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Reproductive Attributes of Polynoid Polychaetes from Hydrothermal Vents on the East Pacific Rise

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REPRODUCTIVE ATTRIBUTES OF POLYNOID POLYCHAETES FROM HYDROTHERMAL VENTS ON THE EAST PACIFIC RISE

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Master of Science

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Jessica Lynn Wallace

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

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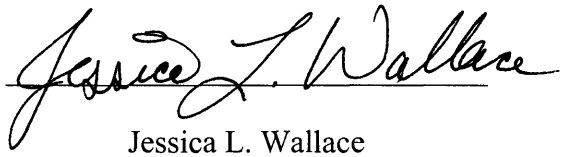

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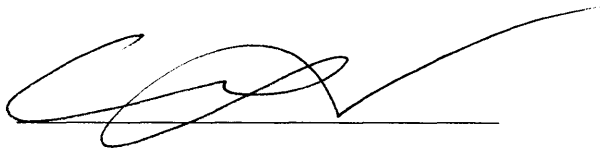

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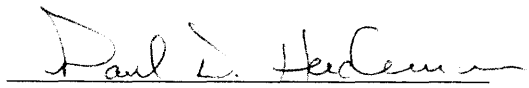

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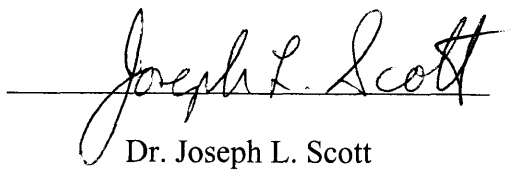

Dr. Joseph L. Scott

To Dad and Ken for inspiring my love of oceanography
To Mom and Stephen for their unending love and support

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ABSTRACT

The internal reproductive anatomy of 2 polynoid species, *Branchipolynoe symmytilida* and *Levensteiniella kincaidi,* from hydrothermal vents on the East Pacific Rise are compared with that of polynoids (*B. seepensis* and *Opisthotrochopodus* sp.) from Mid-Atlantic Ridge vents (Van Dover et al. 1999; Jollivet et al. 2000). In *B. symmytilida*, spermatozoa mature from anterior to posterior and toward the periphery of the body cavity. Masses of spermatocytes with filiform heads are located in sperm sacs that lead to the nephridial papillae. Oogonia mature in a multi-lobed ovary, where vitellogenesis takes place aided by follicle cells and association with blood vessels. Large, lecithotrophic oocytes (max. diameter 300 μm) are stored in a large ovisac and ultimately a spermatheca where packets of sperm are stored and fertilization presumably takes place before release to the outside through the nephridial papillae.

Spermatogenesis is similar in *L. kincaidi* and *B. symmytilida*. *L. kincaidi* also has lecithotrophic development (max. oocyte diameter 300 μm) and sperm storage, but well-developed ovaries and uterus are absent. Oocytes, surrounded by follicle cells, mature in small clusters in close association with blood vessels. All 4 vent polynoid species examined to date share the same basic male reproductive attributes. Differences exist in the female reproductive anatomy, but all 4 species have sperm storage and lecithotrophic development. This contrasts with the reproductive strategies of most shallow-water polynoids, which have broadcast spawning and planktotrophic development.

REPRODUCTIVE ATTRIBUTES OF POLYNOID POLYCHAETES FROM HYDROTHERMAL VENTS ON THE EAST PACIFIC RISE

INTRODUCTION

The ephemeral and unpredictable nature of hydrothermal vents places a premium on the ability of a species to reproduce and colonize new sites before going locally extinct (Corliss et al. 1979, Laubier & Desbruyères 1985). Despite the importance of reproduction in understanding the maintenance of vent communities, life history studies are limited by the logistical difficulties of time-series observations in the deep sea. Fewer than 25 of the more than 500 species described from vents have been investigated primarily for reproductive biology data (Tyler & Young 1999, Young 2003). Soon after the discovery of hydrothermal vents in 1977, vent species were hypothesized to have similar reproductive characteristics, mainly those maximizing long-distance dispersal capabilities such as small, abundant free-swimming planktotrophic larvae (e.g., Desbruyères & Laubier 1983, Turner et al. 1985). More recently, there is evidence that reproductive strategies are often under strong phylogenetic constraints (Eckelbarger & Watling 1995, Tyler & Young 1999, Young 2003). Molluscs (Lutz et al. 1984) and decapod crustaceans (Van Dover et al. 1985, Van Dover & Williams 1991) were among the first taxa specifically shown to employ reproductive strategies dictated by phylogeny instead of by environmental influence.

Phylogenetic constraints are a strong predictor of reproductive attributes in many non-vent invertebrate species as well. For example, echinothuriid echinoids display the same modified gametes and asynchronous gametogenesis in both shallow water and the

deep sea, despite large differences in nutrient availability and environmental conditions (Mori et al. 1980, Eckelbarger et al. 1989). Vent bivalves also show remarkable phylogenetic constraint on reproduction and development. Vesicomyid and solemyid bivalves from vents studied to date have gonads and lecithotrophic larvae (Endow & Ohta 1980, Berg 1985, Fiala-Medioni & Le Pennec 1989, Beninger & Le Pennec 1997) while mytilid bivalves undergo gametogenesis in the mantle and freely spawn their larvae (Berg 1985, Hessler et al. 1988, Le Pennec & Beninger 1997, Comtet & Desbruyères 1998, Tyler & Young 1999). Typical reproductive attributes of polychaetes include coelomic gamete maturation, broadcast spawning, external fertilization, and planktotrophic larvae (e.g., Segrove 1941, Fauchald 1974), but these attributes vary widely and are not necessarily the most common characteristics (Wilson 1991, Rouse & Fitzhugh 1994, Rouse & Pleijel 2001). Within the polynoid polychaetes (scaleworms), all but one shallow-water species examined to date broadcast spawn their eggs, which develop into planktotrophic larvae (Giangrande 1997, Llodra 2002). *Harmothoë imbricata* is the only documented species that differs from this pattern, with females brooding eggs under the elytra (Gremare & Olive 1978). In contrast to most shallow-water species, two vent polynoids, *Branchipolynoe seepensis* and an undescribed species of *Opisthotrochopodus* from vents on the Mid-Atlantic Ridge, show evidence of internal fertilization and lecithotrophic development (Van Dover et al. 1999, Jollivet et al. 2000). It is unknown whether other vent polynoids share these reproductive traits or have attributes in common with shallow-water species. *B. seepensis* and *Opisthotrochopodus* n. sp. maintained similar reproductive characteristics despite contrasting habitats: *B. seepensis* is a commensal inside *Bathymodiolus* sp. mussel mantle cavities, and *L. kincaidi* is free-living among the mussels. The nature of the dependency of *B. seepensis*

on the host mussels is under investigation, with preliminary evidence suggesting that the worm is a kleptoparasite, stealing food from the digestive tract of the host mussel (Britayev et al. 2003).

