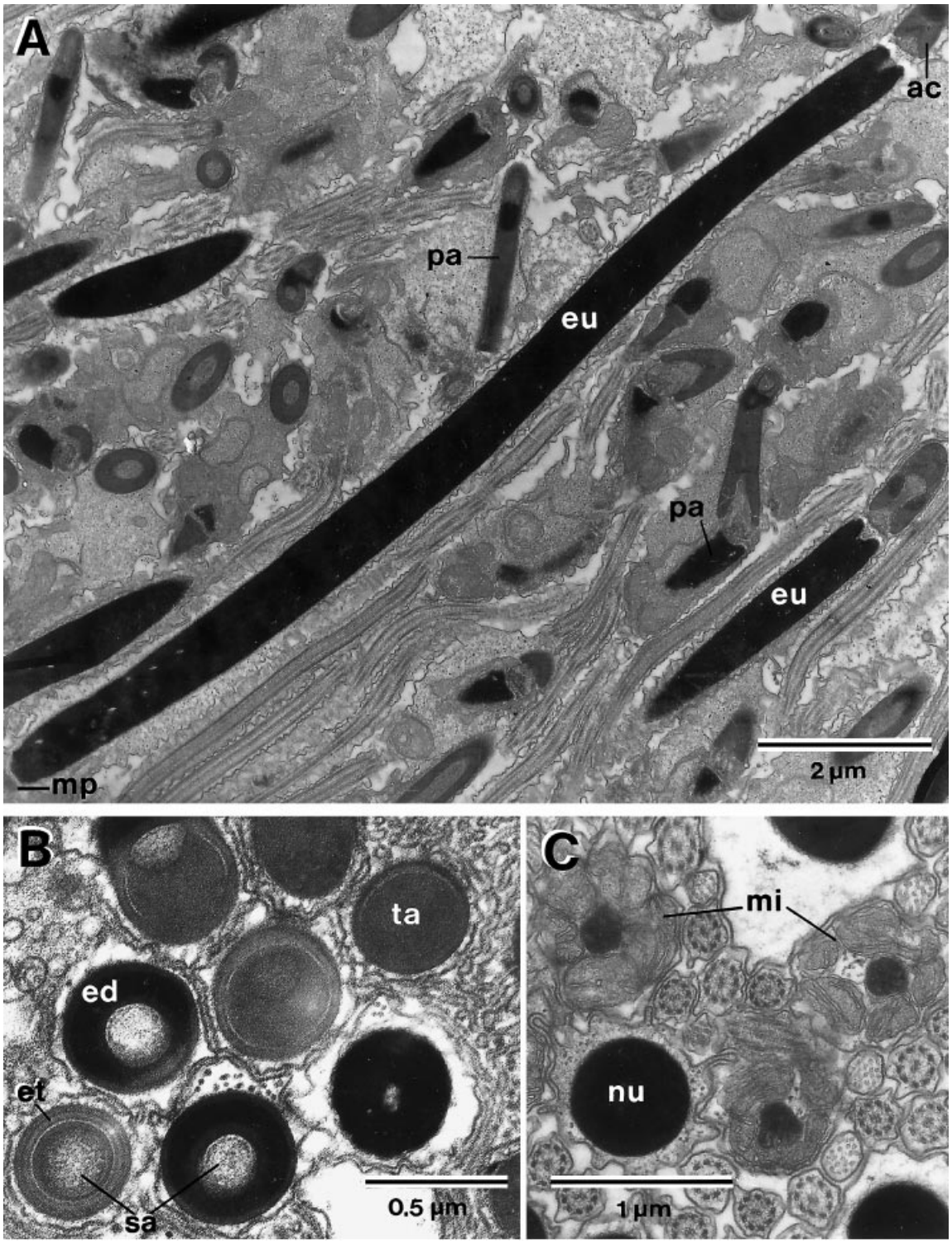


**Fig. 2**—TEM photographs of the testes of *Mysella bidentata*. —**A**, Oblique, transverse and longitudinal sections through several sperm cells. —**B**, Transverse sections through different levels of the acrosomes of euspermatozoa. —**C**, Transverse sections through nucleui and middlepieces of euspermatozoa. ac, acrosome; ed, electron-dense ring-shaped zone; et, electron-translucent ring-shaped zone; eu, euspermatozoa; mi, mitochondria; mp middlepiece; nu, nucleus; pa, paraspermatozoa; sa, subacrosomal material; ta, tip of acrosome.


