

Evolution of larval form in ophiuroids: insights from the metamorphic phenotype of *Ophiothrix* (Echinodermata: Ophiuroidea)

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SUMMARY Comparison of development through metamorphosis in *Ophiothrix* species provided insights into the evolutionary relationships between Type I (ophiopluteus only) and Type II (ophiopluteus and vitellaria) patterns of development in the Ophiuroidea. As typical of Type I developers, the six inner larval arms in *Ophiothrix spongicola* were fully resorbed at metamorphosis and no remnants of ciliated epithelia were retained. The postero-lateral arms function as locomotory organs for the developing juvenile and were discarded at settlement. In contrast, in *O. ciliaris* the epithelia of the inner arms were transformed into ciliated ridges, similar to those seen in vitellariae and the postero-lateral arms were resorbed rather than being discarded. Larval arm resorption in *O. ciliaris* is similar to that in Type II developers. The metamorphic

phenotype of *O. ciliaris* provides a link between Type I and II development. The presence of two types of metamorphosis in congeneric ophiuroids and the variable metamorphic phenotype of *O. ciliaris* was unexpected. It appears that closely related ophiuroids and individual species may have the capacity to metamorphose using either Type I or Type II pathways. Although the phylogenetic distribution of metamorphic phenotypes indicates that Type II development may be the ancestral state, comparative morphology suggests that a developmental dichotomy based on larval arm resorption may not be appropriate for the Ophiuroidea. Until metamorphosis is characterized for more taxa, the ancestral developmental mode for the Ophiuroidea will remain a matter of conjecture.

INTRODUCTION

Morphological evolution in echinoderms has resulted in an array of larval phenotypes. Echinoderm larvae that must feed to complete their development (planktotrophic) are equipped with a ciliary band that is used for swimming and feeding. Development through a feeding larva is considered to be the plesiomorphic condition for living echinoderms (Strathmann 1975; Neilsen 1998). Echinoderm larvae that do not feed to complete their development (lecithotrophic) have a simplified larval form with a reduced ciliary band used for swimming only, or they can be uniformly ciliated.

The basic dichotomy between feeding and nonfeeding larval forms holds well for the best studied echinoderms, the echinoids and asteroids (Raff 1987, 1996; McEdward and Miner 2001; Emler et al. 2002). For ophiuroids, however, development is also categorized according to the phenotype of the metamorphic larva (Mortensen 1921, 1931; Mladenov 1985a; Byrne and Selvakumaraswamy 2002; Cisternas et al. 2004; Cisternas and Byrne 2005). Mortensen (1921) was the first to recognize differences in larval arm resorption and ciliary band rearrangement during ophiuroid metamorphosis. Mladenov (1985a) later suggested that the differences indi-

cated the presence of two developmental types for ophiuroids (Type I and II).

Type I developers have one larval form, the ophiopluteus, whereas Type II developers have the vitellaria larva and may also have an initial ophiopluteal stage (Hendler 1982; Mladenov 1985a; Byrne and Selvakumaraswamy 2002; Selvakumaraswamy and Byrne 2002a, 2004; Cisternas et al. 2004). In Type I developers the six inner larval arms are resorbed at metamorphosis and the postero-lateral arms are retained as locomotory structures to support the developing juvenile (Mortensen 1921, 1931; Mladenov 1979; Hendler 1991). The presence of a rudiment suspended between two arms, a sight often seen in plankton samples, is the defining feature of Type I development. Type I developers with lecithotrophic larvae have yolky plutei with a reduced number of arms and retain the postero-lateral arms to support the rudiment (Mortensen 1921; Fenaux 1963; Mladenov 1979).

In the Type II developer with a feeding ophiopluteus, *Ophiocoma pumila*, all the larval arms are resorbed with the right anterolateral arm being the last to disappear (Mladenov 1985a). During arm resorption in *O. pumila* the ciliary band epithelia are transformed into inter-radial locomotory ciliated ridges around the developing rudiment as the larva trans-

forms into the vitellaria stage (Mladenov 1985a). A similar pattern of arm resorption and formation of ciliary ridges is described for other ophiuroids (Grave 1903; Mortensen 1921, 1931). Most Type II developers have a single larval stage, the nonfeeding vitellaria (Brooks and Grave 1899; Grave 1903; Mortensen 1921, 1938; Fenaux 1969; Stancyk 1973; Komatsu and Shosaku 1993; Cisternas et al. 2004; Cisternas and Byrne 2005). The presence of a reduced nonfeeding pluteal stage prior to the vitellaria in some Type II developers provides a link to an ancestor that had a planktotrophic larva and supports the notion that the ophiuroid vitellaria, while a distinct stage, may be regarded as the rudiment plus ophiopluteal remnants (Hendler 1982; Mladenov 1985a; Selvakumaraswamy and Byrne 2002a, 2004).