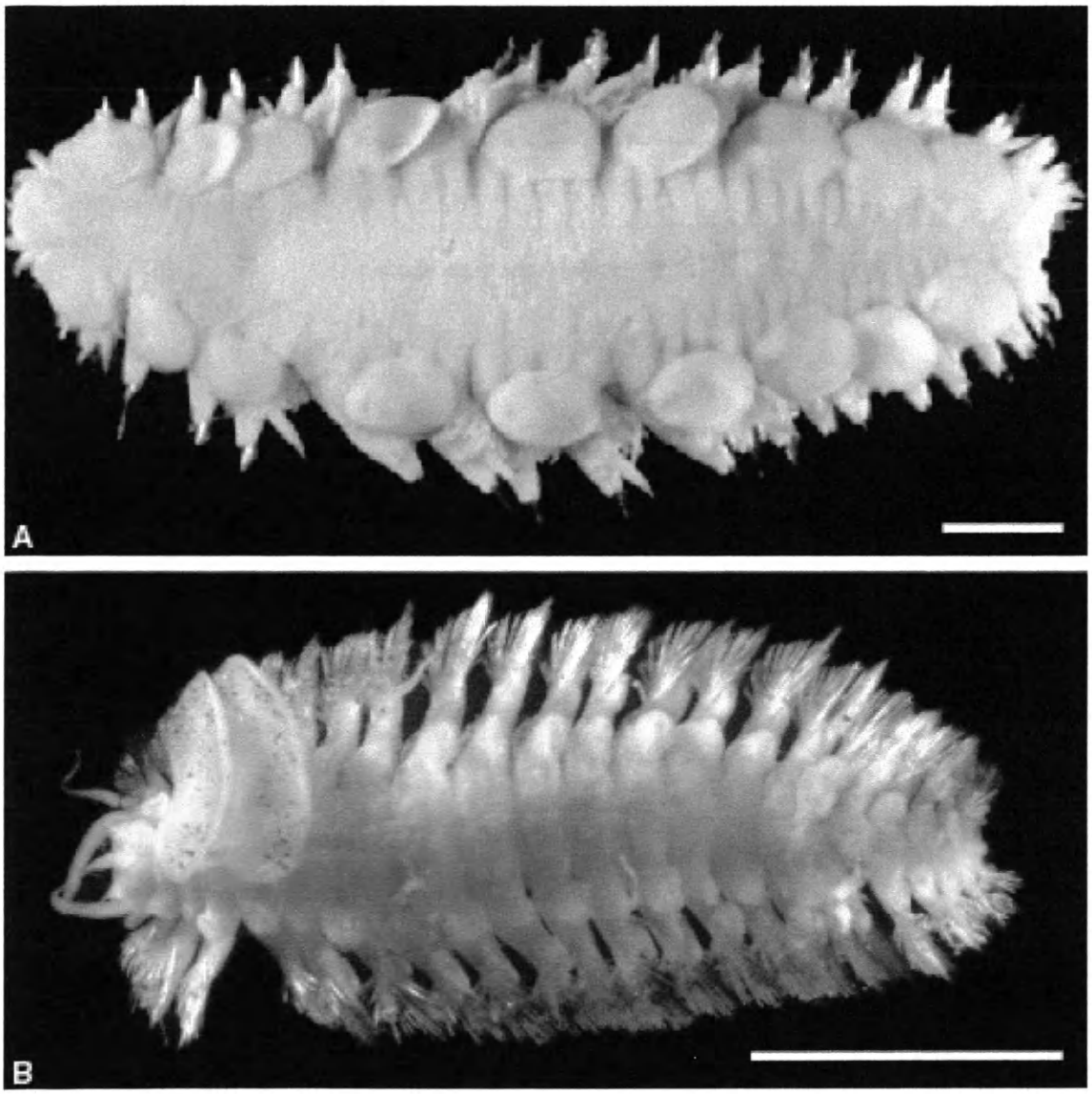
Reproductive attributes are only known for about 3% of all polychaete species, while oogenesis has been described in only 0.3% of polychaete species (Giangrande 1997). Of the numerous described hydrothermal vent polychaete species (Desbruyères & Segonzac 1997, Young 2003), only 13 species in 4 families have been investigated for reproductive life history data, and a variety of strategies are employed. Five of the species examined are vestimentiferan tubeworms, one of the most extensively studied and unusual taxa at hydrothermal vents. All 5 vestimentiferans investigated have sperm storage and internal fertilization (Hilário et al. 2005). Alvinellid polychaetes *Paralvinella grasslei* (Jollivet et al. 1995, Zal et al. 1995), *P. pandorae* (McHugh 1989, McHugh 1995), *Alvinella pompejana* (Jollivet et al. 1995), and *A. caudata* (Jollivet et al. 1995) and *P. palmiformis* have sperm storage (McHugh 1985, McHugh 1995, Copley et al. 2003). The ampharetid polychaetes *Amphisamytha galapagensis* (McHugh & Tunnicliffe 1994) and *Amathys lutzi* (Blake & Van Dover, in press) have freely-spawned lecithotrophic larvae. Polynoid polychaetes are one of the most speciose taxa at chemosynthetic environments including hydrothermal vents (Desbruyères & Segonzac 1997), cold seeps (Sibuet & Olu 1998), whale falls (Smith et al. 2003). The 2 polynoid species investigated make up 15% of the total number of described vent polynoid species, making polynoids proportionally one of the least studied vent polychaete taxa. This lack of reproductive information hinders our understanding of these animals and their ecological niche among cohabitating vent species.

To expand on the current knowledge of vent polynoid reproduction, we investigated the reproductive characteristics of two polynoid polychaete species. The first, *Branchipolynoe symmytilida* Pettibone 1984, is commensal within the mantle cavity of the mussel *Bathymodiolus thermophilus* and endemic to vents and other deep-sea reducing environments (Fig. 1A). One polychaete usually inhabits each mussel mantle cavity, but 2 or more have been found in a single host (Pettibone 1984, pers. obs.). The known geographic range of *B. symmytilida* includes vents along the northern and southern East Pacific Rise and the Galapagos Rift (Chevaldonne 1998, Hurtado et al. 2004, pers. obs.). The second species, *Levensteiniella kincaidi* Pettibone 1985, is free-living in vent mussel beds along the East Pacific rise (Fig. 1B, Pettibone 1985, Van Dover 2003).

Reproductive strategies of *Branchipolynoe symmytilida* and *Levensteiniella kincaidi* were determined from investigations of histological sections and gross dissections. Sperm head shape was noted to infer sperm transfer method: "normal" or primitive sperm with a spherical head shape are often associated with free spawning and external fertilization (Franzen 1977), while elongate or filiform sperm with limited mobility are often indicative of internal fertilization, pseudocopulation in gelatinous egg masses, or fertilization in tubes (Franzen 1956, Jamieson & Rouse 1989). Oocyte size range and distribution was investigated to characterize the level of gametogenic synchronization and type of development (e.g. planktotrophic vs. lecithotrophic, reviewed by Ecklebarger 1994). The presence or absence of sperm storage was also noted.

FIGURE 1

DORSAL VIEW OF *BRANCHIPOLYNOE SYMMYTILIDA* AND *LEVENSTEINIELLA KINCAIDI*



Dorsal surface of (A) *Branchipolynoe symmytilida* and (B) *Levensteiniella kincaidi*. Note that most scales are lost in processing of *L. kincaidi*. Scale bars = 400 μm.

CHAPTER I

MATERIALS AND METHODS

Specimens of *Branchipolynoe symmytilida* and *Levensteiniella kincaidi* were collected in December 2001 by *DSV Alvin* from mussel beds at 9°N on the northern East Pacific Rise [Train Station: 9° 49.645' N, 104° 17.357' W (2491 m); East Wall: 9° 50.534' N, 104° 17.520' W (2499 m); Biovent: 9° 50.992' N, 104° 17.592' W (2494 m)]. Additional specimens of *L. kincaidi* were obtained from February 1999 collections from mussel beds at 17°S on the southern East Pacific Rise [Oasis: 17° 25.394' S, 113° 12.323' W (2582 m); Rehu Marka: 17° 24.940' S, 113° 12.190' W (2581 m)]. Animals were removed from mussel mantle cavities and mussel washings, preserved in 10% buffered seawater formalin, and stored in 70% ethanol.

Thirty intact specimens from each species were dehydrated in an alcohol series and embedded in paraffin wax. Each was serially sectioned (5-7 μm sections) using a microtome, mounted on slides, and stained with Gill's hematoxylin and eosin (H&E; Stevens 1990) for examination under a Zeiss Axioskop 2 compound microscope. For additional study of *Branchipolynoe symmytilida* internal anatomy, 8 preserved specimens from 9°N and 8 fresh specimens from 38°S were dissected and photographed with an Olympus DP11 digital camera. *Branchipolynoe* aff. *symmytilida* were collected in March 2005 at 38°S on the Pacific-Antarctic Ridge (38°47.466'S; 110°54.867'W, 2230 m) for fresh dissection.

