Indian Journal of Experimental Biology
Vol. 58, May 2020, pp. 360-364

Microscopic visualization of regeneration in scale worm *Paralepidonotus* sp. (Grube, 1878)

Bharathidasan V, Rajesh N, Murugesan P* & Sivaraj S

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University,
Parangipettai-608 502, Tamil Nadu, India

Received 15 February 2019; *21 December 2019*

Regeneration of damaged or lost body parts is an ecologically important process in the animal realm. Like many other annelids, segmented worms and bearded scale worm, *Paralepidonotus* sp. is capable of regenerating its anterior elytra and posterior body segments and terminal structures that are lost due to amputation. In aquaculture industry, scale worms have importance as common live feed. In this context, we studied the morphology and organization of tissues in *Paralepidonotus* sp. populations which have ability to regenerate the anterior elytra and posterior region. The study revealed that the process of blastema formation in the anterior (Elytra) and posterior segments of *Paralepidonotus* sp. was normal and got regenerated to its original state during 9th to 12th day of experiment, and thus this species can be used for mass scale production to cater to the demand of aquaculture as suitable live feed for feeding the brooders both in shrimp and ornamental Aquaculture.

Keywords: Anterior elytra, Aquaculture, Broodstock feed, Live feed, Polychaetes

Regeneration is the ability of an organism to restore injured or lost parts of the body. Most animals are capable of healing their wounds but reconstruction of organs and body parts is restricted to only a few groups of metazoans. The most spectacular expression of regenerative powers is seen in the reconstitution of a whole individual out of small body fragments. Both “Oligochaeta” and “Polychaeta” use this ability as a means of asexual reproduction[1]. Polychaetes have a great power to regenerate its lost body parts. The regenerative capacity varies extensively across annelid taxa while some species are capable of regenerating its anterior end[2] (*Polyophthalmus pictus*) and some species its posterior region *Polydora colonia*[3,4], and a few species can reconstitute an entire individual from a single mid-body segment as has been reported from Sabellid family[5,6].

The regeneration ability of annelids is related to cellular-based immunological responses[7,8]. Posterior segment regeneration is dependent on hormone secreted by the supra-oesophageal ganglion or 'brain'[9-11]. The rate of segment production is directly correlated with the number of segments lost. Furthermore, the rate is initially high, but declines slowly as regeneration proceeds[12]. Thus, although the brain hormone provides some indispensable prerequisite for regenerative growth, it cannot be said to 'control' the whole process[13]. Wound healing is a complex process involving, among other aspects, namely de-differentiation, proliferation, migration and phagocytosis of various cell types[14,15]. This process and the events leading up to segment proliferation normally take place at the same time in the same area, and this situation hinders the elucidation of the nature of the structures and processes which are subject to the influence the brain hormone. As there is only meager information available on regeneration in scale worm (Aphroditidae), here, we studied this process in *Paralepidonotus* sp., commonly available in the East coast.

Material and Methods

Specimen collection and experimental setup

Paralepidonotus sp. specimens were collected from two stations (Fig.1), namely (i) newly constructed Bridge at Parangipettai (Lat: 11^{0}29’3.33”N; Long: 79^{0}45’41.03”E) and existing Railway Bridge along the Vellar estuary, south east coast of India (Lat: 11^{0}29’12.62”N; Long: 79^{0}44’26.27”E). Live specimens of *Paralepidonotus* sp. were collected from the selected locations using sediment grab and corer besides in the oyster beds without much damage to the worms. Immediately after collection, the collected worms were brought to the laboratory and the same were acclimatized to the lab conditions by following the modified method of Krishnaprakash[16]. Subsequently, the species was identified using standard references[17-19]. The diagnostic features and key characters of the type specimen are illustrated in Fig. 2. For regeneration

*Correspondence:
E-mail: pmurugesaan74@gmail.com (PM);
vrbharathi1989@gmail.com (VB)

