Fission in *Convolutriloba longifissura*: asexual reproduction in acoelous turbellarians revisited

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Abstract

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Studies of the asexual reproduction of *Convolutriloba longifissura* (Acoela, Acoelomorpha) revealed that there is no longitudinal fission of the whole animal as has been described by Bartolomaeus and Balzer (1997). Instead, the first step is a transverse fission. This results in the detachment of the caudal fourth of the mother animal. The detached part forms what we call the butterfly stage, which initially has no mouth and no eye fields. This stage gives rise to two new individuals by a longitudinal fission. Within 2–3 days the eye fields and a mouth develop in each of the two progenies formed in this way. In the meantime the mother individual grows and develops the three typical caudal lobes. The mother animal can repeat this process resulting in three individuals every fourth day. The finding of this new pattern of reproduction in the Acoela has prompted us to review the various ways by which asexual reproduction occurs in the group. The peculiar combination of few cases but high diversity of asexual reproduction in the Acoela is discussed from an evolutionary point of view.

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Introduction

About 40 years ago asexual reproduction and regeneration of the Acoela (Acoelomorpha, Ehlers 1985) began to fascinate investigators interested in Metazoan evolution; there were seminal papers by Hanson (1960, 1967) and Steinböck (1963, 1967). Hanson's review of 1960, 'Asexual reproduction in acoelous Turbellaria', in particular represents a stimulating summary of the knowledge at that time. The discovery of a new mode of asexual reproduction in the Acoela by Hendelberg and Åkesson (1988) revived interest again.

Acoelous turbellarians show three main categories of asexual reproduction: architomy, paratomy and budding. (1) Architomy is the separation of fragments or whole body parts from the mother animal prior to organ differentiation. Spontaneous fragmentation (autotomy) may represent the most primitive mode of that type of asexual reproduction. (2) Paratomy is a kind of transverse fission during which differentiation of new organs occurs prior to separation from the mother animal. This kind of fission leads to the formation of chains of zooids. (3) Budding is a process of local tissue reorganization that leads to a small outgrowth (bud) on the mother animal which later detaches from it.

The occurrence of both sexual and asexual reproduction within one life cycle is common among the most basal extant Metazoa, that is in the Porifera, the Placozoa and in the Cnidaria (Rieger 1986, 1994). On the other hand, asexual reproduction shows a clear decline among the higher Bilateria (Beklemischev 1969). Therefore, asexual modes of reproduction have to be considered in the discussion of models for the bilaterian stem species. Most often small, vermiform organisms, similar to certain turbellarians (Acoelomorpha, Macrostomorpha), are identified as such models (Ax 1996;

Rieger and Weyrer 1998). One can derive two generalizations from these observations: (1) asexual and sexual reproduction should be a feature of life cycles of basal extant turbellarians if they are to be viewed as models for the bilaterian stem species and (2) extant more derived taxa of turbellarians should show loss of asexual reproduction.

As to the first expectation we find, indeed, that asexual and sexual stages are most often seen in two of the basic branches, Catenulida and Macrostomorpha, although this trait is known also from higher Rhabditophora. In the Acoelomorpha, regarded by some investigators as the most basic branch of the Platyhelminthes (Steinböck 1931; Tyler 2001) only very few examples of asexual reproduction are described, so far only among the derived taxon Acoela. However, the Acoela clearly show a high diversity of modes of asexual reproduction: this is the only taxon among the free-living platyhelminths to show all three basic categories (see above), including examples of budding (Wager 1913; Hendelberg and Åkesson 1988, 1991; Åkesson and Hendelberg 1989). As to the question of loss of asexual reproduction, only one example from the Macrostomorpha has been discussed within the Platyhelminthes (Rieger 1986, 2001).

The possible significance of asexual reproduction for the origin of the Bilateria together with new data concerning the claim of a unique longitudinal fission of the whole animal in *Convolutriloba longifissura* (Bartolomaeus and Balzer 1997) stimulated us to present our new observations on that species together with a review of asexual reproduction in the Acoela.

Previous Records

As pointed out, asexual reproduction is not common among acoelous turbellarians. When Ax and Schulz (1959) reported on the first instance of paratomy in an acoelous species, which was later described by Dörjes (1966) as *Paratomella unichaeta*, they could refer to only one previous description of asexual reproduction in the Acoela (Wager 1913). Wager described that species as a degenerate variety of *Convoluta roscoffensis*, which is included now in the genus *Symsagittifera* Mamkaev and Kostenko (1991). It reproduced by transverse fission in the form of architomy and produced two or several fragments at a time. Each fragment, even if very small, could regenerate missing parts. After 'experimental fissioning', i.e. cutting of an animal into four parts, the fragments reorganized into functional individuals within 2 h. Wager also described how the animals put out gradually elongating protuberances that separated from the mother in about 1 h.