CHAPTER II

RESULTS: *Branchipolynoe symmytilida*

Sexual Dimorphism

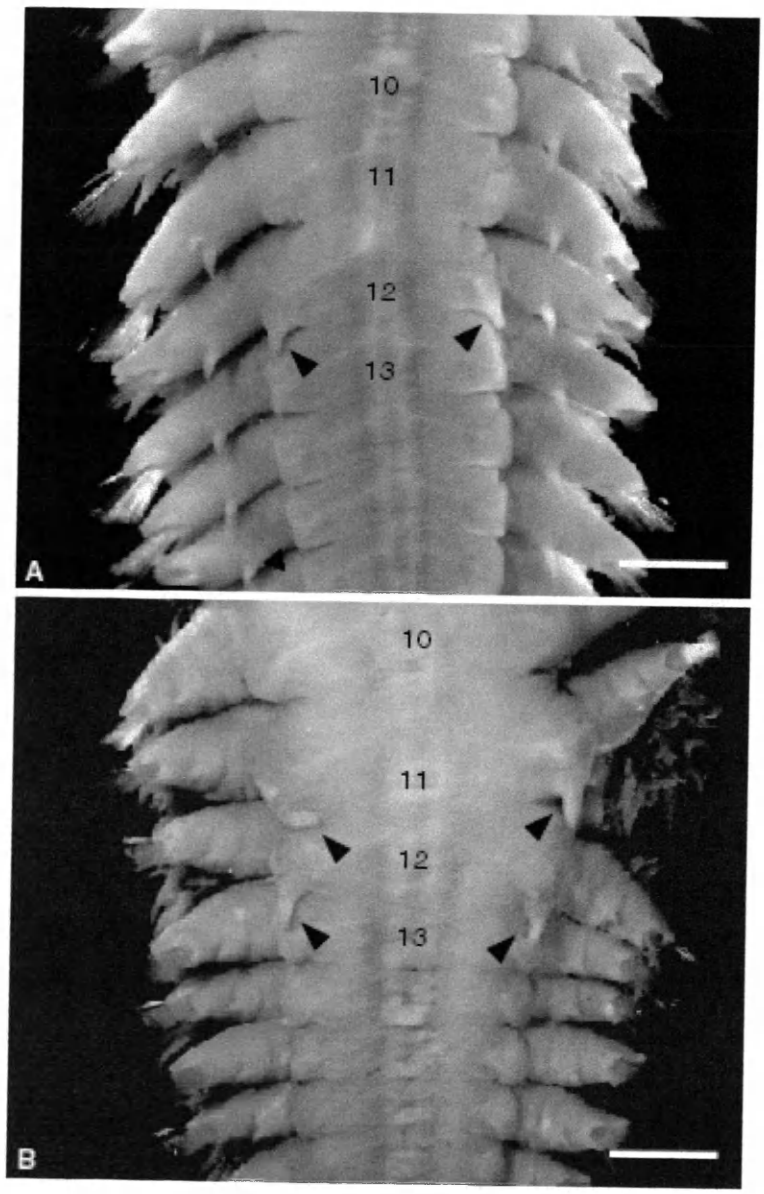
Three morphotypes of *Branchipolynoe symmytilida* are readily distinguished under a dissecting microscope: individuals with 0, 1 or 2 pairs of ventral nephridial papillae. Individuals with 1 pair of papillae (setiger 11) are male (Fig. 2A; n = 10), and those with 2 pairs of papillae (setigers 11 and 12) are female (Fig. 2B; n = 17). Male worms (length $\bar{0}$ = 1.7 cm $\forall$ 0.28 s.d.) were significantly smaller than females (length $\bar{0}$ = 3.0 cm $\forall$ 0.96 s.d., p = 0.020, Fig. 3). Three small worms with no papillae (length $\bar{0}$ = 1.49 cm $\forall$ 0.67 s.d.) contained only early spermatids or early vitellogenic oocytes.

Male Reproductive Anatomy

The male reproductive system in mature *Branchipolynoe symmytilida* consists of an extensive, unsegmented gonad filling the coelomic cavity of setigers ~2 to 9 and of large, lobular, seminal vesicles in setigers 9-11 (Fig. 4A,B). The gut is situated dorsally, so that the bulk of the gonad typically lies in the mid-to-ventral portion of the coelom. Spermatogonia presumably arise from a germinal epithelium associated with the peritoneum, but these immature sperm cells were not visible under light microscopy. Clusters of spherical "early spermatids" are associated with the peritoneum along the

FIGURE 2

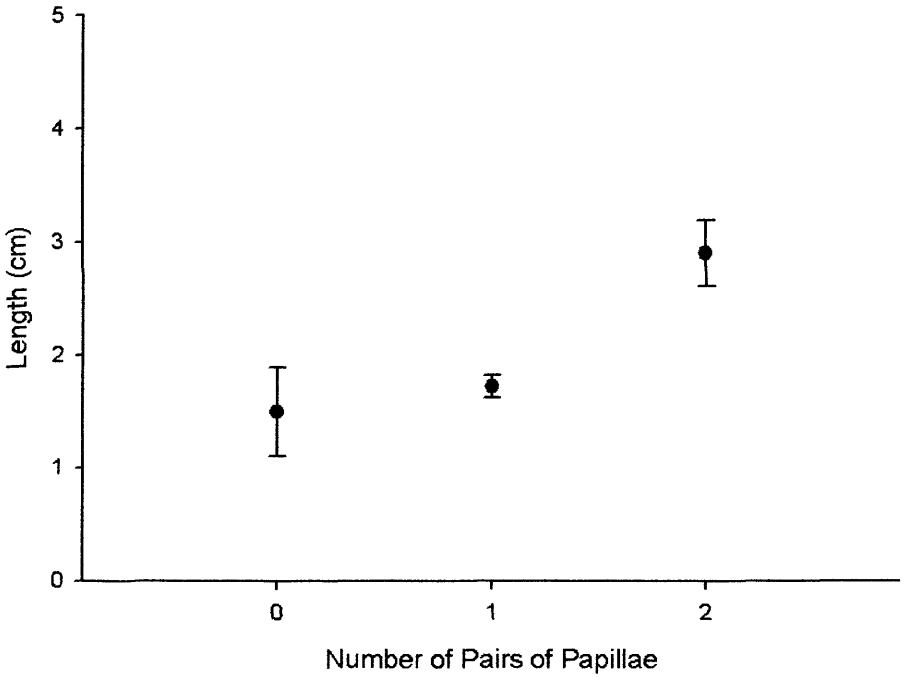
BRANCHIPOLYNOE SYMMYTILIDA, SEXUAL DIMORPHISM



Branchipolynoe symmytilida, sexual dimorphism (ventral views). (A) Male, one pair of nephridial papillae; scale bar = 400 μm. (B) Female, two pairs of papillae; scale bar = 400 μm. arrowheads, nephridial papillae; numbers, setigers.

FIGURE 3

BRANCHIPOLYNOE SYMMYTILIDA,
NUMBER OF PAIRS OF NEPHRIDIAL PAPILLAE VS. WORM LENGTH



Branchipolynoe symmytilida, number of pairs of nephridial papillae vs. worm length. For 0 pairs, n = 3; for 1 pair, n = 8; for 2 pairs, n = 11.

FIGURE 4

BRANCHIPOLYNOE SYMMYTILIDA, MALE REPRODUCTIVE ANATOMY

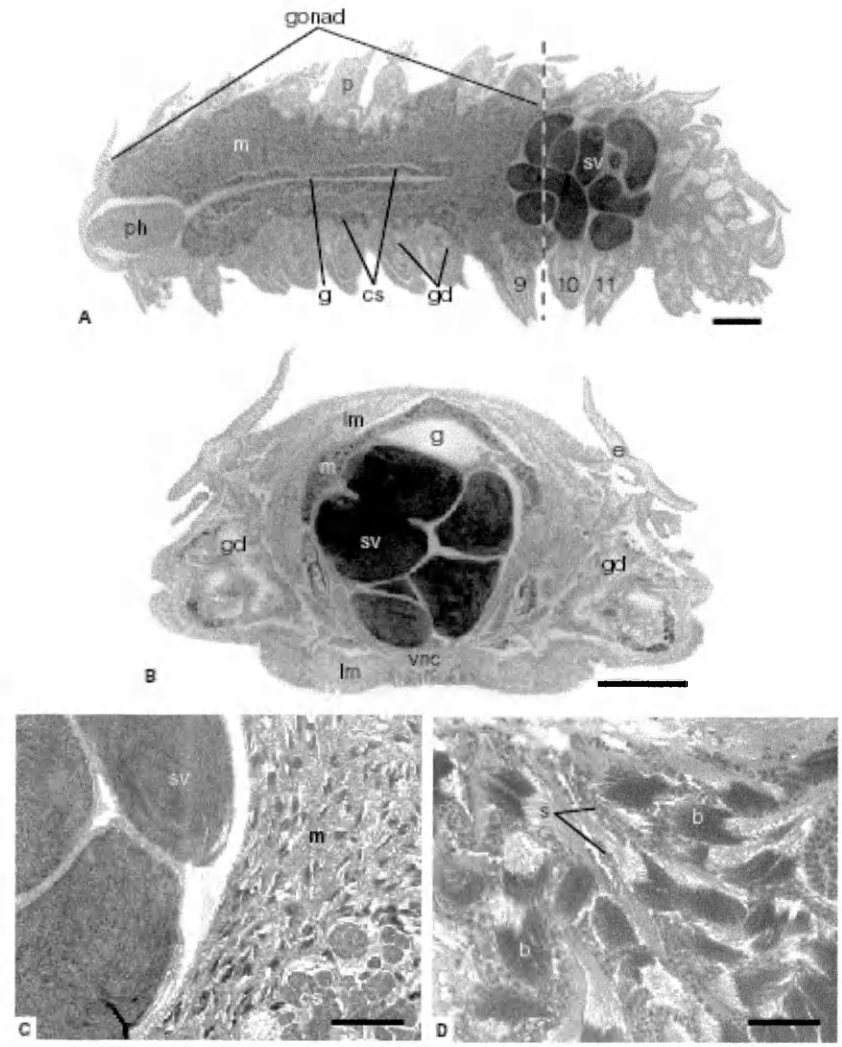


Figure 4. *Branchipolynoe symmytilida*, male reproductive anatomy. (A) Gonad and seminal vesicles; frontal section; scale bar = 1 mm. (B) Gonad and seminal vesicles; cross-section from region of vertical line in (A); scale bar = 1 mm. (C) Clusters of early spermatids associated with the peritoneum, mixed gonadal tissue, and seminal vesicles; scale bar = 200 μm. (D) Mixed gonadal tissue; scale bar = 50 μm. b, bundle of late spermatids; cs, clusters of early spermatids; e, elytron; g, gut; gd, gut diverticulum; lm, longitudinal muscle; m, mixed tissue (early spermatids and bundles of late spermatids); p, parapodium; ph, pharynx; s, early spermatids; sv, seminal vesicle filled with mature filiform sperm; vnc, ventral nerve cord; numbers, setigers.

