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Anterior regeneration in the polychaete *Marenzelleria viridis* (Annelida: Spionidae)

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Abstract. The objective of this study was to examine the regeneration capacity of the spionid polychaete *Marenzelleria viridis* from Long Island, New York. In the field, ~7% of the worms exhibited regeneration of the anterior end. In the laboratory, worms were ablated at the 10th–50th chaetiger and their regeneration documented. Anterior morphogenesis was similar to that previously reported for spionids, with wound healing, blastema formation, differentiation of segments, and formation of feeding and sensory structures (mouth, palps, nuchal organs) occurring within 14 d. Unlike in some spionids, the segments do not appear to all form simultaneously from the blastema; rather, external differentiation of segments was observed from posterior to anterior on the regenerate. The number of segments replaced was equal to the number ablated for up to 10 segments. A maximum of 17 segments were replaced when 20–30 chaetigers were ablated, and the number replaced decreased to 14 when 40–50 chaetigers were ablated. Survival and normal growth of the worms decreased with more chaetigers ablated; a significantly higher number of worms died or grew abnormally with ≥30 chaetigers ablated, compared to worms with ≤20 chaetigers ablated. Members of *M. viridis* could be valuable model organisms in the study of the cellular mechanisms involved in regeneration, and further research on regeneration in the field should be completed.

Additional key words: marine, northwest Atlantic, Polychaeta, regenerate, sublethal predation

The ability to regenerate lost or damaged body parts following injury is found in a range of marine invertebrates and is an important aspect of their ecology and evolution (Bely & Nyberg 2010; Lindsay 2010). Among annelids, regeneration is widespread, and this capacity is believed to be ancestral (Bely 2006, 2010; Fleming et al. 2007; Bely & Nyberg 2010). However, there are entire clades of annelids (e.g., Hirudinea) in which regenerative capability is poor or absent, as well as sporadic instances of loss of regenerative ability in one or a few species within an otherwise regeneration-competent group. Inability to regenerate is due to evolutionary loss likely because in these taxa injury is infrequent and/or the damaged structures are not critical to survival or reproduction (Bely 2006, 2010; Bely & Nyberg 2010). Presumably, loss of regenerative ability affecting one or a few species reflects a relatively recent event, whereas lack of regeneration ability in a large clade such as the leeches represents an older event (Bely 2006, 2010; Bely & Nyberg 2010). Although predation is suggested as the major evolutionary pressure for retention of the ability to regenerate, parasites may also play a role and have been shown to influence rates of fission in some polychaetes (McCurdy 2001).

The abilities to regenerate anterior and posterior body parts are distinct and separable, as shown in numerous examples of annelids that can regenerate posterior but not anterior segments (Bely 2006). Posterior regeneration resembles normal growth in that it involves the establishment of a growth zone just anterior to the new pygidium, and segments are added sequentially from the growth zone. By contrast, anterior regeneration takes place via the process of epimorphosis in which a regenerative bud (blastema) is formed through cell proliferation (Licciano et al. 2012). Typically, ablation is followed by wound healing (including muscle and tissue contraction) and the formation of a blastema (for review of cellular processes during these events, see Bely 2014). The blastema elongates and internal differentiation (neuronal and muscular regeneration) occurs,

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followed by the appearance of segments and segmental structures (Gibson & Harvey 2000; Gibson & Paterson 2003; Lindsay et al. 2007, 2008; Dualan & Williams 2011; David & Williams 2012a,b; Weidhase et al. 2014).

Spionid polychaetes are becoming established as a useful group for the study of regeneration. These worms are key members of marine benthic communities worldwide, where they can reach high abundances in soft bottom and hard substrates (Blake 1996; Radashevsky 2012). Spionids are notable for their effects upon substrates due to their burrowing and feeding behaviors (Luckenbach et al. 1988) and often serve as food for bottom-foraging fish (DeVlas 1979). As such, they are susceptible to sublethal predation, and studies have reported regeneration after cropping of palps, entire heads, and posterior ends by fish and other predators (DeVlas 1979). In addition, spionids may be susceptible to predation when engaging in nocturnal swimming during reproduction (e.g., Dauer et al. 1980, 1982).

Although the capability to regenerate has been documented in many members of the Spionidae (Table 1), experimental studies on regeneration have been conducted on only a few of the 500+ spionid species, mainly members of *Dipolydora* and related genera (i.e., polydorins; see Radashevsky 2012). In addition to regeneration following sublethal predation or physical damage, some spionids have been documented to regenerate anterior ends during asexual reproduction via architomy (transverse fission of parental individual followed by regeneration of the resulting fragments; e.g., Gibson & Harvey 2000) or paratomy (budding of new individuals or stolons from a parental or stock individual; e.g., Williams 2004; see also review of reproduction of spionids in Blake 2006). Although regenerative ability may have been a pre-adaptation for the evolution of asexual reproduction, there is substantial evidence that regeneration and asexual reproduction can be uncoupled, because some species that reproduce asexually do not regenerate (Blake & Arnofsky 1999; Bely 2006). Most spionids that have been evaluated for regeneration ability are able to regenerate anterior segments (and all studied to date can regenerate posterior segments); however, at least one species (*Streblospio benedicti* Webster 1879) is incapable of regenerating even a single anterior segment (S. Lindsay & J. Jackson, unpubl. data, as reported in Bely 2006). Thus, spionids are useful for examining the interrelationships among regeneration, ecology, and reproductive mode.

