

CRUSTACEAN ISSUES 18



Decapod Crustacean Phylogenetics

edited by

Joel W. Martin, Keith A. Crandall, and Darryl L. Felder



CRC Press
Taylor & Francis Group

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CRC Press

Taylor & Francis Group

Boca Raton London New York

CRC Press is an imprint of the
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CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-13: 978-1-4200-9258-5 (Hardcover)

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Library of Congress Cataloging-in-Publication Data

Decapod crustacean phylogenetics / editors, Joel W. Martin, Keith A. Crandall, Darryl L. Felder.
p. cm. -- (Crustacean issues)

Includes bibliographical references and index.

ISBN 978-1-4200-9258-5 (hardcover : alk. paper)

1. Decapoda (Crustacea) 2. Phylogeny. I. Martin, Joel W. II. Crandall, Keith A. III. Felder, Darryl L.
IV. Title. V. Series.

QL444.M33D44 2009

595.3'8138--dc22

2009001091

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<http://www.crcpress.com>

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Spermatozoal Morphology and Its Bearing on Decapod Phylogeny

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ABSTRACT

The use of spermatozoal characters in elucidating animal phylogeny (spermiocladistics) has been successfully applied in the decapod crustaceans. Most of the studies investigating decapod sperm morphology have been published in the last 18 years and cover 100% of the decapod infraorders, 50% of the families, and approximately 10% of the extant genera, but only 2% of the described, extant species. There is great diversity in sperm morphology within the Crustacea, but overall decapod spermatozoa are quite conservative in comparison. Still, it is difficult to describe a typical decapod sperm cell. Decapod sperm are unusual for several reasons: 1) they are aflagellate (lack a true 9 + 2 flagellum), although microtubular processes are often present; 2) there is no reliable record of motility for any individual sperm cell; 3) the acrosome vesicle is not Golgi-derived as in all other described acrosomes of sperm in the animal kingdom, instead being derived from endoplasmic reticulum vesicles; 4) the decapod sperm nuclear protein is unique, with all other animal sperm nuclear proteins falling into four other categories; 5) the sperm nucleus is composed of diffuse, filamentous, heterogeneous chromatin fibers rather than being uniformly dense; and 6) the mitochondria are degenerate in mature sperm cells. I surveyed spermatozoal characters across the investigated decapod crustaceans, highlighting those of phylogenetic utility, such as acrosome vesicle presence, shape, dimensions and size, and internal complexity; nuclear morphology and shape; and microtubular arm presence, number, and origin. Particular spermatozoal characters, or suites of characters, that define various decapod taxa are provided, and their utility to phylogenetic construction is discussed.

1 INTRODUCTION

"The sperm seems never to transgress the few rules which govern the production of its fundamental parts, but in the arrangement of these parts every sperm (flagellate or non-flagellate) seems to be a law unto itself."

Bowen (1925)

Professor Barrie Jamieson coined the term spermiocladistics (Jamieson 1987) and pioneered the use of spermatozoa in decapod phylogenetics (among many other invertebrate and vertebrate groups) using comprehensive datasets based on the ultrastructure of sperm cells from scanning and transmission electron microscopy. Jamieson's contributions to spermiocladistics span two decades, with a significant proportion of this work dedicated to decapod crustaceans. He was not the first to recognize the phylogenetic significance of crustacean spermatozoa, and in fact he was beaten to this claim by 81 years.

The phylogenetic significance of crustacean spermatozoa was first recognized by Koltzoff (1906) and then later by Wielgus (1973). Koltzoff constructed a phylogeny of crustaceans (mostly decapods) based on sperm cell structure observed under the light microscope. He assigned to the

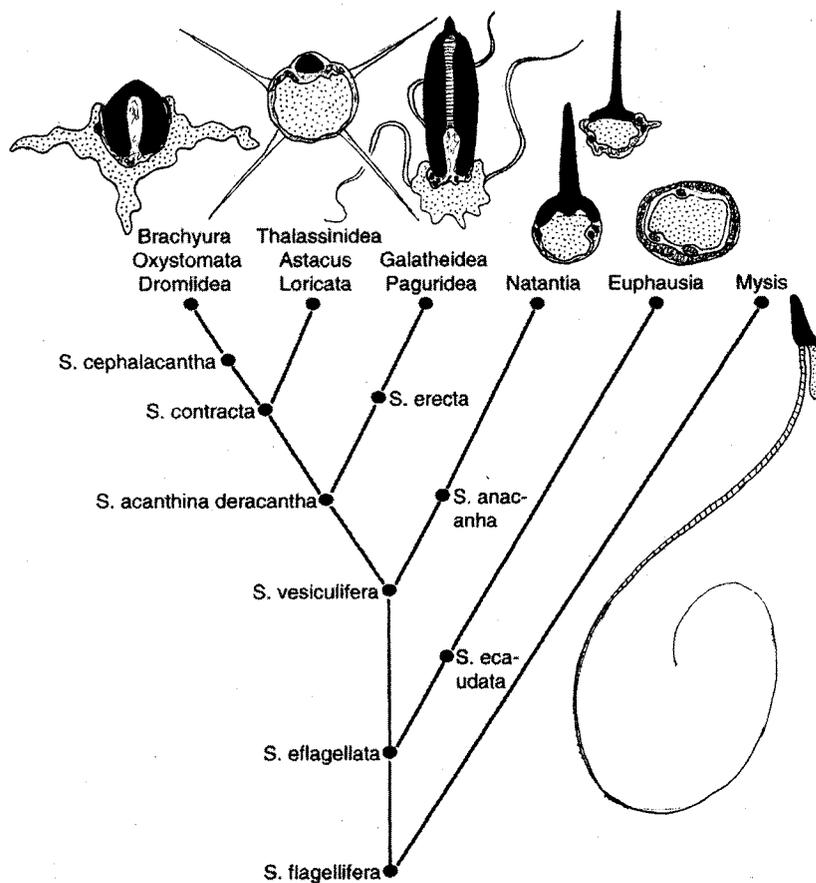


Figure 1. Diagram of the sperm phylogeny of Koltzoff (1906) modified to include a representative sperm morphology for the terminal groups. Spermatozoa not to scale.

different sperm types he encountered the “generic” name *Spermia* and a descriptive “species” name. Some of the significant relationships apparent in Koltzoff’s phylogenetic tree are shown in Figure 1.