length of the gonad, i.e., lining the body wall and surrounding the gut (Fig. 4A). Bundles of tailed, "late spermatids" with darkly staining filiform heads are surrounded by early spermatids (Fig. 4C,D), forming a "mixed tissue" (Fig. 4A,B). In some sections, this mixed tissue does not appear to be part of the gonad proper; it may instead be developing sperm cells that are free (but densely packed) in the coelom. Mature filiform sperm (head length approx. 60 μm) are tightly packed within the seminal vesicles (Fig. 4A-C). The seminal vesicles are formed from a thin layer (15 μm) of epithelial cells, and connect to the exterior via gonoducts through the nephridial papillae of setiger 11 (Fig. 2A). Sperm are presumably transferred to the female as a spermatophore. In dissections of fresh and preserved material, seminal vesicles are iridescent white.

Juvenile male worms (no nephridial papillae, length <2.0 cm) contain a larger proportion of early spermatids to late spermatids than adult males (~80% early spermatids in juveniles vs. ~30% early spermatids in adults). Small bundles of filiform sperm are present among the clumps of spermatogonia in juveniles, but no seminal vesicles or mature sperm are present.

Female Reproductive Anatomy

Oocytes develop anteriorly (setigers ~4-10) in multi-lobed ovaries that arise in association with the peritoneum surrounding the ventral portion of the gut (Fig. 5A,B, 6). The ovaries appear to be segmental and paired, but the number of ovaries, their relationship to specific setigers, and their degree of fusion is uncertain.

Each ovarian lobe (up to 6 per female) consists of a convoluted and furled sheet of oocytes with a continuum of oocyte sizes and vitellogenic stages, from a central region

FIGURE 5

BRANCHIPOLYNOE SYMMYTILIDA,
FEMALE REPRODUCTIVE ANATOMY; VENTRAL VIEW

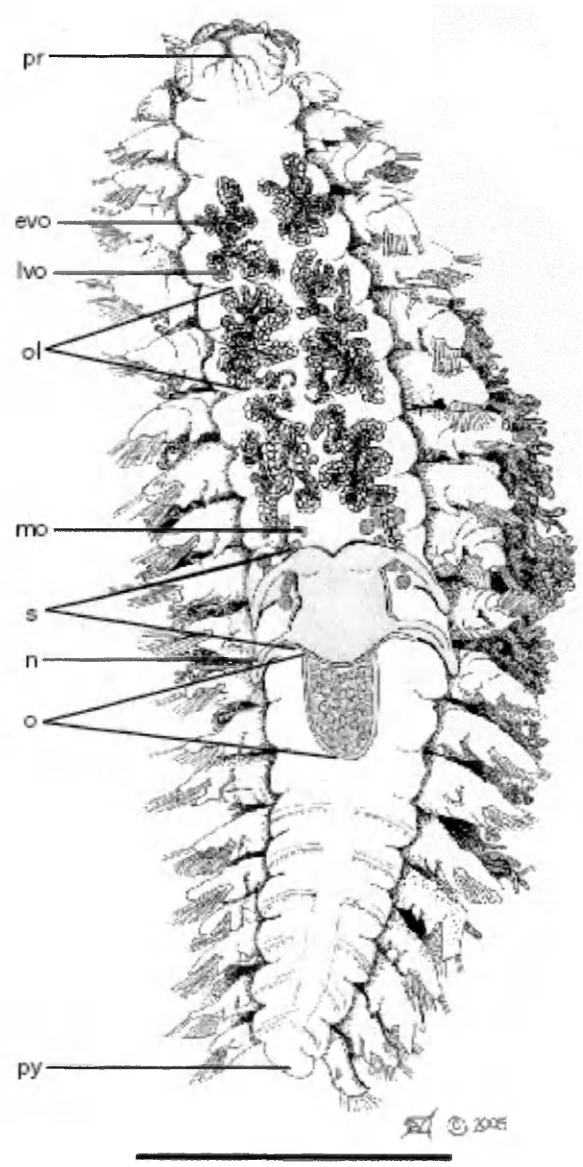


Figure 5. *Branchipolynoe symmytilida,* female reproductive anatomy; ventral view, illustrating location of ovaries, ovisac, spermatheca, and nephridial papillae; scale bar = 1 cm. evo, early vitellogenic oocyte; lvo, late vitellogenic oocyte; mo, mature oocyte; n, nephridial papillae; o, ovisac; ol, ovary lobe; pr, prostomium; py, pygidium; s, spermatheca.

of small, pre-vitellogenic oocytes (<10 μm diameter, Fig. 6) to a region of early vitellogenic oocytes (10-20 μm), to larger, late vitellogenic oocytes (<200 μm diameter) at the periphery of the lobe (Fig. 7). The lobes extend into the parapodia in some specimens. When removed from fresh specimens, the ovarian lobes unfurl, revealing this gradient of oocyte sizes (Fig. 7).

Each adult female *Branchipolynoe symmytilida* observed contained all stages of oocytes (Fig. 5,8A). A single, thin layer of follicle cells surrounds each oocyte (Fig. 8B) and the ovaries are associated with blood vessels (Fig. 8A). Oocytes > ~200 μm are granulated with yolky globules and are free in the coelom (Fig. 5). Mature oocytes (max. diameter 300 μm) fill a central, large, thin-walled ovisac located ventral to the gut in setigers ~11-14 (Fig. 5); ciliated funnels opening into the ovisac are presumed to exist but were not observed.

In preserved dissection, sheets of pre-vitellogenic oocytes are white. Late vitellogenic oocytes (<200 μm) are orange with a white coating (follicle cells?), and mature oocytes (<300 μm) are deeper orange, lack the white coating, and are granular in texture. In fresh dissection, pre-vitellogenic and early vitellogenic oocytes in the central region of each ovarian lobe are transparent. Late vitellogenic oocytes are white, and mature oocytes are whitish and granular in texture (Fig. 7).

Sperm Storage

Mature female specimens possess a well-developed, ventral spermatheca (setigers 11, 12) that opens to the exterior through ducts in the 2 pairs of nephridial papillae (Fig. 5, 9). The ovisac and spermatheca are closely associated with one another, presumably

