Larval Development in *Echinoplana celerrima* (Turbellaria: Polycladida)[1]

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Abstract. Larval development of the polyclad flatworm *Echinoplana celerrima* (Acotylea: Planoceridae) has been described. Before hatching, the embryo reaches the two-eyed and the four-eyed stages. The newly-hatched juvenile worm is ovoid, and dorsoventrally flattened possessing four eyes and six sensory cilia on each side of the body. The juvenile worms fed with unicellular algae and spent part of their time gliding on the glass surface. With aging, they pass more time on the bottom of the dishes and suffer eye loss. The significance of this phenomenon is discussed.

Larval development in polyclads is of considerable phylogenetic interest. In fact, they are the only turbellarians to show spiral determinate cleavage forming quartets of micromeres and lobed larvae, considered to be of a trochophore type (Galleni & Gremigni, 1984). These characters point up additional possible relationships of Turbellaria with other Spiralia. According to Jägersten (1972), lobed larvae characterize the life cycle of primitive turbellarians. In particular, eight-lobed (Müller's) larvae occur in the more primitive suborder Cotylea, but 10-lobed larvae have been described by Dawydoff (1940).

The pattern of larval development in the suborder Acotylea is more diversified (see Table I). Most species analyzed display direct development; i.e., a four-eyed juvenile worm, without lobes and dorsoventrally flattened, hatches from the egg. Juvenile worms having 12 eyes hatch from the eggs of *Hoploplana villosa* and *Planocera reticulata*. Kato (1940) described an intracapsular Müller's larva in the latter species, but recently Teshirogi et al. (1981) observed that some specimens of *Planocera reticulata* also have a free-lobed larva (Kato's larva) similar to Müller's larva. A Müller's larva is present in *Hoploplana inquilina* and *Planocera multitentaculata*, whereas a four-lobed larva (Götte's larva) occurs in some species of the genus *Stylochus* and in *Notoplana australis*. Thus, the Planoceridae are unique among acotyleans, presumably primitive, but Müller's larva being either free-swimming or intracapsular.

Echinoplana celerrima is a rare acotylean polyclad (family Planoceridae, subfamily Gnesiocerinae) first described by Haswell (1907) from animals collected in Port Jackson (Sydney, Australia). A second discovery of a few specimens from the Tyrrhenian Sea, was reported by Galleni (1978). The pattern of development in this species is noteworthy.

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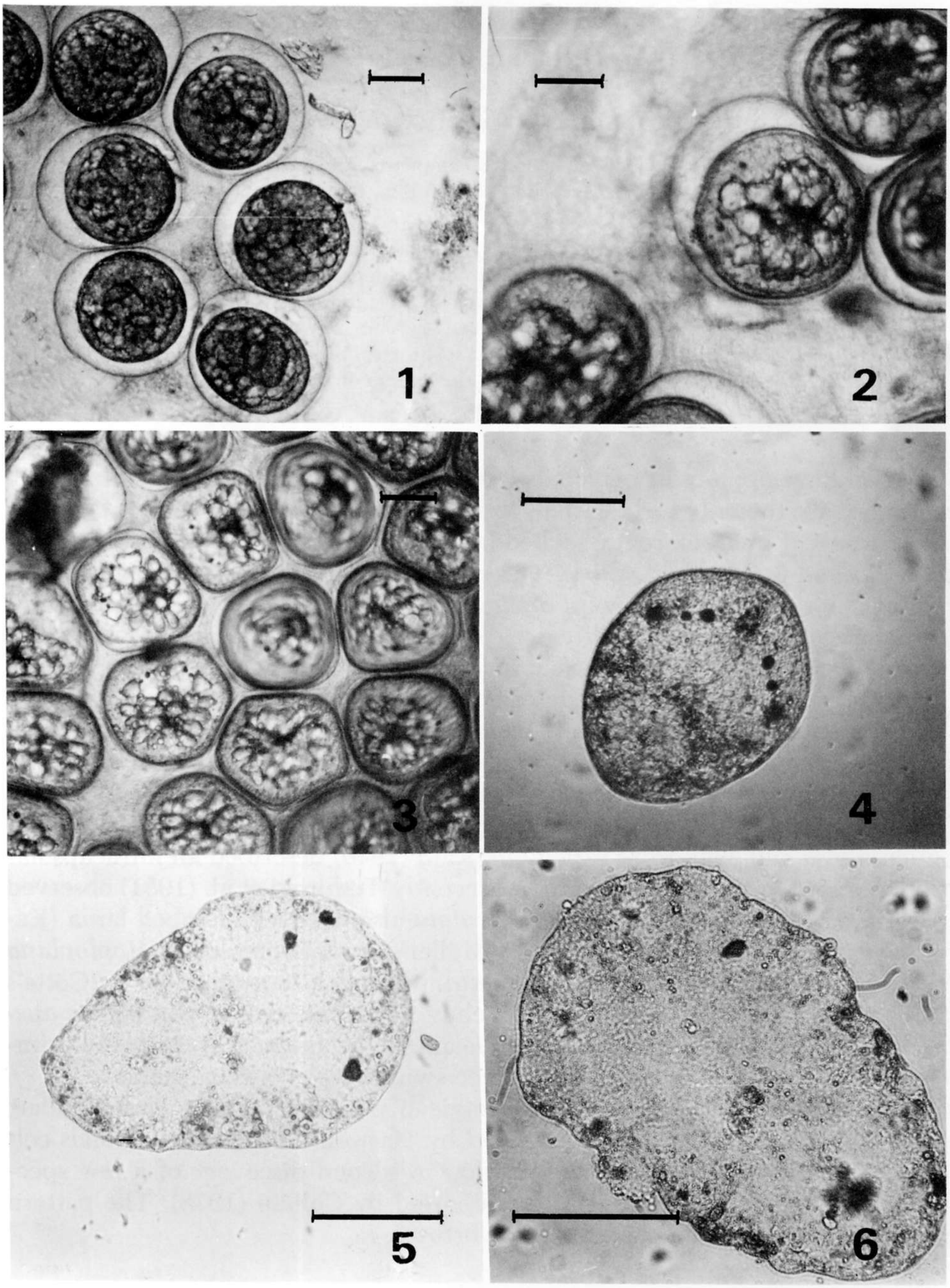

