

Palp growth, regeneration, and longevity of the obligate hermit crab symbiont *Dipolydora commensalis* (Annelida: Spionidae)

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Abstract. The polychaete *Dipolydora commensalis* is an obligate symbiont of hermit crabs and produces a burrow along the columella of the gastropod shells they inhabit. Adults of *D. commensalis* have short palps that they use to feed on particles dropped or brought in by the respiratory currents of hosts. To determine whether hermit crabs influence palp length, specimens of *D. commensalis* were isolated in glass capillary tubes and the growth of palps was measured over a 3-week period. Palp length was also measured in worms isolated in gastropod shells with or without hermit crabs for 2 weeks. In addition, to determine whether adults of *D. commensalis* have regeneration capabilities like those of free-living relatives, worms were cut at the fifth or 15th setiger and then monitored for 35 d. Worms extracted from shells and placed into capillary tubes had initial palp lengths of 1.0 ± 0.4 mm ($n = 17$); after isolation, palps were 40% longer (1.4 ± 0.4 mm, $n = 17$). Worms in gastropod shells with hermit crabs had an average palp length of 0.9 ± 0.4 mm ($n = 31$), whereas worms in shells without hermit crabs had palps that were 33% longer (1.2 ± 0.5 mm, $n = 40$). Adults of *D. commensalis* are capable of regeneration; 35 d after ablation at setigers 5 or 15, the average number of anterior setigers regenerated was 5 ($n = 15$) and 9 ± 1.3 ($n = 13$), respectively. The average number of posterior setigers regenerated from the 15 setiger anterior fragments was 11 ± 6 ($n = 10$). The findings suggest that the palps (and sometimes anterior ends) of the worms are exposed during feeding and are cut during movement of the hermit crab. In the laboratory worms can live for > 4 years, considerably longer than the functional life span of most gastropod shells inhabited by hermit crabs.

Additional key words: symbiosis, commensal, polychaete, pagurid, life span

The Spionidae is one of the largest families of polychaetes (>450 described species and 33 genera). Its members are generally recognized by the pair of palps they use in suspension or deposit feeding (Blake 1996; Rouse 2001). Members of this family occupy a wide range of marine habitats, with some forming symbioses with other marine invertebrates (Blake 1996; Martin & Britayev 1998). Most symbiotic species are found in *Polydora* and related genera that contain a modified fifth segment with major spines or specialized setae. Members of these genera (collectively termed polydorids) are found free living in soft-bottom sediments as well as boring into calcareous substrates (e.g., corals and mollusc shells) or associated with sponges and other invertebrates (Blake & Evans 1973). Members of 24 species of

polydorids are known to bore into gastropod shells inhabited by hermit crabs; of these, 21 are considered facultative associates and five are considered obligate associates (Williams & McDermott 2004).

The spionid *Dipolydora commensalis* (ANDREWS 1891) is an obligate symbiont of hermit crabs, found only in the gastropod shells inhabited by their decapod hosts (Blake 1996; Williams & McDermott 1997). Worldwide, *D. commensalis* is found in association with members of ≥ 16 hermit crab species, from the eastern and western coasts of North America to the Sea of Japan (Williams & McDermott 2004). On the east coast of the United States, the worm is commonly associated with the long-wrist hermit crab, *Pagurus longicarpus* SAY 1817. In this region, between 35% and 50% of gastropod shells inhabited by *P. longicarpus* harbor *D. commensalis* (Williams & McDermott 2004). *Dipolydora commensalis* has a long-shared evolutionary history with hermit crab hosts, from at least the Eocene

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(Ishikawa & Kase 2007). It is assumed that the association between *D. commensalis* and their hermit crab hosts initially evolved because hermit crab shells provided an appropriate substrate for boring; enhanced feeding and protection from predators further reinforced this association (Williams & McDermott 2004). The only other extant obligate polydorid species associated with hermit crabs are an undescribed species of *Diploydora*, *Polydora biocipitalis* BLAKE & WOODWICK 1972, *Polydora nanomon* ORENSKY & WILLIAMS 2009, and *Polydora robi* WILLIAMS 2001 (Williams & McDermott 2004; Ishikawa & Kase 2007; Orensky & Williams 2009).

Although *D. commensalis* has been classified as a commensal, recent research has shown that the symbiotic relationship between the worm and hermit crab needs to be re-evaluated (as do most presumed cases of commensalism; see Zapalski 2011). Members of *D. commensalis* gain multiple benefits from living with their hosts, including a protected substrate, avoidance of stagnant waters, and prevention of burial (Hatfield 1965). In addition, adults of *D. commensalis* find a ready supply of food particles inside their host's shells, including material adhering to the legs of the host (Dauer 1991; Williams & McDermott 1997). However, *D. commensalis* does not appear to be neutral in its effects on hosts. The worms can reduce shell strength (Buckley & Ebersole 1994) and thus can have a negative impact on the survival of hermit crabs through increased chances of shell breakage during predation events. Adults of *D. commensalis* have also been found to feed on hermit crab eggs and larvae (McGuire 2007). Finally, members of *D. commensalis* may also be kleptoparasites, stealing food from the mouthparts of the host, as in the polychaete *Neanthes fucata* (SAVIGNY 1818) (Matthews 1959).

Members of *D. commensalis* are protandric hermaphrodites that exhibit sexual dimorphism. Females (15 mm+ in total length) are larger than males (~2–4 mm) (Hatfield 1965; Radashevsky 1989, 1993; Dauer 1991). Typically, a single female worm will produce a burrow on the columella of the shell (near the inner lip of the shell) that extends to the apex and opens on the inside of the shell; multiple smaller males may be associated with the female's burrow. During reproduction, the female will deposit an egg capsule string in the burrow, producing ~1800–2400 eggs per brood (Hatfield 1965; Blake 1969). Fertilized eggs require ~48 h to develop into trochophore larvae (Hatfield 1965; Blake 1969; Blake & Arnofsky 1999). After ~7 d, the larvae develop to the five segment larval stage and are ready to be released (Hatfield 1965). When the larvae develop to 15–19 segments (~1–1.5 months in the water col-

umn), they are competent to settle on host shells (Hatfield 1965; Radashevsky 1993).

Adults of *D. commensalis* use a pair of peristomial palps to feed and add material to their burrows. These worms capture food particles with their palps; particles are then transported in a ventral ciliated groove (= food groove) to the everted pharynx by a combination of muscular movement and ciliary action (Dauer et al. 1981; Williams & McDermott 1997). While feeding, adults of *D. commensalis* may extend the anterior of their bodies and palps from the burrow opening to capture suspended and deposited food particles (Dauer 1991; Williams & McDermott 1997).

Although the mode of feeding by members of *D. commensalis* is not unique within the Spionidae, several distinct morphological features are associated with their feeding appendages. Specifically, the palps of *D. commensalis* have been reported to be unusually short (~1.0 mm in total length on adult females) compared with members of other species in the polydorid complex (~3–8 mm). In addition, the palp ciliation pattern is distinct from all other polydorids studied to date. Two palp morphology types are typically found in polydorids: Type I has frontal cilia, latero-frontal cirri, lateral cilia, randomly scattered cirri (motile and/or non-motile), and glandular holes; Type II has the same structures as Type I but lacks randomly scattered cirri (motile) and glandular holes (Worsaae 2001). The palp morphology of members of *D. commensalis* does not match either Type I or Type II; this species only has frontal cilia and non-motile cirri on papillae (Worsaae 2001). These non-motile cirri are believed to be involved in sensory function and cover the whole palp except for the food groove, which is covered by frontal cilia (Dauer 1991; Worsaae 2001).