We must also recognize the contributions of others who came before, pioneering the microscopy of spermatozoa in general, including Leeuwenhoek (1678), Swammerdam (1758), Spallanzani (1776), Koltzoff (1906), Retzius (1909), Bowen (1925), Afzelius (1970), and Baccetti (1979), to name a few. We are indebted to their talents, perseverance, foresight, and careful observation.

The considerable decapod sperm literature was ably reviewed by Jamieson (1991), along with the bulk of the crustacean sperm literature to this date. A second review of just the decapod sperm literature from 1991 to 2000 is provided in Jamieson & Tudge (2000). A comprehensive (but not exhaustive) table of subsequent publications (including some missed in the two previous reviews) on spermatozoal descriptions of decapods is provided in Table 1.

Like the animals themselves, spermatozoa of crustaceans are very diverse in their morphology (Pochon-Masson 1983; Jamieson 1989c, 1991). It is therefore difficult to designate sperm features that characterize the entire class. Nevertheless, sperm data are extremely useful in determining relationships among crustacean taxa. Except for the Ascothoracica (Grygier 1982), the Cirripedia (Healy & Anderson 1990), and the Remipedia (Yager 1989), most crustaceans have aflagellate, immotile sperm. The non-caridean, pleocyemate decapods all share a common sperm form consisting of an often large acrosome vesicle (which can be multi-layered), a posterior nucleus of variable

Table 1. Decapods investigated for spermatozoal morphology since Jamieson (1991) and Jamieson & Tudge (2000).

| Suborder/Infraorder, SUPERFAMILY & Family | Species | Reference |
|--|--------------------------------------|---|
| Suborder Dendrobranchiata | | |
| PENAEOIDEA | | |
| Penaeidae | <i>Artemesia longinaris</i> | Scelzo & Medina 2003 |
| | <i>Fenneropenaeus penicillatus</i> | Hong et al. 1993, 1999 (both as <i>Penaeus</i>) |
| | <i>Penaeus chinensis</i> | Lin et al. 1991; Kang et al. 1998; Kang & Wang 2000, Kang et al. 2000 |
| Aristeidae | <i>Aristaeopsis edwardsiana</i> | Medina et al. 2006b |
| | <i>Aristeus varidens</i> | Medina et al. 2006b |
| Solenoceridae | <i>Pleoticus muelleri</i> | Medina et al. 2006a |
| | <i>Solenocera africana</i> | Medina et al. 2006a |
| | <i>Solenocera membranacea</i> | Medina et al. 2006a |
| SERGESTOIDEA | | |
| Sergestidae | <i>Peisos petrunkevitchi</i> | Scelzo & Medina 2004 |
| Suborder Pleocyemata | | |
| Caridea | | |
| PALAEOMOIDEA | | |
| Palaemonidae | <i>Macrobrachium nipponense</i> | Yang et al. 1998 |
| Palinura | | |
| PALINUROIDEA | | |
| Scyllaridae | <i>Thenus orientalis</i> | Zhu et al. 2002 |
| Anomura | | |
| AEGLOIDEA | | |
| Aeglididae | <i>Aegla longirostri</i> | Tudge & Scheltinga 2002 |
| HIPPOIDEA | | |
| Albuneidae | <i>Albunea marquisiana</i> | Tudge et al. 1999 (as <i>Albunea</i> sp.) |
| Hippidae | <i>Emerita talpoida</i> | Tudge et al. 1999 |
| | <i>Hippa pacifica</i> | Tudge et al. 1999 |
| PAGUROIDEA | | |
| Diogenidae | <i>Calcinus tubularis</i> | Tirelli et al. 2006 |
| | <i>Clibanarius erythropus</i> | Tirelli et al. 2007 |
| | <i>Clibanarius vittatus</i> | Matos et al. 1993 |
| | <i>Diogenes pugilator</i> | Manjón-Cabeza & García Raso 2000; Tirelli et al. 2008 |
| | <i>Loxopagurus loxochelis</i> | Scelzo et al. 2006 |
| | <i>Petrochirus Diogenes</i> | Brown 1966a |
| Paguridae | <i>Pagurus stimpsoni</i> | Brown 1966a (as <i>P. bonairensis</i>) |
| Pylochelidae | <i>Pylocheles (Bathycheltes)</i> sp. | Tudge et al. 2001 |
| Brachyura | | |
| MAJOIDEA | | |
| Inachidae | <i>Inachus phalangium</i> | Rorandelli et al. 2008 |
| PORTUNOIDEA | | |
| Portunidae | <i>Scylla serrata</i> | Shang Guan & Li 1994; Wang et al. 1997 |
| Trichodactylidae | <i>Dilocarcinus septemdentatus</i> | Matos et al. 1996 |
| POTAMOIDEA | | |
| Gecarcinucidae | <i>Geithusa pulcher</i> | Klaus et al. 2008 |
| | <i>Heterothelphusa fatum</i> | Klaus et al. 2008 |
| | <i>Oziothelphusa ceylonensis</i> | Klaus et al. 2008 |