FIGURE 6

BRANCHIPOLYNOE SYMMYTILIDA,
FEMALE REPRODUCTIVE ANATOMY; CROSS-SECTIONAL VIEWS

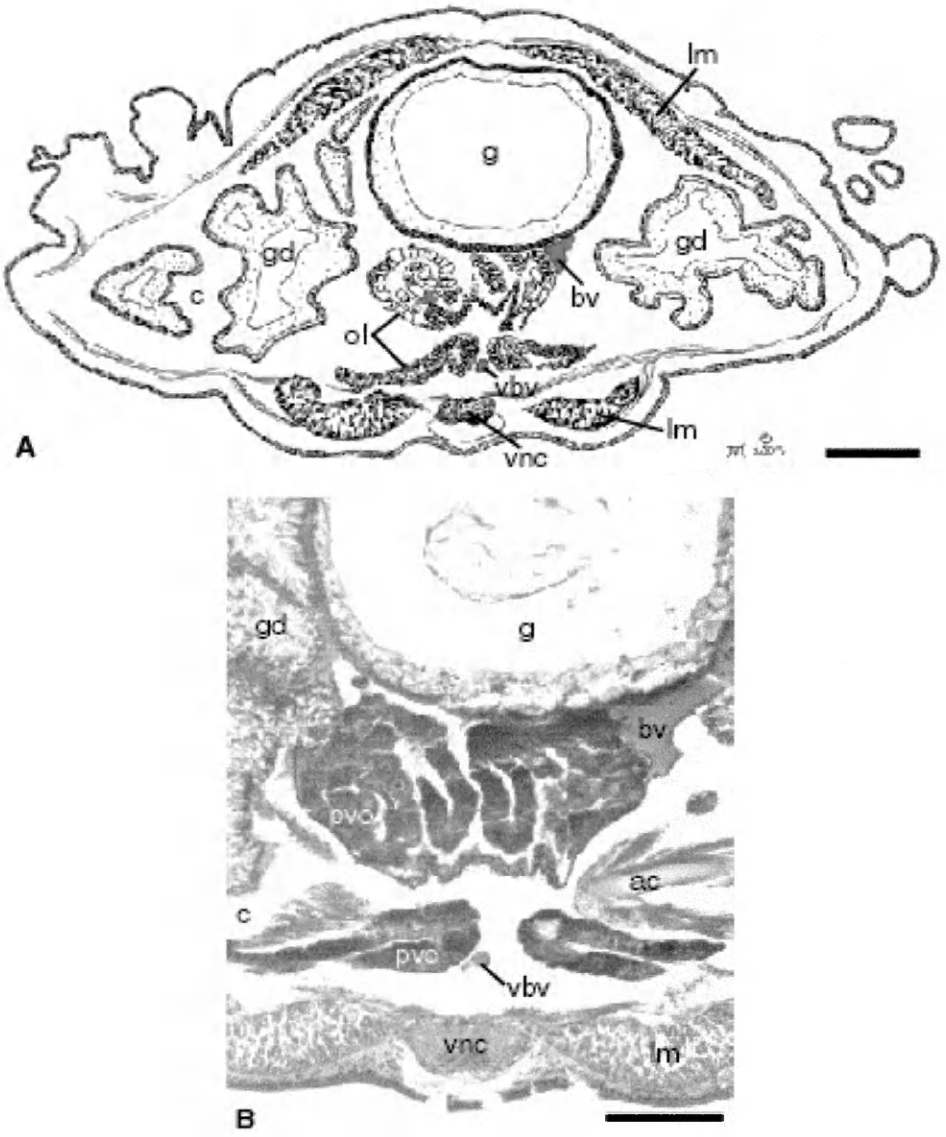


Figure 6. *Branchipolynoe symmytilida*, female reproductive anatomy. (A,B) Cross-sectional views illustrating location of ovarian lobes; scale bars = 200 µm. ac, acicula; bv, blood vessel; c, coelom; g, gut; gd, gut diverticulum; lm, longitudinal muscle; ol, ovary lobe; pvo, pre-vitellogenic oocyte; vbv, ventral blood vessel; vnc, ventral nerve cord.

FIGURE 7

BRANCHIPOLYNOE SYMMYTILIDA,
UNFURLED OVARIAN LOBE

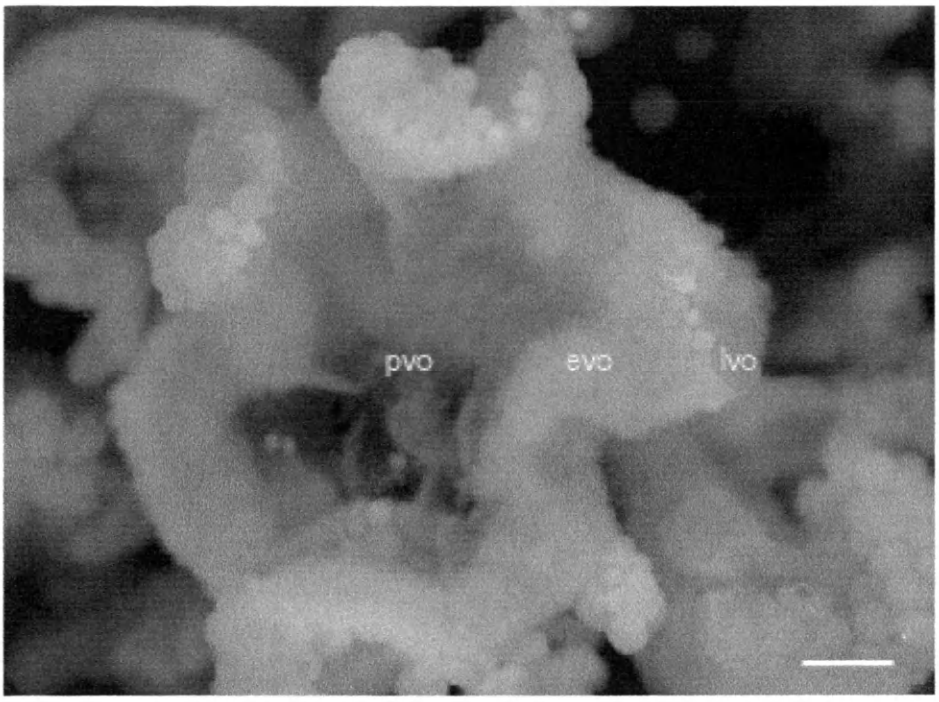


Figure 7. *Branchipolynoe symmytilida*, unfurled ovarian lobe revealing clear, pre-vitellogenic oocytes, white early vitellogenic oocytes, and late vitellogenic oocytes with whitish granular yolky globules; scale bar = 200 µm. evo, early vitellogenic oocyte; lvo, late vitellogenic oocyte; pvo, pre-vitellogenic oocyte.

FIGURE 8

BRANCHIPOLYNOE SYMMYTILIDA,
DEVELOPING OOCYTES IN OVARIAN LOBES

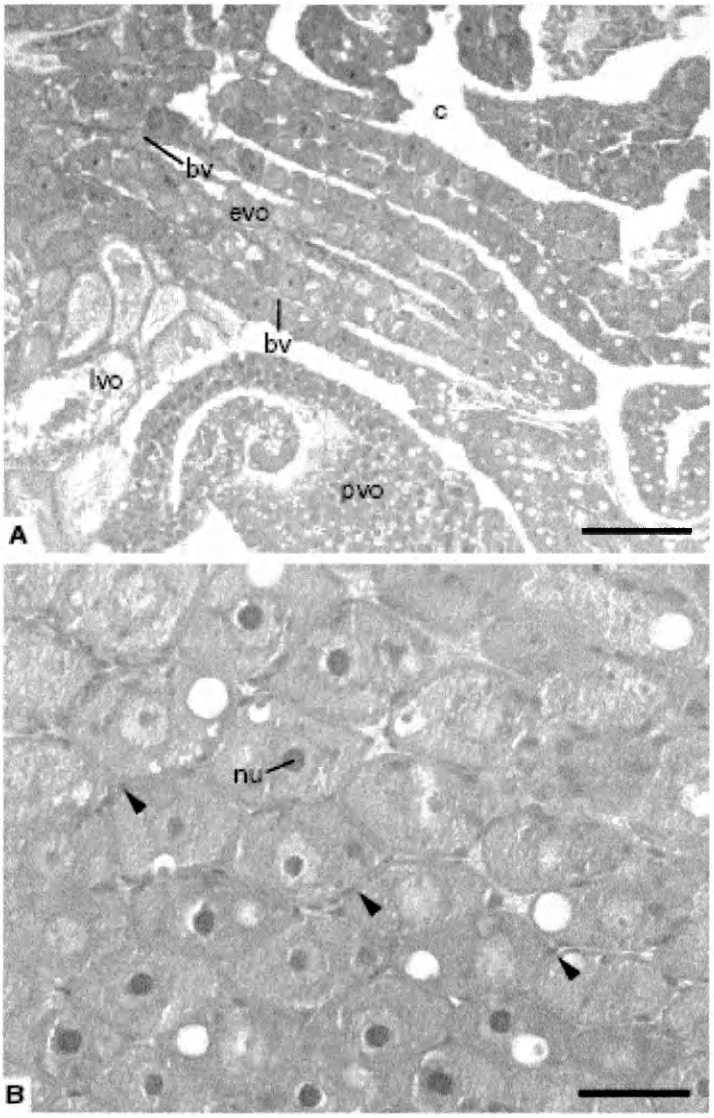


Figure 8. *Branchipolynoe symmytilida,* developing oocytes in ovarian lobes. (A) pre-, early, and late vitellogenic oocytes in a convoluted ovarian lobe, with associated blood vessels; scale bar = 200 μm. (B) Sheet of early vitellogenic oocytes with follicle cells surrounding each oocyte; longitudinal section; scale bar = 50 μm. bv, blood vessel; c, coelom; evo, early vitellogenic oocyte; lvo, late vitellogenic oocyte; nu, nucleus; pvo, pre-vitellogenic oocyte; arrowheads, follicle cells.