FIGS. 1–6. Development of *Echinoplana celerrima*. Fig. 1. Eyeless embryos. Fig. 2. Two-eyed embryos. Fig. 3. Four-eyed embryos. Fig. 4. Just-hatched juvenile worm. Fig. 5. Three-eyed larva (13 days). Fig. 6. One-eyed larva (15 days; distortion due to coverglass pressure). Scale bars represent 50 μm.

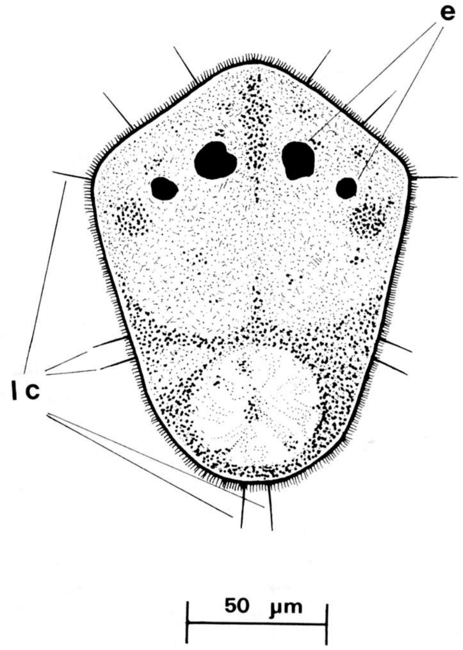

FIG. 7. Semischematic drawing of a newly-hatched juvenile of *Echinoplana celerrima*. e, eyes; lc, lateral sensory cilia. Scale bar represents 50 μm.

MATERIALS AND METHODS

A specimen of *Echinoplana celerrima*, collected from under a stone on a pebble beach near Puntone (Grosseto, Italy), was kept alive for eight weeks at the Institute of Zoology and Comparative Anatomy, University of Pisa. It was reared in a 14 × 25 × 12-cm aquarium containing about 1 liter of artificial seawater (33–35‰) and fed with small moribund crustaceans (amphipods and isopods). The specimen laid some egg plates, containing many eggs, affixed to the wall of the aquarium. Egg plates were removed and kept at room temperature in small dishes containing about 20 ml of artificial seawater. Unfortunately, eggs were collected and observed after cleavage had started (juvenile worms hatch after more than 14 days).

Juvenile worms were reared in the laboratory for 33 days and fed daily with *Tetraselmis* sp., a unicellular algae. Seawater was changed daily for the eggs and every two days for the juvenile worms.

RESULTS

The embryos exhibited typical rotatory (Fig. 1) movements 10 days before hatching, especially when exposed to light. Two symmetrical eyes appeared five days before hatching (Fig. 2) and two days later, embryos had four eyes (Fig. 3); at three days, they hatched (Fig. 4).

