

Ultrastructure of the spermatozoa of the flatworms *Phaenocelis peleca* and *Boninia divae* (Platyhelminthes, Polycladida)

Marcin K. Liana · Marian K. Litvaitis

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Abstract The ultrastructure of spermatozoa of the acotylean *Phaenocelis peleca* and the cotylean *Boninia divae* is described. All spermatozoa are filiform and biflagellate with a 9+“1” microtubular pattern in the axoneme. Sperm characters in *P. peleca* follow the morphologies described for other acotyleans, with axonemes exiting the sperm shaft at the distal end and remaining in close contact with the sperm membrane. The nucleus occupies the proximal region of the shaft, and two types of dense bodies and mitochondria are located at the distal end. Unlike other members of the Cotylea, the axonemes of *B. divae* spermatozoa are incorporated into the sperm shaft, leaving the shaft at some distance from the distal end and then remaining free. This type of morphology is characteristic for acotyleans. Additionally, the spermatozoa of *B. divae* contain only one type of dense bodies plus a unique structure, which we call a central core. The nucleus in this species is unique as well; it shows periodic constrictions and rings of electron-dense granules, characters that further contribute to the distinct status of Boniniidae.

Keywords Platyhelminthes · Polycladida · Spermatozoa ultrastructure · Spermatology · Phylogeny

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M. K. Liana (✉)
Department of Invertebrate Zoology, Institute of Systematics
and Evolution of Animals, Polish Academy of Sciences,
Sławkowska 17, 31-016 Kraków, Poland
e-mail: marcin.liana@gmail.com

M. K. Litvaitis
Department of Natural Resources and Zoology Graduate
Program, University of New Hampshire,
Durham, NH 03824, USA
e-mail: m.litvaitis@unh.edu

Introduction

Morphological differences in the spermatozoa of flatworm taxa have been used to understand phylogenetic relationships within the phylum (Ehlers 1985; Hendelberg 1965, 1969, 1974, 1983, 1986; Rohde 1990; Justine 1995; Bâ and Marchand 1995; Watson and Rohde 1995; Watson 1999; Liana and Litvaitis 2007), and in recent years, knowledge of the ultrastructure of polyclad spermatozoa has greatly increased (Kubo-Irie and Ishikawa 1983; Watson 1999, 2001; Liana and Litvaitis 2007, 2010). In general, polyclad spermatozoa are elongated, biflagellate with a 9+“1” axonemal pattern (common for Trepaxonemata, Ehlers 1985). They contain an elongate nucleus, numerous small and large refractile bodies, and mitochondrial derivatives. In the Acotylea (i. e., polyclads without a ventral sucker, Lang 1884), axonemes remain incorporated in the sperm shaft for some distance. Once exiting the sperm shaft (usually at different levels), the axonemes stay attached to it. The nucleus occupies the proximal part of the sperm body, whereas the distal part contains refractile (dense) bodies and mitochondrial derivatives (distal and proximal parts are defined with respect to the connection to the cytophore). In the Cotylea (i.e., polyclads with a ventral sucker, Lang 1884), the axonemes exit the sperm shaft at the distal end and form free flagella; they are never incorporated into the shaft. The nucleus elongates through the entire sperm body and in the distal part is surrounded by dense bodies and mitochondrial derivatives. Hence, ultrastructural characters of the spermatozoa clearly differentiate the two suborders.

The major goal of this study was to determine the ultrastructure of spermatozoa of the acotylean *Phaenocelis peleca* and the cotylean *Boninia divae* and to compare them to those of previously described polyclads.