Table 1. continued.

| Suborder/Infraorder, SUPERFAMILY & Family | Species | Reference |
|--|--|-----------------------|
| | <i>Oziothelphusa</i> sp. | Klaus et al. 2008 |
| | <i>Parathelphusa convexa</i> | Klaus et al. 2008 |
| | <i>Parathelphusa maindroni</i> | Klaus et al. 2008 |
| | <i>Phricothelphusa gracilipes</i> | Klaus et al. 2008 |
| | <i>Sartoriana spinigera</i> | Klaus et al. 2008 |
| | <i>Sayamia bangkokensis</i> | Klaus et al. 2008 |
| | <i>Siamthelphusa improvisa</i> | Klaus et al. 2008 |
| | <i>Somanniathelphusa</i> sp. | Klaus et al. 2008 |
| | <i>Terrathelphusa kuhli</i> | Klaus et al. 2008 |
| Potamidae | <i>Geothelphusa albogilva</i> | Klaus et al. 2008 |
| | <i>Johora singaporensis</i> | Klaus et al. 2008 |
| | <i>Larnaudia beusekomae</i> | Klaus et al. 2008 |
| | <i>Malayopotamon</i> <i>brevimarginatum</i> | Klaus et al. 2008 |
| | <i>Potamiscus beieri</i> | Brandis 2000 |
| | <i>Pudaengon thatphanom</i> | Klaus et al. 2008 |
| | <i>Sinopotamon yangtsekiense</i> | Wang et al. 1999 |
| | <i>Thaiphusa sirikit</i> | Klaus et al. 2008 |
| Potamonautidae | <i>Hydrothelphusa</i> <i>madagascariensis</i> | Klaus et al. 2008 |
| OCYPODOIDEA | | |
| Ocypodidae | <i>Uca maracoani</i> | Benetti et al. 2008 |
| | <i>Uca thayeri</i> | Benetti et al. 2008 |
| | <i>Uca vocator</i> | Benetti et al. 2008 |
| | <i>Ucides cordatus</i> | Matos et al. 2000 |
| GRAPSOIDEA | | |
| Grapsidae | <i>Metopograpsus messor</i> | Anilkumar et al. 1999 |
| Varunidae | <i>Eriocheir sinensis</i> | Du et al. 1988 |

density, intervening cytoplasm containing some or all of the following organelles — mitochondria, microtubules, lamellar structures and centrioles — and a variable number (from zero to many) of arms or spikes. The arms may be composed of nuclear material, or microtubules, or both. In the Anomura, for example, the arms always contain microtubules, while in the Brachyura they are composed of nuclear material, except for some members of the Majidae that are reported (Hinsch 1969, 1973) to have microtubular elements in the nuclear arms.

Thus, in comparison to the diversity of crustacean spermatozoa, decapods are reasonably conservative, but it is still difficult to describe a typical decapod sperm cell. A taxonomic survey of decapod spermatozoal morphology at this point would be quite extensive, repetitive, and, frankly, dull. Instead, I want to highlight several characteristic and unique spermatozoal characters/features that emphasize the special place that the diverse decapod crustaceans hold within the Crustacea and within the wider animal kingdom.

2 THE UNIQUE DECAPOD SPERM

All decapod spermatozoa are unusual for the following six reasons: 1) they are aflagellate (lack a true 9 + 2 flagellum); 2) there is no reliable record of motility of any individual sperm cell; 3) the acrosome vesicle is not Golgi-derived as it is in all other described acrosomes of sperm in the animal

kingdom; 4) the decapod sperm nuclear protein is unique; 5) the sperm nucleus is composed of diffuse, filamentous, heterogeneous chromatin fibers rather than being uniformly dense; and 6) the mitochondria are degenerate in mature sperm cells. These unique features will be elaborated below.

2.1 Aflagellate sperm cells

Most swimming or flagellate spermatozoa possess a tail(s) with a structured "9 + 2" arrangement of microtubules termed an axoneme. However, in the Crustacea, true flagellate spermatozoa have been recorded only in the Remipedia and in the maxillopodans (Cirripedia, Branchiura, Pentastomida, Mystacocarida, and Ascothoracica). Some apparently flagellate crustacean spermatozoa, such as the long and filamentous ostracod, amphipod, mysid, cumacean, and isopod sperm cells, are considered pseudoflagellate, and their "tail" is most often a long striated extension of the acrosome (see Fig. 1). Jamieson (1987, 1991) referred to this as a pseudoflagellum or striated tail-like appendage and regarded it as a synapomorphy for these peracarids.

Although microtubules are present in many decapod sperm cells, particularly in the long, and often numerous, microtubular arms, no true flagellum has ever been recorded. The entire diverse Decapoda, therefore, possess aflagellate spermatozoa.

2.2 Immotile sperm cells

Taking into account the previous character, it is not at all surprising that all recorded sperm cells in the Decapoda are also non-swimming (immotile). Even though the conspicuous arms (often microtubular) seen emanating from sperm cells seem to indicate motility, it has yet to be recorded in decapods. The absence of a true axoneme, with its inherent complexity, in any sperm cells renders them immobile. Some authors have claimed that the extensive and explosive acrosome reaction seen in decapod sperm cells (Brown 1966a, b; Talbot & Chanmanon 1980) constitutes a form of cell motility, but even though it appears to annex new ground for the expanding cell, it does not qualify as independent swimming motion typically associated with sperm cell motility.

