

Morphogenesis during sexual and asexual reproduction in *Amphipolydora vestalis* (Polychaeta: Spionidae)

GLENYS D. GIBSON*

IAN G. PATERSON

Department of Biology

Acadia University

Wolfville, NS B4P 2R6

Canada

email: glenys.gibson@acadiau.ca

email: ian.paterson@acadiau.ca

Abstract Both sexual and asexual reproduction occur in *Amphipolydora vestalis*, a small, sponge-dwelling spionid polychaete. Sexual reproduction occurs through adelphophagy, where offspring are provided with extra-embryonic yolk in the form of nurse eggs. Ingestion of nurse eggs sustains development until offspring hatch at late larval and early juvenile stages (22 days). Hatched young have an advanced morphology and alternatively crawl and swim, suggesting that a dispersive phase is brief or absent. Asexual reproduction occurs through architomy, in which a parent fragments into 4–6 pieces and each regenerates a complete body plan within 8 days. Asexual propagules remain within the parental tube until growth and differentiation are almost complete. Both reproductive modes were found at the same time in the same population, and asexual fragments often had well developed gametes in the coelom. Both reproductive modes facilitate local recruitment and a reduced dispersal potential. This is the first description of development within the genus *Amphipolydora*. Aspects of morphogenesis during asexual reproduction suggest that *Amphipolydora* is closely related to *Polydorella*, and that architomy in the Spionidae evolved from a widespread ability to regenerate, while paratomy is secondarily derived.

Keywords adelphophagy; embryogenesis; larva; blastema; regeneration; *Amphipolydora*; spionid; *Polydora* complex; architomy; paratomy

INTRODUCTION

The Spionidae are a large group of polychaetes that show an impressive diversity in reproductive modes (see review by Blake & Arnofsky 1999). Sexual reproduction is the most common reproductive mode, and can be planktotrophic (with the release of small, planktonic feeding larvae), adelphophagic (offspring ingest nurse eggs and hatch at an advanced stage) or, less commonly, lecithotrophic (large yolky eggs give rise to large, advanced young, without extra-embryonic nutrition). Poecilogony is also known in this group, and involves the release of more than one morph of offspring (e.g., planktotrophic and adelphophagic young) in a single species (Chia et al. 1996).

Asexual reproduction is less common, and can occur via architomy or paratomy. In architomy, the parent fragments into several pieces and offspring subsequently restore the original body plan through blastemal growth. In paratomy, blastemal growth occurs from parental segments that are still attached to the parent and separation of offspring (e.g., fragmentation) occurs after differentiation is complete. In the Spionidae, architomy is best known in *Pygospio elegans* in which mode of reproduction varies seasonally with sexual reproduction in the winter and asexual in the summer (Rasmussen 1953; Anger 1984). Architomy also occurs in *P. californica* (in Blake & Arnofsky 1999) and is likely in *Amphipolydora abranchiata* (see Blake 1983). Paratomy occurs in *Dipolydora tetrabranchia* (Campbell 1955) and in all four known species of *Polydorella* (Radashkevsky 1996). Although asexual reproduction is not common among spionid polychaetes, many species are capable of regeneration.

The spionid *A. vestalis*, a member of the polydorid complex, inhabits blind-end mud tubes in the sponge *Callyspongia* cf. *ramosa* from New Zealand. Adults are small and range in length from 1.8 to 5.5 mm,

* Author for correspondence.

M02030; Online publication date 31 October 2003

Received 24 April 2002; accepted 27 June 2003

or 20–40 chaetigers (Paterson & Gibson 2003). Our objective is to describe sexual (adelphophagy) and asexual (architomy) reproduction in *A. vestalis*.

MATERIALS AND METHODS

The host sponge, *C. cf. ramosa*, was collected by SCUBA from shallow subtidal sand flats (<10 m depth) on the western side of Kawau Island, New Zealand from November 2001 to January 2002. *A. vestalis* were removed from mud tubes housed within the sponge either by teasing away the sponge and tube material with forceps, or by immersing sponge fragments in MgCl₂ (3% in sea water) to relax the worms, then pulling them from their tubes with suction (with a disposable pipette). After removal, adults and asexual propagules readily made tubes of sediment, as long as their head and palps were intact. Worms lacking a head and palps made tubes upon regeneration of these structures. Worms were cultured in defaunated muddy sand, and kept at 21°C in flowing sea water, and on ambient photoperiod (15 light:9 dark).

Egg strings were removed from the maternal tube and cultured in 1 µm filtered sea water containing antibiotics (50 µg ml⁻¹ penicillin G and streptomycin sulfate; Strathmann 1987) in 2 ml Falcon multi-well plates at 21°C, and at an ambient photoperiod. Each egg string was transferred to a new culture well daily. Advanced larvae were provided with defaunated sediment and short segments of adult tubes in attempts to induce settlement. Sexually produced offspring are referred to as embryos while they are housed within the egg string, and include gastrula (with an open, ciliated mouth), trochophore (prototroch present), and metatrochophore (body elongated but segmentation has not yet occurred), as well as segmented young that were defined by the number of chaetae-bearing or potentially chaetae-bearing segments. After hatching, offspring are

referred to as larvae if they swim or juveniles if they are primarily benthic. Larval traits are those that may appear in embryos and persist in hatched larvae but are lost in adults.

Worms undergoing asexual reproduction were isolated from recently collected *C. cf. ramosa*. A chronology of asexual reproduction was obtained by isolating fragments within 24 h of fission, identified as such by having a smooth blastema in which cellular proliferation was not yet evident. Fragments were cultured as for egg capsules (above) and were observed at 24 h intervals. Field-collected fragments at different stages of asexual reproduction were also examined to check for potential laboratory artefacts. To simplify the description of asexual reproduction, the body plan of *A. vestalis* was categorised into four regions. The head is the most anterior region, and consists of the prostomium, peristomium, mouth, and palps. The thorax consisted of chaetigers 1–11, characterised by a relatively compact (short) appearance and presence of dorsal epithelial bacillary glands. The abdomen began on chaetiger 12, had moniliform chaetigers that were 3–4 times longer than thoracic chaetigers, a large gut, and often contained gametes throughout asexual reproduction. At the posterior abdomen, 6–8 chaetigers were present that were smaller (both in length and width) than those of the abdomen, lacked gametes, and were terminated by the pygidium.

