

## Ultrastructure of paraspermatozoa, euspermatozoa and eusperm-like spermatozoa of *Obtortio* cf. *fulva* (Prosobranchia: Cerithiacea)

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**ABSTRACT:** The cerithiacean *Obtortio* cf. *fulva* produces three distinct types of spermatozoa: (1) paraspermatozoa, (2) euspermatozoa and (3) eusperm-like spermatozoa. Like most mesogastropods, euspermatozoa of *Obtortio* are composed of a conical acrosome, short posteriorly invaginated nucleus, elongate midpiece and glycogen piece, and short terminal region. The midpiece, however, is distinctly cerithiacean in structure and is composed of four non-helical midpiece elements. Eusperm-like spermatozoa closely resemble euspermatozoa, but have a very short nucleus only one half to one third the length of the euspermatozoon nucleus. Paraspermatozoa of this species are composed of (1) "head" (mosaic sheath of dense blocks enveloping multiple axonemes which attach anteriorly to a long apical structure), (2) "midpiece" (multiple axonemes interspersed with elongate mitochondria), and (3) multiple tail tuft (axonemes each ensheathed by glycogen granules). The possible role of eusperm-like spermatozoa is briefly discussed together with the taxonomic implications of the structure of the three sperm types.

### INTRODUCTION

Previous light and electron-microscopic studies have shown that two "types" of sperm are produced in the gonads of many meso- and neogastropods – these commonly being referred to as "atypical spermatozoa" and "typical spermatozoa". Healy & Jamieson (1981) briefly reviewed terminology and proposed "paraspermatozoa" and "euspermatozoa" to replace the misleading terms "atypical spermatozoa" and "typical spermatozoa" whilst retaining the term "paraspermatic cell" of Melone et al. (1980) to refer collectively to both paraspermatozoa and the eusperm-bearing "nurse cells" of *Littorina* (see Buckland-Nicks & China, 1977). Occasionally another type of spermatozoon occurs (referred to as "oligopyrene spermatozoa" by Kohnert [1980] and as "microtypical spermatozoa" by Nishiwaki & Tochimoto [1969]) – this type closely resembling euspermatozoa but possessing a significantly shorter nucleus. In the absence of information concerning the function of these spermatozoa, it is proposed to refer to them as eusperm-like spermatozoa. The present study describes the ultrastructure of paraspermatozoa, euspermatozoa and eusperm-like spermatozoa in the sperm duct and gonadal tissues of *Obtortio* cf. *fulva* and is the first report of all three sperm "types" in any cerithiacean snail.

## MATERIALS AND METHODS

Live, mature *Obortio* cf. *fulva* (Watson) were sieved at low tide from mud-sand at Lota (Moreton Bay, South Queensland). After removal of the shells, the anterior half of sperm-containing individuals (that is, sperm duct and gonadal tissues) were immediately fixed for 2 h in cold, phosphate-buffered (0.1 M) 2.5 % glutaraldehyde (crystalline sucrose added to give fixative in 2.5 % sucrose solution). Subsequently the tissue pieces were (1) washed in phosphate buffer (sucrose added), (2) post-fixed in a 1 % phosphate-buffered osmium tetroxide solution (sucrose added) for 80 m, (3) again rinsed in buffer, (4) dehydrated using a graded series of ethanols and (5) infiltrated and embedded in Spurr's medium. All stages up to and including 70 % ethanol were maintained at 0–4 °C and thereafter at room temperature. Ultrathin sections were cut using an LKB IV Ultratome, collected on uncoated 200 mesh copper grids and stained with uranyl acetate and lead citrate. Grids were examined with a Siemens Elmsikop I transmission electron microscope. Voucher specimens are being deposited in the Australian Museum (Sydney).

## RESULTS

## Euspermatozoa and eusperm-like spermatozoa

*Acrosome*

The acrosomal cone of the euspermatozoon is 0.75  $\mu\text{m}$  in length, deeply invaginated and rests on a thin basal plate capping the nuclear apex (Figs 1, 6, 26b; see also Figs 7, 8). The axial rod lies within the cone invagination and is composed of an electron-dense cylinder (sometimes filled with a granular material).

In longitudinal section, the acrosomal cone of the eusperm-like spermatozoon closely resembles that of the euspermatozoon but is noticeably less deeply invaginated (Figs 2, 3, 9, 26d). In transverse sections through and above the acrosomal cone invagination, it is difficult to determine whether such sections are taken through acrosomes of euspermatozoa or eusperm-like spermatozoa (Figs 7, 8).