Dauer (1991) suggested that the palps of *D. commensalis* are modified for life in the shells of hermit crabs, where they are used to capture particles brought in by the crabs. It has long been assumed that palp length in *D. commensalis* was genetically controlled. However, interactions with hermit crab hosts may affect the feeding behavior and palp size of *D. commensalis*. Williams & McDermott (1997) found that worms isolated in glass tubes for >3 months had longer palps compared with their initial lengths, indicating that the hermit crab hosts may influence palp length. The causes of reduced palp length in this species are unknown but could include action of the chelipeds, walking legs, or ventral portion of the thorax of hermit cutting the palps during the movement and feeding of the host.

Free living spionids are vulnerable to palp loss through sublethal predation events, in which portions

of their palps, anterior ends, or posterior ends can be cut off by predators, typically fish (Zajac 1985; Lindsay et al. 2007; Lindsay 2010). Sublethal predation is common in marine environments and can influence the feeding behavior of polychaetes such as the spionids (Lindsay & Woodin 1992, 1995). Most spionids appear to have the ability to regenerate anterior segments, but at least one spionid (*Streblospio benedicti* WEBSTER 1879) lacks the ability to regenerate even when only a single anterior segment is removed (Lindsay et al. 2007). The spionids *Dipolydora quadrilobata* (JACOBI 1883) and *Pygospio elegans* CLAPARÈDE 1863 have the ability to regenerate anterior segments and palps when five anterior segments, half of the segments with gills, and all of the segments with gills are removed (Lindsay et al. 2007, 2008). Posterior regeneration, unlike anterior regeneration, is found in nearly all annelids (Bely 2006). Although regeneration has been studied in free-living spionids, it is largely unstudied in symbiotic species.

This research tested the hypotheses that (1) host hermit crabs play a role in maintaining the short palp lengths of members of *D. commensalis*, and (2) members of *D. commensalis* are able to regenerate anterior and posterior segments. Although chances of sublethal predation on adults of *D. commensalis* are low due to its protected habitat, anterior segments could be cut by the hermit crab. As the hermit crab feeds, worms could be damaged if extended from their burrows. Because the worm often feeds at the same time as hosts (Dauer 1991; Williams & McDermott 1997), there is a considerable chance for such interactions and loss of palps or anterior segments. While examples of regeneration in free-living spionids have been well studied, this is the first to examine regeneration in the obligate symbiont *D. commensalis*. In addition, we report on the longevity of *D. commensalis* when isolated from its host.

Methods

Specimen collection and measurements

Specimens of the hermit crab *Pagurus longicarpus* ($n = 120$) were collected by hand from Tappan Beach, New York (40°35'39"N, 73°30'11"W) and Welwyn Preserve, Glen Cove, New York (40°38'21"N, 73°17'20"W) in June and July of 2006 for initial measurements of the palps of *Dipolydora commensalis*. From June–November 2007, specimens of *P. longicarpus* ($n = 120$) were collected by hand from the Boat Basin in Jones Beach, New York (40°35'39"N, 73°30'11"W) and Oak Beach, Babylon, New York (40°38'21"N, 73°17'20"W) in order to isolate adults of *D. commensalis* for palp measurements and anterior

regeneration studies. Shells with burrows of *D. commensalis* visible or with other symbionts that provide evidence of occupancy by worms (Williams & McDermott 2004) were collected preferentially to increase likelihood of collecting the worms. The hermit crabs were either examined immediately or kept in aerated seawater (salinity 32‰) in plastic containers (102 cm × 63.5 cm × 51 cm) at 14°C before study. The following were recorded for each hermit crab: sex and size (shield length), presence of hermit crab eggs, shell species, total length and aperture length of the shell, damage to the shell, presence and diameter of burrows of *D. commensalis*, and presence of *Hydractinia symbiolongicarpus* BUSS & YUND 1989. The measurements of hermit crab size, shell aperture, and shell length were taken using vernier calipers. Hermit crabs isolated from their shells (by grasping their chelipeds and walking legs and gently pulling them out before cracking the shells with stainless-steel bone cutters) were returned to their collection site.

In order to remove adults of *D. commensalis* from shells, the apex of the shell was snipped off with cutters, exposing the lumen of the uppermost whorl. Pipetting a stream of seawater through the hole in the apex helped remove the worm. Isolated worms were placed in glass capillary tubes (0.9–1.8 mm in diameter), which were open at both ends. Most worms (90%) stayed in their capillary tubes; those that did not typically died within 1 week. Isolated worms in capillary tubes were stored in Petri dishes (60 mm × 15 mm) filled with artificial seawater (salinity 32‰). The water was changed weekly and specimens of *D. commensalis* were fed ground Shrimp Pellets Formula™ (Wardley®, The Hartz Mountain Corporation, Secaucus, NJ, USA) weekly. A group of worms ($n = 14$) were maintained under these conditions and followed for >4 years to estimate their maximal life spans in the laboratory.

Images of worms (palps and body) in glass capillary tubes and burrow diameters were taken using an Olympus SZX12 dissecting microscope and an Olympus DP 11-N camera (Olympus, Center Valley, PA, USA). No relaxant (e.g., MgCl₂) was used to sedate the worms because MgCl₂ was found to increase mortality of ablated polydorid worms (Lindsay et al. 2007). After calibration with digital images of micrometer scales, Image J (National Institutes of Health, Bethesda, MD, USA) was used to measure palp length and total body length (anterior margin of prostomium to end of pygidium).

Palp length with and without hermit crabs

To assess palp growth in worms held in glass capillary tubes, worms ($n = 28$) were isolated from

hermit crab shells, placed into glass capillary tubes, and maintained in glass Petri dishes that were submerged in a plastic tub (102 cm × 63.5 cm × 51 cm) filled with aerated artificial seawater. Measurements of palp and body lengths were taken immediately after isolation and each week for 3 weeks after isolation from hosts, as described above. Worms were fed *ad libitum* weekly with ground shrimp pellets. Sex of the worms was not determined due to difficulty in distinguishing males and juvenile female worms. However, the data were analyzed after excluding small worms (< 50 setigers) to determine if inclusion of males/juvenile females affected the results.

The influence of host presence on palp length of the worms was examined by holding worms in gastropod shells with and without hermit crab hosts. Differences in palp morphology between worms with and without hermit crab influence were also observed with scanning electron microscopy (SEM). Shells were divided into two groups: 30 shells with hermit crabs removed, and 27 shells with hermit crabs present. All specimens were placed in a 189.3-L aquarium, but those shells with hermit crabs removed were put on a plastic grid that was raised 30.5 cm from the bottom of the aquarium; the rest of the hermit crabs were allowed to move freely on the bottom. The aquarium was maintained at a temperature of 20–21°C, and was exposed to ambient light conditions. Each week, ~12 shrimp pellets were added to the bottom of the tank containing the hermit crabs. A pipette was used to feed ground shrimp pellets to the specimens of *D. commensalis* in isolated shells; the pipette was also used to remove any debris on the isolated shells. After 2 weeks, the palp and body lengths of members of *D. commensalis* found in shells with hermit crabs removed and shells with hermit crabs present were measured as described above. Any empty shells found on the bottom of the aquarium tank (due to hermit crab death or shell eviction) were removed from the analysis. Sex of the worms was not determined; the effects of small male/juvenile female worms (< 50 setigers) on the results were analyzed as described above.

