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A rare condition of budding in bipinnaria larva (Asteroidea)

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A rare situation of budding in bipinnaria larva was encountered in the plankton samples collected from coastal waters of Visakhapatnam in 1987. Elsewhere, paratomy is a regular feature of adult flatworms and annelids. The phenomenon seems to be rather frequent and widespread. However, the chances of encountering the larvae actually may have been minimal. It may also be that very few have had enough opportunities to discern them cloning. The present paper reports asexual reproduction through budding in echinoderm larvae.

AMONG invertebrates the phenomenon of asexual reproduction is well established in several phyla. Turbellarians and some annelids like earthworms or aeolosomatids have remarkable power of regeneration and cloning. In some parasitic flatworms there is normal larval propagation by asexual reproduction, whereas in other groups like coelenterates larval propagation takes place by transverse fission, as in scyphistoma stage of *Aurelia*, producing ephyrae larvae. In echinoderms, asteroids reproduce by asexual method. A sea star may break into two by what is known as fissiparity, each piece regenerating into a complete individual. In our study on planktotrophic larvae in the coastal waters of Visakhapatnam we came across several specimens of bipinnaria larvae presenting evidence of budding.

The first report on asexual reproduction in echinoderm larvae is that of Bosch *et al.*[1]. They reported the presence of highly modified posterolateral arms in the bipinnaria larvae (of the sea star *Ludia* sp.) of the Gulf stream and the western Sargasso sea in June–July 1987. We have found the same (Figure 1) in several specimens of bipinnaria larva encountered in our samples. The asteroid identity of the bipinnaria in our plankton collections (made in 1987) is, however, not clear at present. Typically, each larva has two

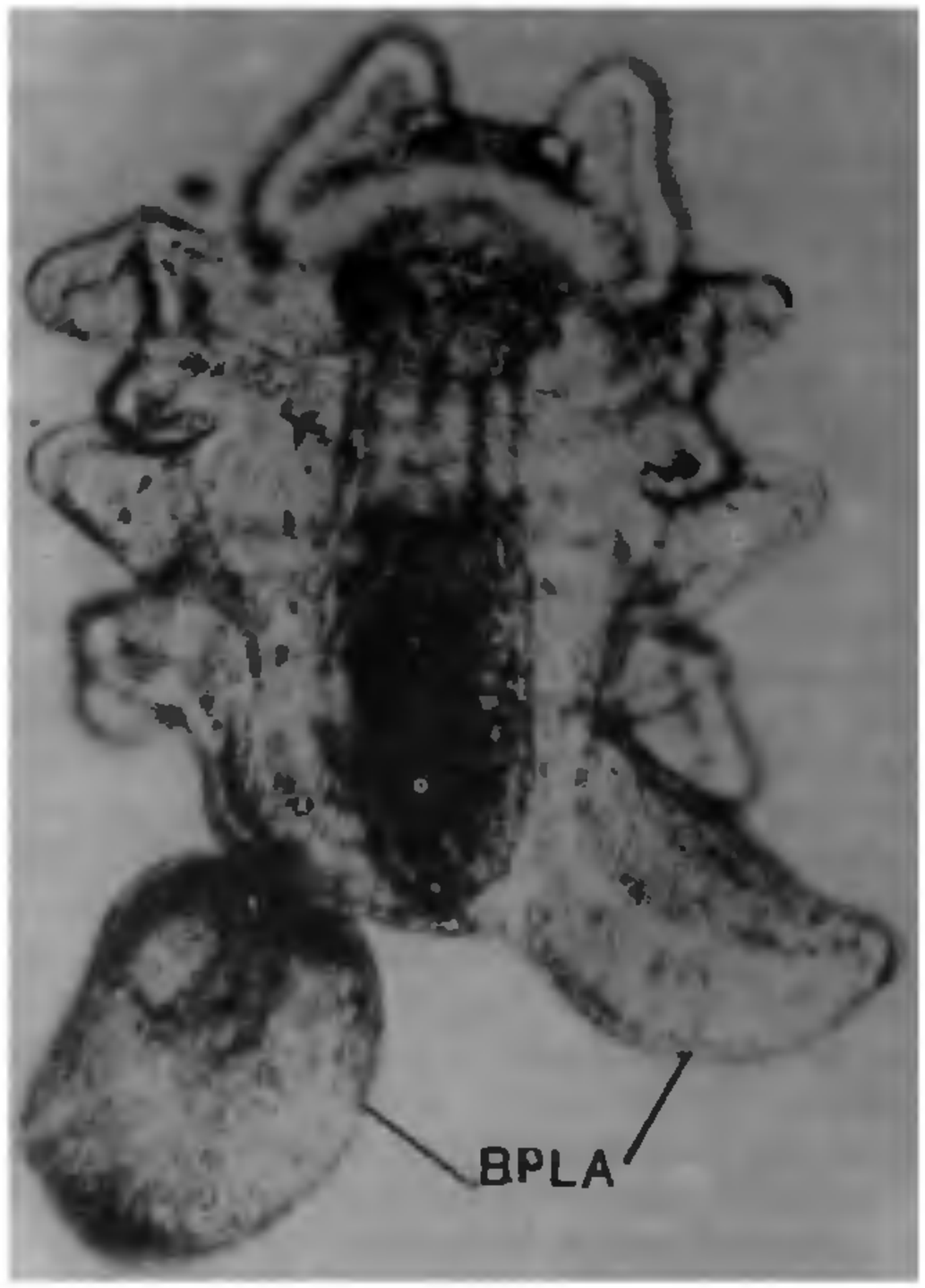

Figure 1. Bipinnaria larva showing budding of postero-lateral arms (BPLA)

fission zones, one on each postlateral arm. There are no ciliated bands, but rudiments of as-yet undifferentiated gut could be seen in each. Bosch *et al.* have discussed at length the details pertaining to the origin, formation and release of these fission products. It has been stated that at a stage when the stomodael invagination breaks through the undifferentiated digestive tract, the epithelium joining the primary and secondary larvae separates and the secondary larva is released.