The objective of this study was to document the process of anterior morphogenesis in *Marenzelleria viridis* (Verrill 1873), a large deposit-feeding spionid native to the east coast of North America. *Marenzelleria viridis* has been reported to regenerate under field and laboratory conditions (Stock 1965; Essink & Kleef 1993; David & Williams 2016), but little data exist on the occurrence of regeneration in the field, and the process of regeneration has not been examined in detail. In addition, we show that increasing the number of ablated anterior chaetigers impacts the number of segments replaced and the survival of worms. This work has important implications because species in the genus *Marenzelleria* are highly invasive, and *M. viridis* has been introduced into the North and Baltic Seas, where they have attained high population densities and drastically altered benthic communities (e.g., Zettler et al. 1995, Zettler 1997a,b; Kotta et al. 2001; Bastrop & Blank 2006; Thomsen et al. 2009; Delefosse et al. 2012). Recently, David & Williams (2016) examined the impacts of salinity on regeneration and survival of *M. viridis*. However, the present study is the first to provide a detailed description of morphogenesis with scanning electron microscopy following ablation in the laboratory. The ability to persist under harsh conditions and regenerative capabilities are likely important factors in the success of *M. viridis,* and therefore studies on these aspects of their biology could be critical in understanding and making predictions about their impacts following introduction to non-native areas. Finally, we provide a review of regeneration in spionid species, with discussion of the maximum number of anterior segments replaced following ablation and potential factors that control this aspect of regeneration.

Methods

Collection and maintenance

Worms were collected from Hempstead Harbor in Sea Cliff, New York (40°50′27.09″N, 73°39′11.46″ W), from October–December 2010 and March–June 2011 (Table 2). Collections were made on sand flats at low tide using a shovel and sieve, and animals were transported to the laboratory at Hofstra University where they were sorted into 10-cm glass finger bowls containing approximately 100 mL of artificial seawater (salinity 25 ppt). The mean salinity at the collection site was 24.8 ppt. Individual worms were examined to determine whether the posterior and anterior ends were intact, and the number of animals already in the process of regenerating the anterior was determined. Worms were scored as regenerating if there was a clear reduction in size

Table 1. Regeneration in spionids following division resulting from asexual reproduction via architomy (AA), asexual reproduction via paratomy (AP), or physical damage (PD). Plus sign (+) indicates ability to regenerate, minus sign (−) indicates inability to regenerate. Max # of chaetigers produced indicates maximum numbers of chaetigers produced during regeneration after ablation posterior to segment 5 or during asexual division (some estimated from figures in references).

Species	Type	Anterior regeneration	Max# of chaetigers produced	Posterior regeneration	References
Amphipolydora abranchiata (Hartman 1953)	AA	+	Unk	+	(Blake 1983, 2006; Gibson & Paterson 2003)
Amphipolydora vestalis Paterson & Gibson 2003	AA	+	Unk	+	(Gibson & Paterson 2003; Bely 2006; Blake 2006)
Dipolydora armata (Langerhans 1880)	AA	+	10	+	(Bick 2001; Radashevsky & Nogueira 2003; Blake 2006)
Dipolydora caulleryi (Mesnil 1897)	PD	+	10	+	(Stock 1965)
Dipolydora commensalis (Andrews 1891)	PD	+	9	+	(Dualan & Williams 2011)
Dipolydora flava (Claparède 1870)	PD	+	8	+	(Abeloos 1954; Abeloos & Thouveny 1957)
Dipolydora quadrilobata (Jacobi 1883)	PD	+	10	Unk	(Lindsay et al. 2007)
Dipolydora socialis (Schmarda 1861)	PD	+	8	+	(Stock 1965)
Dipolydora tetrabranchia (Hartman 1945)	PA	+	10	+	(Campbell 1955)
Marenzellaria viridis (Verrill 1873)	PD	+	17	+	(Stock 1965; Present study)
Polydora ciliata (Johnston 1838)	PD	+	9	+	(Abeloos 1954)
Polydora colonia Moore 1907	AA	+	8	+	(David & Williams 2012b)
Polydora cornuta Bosc 1802	PD	Unk[a]	Unk	+	(Zajac 1985; Radashevsky 2005; Hentschel & Harper 2006)
Polydora ecuadoriana Blake 1983	PD	+	8	+	(Radashevsky et al. 2006)
Polydora sp.	PD	+	6	Unk	(Iwanoff 1928)
Polydorella dawydoffi Radashevsky 1996	PA	+	10	+	(Radashevsky 1996)
Polydorella kamakamai Williams 2004; [b]	PA	+	10	+	(Williams 2004)
Pygospio californica Hartman 1936	AA	+	Unk	+	(Blake 2006)

(continued)

Table 1 (continued)