Body regeneration

Members of *D. commensalis* isolated in glass capillary tubes were used for examination of regeneration. Each specimen of *D. commensalis* was cut using micro-scissors or a scalpel at the posterior border of the fifth setiger, or the posterior border of the 15th setiger (occasionally setigers 14 or 16 were cut). After worms were cut, the posterior ends (of both 5 and 15 setiger cut individuals) and anterior ends (of 15 seti-

ger cut individuals) were put into capillary tubes (as described in Williams & McDermott 1997). Anterior ends of worms cut at the fifth setiger were too small to stay within capillary tubes and therefore were maintained free on the bottom of Petri dishes. All worms were maintained individually in plastic Petri dishes with ~7 mL of artificial seawater (salinity 32‰) in a refrigerator at 14°C in the dark. Digital pictures of worms were taken before cutting, and on a weekly basis for 5 weeks after ablation. The worms were fed ground shrimp pellets *ad libitum*, and the artificial seawater was changed weekly. Any growth abnormalities were recorded.

A subset of worms ($n = 12$) was used to examine regeneration with SEM. These worms were isolated from their gastropod shells and were kept in glass capillary tubes for ~4–5 months before ablation at the 15th setiger. Pairs of worms were fixed at 1, 6, 14, 21, 28, and 35 d after ablation.

SEM

Specimens were fixed in 4% seawater/formalin solution (1 part 40% formalin: nine parts seawater) for ≥ 24 h before immersion in warm tap water to remove salts. Specimens were then transferred to 70% ethyl alcohol. Specimens were placed into a Pelco Sample Holder and dehydrated in an ascending ethyl alcohol series (75%, 80%, 85%, 90%, and 95% ethanol) for 10 min each, followed by 100% ethanol three times for 15 min each. Following critical point drying in CO₂ (Samdri-795 critical point dryer, Tousimis, Rockville, MD, USA), worms were mounted with adhesive tape onto numbered aluminum stubs. The specimens were sputter coated with gold (EMS-550 sputter coater, Electron Microscopy Sciences, Hatfield, PA, USA) and examined using an S-2460 Hitachi SEM (Hitachi, Pleasanton, CA, USA).

Statistical analysis

SPSS (SPSS Inc., 2008; IBM Corporation, Somers, NY, USA) was used for statistical analyses. The Shapiro–Wilk test was used to determine if palp and total body lengths examined in both the capillary tube and gastropod shell isolation experiments were normally distributed. A t-test was used to compare initial palp length to final palp length and initial total body length to final total body length in both experiments. For the experiment with *D. commensalis* in gastropod shells with or without hosts, total body lengths were log transformed because these measures were not normally distributed. All means of length measures are reported with standard deviation.

Results

The average palp length of specimens of *Dipolydora commensalis* isolated in 2006 was 1.0 ± 0.4 mm ($n = 72$). Of the 120 shells inhabited by *Pagurus longicarpus* collected in 2007, 79% ($n = 95$) contained *D. commensalis*. The worms were found in the following species of gastropod shells (plus one unidentified shell): *Ilyanassa trivittata* (SAY 1822) ($n = 51$, 54%), *Nassarius obsoletus* SAY 1822 ($n = 35$, 37%), and *Urosalpinx cinerea* (SAY 1822) ($n = 8$, 8%). When present, typically one to two large female and one to two male worms were found per shell. Of the shells inhabited by individuals of *D. commensalis*, 87% ($n = 83$) were also covered by *Hydractinia symbiolongicarpus*. Of the 120 hermit crabs examined, 76% were male ($n = 91$, shield length 3.7 ± 2.2 mm) and 24% were female ($n = 29$, shield length 2.9 ± 0.4 mm). No gravid female hermit crabs were found.

Palp length with and without hermit crabs

For worms isolated in glass capillary tubes, mean palp length on the first day of isolation was 1.0 ± 0.4 mm ($n = 17$; Fig. 1A), and mean total length was 8.7 ± 5.1 mm ($n = 17$). The average palp length after 3 weeks of isolation in capillary tubes was 1.4 ± 0.4 mm ($n = 17$; Fig. 1B), and average total length of the worms was 8.4 ± 4.4 mm ($n = 17$). The increase (~40%) in palp length after isolation from hermit crabs was significant (paired t-test 3.723, $p = 0.002$, $df = 16$), but initial and final body lengths were not significantly different (paired t-test 0.411, $p = 0.69$, $df = 16$). When small worms (<50 setigers) were removed from analyses, the difference in palp length remained significant (paired t-test 3.614, $p = 0.004$, $df = 12$) and body lengths were still not significantly different (paired t-test 0.636, $p = 0.54$, $df = 12$).

After a 2-week period, the palps of *D. commensalis* reared in shells without hermit crabs were, on average, 33% longer (average palp length 1.2 ± 0.5 mm, $n = 40$; Fig. 2) than those in shells occupied by hermit crabs (average palp length 0.9 ± 0.4 mm, $n = 31$; Fig. 2). The difference in palp length between worms reared with and without hermit crabs was significant (unpaired t-test 3.054, $p = 0.003$, $df = 69$). The average body length was not significantly different between the two groups of worms (unpaired t-test 1.367, $p = 0.176$, $df = 69$). When small worms (<50 setigers) were removed from the analysis, the increase in palp length remained significant (unpaired t-test 3.882, $p = 0.0003$, $df = 53$) and body lengths were not significantly different (unpaired t-test 0.586, $p = 0.561$, $df = 53$).

Differences in palp morphology between worms reared in shells with and without hermit crabs were