Type I development in ophiuroids through a two-armed metamorphic stage is typified by the genus *Ophiothrix*, as illustrated by the six species that have been reared through metamorphosis (MacBride 1907; Mortensen 1921, 1937, 1938; Mladenov 1979, 1985b; Selvakumaraswamy 2002; Morgan and Jangoux 2005). Our observations with *O. ciliaris* indicated that metamorphosis in this species did not follow the *Ophiothrix* pattern and that the distinction between Type I and II development may need re-examination (Selvakumaraswamy 2002). In this study we use the comparative approach to assess the evolution of development in *Ophiothrix* by exploring how changes to the metamorphic stage have influenced the larval phenotype. Particular attention was paid to the pattern of larval arm resorption in *O. spongicola* and *O. ciliaris* because this feature is used to distinguish between Type I and II development (Mortensen 1921). Our results revealed an unexpected variation in the metamorphic phenotype between congeneric species and variation in metamorphosis within a single species. We revisit the hypothesis (Mortensen 1921; Mladenov 1985a) that the Ophiuroidea has two developmental modes (Type I and II), distinguished by differences in larval arm resorption and ciliary band rearrangement at metamorphosis. We used inferences gained from *Ophiothrix* to suggest potential pathways involved in the evolution of larval form and examined the phylogenetic distribution of the two types of development in the Ophiuroidea for insights into the ancestral state.

MATERIALS AND METHODS

Specimens of *O. spongicola* and *O. ciliaris* were collected on snorkel from several sites in NSW, Australia including Sydney (33°54'S; 151°17'E) and Shell Harbour (34°36'S; 151°54'E). Both species were found under boulders, with *O. spongicola* being found only under sponge covered boulders. Specimens spawned spontaneously after collection or were induced to spawn using temperature and light shock (see Selvakumaraswamy and Byrne 2000b). *O. spongicola* and *O. ciliaris* larvae were cultured at $21 \pm 2^\circ\text{C}$ and

$19.5 \pm 1.5^\circ\text{C}$, respectively. Larvae were cultured in replicate glass beakers (500 ml or 3.5 l) in 1 μm filtered sea water (FSW) at densities of approximately 1/ml and kept constantly stirred. Larvae were fed the diatom *Chaetoceros calcitrans* every 2 days at concentrations of 2×10^4 cells/ml and the FSW was changed every 4 days by reverse filtration. Both species underwent metamorphosis without the need to introduce a settlement cue.

For *O. spongicola*, development through metamorphosis was documented from five independent fertilizations (April–May 1997 and March 1998) each consisting of two to four females and at least two males. Two of these fertilizations generated cultures that developed to the settled juvenile stage. The other three fertilizations generated cultures that developed to metamorphosis but never settled. For *O. ciliaris* complete development through metamorphosis to settlement was documented from two independent fertilizations (March and June 2001) each of which was from one male and one female pair. Larvae were examined live with light microscopy (LM) or fixed for 1 h in 2% paraformaldehyde in 0.2 μm FSW or 2.5% glutaraldehyde in 0.2 μm FSW and stored in 70% ethanol, followed by dehydration and examination as whole-mounts. Larvae were examined every 2–4 days during development and closer to metamorphosis were examined daily.