Wager had collected his material in the intertidal of the Natal coast, South Africa. The species had previously been described by Warren (1906) from the same region. According to Warren, the animals fed on diatoms. He also described sexual organs resembling those found in *S. roscoffensis*. But Wager (1913) reported that the animals had no sexual organs and were not feeding holozoically. According to Wager they depend entirely on their symbiotic algae.

Later on, Marcus and Macnae (1954) confirmed both Wager's (1913) description of fissiparous reproduction and Warren's (1906) report on sexual reproduction. Marcus and Macnae also maintained that the Natal species constituted a new species. It was redescribed by Bois-Reymond Marcus (1957) as *C. macnaei* and is now included in the genus *Pseudohaplogonaria* Dörjes (1968). Two years earlier (1955) she had described a species from South America, *C. cenata*, where oviposition causes a rupture of the body wall resulting in a transverse fission. Each part regenerates missing structures. The species is now included in the genus *Adenopea* (Antonius 1968).

An unequal transverse fission of the architomy type has been described by Hanson (1960) in *Amphiscolops langerhansi*. He reported: 'Odd-shaped little worms appeared in each of the culture depressions containing a single immature worm. These worms appeared one at a time, that is – within any period of observation only one new worm was discovered in a culture.' The fission occurs near the posterior end so that about 20–25% of the volume will be detached as an odd-shaped quiescent fragment. It begins to move after a day and eventually assumes the adult shape.

In three papers, Hendelberg and Åkesson (1988, 1991) and Åkesson and Hendelberg (1989) have reported on *Convolutriloba retrogemma*, so far the only known species reproducing by budding in a way similar to the budding described by Wager (1913). In early stages, the buds, one or two at a time, are inactive, but soon the cells organize into all tissues and organs (except those for sexual reproduction) present in the adult animal. Prior to detachment, the buds receive nutrients from the abundant algal cells that had been transported to the bud by the mother. When such algae have been digested, their remnants are seen as a dark spot. The most remarkable property of *C. retrogemma*, however, is that the polarity of the bud is reversed 180° relative to that of the mother animal.

The same species (or at least members of the same genus) has been reported from Okinawa (Ishikawa and Yamasu 1992) and from north Queensland waters in Australia (Winsor 1990). Ishikawa and Yamasu reported on two different species. Both could be collected in the low intertidal zone as well as in an outdoor aquarium. The larger species is similar to *C. retrogemma* whereas the smaller one, characterized by the absence of sexual organs and eye fields, seemed to be a different species. The small species is reported to release progeny from the ventral caudal part.

Winsor (1990) obtained his material of *C. cf retrogemma* in an aquarium where they were found on juvenile *Tridacna gigas* clams. Winsor also described a new species, *C. hastifera*. The name refers to prominent anterior batteries of sagittocysts. *C. hastifera* has well-developed male and female sexual organs. By comparing the mature male organs in *C. hastifera* with the male organs in *C. retrogemma* as described by Hendelberg and Åkesson (1988) Winsor concluded that the genus can be assigned to Haploposthiidae. No observations

on asexual reproduction was reported in *C. hastifera*. More recently, Gschwentner *et al.* (1999) provided new evidence that warranted placing the genus *Convolutriloba* into the Sagittiferidae.

All three of the budding species lack a statocyst, and that seems also to be true of progeny produced by architomy. On the other hand, *Paratomella unichaeta* has a statocyst in every individual produced by paratomy (Ax and Schulz 1959), and so have all sexually produced progeny of *A. langerhansi*, even in those worms originating from eggs produced by statocyst-less forms (Hanson 1960).

Yet another new species, *C. longifissura*, was recently described by Bartolomaeus and Balzer (1997). Their beautiful pictures and photographs, some of them shown in colour, leave no doubt that the new species belongs to the genus *Convolutriloba*. The similarities are remarkable as long as the comparison is confined to nonreproductive stages. As in *C. retrogemma*, no sexually mature individuals were observed, but motile sperm with a shape similar to that of sperm of *C. retrogemma* were recorded. As far as asexual reproduction is concerned, there are, however, fundamental differences. *Convolutriloba retrogemma* produces propagules by budding from the posterior margin, one or two at a time. Bartolomaeus and Balzer (1997) describe a longitudinal fission along the medial plane of the body. One eye field and half the brain as well as one half of other internal organs are reported to go to each daughter individual. Such a longitudinal fission followed by regeneration is stated to be the first one recorded in Plathelminthes and also the first one in vagile Bilateria.