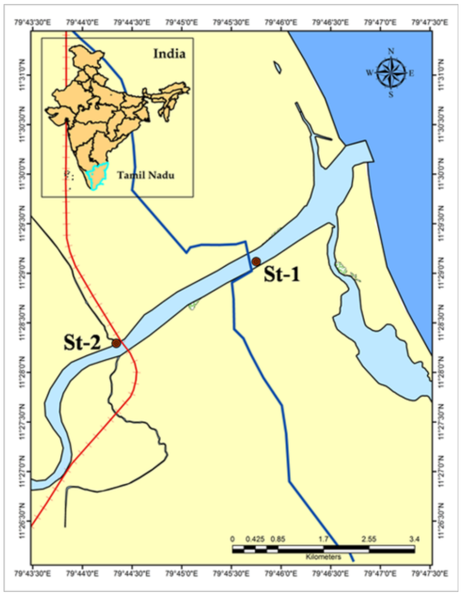

Fig. 1 — Map showing the sampling stations

Table 1 — The results of water quality parameters in field and lab condition			
Parameters/Unit	Station-1	Station-2	Lab condition
Salinity (ppt)	26.2	27.1	25.8
pH	8.0	7.9	8.1
Dissolved oxygen (mg/L)	4.9	5.6	5.4
Total Organic Carbon (mg C/g)	5.41	4.58	5.23

experiment, glass tanks (30×20 cm) with appropriate muddy sand bed were used. In each set of experiment, 10-15 worms in separate glass tank (30×20 cm) were maintained. Before amputation, specimens were relaxed with 0.37 M $MgCl_2$. After 10-15 min, 6-7 lamellas (segments 1-5) were amputated in the posterior side and similarly, the elytra from 2nd to 13th segments were amputated in the anterior end using sterile dissecting needle. Subsequently, the amputated worms were allowed to regenerate and the growth progress was photographed using Sony ZEISS 1080 HD camera. The level of water quality parameters were maintained similar to that of sampled environment condition (Table 1).

Results and Discussion

Morphological observations of *Paralepidonotus* sp.

The *Paralepidonotus* species were identified by following the key characters described by Read[18]. Head without cephalic peaks, with lateral antennae arising termino-ventrally below median antenna. Apparent short midline prostomial groove with post median antenna. Two pairs of black eyes, anterior-most at widest part of prostomium, posterior-most near posterior margin (Fig. 2 A and B). Mouth oval shape from the ventral view (Fig. 2C). Elytra thick with baloon-shaped macrotubercle vesicles variably scattered smooth-surfaced but densely blotched with dark pigment (Fig. 2 D and E). On ventral surface, small nephridial papillae present (most segments) and ventral lamellae present (Fig. 2F). These characters confirmed the type species (*Paralepidonotus* sp.).

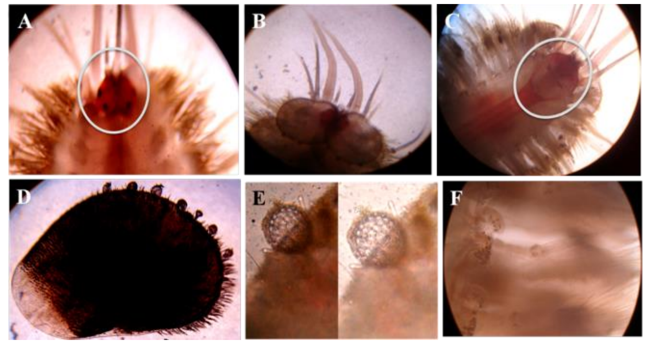

Fig. 2 —– Morphological descriptions of *Paralepidonotus* sp. (A) Dorsal view of anterior end showing pair of eyes; (B) anterior end showing elytron; (C) ventral view of the anterior end showing mouth; (D) elytron from anterior end with tubercles; (E) ampullae on elytra pigment of macrotubercle; and (F) lamellae and nephridial papillaeof parapodial base

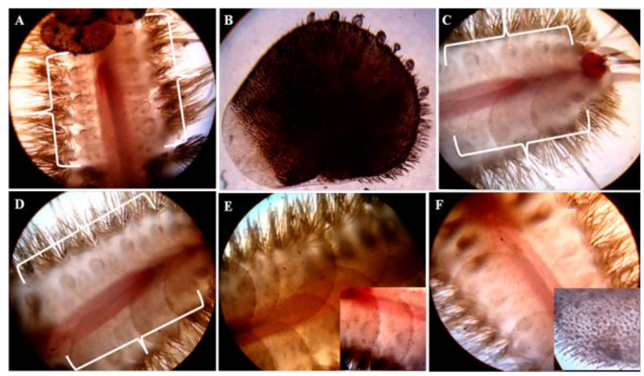

Fig. 3 — Regeneration process of amputated elytra in the anterior end at various time intervals. (A) Amputated anterior end dorsal view without elytron at 0hr; (B) amputated elytron showing binding region; (C) 3rd day observation elytra started regeneration; (D) 6th day observation; (E) 9th day observation; and (F) 12th day observation