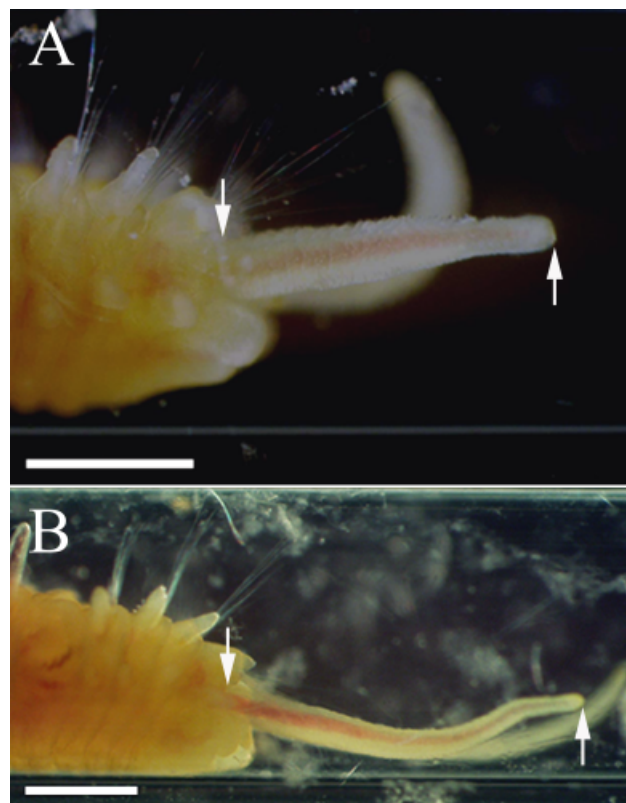


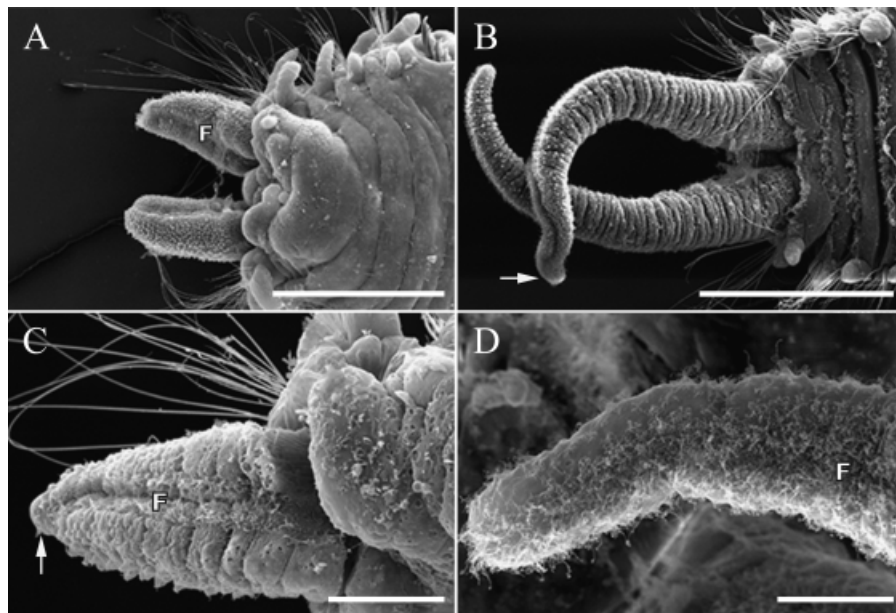
Fig. 1. Light micrographs of adults of *Dipolydora commensalis* isolated in glass capillary tubes. **A.** A representative worm immediately after removal from gastropod shell, in lateral view (palp length = 1.0 mm). Scale bar = 500 μ m. **B.** The same worm 3 weeks after isolation, in lateral view (palp length = 1.7 mm). Scale bar = 500 μ m. Downward facing arrows show insertion of palps, and upward facing arrows show palp tips.

observed with SEM (Fig. 2). Some worms in shells with hermit crabs had palps with reduced tips and lacking cirri, suggestive of a regenerating distal end (Fig. 2C, arrow). The palps of worms from shells with hermit crabs were on average 0.13 ± 0.02 mm wide, and the food groove was 0.02 ± 0.004 mm in width ($n = 5$). However, the tips of the palps of worms extracted from shells without hermit crabs showed cirri on the tips and throughout the entire length of the palp (Fig. 2D). The palps of worms isolated from shells with hermit crabs were on average 0.11 ± 0.005 mm wide, and the food groove was 0.01 ± 0.001 mm in width ($n = 5$).

Body regeneration

Field-collected specimens. One specimen of *D. commensalis* from field collections (1/120, 0.8%) was found exhibiting anterior regeneration (Fig. 3A).

Fig. 2. Scanning electron micrographs of adults of *Dipolydora commensalis* isolated from gastropod shells after 2 weeks with or without hermit crab hosts. **A.** The short palps of a worm with hermit crab influence. Ventral view showing food groove (F). Scale bar = 500 μ m. **B.** The long palps of a worm without hermit crab influence. Dorsal view. The arrow shows the tip of a palp that is twisted and has the ventral food groove exposed toward end. Scale bar = 400 μ m. **C.** The palp of a worm with hermit crab influence, in ventral view, showing the food groove (F) and presumably regenerating tip (arrowhead). Scale bar = 100 μ m. **D.** The palp of a worm without hermit crab influence. Ventrolateral view showing food groove (F). Scale bar = 50 μ m.



This worm had 56 setigers total (including nine regenerated anterior setigers and palps) and was extracted from a shell of *N. obsoletus* (aperture length 9.6 mm, shell length 20.7 mm). The burrow of the worm was located in the typical position on the columella of the shell. When the worm was extracted from its shell (after 2 weeks of isolation with no hermit crab present), the regenerated portion of the worm was a lighter color and the regenerating setigers were $\sim 50\%$ smaller in diameter compared with the rest of the body.

Anterior regeneration after fifth setiger ablation. Of the worms cut at the fifth setiger ($n = 15$), 100% survived and regenerated an anterior end. Figure 3(B–I) shows anterior and posterior regeneration in *D. commensalis*. Anterior regeneration was visible as early as day six, with the formation of a small blastema (Fig. 3B). Three worms started to grow small palps at day six. By day six, some worms ($n = 4$) also began showing distinct setigers with minute setae. Between days 12 and 15, the anterior regenerating portion had more distinct segmentation, and worms also showed more palp development (Fig. 3C). By 19 and 21 d after ablation, the anterior end was fully formed with a functional mouth, but the segments were smaller and paler than posterior segments (Fig. 3D, E). All worms ($n = 15$) regenerated only five setigers. The average palp length between 35 and 36 d after ablation was 1.5 ± 0.5 mm ($n = 15$) (Fig. 3E). The worms were first observed to feed at 19 d after ablation, but ingestion of fine food particles before complete regeneration of mouth may have occurred.

Anterior regeneration after 15th setiger ablation. Of the worms cut at the 15th setiger ($n = 15$), two died. Of those that survived, 70% ($n = 9$) regenerated a normal anterior end; an additional 30% ($n = 4$) survived but exhibited abnormal growth. The worms cut at the 15th setiger (Fig. 4A) began to exhibit anterior regeneration at day seven (Fig. 4B). No worms had observable palp growth until day 14, when small palp buds were observed and average length of the anterior regenerating portion was 0.09 ± 0.02 mm ($n = 9$) (Fig. 4C). By day 21, the anterior regenerating portion had more distinct segmentation and worms showed additional palp development (Fig. 4D). By day 28, worms had regenerated an average of 8 ± 1.5 setigers ($n = 9$) (Fig. 4E). At day 35, worms had regenerated an average of 9 ± 1.3 setigers ($n = 9$) (Fig. 4F). The worms were first observed to feed 21 d after ablation, but ingestion of fine food particles before formation of mouth may have occurred.

Posterior regeneration. When specimens of *D. commensalis* were cut at setiger 5, no anterior ends survived ablation ($n = 5$). The dead anterior ends were almost always found outside glass capillary tubes a few days after being placed in the tubes.

When specimens of *D. commensalis* were cut at setiger 15, the majority of anterior ends survived (67%) and regenerated posterior ends. Five weeks after the ablation, the average number of posterior setigers regenerated was 11 ± 6 ($n = 10$) plus pygidium (Figs. 3F–I, 5).