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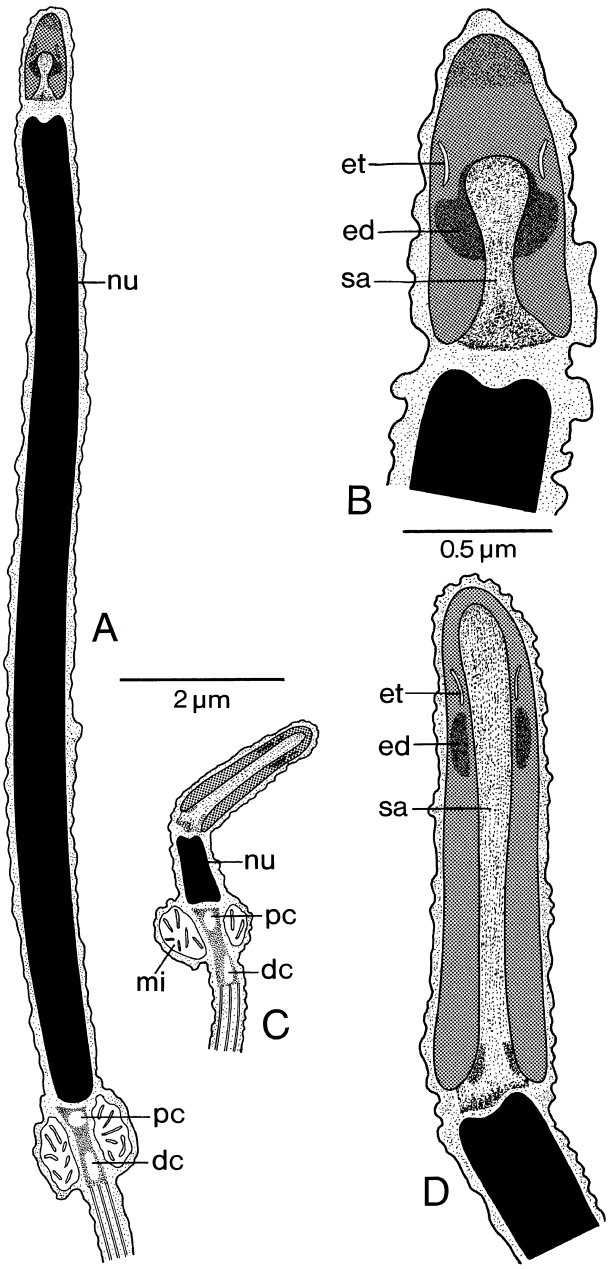


**Fig. 3**—Scheme of the sperm of *Mysella bidentata*. —**A** and **B**, Longitudinal sections through euspermatozoa, —**C** and **D**, similar sections through paraspermatozoa, dc, distal centriole; ed, electron-dense ring-shaped zone; et, electron-translucent ring-shaped zone; mi, mitochondrion; nu, nucleus; pc, proximal centriole; sa, subacrosomal material. Drawn by Beth Beyerholm.

*Ultrastructure of the seminal receptacle and the stored sperm*

The ciliated epithelium consists of 4.5–6.0 µm high semi-cuboidal cells with flattened nuclei (Fig. 5A). The cilia are abundant, close-set and regularly arranged. The apical zone of the cells contains many small electron-dense inclusions and numerous spherical mitochondria. Throughout the cytoplasm, but especially basally, there are smaller or larger profiles of glycogen granules.

The non-ciliated epithelium consists of 6.0–8.0 µm high cuboidal cells. The apical plasma membrane is thrown into many, sometimes bifurcate, regularly spaced, mostly equally long (≈ 1 µm) and fairly thick (0.10–0.15 µm) microvilli (Fig. 5C). The microvilli, which have no microfilament core, are separated by a flocculent material.