2.3 Acrosome vesicle

The acrosome vesicle, probably more correctly termed "acrosomal complex" (Baccetti & Afzelius 1976), refers to the often large, concentrically zoned, electron-dense vesicle at the apical end, or constituting the apical portion, of the sperm cell of all decapods (see Figs. 3A, 4A, B). The term acrosome ("akrosoma") was first introduced by Lenhossek (1898) and was later applied to the "capsule" of decapod sperm by Bowen (1925), who also postulated that acrosomal material is formed in close association with the Golgi complex (Figs. 2A, B). Although the typical definition of an acrosome states that its origin is clearly from the Golgi complex, this does not apply to the acrosome of all decapod crustaceans studied to date. It has been shown in a wide range of decapods, including the dendrobranchiate shrimp *Parapenaeus longirostris* (Medina 1994), the caridean shrimp *Palaeomonetes paludosus* (Koehler 1979), the crayfish *Procambarus clarkii* (Moses 1961a, b) and *Cambaroides japonicus* (Yasuzumi et al. 1961), the hermit crab *Pagurus bernhardus* (Pochon-Masson 1963, 1968) and the brachyurans *Eriocheir japonicus* (Yasuzumi 1960), *Menippe mercenaria*, *Callinectes sapidus* (Brown 1966a), *Carcinus maenas* (Pochon-Masson 1968), *Uca tangeri* (Medina & Rodriguez 1992), and *Cancer* species (Langreth 1969), that no typical Golgi complex is involved during acrosomal differentiation. Some recent authors (e.g., Yang et al. 1998; Wang et al. 1999) have suggested the presence of Golgi-derived acrosomes in certain decapods, but careful examination of their micrographs indicate that their "Golgi bodies" are complex membrane arrays (admittedly looking remarkably Golgi-like in appearance) and probable extensions of abundant endoplasmic reticulum. The acrosome vesicle of decapods is therefore defined as an acrosome by its position and function and not strictly by its cellular origin.

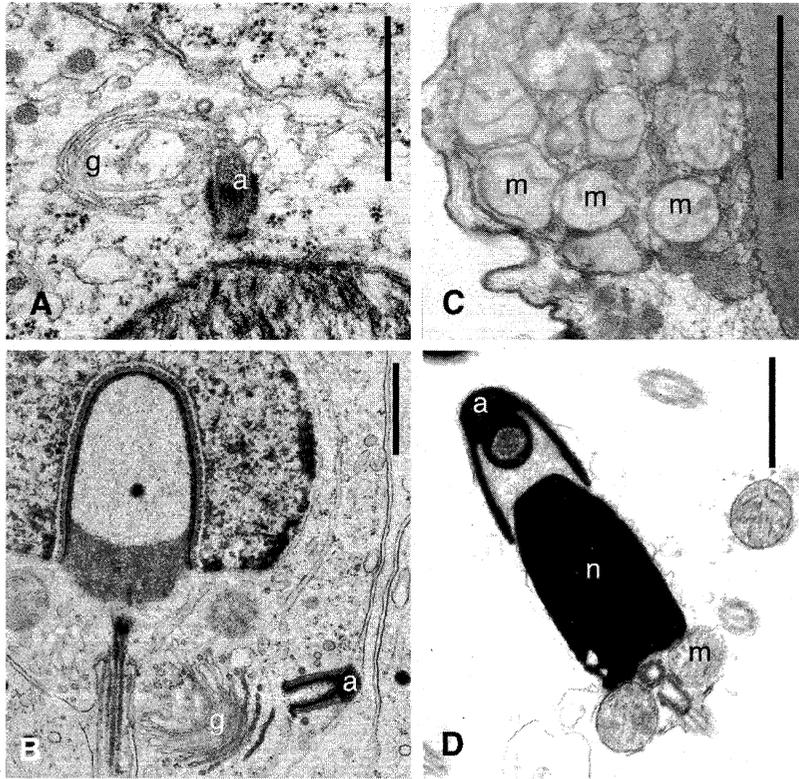


Figure 2. Transmission electron micrographs. (A) Golgi body (g) participating in acrosome development during spermiogenesis in the gastropod mollusc *Littorina sitkana*. Modified from Buckland-Nicks & Chia (1976). (B) Golgi body adjacent to developing acrosome (a) in the gastropod mollusc *Nerita picea*. Modified from Buckland-Nicks & Chia (1986). (C) Poorly cristate or acristate mitochondria (m) in the mature spermatozoa of the coconut crab, *Birgus latro*. (D) Typical electron-dense nucleus (n) in the mature spermatozoa of a limpet mollusc, *Cymbula concolor* (note the complex, cristate mitochondria (m) at the base of the nucleus). Photos courtesy of John Buckland-Nicks (A & B) and Alan Hodgson (D). Scale bars = 1 μm .

2.4 Sperm nuclear proteins

In the nucleus of all sperm cells the DNA is closely associated with a collection of proteins referred to as sperm nuclear basic proteins or SNBPs (Bloch 1969). These sperm-specific nuclear proteins appear in late spermiogenesis and are associated with highly compacted and inactive DNA. Unlike the evolutionarily conservative histones in somatic cell nuclei, SNBPs are highly diverse. There are five categories of these SNBPs spread across all the animal kingdom (both protostomes and deuterostomes) (Bloch 1969; Ausio 1995; Kasinsky 1991, 1995). The arthropods, for example, have representatives with all five types of SNBPs: H, P, PL, KP, and O:

- H-type (histones) *Rana* type (named for the animal in which it was first described)
- P-type (protamines) Salmon type (also in plants and the cirripede barnacle, *Balanus*)
- PL-type (protamine-like) *Mytilus* type
- KP-type (keratinous proteins) Mouse type
- O-type (absence of any sperm basic proteins) Crab type

Type “O,” as you would expect, is found only in the decapods. Decapods have no SNBPs (but see Kurtz et al. 2008 for new, contrary information) but instead have extra-nuclear basic proteins, first termed “decapodine” by Chevaillier (1967) in *Nephrops*, *Pagurus*, and *Carcinus*. These unique decapodines are found in the large, electron-dense, and often voluminous acrosome vesicle, and migrate there from the nucleus during spermiogenesis (Chevaillier 1968; Vaughn et al. 1969).