Embryos, larvae, and asexual propagules were observed and photographed daily with a Nikon AFX. Negatives were scanned and plates composed in Corel Photo Paint 6.0 and Corel Draw 6.0.

RESULTS

Sexual reproduction

Sexually reproductive females were an average of 3.5 mm in length, and produced egg strings that were almost the same length (Table 1). Egg strings

Table 1 Summary of brood data for *Amphipolydora vestalis*. Diameter of an early embryo is given for a 16-cell stage.

Trait	<i>n</i>	$\bar{x} \pm SD$	Min.	Max.
Female length (mm)	7	3.57 ± 0.66	2.79	4.94
Brood length (mm)	9	2.91 ± 1.09	1.44	4.95
No. capsules/brood	10	6.8 ± 4.56	3	16
Av. capsule volume/brood (mm ³)	9	0.043 ± 0.031	0.015	0.114
No. embryos/brood	6	10.17 ± 13.35	2	37
No. nurse eggs/brood	7	323.17 ± 191.77	131	661
Diam. of early embryos (µm)	11	74.54 ± 7.89	60	90
Diam. of nurse eggs (µm)	64	58.83 ± 15.45	30	80

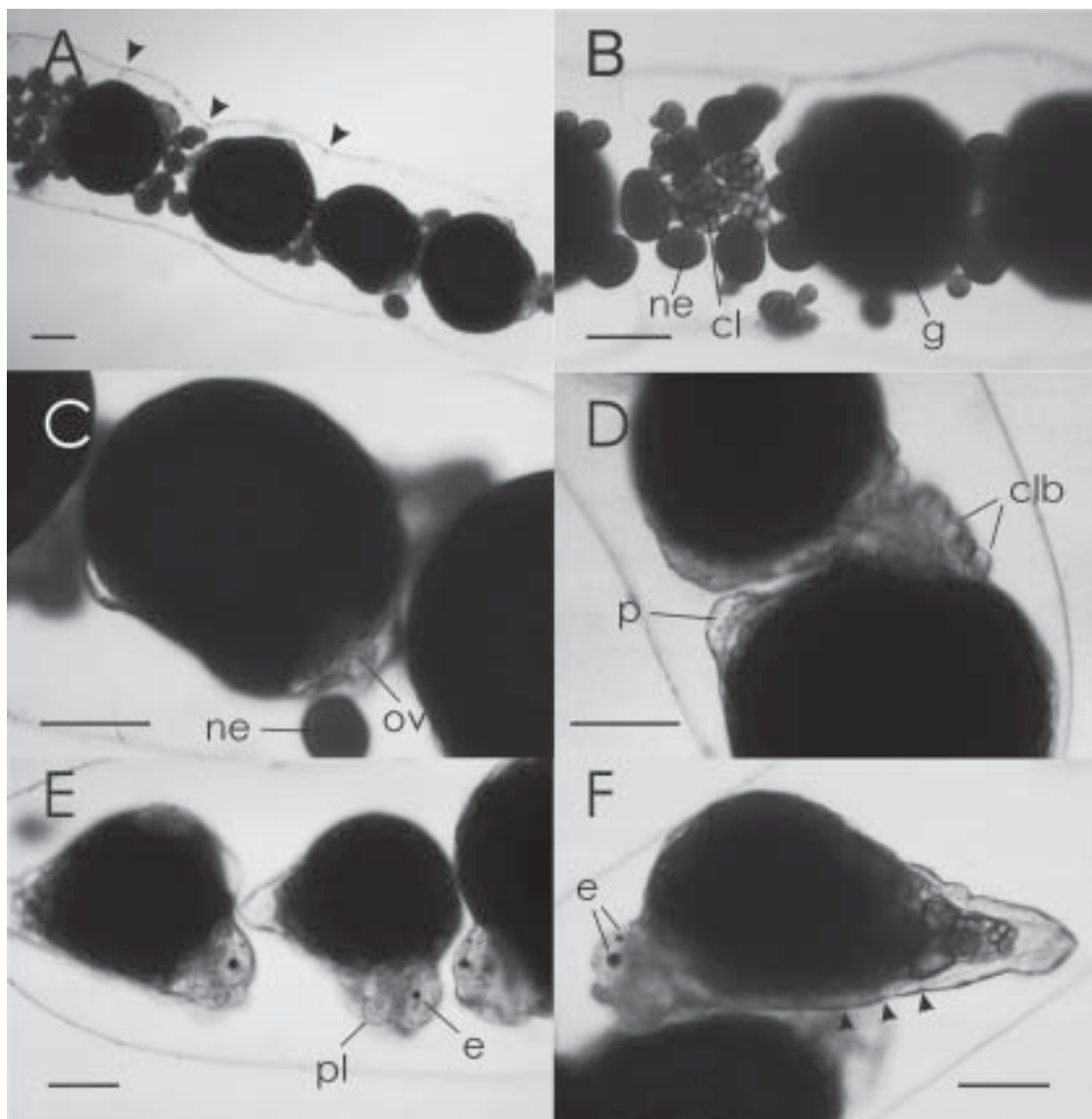


Fig. 1 Early embryogenesis in *Amphipolydora vestalis*. (Scale bar = 100 μ m.) **A**, Egg string. Capsules are closely oppressed laterally and divisions are thin (arrows). Embryos (trochophores) and the much smaller nurse eggs are visible. **B**, Nurse eggs in an early brood. Some nurse eggs are still intact (ne) whereas others have undergone cleavage (cl) to form a loose ball of cells. Gastrulae (g) are also present in the egg string. **C**, Trochophore ingesting nurse egg (ne) by rotating it rapidly in the ciliated oral vestibule (ov). **D**, Embryos at the metatrochophore stage with ciliated lobes (clb) on the head and a small pygidium (p). **E**, Five- and six-chaetiger embryos with a single pair of eyes (e) and well developed ventral lobes of the peristomium (pl). Note the hunchbacked appearance of the embryos because of the large amount of yolk in the gut. **F**, Eight-chaetiger embryo with two pairs of eyes (e) and chaetigers that are visible ventrally (arrows) and posteriorly.

contained, on average, seven thin-walled capsules that were closely compressed laterally (Fig. 1A). Advanced young broke through lateral capsule walls and moved freely throughout the entire string. Egg

strings lacked stalks and were attached within the female's tube by mucus. Capsules varied greatly in size within a single string; for example, within a single, recently spawned brood, capsules ranged

from 180 to 1110 μm in length. Early cleavage stages (16c) and nurse eggs were 75 μm and 59 μm in diameter, respectively (Table 1). Both were bright orange in colour.