All of the six sectioned receptacles contained euspermatozoa, while paraspermatozoa were absent. The euspermatozoa are generally orientated with their long axis perpendicular to the non-ciliated epithelium (Figs 1, 5B). Their acrosomes are never in direct contact with the epithelial microvillar surface but are separated from it by a 0.5–1.0 µm thick zone of intertwined, straight or twisted and extremely thin (0.03 µm) microvilli (Fig. 5B,C). High-resolution electronmicrographs show that these microvilli arise as extensions from the sperm cell membrane of the acrosome, especially its tip (Fig. 5B). No such microvilli were ever seen in either euspermatozoa or paraspermatozoa while in the testis. Membrane fusion between the acrosomal microvilli and the much thicker epithelial microvilli was never observed with certainty. However, there are often close contacts between the two. There is no indication that either of the types of epithelia are engaged in resorption of the sperm cells.

## Discussion

According to observations on live material by Ockelmann and Muus (1978), the sperm of *M. bidentata* are encapsulated within sperm bags that have a 2–4-µm thick flexible wall and a single opening. The transfer of such bags from one to another individual was never observed, but presumably nevertheless occurs since the purpose of producing such bags otherwise becomes puzzling and because similar sperm bags were seen to be inhaled by specimens of *M. tumida* (Carpenter) (Ó Foighil 1985b). In *M. bidentata* the bags attach to the inner lamella of one of the demibranchs. When emitted from the bags, the sperm first settle upon the gills but ultimately enter the seminal receptacle. In *M. tumida* there is no seminal receptacle and the sperm cells are stored upon the ascending lamellar gill filaments of the recipient animal (Ó Foighil 1985b).

Seminal receptacles are of rare occurrence among bivalves. With the possible exception of *Xylophaga dorsalis* Turton (superfamily Pholadoidea), species with sperm receptacles fertilize and incubate the ova within the mantle cavity or suprabranchial cavity. They are either known to reproduce by outcrossing, or at least believed to do so. The species in which receptacles occur, or are justly suspected to occur, are listed by Jespersen and Lützen (2000). They all belong to the family Montacutidae of the superfamily Galeommatoidea, but we have recently found them also in a species of *Pseudopythina* (family Kellidae, also superfamily Galeommatoidea)

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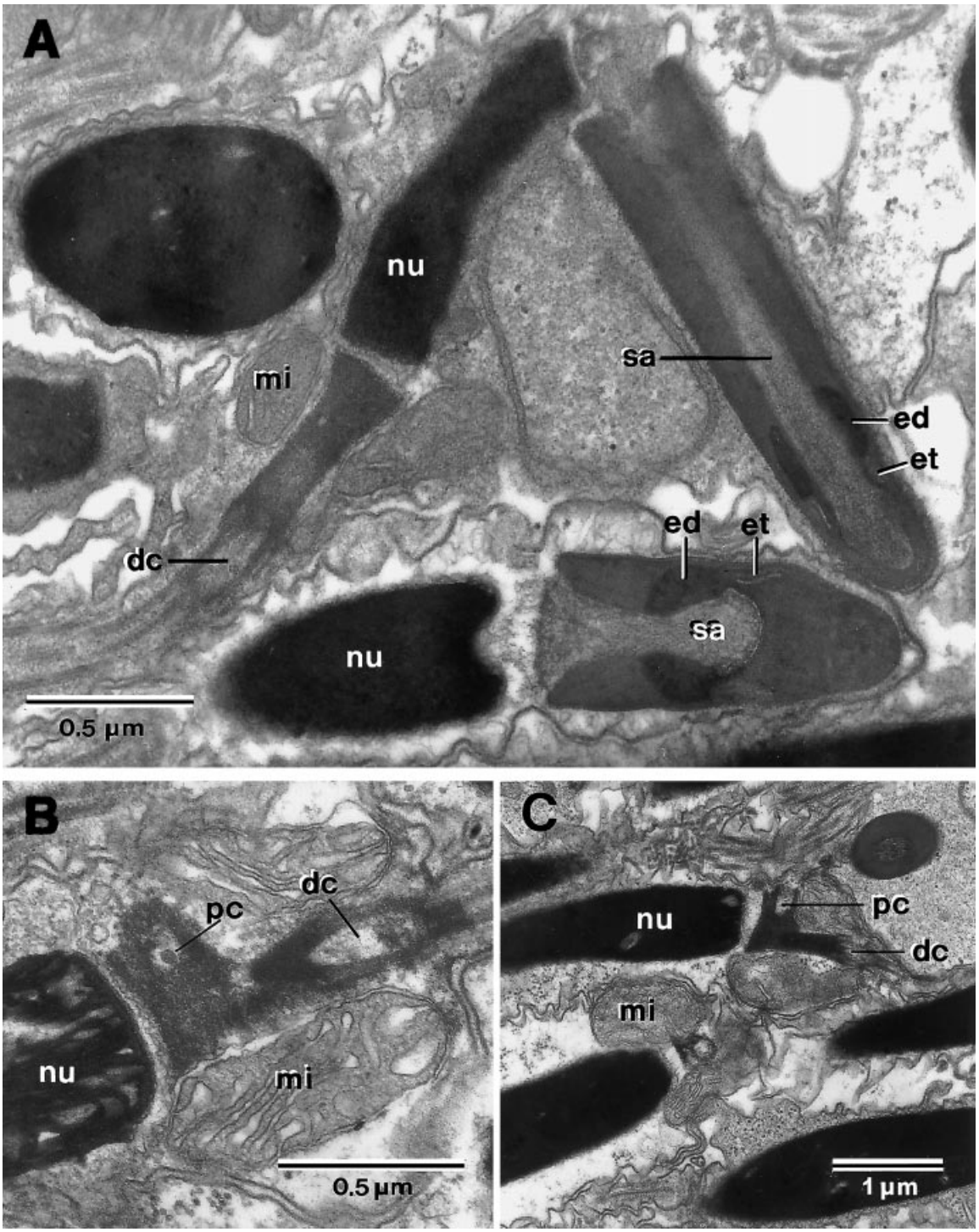