Anterior elytra regeneration

Once the worms were acclimatized to lab condition, elytral structure from 2nd to 13th segments in the anterior end were amputated (Fig. 3 A and B) and the amputated worms were allowed to grow. On 3rd day, budding of elytron regeneration was observed but fringing papilla and surface papillae were not observed (Fig. 3C). By 6th day, a clear visibility of macrotubercle pigment, fringing papillae and surface papillae were

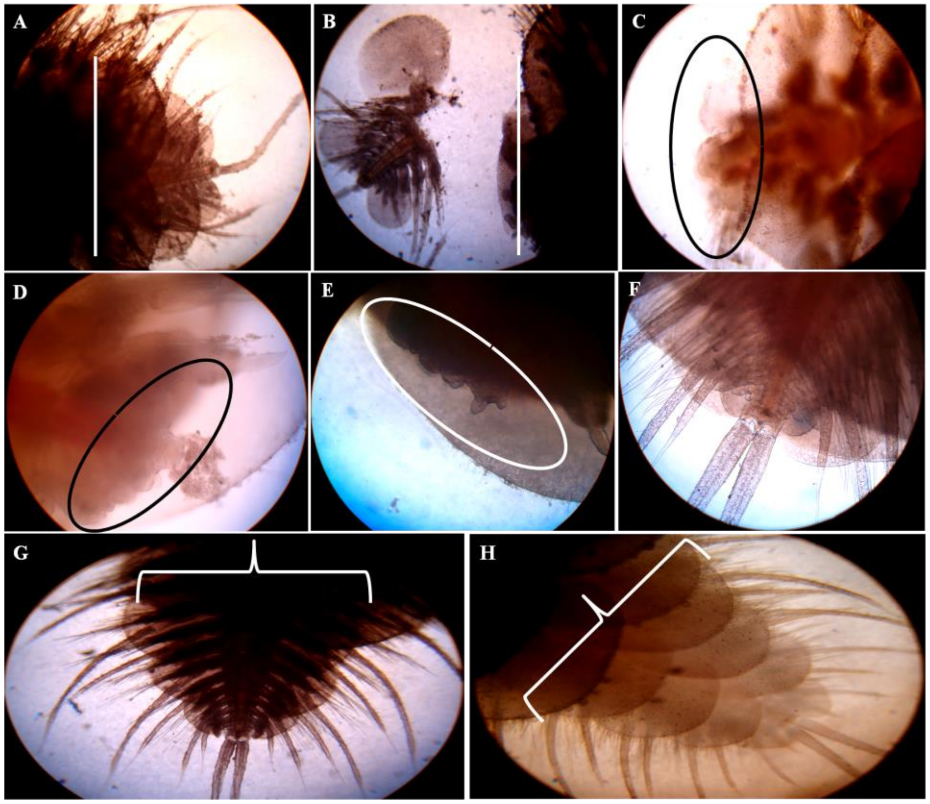

Fig. 4 — Regeneration process in posterior end during various time intervals. (A) Posterior end before amputation; (B) posterior end after amputation at 0 h; (C) At 12 h observation; (D) observation at 24 h; (E) on 3rd day Budding of elytral structure; (F) ventral view on 7th day observation; (G) on 9th day observation; and (H) a complete elytra formation in 12th day

noticed (Fig. 3D). During 9th to 12th day, elytra got regenerated to its original state (Fig. 3 E and F).

Regeneration in posterior end (segments)

With respect to posterior end, as done for elytra in the anterior end, 5 segments anterior to pygidium (posterior end) were amputated (Fig. 4 A & B). A clear swelling was observed within 12 to 24 h on the amputated region with division of mass of undifferentiated cells (blastema) (Fig. 4 C and D). On 3rd to 7th day, formation of elytra and parapodia was noticed (Fig. 4 E & F) and during 9th to 12th day, a complete regeneration of structure with segments was formed in the posterior side (Fig. 4 G & H).