*Nucleus*

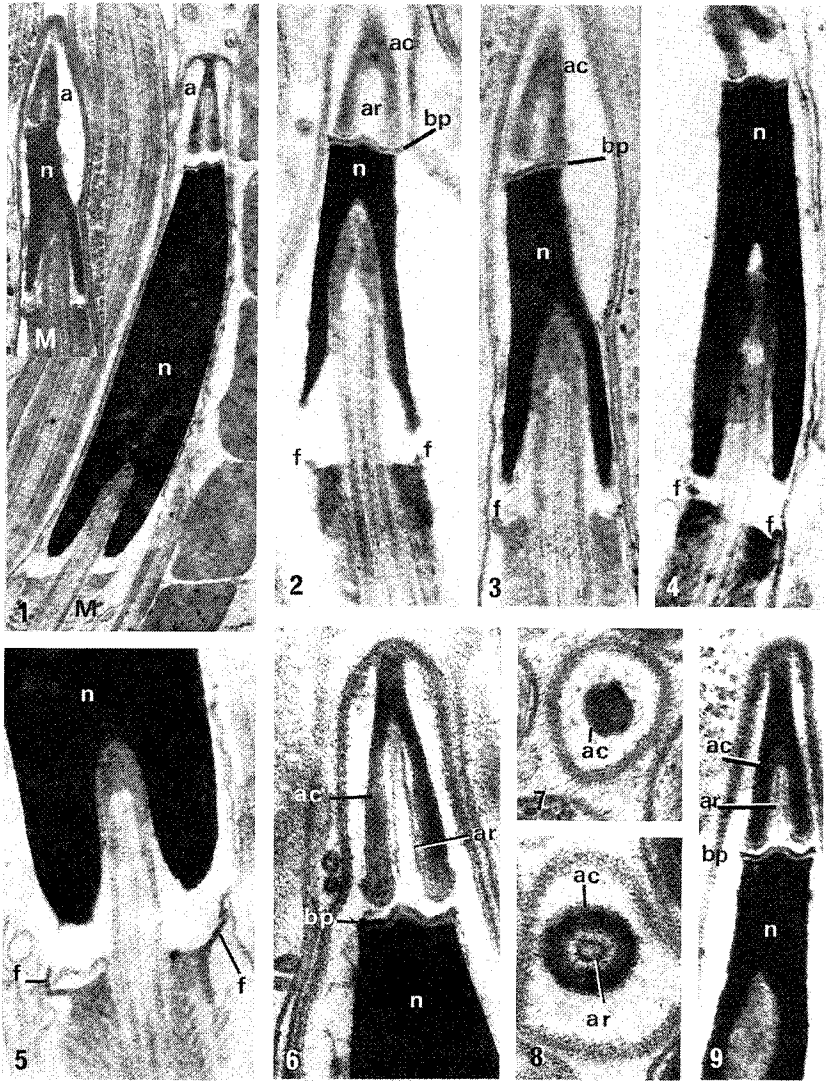
The nucleus of the euspermatozoon is approximately 3  $\mu\text{m}$  in length, invaginated posteriorly and oval in transverse section (Figs 1, 5, 10, 11). Both anteriorly and posteriorly the nucleus tapers slightly. Only the 9+2 microtubular pattern could be detected within the nuclear invagination – this becoming obscured within the innermost portion of the invagination by the dense attachment matrix (Figs 5, 10, 11).

In comparison to euspermatozoa, the nuclei of eusperm-like spermatozoa are very short (see Fig. 1, 26d). There appears to be some variation in the length of nuclei of eusperm-like spermatozoa (1–1.4  $\mu\text{m}$  in length) and consequently the relative depth of the posterior invagination (see Figs 2–4). The posterior invagination of the nucleus contains the proximal portion of the axoneme and dense attachment material (Figs 12, 13) as observed in euspermatozoa.