FIGURE 9

BRANCHIPOLYNOE SYMMYTILIDA, SPERM STORAGE

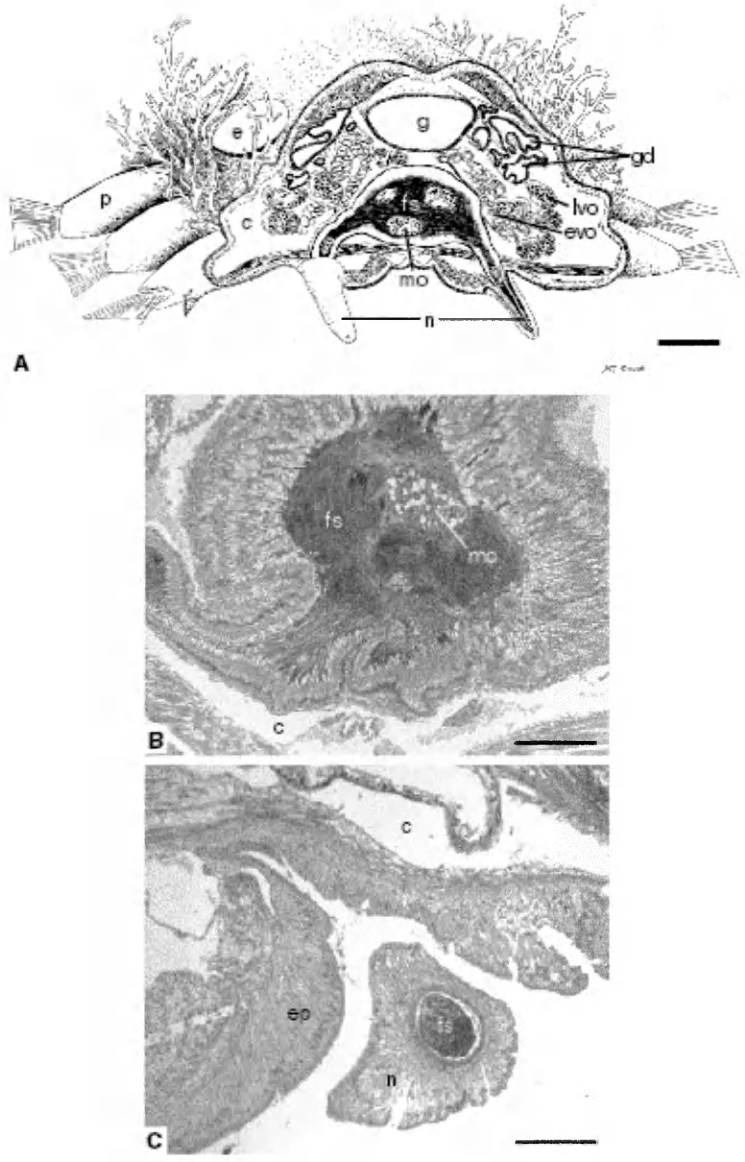


Figure 9. *Branchipolynoe symmytilida,* sperm storage. (A) Spermatheca with filiform sperm, and nephridial papillae; cross-section; scale bar = 200 μm. (B) Mature oocyte surrounded by sperm in spermatheca; cross section; scale bar = 200 μm. (C) Nephridial papilla packed with sperm; cross-section; scale bar = 200 μm. c, coelom; e, elytron; ep, epithelium; evo, early vitellogenic oocyte; fs, filiform sperm; lvo, late vitellogenic oocyte; mo, mature oocyte; n, nephridial papilla; p, parapodium.

connected by a valve or duct. The spermatheca is filled with masses of filiform sperm; heads of many sperm cells are embedded in the thick (100 μm) epithelium of the spermatheca (Fig. 9A,B). Up to 10 mature oocytes are in the sac (Fig. 9A,B), and, in one female specimen, mature oocytes had been partially extruded through a nephridial papilla. This papilla was packed with sperm (Fig. 9C) and several mature oocytes. Germinal vesicles are not visible in oocytes within the spermatheca or papillae, suggesting that fertilization occurs internally. No evidence of zygote cleavage was observed.

CHAPTER III

RESULTS: *Levensteiniella kincaidi*

Sexual Dimorphism

Levensteiniella kincaidi shows no sexual dimorphism by size (male length $\bar{0}$ = 0.62 cm $\forall$ 0.16 s.d., n = 6; female length $\bar{0}$ = 0.83 $\forall$ 0.40 s.d., n = 5; p = 0.708). Two morphotypes are apparent under a dissecting scope: males have 2 pairs of long, thin ventral nephridial papillae on setigers 11 and 12 and females have no papillae (Fig. 10).

Male Reproductive Anatomy

The male reproductive system in mature *Levensteiniella kincaidi* is limited to the coelomic cavity in setigers ~8-12 (Fig. 11A). Spermatogonia presumably arise from a germinal epithelium but were not visible under light microscopy. Clusters of spherical, early spermatids occupy ~20% of the coelomic space in setigers ~8-11 (Fig. 11), but are not always so closely associated with the peritoneum as they are in *Branchipolynoe symmytilida*. A mixture of early spermatids and tailed, late spermatids partially fills the coelomic volume (Fig. 11A-C) and sometimes surrounds clusters of early spermatids (Fig. 11B). Bundles of late spermatids were not observed. In segments 10-12, relatively small, thick-walled (40 μm) seminal vesicles are packed with mature spermatids with elongated, filiform heads (~40 μm head length, Fig. 11A,D). The seminal vesicles

FIGURE 10

LEVENSTEINIELLA KINCAIDI, SEXUAL DIMORPHISM

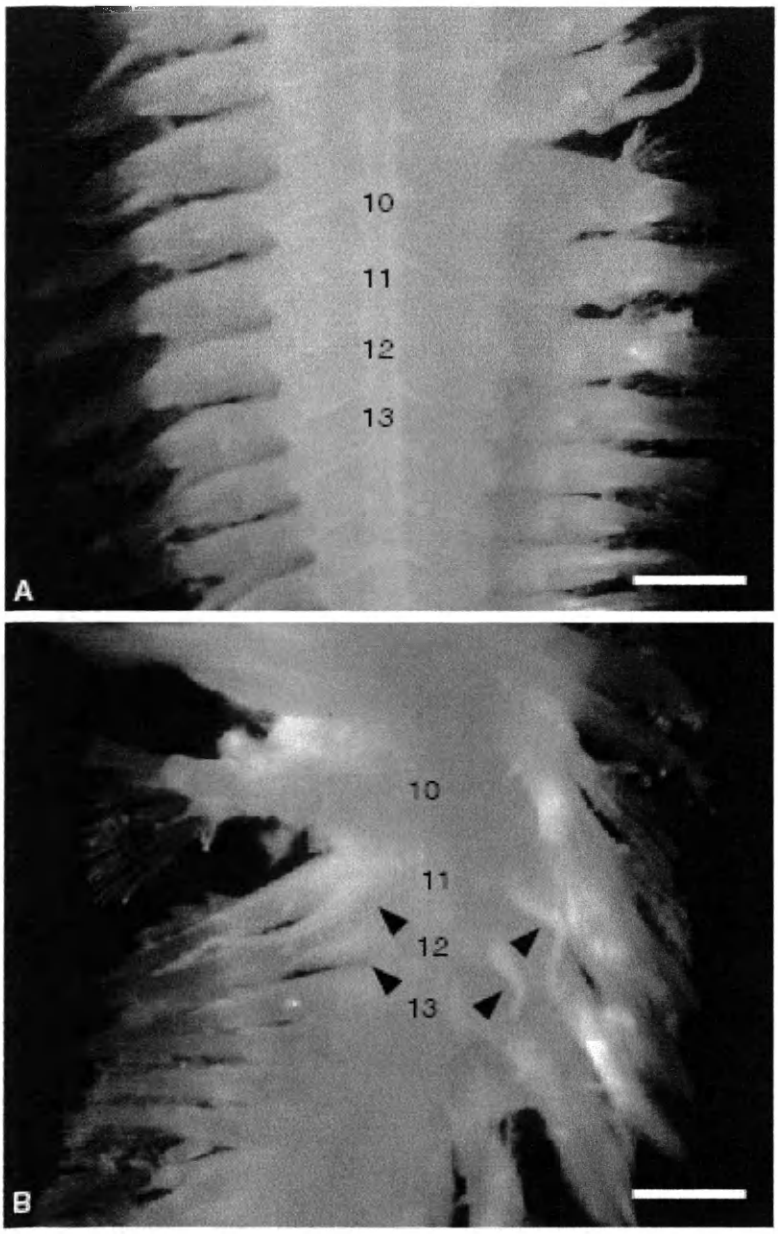


Figure 10. *Levensteiniella kincaidi,* sexual dimorphism (ventral views). (A) Female, without nephridial papillae; scale bar = 200 μm. (B) Male, two pairs of nephridial papillae; scale bar = 200 μm. arrowheads, nephridial papillae; numbers, setigers.