The regeneration ability of the lost body region has been investigated in a wide range of annelid taxa[20]. Among annelids, regeneration of the posterior segment after amputation is common[8,20]. This might be due to the fact that posterior regeneration is similar to growth of adults by segment addition[21]. Many annelids have the capability of regenerating anterior segments, but this ability is less common than posterior end regeneration[20]. The regeneration in polychaetes is common and the present study on scale worm *Paralepidonotus* sp. forms baseline information in the Indian context, especially in relation to regeneration of elytral structures.

The morphology of elytral structure in anterior end and posterior end regeneration were studied in various species of polychaetes during yesteryears by various researchers. The regeneration of anterior and posterior end in *Platynereis dumerilii* and in *Typosyllis antoni* was studied by Pfeifer *et al*.[22] and Weidhase *et al*.[23], respectively. Simthi[24] also reported dislocation of about one third of the main ventral ganglia in anterior end of *N. virens.* Similarly, *Alitta (Nereis) virens* is able to regenerate its posterior as well as the anterior part of the body[25,26] and similarly Dualan & Williams[27] reported that the spionidae genus, *Dipolydora* sp. can regenerate number of anterior and posterior segments after amputation. The filter feeding polychaetes, namely *Dipolydora quandrilobata* and *Pygospio elegans* were found to regenerate anterior tissues and even palps[28]. Likwise, Whitford & Williams[29] also reported anterior regeneration in *Marenzelleria viridis*, spionid polychaete, corroborating the findings of the present study.

Licciano *et al*.[30] carried out an amputation experiments in different body parts of *Sabella spallanzanii* and *Branchiomma luctuosum,* and the results revealed that these species are capable of reconstructing their lost body parts completely. Szabo & Ferrier[31] described opercula regeneration and cell proliferation patterns of regenerating opercula filament in serpulid polychaete *Pomatoceros lamarckii*. The spionid Polychaete *Polydora ciliata* and *P. flava*can are known to regenerate completely even if six or eight anterior chetigers are removed but regeneration from more posterior ablations yields only 8-9 chetiger. Similarly, Stock[32] found that *Polydora caulleryi* (30-120 chetigers) can regenerate posterior segments (10-14 setiger anterior to pygidium) at nearly all levels. The results of above studies are in close agreement with the results of the present study, since in the present study also regeneration of elytral structures both in anterior and posterior ends was found to regenerate completely.

Giani *et al*.[33] reported that *Capitella teleta* got regenerated by 20 segments on 18th day after amputation. However, in *Eisenia fetida,* (earth worm) amputation of anterior end resulted in 20-30 segments whereas 50-60 segments in posterior end after amputation[34]. The amputation of posterior region of *Lamellibrachia satsuma* showed earlier blastema development in 0-20 days and after 40 days single chaetae regenerated[35]. The regeneration ability in posterior end of same size and age *Ophryotrochanoto glandulata* was studied and the results revelaed that blastema got developed on second day of amputation[36]. Bely & Wray[37] found that both the anterior and posterior ends got regenerated on 5th day in Oligochaete *Pristina leidyi*. Similarly, Matthews & Hentschel[38] found that palp structure got regenerated during 3-6 days interval in *Polydora cornuta*. In his study, Hofmann[39] carried out regeneration experiment in *Eunice siciliensis* and found that the regeneration bud started from 5th day onwards. The observations of above referred works lend support to the findings of the present study since regeneration bud of elytral structures especially blastema formation of *Paralepidonotus* sp., was observed from 3rd day onwards.In the light of findings of above referred studies, the present experimental species is also capable of displaying robust regeneration activity following amputation of both anterior and posterior segments suggesting an effective model species for regeneration and thereby mass multiplication of these worms could be done as suitable brooder feed targeting aquaculture industry.

Conclusion

The polychaete scale worm *Paralepidonotus* sp. forms suitable live feed/fresh feed for brooders in shrimp besides ornamental aquaculture sectors. Mass scale production of these polychaete worms require thorough knowledge on the special regeneration ability of these worms. The present observations yield interesting information on the morphology of regenerated elytral structures in the anterior and posterior end of *Paralepidonotus* sp. Based on the findings of the present study, the mass scale production of these polychaete worms can be done through regeneration as the shrimp brooders fed on polychaetes showed better results in terms of growth and maturation and thus the findings will go a long way in serving as bench mark information to the researchers who work in this line.