*Midpiece*

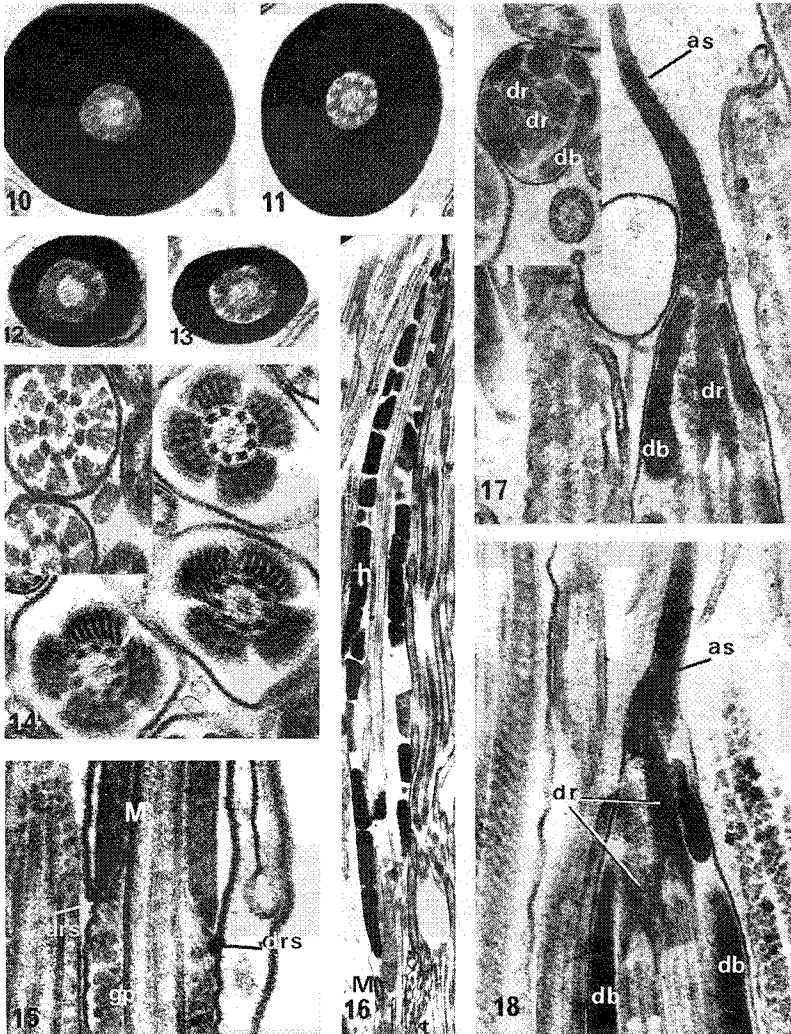
Midpieces of both euspermatozoa and eusperm-like spermatozoa are composed of four, equal-sized midpiece elements (each constructed of curved, parallel cristal plates), non-helicly arranged around the axoneme (Fig. 14). In both euspermatozoa and eus-

perm-like spermatozoa, flange structures form the initial portion of the midpiece elements but do not exhibit any cristal plate component. These flanges are more prominent in euspermatozoa (Figs 5, 26b) than in eusperm-like spermatozoa (Figs 2–4). It does not appear to be possible to differentiate between midpieces of euspermatozoa and eus-



Figs 1–9. 1. Acrosome and nucleus of euspermatozoon. Inset – Acrosome and nucleus of eusperm-like spermatozoon ( $\times 18700$ ); 2–4. Acrosome, nucleus and proximal region of midpiece of eusperm-like spermatozoa showing variation in nuclear length ( $\times 37300$ ); 5. Base of euspermatozoon with attached axoneme. Note midpiece flange structures ( $\times 37300$ ); 6. Acrosome of euspermatozoon ( $\times 42700$ ); 7–8. Transverse sections through anterior (7:  $\times 53300$ ) and posterior (8:  $\times 66700$ ) of acrosomes of either euspermatozoa or eusperm-like spermatozoa; 9. Acrosome and nucleus of eusperm-like spermatozoon ( $\times 37300$ ). Abbreviations: a – acrosome, ac – acrosomal cone, ar – axial rod, bp – basal plate, f – midpiece flange structures, M – midpiece, n – nucleus

perm-like spermatozoa in transverse sections (Fig. 14, 26e) but from phase-contrast light microscopy the length of the eusperm-like spermatozoon midpiece is usually longer (50–60  $\mu\text{m}$ , Fig. 26c) than that of the euspermatozoon (40–45  $\mu\text{m}$ , Fig. 26a).



Figs 10–18. 10–11. Transverse sections of euspermatozoa at anterior (10) and posterior (11) levels of nuclear invagination ( $\times 40000$ ); 12–13. Transverse sections of eusperm-like spermatozoa at anterior (12) and posterior (13) levels of nuclear invagination ( $\times 40000$ ); 14. Transverse section of midpieces of euspermatozoa or eusperm-like spermatozoa ( $\times 27700$ ), Inset – transverse sections of glycogen pieces of euspermatozoa or eusperm-like spermatozoa ( $\times 33300$ ); 15. Junction of midpiece and glycogen piece of euspermatozoon or eusperm-like spermatozoon ( $\times 40000$ ); 16. Complete head of paraspermatozoon ( $\times 6700$ ); 17. Apex of paraspermatozoon ( $\times 27700$ ), Inset – transverse section of dense rods of axonemal attachment complexes ( $\times 24700$ ); 18. Apex of paraspermatozoon showing at least two attached axonemes ( $\times 32000$ ). Abbreviations: as – apical structure of paraspermatozoon, db – dense blocks of mosaic sheath, dr – dense rods of axonemal attachment complexes, drs – dense ring structure, gp – glycogen piece, h – head of paraspermatozoon, M – midpiece