RESULTS

O. spongicola and *O. ciliaris* have small eggs, 128 (range: 123–140; SE = 1.2, 20 eggs from two females) and 98 μm (range: 88–121; SE = 1.89, 22 eggs from two females) diameter, respectively. They developed into typical eight-armed ophioplutei with straight, long and thorny postero-lateral arms, as characteristic of ophiotrichid ophioplutei (Fig. 1A). The earliest sign of metamorphosis, 20 days in *O. spongicola* and 25 days in *O. ciliaris*, was marked by the onset of resorption of the six inner larval arms and the formation of the five lobed hydrocoel as a linear structure on the left side of the oesophagus (Fig. 1B). The timing of the start of metamorphosis was variable both within and between independent cultures for both species. Development times in Table 1 reflect the earliest sign of each stage observed. The hydrocoel twisted behind the oesophagus in a dorsal direction and eventually encircled the oesophagus. The most anterior lobe of the hydrocoel was the first to shift behind the oesophagus and therefore when viewed ventrally appeared smaller than the posterior lobes (Fig. 1B). Prominent growth of the hydrocoel on the left side caused the oesophagus to shift slightly to the right of the anterior/posterior (A/P) axis (Fig. 1B). After hydrocoel development, the larval body and arms continued to be resorbed. As the oral hood was resorbed, the oesophagus and associated circular hydrocoel rotated from the plane of the A/P axis into the plane of the juvenile oral/aboral (O/A) axis. Feeding ceased and the buccal cavity was reduced. The floor of the mouth bordered by the adoral ciliary band was retained (Fig. 1B) and contributed to formation of the juvenile mouth.