Species	Type	Anterior regeneration	Max# of chaetigers produced	Posterior regeneration	References
Pygospio elegans Claparède 1863	AA, PD	+	13	Unk	(Rasmussen 1953, 1973; Hobson & Green 1968; Malikova 1975; Armitage 1979; Anger 1984; Gibson & Harvey 2000; McCurdy 2001; Blake 2006; Lindsay et al. 2007)
Scolelepis hutchingsae Dauer 1985	PD	Unk	N/A	+	(Williams 2007)
Scolelepis squamata (Müller 1806)	PD	+	Unk	+	(Michaelis & Vennemann 2005)
Scolelepis villosivaina Williams 2007	PD	Unk	N/A	+	(Williams 2007)
Scolelepis sp.	PD	+	13	Unk	(Iwanoff 1928)
Spio filicornis (Müller 1776)	PD	+	Unk	+	(Stock 1965)
Spio setosa Verrill 1873	PD	+	Unk	+	(Stock 1965)
Spio sp.	PD	+	12	Unk	(Iwanoff 1928)
Streblospio benedicti Webster 1879	PD	–	N/A	Unk	Lindsay & Jackson (unpublished data) in Bely (2006)

[a]Although regeneration of the anterior end is not known in this species, it readily regenerates feeding appendages (palps), as has been documented in other spionids (e.g., Dualan & Williams 2011; Hentschel & Harper 2006).
[b]Other *Polydorella* (e.g., *P. prolifera* Augener, 1914) appear to regenerate 10–11 segments during paratomic division, but this needs to be confirmed (see Radashevsky 2015; Williams 2004).
Unk, unknown; N/A, not applicable.

and/or pigmentation between new anterior and non-regenerating posterior segments when observed externally. Ninety-three percent of the worms examined had recently broken at the posterior end, most likely by fission during collection. Worms with broken posteriors were included in the study because of the difficulty in obtaining sufficient intact specimens. To allow the posterior end to heal, animals were maintained in ~24 ppt artificial seawater at 15°C for at least 1 week prior to use in experiments. The mean size of specimens in a representative collection from 27 May 2011 was 141.7±32.9 chaetigers (n=15; range=82–207 chaetigers), although members of the species can reach a total of up to 247 chaetigers (Sikorski & Bick 2004).

Table 2. Frequency of anterior regeneration in *Marenzellaria viridis* collected in Hempstead Harbor, New York, during 2010–2011.

Collection date	Number of individuals with anterior regeneration	Number of specimens	Regeneration (%)
20 October 2010	4	78	5.1
12 November 2010	4	51	7.8
6 & 14 April 2011	6	66	9.1
8 May 2011	3	57	5.3
Total	17	252	6.75

Anterior morphogenesis

Worms were anaesthetized in 7% $MgCl_2$ for 30–60 min. Prolonged anaesthetization was required for adequate relaxation, and worms recovered well with no apparent deleterious effects. Once the animals were minimally responsive, the anterior ten chaetigers were ablated with a scalpel (see Fig. 1A–C for morphology of *Marenzelleria viridis* prior to