*Dense ring structure, glycogen piece*

From phase-contrast light microscopical studies, both euspermatozoa and eusperm-like spermatozoa possess a glycogen piece proceeding posteriorly from the midpiece. In euspermatozoa, the length of the glycogen piece is approximately 20  $\mu\text{m}$  while in eusperm-like spermatozoa, the glycogen piece is slightly longer (24–30  $\mu\text{m}$ , Figs 26a, c). Occasionally much longer glycogen pieces were observed in eusperm-like spermatozoa (60  $\mu\text{m}$ ). Under the electron microscope it is impossible to differentiate between glycogen pieces of euspermatozoa and eusperm-like spermatozoa – both consisting of nine longitudinal tracts of granules associated with the nine axonemal doublets (Fig. 14, inset; see also Figs 18, 26g). Similarly, the dense ring structure observed at the junction of midpiece and glycogen piece (Fig. 15, 26f) is probably found in both euspermatozoa and eusperm-like spermatozoa. A tail region forms the posterior region of both euspermatozoa (10  $\mu\text{m}$ ) and eusperm-like spermatozoa (20–80  $\mu\text{m}$ ).

## Paraspermatozoa

Each mature *Obtortio* paraspermatozoon is divisible into "head", "midpiece" and "tail tuft" (Figs 16, 22, 26h, i).

*"Head"*

The "head" consists of a variable number of axonemes (5–10, usually 5), surrounded by a mosaic sheath of dense blocks. An elongate, apparently flexible structure forms the apex of the head. Commonly two or three electron-dense attachment rods are observed fixed to this apical structure, anchoring their respective axonemes (Figs 17, 18, 26i, j). Transverse and longitudinal sections through the anterior portion of the head (Figs 17, 19, 26j, k) reveal that the attachment complexes are structurally similar to those demonstrated by Healy & Jamieson (1981) for *Pyraxus ebeninus*, with the exception that in *Obtortio* the 9+2 microtubule pattern of each axoneme persists into the base of each attachment rod. Transverse sections through the middle and lower regions of the head most commonly show four or five axonemes (Figs 20, 11) but occasionally (Fig. 25) up to ten axonemes may be present.

*Midpiece, tail tuft*

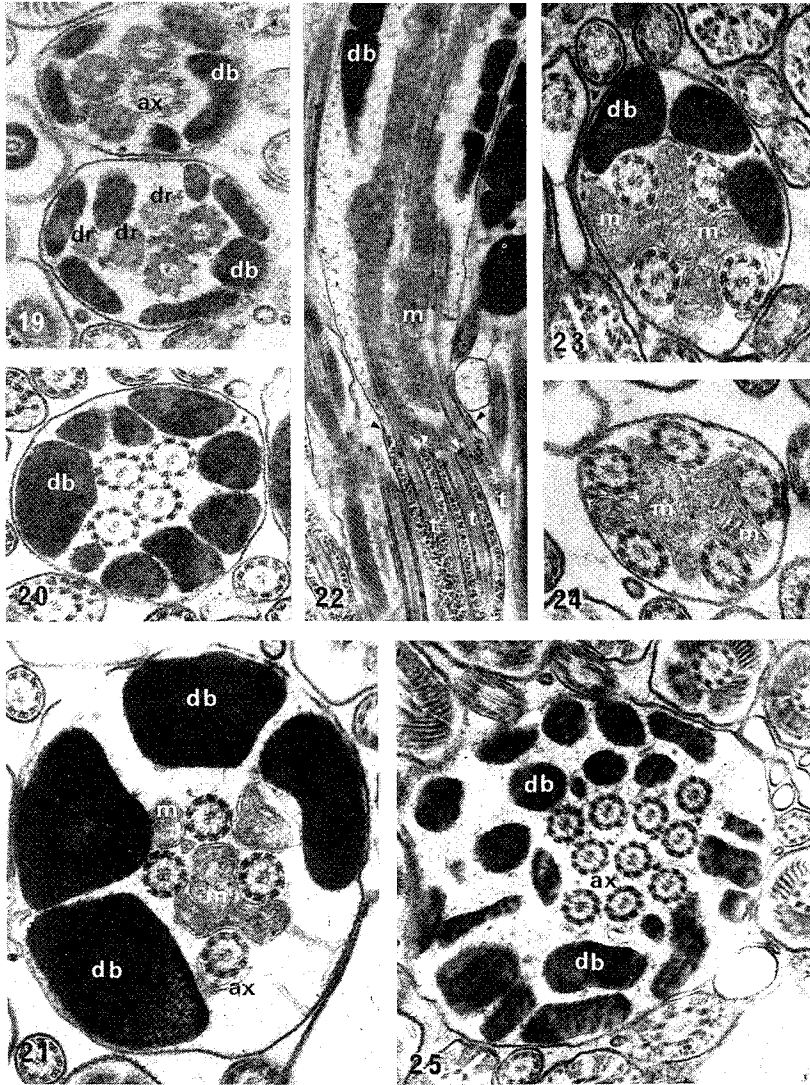
The multiple axonemes in the most posterior region of the head become interspersed with elongate mitochondria, indicating that (sometimes considerable) overlap between "midpiece" and "head" regions does occur (Figs 21–24, 26l). The multiple axonemes emerge from the midpiece region as the tail tuft (Figs 22, 26i, m) – each axoneme ensheathed by a (usually) singular layer of electron-dense (presumably glycogen) granules. Dense ring structures are associated with each emergent tail and are probably homologous to dense ring structures of euspermatozoa and eusperm-like spermatozoa (Fig. 26m).