**Fig. 4**—TEM photographs of *Mysella bidentata*. —**A**, Longitudinal section through head of a paraspermatozoon (top) and a euspermatozoon (bottom). —**B**, Longitudinal sections through a middlepiece of a spermatid. —**C**, Longitudinal sections through middlepieces of two euspermatozoa. dc, distal centriole; ed, electron-dense ring-shaped zone; et, electron-translucent ring-shaped zone; mi, mitochondrion; nu, nucleus; pc, proximal centriole; sa, subacrosomal material.


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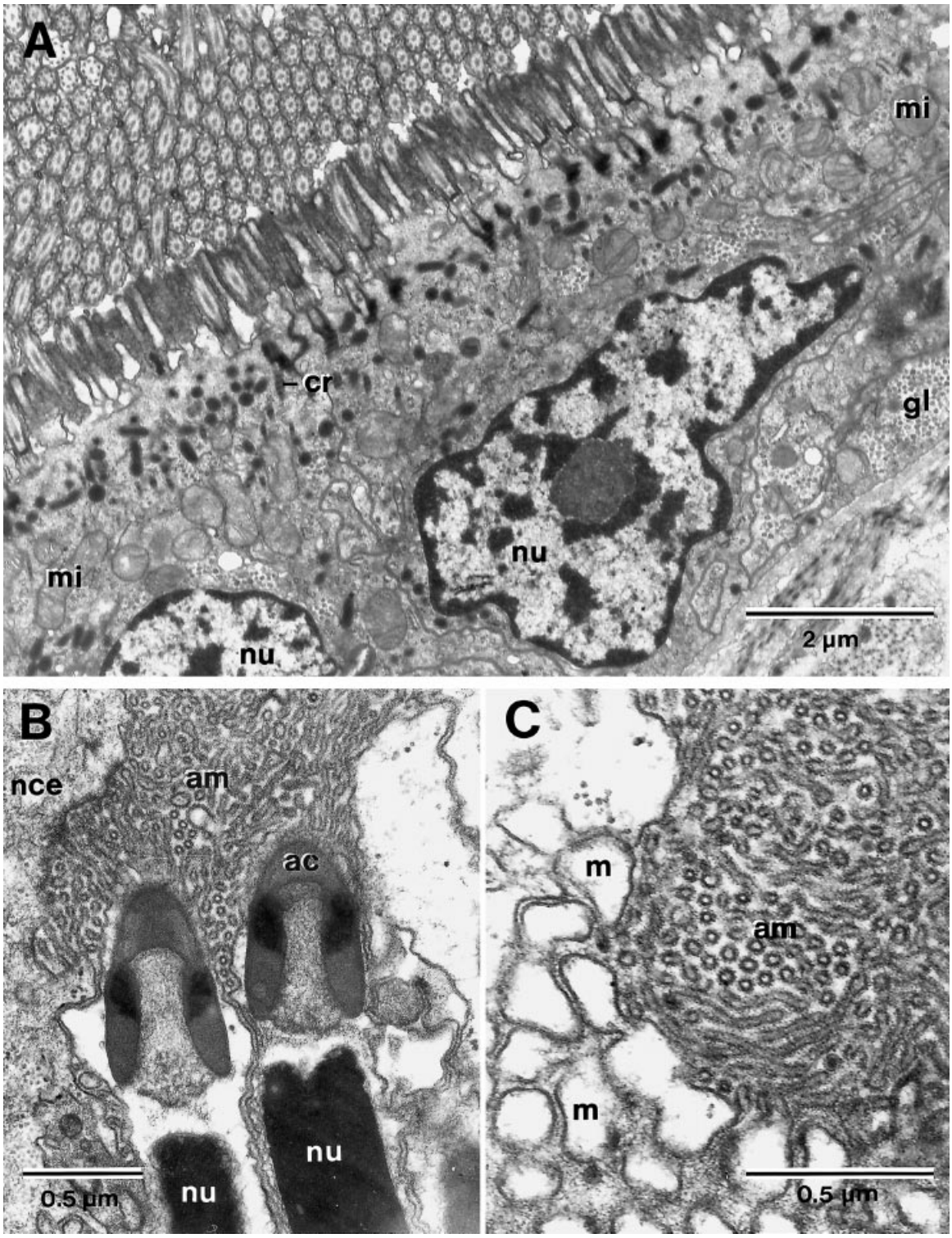


