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

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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 9<sup>th</sup> to 12<sup>th</sup> 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<sup>1</sup>. 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<sup>2</sup> (*Polyphthalmus pictus*) and some species its posterior region *Polydora colonia*<sup>3,4</sup>, and a

few species can reconstitute an entire individual from a single mid-body segment as has been reported from Sabellid family<sup>5,6</sup>.

The regeneration ability of annelids is related to cellular-based immunological responses<sup>7,8</sup>. Posterior segment regeneration is dependent on hormone secreted by the supra-oesophageal ganglion or 'brain'<sup>9-11</sup>. 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<sup>12</sup>. Thus, although the brain hormone provides some indispensable prerequisite for regenerative growth, it cannot be said to 'control' the whole process<sup>13</sup>. Wound healing is a complex process involving, among other aspects, namely de-differentiation, proliferation, migration and phagocytosis of various cell types<sup>14,15</sup>. 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°29'3.33"N; Long: 79°45'41.03"E) and existing Railway Bridge along the Vellar estuary, south east coast of India (Lat: 11°29'12.62"N; Long: 79°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<sup>16</sup>. Subsequently, the species was identified using standard references<sup>17-19</sup>. The diagnostic features and key characters of the type specimen are illustrated in Fig. 2. For regeneration

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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<sub>2</sub>. After 10-15 min, 6-7 lamellas (segments 1-5) were amputated in the posterior side and similarly, the elytra from 2<sup>nd</sup> to 13<sup>th</sup> 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<sup>18</sup>. 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

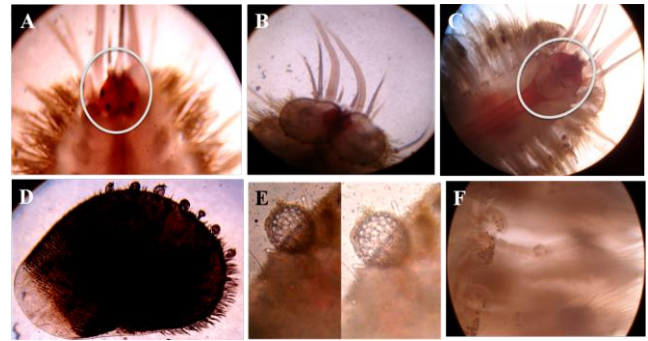


Fig. 2 — Morphological descriptions of *Paralepidonotus* sp. (A) Dorsal view of anterior end showing pair of eyes; (B) anterior end showing elytron; (C) ventral view of the anterior end showing mouth; (D) elytron from anterior end with tubercles; (E) ampullae on elytra pigment of macrotubercle; and (F)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

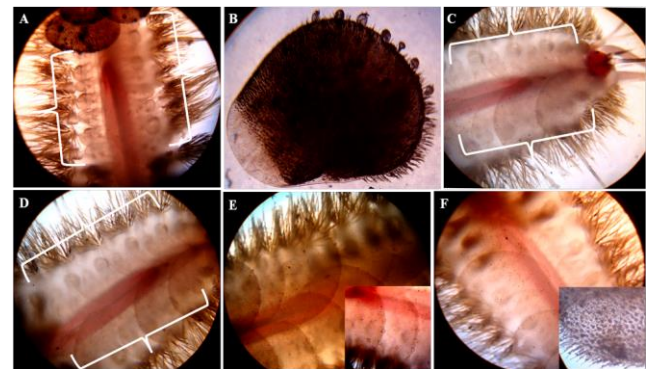


Fig. 3 — Regeneration process of amputated elytra in the anterior end at various time intervals. (A) Amputated anterior end dorsal view without elytron at 0hr; (B) amputated elytron showing binding region; (C) 3<sup>rd</sup> day observation elytra started regeneration; (D) 6<sup>th</sup> day observation;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

posterior margin (Fig. 2 A and B). Mouth oval shape from the ventral view (Fig. 2C). Elytra thick with balloon-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.).