Abnormal regeneration. None of the worms undergoing posterior regeneration exhibited any obvious

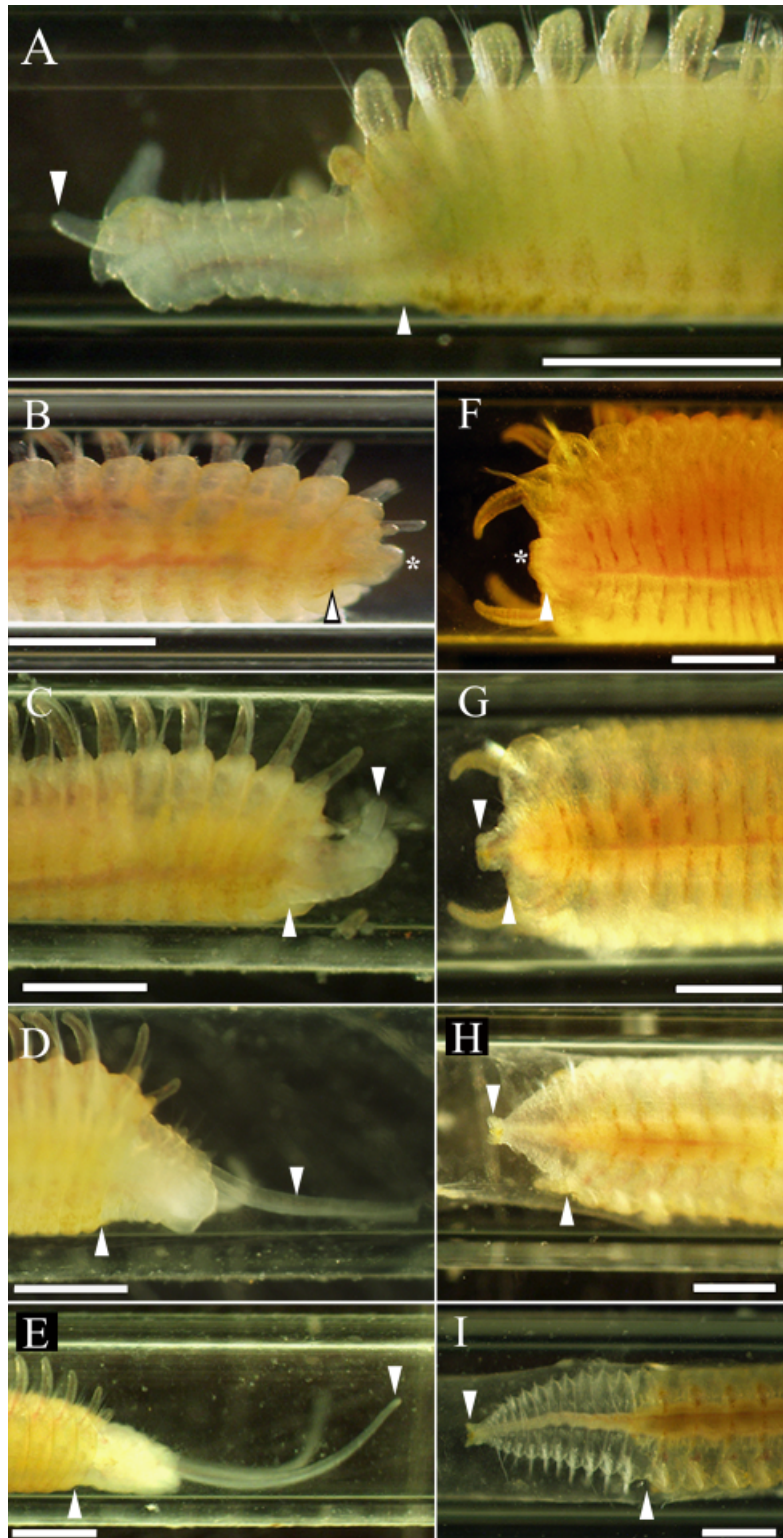
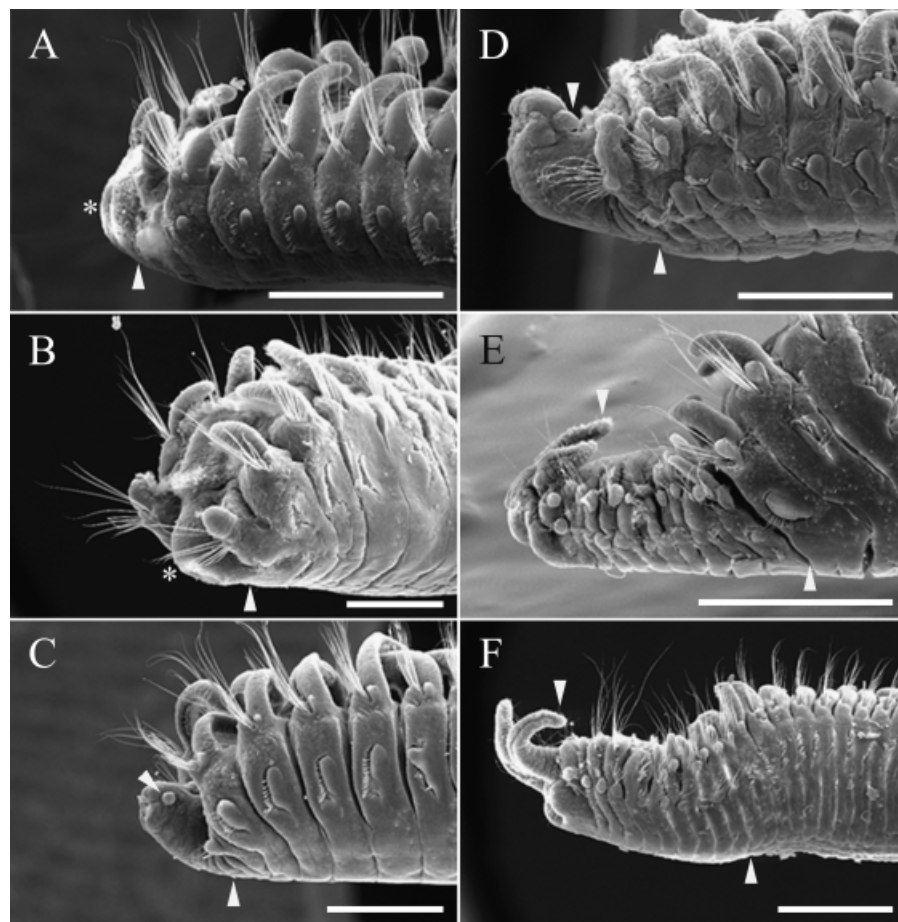