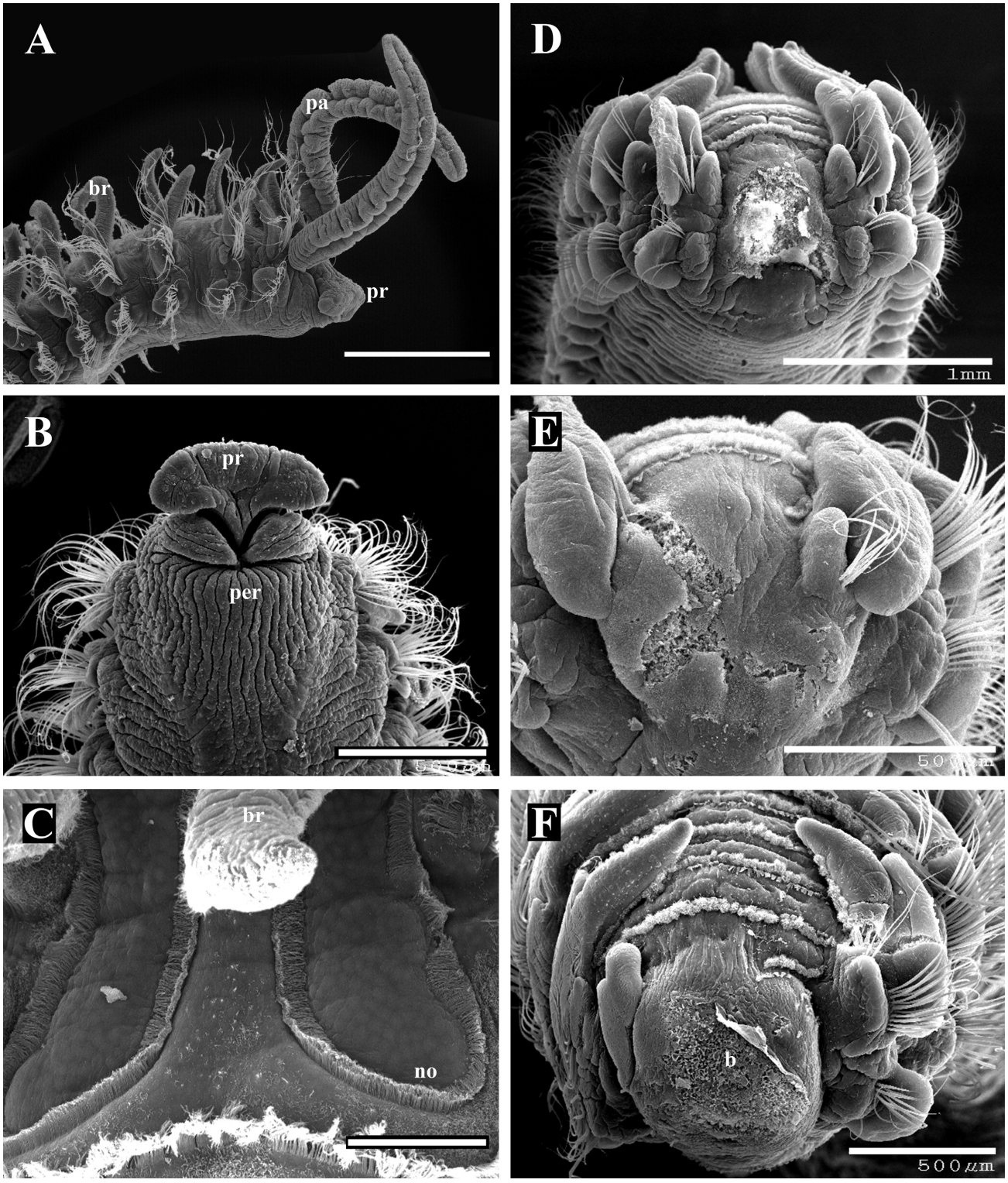

Fig. 1. SEM of *Marenzelleria viridis* showing anterior features of normal (non-regenerating) specimen (A–C) and specimen post-ablation of ten anterior chaetigers (D–F). **A.** Lateral view showing parapodia, branchia, and palps. **B.** Ventral view showing prostomium and peristomial lips. **C.** Dorsal view showing nuchal organ. **D.** Unhealed wound showing transverse constriction. **E.** Healing wound covered by thin epithelium. **F.** Blastema formed 2 d post-ablation. Scale: A=1 mm; B,E,F=500 μm; C=100 μm; D=1 mm. b, blastema; br, branchia; m, mouth; no, nuchal organ; pa, palp; per, peristomium; pro, prostomium.

ablation of anterior structures). The posterior portion was placed in 8 mL of artificial seawater (salinity 24 ppt), and allowed to regenerate at 22–24°C. On each day for 1–15 d post-ablation (dpa), regenerating worms were fixed in 3% glutaraldehyde with 0.1 mol L^{-1} phosphate buffer (pH 7.2), washed in phosphate buffer, dehydrated by stepwise series of ethanol (70% to 100%), and dried in a Samdri-550 critical point dryer. Worms were coated with gold (EMS-550 Sputter Coater) and viewed with a scanning electron microscope (Hitachi 2460-N). Five separate time course experiments were performed, and at least four worms were observed by SEM for each day post-ablation.

Impact of ablation position on number of segments regenerated and survival

Five groups of worms were established in which to vary the total number of chaetigers ablated (10, 20, 30, 40, or 50 anterior chaetigers ablated) and quantify the number of regenerated segments. Worms were anaesthetized in 7% $MgCl_2$ and 10–50 anterior chaetigers were targeted for ablation with a scalpel. The worms were anaesthetized but still mobile, and as a result cuts made as close as possible to the targeted chaetigers produced slightly different numbers of chaetigers that were actually ablated for worms in the same treatment group; see Table 3 for range of chaetigers ablated in each group (average numbers of chaetigers ablated correspond to the groupings above). Anterior portions were placed in 70% EtOH for counts of chaetigers ablated. The posterior portion was placed in ~8 mL of artificial seawater (salinity 24 ppt), and allowed to regenerate at 22–24°C. Survival of the worms and any abnormalities were recorded. After ablation, worms were allowed to regenerate for 14 or 21 d, transferred to 70% EtOH, and the number of regenerated segments counted. Segment counts were done at 14 dpa in initial experiments addressing the numbers of segments replaced (n=2 for 10 chaetigers ablated, n=2 for 20 chaetigers ablated, and n=2 for 30 chaetigers ablated). Because regenerating chaetigers were small and closely spaced, particularly when 30–50 chaetigers were ablated, counts of regenerated segments were also done on day 21 post-ablation (n=8 for 10 chaetigers ablated, n=8 for 20 chaetigers ablated, n=7 for 30 chaetigers ablated, n=6 for 40, and n=9 for 50 chaetigers ablated).

Results

Frequency of regeneration in the field

A total of 252 worms from five collection dates in 2010–2011 were examined for evidence of regeneration occurring in the field (Table 2); in total, 17 of these worms (6.7%) were found at some stage of anterior end regeneration. Among the collections, 5.1–9.1% of worms exhibited regeneration (mean=6.83±1.95%; n=5).