Fig. 1 Spermatozoon of *Phaenocelis peleca*. **a** Diagrammatic representation of a mature spermatozoon. **b–h** Electron micrographs of sections through the spermatozoon at the levels indicated in **a**. *a* axoneme, *icb* intercentriolar body, *l* large dense bodies, *m* mitochondrial derivatives, *n* nucleus, *s* small dense bodies, *arrowhead* microtubules

Materials and methods

Specimens of *Boninia divae* Marcus and Marcus, 1968 (Boniniidae, Polycladida) and *Phaenocelis peleca* Marcus and Marcus, 1968 (Cryptocelidae, Polycladida) were collected from the intertidal and subtidal zones on St. John, US Virgin Islands during May and June 2006. Animals were fixed in 2.5% glutaraldehyde in PBS for 24–48 h and postfixed for 12 h in 1% OsO₄. Samples were dehydrated in a graded ethanol series followed by a final dehydration step in propylene oxide. Fragments of the vasa deferentia containing mature spermatozoa were embedded in EMBED 812 resin. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a LEO 922 Omega transmission electron microscope.

Results

The proximal part of the spermatozoon of *Phaenocelis peleca* contains an elongated nucleus, which tapers to a very thin extension distally. This thin taper occupies the center of the shaft (Fig. 1a–e) and is surrounded by large (~0.2 μm in length) dense bodies (Fig. 1c–e). Distally, the spermatozoon is filled with mitochondrial derivatives (0.4 μm in diameter), which are surrounded by large dense bodies (Fig. 1f). The very distal end of the spermatozoon is filled with small dense bodies (0.1–0.15 μm in length) only (Fig. 1g). The sheet of microtubules is located beneath the cell membrane and extends through the entire sperm shaft. Two axonemes typical of Trepaxonemata extend from basal bodies that are separated by an intercentriolar body (ICB; Fig. 1h) located at the distal end of the sperm shaft. The axonemes exit the shaft at the distal end and form flagella that remain in close contact with the membrane of the spermatozoon (Fig. 1c–h).

The spermatozoa of *Boninia divae* are elongated and biflagellate (Fig. 2a). The nucleus extends throughout the length of the sperm shaft, completely filling the proximal portion (Fig. 2c–e) and tapering to a thin, peripherally displaced extension in the distal part (Fig. 2f–k). The proximal section of the nucleus is constricted every 0.3 μm, giving it the appearance of a string of pearls (Fig. 2e). In these constriction areas, the nucleus is surrounded by small granules of electron-dense material (Fig. 2d–e). The distal section of the sperm shaft contains