Most eggs per string were nurse eggs (93–97% of the eggs per brood). Nurse eggs cleaved unequally over the first 3 days of development to form a loose ball of blastomeres (Fig. 1B) that later dissociated, releasing individual blastomeres. Embryos ingested small fragments of nurse eggs by twirling them with their oral cilia (Fig. 1C). Adelphophagy continued until the metatrochophore stage (Day 8; Table 2) when all nurse egg fragments had been consumed. During this time, embryos grew quickly and increased in length from 95 to 380 μm from the gastrulae to the late metatrochophore stage (Fig. 1C,D; Table 2). Yolk ingested within this 5-day period was sufficient to support embryogenesis for the remaining 14 days before hatching.

The earliest brood isolated was at the 16-cell stage and became the reference for the day of spawning (Day 1). Early cleavage was spiral and unequal. A blastula had formed by Day 2, and gastrulation occurred over the next 24 h (Table 2). Gastrulae were very yolky with a small, clear oral vestibule and ciliated mouth. Early trochophores (Day 3) had tufts of short prototrochal cilia with a dorsal gap, and two ventral ciliary patches. Over the next few days, trochophores rapidly grew into yolky balls with a thin ectomesoderm (Fig. 1C). By Day 7, slight wrinkles on the ventral surface indicated the onset of segmentation in metatrochophores, whereas the dorsal, non-segmented surface had a hunchbacked appearance caused by the large mass of yolk (Fig. 1D–F). As the yolk was digested, body regions became visible (Day 10), followed more slowly by the completion of segmentation on the dorsal surface (Day 10–12). Development of ectomesodermal structures appeared to be minimal until this fairly advanced stage. Once segmentation was dorsally complete, addition of new chaetigers proceeded fairly rapidly until c. Day 16. Subsequently, offspring grew primarily by increasing in chaetiger size and morphological complexity and few additional chaetigers were formed until young hatched at 14–17-chaetiger stages (Day 22).

Cilia

The first cilia to develop were the oral cilia and two post-oral, ventral patches, both visible in gastrulae, followed by short tufts of prototrochal cilia in trochophores (Table 2). Cilia of the telotroch did not appear until after segmentation had begun (Day 10).

Late stage trochophores (Day 6) had a pair of ciliated lobes located just anterior to the dorsal prototroch and as the first segments became visible ventrally, two additional pairs of ciliated lobes formed (Fig. 1D) that persisted until the 10–11-chaetiger stage. The gastrotroch and nototroch were late to develop and were not observed until the 10–11-chaetiger stage. As segments differentiated, the nototroch appeared from chaetiger 4 to the terminal chaetiger, while the gastrotroch appeared ventrally on chaetigers 3, 5, 7, 10, and 13.

Chaetae

Chaetal development slowly followed segmentation. The first short, non-serrated notochaetae were observed on chaetiger 1 (3–4 per side) shortly after the first segments had appeared (Day 10; Table 2). Subsequent development of notochaetae was slow until the 12 chaetiger stage (Day 16) when embryos took on a bristly appearance, as notochaetae increased both in number (e.g., to 28 notochaetae per side on chaetiger 1) and also length. The notopodial lobes increased in length shortly before hatching of late-stage young (Fig. 2A,B). Capillary neurochaetae were first observed in 12–13-chaetiger embryos, when they appeared in most chaetigers almost simultaneously. Hooded hooks did not appear until shortly before hatching (14-chaetiger stage, Day 20); they first emerged in chaetigers 10–12 (1–2 per side), with subsequent development of 3–4 hooks per side on chaetigers 7–14. Anterior modified spines of the 5th chaetiger were visible within the chaetal sacs, but had not yet emerged at the 12–13-chaetiger stage (Day 16–18). At 15 chaetigers (Day 21), one modified spine had emerged on chaetiger 5, with a second to follow on Day 22. The 5th chaetiger retained capillary noto- and neurochaetae (Fig. 2C) at this time.

Sensory and other structures

Young were characterised by black eyes and prominent clear cells on the prostomium (Fig. 2A,E). A single pair of round, black eyes appeared in 3–4-chaetiger embryos and were joined by a bilobed, lateral pair in 7-chaetiger young (Table 2). Large clear cells appeared on the prostomium in the early 12-chaetiger stage (Fig. 2A,E). A pair of prostomial papillae were present anteriorly in the 10–12-chaetiger stages only, followed by the development of a second, dorsal pair (12–14 chaetigers only). Palp buds formed fairly early (11–12 chaetigers) but were not ciliated until just before hatching. The caruncle and nuchal organs formed shortly after the palps

Table 2 Summary of morphogenesis during embryonic development in *Amphipolydora vestalis* highlighting the onset, presence or loss of the indicated traits. Young are classified as: B, blastula; G, gastrula; T, trochophore; M, metatrochophore; and by the number of chaetigers (c). For traits found on more than one segment, the number of chaetigers showing that trait is also given for each developmental stage. For ciliated lobes and eyes, data given are number of pairs. Cilia: CL, ciliated lobes (No. pairs); Gastr, gastrotrich; Noto, nototroch; Pr, prototroch; Telo, telotroch; VP, ventral patches. Chaetae: Mod, 5th, modified spines of the 5th chaetiger; Neuro, neurochaetae, both capillary and hooded hooks; Notoch, notochaetae. Other: Bacillary glands, on pygidium or dorsal chaetigers; Car, caruncle; Eyes, No. pairs eyes; Prost gl, prostomial glands. Mean (\pm SD) length is given for up to 8 embryos/stage.