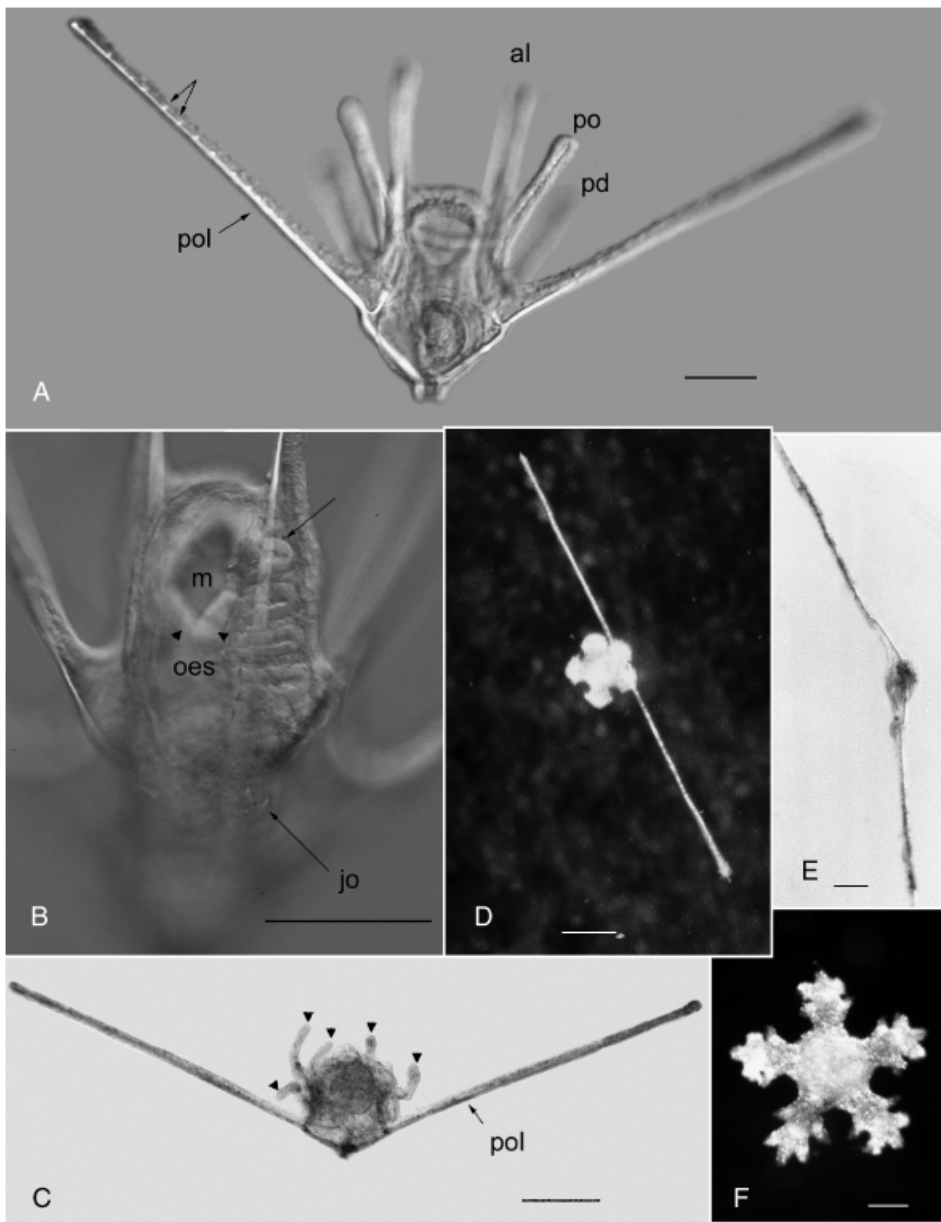


Fig. 1. *Ophiothrix spongicola*. Light microscopy images of metamorphosing larvae with anterior toward the top of the page. (A) Typical eight-arm *Ophiothrix* ophiopluteus showing straight postero-lateral arms (pol) with thorns along the inner edge of the arm rod (arrows). (B) Ventral view showing the 5-lobed hydrocoel alongside the oesophagus (oes). The most anterior lobe appears smallest (arrow) because it has started to rotate dorsally to encircle the oesophagus. Resorption of the larval mouth (m) is underway, but the posterior rim associated with the adoral ciliary band is retained (arrowheads). (C) The resorbing larval arms are no longer supported by skeleton (arrowheads) and have collapsed around the rudiment. The postero-lateral arms are retained to support the rudiment and as locomotory structures. (D) Swimming metamorphosing larva with fully formed juvenile rudiment suspended between the postero-lateral arms. (E) Released postero-lateral arms swimming in culture. (F) Settled juvenile with 2 arm segments. al, antero-lateral arm; jo, juvenile ossicle; pd, postero-dorsal arm; po, post-oral arm. Scale bars (A–C, E, F) = 100 μ m, (D) = 250 μ m.

The postero-dorsal, post-oral and antero-lateral arms of the plutei of both species, degenerated distal end first and were resorbed into the rudiment (Figs. 1C and 2A). The skeletal rods fragmented or became split along their length during resorption (not illustrated) and were not incorporated into the juvenile skeleton. The rods were resorbed before the arm epithelia, causing the larval arms to collapse to form small protrusions extending from the rudiment (Figs. 1C and 2, A–C).

In *O. spongicola*, the antero-lateral, post-oral and postero-dorsal arms were reduced synchronously forming small bumps in variable positions around the rudiment (Fig. 1C). These were fully resorbed, as is typical of Type I developers.

There were no remnants of ciliary band epithelium. The rudiment developed a pentamerous shape as the arms were resorbed (Fig. 1C). Resorption of the three inner arm pairs was complete 2–3 days following the initiation of metamorphosis (Table 1). The postero-lateral arms were retained as locomotory structures to support the developing rudiment, this stage being termed the suspended rudiment stage (Fig. 1, C and D). The position where the rudiment developed was slightly (10°) offset to the left of the larval A/P axis. The suspended rudiment stage swam in culture for 2–3 days before discarding the postero-lateral arms. These larvae had two arm segments and settled to the bottom of the culture dish by 31 days, with

Table 1. Chronology of development in *Ophiothrix spongicola* and *O. ciliaris* reared at $21 \pm 2^\circ\text{C}$ and $19.5 \pm 1.5^\circ\text{C}$, respectively

Developmental stage	Times	
	<i>O. spongicola</i>	<i>O. ciliaris</i>
Hatched blastulae	16 h	10–12 h
Gastrulae	22 h	16–18 h
Early prism	35 h	36 h
Two-arm plutei	40 h	40 h
Six-arm plutei and mouth open	60 h	4 days
Eight-arm plutei	4 days	6.5 days
Start of resorption of three inner arm pairs	20–24 days	18 days
Five-lobed hydrocoel	20 days	21 days
Suspended rudiment stage +PL arms	28 days	25 days
Benthic juvenile with two-arm segments +PL arms	31 days	31 days
Benthic juvenile	33 days	32 days

Times indicate the first appearance of the developmental stage.

their attached postero-lateral arms. The postero-lateral arms could be maneuvered around the dorsal surface of the juvenile so as to not inhibit movement. The postero-lateral arms in *O. spongicola* were discarded by benthic juveniles after 1–2 days and these arms swam in culture for 1–2 days before they degenerated (Fig. 1E). Newly settled juveniles had two arm segments with claw-like arm spines (Fig. 1F) and a mean disc diameter of $195 \mu\text{m}$ (range: $151\text{--}250 \mu\text{m}$, $n = 6$).