Materials and Methods

In both our laboratories in Göteborg and Innsbruck we have maintained cultures of a *Convolutriloba* identified as *C. longifissura* Bartolomaeus and Balzer, 1997. In Sweden the first individuals were collected in a hobby aquarium in Karlstad in 1993. Later on, the same species was also found in two aquaria in Göteborg. When the first culture was established in 1993, we also had a culture of *C. retrogemma* available for comparison.

In Innsbruck the first populations of *C. longifissura* were established from marine aquaria in the 'Haus der Natur' in Salzburg in 1998. Since that time several populations have been obtained from hobby aquaria in Austria. *Convolutriloba retrogemma* has been collected sporadically since 1990, and cultures were established in February 2000 from marine aquaria in 'Haus des Meeres' in Vienna.

Material has been exchanged between the laboratories in Göteborg and Innsbruck, and there is no doubt that we are working with the same two species in both laboratories.

The culture methods were slightly different in the two laboratories, but those differences do not seem to have affected the outcome of our experiments. In Göteborg, worms were kept in 80-ml semispherical bowls; in Innsbruck they were either in large aquaria or individually isolated in plastic tubes (8 cm in diameter) with a plankton-net bottom. The tubes were floated in the large aquaria. The animals were fed freshly hatched *Artemia* larvae and harpacticoid copepods. For details see Åkesson and Hendelberg (1989) and Gschwentner *et al.* (1999). Hanson (1960) was followed in isolating immature worms individually for studying the progeny of individuals.

The specimens were studied under dissecting microscope either in their culture bowls or after transfer with a pipette into flat-bottom bowls. Isolated specimens were inspected daily. Pictures used as a basis for the diagram of the asexual reproduction were taken with a Leitz Aristophot using an electronic flash. In addition, video recording (VHS) was performed on an inverted microscope. Internal structures were studied in squeezed specimens under light microscope. Such whole-mounts were also prepared for confocal microscopy according to Gschwentner *et al.* (1999).

A 4-day workshop in Göteborg resulted in this paper for which the authors share equal responsibility.

Results

Convolutriloba longifissura had a maximum length of about 6 mm, which is the same as reported for *C. retrogemma*. However, specimens of *C. longifissura* were on average somewhat smaller; most of them were not sexually mature (Fig. 1A). In some of them, two genital openings in the ventral side between the mouth and the caudal median lobe could be seen, the female one in front of the male one (Fig. 1B). In these specimens a single medially placed, straight bursal nozzle (50–60 μm long) was observed (Fig. 1C). The nozzle was comparatively shorter and broader than in *C. retrogemma*, but it had the same characteristic zigzag-shaped contour. In maximally squeezed specimens, spermatozoa (130 μm long) resembling those reported from *C. retrogemma* were observed (Fig. 1D).

Following Hanson (1960), 24 medium-sized to large specimens of the Göteborg cultures were isolated. We obtained within 3 days in 19 of the bowls a large individual devoid of caudal lobes, and in addition either a 'twin' progeny at the butterfly stage (see below) or two small worms. After one more day, the longitudinal fission of the butterfly stage had been completed and separated the twin progeny. Reproduction was delayed 2–3 days in the remaining five bowls. In similar experiments in Innsbruck, 33 of 45 isolated individuals featured asexual reproduction. Butterfly stages occurred after 1–11 days, the separation of this stage into two individuals was completed 2–3 days later also here.