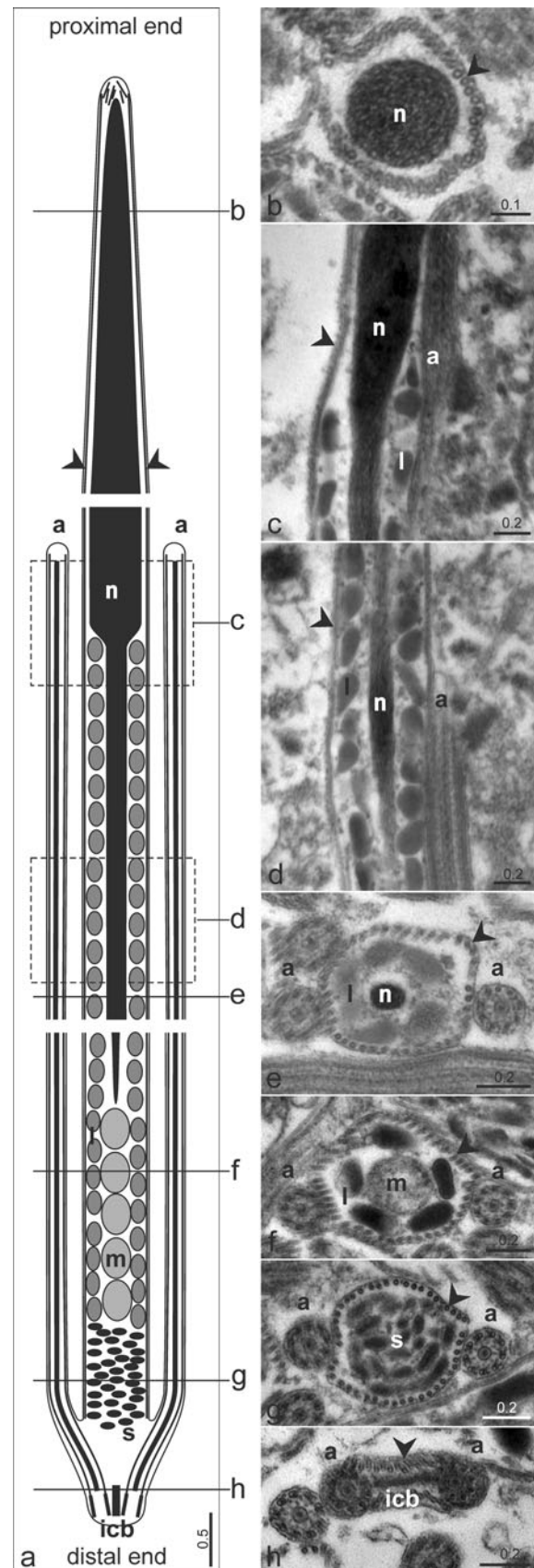
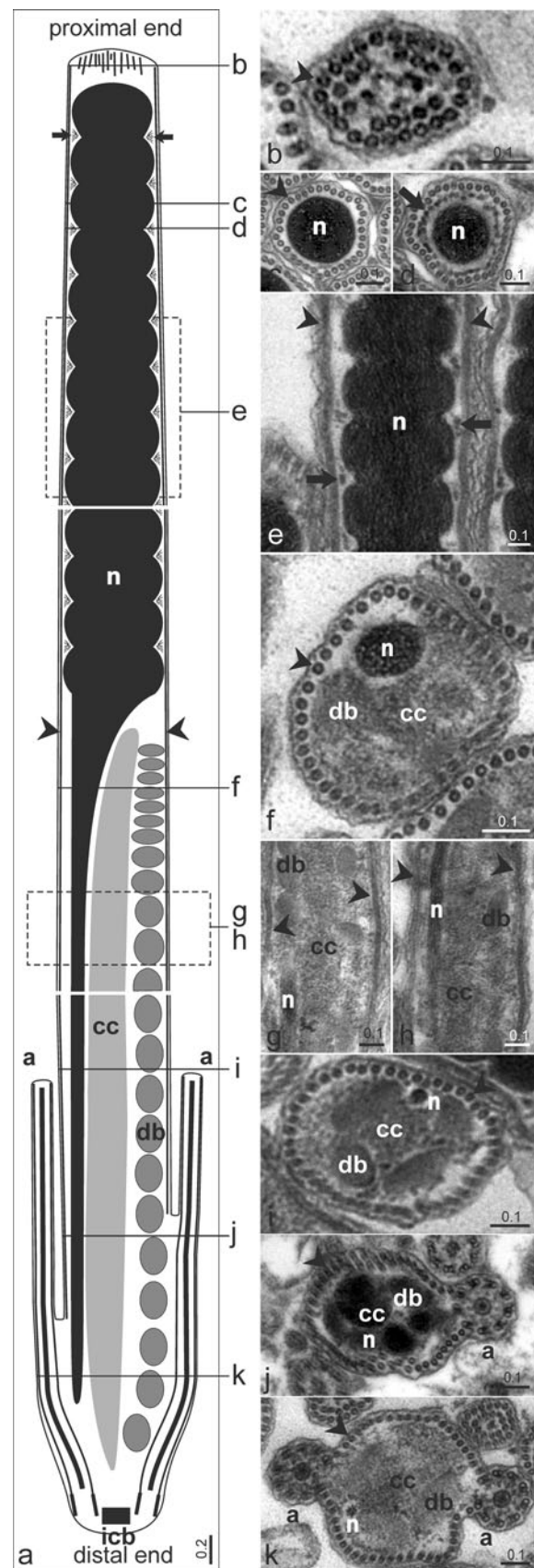