Acknowledgement

The authors thank the National Centre for Coastal Research (NCCR, Ministry of Earth Sciences, Govt. of India for financial support.

Conflicts of interest

Authors have declared no conflict of interests.

References

1 Hyman LH, Aspects of regeneration in annelids. *Am Nat*, 74 (1940) 513.

2 Stolte HA, Regeneration und Wachstum von *Polyophthalmus pictus* Duj. in Beziehung zum Problem der Zellkonstanz. *Dev Genes Evol*, 117 (1929) 562.

3 David AA & Williams JD, Asexual reproduction and anterior regeneration under high and low temperatures in the sponge associate *Polydora colonia* (Polychaeta: Spionidae). *Invertebr Rep Dev*, 56 (2012) 315.

4 Pires A, Freitas R, Quintino V & Rodrigues AM, Can Diopatra neapolitana (Annelida: Onuphidae) regenerate body damage caused by bait digging or predation? *Estuar Coast Shelf Sci*, 110 (2012) 36.

5 Yoshida Noro C & Tochinai S, Stem cell system in asexual and sexual reproduction of *Enchytraeus japonensis* (Oligochaeta, Annelida). *Dev Growth Differ*, 52 (2010) 43.

6 Prentiss NK, Tyler MS & Dean D, A morphological and histological investigation of the regeneration in *Myxicola infundibulum* (Montagu, 1808) (Sabellida, Annelida). *J Mar Biol Assoc UK*, 97 (SI5) (2017) 1155.

7 Nusetti O, Zapata E, Venes V, Esclap MM & Rojas A, Antioxidant enzymes and tissue regeneration in *Eurythoe complanata* (Polychaeta: *Amphinomidae*) exposed to used Vehicle Crankcase Oil. *Arch Environ Con Tox*, 48 (2005) 509.