Day	Stage	Length (μ m)	Cilia				Chaetae				Other					
			Pr	Gastr	Noto	Telo	VP	CL	Notoch	Neuro	Hook	Eyes	Palps	Prost gl	Bacillary gl	Car
1	16-cell	75 \pm 8														
2	B, G															
3	T	95	*													
Adelphophagy begins																
4	T	230	*													
5	T		*													
6	T	325 \pm 32	*													
7	M		*													
8	M	380	*													
Adelphophagy ends, all nurse eggs consumed																
9	3-4c	330 \pm 64	*													
10	3-4c	336 \pm 54	*													
11	5-6c	380 \pm 25	*													
12	7-8c		*													
13	10-11c	550 \pm 26	*													
14	11-12c	653 \pm 25	*													
15	11-12c		*													
16	12-13c		*													
17	12-14c	786 \pm 111	*													
18	12-14c		*													
19	12-14c	813 \pm 151	*													
20	12-14c		*													
21	14-16c	876 \pm 187	*													
22	14-17c	910 \pm 23	*													
Hatching																

* Indicates that a trait is present.

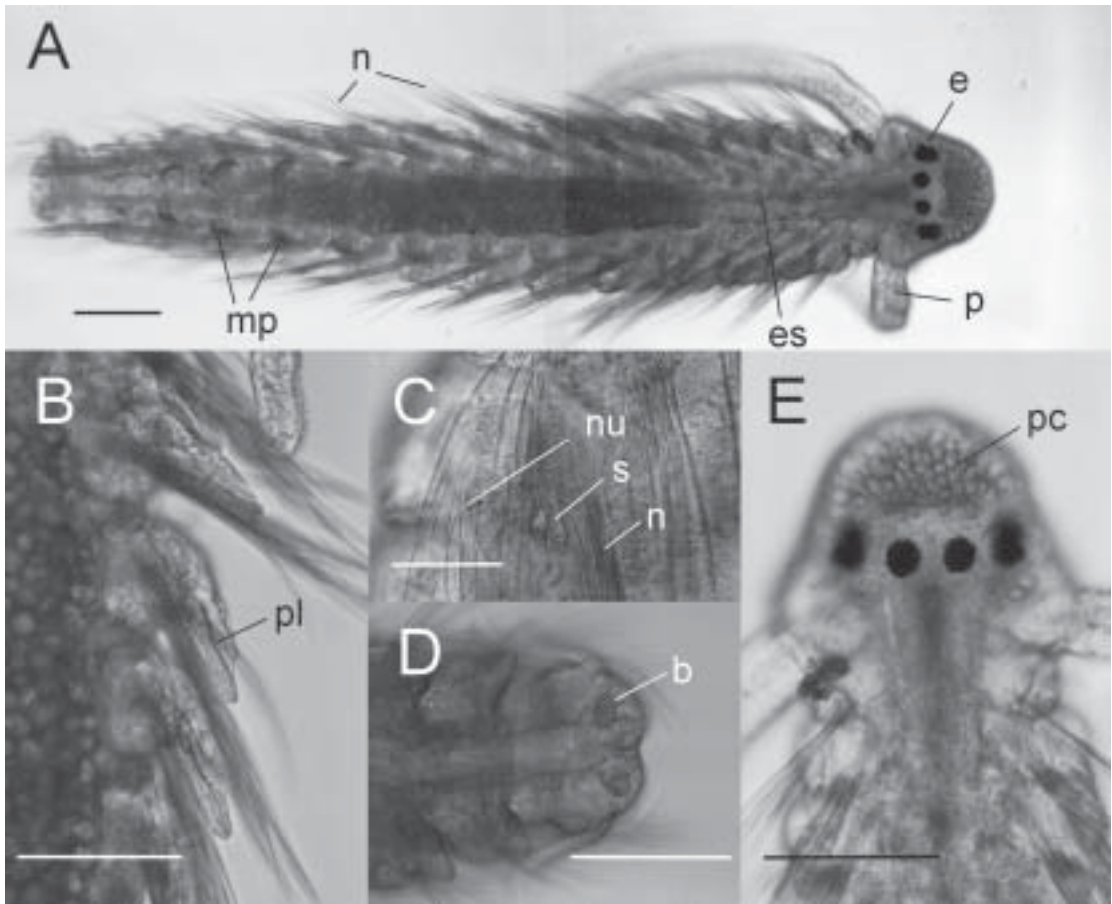


Fig. 2 Late-stage *Amphipolydora vestalis* offspring at hatching. (Scale bar: A, B, D, E = 100 μ m; C = 50 μ m.) **A**, Late-stage larva (15-chaetiger) with characteristic clear cells on the prostomium and sooty melanophores (mp) on the posterior chaetigers. Note that the larva also has two pairs of eyes (e), well developed palps (p), and that the notochaetae (n) are similar in length throughout. Esophagus (es) is easily distinguished from the yolky, posterior gut. **B**, Long post-chaetal lamellae on the notopodium (pl) characteristic of advanced larvae (e.g., 16-chaetiger as shown here). **C**, Modified 5th chaetiger of late-stage (16-chaetiger) larva with two modified spines (s), notochaetae (n), and neurochaetae (nu). **D**, Pygidium of 15-chaetiger larva with bacillary glands (b). **E**, Head of a 15-chaetiger larva with large clear prostomial cells (pc).

appeared. At 13 chaetigers, embryos had small dorsal melanophores visible as paired, lateral regions of sooty black pigment on chaetigers 11–13; melanophores appeared on additional posterior chaetigers until at least the 17-chaetiger stage (Fig. 2A). Embryos had large bacillary glands that first appeared on the pygidium at the 11–12-chaetiger stage (Fig. 2D). Shortly before hatching, additional bacillary glands developed in the dorsal epithelium of the thoracic chaetigers (14–15-chaetiger stage). Both groups of bacillary glands persisted through metamorphosis.

Hatching

In laboratory culture, larvae hatched at the 14–15-chaetiger stage (Day 22). Advanced larvae (15–17 chaetigers) also hatched during dissection of tubes from the host sponge in the laboratory. Hatched offspring were capable of short bouts of swimming activity, but generally crawled in the culture dish and could catch suspended particles with their palps, suggesting that they were close to settlement. Attempts to induce settlement were not successful.