Morphogenesis during anterior regeneration

Shortly following anterior ablation, transverse constriction took place at the cut site, potentially reducing blood loss (Fig. 1D). Over the first 2 d post-ablation (dpa) the wound healed and formed blastema tissue (Fig. 1E,F).

At around 3 dpa, the blastema began to elongate by outgrowth from the ventral portion, and as the regenerate elongated over the next several days, it recurved dorsally (Fig. 2A,D–F). A transient midventral band (mvb) appeared at 3–4 dpa, and formation of intersegmental furrows was initiated at the lateral margins of the mvb (Fig. 2A–C). The oral invagination formed at about 4 dpa (Fig. 2B).

Segment boundaries were established by about 7 dpa, and external segmental features developed from posterior to anterior on the regenerate (Fig. 2D–F). The mean number of segments regenerated

Table 3. Pattern of segment replacement during anterior regeneration in *Marenzellaria viridis*.

Mean no. of segments ablated (Range)	Number cut	Dead	Non-regenerating or abnormal	Regenerating	Mean no. of segments regenerated (Range)
10.2 (8–12)	11	1	1	9	10.3 (8–12)
20.8 (19–22)	12	2	1	9	15.1 (13–17)
30.8 (29–32)	12	3	4	5	15.6 (14–17)
40.4 (38–42)	10	4	5	1	14
51.0 (49–52)	10	1	8	1	14