The asexual reproduction in *C. longifissura* was observed to begin with a transverse fission (Fig. 2). In the initial stage the indentation was unilateral, either on the left (as in Fig. 2) or on the right side. The fission divided the animal unequally, with about three-quarters of the body in front of the fission plane (line a in Figs 1, 2). Already before the detachment of the posterior part a new indentation appeared in its frontal
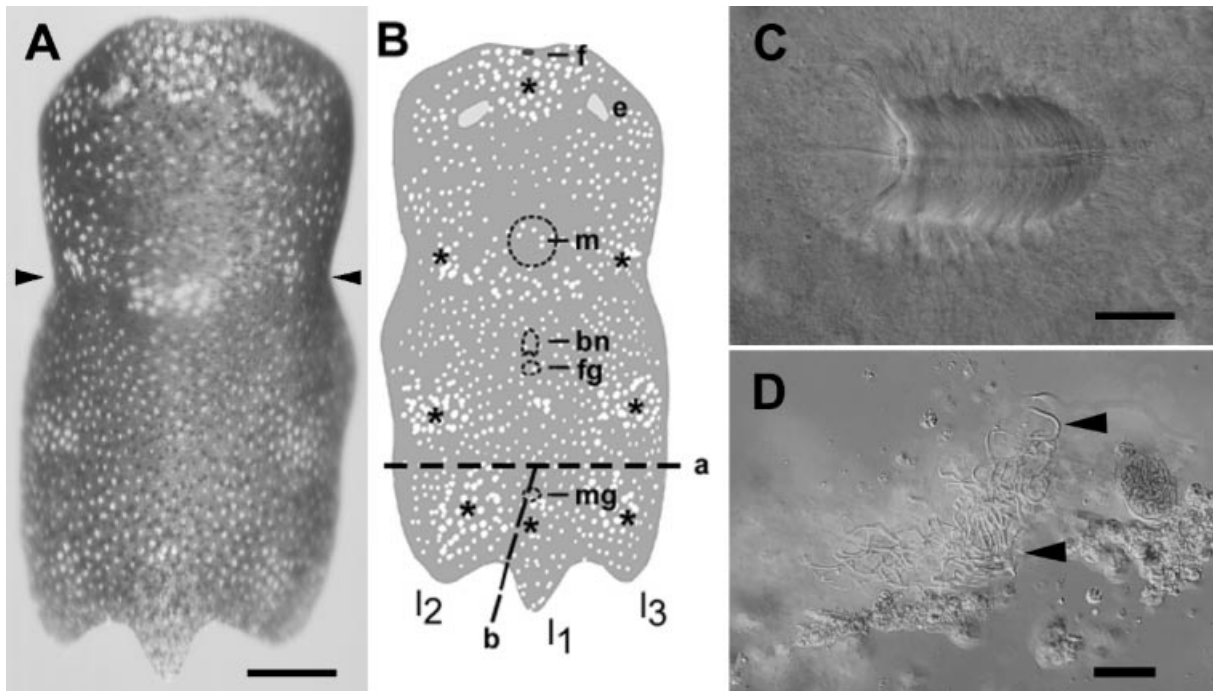
Fig. 1—*Convolutriloba longifissura* —**A**, Dorsal view of the whole animal. Note indentations at about one third of the animal (arrowheads). Scale bar: 160 μm —**B**, Diagrammatic illustration of adult specimen showing line of transverse fission (broken line a) and line of longitudinal fission (broken line b). (bn) bursal nozzle, (e) eye-field, (f) frontal organ, (fg) female genital opening, (l$_1$) median lobe, (l$_2$, l$_3$) lateral lobes, (m) mouth, (mg) male genital opening, (*) white iridescent concrements —**C**, Bursal nozzle of squeezed animal, anterior towards right. Scale bar, 20 μm —**D**, Spermatozoa (arrowheads) in the right lateral side of squeezed animal. Scale bar: 40 μm.

margin. As this indentation deepened, the posterior fragment assumed the shape of a butterfly ('butterfly stage', Figs 2, 3D, 5A–C). Finally, a longitudinal division (line b in Figs 1, 2) split the detached part into two individuals.

After the transverse fission, the mother animals were proportionally shorter and featured for some time a characteristic caudal margin lacking median and lateral caudal lobes (Figs 2D, 3C, 4A). The lobes regenerated within 4 days (Fig. 4).

No mouth openings or eye fields were noted in the butterfly stage immediately after separation from the mother animal (Figs 2, 3, 5). One day after the transverse fission, a regeneration blastema – distinguishable by its lack of symbiotic algae – could be observed at the anterior tip of both forming daughter individuals (Fig. 5B). Often a round dark spot was visible in each of them (Fig. 5B). A frontal organ was visible after the first day. A mouth and two eye fields occurred after about two days in each of the two forming individuals. The longitudinal fission of the butterfly stage resulted in an asymmetric distribution of the sagittocysts and the characteristic red glands (Fig. 6). This asymmetry persisted for several days. Only one of the progeny retained the whole median lobe of the mother animal.

Mother animals have been observed to repeat the described kind of reproduction several times, with 4-day intervals between events. Even in animals exhibiting sexual organs, this kind of asexual reproduction was observed. The transverse fission then occurred between the two gonopores (Fig. 1B).

As long as the posterior fragment was attached to the mother animal, it was rather immobile and followed the 'mother's' movements. Similarly, during the first 24 h after detachment, when still at the butterfly stage (Figs 2E,F, 3D, 5A,B), the progeny showed low motility mainly restricted to ciliary movements. It seemed that this was the time the cells of the fragment require to organize tissues and new organs.