**Fig. 5**—TEM photographs of epithelial walls of seminal receptacle of *Mysella bidentata*. —**A**, Ciliated epithelium. —**B**, longitudinal sections through two euspermatozoa with their acrosomal microvilli adjacent to surface of a non-ciliated epithelial cell. —**C**, acrosomal microvilli (right) in close contact with microvilli of a non-ciliated cell (left). ac, acrosome; am, acrosomal microvilli; cr, ciliary roots; gl, glycogen granules; m, microvilli of non-ciliated epithelial cell; mi, mitochondria; nce, non-ciliated epithelium; nu, nucleus.

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(Jespersen *et al.* 2001). The receptacles of these species are most often pouches in the visceral mass with narrow ciliated openings into the gill chamber near the genital apertures. Though normally paired, in *Mysella cuneata* (Verrill and Bush) and *M. bidentata* they are single pouches.

Pérès (1937) and Deroux (1961) incorrectly described the seminal receptacle ('annexe séminale') of *M. bidentata* as a transverse, ciliated fold in the roof of the mantle cavity connecting the posterior extremity of the inner lamella of right and left demibranchs. The present study supports the view of Ockelmann and Muus (1978) that the receptacle forms a closed sac except for its left and right openings. Deroux (1961) doubted whether it functioned as a true receptacle and suggested that it might represent the production site for the bags ('spermatophores') encapsulating what would then have to be autosperm. This can be rejected for two reasons: (1) there is no glandular epithelium within the receptacle that could possibly manufacture such bags and (2) while the bags contain both types of sperm, the receptacle only contains euspermatozoa.

There is probably not a single answer to the question why some brooding species have evolved seminal receptacles and other have not. However, true receptacles in which the sperm are protected and can be cared for, presumably satisfy the need for long-term sperm storage better than unspecialized storage sites. Ó Foighil (1985b) noted that the advent of sperm storage preceded by only one month the onset of brooding in *M. tumida*, which has no true seminal receptacle. By contrast, ripe sperm in the testes and sperm bags on the gills are prevalent in *M. bidentata* almost all year round while brooding is restricted to four summer months (Ockelmann and Muus 1978). The sperm found in the receptacles in most of our specimens from December would have to survive for almost 6 months to fertilize the first ova spawned next June. It has been calculated that in the commensal bivalve *Peregrinamor ohshimai* Shôji, sperm remain active and viable for a minimum of 3–4 months in the female's seminal receptacles (Lützen *et al.* in press).