For *O. ciliaris*, the sequence of larval arm resorption varied among plutei in all cultures. In most larvae (ca. 80%), the postero-dorsals, post-orals and the left antero-lateral arm were resorbed first, with the right antero-lateral arm being resorbed last (Fig. 2A). This pattern of arm resorption is similar to that described for Type II developers (Mortensen 1921, 1931; Mladenov 1985a). In the remaining larvae (ca. 20%) the six inner arms resorbed synchronously, as seen in *O. spongicola*.

In most *O. ciliaris* larvae the epithelia of the inner larval arms were retained as small ciliated ridges positioned in the inter-radii of the developing juvenile (Fig. 2C). These ridges were present for 2–3 days and were strikingly similar to the inter-radial ciliary ridges of the vitellariae of Type II developers (Fig. 2, C and D). In contrast to *O. spongicola*, the postero-lateral arms were not discarded by the larvae of *O. ciliaris* (Fig. 2B). Resorption of the postero-lateral arms occurred in pre-settlement swimming larvae (Fig. 2B) or in newly settled juveniles. In settled juveniles, the postero-lateral arms appeared as spikes extending from the side of the disc as they were resorbed (Fig. 2E). Like *O. spongicola*, the rudiment in *O. ciliaris* also developed to the left of the larval A/P axis. Newly settled juvenile *O. ciliaris* had two arm segments with

claw-like arm spines and a mean disc diameter of $122 \mu\text{m}$ (range: $90\text{--}147 \mu\text{m}$, $n = 4$).

DISCUSSION

As typical of Type I development in ophiuroids the six inner larval arms in *O. spongicola* were fully resorbed in a synchronous pattern whereas the two outer arms, the postero-laterals, were retained as locomotory structures until settlement. This Type I metamorphosis occurs in other *Ophiothrix* species (MacBride 1907; Mortensen 1937, 1938; Mladenov 1979, 1985b; Morgan and Jangoux 2005). Although the discarded postero-lateral arms swam in culture for 1–2 days before they degenerated, there was no indication that they had the capacity to regenerate into new larvae as occurs in *Ophiopholis aculeata* (Balsler 1998).

Metamorphosis in *O. ciliaris* differed from that seen in *O. spongicola* and other *Ophiothrix* species in the following features: (1) asynchronous resorption of the inner arms, (2) transformation of the larval arm epithelia into inter-radial ciliated ridges around the rudiment, and (3) resorption of the postero-lateral arms. These features occur in *Ophiocoma pumila* and other ophiuroids with Type II development (Mortensen 1921, 1931; Mladenov 1985a). Like *O. pumila*, the right antero-lateral larval arm was the last to be resorbed in *O. ciliaris*. In addition, the inter-radial ridges in *O. ciliaris* developed in the same location as ciliary ridges do in vitellaria larvae. Possession of a larva with vitellaria-like features in *O. ciliaris* suggests a previously undetected link between Type I and II development in the Ophiuroidea.

Are Type I and II developmental pathways divergent?

The suspended rudiment of Type I developers and the vitellaria of Type II developers both serve as pre-settlement stage larvae. There has been much speculation on the affinities of one versus two larval phases in ophiuroid development and whether they represent separate lines of evolution (Review, Hendler 1991). Hypothetical pathways for the evolution of larval forms in the Ophiuroidea are shown in Fig. 3. If Type I development (ophiopluteus only) was ancestral (Fig. 3A), then evolution of the vitellaria would have arisen through retention of cilia during larval arm resorption on epithelial ridges (Hendler 1982; Mladenov 1985a). In this scenario, a relatively minor change in development, retention of cilia on ridges, would have been involved in the evolution of the vitellaria (Fig. 3A). In contrast, if Type II development (ophiopluteus and vitellaria) was ancestral (Fig. 3B), then Type I development would have arisen through loss of the vitellaria stage, as suggested by McEdward and Miner (2001). In this case the vitellaria-like ridges in metamorphosing *O. ciliaris*