Fig. 3. Light micrographs showing regeneration in a field collected specimen of *Dipolydora commensalis*, a laboratory-reared worm after ablation at the fifth setiger, and a laboratory-reared worm with posterior regeneration. **A.** A field-collected specimen with nine anterior regenerated setigers and palps (palp length = 0.2 mm), in lateral view. Scale bar = 1 mm. **B–E.** Anterior regeneration in a laboratory-reared worm after ablation at the fifth setiger. Scale bars = 500 μ m. **B.** Six days after ablation, ventral view; asterisk shows initial blastema formation. **C.** Twelve days after ablation, in lateral view. **D.** Nineteen days after ablation, in lateral view. **E.** Twenty-seven days after ablation, in lateral view. Five setigers have regenerated. **F–I.** A laboratory-reared worm in ventral view, showing posterior regeneration. Scale bars = 500 μ m. **F.** Seven days after ablation; asterisk shows initial blastema formation. **G.** Fourteen days after ablation. **H.** Twenty-one days after ablation. **I.** Twenty-eight days after ablation. Eleven setigers have regenerated. Upward-facing arrowheads show point of ablation, downward-facing arrowheads show regenerating palp (C–E) or pygidium (G–I).

abnormalities. However, four individuals of *D. commensalis* (27%) exhibited abnormal growth during anterior regeneration in the laboratory after 15 setigers were removed (Figs. 6, 7). Two of the four

worms regenerated supernumerary palps. One of these worms appeared to have regenerated two heads, each with a mouth and two palps evident by day 28 (Fig. 6A). The other worm initially had four

Fig. 4. Scanning electron micrographs of adults of *Dipolydora commensalis* showing anterior regeneration after ablation at the 15th setiger. All images are lateral views. **A.** Day of ablation; asterisk shows area of wound healing. Scale bar = 500 μ m. **B.** Six days after ablation; asterisk shows initial blastema formation. Scale bar = 250 μ m. **C.** Fourteen days after ablation; downward-facing arrowhead indicates blastema with initial palp buds. Scale bar = 500 μ m. **D.** Twenty-one days after ablation. Scale bar = 500 μ m. **E.** Twenty-eight days after ablation. Scale bar = 500 μ m. **F.** Thirty-five days after ablation. Ten setigers have regenerated. Scale bar = 500 μ m. Upward-facing arrowheads show point of ablation, downward-facing arrowheads show regenerating palp.



palps and only one functional mouth (Fig. 6B). However, by 180 d after ablation, the two medial palps had fused along their length, with the food grooves coalescing at the middle of the palps (Fig. 7C, D). Another abnormal worm developed modified spines on the sixth setiger (Figs. 6C, 7A); the right side of the sixth setiger contained modified spines and a branchium (Fig. 7B). Modified spines are normally present on the fifth setiger and branchiae begin on the sixth setiger. The final abnormality was an anterior end and a posterior end both growing from the ablation site (Fig. 6D). The anterior end contained three distinct anterior setigers and two palps; the posterior end contained approximately four distinct setigers and pygidium.

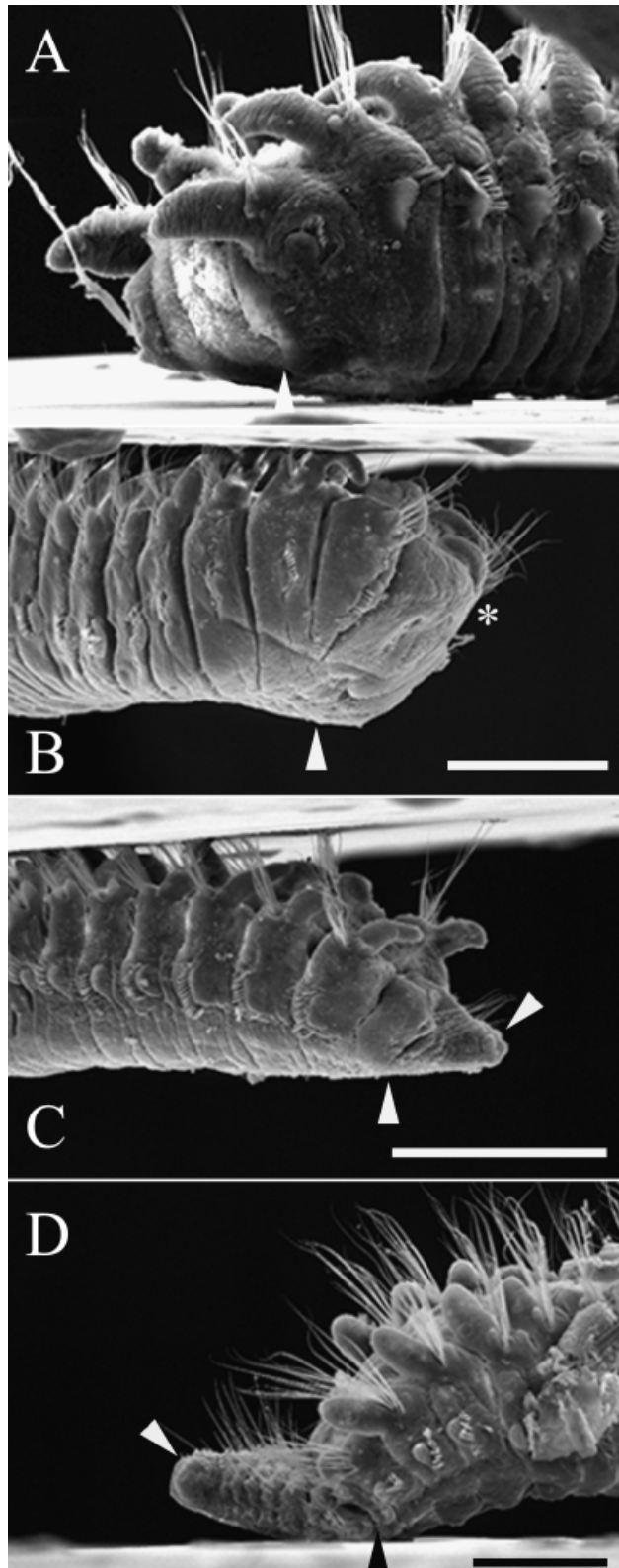
Longevity of isolated *D. commensalis*

Of the 14 specimens of *D. commensalis* isolated in glass capillary tubes and maintained in the laboratory, three died or were sacrificed due to poor condition after 1–3 years in isolation. The rest of the specimens (79%) remained alive past 3 years, with

the five oldest specimens 4.75 years in age at the time of this writing.

Discussion

The palp length of *Dipolydora commensalis* is influenced by its hermit crab host. In general, individuals of *D. commensalis* removed from exposure to hermit crab hosts had palps that were 33–40% longer than those with hosts (Figs. 1, 2). As the hermit crab feeds, worms extend their palps to retrieve food drawn in or dropped by the hermit crab. While the palps and anterior end of *D. commensalis* are extended, the worms are susceptible to being cut by the hermit crab host during its movement across the columella. Following loss of body tissue, *D. commensalis* has the ability to regenerate palps, anterior setigers and posterior setigers. Although the benefits to the worms from their association with hermit crabs have been studied and their negative effects on hosts are known (Williams & McDermott 2004), this is the first study to show potential negative effects on the worms.