Fig. 2 Spermatozoon of *Boninia divae*. **a** Diagrammatic representation of a mature spermatozoon. **b–k** Electron micrographs of sections through the spermatozoon at the levels indicated in **a**. *a* axoneme, *cc* central core, *db* dense bodies, *icb* intercentriolar body, *n* nucleus, *arrow* granules of electron-dense material, *arrowhead* microtubules

the tapered part of the nucleus and numerous oval refractile (dense) bodies ($\sim 0.1 \mu\text{m}$ long). The dense bodies lie in three or four rows located peripherally (Fig. 2g–i). Electron-dense, granular material forming a core fills the central portion of the distal sperm shaft (Fig. 2g–i). A complete sheet of microtubules is located just beneath the cell membrane and extends along the entire sperm shaft (Fig. 2a–k). It is doubled in the proximal end of the shaft (Fig. 2b). The distal part of the shaft contains two axonemes that originate from basal bodies separated by an intercentriolar body (ICB). The axonemes extend inside the shaft and leave it at about one-third of the length of the sperm shaft (Fig. 2a, j, k) to form free flagella.

Discussion

The ultrastructure of the axoneme in both species examined is characteristic of Trepaxonemata in general (Hendelberg 1986). A comparison of the spermatozoon of *Phaenocelis peleca* with that of other representatives of Acotylea reveals morphological congruence with regard to the arrangement of dense bodies and mitochondrial derivatives. The tapering extension of the nucleus though differs from previously described acotylean sperm nuclei, which tend to be more spindle shaped (Liana and Litvaitis 2007). The most surprising difference is the early exit of the axonemes at the distal end of the sperm shaft. Generally, acotylean sperm flagella remain incorporated into the sperm shaft and exit at various distances from the distal end. Once outside the sperm shaft, the flagella tend to remain in close contact with the sperm membrane (Liana and Litvaitis 2007). So far, an early exit of the flagella from the distal end has been described for only two acotylean species, namely *Notoplana japonica* Kato, 1937 (Kubo-Irie and Ishikawa 1983) and *Pleioiplana atomata* (O.F. Müller 1976) (Liana and Litvaitis 2010). The genus *Notoplana* is very heterogeneous and historically included *N. atomata* (O.F. Müller 1976). Faubel (1983) removed *N. atomata* and placed it into a newly erected genus *Pleioiplana* in the new family Pleioiplanidae. However, it seems then that morphological similarities at least of sperm ultrastructure reflect a close affinity of the two species as well as with *Phaenocelis peleca*. *P. peleca* is a member of Cryptocelidae (although Faubel (1983) places it into a newly erected taxon Stylochoplanidae). Although so far no comprehensive hypothesis regarding the phylogenetic relationships of



acotylean families exists, our findings of ultrastructural similarities of spermatozoa between Cryptocelidae and Pleioplanidae (and other species of *Notoplana*) may reflect an evolutionary relationship.

Free flagella emerging from the distal end of the spermatozoon are a characteristic of Cotylea and have been observed in *Pseudoceros bicolor* Verrill, 1909 *Phrikoceros mopsus* (Marcus, 1952), and *Enchirdium evelinae* Marcus, 1949 (Liana and Litvaitis 2007). Hence, flagella incorporated into the sperm shaft as observed here in the cotylean *Boninia divae* represent a unique exception; in fact, this character state so far has been described only in Acotylea (Liana and Litvaitis 2007). Additional unique features of the spermatozoon of *B. divae* include the nucleus with its periodic constrictions, the presence of only one type of dense bodies, and the lack of mitochondrial derivatives. We suspect that the electron-dense material filling the central core of the distal sperm shaft may be a group of highly modified mitochondria, which fuse into a single structure. However, to support this hypothesis, a study of spermiogenesis is needed.

Currently, Boniniidae is a monophyletic taxon, assigned to Cotylea (Faubel 1984; Rawlinson and Litvaitis 2008). However, the taxon shares morphological characters (e.g., presence of a Lang's vesicle [a sac-like expansion of the terminal end of the vagina used for sperm storage], incorporated sperm flagella) with representatives of Acotylea, and Rawlinson and Litvaitis (2008) showed that it represents a basal lineage within the suborder. The unique status of the taxon is further supported by characters of the epidermal, nervous and muscular systems (unpublished data), and by distinct developmental processes. Finally, a preliminary molecular analysis indicates that Boniniidae occupies an intermediate position between Acotylea and Cotylea. Clearly, the taxonomic position of Boniniidae will be further substantiated by future research.