## DISCUSSION

## Paraspermatozoa

The structure of *Obtortio* paraspermatozoa shows features observed in cerithiacean paraspermatozoa (Healy & Jamieson, 1981; Melone et al., 1980; Yasuzumi et al., 1962), viviparacean paraspermatozoa (Anderson & Personne, 1970, 1976; Gall, 1961; Hachiri &

Higashi, 1972, 1974; Ishizaki & Kato, 1958; Tanaka, 1958; Yasuzumi & Tanaka, 1958; Yasuzumi et al., 1970), and cyclophoracean paraspermatozoa (Selmi & Giusti, 1980). Most possess a mosaic (head) sheath of dense blocks (in paraspermatozoa of *Viviparus*



Figs 19–25. 19. Transverse section through anterior region of heads of two paraspermatozoa ( $\times 33300$ ); 20. Transverse section through middle region of paraspermatozoan head ( $\times 33300$ ); 21. Transverse section of posterior region of paraspermatozoan head showing infiltration of midpiece mitochondria ( $\times 33300$ ); 22. Longitudinal section of posterior portion of head, midpiece and tail tuft. Arrows indicating position of dense ring structures ( $\times 13700$ ); 23. Head-midpiece overlap zone ( $\times 33300$ ); 24. Transverse section of midpiece ( $\times 33300$ ); 25. Transverse section of paraspermatozoan head showing ten axonemes ( $\times 21300$ ). Abbreviations: ax – axoneme, db – dense blocks of mosaic sheath, dr – dense rod of axonemal attachment complexes, m – mitochondrion, t – multiple tails

