



# Ultrastructure of spermatozoa in four species of Alvinellidae (Annelida: Polychaeta)

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## Introduction

Polychaete species from the family Alvinellidae are exclusively found at deep-sea hydrothermal vents in the Pacific Ocean. Twelve species have been described so far, occurring in different microhabitats of the vent ecosystem. Comparative studies of the genital apparatus and ultrastructure of spermatozoa in these species can be used to better understand the reproductive biology of these species and their phylogenetic relationships within the order Terebellida.

The single pair of gonoducts in alvinellids, and the presence of spermathecae in females and seminal vesicles in males were first described in *Paralvinella grasslei* Desbruyères & Laubier, 1982 (Zal et al., 1994). Investigations of *Alvinella pompejana* Desbruyères & Laubier, 1980 revealed a similar organization in the genus *Alvinella* (Jouin-Toulmond et al., 1997; Desbruyères et al., 1998; Zhadan et al., 2000). In *P. grasslei* and *A. pompejana*, spermatozoa transferred from males to females during a probable pseudocopulatory process are stored in the spermathecae located anteriorly, just below the genital pore opening, at the base of the gills.

We conducted similar anatomical and ultrastructural investigations in *Paralvinella palmiformis* Desbruyères & Laubier, 1986, *P. pandorae irlandei* Desbruyères & Laubier, 1986 and *Alvinella caudata* Desbruyères & Laubier, 1986. The aim of this study was to examine specific characters of the spermathecae and spermatozoa, and eventual ultrastructural similarities between closely related species and sub-species.

## Material and methods

### Animal collection

Specimens of *Paralvinella palmiformis* were collected from the Endeavour Segment, Juan de Fuca Ridge (JDF) (47°57'N, 129°05'W; 2195 m depth) and provided by Dr. V. Tunnicliffe: four females were studied. Specimens of *P. pandorae irlandei* were collected by Dr. D. Desbruyères from the East Pacific Rise (EPR) (12°48.8'N, 103°56.5'W; 2635 m depth): six specimens were studied. Specimens of *Alvinella caudata* were collected by C. J-T. during the HERO 91 cruise, from the EPR, (12°48.66'N, 103°56.43'W): four specimens were examined. All material was initially fixed in a solution of 10% neutral formalin.

### Tissue preparation

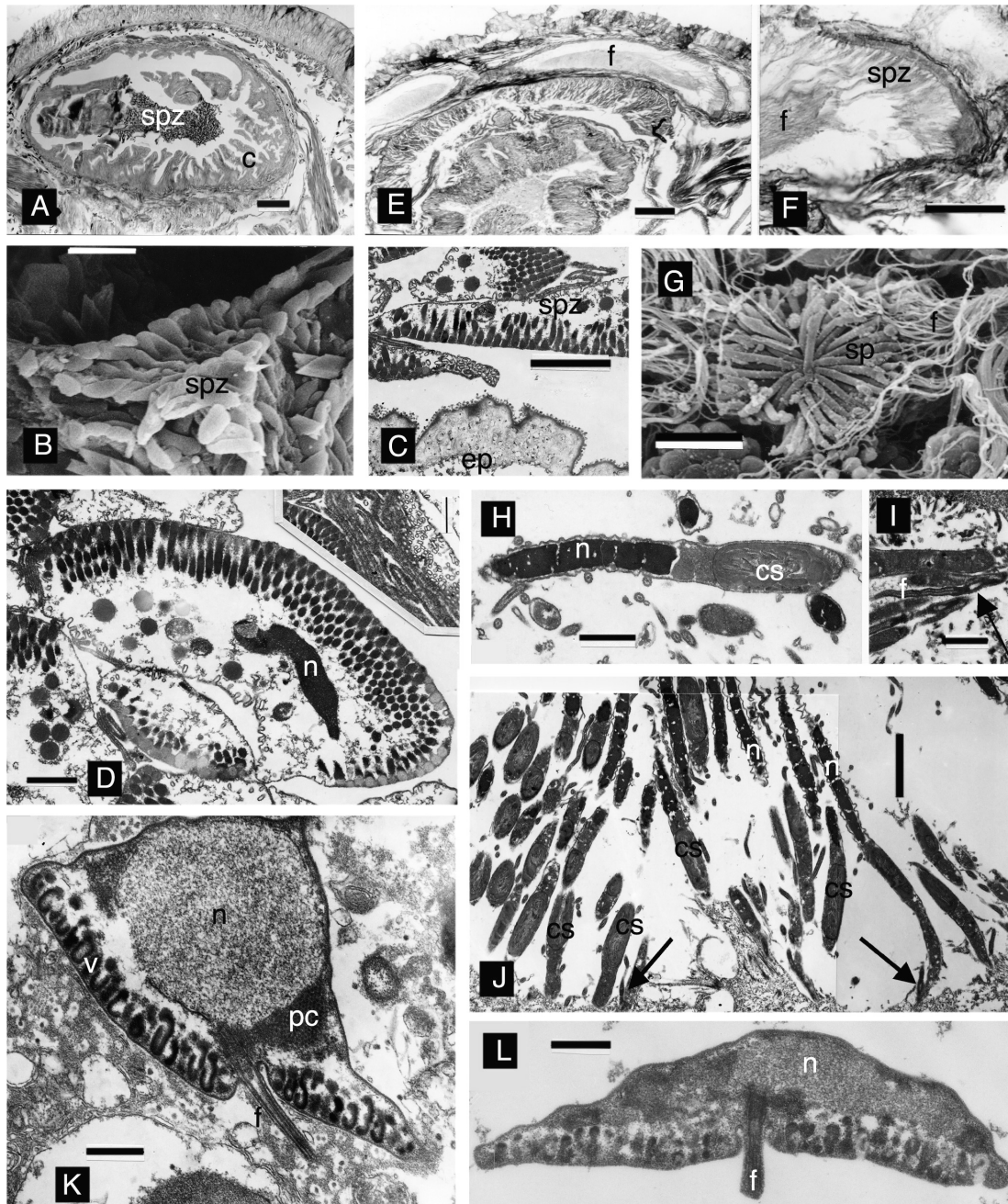
Tissues were prepared as described in Zal et al. (1994). Ultrathin sections were examined with a Jeol JEM 1200 EX.