In a few occasions, exceptions from the described way of fissioning in *C. longifissura* were observed. In one instance, the longitudinal fission divided the caudal fragment shortly after its detachment from the mother animal into a right and a left piece without going through the butterfly stage. Both fragments developed into daughter individuals. In a few other cases the transverse fission had not been completed when the longitudinal fission occurred, and only half of the caudal end was detached from the mother individual (J. Müller, unpublished observations).
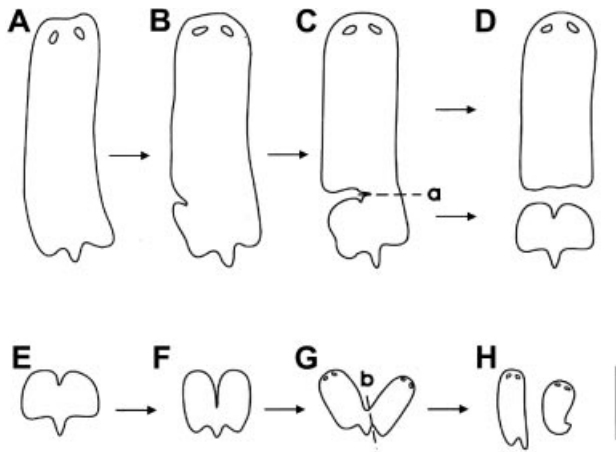
Fig. 2—Diagram showing the sequence of stages in the asexual reproduction of *Convolutriloba longifissura*. The top row (**A–D**) shows the first fission, a transverse one (broken line a), of the mother animal, giving rise to a new individual with a typical W-shaped hind end and a short so-called butterfly stage. The bottom row (**E–H**) shows how the butterfly stage forms two progenies which are separated from each other by a longitudinal fission (broken line b). Note that the eye fields are absent from early butterfly stages. They are later newly developed. The mother individual has the form observed in electron flash photographs of specimens in gliding motion resulting from the activity of the epidermal cilia. In basking position the mother animals are somewhat shorter and much broader. For other explanation see the text. Scale bar: 1.0 mm.
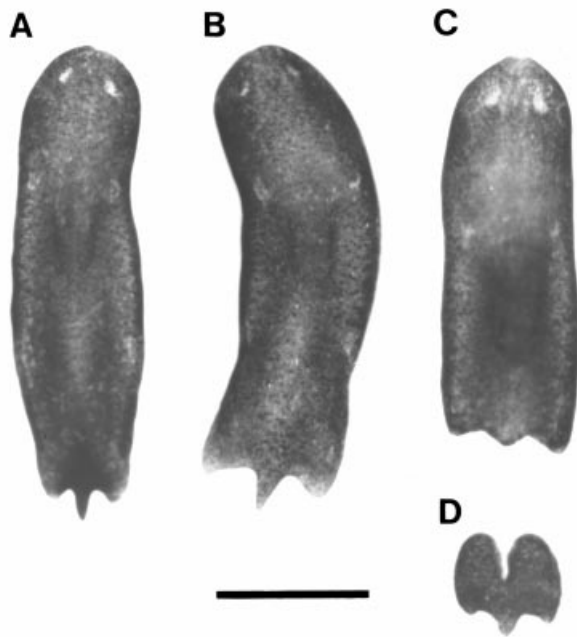

Fig. 3—Photographs of *C. longifissura* obtained by electronic flash. Free-moving animals before (**A, B**) and after (**C**) fission. —**D**, butterfly stage. Scale bar: 1.0 mm.
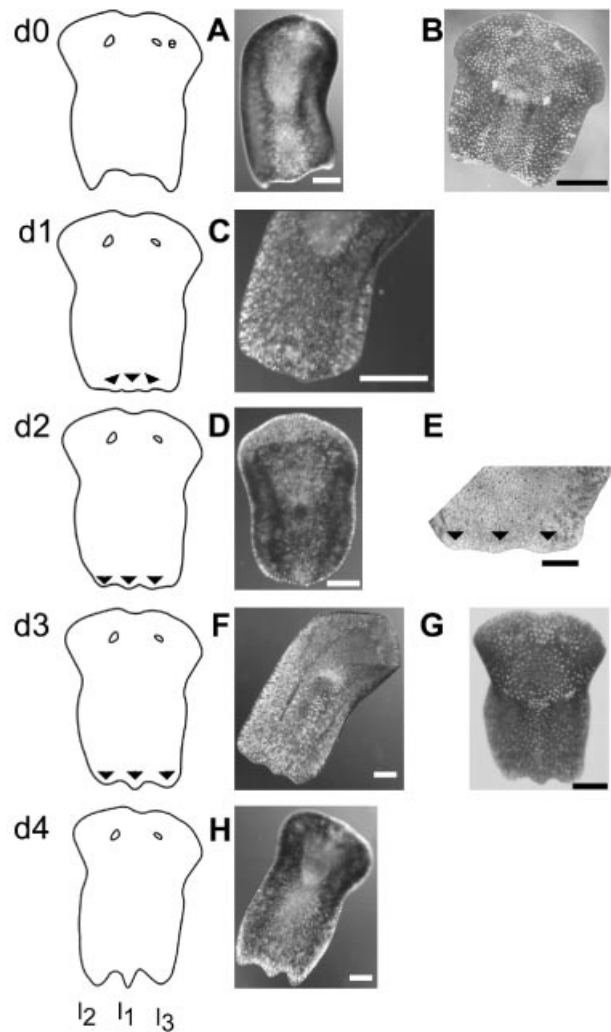