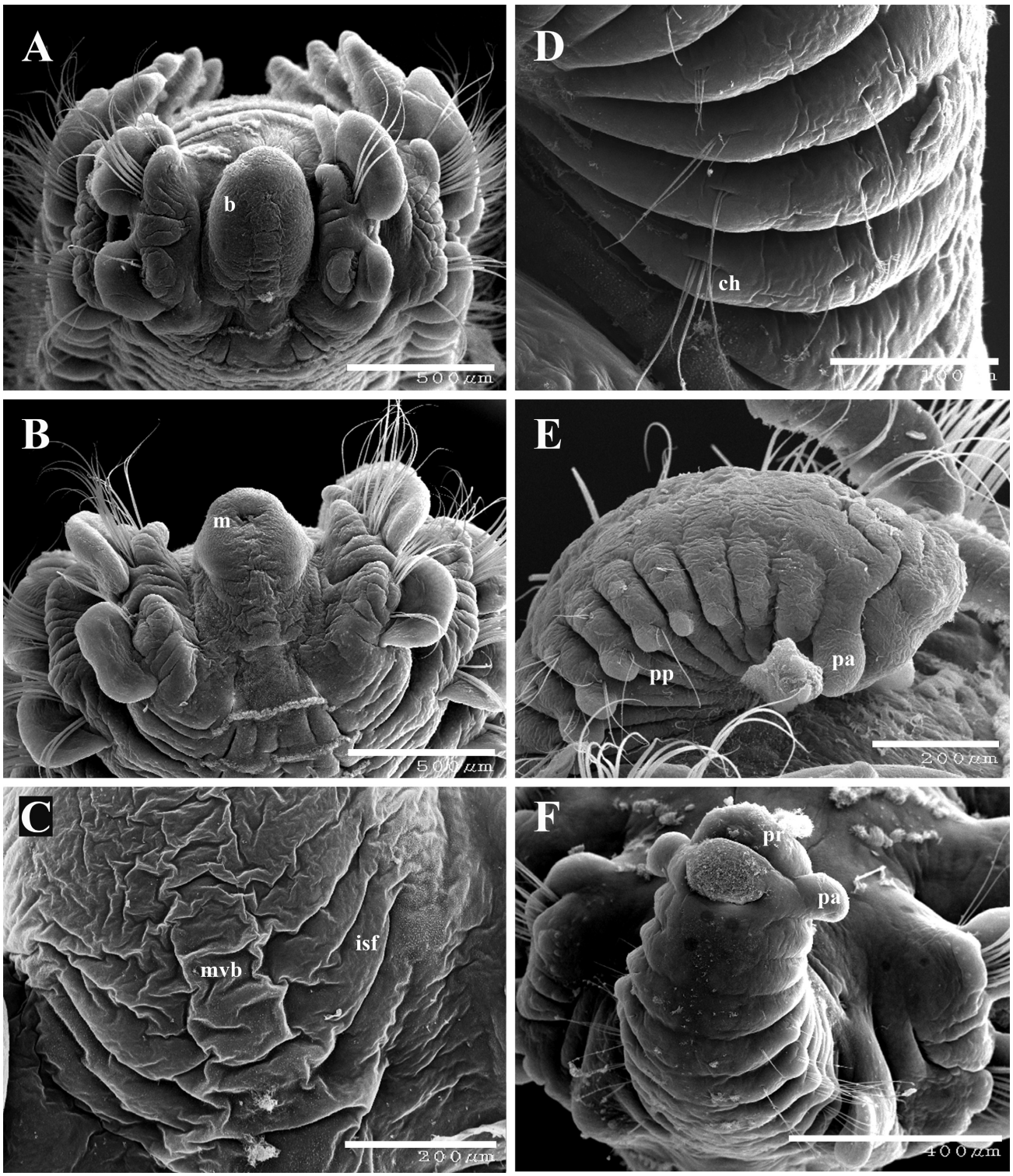

Fig. 2. SEM of *Marenzelleria viridis* showing blastema elongation and formation of segmental structures over 3–6 d post-ablation (dpa). **A.** Blastema elongation, 3 dpa. **B.** Ventral view of elongating blastema at 4 dpa showing the mouth and the region where intersegmental furrows initiate. **C.** Detail of specimen in B showing the intersegmental furrows extending from the midventral band. **D.** Chaetae on an 8-dpa regenerating individual. **E.** Regenerating individual at 7 dpa, with parapodial buds and chaetae. **F.** Regenerating individual at 8 dpa. Note absence of anterior chaetae and parapodial buds. Scale: A,B=500 μm; C,E=200 μm; D=100 μm; E=400 μm. b, blastema; ch, chaetae; isf, intersegmental furrows; mvb, midventral band; m, mouth; pa, palp; pp, parapodium; per, peristomium.

following the ablation of 10 chaetigers was 10.2±1.09 (range 8–12) (see section below and Table 3). Chaetal development preceded the formation of parapodia buds (Fig. 2D,E). Features of the head including the prostomium, lateral and ventral peristomial lips, palp buds, and rudimentary nuchal organs appeared around 7 dpa.

The regenerate elongated over days 7–14, and by about 13–15 dpa, major anterior features were present and similar in appearance to the original, although smaller. Palp buds appeared at about 7 dpa (Fig. 3A,B). By 13–14 dpa, the food groove was present and the frontal surface of the palps had the asymmetric ciliation pattern previously described for *Marenzelleria viridis* (Dauer 1997) (Fig. 3C). At their first appearance around 7 dpa, nuchal organs were ciliated crescent-shaped indentations that elongated into loop-like structures (as found in fully formed adults) by about 13 dpa (Fig. 3D–F).

Impact of ablation position on number of segments regenerated

The number of segments replaced was equal to the number ablated when 8–12 chaetigers were ablated, but increased to a maximum of 17 segments when 20–30 chaetigers were ablated, and decreased to 14 when 40–50 chaetigers were ablated (Table 3). Survival and normal growth of the worm decreased with increasing number of chaetigers ablated; the number of worms that died or abnormally regenerated following ablation was significantly higher in worms with 30 or more chaetigers ablated (25% died, 53.1% abnormal) than in worms with 20 or fewer chaetigers ablated (13.0% died, 8.7% abnormal) (χ^2_1=17.6, p<0.001; Table 3). Non-regenerating animals include those in which the wound failed to close properly, such that the edge of the gut tube was visible, and those that appeared to form a blastema but did not progress further. Animals with abnormal regeneration included those in which a blastema formed and extended, but failed to form anterior structures such as palp buds or to develop intersegmental furrows. Duplicated structures such as palps or heads were never observed (in ~50 individuals that had 10 anterior chaetigers ablated).

Discussion

Regeneration following injury is ecologically important for benthic invertebrates such as polychaetes, and quantifying the frequency of regeneration found in the field is important for examining factors that might play a role in maintaining this trait (Lindsay 2010). About 7% of individuals of *Marenzelleria viridis* collected in Sea Cliff, New York, were in the process of regenerating the anterior end. This is similar to the frequency of anterior regeneration observed in *Dipolydora quadrilobata* (Jacobi 1883) (7.6%) and *Pygospio elegans* Claparède 1863 (6.5%) (Lindsay et al. 2008). Regenerating individuals could be overlooked (Lindsay 2010), so our numbers likely represent a conservative estimate in this population. The frequency of anterior regeneration reported in field-collected specimens of *M. viridis* in the Ems Estuary, Netherlands was 2.8% (Essink & Kleef 1993). *Marenzelleria viridis* is known to be a second intermediate host for three species of trematodes (Phelan et al. 2016). Some of the trematode species found in *M. viridis* use fish (flounder and eels) and birds (gulls and others) as definitive hosts (Phelan et al. 2016), providing evidence that these species are likely responsible for some of the sublethal predation found in the field. Although not documented as a prey item of these birds, individuals of *M. viridis* have been found in the gut of several fish species (Essink & Kleef 1993; Winkler & Debus 1996; Derrick & Kennedy 1997; Sardá et al. 1998), explaining the relatively common occurrence of regeneration. *Marenzelleria viridis* is known to exhibit nocturnal swimming in the water column (Dauer et al. 1980, 1982), during which they might be susceptible to predation.

The overall process of anterior morphogenesis in *M. viridis* was similar to previously reported studies on other spionids (Stock 1965; Gibson & Harvey 2000; Lindsay et al. 2008; David & Williams 2012b). Ablation of anterior segments is followed by wound healing and blastema formation. The blastema extends and the prostomium starts to differentiate at the distal end. However, some aspects of regeneration were distinct in *M. viridis* compared to other spionids, as discussed below.