## Results

### Spermathecae and spermatozoa of *Paralvinella palmiformis*

As in *Paralvinella grasslei*, two spermathecae, located in the dorsal part of segment 4, form the terminal part of two oviducts. They communicate with the exterior by a short common canal, located medially at the base of the most posterior gills, that opens at the genital pore. The spermathecae have a muscular peripheral layer and a luminal epithelium forming cristae (Fig. 1A-C). The spermatozoa, clustered together on the cristae or in the lumen, are just adjacent to the cristae and do not penetrate the spermathecal epithelium (Fig. 1C).

The spermatozoon of *P. palmiformis* (Fig. 1D) is very similar to that of *P. grasslei*: it has no acrosome, no

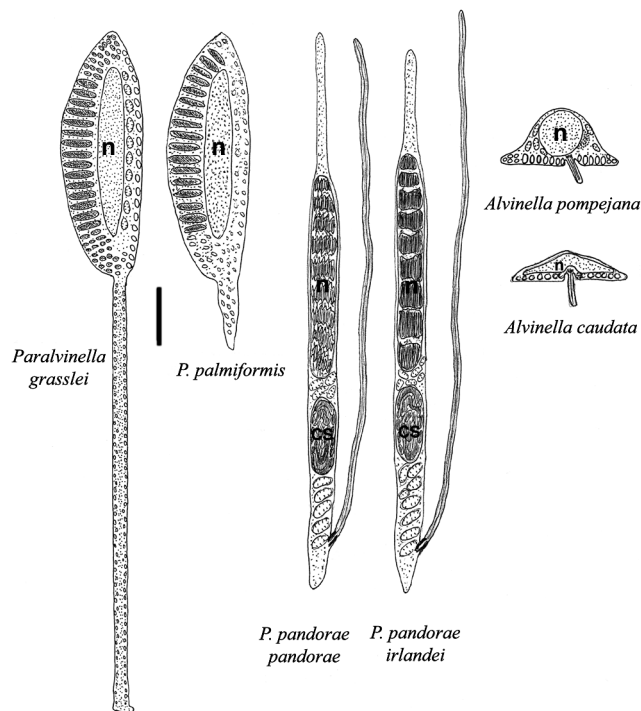


**Figure 1.** A–D: *Paralvinella palmiformis*. A. Histological section through a spermatheca, showing cristae of the luminal epithelium and spermatozoa clustered in the cavity. B. Scanning electron microscopy, SEM, view of a crista covered with aflagellate spermatozoa. C. Transmission electron microscopy, TEM, micrograph showing spermatozoa not attached to the spermathecal epithelium. D. A spermatozoon showing a convex cortical layer of electron-dense grains, an opposite vesicular side, part of the central nucleus, few mitochondria and granules. Inset: plasma membrane protrusions between the spermatozoa. E–J: *Paralvinella pandorae irlandei*. E–F. Histological sections through the spermathecae, showing a smooth luminal epithelium, abundant spermatozoa attached to the spermathecal wall and long flagella in the spermathecal lumen. G. SEM view of morulae of spermatids from the coelomic fluid of a male; long flagella are at the periphery of the morulae. H. Middle part of a spermatozoon with part of the nucleus and convoluted structure. I. Posterior part of spermatozoa in the spermduct of a male. The flagella are retro-oriented (toward anterior end); basal body (arrow). J. Spermatozoa attached to the spermathecal wall by their posterior ends; basal bodies (arrows). K. *Alvinella pompejana*: spermatozoon from a spermatheca. L. *Alvinella caudata*: spermatozoon from a spermduct. Note the proximal centriole adjacent to the distal one.