There is no doubt that an exactly similar situation occurs in the bipinnaria encountered in our samples. This is the second report from the world and the first from India on paratomy, or larval propagation by fission, in echinoderm larvae. Both reports of budding in the bipinnaria are from warm waters (The Gulf stream and The Bay of Bengal). An evolutionary significance from the point of view of hazards in the completion of life cycle and survival of race may be implicated.

In the parasitic flukes, for instance, asexual reproduction in the larval stages (sporocysts and rediae) which are intramolluscan stages has definitely survival value for the species, and this could be directly related to hazards in the completion of life cycle. Mortensen[2] had already implied proliferation by budding in echinoderm larvae.

Many investigators may have noticed this and may have dismissed it as an abnormality or something not warranting serious consideration. Rao[3] had stated that detailed studies are very essential to understand the ecological aspects of tropical plankton. We emphasize that living plankton should be subjected to closer scrutiny, and developmental and physiological studies need to be improved.

This case emphasizes the fact that a closer scrutiny of as fresh a plankton collection as possible will amply reward the keen observer in unexpected ways.

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Regeneration of *Piper betle* from callus tissue

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A protocol for *in vitro* propagation of *Piper betle* L. has been standardized. Callus tissue was raised from shoot tip explants by culturing them on MS medium supplemented with 3 mg/l 1-naphthaleneactic acid and 0.05 mg/l 6-benzylamino purine. Well established callus tissue was differentiated into shoots as well as roots in MS medium containing 0.9 mg/l kinetin and 0.5 mg/l indole-3-acetic acid. Tissue culture raised plantlets were successfully transplanted into pots. Chromosomal variation has been observed in *in vitro* raised plantlets.

PIPER BETLE L., commonly known as betelvine, is widely grown in India for its leaves, under varying climatic conditions. Productivity of betel leaves can be increased through rapid introduction of improved technology and superior planting materials. The existing commercial cultivars of betelvine are mostly susceptible to diseases such as foot and leaf rot (*Phytophthora palmivora*) and bacterial leaf spot (*Xanthomonas campestris* pv. *bethicola*). Betelvine has been vegetatively propagated by stem cuttings for centuries and contains systemic bacteria. It is, therefore, desirable to produce plants which are free from such debilitating organisms. Secondly, genetic variability has been limited in the existing germplasm of *P. betle* as it is propagated by conventional means. Occurrence of variation is an essential prerequisite for improvement of this crop. Tissue culture techniques are proving to be a rich source of variability with potential in crop improvement. Therefore, an attempt was made to establish aseptic cultures, induce callus tissue from stem tip explants, and regenerate them into plantlets.

Shoot tips from field-grown betelvine (*Piper betle* cv. Desi-Bangla) were collected from Banthra Research Station of National Botanical Research Institute, Lucknow. The explants were washed thoroughly with Teepol (5%, 5 min) and then incubated overnight in a solution containing salts of Murashige and Skoog's[1] medium (MS) supplemented with antibiotics 5 mg/l each of chloramphenicol and oxytetracycline, 50 mg/l nystatin, 150 mg/l citric acid and 100 mg/l ascorbic acid[2]. pH of the solution was adjusted to 4.5. Thereafter the material was washed once in sterile water and twice in ascorbic acid (1%). Shoot tip explants (0.5–1.0 cm long) were excised on a filter paper soaked in ascorbic acid and then aseptically inoculated in various media separately supplemented with different concentrations/combinations of indole-3-acetic acid (IAA)/kinetin (Kn); 2,4-dichlorophenoxyacetic acid (2,4-D); and 1-naphthaleneacetic acid (NAA)/6-benzylamino purine (BAP) for callus induction (Table 1). The medium was also fortified separately or in combination with antioxidants, i.e. polyvinylpyrrolidone (PVP), ascorbic acid and citric acid. For rhizogenesis, newly emerged shoots were subcultured on various rooting media (Table 1). Sucrose concentration was reduced to 20 g/l as against 30 g/l initially used in callus induction. Rooted plantlets were transferred to nutrient solution containing KH_2PO_4, $MgSO_4$, KNO_3 125 mg/l, $Ca(NO_3)_2$ 500 mg/l and 1 ml/l each iron stock and trace elements of MS medium. Peat or soil was mixed with vermiculite and sand for the greenhouse potting of regenerated plants. The greenhouse was adjusted for conditions of 90% relative humidity and 25°C temperature until potted plants were well established.

For cytological investigation root tips from *in vitro* raised regenerants, acclimatized in nutrient solution were pretreated in 0.002 M 8-hydroxyquinoline for 4 h at 12°C, fixed in glacialacetic acid and ethanol (1:3) for 24 h at room temperature and thereafter stored in 70% ethanol. For mitotic examination, root tips from each plant were hydrolysed in 1N HCl for 15 min at 60°C and then treated with 4% iron alum for 15 min. These were stained in 2% acetohaematoxylin. The meristematic region was excised and squashed in a drop of glacialacetic acid (45%) on a slide. At least five root tips from each regenerated plant were examined to