Although the mechanism for tissue loss in members of *D. commensalis* may be different, it is comparable to the sublethal predation found in free living

Fig. 5. Scanning electron micrographs of adults of *Dipolydora commensalis* showing posterior regeneration. All images are lateral views. **A.** Day of ablation. Scale bar = 250 μ m. **B.** Six days after ablation; asterisk shows initial blastema formation. Scale bar = 500 μ m. **C.** Fourteen days after ablation. Scale bar = 500 μ m. **D.** Twenty-eight days after ablation. Scale bar = 250 μ m. Upward-facing arrowheads show point of ablation, and downward-facing arrowheads show regenerating pygidium.

spionids (see Zajac 1985 for review of spionids as prey of epifaunal and infaunal organisms). The impacts of sublethal predation can be considerable. For example, Hentschel & Harper (2006) studied *Polydora cornuta* Bosc 1802 and found a significant difference in relative growth rates between worms that had lost both palps versus worms that had lost one or no palps. Individuals of *P. cornuta* with damaged palps often altered their feeding behaviors (deposit feeding using the mouth), which increased the rate of palp regeneration (Hentschel & Harper 2006). Lindsay & Woodin (1995) also documented changes in feeding behavior following palp loss in several spionid species. In addition, Zajac (1985) found that adults of *P. cornuta* experienced a decrease in fecundity, gametogenic segments, and number of eggs per capsule after palps or posterior segments were removed. However, regeneration of long palps may reduce feeding efficiency if worms are in areas of high flow and contact rate of food particles is constrained by the coiling of these appendages (Shimeta 2009). Palp coiling is not observed in *D. commensalis* (J.D. Williams, pers. obs.) and it is possible that reduced palp lengths could enhance the feeding of *D. commensalis*, but that remains to be tested. In the present study, there was no significant difference in palp and food groove widths between worms that were exposed to hermit crabs and those that were isolated.

Spionids also rely on chemoreception after food particle contact, using their palps to assess the nutritional quality of food particles (Lindsay et al. 2008; Lindsay 2009). Such chemoreception appears to be important in the feeding and behavior of adults of *D. commensalis*. Dauer (1991) documented the ciliation patterns of palps of *D. commensalis* and hypothesized that the papillae of the palp were primarily sensory organs because: (1) the cirri of the papillae were non-motile and (2) the abfrontal and lateral surfaces of the palp, where the papillae were located, continually made contact with the hermit crab appendages. Individuals of *D. commensalis* presumably make contact with hermit crab appendages to locate

food particles adhering to the setae of the hermit crab, and to sense potential danger when the hermit crab retreats back into its shell (Dauer 1991).

Whereas the palps of many free-living polydorids are deciduous (easily removed or drop off when agitated/pulled by predators), those of *D. commensalis* are difficult to remove. In species with deciduous palps, such as *Rhynchospio glutaeus* (EHLERS 1897), *Pseudopolydora kempji japonica* IMAJIMA & HARTMAN 1964, *Pygospio elegans*, and *P. cornuta*, field populations often exhibit 7–20% palp loss; members of these species also have palps two to eight times the length of that found in *D. commensalis* (Woodin 1982; Radashevsky 1989, 1993, 2005; Dauer 1991; Lindsay & Woodin 1992; Zajac 1995; Williams & McDermott 1997; Williams 2001; Worsaae 2001; Lindsay et al. 2007, 2008). The palps of *D. commensalis* are strongly attached and can be clasped with forceps and pulled without removal; <1% of the individuals in the present study exhibited palp loss in the field (similar to populations in other localities along the east coast of the United States; J.D. Williams, pers. obs.). In the present study, hermit crabs were never observed to directly attack the worms with their chelipeds, further suggesting that the palps could be damaged when the hermit crab accidentally cut worms while they are feeding.

Results from the present study also demonstrate that members of *D. commensalis* are capable of anterior and posterior regeneration, and that damage to worms can occur in the field (Fig. 3). The presence of the hermit crab host limits the amount of time that members of *D. commensalis* are able to extend their anterior ends from their natural burrows in the gastropod shell (Williams & McDermott 1997). However, when individuals of *D. commensalis* sense food, they not only extend their palps, but can also extend anterior segments from the burrow opening. When worms feed in this way, they could increase the chance that anterior portions of the body (e.g., palps and anterior setigers) will be damaged by the hermit crab.

The anterior regeneration capabilities of two free-living spionids (*Dipolydora quadrilobata* and *P. elegans*) have been studied in detail. When more segments were removed from these worms, they were slower at regenerating the segments (Lindsay et al. 2007). Regardless of the number of anterior segments removed from members of both species, wound healing was observed, the formation of a blastema with a recognizable prostomium and peristomium occurred by 6 d after ablation, and worms eventually regenerated palps and anterior segments (Lindsay et al. 2007). Members of *D. quadrilobata* regenerated nine

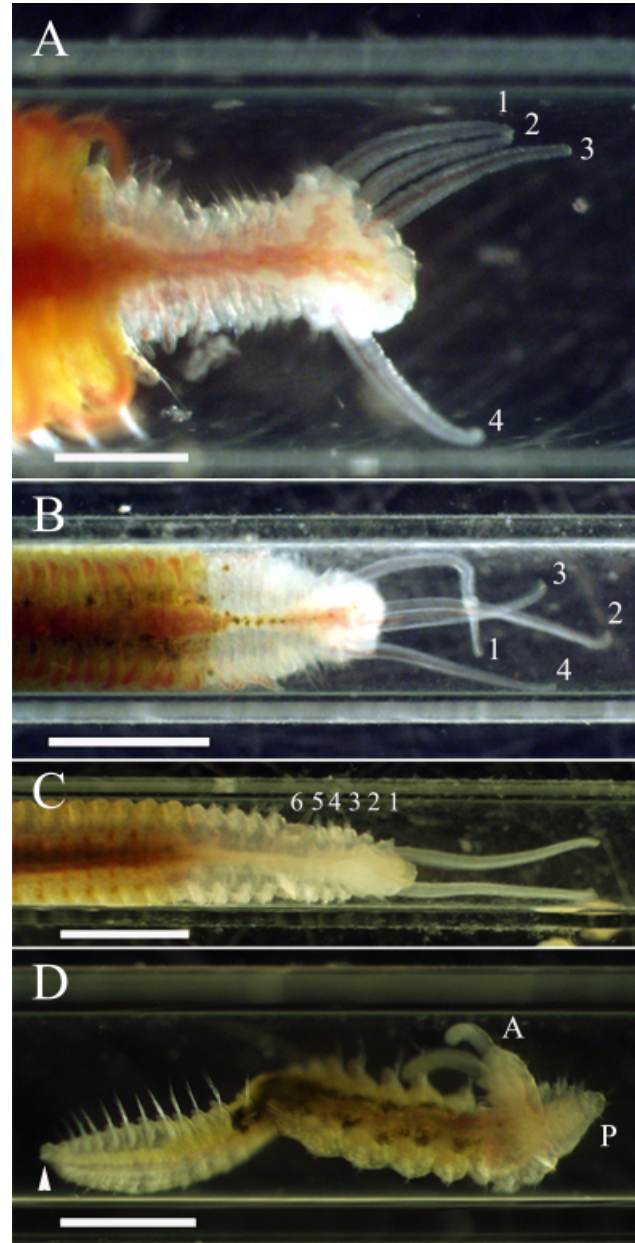


Fig. 6. Light micrographs of abnormal regeneration in adults of *Dipolydora commensalis*. **A.** A worm with two heads and four palps, in dorsal view (tips of palps labeled 1–4 from left to right). Scale bar = 500 μ m. **B.** A worm with four palps and one functional mouth, in dorsal view (tips of palps labeled 1–4 from left to right). Scale bar = 1 mm. **C.** A worm with modified spines on setiger 6, in ventral view (setigers 1–6 of left side labeled). Scale bar = 500 μ m. **D.** A worm with both an anterior end (A) and an extra posterior end (P) formed at the ablation site, in dorsal view; the original pygidium is marked with an arrowhead. Scale bar = 1 mm.