8 de Jong DM & Seaver EC, Investigation into the cellular origins of posterior regeneration in the annelid *Capitella teleta*. *Regeneration*, 5 (2017) 61.
9 Hofmann DK, Studies on the regeneration of the posterior end at *Platynereis dumerilii* (Annelida, Polychaeta). *Zool J B Physiol*, (Zool. und Physiol.), 72 (1966) 374.
10 Golding DW, Regeneration and growth control in *Nereis*. I. Growth and regeneration. *J Embryol Exp Morphol*, 18 (1967) 67.
11 Durchon M, Endocrinologie Worms and Mollusks. Paris: Masson. M. E. Clark and R. B. Claark, (1962). Growth and regeneration in *Nephtys. Zool J B Physiol*, 70 (1967) 24.
12 Golding DW, Neurosecretion and regeneration in *Nereis*. I. Regeneration and the role of the supra-oesophageal ganglion. *Gen Comp Endocrinol*, 8 (1967) 348.
13 Golding DW, Neurosecretion and regeneration in *Nereis*. II. Prolonged secretory activity of the supra-oesophageal ganglion. *Gen Comp Endocrinol*, 8 (1967) 356.
14 Herlant MH & Nokin A, Early stages of healing and regeneration in *Nereis*. (1962).
15 Boilly B, Origine des cellules regeneratrices chez *Nereis diversicolor*. O. F. Muller (Anne1ide Polychete). *Wilhelm Roux' Archiv*, 162 (1969) 286.
16 Krishnaprakash R, Hatchery production of polychaete Perinereis cultrifera (Grube, 1840) as a potential live feed for aquaculture, (PhD Thesis Annamalai University, Chidambaram), 2010.
17 Hanley JR, Revision of the genus *Parlalepidonotus* Horst, 1915 (Polychaeta: Polynoidae), *Invertebr Syst*, 4 (1991) 1053.
18 Read GB, Adventive occurrence in New Zealand of the scale worm *Paralepidonotus ampulliferus* (Annelida: Polychaeta: Polynoidae). *New Zeal J Mar Fresh*, 40 (2006) 643.
19 Naeini AB & Rahimian H, Intertidal scale worms (Polychaeta, Polynoidae and Sigalionidae) from the northern coasts of the Persian Gulf and Gulf of Oman. *ZooKeys*, 31 (2009) 53.
20 Bely AE, Distribution of segment regeneration ability in the Annelida. *Integr Comp Biol*, 46 (2006) 508.
21 Bely AE & Wray GA, Evolution of regeneration and fission in annelids: insights from engrailed-and orthodenticle-class gene expression. *Development*, 128 (2001) 2781.
22 Pfeifer K, Dorresteijn AWC & Fröbius AC, Activation of Hox genes during caudal regeneration of the polychaete annelid *Platynereis dumerilii*. *Dev Genes Evol*, 222 (2012) 165.
23 Weidhase M, Beckers P, Bleidorn C, Aguado MT, Nervous system regeneration in *Typosyllis antoni* (Annelida: Syllidae). *Zool Anz*, 269 (2017) 57.
24 Smith JE, Some observations on the neuron arrangement and fibre patterns in the nerve cord of nereid polychaetes, *Pub Stn Zool Napoli*, 27 (1956) 168.
25 Novikova EL, Bakalenko NI, Nesterenko AY & Kulakova MA, Expression of Hox genes during regeneration of nereid polychaete *Alitta (Nereis) virens* (Annelida, Lophotrochozoa). *Evol Dev*, 4 (2013) 1.
26 Kozin VV, Filippova NA, Kostyuchenko RP, Regeneration of the nervous and muscular system after caudal amputation in the polychaete *Alitta virens* (Annelida: Nereididae). *Russ J Dev Biol*, 48(3) (2017) 198.
27 Dualan IV & Williams JD, Palp growth, regeneration, and longevity of the obligate hermit crab symbiont *Dipolydora commensalis* (Annelida: Spionidae). *Invertebr Biol*, 130 (2011) 264.
28 Lindsay SM, Jackson JL & He SQ, Anterior regeneration in the spionid polychaetes *Dipolydora quadrilobata* and *Pygospio elegans*. *Mar Biol*, 150 (2007) 1161.
29 Whitford TA, Williams JD, Anterior regeneration in the polychaete *Marenzelleria viridis* (Annelida: Spionidae). *Invertebr Biol*, 135 (4) (2016) 357.
30 Licciano M, Murray JM, Watson GJ & Giangrande A, Morphological comparison of the regeneration process in *Sabella spallanzanii* and *Branchiomma luctuosum* (Annelida, Sabellida). *Invertebr Biol*, 131 (2012) 40.
31 Szabo R & Ferrier DEK, Cell proliferation dynamics in regeneration of the operculum head appendage in the annelid *Pomatoceros lamarckii*. *J Exp Zool Part B*, 322 (2014) 257.
32 Stock MW, *Anterior regeneration in Spionidae*, *M. Sc., thesis*, University of Connecticut, Storrs, CT, (1965).
33 Giani VC, Yamaguchi E, Boyle MJ & Seaver EC, Somatic and germline expression of piwi during development and regeneration in the marine polychaete annelid *Capitella teleta*. *Evol Dev*, 2 (2011) 1.
34 Xiao N, Ge F & Edwards CA, The regeneration capacity of an earthworm, *Eisenia fetida*, in relation to the site of amputation along the body. *Acta Ecol Sinica*, 31 (2011) 197.
35 Miyamoto N, Shinozaki A & Fujiwara Y, Segment Regeneration in the Vestimentiferan Tubeworm, *Lamellibrachia satsuma*. *Zool Sci*, 31 (2014) 535.
36 Pfannenstiel HD, Regeneration in the gonochoristic polychaete *Ophryotrocha notoglandulata*. *Mar Biol*, 24 (1974) 269.
37 Bely AE & Wray GA, Evolution of regeneration and fission in annelids: insights from engrailed-and orthodenticle-class gene expression. *Development*, 128 (2001) 2781.
38 Matthews JA & Hentschel BT, In situ rates of body growth and palp regeneration of a spionid polychaete following simulated sublethal predation. *Mar Biol*, 159 (2012) 1039.
39 Hofmann DK, Maturation, epitoky and regeneration in the polychaete *Eunice siciliensis* under field and laboratory conditions. *Mar Biol*, 25 (1974) 149.