2.5 Sperm nucleus

Associated with these sperm nuclear basic proteins and their unique absence (once again see Kurtz et al. 2008 for new, contrary information) in the decapod sperm nucleus is the fact that decapod sperm nuclei are also diffuse, electron-translucent, and filamentous in appearance (Fig. 3B) rather than being typically condensed, electron-dense, and granular (Fig. 2D). Condensation of the sperm head (nucleus) is characteristic of most animals regardless of the type of SNBPs they contain, except for decapod crustaceans. In fact, the densest part of the spiked decapod sperm cell is the acrosome vesicle, while the nucleus is electron-lucent and lightly granular or more usually filamentous.

If you look more closely at the structure of the nuclear filaments in decapod sperm under transmission electron microscopy (Fig. 3B), the nucleus has dense fibers ranging from 20 to

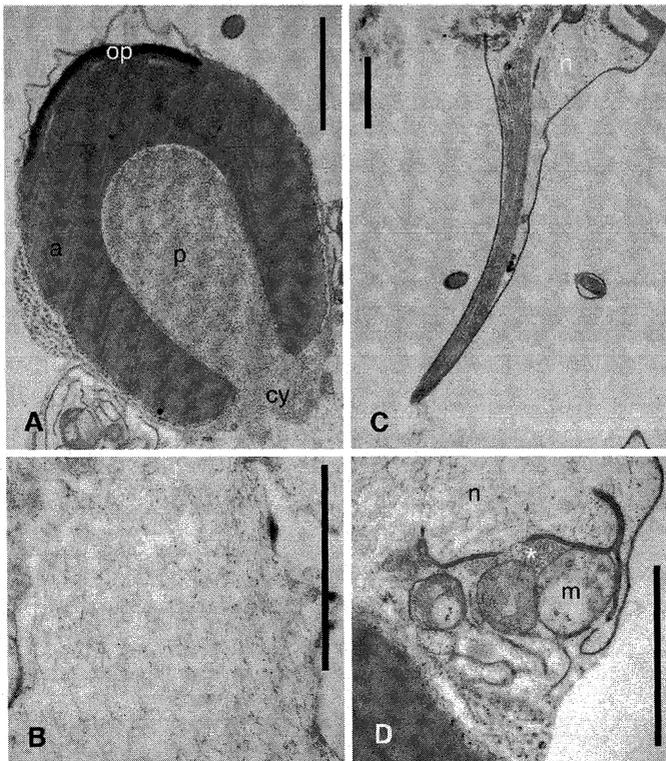


Figure 3. Transmission electron micrographs of the mature spermatozoa of the hermit crab *Loxopagurus loxochelis* (Diogenidae). (A) Electron-dense and complexly zoned acrosome vesicle (a). (B) Chromatin fibers in the electron-lucent nucleus (n). (C) Longitudinal section through an external microtubular arm. (D) Internalized microtubular arm (*), in cross-section, adjacent to degenerate mitochondria (m). Other abbreviations: cy, cytoplasm; op, operculum; p, perforatorial chamber. Photos courtesy of Marcelo Scelzo. Scale bars = 1 μ m.

200 angstroms (Å) in width. These were argued by Chevaillier (1966b, 1991) to be bare DNA fibers. He also stated that all the SNBPs migrated during spermiogenesis from the sperm nucleus into the acrosome vesicle, where they associated with other proteins to form the characteristic decapodine.

2.6 Mitochondria

The last of our six unique decapod sperm characters is the presence in the mature sperm of only degenerate (or nearly so), non-cristate, non-functional mitochondria. In general, decapod sperm have only small amounts of cytoplasm and, therefore, often low numbers of recognizable organelles. Mitochondria can even appear to be totally absent in mature sperm (the Brachyura are a good example of this). What few mitochondria there are usually have few recognizable cristae or are devoid of them (Figs. 2C, 3D).

Studies conducted in the mid-1970's (Pearson & Walker 1975) showed that cytochrome C oxidase activity (an indicator of oxidative phosphorylation and confined to mitochondrial cristae) diminished as mitochondrial morphology changed over spermiogenesis in the crab *Carcinus maenas*. As decapod spermatids mature, most mitochondria are lost or lose their cristae. By the time the sperm cell is mature, it does not show this enzyme activity. This should not be surprising considering that we already established that all decapod sperm are immotile, and so mitochondria are used to power the dynamic process of spermiogenesis only, rather than in cell motility. But aspects of mitochondrial morphology and function in those decapods that store sperm for long periods (e.g., Cheung 1968; Paul 1984) may be worth investigating.

The above six characteristics demonstrate that decapods are unique spermatologically but do not provide much useful information for elucidating phylogenetic relationships within the Decapoda. Of the large suite of spermatozoal characters described in the literature for various decapod sperm, there is only a subset that has any potential phylogenetic utility.

3 SPERM AND DECAPOD PHYLOGENY

"that one may often safely venture to infer from the specific shape of these elements (spermatozoa) the systematic position and the name of the animals investigated."

Wagner & Leuckhart (1852)

The use of spermatozoal ultrastructure in taxonomy and phylogeny is well established in various animal groups. Examples include: Oligochaeta (Jamieson 1983); Pentastomida (Storch & Jamieson 1992); Insecta (Jamieson et al. 1999); Anura (Jamieson 2003); Annelida (Rouse & Pleijel 2006); and Aves (Jamieson 2007).