TABLE I

Development in Acotylea

Polyclad species	Type of development	Source
Cryptocelis alba	Juvenile worm	Lang (1884)
Discocelis tigrina	Juvenile worm	Lang (1884)
Euplana gracilis	Juvenile worm	Christensen (1971)
Hoploplana inquilina	Müller's larva	Surface (1907)
H. villosa	12-eyed cylindrical juvenile worm	Kato (1940)
Leptoplana tremellaris	Juvenile worm	Keferstein (1868); Vaillant (1868); Hallez (1879); Selenka (1881)
Notoplana alcinoi	Juvenile worm	Selenka (1881); Galleni & Gremigni (1983)
N. atomata	Juvenile worm	Remane (1929)
N. australis	Götte's larva	Anderson (1977)
N. delicata	Juvenile worm	Kato (1940)
N. humilis	Juvenile worm	Kato (1940)
Planocera multitentaculata	Müller's larva	Kato (1940)
P. reticulata	Intracapsular; Müller's larva; Kato's larva	Kato (1940); Teshirogi et al. (1981); Teshirogi et al. (1981)
Pseudostylochus elongatus	Juvenile worm	Kato (1940)
P. obscurus	Juvenile worm	Teshirogi et al. (1981)
P. ostreophagus	Juvenile worm	Smith (1955); Woelke (1956)
Pseudostylochus sp.	Juvenile worm	Teshirogi et al. (1981)
Stylochoplana maculata	Juvenile worm	Remane (1929)
S. parasitica	Juvenile worm	Kato (1940)
S. pusilla	Juvenile worm	Teshirogi et al. (1981)
Stylochus aomori	Götte's larva	Kato (1940)
S. ellipticus	Miniature worm; pelagic ciliated larva	Arvy & Nigrelli (1969); Provenzano (1959)
S. flevensis	Götte's larva	Hofker (1930)
S. frontalis (=*S. inimicus*)	Juvenile worm	Pearse & Wharton (1938)
S. ijimai	Götte's larva	Rho (1976)
S. mediterraneus (Galleni)	Götte's larva	Bytinski-Salz (1935); see also Galleni (1976)
S. neapolitanus	Juvenile worm	Lang (1884)
S. pilidium	Götte's larva	Götte (1878, 1881); Lang (1884)
S. tripartitus	Pelagic larval stage	Hurley (1975)
S. uniporus	Götte's larva	Kato (1940)
S. zebra	Juvenile worm	Lytwyn & McDermott (1976)

The newly-hatched juvenile worm is ovoid and dorsoventrally flattened (Figs. 4, 7). The body is covered with cilia, including six sensory cilia (longer than the usual ones) on each side of the body (Fig. 7). A pharynx occurs in the caudal one-half of the body, posterior to the branched gut (Fig. 7).

The morphology of newly-hatched specimens is similar to that of the juveniles of many species in the suborder, but never has been reported previously for the Planoceridae.

By contrast with the lobed larvae of *Stylochus mediterraneus* reared in our laboratory, juveniles of *Echinoplana celerrima* spent part of their time gliding on the glass surface and the remaining time swimming freely in the water. With aging, swimming periods were shortened progressively.

Contrary to what happens in larval stages of *S. mediterraneus,* we observed a tendency for eyes to degenerate with age and, consequently, a reduction of eye number occurred. Of particular interest is the fact that 13 days following hatching, three-eyed (Fig. 5) and two-eyed juvenile worms were observed. Some of these specimens also had two or three well-formed eyes and one or two eyes (always the anterior ones) with less pigment; one-eyed specimens were observed 15 days post-hatching (Fig. 6). A five-eyed specimen also was observed 20 days after hatching.

Discussion

As proposed by Jägersten (1972), the primitive turbellarian life cycle involves a planktonic lobed larva of the trochophore type, whose representative among Turbellaria is the lobed larva of polyclads. This life cycle occurs among all cotylean polyclads examined (see Galleni & Gremigni, 1984), while in the acotylean suborder a diversified situation obtains (Table I). For this reason, early acotylean stages are phylogenetically important for evaluation of the evolution of turbellarian development. Particularly significant is the loss of planktonic lobed larva (see Strathmann, 1978, for a discussion of this topic in other invertebrate phyla).

Echinoplana celerrima is characterized by some phylogenetically primitive characters: (1) the cirrus with numerous identical spines decreasing in size from the base to the apex (Galleni, 1978; Haswell, 1907; see Karling, 1963, for a discussion of primitive states in male copulatory structures among Turbellaria); and (2) a primitive mode of insemination (hypodermic impregnation; see Haswell, 1907).

The occurrence of direct development in *Echinoplana celerrima* suggests early establishment of direct development in the Acotylea and further, the coexistence in reproductive patterns of both primitive and secondary characters. Moreover, diversity of development among acotyleans also may suggest the absence of phyletic homogeneity in the suborder.

Our observations of feeding indicate a precocious planktotrophic habit, which also seems probable for lobed larvae (Ruppert, 1978). Clearly, the juvenile worm is a benthic form, different from planktonic lobed larva; however newly-hatched specimens spent some considerable time swimming freely.

Eye loss during development of juvenile worm has not been described previously. However, eye appearance and numbers are in accordance with descriptions of other polyclads with direct life cycles (Christensen, 1971; Kato, 1940; Teshirogi et al., 1981). The paucity of adults and larval material does not permit us to make conclusive statements relative to the following hypotheses: (1) that eye loss during larval metamorphosis of *E. celerrima* is the only degenerative event, whereas lobed larvae of *Stylochus mediterraneus* reared under the same conditions do not display such alterations—juvenile forms among polyclads are stenohaline and optimal conditions might not be the same for different species (Bytinsky-Salz, 1935; Pearse & Wharton, 1938); (2) that the developmental pattern of eye loss is peculiar to the species; or (3) that eye loss is part of the general developmental pattern among polyclads. Additional and more complete investigations of larval development must be made to elucidate information relevant to these hypotheses.

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