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References

Bâ CT, Marchand B (1995) Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. In: Jamieson BG, Ausio J, Justine J-L (eds) *Advances in spermatozoal phylogeny and taxonomy*, vol 166. Museum National D' Histoire Naturelle, Paris, pp 87–95

- Ehlers U (1985) Phylogenetic relationships within the Platyhelminthes. In: Morris SC, George JD, Gibson R, Platt HM (eds) *The origins and relationships of lower invertebrates*. Oxford University Press, Oxford, pp 143–158
- Faubel A (1983) The Polycladida, Turbellaria proposal and establishment of a new system. Part I the Acotylea. *Mitt hambg zool Mus Inst* 80:17–121
- Faubel A (1984) The Polycladida, Turbellaria proposal and establishment of a new system. Part II the Cotylea. *Mitt hambg zool Mus Inst* 81:189–259
- Hendelberg J (1965) On different types of spermatozoa in Polycladida, Turbellaria. *Ark Zool* 18:267–304
- Hendelberg J (1969) On the development of different types of spermatozoa from spermatids with two flagella in the Turbellaria with remarks on the ultrastructure of the flagella. *Zool Bidrag Uppsala* 38:1–50
- Hendelberg J (1974) Spermiogenesis, sperm morphology and biology of fertilization in the Turbellaria. In: Riser NW, Morse MP (eds) *Biology of the Turbellaria*. McGraw-Hill, New York, pp 148–164
- Hendelberg J (1983) Platyhelminthes: Turbellaria. In: Adiyodi KG, Adiyodi RG (eds) *Reproductive biology of invertebrates*, Vol. II: Spermatogenesis and sperm function. Wiley, New York, pp 75–104
- Hendelberg J (1986) The phylogenetic significance of sperm morphology in the Platyhelminthes. *Hydrobiologia* 132:53–58
- Justine J-L (1995) Spermatozoal ultrastructure and phylogeny in the parasitic Platyhelminthes. In: Jamieson BG, Ausio J, Justine J-L (eds) *Advances in spermatozoal phylogeny and taxonomy*, vol 166. Museum National D' Histoire Naturelle, Paris, pp 55–86
- Kubo-Irie M, Ishikawa M (1983) Spermiogenesis in the flatworm, *Notoplana japonica* with special attention to the organization of an acrosome and flagella. *Dev Growth Differ* 25:143–152
- Lang A (1884) *Die Polycladen (Seeplanarien) des golfes von neapel und der angrenzenden meeresabschnitte, eine monographie*. Fauna Flora Golfes v Neapel, Leipzig
- Liana KM, Litvaitis KM (2007) Comparative spermatology of selected polyclad flatworms (Platyhelminthes). *J Morphol* 268:891–897
- Liana KM, Litvaitis KM (2010) Anatomy and ultrastructure of the male reproductive system of *Pleioplana atomata* (Platyhelminthes: Polycladida). *Invertebr Biol* 129:129–137
- Rawlinson KA, Litvaitis MK (2008) Cotylea (Polycladida): a cladistic analysis of morphology. *Invertebr Biol* 127(2):121–138
- Rohde K (1990) Phylogeny of Platyhelminthes, with special reference to parasitic groups. *Int J Parasitol* 20:979–1007
- Watson NA (1999) Platyhelminthes. In: Adiyodi KG, Adiyodi RG, Jamieson BGM (eds) *Reproductive biology of invertebrates*, vol. 9A: progress in male gamete ultrastructure and phylogeny. Wiley, New York, pp 97–142
- Watson NA (2001) Insights from comparative spermatology in the 'turbellarian' Rhabdozoa. In: Littlewood DTJ, Bray RA (eds) *Interrelationships of the platyhelminthes*. Taylor and Francis, London, pp 217–230
- Watson NA, Rohde K (1995) Sperm and spermiogenesis of the "Turbellaria" and implications for the phylogeny of the phylum Platyhelminthes. In: Jamieson BG, Ausio J, Justine J-L (eds) *Advances in spermatozoal phylogeny and taxonomy*, vol 166. Museum National D' Histoire Naturelle, Paris, pp 37–54