Similarly, in the decapod crustaceans spermatozoal ultrastructure has been successful in elucidating phylogenetic relationships (e.g., Jamieson & Tudge 1990; Jamieson 1994; Jamieson et al. 1995; Medina 1995; Tudge 1997; Medina et al. 1998). Spermatozoal characters have also been used in conjunction with existing morphological character sets in recent phylogenetic analyses (Ahyong & O'Meally 2004) or to support taxonomic or systematic works (Scholtz & Richter 1995; Brandis 2000).

Some spermatozoal characters with relevance to phylogenetic reconstruction of decapod crustaceans include the following, with examples from investigated taxa.

3.1 The acrosome vesicle

Presence/absence: As previously mentioned, the acrosome vesicle is an electron-dense structure, usually used to help define the apical end or pole of the decapod sperm cell, that contains most of the cell's proteins and is therefore often complexly structured. An apical acrosome vesicle (variously

sized and shaped) is present in all decapods studied to date, with the notable exception of some of the dendrobranchiate shrimp (families Aristeidae and Sergestidae) and the basal pleocyemate shrimp *Stenopus*, in the family Stenopodidae. Interestingly, several investigated genera in the order Euphausiacea also possess acrosome-less spermatozoa. See Jamieson & Tudge (2000) for a brief review of the supposedly plesiomorphic acrosome-less spermatozoa in the decapods and the novel development and origin of the malacostracan acrosome vesicle (also mentioned above). The loss of the “Golgi-derived” acrosome, common in the rest of the Crustacea, the absence of any acrosome in the above-mentioned basal shrimps, and the independent development of the “ER-derived” malacostracan acrosome vesicle could be important characters for helping to define the early branching patterns in the evolution of the Decapoda.

Shape: When present, the decapod acrosome vesicle is either embedded into the sperm cell (Fig. 4) or sits prominently atop the rest of the cell components (cytoplasm and nucleus) (Fig. 3A). The acrosome vesicle also assumes a large variety of shapes including straight spikes, curved spikes, flat discs, hollow domes, ovals (depressed or elongate), hemispheres, spheres (both slightly depressed or slightly elongate), and elongate cones or cylinders. Differences in shape can also occur because of apical perforations (through the operculum, for example) or basal perforations or invaginations, usually termed the perforatorial chamber (Figs. 3A, 4). This term refers to the

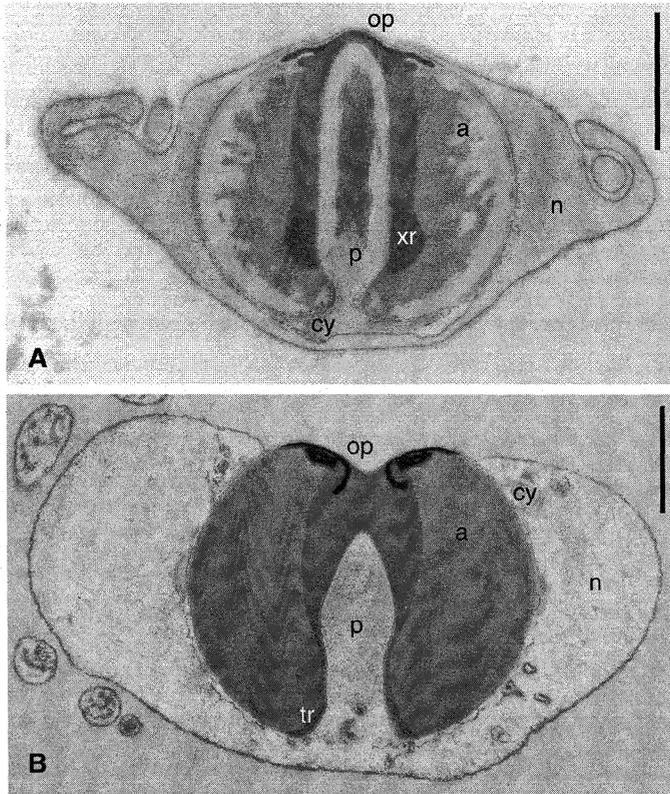


Figure 4. Transmission electron micrographs of a longitudinal section through the mature spermatozoa of two brachyuran crabs. (A) *Pilodius areolatus* (Xanthidae). (B) *Camposcia retusa* (Inachidae). Abbreviations: a, acrosome vesicle; cy, cytoplasm; n, nucleus; op, operculum; p, perforatorial chamber; tr, thickened ring; xr, xanthid ring. Photos courtesy of Barrie Jamieson. Scale bars = 1 μm .

invagination of the posterior end of the acrosome vesicle into a column or tube that penetrates the acrosome vesicle to various depths. The term “perforatorium” was first used by Waldeyer (1870) for a rod of fibrous material between the acrosome and nucleus in an amphibian sperm cell. It was later shown that the vertebrate perforatorium is homologous to equivalent invertebrate acrosomal structures (Dan 1967), and it was convenient to extend the term perforatorium to any subacrosomal material that projects outward at the time of the acrosome reaction (Baccetti 1979). The term perforatorial chamber reflects the fact that it is an invagination of the acrosome vesicle membrane (forming a chamber) that, with its contents, carries out the function of a perforatorium (*sensu* Baccetti 1979) without necessarily being rod-like or fibrous.

Dimensions and size: With the diversity of acrosome vesicle shapes comes an equally diverse array of sizes and dimensions for this organelle. A figure plotting acrosome length versus width for a variety of decapod sperm cells was provided by Jamieson (1991: 121), and a similar figure for the Anomura alone can be found in Tudge et al. (2001: 126) showing basic trends of spherical, elongate, or depressed, and any obvious deviations of individual species or groups of taxa. Often, the unusual size and shape differences of some taxa become clearer when plotted in this manner (see the example of the anomuran *Pylocheles* sperm in Tudge et al. 2001).