FIGURE 11

LEVENSTEINIELLA KINCAIDI, MALE REPRODUCTIVE ANATOMY

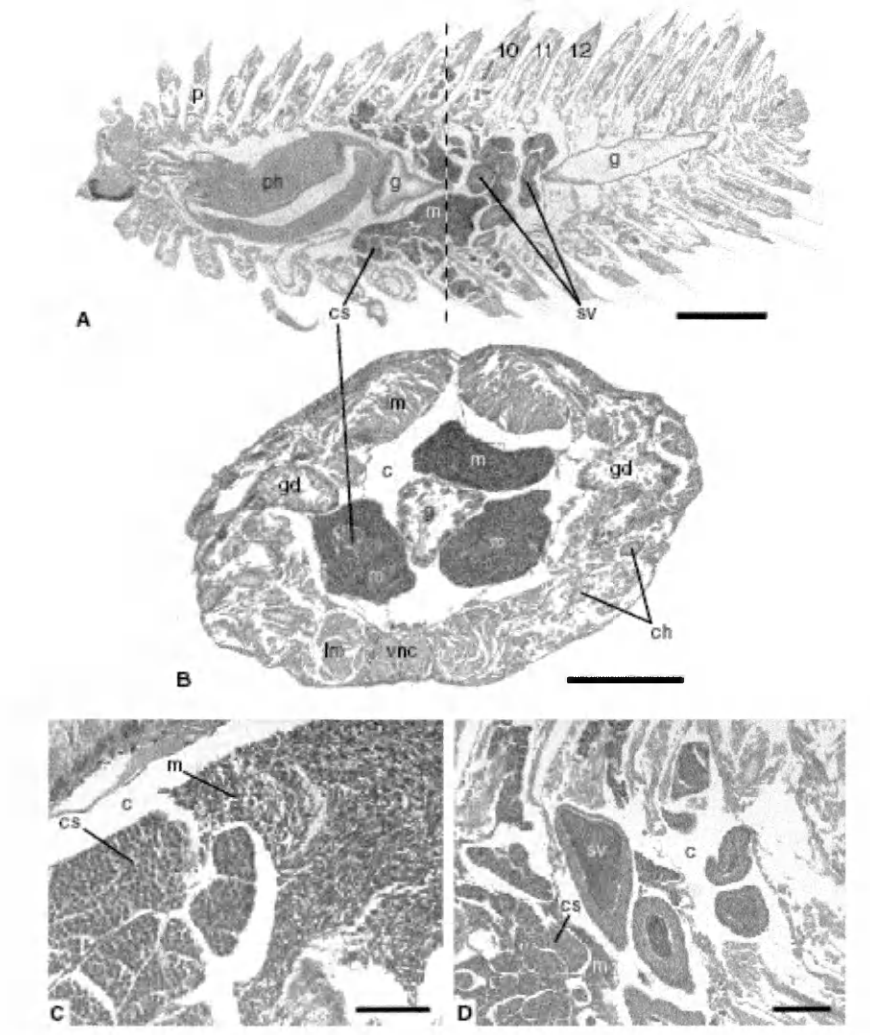


Figure 11. *Levensteiniella kincaidi*, male reproductive anatomy. (A) Gonad and seminal vesicles; frontal section; scale bar = 1 mm. (B) Gonad; cross-section from region of vertical line in (A); scale bar = 200 μm. (C) Clusters of early spermatids and mixture of early spermatids and tailed, late spermatids; longitudinal section; scale bar = 200 μm. (D) Seminal vesicles filled with mature filiform sperm; frontal section; scale bar = 200 μm. c, coelom; ch, chaeta; cs, clusters of early spermatids; g, gut; gd, gut diverticulum; lm, longitudinal muscle; m, mixed tissue (early spermatids, late spermatids); p, parapodium; ph, pharynx; sv, seminal vesicles; vnc, ventral nerve cord; numbers, setigers.

connect to the exterior via gonoducts through 2 pairs of nephridial papillae on setigers 11 and 12 (Fig. 10B).

Female Reproductive Anatomy

The coelomic cavity of adult female *Levensteiniella kincaidi* is segmented internally, with most body segments posterior to the pharynx containing ovaries (Fig. 12A). Oogonia were not observed, but we infer that they arise from germinal epithelia associated with blood vessels. Immature oocytes are grouped around blood vessels (12B) and represent a continuum of vitellogenic states distinguished by size and/or by the density and texture of the cellular contents (pre-vitellogenic oocytes: <30 μm diameter, with homogeneous ooplasm; early vitellogenic oocytes: ~100 μm diameter; late vitellogenic oocytes: ~300 μm diameter, with granular, yolky appearance). At least some part of the envelope of each of these oocytes is in contact with the wall of a blood vessel (Fig. 12B). A single, dense layer of follicle cells surrounds each pre-vitellogenic and vitellogenic oocyte (Fig. 12B,C). Mature oocytes that have separated from the blood vessels are large (max. diameter 300 μm) and lack follicle cells. Some segments contain numerous oocytes at all stages of development, while other segments contain only 1 or very few mature oocytes (Fig. 12C).

Sperm Storage

Adult female *Levensteiniella kincaidi* have several (up to 3 pairs) small, segmental spermathecae containing filiform sperm (Fig. 13A). The spermathecae appear as invaginations of the ectodermal epithelium and are comprised of a bulbous ampulla, a

FIGURE 12

LEVENSTEINIELLA KINCAIDI, FEMALE REPRODUCTIVE ANATOMY

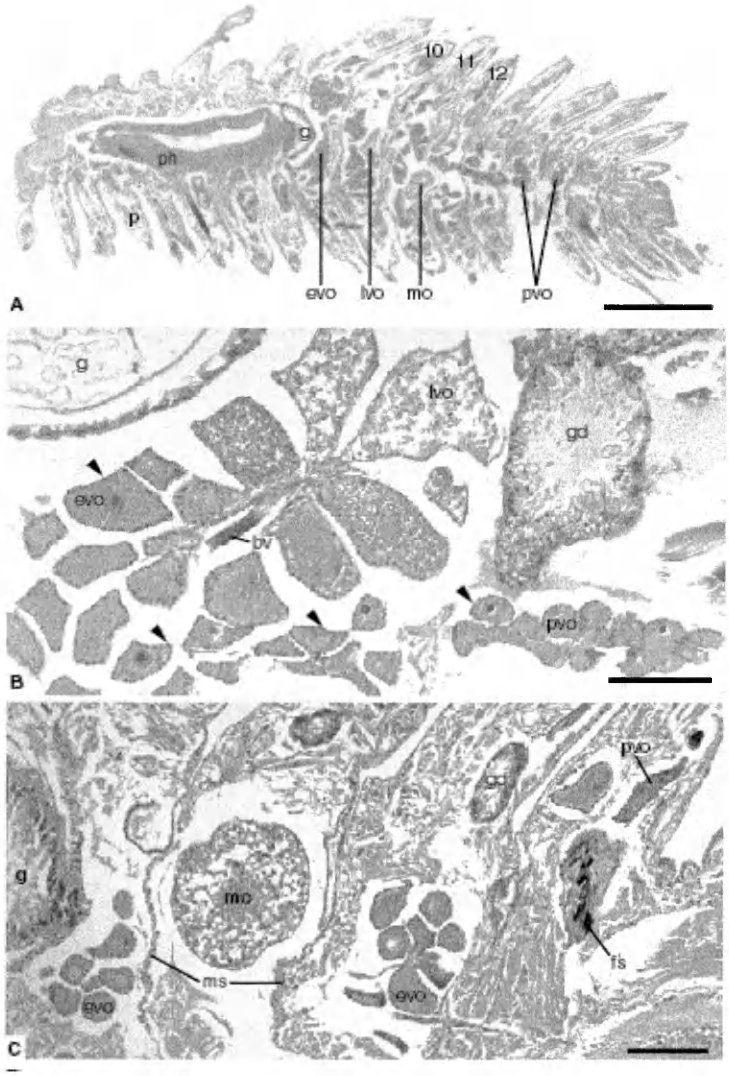


Figure 12. *Levensteiniella kincaidi*, female reproductive anatomy. (A) Ovaries associated with blood vessels in body segments posterior to the pharynx; frontal section; scale bar = 1 mm. (B) Pre-vitellogenic, early vitellogenic, and late vitellogenic oocytes surrounded by follicle cells and associated with blood vessels; cross-section; scale bar = 200 µm. (C) Developing oocytes of various vitellogenic states (including a mature oocyte lacking follicle cells) between complete mesenteries and a spermatheca filled with filiform sperm; scale bar = 200 µm. bv, blood vessel; evo, early vitellogenic oocyte; fs, filiform sperm; g, gut; gd, gut diverticulum; lvo, late vitellogenic oocyte; mo, mature oocyte; ms, mesenteries; p, parapodium; ph, pharynx; pvo, pre-vitellogenic oocyte; arrowheads, follicle cells; numbers, setigers.