**Abbreviations:** (c) cristae; (cs) convoluted structure; (ep) spermathecal epithelium; (f) flagella; (n) nucleus; (pc) paracrystalline body; (sp) spermatids; (spz) spermatozoa; (v) vesicles with an electron lucent core.

Scale bars. A: 100  $\mu$ m. B: 10  $\mu$ m. C: 2  $\mu$ m. D: 1  $\mu$ m. E: 100  $\mu$ m. F: 50  $\mu$ m. G: 10  $\mu$ m. H: 1  $\mu$ m. I: 1  $\mu$ m. J: 2  $\mu$ m. K: 0.5  $\mu$ m. L: 0.5  $\mu$ m.

midpiece, no flagellum and it comprises an oval “head”, (ca. 10 x 4 µm) containing the central nucleus and a few mitochondria. It has a convex-shaped cortical layer of electron-dense granules, very similar to that of *P. grasslei*. On the opposite vesicular side, the plasma membrane can form elongate extensions between the spermatozoa (Fig. D, inset) which could be adhesive material favouring the clustering and storage of the male gametes in the spermathecae. This spermatozoon possess a short process different from the long “caudal” process present in *P. grasslei* (Fig. 2).



**Figure 2.** Schematic drawings of spermatozoa of six species of Alvinellidae highlighting the ultrastructural diversity of spermatozoa in the genus *Paralvinella* and the morphological similarities of sperms of two closely related species, of two sub-species and of the two species of *Alvinella*. All species at the same scale : 2 µm. Abbreviations as in Figure 1.

*Spermathecae and spermatozoa of Paralvinella pandorae irlandei*

The anatomy of the genital apparatus is similar to that of *P. grasslei* and *P. palmiformis* : one pair of spermathecae is present in the females, located in the dorsal part of setigerous segment 4 and connected laterally to one pair of oviducts (Fig. 1 E).

The flagellate spermatozoa are quite different from that of *P. grasslei* and *P. palmiformis*, and very similar to that of *P. pandorae pandorae* described by McHugh (1995). Groups of late spermatids in the coelom of males show long flagella at the periphery of the morulae (Fig. 1G). Each spermatozoon is an elongate cell (about 19 µm in length) comprising several parts which are, following the nomenclature introduced by McHugh (1995): an apical

elongation, an electron-dense nucleus, a midpiece comprising two mitochondrial areas anterior and posterior to a convoluted structure (Fig. 1 H, J), then a short area bearing the basal body of a long flagellum forming an angle with the cell and directed anteriorly (Fig. 1, I, J ; Fig. 2).

In all the females examined, the spermathecae harboured bundles of spermatozoa attached to the spermathecal wall by their posterior (flagellate) end (Fig. 1 J), of which about 1 µm penetrates the epithelial surface; the basal bodies are superficially inserted in the epithelium. The flagella, along with the rest of the sperm cell, are free, but all aligned in the spermathecal lumen (Fig. 1 E, F, I, J).

*Spermatozoa of Alvinella pompejana and A. caudata*

The anatomy of the gonoducts, and the ultrastructural features of the spermatozoa have already been described for *A. pompejana* (see Introduction). The arrangement of the genital apparatus is similar in *A. caudata*. In both species, the spermathecae are located in the dorsal part of setigerous segment 1.

Numerous spermatozoa are present in the spermathecae of *A. pompejana*, free in the spermathecal lumen. They have a small size, a conical shape, and a rather flat (ca. 4 µm diameter), vesicular surface, in the middle of which is inserted a short (ca. 1 µm) flagellum (Fig. 1K). The central nucleus is round (ca. 2 µm in diameter) and surrounded by few mitochondria; adjacent to the nucleus there is a paracrystalline body, made of tubules and lying always against the nucleus, but with a variable position (near the flagellum or opposite to it) (Fig. 1K). The distal centriole (basal body) is clearly visible, but the proximal centriole is lacking.

In *A. caudata*, spermatozoa have only been examined in the sperm ducts of a male specimen, the spermathecae examined being empty. The spermatozoa are similar to those of *A. pompejana*: a cell comparatively more flattened than that of *A. pompejana*, a slightly elongate nucleus (Fig. 1 L), a short flagellum (ca. 1 µm in length) in the middle of the flat vesicular surface (about 4.5 µm in diameter). A proximal centriole is present next to the distal centriole in *A. caudata*, but the paracrystalline body is lacking.