Internal complexity: The decapod acrosome vesicle in its various shapes and sizes also exhibits a range of internal ultrastructural complexity from simple to concentrically arranged in multiple layers or zones, each with its own electron density and morphology (Fig. 3A, 4). The exact biochemical nature and cellular function of most of these acrosomal zones are unknown, beyond their being composed of an array of PAS+ complex polysaccharides (e.g., Pochon-Masson 1965; Brown 1966b; Chevaillier 1966a), migrated sperm nuclear proteins (see above), and cytoskeletal proteins (Jamieson & Tudge 2000). These acrosome vesicle zones are intimately involved in the dynamic acrosome reaction that delivers the posterior nuclear material across the egg membrane at fertilization (see Jamieson & Tudge 2000 for review). Although their exact composition and function are still mysterious, their unique density, granularity, and morphology under TEM have provided a wealth of acrosomal characters for comparison of decapod sperm cells, particularly within the major infraorders. The complexly zoned and morphologically distinct acrosome vesicles have yielded a suite of characteristic and consistent traits identifying and unifying different groups of decapod taxa.

Some notable examples of these acrosome vesicle character traits include: the “dense perforatorial ring” in the hermit crab genus *Clibanarius* (Tudge 1997), the “xanthid ring” (Fig. 4A) common to all investigated members of this heterotreme brachyuran family (Jamieson 1989a, 1991), the distinctive structure of the flattened, centrally depressed, and often perforated majoid operculum (Fig. 4B) (Jamieson 1991; Jamieson et al. 1998; Jamieson & Tudge 2000) seen in this basal eubranchyuran group, and, finally, both the “apical button” perched on top of the operculum and the concentric lamellae present in the outer acrosome zones seen in nearly all thoracotreme crabs (Jamieson & Tudge 2000).

3.2 The nucleus

Membrane-bound: A defining feature separating the dendrobranchiate shrimp from the remaining pleocyemate decapods is that the nuclear region in the sperm cell of the former is not membrane-bound, while it is always membrane-bound in the latter (Medina 1995; Jamieson & Tudge 2000).

Morphology and shape: The basal or posterior sperm nucleus (if the acrosome vesicle is considered apical or anterior) can assume many different shapes throughout the Decapoda. It is spherical or globular in most of the dendrobranchiate shrimp (Medina et al. 1998) and the achelate lobsters (Tudge et al. 1998); triradiate in some of the podotreme brachyuran crabs, such as *Ranina*

(Jamieson 1989b) and *Dromidiopsis* (Jamieson et al. 1993) and the heterotreme brachyurans in the family Leucosiidae (Felgenhauer & Abele 1991; Jamieson & Tudge 2000); amorphous with multiple, pseudopodia-like lateral extensions or arms in many anomurans (Tudge & Jamieson 1991; Tudge 1995) and brachyurans; and secondarily cup-like in overall shape in all the brachyuran crabs where the spherical acrosome vesicle is embedded deeply into the cytoplasm and nuclear material (Jamieson & Tudge 2000).

Sometimes the nucleus is posteriorly extended as a distinct, single, thickened elongation (termed the "posterior median process"), and this has been recorded in the spermatozoa of some homolid and basal heterotreme brachyurans (Hinsch 1973; Jamieson & Tudge 2000). A fundamental difference in spermatozoal nuclear shape has also been used to support a division between the genera within the anomuran family Porcellanidae (Haig 1965; Sankolli 1965; Van Dover et al. 1982). Some genera (e.g., *Petrolisthes*) possess a spherical, more globular nucleus below the large complex acrosome vesicle, while others (e.g., *Aliaporcellana*, *Pisidia* and *Polyonyx*) have the sperm nucleus extended out into a long thick "tail," with a dense microtubular core, splitting terminally to yield multiple microtubular arms (Tudge & Jamieson 1996a, b). This unusual, superficially flagellate, decapod sperm morphology was first illustrated by Retzius (1909) for *Pisidia* (as *Porcellana*).

3.3 Microtubular arms

Presence/absence: As previously stated, all decapod spermatozoa are aflagellate, lacking a true "9+2" flagellum, but many do possess microtubular extensions from the sperm cell, which are often collectively called microtubular arms (Fig. 3C, D). The few decapod groups where no microtubular arms have been recorded include all the dendrobranchiate, caridean, and stenopodidean shrimps and the Brachyura (secondary loss), with the doubtful exception of some lower heterotremes in the majoid group (Jamieson & Tudge 2000). In these latter crabs the lateral arms are nuclear in origin (as they are in all brachyurans) but are said to contain a microtubular core inside them (Hinsch 1973). No independent, "naked," microtubular arms are present in any brachyuran investigated for sperm ultrastructure to date, although microtubules may be evident in sperm cell lateral arms and nuclei under certain conditions (Jamieson & Tudge 2000).

Number: In the Decapoda with sperm cells possessing true microtubular arms, the number is highly variable (see Table 2), but it can be simplified into a system whereby four or more arms appear to be plesiomorphic (Astacidea, Thalassinidea, and Palinura). A reduction to three occurs in enoplometopid and nephropid lobsters and most groups in the Anomura (12 of 15 families), and then a further reduction to total loss (as mentioned above) occurs in the Brachyura (Tudge 1997). It is interesting to note that in the podotreme brachyurans, some have sperm cells that exhibit three nuclear arms or extensions (the triradiate condition previously mentioned), and in the few heterotremes with microtubules still present in their nuclei, three lateral nuclear vertices are often apparent (Jamieson & Tudge 2000).