We clearly observed formation of intersegmental furrows initiating near the ventral midline and proceeding from posterior to anterior of the regenerate. The formation of intersegmental furrows in *Amphipolydora vestalis* Paterson & Gibson 2003 also initiated on the ventral side and progressed dorsally along the regenerated region (Gibson & Paterson 2003). Formation of intersegmental furrows and the appearance and development of segmental features such as parapodia occurs from posterior to anterior on the regenerate, whereas all 11 thoracic segments appeared at the same time in *Amphipolydora* (Gibson & Paterson 2003). The formation of segments was also described as "essentially simultaneous" in oligochaetes (Bely & Wray 2001) and in

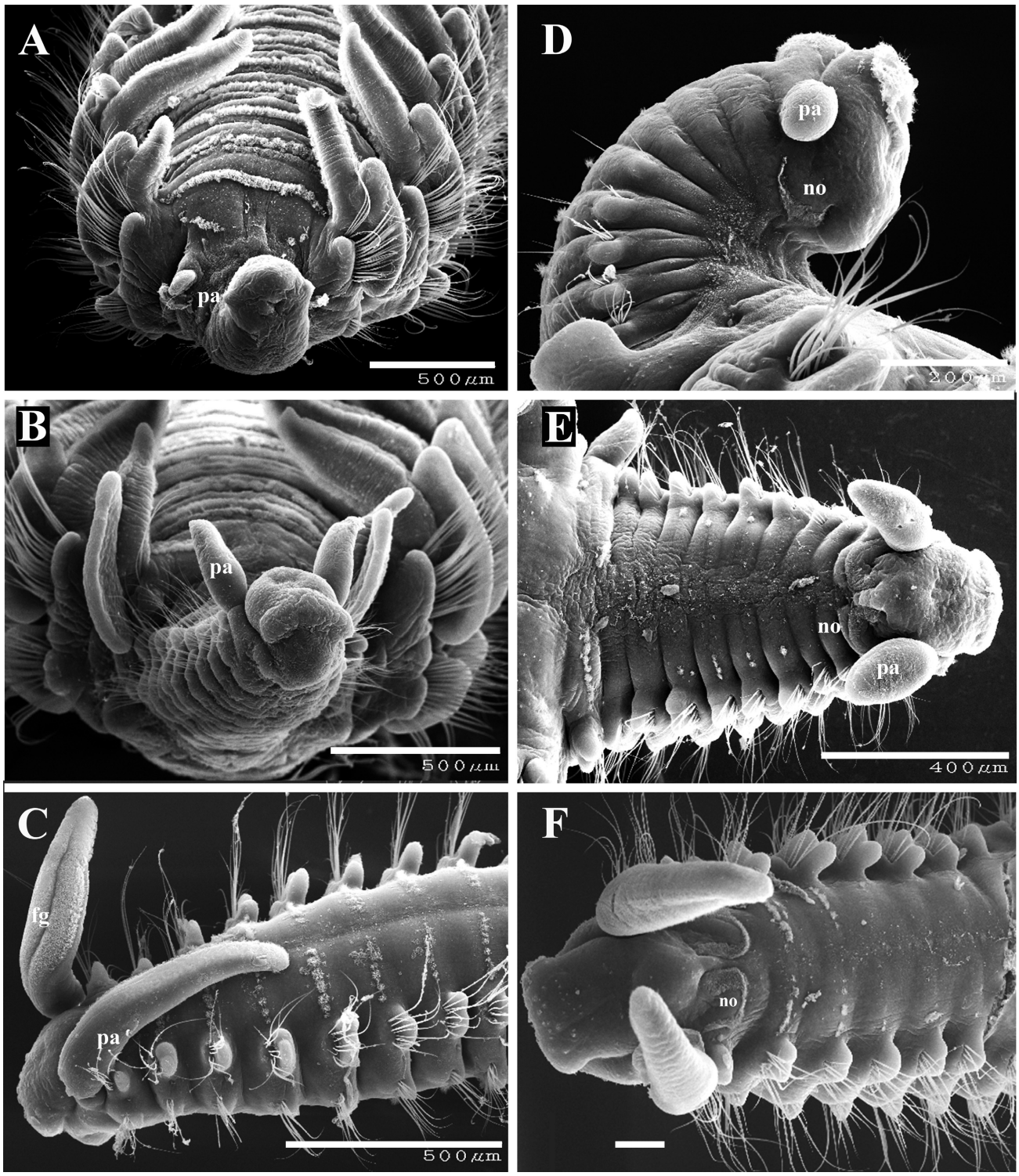

Fig. 3. SEM of *Marenzelleria viridis* showing regeneration of palps and nuchal organs over 7–14 d post-ablation (dpa). **A.** Palp buds on a specimen at 7 dpa. **B.** Elongated cylindrical palps at 9 dpa. **C.** Palps with food groove at 14 dpa. **D.** Nuchal organ on an 8-dpa regenerating individual. **E.** Nuchal organ 9 dpa. **F.** Nuchal organ at 13 dpa. Scale: A,B, C=500 μm; D=200 μm; E=400 μm; F=100 μm. fg, food groove; no, nuchal organ; pa, palp.

the spionids *Spio setosa* Verrill 1873 and *Dipolydora caulleryi* (Mesnil 1897) (Stock 1965). The apparent discrepancy in segment formation (posterior to anterior progression vs. simultaneous) may be due to differences in the frequency of observation. In other words, some species in which "simultaneous" formation has been purportedly found could represent cases in which the progression of segment formation was not observed. Alternatively, this apparent discrepancy could be attributed to better resolution of surface details such as faint furrows by SEM as compared to light microscopy. Since formation of the intersegmental furrows is initiated at the ventral midline, these structures would not be visible in the lateral views. Thus, additional research (including immunohistological and molecular investigations) is needed to more accurately describe progression of segment formation and differentiation both internally and externally.