An earlier description of the sperm of *M. bidentata* by Ockelmann and Muus (1978) based on light microscopy in most details corresponds with the present investigation. The size of the acrosome was underestimated by Ockelmann and Muus and we found no dictyosomes (Golgi stacks) associated with the acrosome in the mature euspermatozoa. Among sperm of other montacutid species studied ultrastructurally, the euspermatozoa of *M. bidentata* show a striking similarity with the sperm of *Mysella tumida* (see Ó Foighil 1985a). The same internal structure of the acrosomal vesicle occurs in both species and differs only in dimensions. The nucleus of *M. bidentata* is more elongate ($\geq 13$ μm) and slender ($< 0.8$ μm) than in *M. tumida* (6.1 and 1.1 μm, respectively), which allows packing of a larger number of sperm within the restricted area of the seminal receptacle.

The middlepieces in the two species are identical, except that the cell membrane in this part of the mature sperm of *M. tumida* shows numerous finger-like and sometimes bifurcate microvilli (Ó Foighil 1985a). They are absent in *M. bidentata*. However, the two species' sperm share the unusual feature that, having arrived at the storage site, the plasma membrane develops numerous microvilli at the acrosomal end (Ó Foighil 1985b). In both species, sperm attachment is accomplished when the acrosomal microvilli enter into close contact with the shorter and thicker microvilli of the epithelium of the storage area. Ó Foighil (1985b) speculated that sperm adhesion may be achieved by glycoprotein crosslinking of the epithelial cells and sperm glycocalices. Ockelmann and Muus (1978) noted that, if squeezed out of the receptacle, the sperm of *M. bidentata* were attached to 'plate-like cell material' (probably part of the non-ciliated epithelium loosened and torn off in the process). The sperm remained attached to the plates for some time and with their tails beating were able to propel the plates through the water. Subsequently, but only slowly, individual sperm cells broke away from the plates.

The fine structure of the euspermatozoa of *M. bidentata* and *M. tumida* provides convincing evidence of a close taxonomic relationship between these two species. This is best expressed in the structure of the acrosome and in the presence of acrosomal microvilli used for attaching the sperm head to the storage epithelium. The only other montacutids whose sperm have been studied with the electron microscope, *Montacuta phascolionis* (Dautzenberg) and *Nipponomysella subtruncata* (Yokoyama 1927), have the acrosome organized differently and never develop acrosomally placed microvilli (Jespersen and Lützen 2000; Lützen *et al.* in press).

The fine structure of the paraspermatozoa of *M. bidentata* offers no clue to their possible function in reproduction. They are hardly fortuitous aberrations from the typical process of spermiogenesis since the ultrastructure of the mature sperm is always constant and because they occur in a number comparable to that of the euspermatozoa. They have an apparently normal acrosome and a condensed, although diminutive, nucleus, yet from their total absence from the receptacle [also noted by Ockelmann and Muus (1978)] it is unlikely that they function genetically in fertilization. Paraspermatozoa are much more common among prosobranch gastropods than bivalves but in spite of many studies the answers to their function(s) are mere guesses. However, in some aphallic prosobranch species, especially large and mobile paraspermatozoa serve as carriers of numerous euspermatozoa between specimens of opposite sex. In a species of the bivalve *Pseudopythina* giant paraspermatozoa and small euspermatozoa aggregate to form balls with the sperm filaments projecting from the surface (Jespersen *et al.* 2001). In live sperm from the prosobranch *Goniobasis* laqueta say, (Woodard 1940) demonstrated that the euspermatozoa became entangled in the multiple tails of the paraspermatozoa, which caused clumping and prevented their dispersal. In *M. bidentata*, Ockelmann and Muus (1978) observed that mature sperm consisting of both types in a slightly viscid


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fluid did not disperse, but soon formed a dense sperm bag. It is thus possible that the paraspermatozoa function in promoting sperm aggregations as a means of reducing euspermatozoan diffusion during sperm transfer. It is also possible that beating of the filaments may propel the sperm bags or at least keep them floating in the water of the microenvironment until they are sucked in by a recipient animal. When found on the gills, the sperm bags of *M. bidentata* are immobile. However, newly produced sperm bags have not been seen. Deroux (1961) illustrated a *M. bidentata* bag with some of the sperm filaments protruding from it. If this represents a stage previous to its uptake, a possible function of the paraspermatozoa, which have much longer filaments than the euspermatozoa, could be to keep the bags in suspension.

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