Origin: In the Decapoda that have sperm cells possessing true microtubular arms, these are externalized from the cell either from within the cytoplasm or from the nuclear material (Fig. 3C, D). Initially, all microtubules are grown from centrioles in the cytoplasm of the developing sperm cell, but once they become externalized they appear as either originating from the cytoplasm (e.g., all anomurans studied to date) or from the nucleus (e.g., Thalassinidea, Astacidea, and Palinura). This differing "origin" may have some phylogenetic significance (Tudge 1997).

An example of a spermatological character that does not appear to have any phylogenetic significance in the decapods investigated to date is the presence or absence of one or more centrioles in the mature sperm cell. In many decapod sperm cells, the pair (usually) of centrioles is observed

Table 2. The number of microtubular arms recorded in spermatozoa across the investigated decapod families, with indications of where the data are not available (NA) or need confirmation (?).

| | |
|-----------------------------------|--|
| Dendrobranchiata = 0 | Palinura |
| Pleocyemata | Palinuridae = 3–12 |
| Stenopodidea = 0 | Polychelidae = NA |
| | Scyllaridae = 6 |
| Caridea = 0 | Synaxidae = NA |
| Astacidea | Anomura |
| Astacidae = 5–8, 15–20 | Aeglididae = 3? |
| Cambaridae = 4–7, 20 | Albuneidae = >4 |
| Enoplometopidae = 3 | Chirostylidae = 3 |
| Glyphidae = NA | Coenobitidae = 3 |
| Nephropidae = 3 | Diogenidae = 3 |
| Parastacidae = 0? (nuclear only?) | Galatheidae = 3 |
| Thaumastocheilidae = NA | Hippidae = >4, 3–9 |
| Thalassinidea | Kiwaiidae = NA |
| Axianassidae = 5 | Lithodidae = 3 |
| Axiidae = NA | Lomisidae = 3? (3 nuclear vertices) |
| Callianassidae = 3?, 4–7 | Paguridae = 3 |
| Callianideidae = NA | Parapaguridae = 3 |
| Calocarididae = 4–5 | Porcellanidae = >4 |
| Ctenochelidae = NA | Pylochelidae = 3 |
| Laomediidae = NA | Pylojacquesidae = NA |
| Micheleidae = NA | Brachyura = 0 (sometimes 3 nuclear vertices) |
| Strahlaxiidae = 4 | |
| Thalassinidae = 3–5? | |
| Thomassinidae = NA | |
| Upogebiidae = NA | |

in the cytoplasm below the acrosome vesicle in mature spermatozoa, but their occurrence seems erratic and may be more dependent on the state of maturity of the cell, or even on fixation procedures (Jamieson & Tudge 2000). Often, closely related taxa (two species in a genus, for example) will differ in this character state. It should be expected that all sperm cells exhibiting microtubules should have one or more obvious centrioles, but this is not the case, and in fact many brachyuran crab spermatozoa (which mostly do not retain microtubules in the mature sperm cell) show a pair of orthogonally arranged centrioles beneath the acrosome vesicle. Recently, though, the number of centrioles (Benetti et al. 2008) or their unusual arrangement in a parallel pair (Jamieson 1993; Guinot et al. 1997; Klaus et al. 2008) has been suggested to have taxonomic and/or phylogenetic importance in the Ocypodidae and Potamoidea, respectively.

4 CONCLUSIONS

Spermatozoal characters have proven to be, and continue to be, useful tools in helping to elucidate phylogenetic relationships in the decapod crustaceans. Their greatest utility, though, does not lie in generating phylogenetic trees using only spermatozoal (and spermatophore) characters (e.g., Jamieson 1994; Tudge 1997), but in providing additional character states for establishing robust

nodes and clades in trees generated from more comprehensive datasets. Decapod species investigated for spermatozoal (and spermatophore) morphology will always be a smaller subset (currently 50% of the families, about 10% of genera, and only 2% of species) of those whose somatic morphology or gene sequences are known. Reproductive data, such as spermatozoal structure, can be used to supplement the initial matrices of characters for phylogenetic analysis or can be plotted *a posteriori* onto trees generated by morphological and molecular data to increase support for clades and trace the evolutionary history of the changing reproductive biology of decapod crustaceans. Similar evidence from reproductive biology may also help to confirm the most recent sister group of the Decapoda.

Continued research into the reproductive biology of decapod crustaceans is needed to fill the current gaps in our knowledge of this group, especially representatives from the families and superfamilies whose reproductive biology remains largely or totally unknown (e.g., Glypheidae, Micheleidae, Polychelidae, and Kiwaiidae). Also, further investigation is required on the taxa, and their congeners if available, for which only single species have been investigated for spermatozoal and spermatophore morphology and where they still provide only incomplete or enigmatic results (e.g., *Lomis* and *Aegla* in the Anomura, *Thalassina* in the Thalassinidea, and *Cherax* in the Astacidea).

ACKNOWLEDGEMENTS

I would like to acknowledge the constant support and guidance of my friend and colleague Professor Barrie Jamieson. His encouragement and academic fervor for this field are directly responsible for my own interest in, and association with, crustacean gamete biology. For this I am eternally grateful, and I dedicate this review paper to him. Without his significant contributions to crustacean and decapod spermatozoal ultrastructure and spermiocladistics, the field would not be in the prominent, dynamic, and advanced state that it currently enjoys. I also wish to acknowledge the support and assistance of Rafael Lemaitre (Chair, Invertebrate Zoology) and his excellent support staff team at the National Museum of Natural History, Smithsonian Institution, Washington, D.C. Barrie Jamieson, Alan Hodgson, John Buckland-Nicks, and Marcelo Scelzo are thanked for providing figures, and Rose Gullledge was invaluable to figure assembly.

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