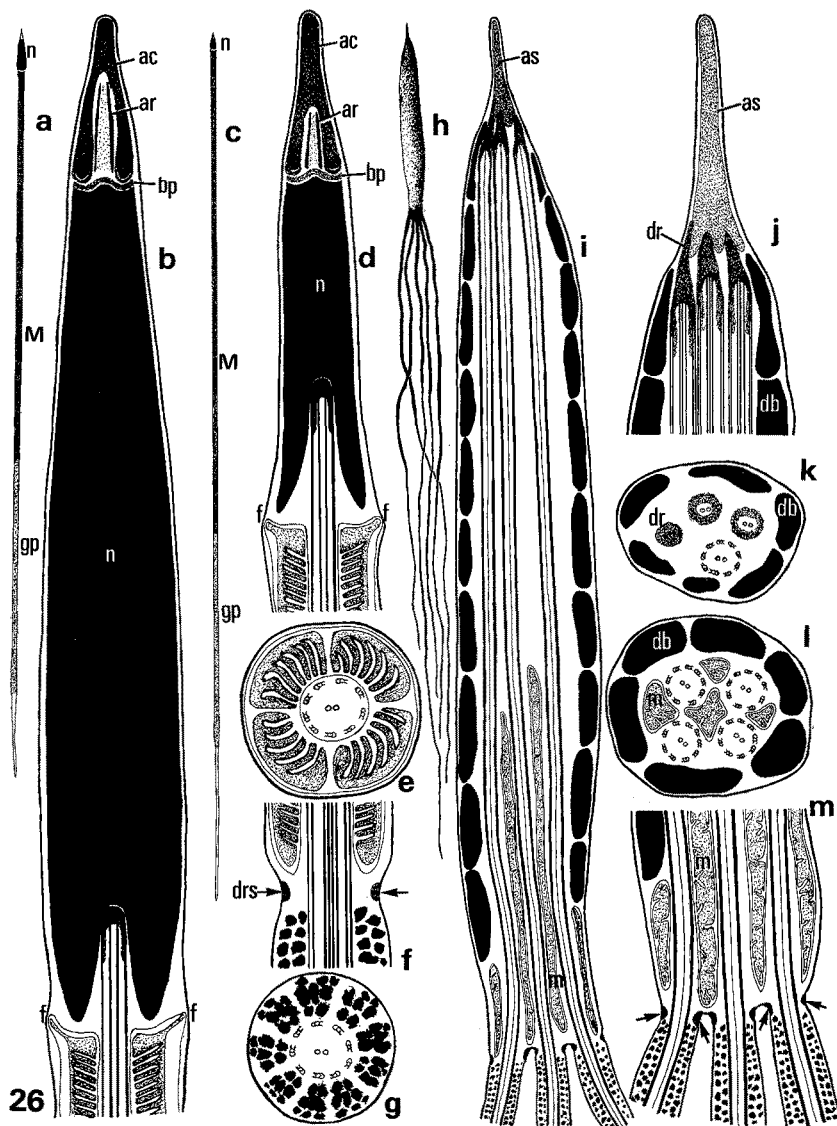


Fig. 26. Euspermatozoa, eusperm-like spermatozoa and paraspermatozoa of *Obtortio cf. fulva* (semi-diagrammatic). a, b: Euspermatozoa. Entire euspermatozoon (a,  $\times 1500$ ), acrosome, nucleus and proximal portion of midpiece (b,  $\times 40000$ ). c, d: Eusperm-like spermatozoa. Entire eusperm-like spermatozoon (c,  $\times 1500$ ), acrosome, nucleus and proximal portion of midpiece (d,  $\times 40000$ ). e: Transverse section of midpiece of euspermatozoon/eusperm-like spermatozoon simultaneously showing the cristal plate structure of all four midpiece elements ( $\times 50000$ ). f: Junction of midpiece and glycogen piece of euspermatozoon/eusperm-like spermatozoon ( $\times 50000$ ). g: Transverse section of glycogen piece showing nine tracts of granules associated with axonemal doublets ( $\times 50000$ ). h-m: Paraspermatozoa. Entire paraspermatozoon (h,  $\times 1500$ ), longitudinal section of head, midpiece and tail tuft (i,  $\times 15000$ ), apical structure with attached axonemes (j,  $\times 30000$ ), transverse section near apex of mosaic sheath of head (k,  $\times 30000$ ), transverse section base of mosaic sheath of head showing intrusion of mitochondria (l,  $\times 30000$ ), detail of midpiece and proximal portion of emergent tails (m,  $\times 20000$ ), arrows showing dense ring structure. Abbreviations: a - acrosome, ac - acrosomal cone, ar - axial rod, as - apical structure (of paraspermatozoon), ax - axoneme, bp - basal plate, dr - dense rod of attachment complex (of paraspermatozoon), f - flange structures of midpiece, gp - glycogen piece, m - mitochondria, M - midpiece, n - nucleus

*malleatus*, these blocks are composed of glycoprotein – Yasuzumi et al., 1970) and all possess multiple axonemes which form a tail tuft. A distinct "midpiece" has also been observed in paraspermatozoa of *Pyrazus ebeninus* (Healy & Jamieson, 1981), other cerithiaceans (Nishiwaki, 1964; personal observations) and may be present in paraspermatozoa of *Turritella communis* (see Melone et al., 1980). In paraspermatozoa of *Pyrazus* (Healy & Jamieson, 1981), *Turritella* (Melone et al., 1980) and *Melania libertina* (Yasuzumi et al., 1962), nuclear material is present in the form of a highly condensed nuclear core which runs almost the full length of the head region and is surrounded by the dense blocks of the mosaic sheath. In paraspermatozoa of *Cochlostoma montanum* (Selmi & Giusti, 1980) the nuclear core is surrounded by axonemes and scattered dense blocks. No nuclear core has been detected in *Obtortio* paraspermatozoa. While the multiple axonemes of *Pyrazus* and *Turritella* paraspermatozoa attach to the nuclear core at or near the base of the head, axonemes of *Obtortio* paraspermatozoa penetrate deep into the mosaic sheath and attach anteriorly to the dense elongate apical structure.