FIGURE 13

LEVENSTEINIELLA KINCAIDI, SPERM STORAGE

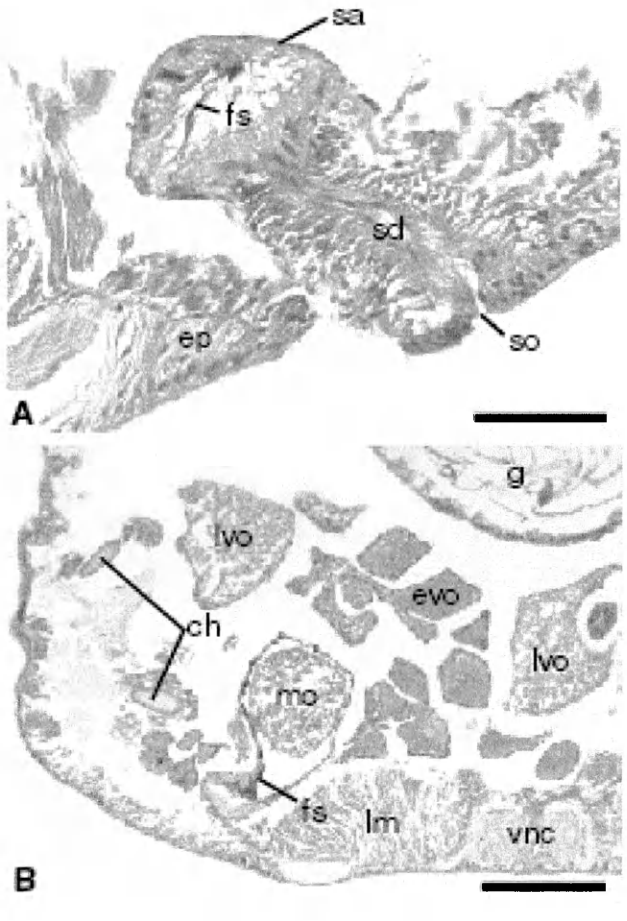


Figure 13. *Levensteiniella kincaidi*, sperm storage. (A) Spermatheca containing filiform sperm; cross-section; scale bar = 100 μm. (B) Spermatheca containing a mature oocyte and filiform sperm; cross-section; scale bar = 200 μm. ch, chaetae; ep, epithelium; evo, early vitellogenic oocyte; fs, filiform sperm; g, gut; lm, longitudinal muscle; lvo, late vitellogenic oocyte; mo, mature oocyte; sa, spermathecal ampulla; sd, spermathecal duct; so, spermathecal opening; vnc, ventral nerve cord.

short duct, and an opening to the exterior. Spermathecal ampullae were expanded in several specimens (up to ~400 μm) to contain large oocytes surrounded by stored sperm (Fig. 13B). Germinal vesicles were not observed in oocytes inside the spermathecae, suggesting that fertilization occurs in the spermathecae. Cleavage of zygotes in the spermathecae was not observed.

CHAPTER IV

DISCUSSION

Although both male and female gametes are found in adult *Branchipolynoe symmytilida* and *Levensteiniella kincaidi*, hermaphroditism was ruled out due to the lack of developing sperm stages (e.g. spermatogonia) within females. In *B. symmytilida*, protandric hermaphroditism was suspected due to the observed size dimorphism (females are on average 1.3 cm larger than males) but the observation of immature worms with developing oocytes suggested that this species has separate sexes. Gonochorism is consistent with observations of the only other vent polynoids studied to date, *B. seepensis* and *Opisthotrochopodus* n. sp. from the Mid-Atlantic Ridge (Van Dover et al. 1999, Jollivet et al. 2000).

Observations of oocyte size range indicate that both *Branchipolynoe symmytilida* and *Levensteiniella kincaidi* undergo asynchronous gametogenesis, both within individual worms and among worms in the population. All adults in both species contain all stages of oocytes; no worms appeared to be overly ripe with mature gametes, or to be "spent" of mature gametes, as would indicate proximity of a spawning event. The unusual ovaries in *B. symmytilida* all had small oocytes in the central region and larger vitellogenic oocytes closer to the periphery of each lobe. Similarly, individual body segments of *L. kincaidi* contained varying ranges of oocyte sizes, but each female

contained the entire range of observed oocyte diameters. Additionally, mature *B. symmytilida* all have complete nephridial papillae, showing no signs of papilla regression between spawning events, as observed in the polynoid *Harmothoe imbricata* (Daly 1972). This evidence supports continuous ("dribble") spawning of both species.

The level of internal segmentation of the coelom differs between the 2 species. In *Branchipolynoe symmytilida*, neither males nor females are segmented internally, which could aid in the rapid development and transfer of gametes through the body. In contrast, female *Levensteiniella kincaidi* have complete mesenteries dividing the coelomic cavity, suggesting slower oocyte development in this species. Male *L. kincaidi* are completely segmented except in the few segments (segments 8-11) that contain gametes. The lack of mesenteries in this area of the body presumably aids the collection of sperm to the nephridial papillae at the time of gamete release.

The proximity of follicle cells and blood vessel associations in developing oocytes of *Branchipolynoe symmytilida* and *Levensteiniella kincaidi* is consistent with rapid vitellogenesis, as these attributes would make a large amount of yolk precursor material available to the growing egg. In *L. kincaidi*, more follicle cells per developing oocyte are observed than in *B. symmytilida* (Fig. 8B and Fig. 12B). This supports rapid vitellogenesis in *L. kincaidi*, possibly to counteract the presumed retarding of vitellogenesis by the complete mesenteries segmenting adult female coelomic cavities.

Elongate filiform sperm heads and the presence of stored sperm in females indicate some form of specialized sperm transfer from male to female (Jamieson & Rouse 1989). Although spermatophores were not observed in this study, they are occasionally found in washings from mussel-bed samples that contain abundant *Branchipolynoe*

symmytilida, and polynoids with partially extruded spermatophores have been observed (Van Dover, pers. comm.). We infer that *B. symmytilida* and *Levensteiniella kincaidi* have introsperm, although ultrastructural characterization of the sperm remains to be undertaken. The obligate passage of mature oocytes through sperm-lined spermathecae of adult females indicates that fertilization probably takes place internally. The absence of germinal vesicles in oocytes within spermathecae also supports internal fertilization, although the exact location of fertilization is unknown. Although both *Branchipolynoe symmytilida* and *Levensteiniella kincaidi* had spermathecae, morphology of sperm storage organs in the 2 species was markedly different. With the loss of internal segmentation in *B. symmytilida*, this species is able to store sperm in a large central cavity that empties out of 4 ventral nephridial papillae. In contrast, females of *L. kincaidi* possess comparatively small spermathecae along the ventral surface presumably derived from invaginations of the epidermis. The observed number of these spermathecae varied, but due to the complete segmentation of the body cavity in females, several small sperm storage organs were utilized instead of one larger central one as seen in *B. symmytilida.*

Members of at least 12 polychaete families have spermathecae (Westheide 1988), and sperm storage is a successful strategy for at least 3 clades found at hydrothermal vents. Alvinellids (Zal et al. 1994), siboglonid vestimentiferans (Hilário et al. 2005), and polynoids (Van Dover et al. 1999, Jollivet et al. 2000, this study) observed to date have elongate, modified sperm that is stored in spermathecae in adult females, although the details of this process vary among the different groups. The scarcity of strong environmental cues for gametogenesis and spawning at hydrothermal vents increases selection for sperm storage (Young et al. 1999). This strategy generally ensures higher

fertilization rates than broadcast spawning and eliminates the need for members of a population to be synchronized in reaching reproductive maturity and spawning readiness (Young et al. 1999).

The large, yolky oocytes found in *Branchipolynoe symmytilida* and *Levensteiniella kincaidi* support lecithotrophy as a developmental strategy. Although lecithotrophic larvae were initially thought to have more limited dispersal than planktotrophic larvae, this paradigm has been challenged by findings of lecithotrophic species with very large habitat ranges at hydrothermal vents (Lutz 1984). *Branchipolynoe symmytilida* exhibits an enormous range from the northern East Pacific Rise to the Galapagos Rift and Southern East Pacific Rise with high rates of gene flow (Hurtado et al. 2004). The large range of this species is maintained despite the varying distance between suitable vent habitat (up to 1000s of km) and presence of large dispersal barriers in the topography of the seafloor (e.g. transform faults, Hess deep, Easter microplate region, Hurtado et al. 2004). Sperm storage and lecithotrophic larvae allow this species to ensure a high rate of fertilization without sacrificing long-distance dispersal potential.

The surprisingly wide range of reproductive strategies of hydrothermal vent species, and deep-sea species in general, have been attributed to the large role of phylogenetic constraints in determining these characteristics (e.g. Ecklebarger & Watling 1995). The sperm storage and lecithotrophy reported for *Branchipolynoe symmytilida* and *Levensteiniella kincaidi* reveal that these characteristics are present in vent polynoids from different ocean basins and from different microhabitats (commensal vs. free-living) but not in any shallow-water polynoid studied to date.

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