**Anterior elytra regeneration**

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

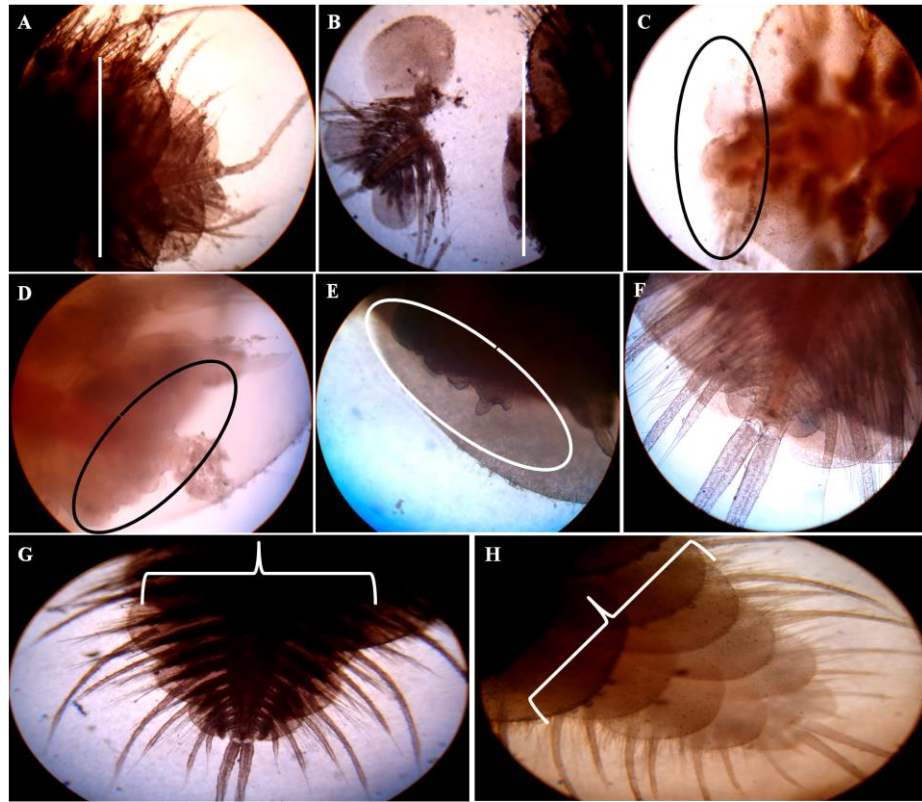


Fig. 4 — Regeneration process in posterior end during various time intervals. (A) Posterior end before amputation; (B) posterior end after amputation at 0 h; (C) At 12 h observation; (D) observation at 24 h; (E) on 3<sup>rd</sup> day Budding of elytral structure; (F) ventral view on 7<sup>th</sup> day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

noticed (Fig. 3D). During 9<sup>th</sup> to 12<sup>th</sup> 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 3<sup>rd</sup> to 7<sup>th</sup> day, formation of elytra and parapodia was noticed (Fig. 4 E & F) and during 9<sup>th</sup> to 12<sup>th</sup> 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<sup>20</sup>. Among annelids, regeneration of the posterior segment after amputation is common<sup>8,20</sup>. This might be due to the fact that posterior regeneration is similar to growth of adults by segment addition<sup>21</sup>. Many annelids have the capability of regenerating anterior segments, but this ability is less common than posterior end regeneration<sup>20</sup>. 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.*<sup>22</sup> and Weidhase *et al.*<sup>23</sup>, respectively. Simthi<sup>24</sup> 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<sup>25,26</sup> and similarly Dualan & Williams<sup>27</sup> reported that the spionidae genus, *Dipolydora* sp. can regenerate number of anterior and posterior segments after amputation. The filter feeding polychaetes, namely *Dipolydora quadrilobata* and *Pygospio elegans* were found to regenerate anterior tissues and even palps<sup>28</sup>. Likewise, Whitford & Williams<sup>29</sup> also reported anterior regeneration in *Marenzelleria viridis*,

spionid polychaete, corroborating the findings of the present study.

Licciano *et al.*<sup>30</sup> 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<sup>31</sup> described opercula regeneration and cell proliferation patterns of regenerating opercula filament in serpulid polychaete *Pomatoceros lamarckii*. The spionid Polychaete *Polydora ciliata* and *P. flavacan* 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<sup>32</sup> 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.*<sup>33</sup> reported that *Capitella teleta* got regenerated by 20 segments on 18<sup>th</sup> 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<sup>34</sup>. The amputation of posterior region of *Lamellibrachia satsuma* showed earlier blastema development in 0-20 days and after 40 days single chaetae regenerated<sup>35</sup>. The regeneration ability in posterior end of same size and age *Ophryotrochanoto glandulata* was studied and the results revealed that blastema got developed on second day of amputation<sup>36</sup>. Bely & Wray<sup>37</sup> found that both the anterior and posterior ends got regenerated on 5<sup>th</sup> day in Oligochaete *Pristina leidyi*. Similarly, Matthews & Hentschel<sup>38</sup> found that palp structure got regenerated during 3-6 days interval in *Polydora cornuta*. In his study, Hofmann<sup>39</sup> carried out regeneration experiment in *Eunice siciliensis* and found that the regeneration bud started from 5<sup>th</sup> 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 3<sup>rd</sup> 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.

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## Conflicts of interest

Authors have declared no conflict of interests.

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