The association of multiple axonemes and nuclear core (or remnant) in *Pyrazus* (Healy & Jamieson, 1981), *Turritella* (Melone et al., 1980), cerithiids (Healy, in preparation), vivipariids (Gall, 1961; Ishizaki & Kato, 1958; Tanaka, 1958; Yasuzumi & Tanaka, 1958) and the cyclophoracean *Cochlostoma montanum* (Selmi & Giusti, 1980) suggests that the apical structure of *Obtortio* paraspermatozoa is a nuclear remnant and not therefore comparable with the "acrosome-like structure" demonstrated by Healy & Jamieson (1981) in paraspermatozoa of *Pyrazus ebeninus*. Similarly, the apical body (to which multiple axonemes are attached) observed in paraspermatozoa of *Fusinus pulchellus* (and probably *Trunculariopsis trunculus*) by Melone et al. (1980) may also represent nuclear remnants. The possibility that the apical structure of *Obtortio* paraspermatozoa is a dense block modified for axoneme attachment cannot, as yet, be dismissed.

As observed in paraspermatozoa of viviparaceans (see Anderson & Personne, 1970, 1976; Hachiri & Higashi, 1972, 1974; Ishizaki & Kato, 1958) all tails of *Obtortio* paraspermatozoa are ensheathed by electron-dense (glycogen) granules as distinct from paraspermatozoa of *Pyrazus* (Healy & Jamieson, 1981) in which only a single tail of the tail tuft is ensheathed by granules.

From an investigation of cerithiacean paraspermatozoa (Healy, in preparation), it appears that deep penetration of the multiple axonemes into the mosaic sheath is common in potamidids and not limited to paraspermatozoa of *Obtortio* and viviparaceans.

### Euspermatozoa

Although euspermatozoa of *Obtortio* loosely resemble those of some mesogastropods, notably in the shape of the nucleus and/or acrosome (see Bulnheim, 1962; Giusti, 1969, 1971; Giusti & Mazzini, 1973; Griffond, 1980; Kohnert, 1980; Koike & Nishiwaki, 1980) the structure of the midpiece clearly indicates a close relationship with other cerithiaceans, in particular, the Potamididae (Healy, 1982; Healy, in preparation). All cerithiacean euspermatozoa that have, to date, been examined ultrastructurally, possess four midpiece elements arranged non-helically around the axoneme (Healy, 1982; see also Giusti, 1971 [*Cerithium*] and Melone et al., 1980 [*Turritella*]). Midpiece elements of these cerithiaceans, viviparaceans (Anderson, 1970; Anderson & Personne, 1970, 1976; Gall, 1961; Griffond, 1980; Kaye, 1958; Tanaka, 1958; Yasuzumi, 1962;



Yasuzumi & Tanaka, 1958) and the cyclophoracean *Cochlostoma* (Selmi & Giusti, 1980) are composed of parallel cristal plates as distinct from the structure of euspermatozoa of other mesogastropods (Buckland-Nicks, 1973; Bulnheim, 1962; Giusti, 1969, 1971; Giusti & Mazzini, 1973; Kohnert, 1980; Koike & Nishiwaki, 1980) and of neogastropods (Walker & MacGregor, 1968; West, 1978). Selmi & Giusti (1980) have demonstrated the presence of "crystalline deposits" between cristae in *Cochlostoma montanum*. Such deposits have not been reported in euspermatozoa of any viviparacean or cerithiacean (including *Obtortio*).

### Eusperm-like spermatozoa

Both euspermatozoa and eusperm-like spermatozoa have been demonstrated in epitoniids (these being attached exclusively to different "types" of paraspermatozoa – Nishiwaki & Tochimoto, 1969), in *Bithynia tentaculata* (Ankel, 1924, 1933; Kohnert, 1980) and interestingly, in the neritacean *Waldemaria japonica* (Tochimoto, 1967). Eusperm-like spermatozoa of epitoniids, *Bithynia*, *Waldemaria* and *Obtortio* all have nuclei much shorter than their (respective) euspermatozoa. In *Obtortio*, the midpiece and glycogen piece regions are longer in eusperm-like spermatozoa than in euspermatozoa, while eusperm-like spermatozoa of epitoniids, *Bithynia* and *Waldemaria* possess midpieces/glycogen pieces shorter than those of euspermatozoa (phasecontrast light microscopic observations).