At hatching, 15–17-chaetiger offspring had both larval and juvenile traits. Larval traits included well

developed notochaetae that were longer (200–250 μm) and more numerous (15–20 notochaetae per side on chaetiger 2) than occurred in adults (adult notochaetae 150–200 μm , 4–6 per side on chaetiger 2; Paterson & Gibson 2003). Other larval traits included a pair of stereocilia on the prostomium, large prostomial cells, paired melanophores on posterior chaetigers, and long notopodial lobes (Fig. 2A,B,E); these traits were not observed in adults. All hatchlings had two pairs of eyes which were seldom found in adults. Otherwise, hatchlings had well developed juvenile traits: almost all of the cilia of the prototroch, gastrotroch, and telotroch had been shed; the caruncle extended to the end of chaetiger 1; nuchal organs and long, ciliated palps were well developed; neurochaetae were typical of adults; and the pygidium had four unequal lobes. Chaetiger 5 showed structures characteristics of both advanced larvae (elongate notochaetae) and adults (major modified spines and a small neurochaetal fascicle).

Asexual reproduction

Asexual reproduction occurred through architomic fragmentation of the parent into several fragments by transverse fission. Most fragments contained two or three original chaetigers except when the fragment included the original head and thoracic region (the thorax typically contains 11 chaetigers) or original posterior chaetigers plus pygidium (up to 19 chaetigers). Many asexual propagules (46%) also contained gametes in the parental abdominal chaetigers. Numerous tubes (>50) were dissected that contained multiple fragments (3–6), all at the same stage, from the original parent worm. Morphogenesis involved three phases: extension of the blastema to form an entire body region, segment formation, and the formation of segment-specific features such as chaetae (Fig. 3A–I, Table 3). Subsequent growth involved addition of individual setigers anterior to the pygidium.

Development of the anterior blastema

On the day of division (Day 1), the blastema surface was flat and smooth. Within the next 24 h (Day 2), the blastema elongated to form the anlage of the thoracic region and head, and the mouth invaginated (Fig. 3A, Table 3). The thorax developed faster on the ventral surface than on the dorsal surface and the head was deflected dorsally. All 11 thoracic chaetigers appeared simultaneously along the anterior-posterior axis, but segmentation proceeded as a wave from the ventral (onset on Day 2) to dorsal surface (completion by Days 3–4). On Day 3, the

blastema had greatly elongated and the palp buds were evident (Fig. 3B). Segmentation of the body wall was complete by Day 4, and the head showed greater differentiation with larger palps and well developed prostomium extending as a caruncle to the posterior of chaetiger 1. Also by Day 4, the coelomic cavity was visible throughout the anterior blastema and the gut began to show regional differentiation, with the formation of the oral cavity and the esophagus in the first two chaetigers (Fig. 3C).

Notochaetae appeared externally on Day 5. Chaetal formation began as the invagination of the body wall to form the chaetal sacs, followed within 12 h by extension of short, sparse notochaetae. Organogenesis was also evident as the extension of blood vessels into the palps, completion of the gut tube, and differentiation and growth of the muscular esophagus. The circumesophageal nerve ring was visible as a thin ring of cells posterior to the oral cavity. The mesothelium of the inner body wall began to extend into the coelom to form thin septa. The body wall became more defined and the epithelial bacillary glands were visible as a few thick cells on the dorsal surface of chaetigers 5–8. By Day 6 (Fig. 3D), two rows of notochaetae (one short, one long) were evident on chaetigers 2–11. A single tuft of short neurochaetae was also evident on chaetigers 1–11. Also on Day 6, the first modified spines were visible on chaetiger 5, as one distally expanded spine and one smaller falcate spine, and the long notochaetae were lost. The first hooded hooks were visible on Day 7 on chaetigers 10 and 11. By Day 8, the hooded hooks were also visible on chaetigers 7 through 11, with subsequent development from chaetiger 12 on posterior segments.

By Day 8, most of the original body plan had been restored. The ciliated feeding groove was evident on the palps, and the nuchal organs were prominent on either side of the caruncle. The adult chaetal arrangement was complete, although the chaetae were small. The chaetal sacs had enlarged to fill most of the coelomic cavity, and ciliated nephridia were also visible in chaetiger 11. Blastemal chaetigers grew to the same size as those lost during fission within the next 4–5 days, and the original body plan was restored with the exception of eyes. Although eyes were always observed in embryos, and seldom in adults, they were not observed in progeny resulting from architomy.

Development of the posterior blastema

Overall, the posterior blastema developed according to the basic pattern described above. One exception