Fig. 4—Left column: scheme of regeneration (arrowheads) of the caudal lobes of the mother individual of *C. longifissura* from the day of transverse fission (d0) to day 4 after transverse fission (d4). (l_1) median lobe, (l_2, l_3) lateral lobes —**A–H**, Photographs of increasing regeneration of the lobes from day 0 (**A**) to day 4 (**H**) of the mother individual. Scale bars: 250 μm.

Discussion

The unique fission of Convolutriloba longifissura

The identification of the species from which we report this new type of asexual reproduction as *C. longifissura* is based on the identical form of our specimens with that shown in the photos of fully developed specimens and of all stages of asexual reproduction published by Bartolomaeus and Balzer (1997). That the species is closely related to *C. retrogemma* is evident not only from the great similarities in general appearance of fully grown individuals of the two species but also from the similarities in genital organs, including the single, straight median bursal nozzle. Thus, even though asexual
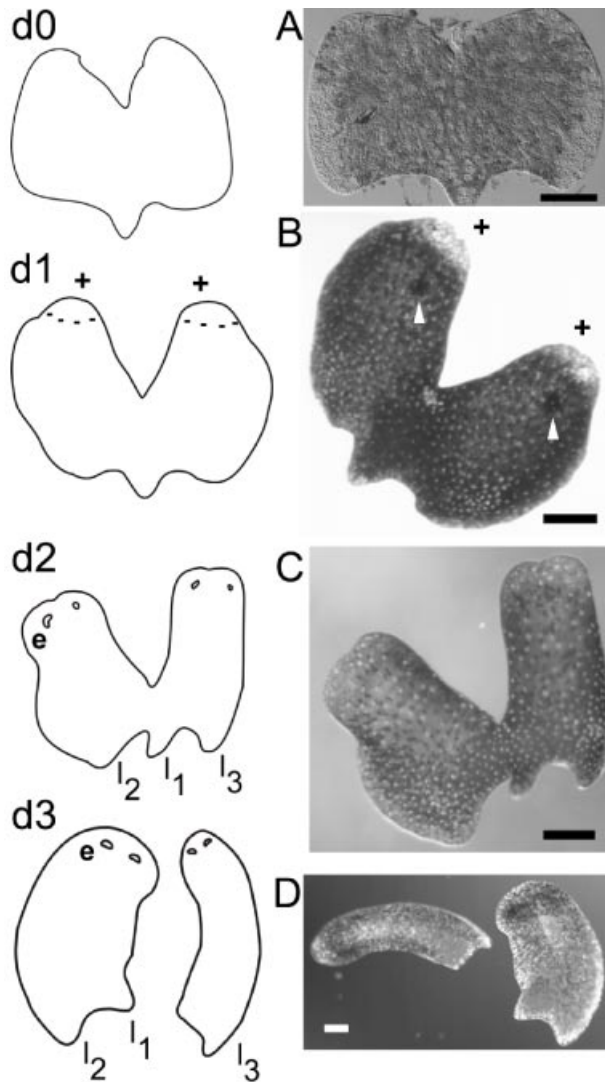
Fig. 5—Left column: scheme of development from butterfly stage to two daughter individuals of *C. longifissura* from day of transverse fission (d0) to day 3 after transverse fission (d3). (e) eye-field, (l_1) median lobe, (l_2, l_3) lateral lobes —**A–D**, Photographs of development from butterfly stage to two daughter individuals from day 0 (**A**) to day 3 (**D**). Note dark round spots (white arrowheads) and regeneration blastema (+). Scale bars: 250 μm.
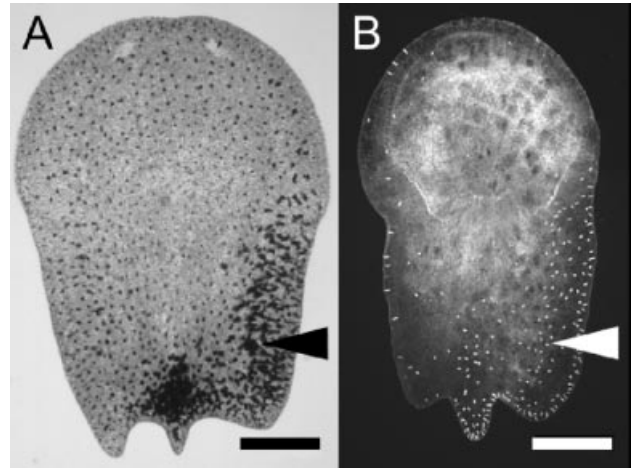