Nishiwaki & Tochimoto (1969) report that both "macro- and micro-typical spermatozoa" (euspermatozoa and eusperm-like spermatozoa) of epitoniids react positively to Feulgen ("head") and PAS ("tail") tests and conclude (p. 43) that "it is difficult to think that micro-typical spermatozoa have no fertilizing capability, because it seems highly improbable that non-functional spermatozoa are produced more numerous than functional ones". The results presented in this study on *Obtortio* lend support to this conclusion. Nishiwaki & Tochimoto (1969) also point out that since epitoniids are protandric gastropods, a sex-determining role for "micro-typical spermatozoa" (eusperm-like spermatozoa) is not possible (at least, presumably, within the Epitoniidae).

From studies of a large number of mesogastropods (including many cerithiaceans) the present author has, to date, only observed eusperm-like spermatozoa in *Obtortio* cf. *fulva* and must therefore conclude that it is most unlikely that eusperm-like spermatozoa are involved with sex-determination. Euspermatozoa of *Obtortio* are structurally very similar to euspermatozoa of other cerithiaceans (Giusti, 1971; Healy, 1982; Healy, in preparation) while the small size of the nucleus of eusperm-like spermatozoa suggests that less than the haploid number of chromosomes is present in these sperm. Although the role of paraspermatozoa is presumably one of nutritional support of euspermatozoa (see Healy & Jamieson, 1981), the function of eusperm-like spermatozoa cannot, as yet, be satisfactorily determined.

It is difficult to suggest a plausible role (or roles) for eusperm-like spermatozoa, but presuming that they are functional cells, the following roles are possible: (1) fertilization of eggs, (2) parthenogenetic stimulation of egg development or (3) that eusperm-like spermatozoa are paraspermatozoa and provide nutrients for euspermatozoa before or after entry into the reproductive tract of the recipient female. The presence of an acrosome and motile midpiece and glycogen piece in all eusperm-like spermatozoa

suggests that penetration of eggs (that is [1] and [2]) is possible, and, assuming that eusperm-like spermatozoa are capable of (1) or (2), they may be intended for long-term storage in the recipient snail (enhancing the reproductive flexibility of the species?). An ultrastructural examination of the eggs of *Obtortio*, *Bithynia*, epitoniids and *Waldemaria* may provide useful information concerning the true purpose of eusperm-like spermatozoa.

#### Taxonomic significance of *Obtortio* spermatozoa

The genus *Obtortio* Hedley, 1899, has been placed into various families including Rissoidae (Laserson, 1950), Finellidae (Thiele, 1931), Cerithiidae (Ludbrook, 1941), Diastomatidae (Diastomidae – Ludbrook, 1957) and placed into a separate family – Obtortionidae – by Iredale & McMichael (1962). According to Houbrick (1981) the relationship of *Obtortio* to other cerithiaceans is uncertain. The present study provides very useful information concerning the taxonomic position of *Obtortio* cf. *fulva* (Fig. 27) and

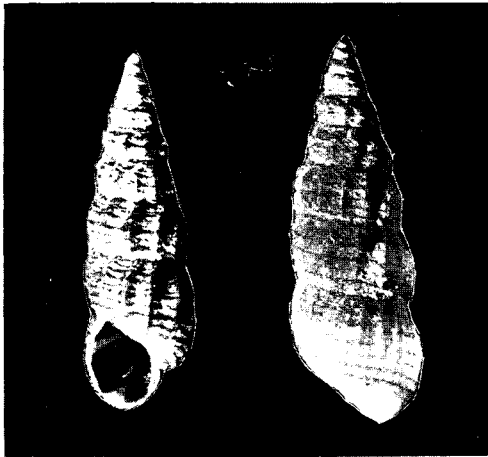


Fig. 27. Shells of *Obtortio* cf. *fulva* ( $\times 13$ )

presumably other species of *Obtortio*. (1) The structure of the euspermatozoon (and eusperm-like spermatozoon) midpiece is as observed in many potamidid species (Healy, in preparation) – that is, composed of four equal-sized midpiece elements. However, the acrosome differs in structure from these potamidids. (2) Although the paraspermatozoon most closely resembles those of many potamidids (Healy, in preparation) the structure of the paraspermatozoon apex is not similar to any studied cerithiacean. (3) In the Cerithiacea, eusperm-like spermatozoa have only been observed in *Obtortio* (this study). These significant differences, in particular the possession of eusperm-like spermatozoa, may indicate the need for a separate family (Obtortionidae of Iredale & McMichael, 1962) for these minute cerithiaceans, but this will depend on the results of further ultrastructural studies on other species of *Obtortio* and (more generally) other cerithiaceans.

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