**Discussion**

The six alvinellid species investigated so far possess spermathecae which harbour spermatozoa transferred from males to females, by the way of spermatozeugmata, during a probable pseudocopulatory process. The ultrastructural similarities of spermatozoa of the two sub-species *Paralvinella pandorae pandorae* and *P. p. irlandei* strongly suggest that, although not seen by McHugh (1995), the spermathecae are also present in *P. p. pandorae*. The gonoducts and spermathecae of this species have been studied by Zhadan et al. (2000), who also described a similar anatomy for the genital organs in *Paralvinella dela.*

The Alvinellidae is, at present, the only taxon in the order Terebellida known to possess spermathecae, a feature which may represent an apomorphy for this family. The luminal wall of the spermathecae have either internal cristae (e.g. *P. palmiformis*, *P. grasslei* and the two species of *Alvinella*) or a smooth surface (e.g. *P. pandorae irlandei*) and the

storage of spermatozoa differs according to the sperm morphology of the species. In *Alvinella pompejana*, *A. caudata*, *Paralvinella grasslei* and *P. palmiformis*, the sperm cells, almost immobile, with flagella reduced or absent, are stored without any special attachment at the surface of cristae in the spermathecae. In contrast, in *P. pandorae irlandei*, the elongate spermatozoa displaying long flagella are firmly implanted in the smooth spermathecal epithelium by their "posterior" flagellate end. In the Alvinellidae, it is still unknown whether fertilization occurs in the spermathecae or at spawning, when oocytes and spermatozoa, both mature, are emitted through the female genital pore.

There are three types of spermatozoa in the species investigated (Fig. 2). All belong to the introsperm category and display modified structures (see Jamieson & Rouse, 1989) being flagellated or not, having no acrosome and an atypical or absent midpiece. In *P. p. pandorae* and *P. p. irlandei*, the elongate spermatozoa exhibit an atypical midpiece and a long flagellum, with a typical pattern, but a reverse orientation, producing a movement of the sperm cells with the flagellar part ahead, since this part is implanted in the spermathecal wall after the sperm transfer.

The aflagellate and oval shaped spermatozoa of the two closely related species *P. grasslei* and *P. palmiformis* represent another type of modified sperm having no acrosome, no midpiece, and no flagellum. The role of the cortical layer of dense granules remains unknown, but in *P. palmiformis* the cell membrane of the vesicular side produces some material that could be adhesive, and thus favour clustering and storage in the spermathecae.

*Alvinella pompejana* and *A. caudata* also have modified spermatozoa, conical in shape and very small sized. They have no acrosome, no midpiece and a short flagellum with a typical pattern. Their vesicular surface could be also involved in adhesiveness in the spermathecae.

In a study of phylogenetic relationships of eleven species of Alvinellidae using allozymes, Jollivet et al. (1995) showed that paralvinellid species are separated into three major clades that reflect an old habitat radiation. This radiation seems to have occurred before the ridge subduction event that led to the emergence of sibling species in the EPR and JDF vent fauna. In this study, species from the pairs *Paralvinella grasslei* (from EPR) and *P. palmiformis* (from JDF), and *P. pandorae pandorae* (JDF) and *P. pandorae irlandei* (EPR) inhabit similar environments on both ridge systems and are genetically closely related. A similar relationship is found in our study in which *P. grasslei* and *P. palmiformis*, on one hand, *P. pandorae pandorae* and *P. p. irlandei*, on the other hand, share great similarities in their sperm fine structure.

On the basis of morphological studies, Desbruyères & Laubier (1993) erected three subgenera of *Paralvinella*:

1. *Paralvinella* (*Paralvinella*) *grasslei*; *P. (P.) palmiformis*; *P. (P.) fijiensis*;
2. *Paralvinella* (*Miralvinella*) *dela*; *P. (M.) hessleri*; *P. (M.) bactericola*;
3. *Paralvinella* (*Nautalvinella*) *pandorae*; *P. (N.) unidentata*.

Although not all alvinellid species have been studied to

date, neither a molecular phylogeny analysis of five alvinellid species (Féral et al., 1994) nor our ultrastructural data on the spermatozoa of three species of *Paralvinella* are at variance with the proposed subgeneric taxonomy. Further studies of the remaining species, especially the ones belonging to the subgenus *Miralvinella*, should prove useful to test the validity of the three subgenera.

All alvinellids described to date are endemic to deep-sea hydrothermal vents of the Pacific Ocean and for those whose reproductive structures were studied, specific features are notable: the single pair of gonoducts and the regular presence of spermathecae and seminal vesicles in six of twelve species suggest that these structures were present in the common ancestor that colonized the vent environment.

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