to ten segments whereas members of *P. elegans* regenerated ten to 13 segments after an average of 29 setigers and an average of 32 setigers, respectively,

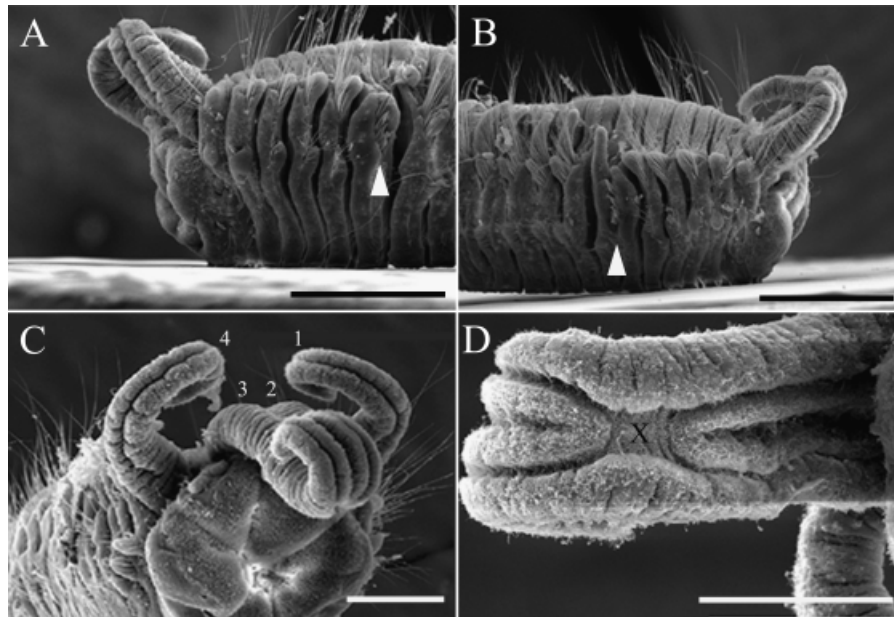


Fig. 7. Scanning electron micrographs of adults of *Dipolydora commensalis* showing abnormal regeneration. **A.** A worm with modified spines on the left side of the sixth setiger, in lateral view (modified spines shown with arrowhead). Scale bar = 500 μ m. **B.** The same worm as in (A), showing modified spines on the right side of the setiger 6, and a branchium on same setiger (modified spines shown with arrowhead). Scale bar = 500 μ m. **C.** An oblique view of the anterior end of a worm with four palps (palps labeled 1–4 from left to right). Scale bar = 250 μ m. **D.** Closeup of two fused medial palps of the same worm as in (C), in ventral view. The X indicates where the

food grooves of the fused palps have merged. See Fig. 6B for a light micrograph of this same worm, before fusion of the palps. Scale bar = 200 μ m.

were ablated (Lindsay et al. 2007). Similarly, Stock (1965) showed that individuals of the spionids *Dipolydora caulleryi* (MESNIL 1897), *Dipolydora socialis* (SCHMARD 1861), and *Polydora ciliata* (JOHNSTON 1838) regenerated 10, 8, and 7 anterior setigers, respectively, following ablation. As in most of these cases, members of *D. commensalis* regenerated no more than ten anterior setigers. When cuts were made more anteriorly (at setiger 5), the worm regenerated only five setigers. Members of *D. commensalis* appear to share a genetic constraint in regeneration with other spionids; in all the worms of this family that have been studied to date, no more than 13 anterior setigers regenerate, regardless of how many are removed. Genetic studies are needed to determine how regeneration is regulated and controlled in *D. commensalis*. Such studies have been completed on some annelids, but little is known of worms within the Spionidae (Carroll 1995; Bely & Wray 2001; Irvine & Martindale 2001; Bely 2006; Bely & Sikes 2010; Zattara & Bely 2011).

Abnormal development during regeneration in spionids, as found here in some individuals of *D. commensalis*, has rarely been reported. However, Stock (1965) observed abnormalities in several spionid worms during anterior regeneration. Abnormalities included segmental and branchial abnormalities, lateral heads, an indeterminate regenerate, an axially twisted regenerate, bent regenerates, and the growth

of four palps. Specimens of *D. commensalis* exhibited abnormal regeneration similar to that of *D. caulleryi*, with a lateral head and a posterior end growing from the same segment (Stock 1965). Furthermore, *D. commensalis* regenerated four palps, as was also observed in *Spio setosa* VERRILL 1873 (Stock 1965). These examples of abnormal regeneration, and those found in *D. commensalis*, appear to be the result of the worms being cut diagonally across two or more segments, leading to the formation of multiple regenerating blastemas (Stock 1965).

Hermit crabs must inhabit shells that contain associates like *D. commensalis* because empty gastropod shells are often limited in the field (Williams & McDermott 2004). Hermit crabs need to move to larger shells as they grow and they typically exchange shells with one another in a shell vacancy chain (Williams & McDermott 2004). As a result, individuals of *D. commensalis* are rarely found in shells without a host. However, when the gastropod shell is temporarily uninhabited by a hermit crab host, nutrient granules in the stomach wall of *D. commensalis* may help to ensure the survival of the worms (Radashevsky 1993). Larger individuals of *D. commensalis* are predicted to be able to survive longer during times when a hermit crab does not occupy their shells, due to a larger store of nutrient granules. It is likely that worms can deposit feed when in uninhabited shells (at least until they become totally buried), or during times when host crabs

burrow into the sediment; however, no studies have tested this ability. Energy reserves are assumed to be important for worms that may have had portions of their anterior body cut and must rely on these stores for regeneration. However, studies showing impacts of energy reserves (e.g., glycogen stores) on regeneration in polychaetes are limited (Buongiorno-Nardelli & Thouveny 1966; Page & Lindsay 2010).

The maximal life span of spionids is known only for a few species (see Zettler 1997), and this is the first study to document that a polydorid can survive for nearly 5 years, at least under laboratory conditions. Other polydorids live 1–2.5 years or less (Anger et al. 1986; Sato-Okoshi et al. 1990). Outside of the family Spionidae, some polychaetes have been aged based on growth rings of their jaws or chaetal patterns and size (Estcourt 1975; Olive 1977; Duchêne & Bhaud 1988). These studies have shown that members of the Nephtyidae and Terebellidae may live 5 years or more. In the field it is unlikely that the *D. commensalis* would survive for this long because the shells they inhabit are often eroded to the point that they are no longer inhabitable by hermit crabs in < 1 year (Walker & Carlton 1995). This study suggests that the worms can survive longer than the functional life of inhabited shells, and likely die when the shells are worn to the point that hermit crabs abandon them and they become permanently buried, or during a predation event on the host hermit crabs.

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