We observed that the number of segments replaced was equal to the number ablated for up to 10 segments. However, ablation of 20–30 anterior chaetigers resulted in the formation of a maximum of 17 segments (range 13–17). The number of segments regenerated decreased to 14 when 40–50 chaetigers were ablated. The number of segments replaced in *M. viridis* was larger than previously reported for other spionids (Table 1). *Pygospio elegans* regenerates a maximum of 13 segments (Gibson & Harvey 2000; Lindsay et al. 2007), whereas all polydorins appear to produce 10 or fewer segments. This limited number of segments formed is found even during asexual reproduction via paratomy in the genus *Polydorella* (Williams 2004). During paratomic division, the growth zone of the developing stolon gives rise to 10 segments simultaneously (see fig. 5 in Williams 2004). Other annelids are also limited in the maximum number of regenerated anterior segments (e.g., Müller 2004; Zattara & Bely 2011). Additional studies should investigate the underlying mechanisms that control the maximum number of segments replaced during regeneration and asexual reproduction. Research combining molecular work on genes involved in anterior regeneration (e.g., Hox genes) and use of immunohistological staining to track redevelopment of internal features (e.g., muscular and nervous system) will help to identify the mechanisms involved. Such data have been collected in some polychaetes but mostly in studies focused on caudal regeneration or postlarval development (e.g., Irvine & Martindale 2000; Bakalenko et al. 2013; Novikova et al. 2013; Kozin & Kostyuchenko 2015).

Stock (1965) observed complete regeneration of a spontaneously produced three-segment fragment from an intact 180-chaetiger individual of *M. viridis* (Stock 1965). While we did not directly test the minimum fragment required for complete regeneration, we did observe complete regeneration from a small piece of a worm that fragmented in culture. The worm apparently broke across two segments since the fragment had five segments on one side and seven on the other. Other polychaetes of the canalipalpata, including members of the genus *Chaetopterus,* are known to have extensive capabilities in regeneration including formation of individuals from single segments (Berrill 1928). This is intriguing considering that chaetopterids appear to represent a basal group within the polychaetes (e.g., Kvist & Siddall 2013; Weigert et al. 2014; Bleidorn et al. 2015). For *M. viridis* it is unclear whether such extensive fragmentation takes place in the field and if it does, what environmental factors may trigger its occurrence. Nearly all specimens are broken at the posterior at the time of collection, although the cause for this breakage has not been observed directly and is likely a result of the collecting process. Although *M. viridis* is known to be a host species for trematode metacercaria (Phelan et al. 2016), these cysts do not appear to induce fragmentation as they do in other spionids (McCurdy 2001). However, the cysts do cause a cellular immune response that involves coelomocytes and other cell types (see refs in Phelan et al. 2016), and some of these cell types are also implicated in wound healing during regeneration (Bely 2014). Considering the relative ease in culturing worms of *M. viridis* (e.g., the worms can be maintained in dishes without running seawater) and the fact that cysts can be observed externally, members of this species could act as models for research on the activity of coelomocytes during cellular immunity and regeneration.

Regeneration abnormalities such as duplication of structures have been reported for a number of annelids. For example, Dualan & Williams (2011) described duplication of palps following their ablation in *Dipolydora commensalis* (Andrews 1891). Other polydorins have been observed with duplicated functional heads following ablation (Stock 1965; Gibson & Harvey 2000; David & Williams 2012b). David & Williams (2012b) suggested that duplications may be the result of oblique cuts across two segments potentially causing the formation of two blastemas, but this remains to be tested. Duplication of anterior ends during regeneration was never observed in *M. viridis* (~50 specimens ablated at the 10th chaetiger), even when diagonal ablations

were made (unpubl. data), as was recorded for *Polydora* spp. (Abeloos & Thouveny 1957).

In conclusion, we report on morphogenesis during regeneration of *M. viridis*, providing a comparison with all other species studied to date in the Spionidae. This species could be further used in comparative studies to develop a clearer picture of the selection pressures that maintain regeneration ability and for elucidating the genetic mechanisms involved in regeneration. *Marenzelleria viridis* is an ecologically important polychaete that has been introduced to the North and Baltic Seas. Researchers have indicated that its invasion success is, at least in part, due to its ability to reach high densities even under multiple environmental stressors such as pollution, hypoxia, and low salinity conditions (Bochert & Bick 1995; Bochert et al. 1996; Bastrop and Blank 2006; David & Williams 2016). In addition, its strong capabilities in regeneration could also play a role in its invasion success, and future studies should examine this possibility through field research and manipulative experiments.

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