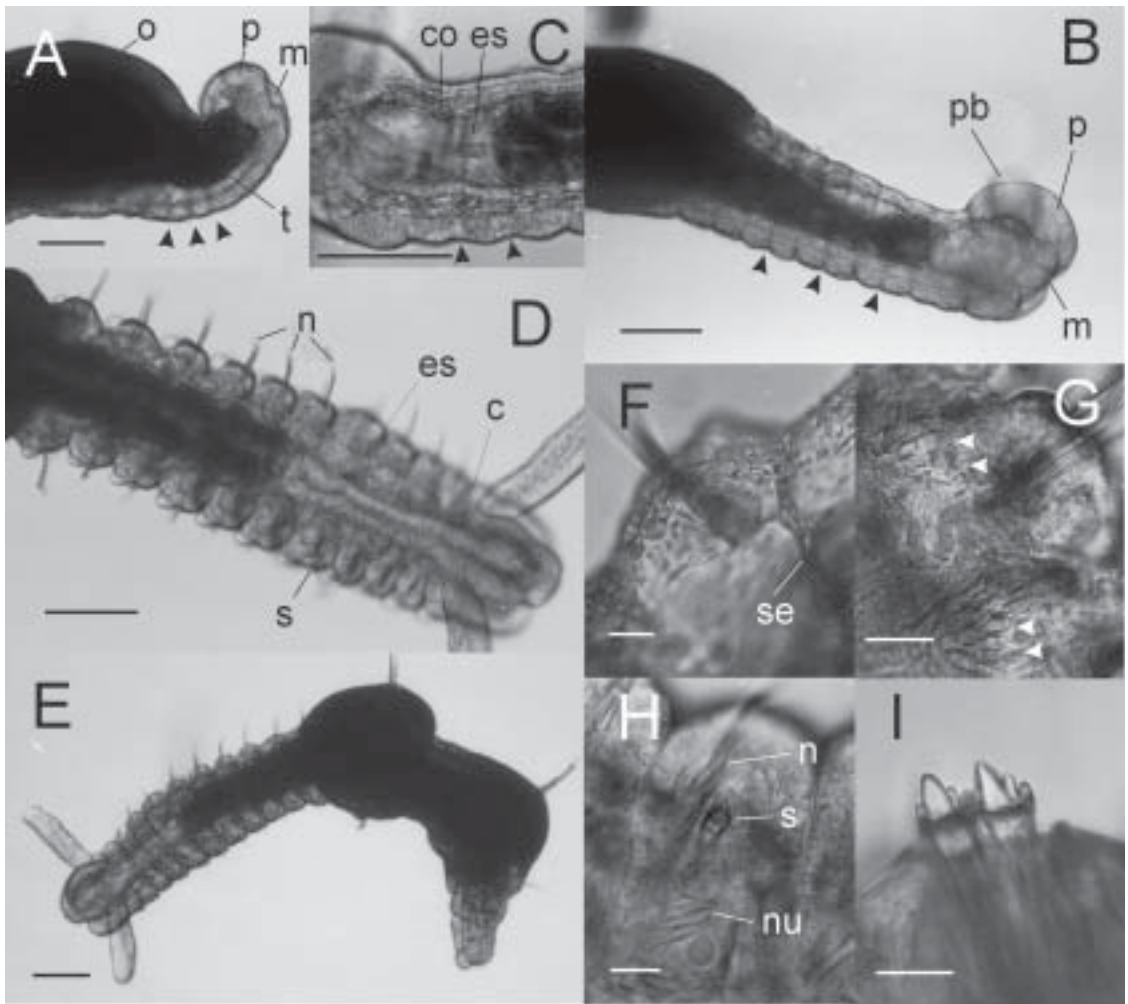


Fig. 3 Asexual reproduction in *Amphipolydora vestalis*. (Scale bars: A–E = 100 μ m; F–I = 25 μ m.) **A**, Anterior blastema on Day 2, with the large, original chaetigers (o) on the left, and the smaller blastema on the right. Blastema is divided into the thorax (t) and the head, with the prostomium (p) and mouth (m). Onset of segmentation is visible on the ventral blastema (arrows). **B**, Anterior blastema on Day 3. Divisions (arrows) between well developed chaetigers are visible, as are the mouth (m) and prostomium (p). Palp buds (pb) are also visible but are out of the plane of focus. **C**, Anterior blastema on Day 4, showing the interface between the head (left) and thorax (right). Segmentation is clearly visible in the body wall (arrows), with the gut showing internal differentiation into the oesophagous (es) and posterior gut within the coelomic cavity (co). **D**, Anterior blastema on Day 6, with caruncle (c), notochaetae (n), and regionalised gut with a muscular esophagus (es). Modified spines (s) have emerged on chaetiger 5. **E**, Late Day 6 asexual propagule, with anterior and posterior blastema that have formed from a fragment of two large, parental (i.e., original) chaetigers. **F**, Part of a chaetiger from the anterior blastema on Day 6 with well developed septa (s). **G**, Epithelial bacillary glands on the anterior blastema of a Day 6 asexual propagule. Arrows indicate the clusters of glandular cells. **H**, Modified 5th chaetiger of a Day 6 asexual propagule. Note that the first modified spine (s) has emerged and the notochaetae (n) and neurochaetae (nu) are present. **I**, Two modified spines of the 5th chaetiger on Day 8 of asexual reproduction, with a prominent central tooth surrounded by a ring of shorter teeth.

is that the posterior blastema gave rise to only three chaetigers, which formed simultaneously (Fig. 3E), and additional chaetigers arose from the growth zone just anterior to the pygidium. Cell proliferation extended the blastema on Days 2–3 after fission, and three segments differentiated simultaneously on Days 3–4 (Table 3). Both notochaetae (long capillary) and neurochaetae (short capillary and hooded hooks) appeared externally on Days 5–6 on the most anterior chaetiger of the posterior blastema, followed by chaetal development on the other blastemal chaetigers 1 day later. The gut tube extended from the original chaetigers early (Days 1–3) and differentiated, with internal ciliation, on Days 4–5. The pygidium differentiated early, and four unequal lobes were visible on Day 3. The subterminal growth zone was present by Day 6, and growth began within the next 2 days as single chaetigers formed, and

subsequently differentiated (e.g., chaetal development) as a second chaetiger began to extend.

Regeneration following collection

Although great care was taken, *A. vestalis* occasionally fragmented during collection or isolation. Morphogenesis following stress-induced fragmentation was similar to that of asexual reproduction. Worms divided into two, or sometimes into several (5–6) fragments. The terminal body regions generally remained intact (i.e., the head and 10–11 thoracic chaetigers, and the pygidium with 6–10 posterior segments) while the abdomen broke into several subunits. Fragments that lacked abdominal tissue (i.e., terminal body regions; $n = 12$) often had delayed regeneration or failed to regenerate altogether, and died after 5–7 days of laboratory culture. Terminal fragments undergoing asexual

Table 3 Summary of morphogenesis during asexual reproduction in *Amphipolydora vestalis*. (p, body region present; v, differentiated on ventral surface only.)

Structure	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Anterior blastema								
Head		*	*	†	†	†	†	†
Palps			*	*	†	†	†	†
Caruncle				*	†	†	†	†
Nuchal organ								†
Mouth		*	*	*	†	†	†	†
Eyes								
Prostomium		*	*	*	†	†	†	†
Thorax		*	*	†	†	†	†	†
Chaetigers		v	*	†	†	†	†	†
Notochaetae					*	†	†	†
Neurochaetae						*	†	†
Modified spines on chaetiger 5						*	†	†
Gut, present		*	*	†	†	†	†	†
Gut, regional differentiation				*	*	†	†	†
Dorsal bacillary glands					*	†	†	†
Posterior blastema								
Tail		p	*	†	†	†	†	†
Chaetigers			v	*	†	†	†	†
Notochaetae					*	†	†	†
Neurochaetae						*	†	†
Gut, present			*	*	†	†	†	†
Gut, regional differentiation					†	†	†	†
Growth zone						*	†	†
New chaetigers							*	†
Pygidium								
Dorsal lobes			*	†	†	†	†	†
Ventral lobes			*	†	†	†	†	†

* Anlage visible.