Fig. 6—Asexual juveniles after 7 days still exhibiting the asymmetry in red pigment (**A**, see arrow) and the distribution of spiral muscles surrounding the sagittocysts (**B**, see arrow). —**A**, Squeeze preparation of live animal —**B**, Whole-mount phalloidin-stained preparation, confocal microscope. Scale bars: 250 μm.

longitudinally. Both of the two eye fields of the new offspring are newly developing structures. The butterfly stage lacks mouth openings in the beginning. Evidently energy is provided by the symbionts. The dark spot in each of the two developing individuals represents, according to our interpretation, a cluster of remnants of the symbiotic algae that have been digested.

If there were a longitudinal, medial fission of the whole animal, there should be stages of regeneration that have only one half of the animal fully developed. No such photographs are shown in Bartolomaeus and Balzer (1997). The relation of the size of the large individual and the twin fragment (their Fig. 1, at arrow) supports our version of the course of events. It is also hard to imagine how a sequence of events during evolution could produce a vagile metazoan organism with bilateral symmetry that undergoes asexual reproduction by dividing all organs in medial position, including the brain, into symmetrical halves, each of which regenerates missing parts. As mentioned earlier, such a fission is unknown in the Bilateria.

In *C. retrogemma*, it was observed that the mother animal is separated from its buds by a tearing away, resulting in wounds where the individuals were once connected (Hendelberg and Åkesson 1991). Also in *C. longifissura* there is a mechanical process that tears the mother animal away from the butterfly stage, which adheres to the substratum. Thus, even if there are great differences in the patterns of asexual reproduction in these two evidently closely related species, the mechanical process of tearing away the mother animal from its progeny implies a similar mechanism.

How can the double fission observed in *C. longifissura* be related to other types of asexual reproduction? Åkesson and Hendelberg (1989) suggested that the low motility in

reproduction of the two species is very different, there seems to be good support for referring them to one and the same genus.

What is new in our observations of *C. longifissura* is how the offspring of the asexual reproduction are formed (Gschwentner *et al.* 1999; unpublished observations by B. Åkesson and J. Hendelberg). There is no evidence in our material of a longitudinal fission of the whole mother individual, as suggested by Bartolomaeus and Balzer (1997). We did not observe specimens with only one eyefield as constructed in the diagram in their Fig. 4 (this point is not illustrated in their photographs). Only the detached posterior end divides

newly detached propagules of *A. langerhansi* (Hanson 1960) reflects the incomplete regeneration of this stage. Also the newly detached fragment at the butterfly stage of *C. longifissura* rests motionless on the bottom of the culture bowls. Usually it adheres tightly to the bottom. In *C. longifissura* there is a scenario opposite to that in fissiparous organisms with paratomy, where future organs are preformed ahead of the final fission. The mode of asexual reproduction described by Hanson (1960) for *A. langerhansi* can serve as a model for the ancestral stage from which the fission process in *C. longifissura* can be derived.

Both in *A. langerhansi* and *C. longifissura* dedifferentiation may occur to a certain degree before the fission. On the other hand, we know that the stem-cell system (neoblasts), a prerequisite for platyhelminth regeneration, growth and development (see literature in Baguñà *et al.* 1989; Rieger *et al.* 1999; Ladurner *et al.* 2000; see also Hori *et al.* 1999 who use the designation 'regenerative cells'), is well developed in *C. longifissura* prior to the transverse fission as well as in the butterfly stage (R. Gschwentner, unpublished observations). Regeneration in these animals is therefore largely an epimorphic process. There is good evidence that the algal symbionts are actively transported into the regenerating area as found in *C. retrogemma* by Hendelberg and Åkesson (1991).