† Structure well developed.

reproduction (versus lab-induced fragmentation) often contained one or two abdominal chaetigers and were capable of normal regeneration.

DISCUSSION

In *A. vestalis*, both sexual and asexual reproduction occur simultaneously within the population. Both egg strings and asexual propagules were collected in all samples and many asexual propagules contained gametes in the parental chaetigers. Sexual reproduction in *A. vestalis* involves the release of advanced young that were capable of short periods of swimming activity, but spent most of their time crawling (laboratory observations). Both patterns of reproduction suggest that dispersal is limited and that most offspring recruit locally. Local recruitment may be successful over the short term as *A. vestalis* appear to specialise on a common, patchy and presumably long-lived host sponge. Sexual and asexual reproduction are reported to co-occur in only one other spionid, *Polydorella smurovi* (Tzetlin & Britayev 1985). Both asexual and sexual reproduction may be simultaneous in populations of *A. abranchiata*, as Hartman noted that “individuals are minute though ovigerous” (1953, p. 44) and Blake (1983), examining Hartman’s specimens, suggested that architomic asexual reproduction occurred since some specimens were in the process of regeneration.

Asexual reproduction is not common in the Spionidae and can occur through architomy or paratomy. Architomy occurs in *Pygospio elegans* (Rasmussen 1953), *P. californica* (Blake unpubl. in Blake & Arnofsky 1999), and is also likely in *A. abranchiata* (Blake 1983). Morphogenesis during architomy has been described for *P. elegans* (Gibson & Harvey 1999) and is very similar to that described here for *A. vestalis*. In both species, adults fragment into several 2–3-chaetiger long pieces. Morphogenesis in both involved formation of body regions, simultaneous formation of a fixed number of chaetigers across a body region, followed by differentiation of segment-specific structures (e.g., chaetae). Also, for both species, survivorship was highest if the fragment contained chaetigers from the parent’s abdomen, and lowest for posterior fragments.

Paratomy occurs in a few species of Spionidae including *Dipolydora tetrabanchia* (Campbell 1955, as *Polydora*) and all four species of *Polydorella* (Blake & Kudenov 1978, as *Pseudopolydora*; Tzetlin & Britayev 1985; Radashevsky 1996). Paratomy in *Polydorella*

prolifera begins as a growth zone within the parental body and gives rise anteriorly to a stolon body. The stolon differentiates before the progeny detach with a “new” anterior region and a “parental” posterior region (Radashevsky 1996). Although time of fission differs, paratomy is similar to architomy in several ways. First, the anterior growth zone gives rise to the head and thorax; this is in contrast to regeneration in some species of Sabellidae, where existing anterior abdominal chaetigers reorganise to become thoracic, and the anterior blastema gives rise to the head only (Abeloos 1965). Second, the same sequence is observed: formation of a body region, segmentation, with subsequent differentiation. This sequence also occurs during anterior regeneration, which is relatively common within the polydoridae complex, but differs from growth where individual chaetigers are added from a subterminal growth zone. Although phylogenetic analyses are needed, the existing data suggest that within the Spionidae, architomy has evolved in a few lineages from a widespread ability to regenerate and that paratomy is derived from architomy through a decoupling of timing of morphological change from time of fission. This is consistent with Bely & Wray (2001) who argue that asexual regeneration has evolved from regeneration in oligochaetes.

Sexual reproduction is the norm in the Spionidae and adelphophagy is common. Embryonic development in *A. vestalis* is typical of polydoridae species in many ways (see Wilson 1928; Hannerz 1956; Blake 1969). Brood structure differs from Blake’s (1969) classification of polydoridae egg capsules into three types based on stalk morphology and degree of separation of individual capsules. Egg strings of *A. vestalis* are smooth and stalkless which is likely a derived state in this group. Also, embryos rupture the internal divisions between capsules converting the discrete capsules into an elongate cylinder, as happens in some adelphophagic species. Differences in offspring morphology include the presence of ciliated lobes anterior to the prototroch, observed only in *Polydora quadrilobata* (Blake 1969) and *Boccardia androgyna* (Gibson pers. obs.), and the small dorsal melanophores.

Embryonic development in *A. vestalis* is also unusual in that early morphogenesis is delayed, mid-stage embryos increase in morphological complexity without an increase in chaetiger number, and late-stage larvae acquire several traits that are not found in adults. In polychaetes generally, larval traits appear early in morphogenesis and are lost in late larval development, while many juvenile traits form

throughout the late larval phase resulting in a gradual metamorphosis (e.g., Hannerz 1956; Blake 1969; Pernet et al. 2002). In *A. vestalis*, formation of many larval structures is delayed, possibly as resources are diverted to rapid growth rather than morphological differentiation. Mid-stage embryos (11–13 chaetigers) greatly increase in morphological complexity over several days while chaetiger formation has ceased. This differs from larval development in most polydorid species where morphological complexity is closely linked to developmental stage (i.e., chaetiger number). Late-stage larvae of *A. vestalis* acquire new structures, including many notochaetae, well developed eyes, long notopodial lobes, and prominent prostomial glands. These structures are reduced (e.g., notochaetae and notopodial lobes), lost (e.g., prostomial glands), or rarely seen (e.g., eyes) in adults (Paterson & Gibson 2003). A similar phenomenon was noted by Radashevsky & Fauchald (2000) who described the formation and loss of provisional modified spines on chaetiger 5 in late-stage larvae of a few polydorid species.

It is not known if the morphology of late-stage larvae and juveniles of *A. vestalis* supports a short dispersive phase or colonisation of a host sponge during recruitment. As we were unable to induce settlement, we could not determine when these structures were lost but they are likely shed at settlement or, if retained by adults, may fail to regenerate after architomic fission. For example, eyes were infrequently found in adult *A. vestalis* suggesting they may not be regenerated, as is known in several species of *Polydora* (Dean 1969 in: Rice & Simon 1980).