Efficiency of reproduction

From the literature we have only scanty information about the reproductive rate associated with the various kinds of asexual reproduction. It seems as if the smaller species reported by Ishikawa and Yamasu (1992) is the best reproducer with a reproductive rate, RR, of 0.8 progeny per individual per day followed by *C. retrogemma* with 0.52 progeny per individual per day. Hanson (1960) reports a fast initial reproduction in *Amphiscolops langerhansi*, one new progeny per day for the first two days and then a slowdown to an RR of 0.5 progeny per individual per day recorded over 18 days.

Reproduction as described above for *C. longifissura* proved to be slower. It has an RR of 0.32. Data on the intrinsic rate of increase, *r*, a commonly used measure of fitness, have not yet been obtained. In *C. retrogemma*, Åkesson and Hendelberg (1989) recorded an *r* of 0.655, a remarkably high figure relative to fragments produced by polychaetes of the same size (Paavo *et al.* 2000).

Asexual reproduction of *Convolutriloba* species can be very effective in marine aquaria, leading to explosive growth of the population. The organisms then form coats over large aereas in the aquaria (see also http://www.marin.de/salzwasser/deutsch/Berichte/Planarien/planarien.htm). However, little is known of conditions affecting their reproduction.

Diversity of asexual reproduction in the Acoelomorpha

As mentioned in the introduction, asexual reproduction is rare in the Acoelomorpha. It is not known in one of the two

Table 1 Variation in asexual reproduction in acoelous turbellaria

Species	Architomy	Paratomy	Budding
Adenopea cenata	x		
Amphiscolops langerhansi	x		
Convolutriloba longifissura	x		
Convolutriloba retrogemma			x
Convolutriloba sp. (Okinawa)			x
Paratomella rubra	x		
Paratomella unichaeta		x	
Pseudhaplogonaria macnaei	x		x

subtaxa, the Nemertodermatida. Within the Acoela it is described only from certain genera of 4 out of the 17 known families: (1) in the genus *Convolutriloba* (recently moved to the Sagittiferidae because of the discovery of sagittocysts – see Gschwentner *et al.* 1999); (2) in certain species of the genera *Amphiscolops* and *Adenopea* of the Convolutidae; (3) in *Pseudohaplogonaria macnaei* of the Haploposthiidae; and (4) in *Paratomella unichaeta* of the Paratomellidae. Ehlers (1992) mentions the occurrence of paratomy also for the only other described species of the genus *Paratomella*, *P. rubra* (see also Crezee 1982).

Even if there is no longitudinal fission of the whole animal as described by Bartolomaeus and Balzer (1997) there is still a remarkable variation in asexual reproduction in acoelous turbellarians (Table 1). Fission by architomy can result in a variable number of progeny: one to several in *Pseudohaplogonaria macnaei*, two in *Convolutriloba longifissura*, one in *Convoluta cenata* and in *Amphiscolops langerhansi*. In *Adenopea cenata* asexual reproduction seems to be obligatorily connected with sexual reproduction. Fission by paratomy is only reported for *Paratomella* where it results in chains of up to three zooids. Reproduction by budding is now known from *Pseudohaplogonaria macnaei*, *Convolutriloba retrogemma* and *Convolutriloba* sp. (Okinawa).

Because the majority of species with asexual reproduction also harbour algal symbionts, it would be useful to study possible correlations between these two traits.

The combination of infrequent occurrence and high diversity of modes of asexual reproduction is difficult to interpret in evolutionary terms. On one hand, one could argue that the high diversity of asexual strategies is a primitive trait, characteristic of many early metazoans and present in the extant platyhelminth fauna only in disjunct taxa. We have addressed this argument in the introduction of this paper. On the other hand, the rare cases of asexuality observed in the Acoela could be interpreted as a sign of multiple origins of asexual reproduction in response to certain environmental needs, as has been suggested earlier by Ax and Schulz (1959). However, Smith and Tyler (1985) and Ehlers (1992) have provided structural evidence for the Paratomellidae to be the most primitive member of the Acoela. The Acoela, and

especially *Paratomella*, were shown to be the basal-most side branch in the phylogenetic tree of the Bilateria in a recent analysis of 18S rDNA in the free-living Platyhelminthes (Ruiz-Trillo *et al.* 1999). Asexual reproduction may therefore indeed be plesiomorphic in the Acoela.

Acknowledgements

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