The genus *Amphipolydora*, previously known by one species, *A. abbranchiata*, has been variously associated with *Polydora* (e.g., Hartman, 1953), *Boccardia* (e.g., Blake 1983), and *Polydorella* (e.g., Radashevsky 1996). Our observations of adult morphology, habitat (Paterson & Gibson 2003) and reproduction support Radashevsky (1996) in proposing a close relationship between *Amphipolydora* and *Polydorella*. Asexual reproduction (via paratomy) occurs in *Polydorella* species and one *Dipolydora*. Architomy is only known (in the Spionidae) in *Amphipolydora* and the non-polydorid *Pygospio*. Morphogenesis of architomy and paratomy is very similar and we predict a common developmental origin. In terms of larval development, *A. vestalis* agrees with *Polydora* spp. in the presence of paired dorsal melanophores (not single as in *Boccardia*; Blake & Arnofsky 1999), although these are much reduced.

A comparison of larval morphology with *Polydorella* is not yet possible. Other reproductive traits distinguish *Amphipolydora* from other polydorids, including lack of stalks on egg capsules.

ACKNOWLEDGMENTS

This work was conducted at the Leigh Marine Laboratory, University of Auckland. We thank the staff at Leigh Labs, especially Jo Evans, and also the University of Auckland librarians. Thanks to Vasily Radashevsky, Geoff Read, Merritt Gibson, and an anonymous reviewer who provided valuable comments on the manuscript, and to James Williams for help collecting samples. This research was funded by a Natural Sciences and Engineering Research Council of Canada grant to G. D. Gibson.

REFERENCES

- Abeoos, M. 1965: La régénération des Annélides. *In*: Kiortsis, V.; Trampusch, H. ed. Regeneration in annelids and related problems. Amsterdam, North-Holland Publishing Company. Pp. 207–215.
- Anger, V. 1984: Reproduction in *Pygospio elegans* (Spionidae) in relation to its geographical origin and to environmental conditions: a preliminary report. *Fortschritte der Zoologie* 29: 45–51.
- Bely, A.; Wray, G. 2001: Evolution of regeneration and fission in annelids: insights from engrailed- and orthodenticle-class gene expression. *Development* 128: 2781–2791.
- Blake, J. 1969: Reproduction and larval development of *Polydora* from northern New England (Polychaeta: Spionidae). *Ophelia* 7: 1–63.
- Blake, J. A. 1983: Polychaetes of the family Spionidae from South America, Antarctica and adjacent seas and islands. *Biology of the Antarctic Seas XIV Antarctic Research Series* 39: 205–288.
- Blake, J. A.; Arnofsky, P. L. 1999: Reproduction and larval development of the spioniform polychaeta with application to systematics and phylogeny. *Hydrobiologia* 402: 57–106.
- Blake, J. A.; Kudenov, J. D. 1978: The Spionidae (Polychaeta) from southeastern Australia and adjacent areas with a revision of the genera. *Memoirs of the National Museum of Victoria* 39: 171–280.
- Campbell, M. 1955: Asexual reproduction and larval development in *Polydora tetrabanchia* Hartman. Unpublished PhD thesis, Duke University, Durham, NC, United States. 67 p.

- Chia, F.-S.; Gibson, G.; Qian, P.-Y. 1996: Poecilogony as a reproductive strategy of marine invertebrates. *Oceanologica Acta* 19: 203–208.
- Dean, D. 1969: Relationship between eyes and anterior regeneration in spionid polychaetes. *American Zoologist* 9: 1146.
- Gibson, G.; Harvey, J. 1999: Morphogenesis during asexual reproduction in *Pygospio elegans* Claparede (Annelida, Polychaeta). *Biological Bulletin* 199: 41–49.
- Hannerz, L. 1956: Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil, and Poecilochaetidae n. fam. in the Gullmar Fjord (Sweden). *Zoologiska bidrag från Uppsala* 31: 1–204.
- Hartman, O. 1953: Non-pelagic polychaeta of the Swedish Antarctic Expedition 1901–1903. *Further Zoological Results of the Swedish Antarctic Expedition 1901–1903* 4: 1–83.
- Paterson, I. G.; Gibson, G. D. 2003: A new species of *Amphipolydora* (Polychaeta: Spionidae) from New Zealand. *New Zealand Journal of Marine and Freshwater Research* 37: 733–740.
- Pernet, B.; Qian, P.-Y.; Rouse, G.; Young, C.; Eckelbarger, K. 2002: Chapter 12. Phylum Annelida: Polychaeta. In: Young, C.; Sewell, M.; Rice, M. ed. Atlas of marine invertebrate larvae. London, Academic Press. Pp. 209–244.
- Radashevsky, V. I. 1996: Morphology, ecology and asexual reproduction of a new *Polydorella* species (Polychaeta: Spionidae) from the South China Sea. *Bulletin of Marine Science* 58: 684–693.
- Radashevsky, V. I.; Fauchald, K. 2000: Chaetal arrangement and homology in spionids (Polychaeta: Spionidae). *Bulletin of Marine Science* 67: 13–23.
- Rasmussen, E. 1953: Asexual reproduction in *Pygospio elegans* Claparede (Polychaeta sedentaria). *Nature* 171: 1161–1162.
- Rice, S.; Simon J. 1980: Intraspecific variation in the pollution indicator polychaete *Polydora ligni* (Spionidae). *Ophelia* 19: 79–115.
- Strathmann, M. F. 1987: Reproduction and development of marine invertebrates of the northern Pacific coast. Data and methods for the study of eggs, embryos, and larvae. Seattle, University of Washington Press.
- Tzvetlin, A. B.; Britayev, T. A. 1985: A new species of the Spionidae (Polychaeta) with asexual reproduction associated with sponges. *Zoologica Scripta* 14: 177–181.
- Wilson, D. 1928: The larvae of *Polydora ciliata* Johnston and *Polydora hoplura* Claparède. *Journal of the Marine Biology Association of the United Kingdom* 15: 567–603.