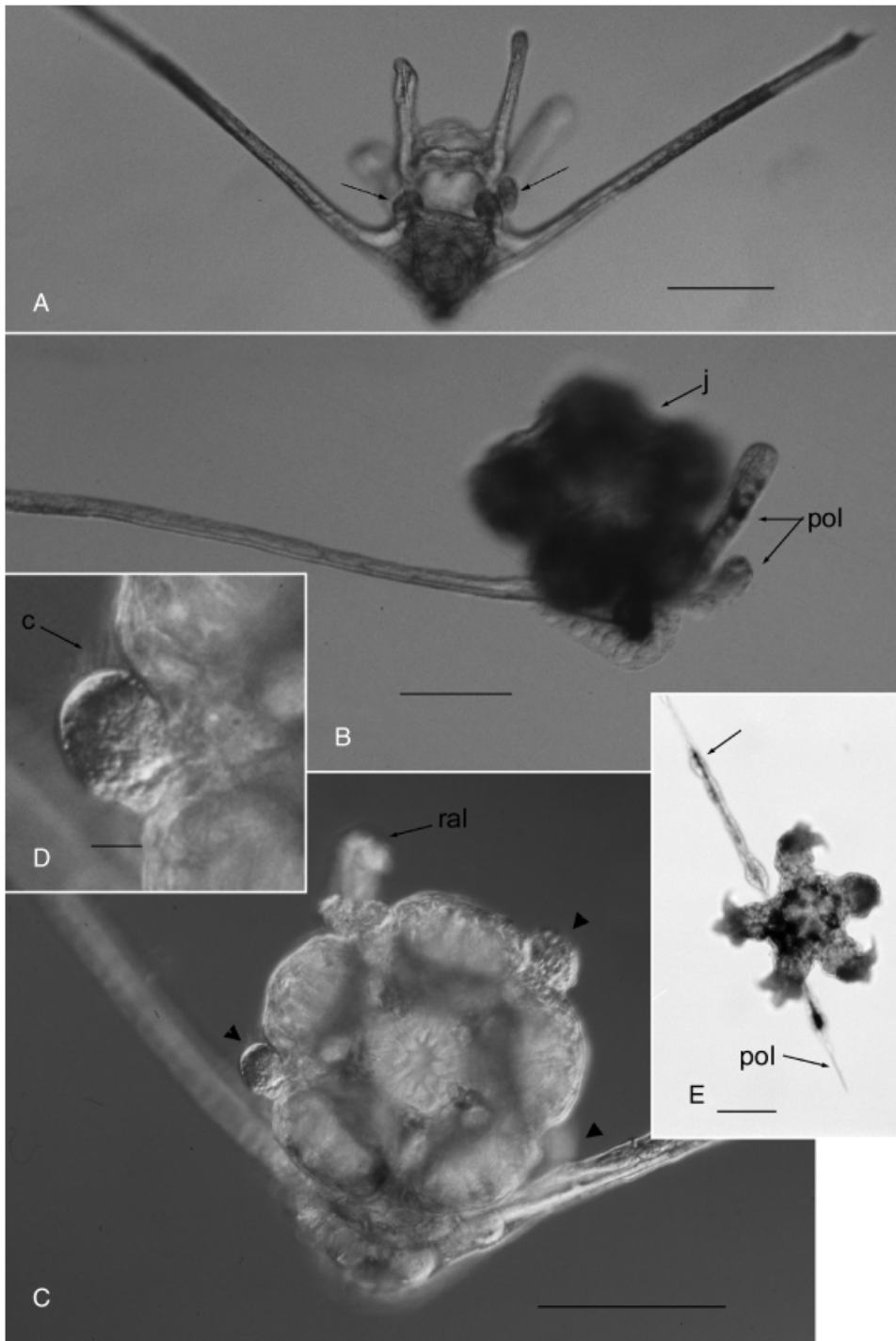


Fig. 2. *Ophiothrix ciliaris*. Light microscopy images of metamorphosing larvae with anterior toward the top of the page. (A) Each larval arm resorbs in a distal-proximal direction. The post-oral arms have resorbed first (arrows). (B) Pentaradiate juvenile (J) in between the postero-lateral arms with left one being resorbed. (C) The inner pairs of larval arms have transformed to inter-radial ridges (arrowheads), similar to the ciliated ridges in vitellariae. The right antero-lateral arm is last of the inner arms to be resorbed. (D) Higher power of (C) showing cilia on the inter-radial ridges. (E) Benthic juvenile resorbing the postero-lateral arms. One arm has nearly fully resorbed into the rudiment. c, ciliary ridge; j, juvenile; m, mouth; pol, postero-lateral arm; ral, right antero-lateral arm. Scale bars (A–C, E) = 100 μm ; (D) = 25 μm .

would be vestigial structures retained from an ancestor with a vitellaria larva. Alternatively, the ancestral form could have resembled *O. ciliaris*, exhibiting phenotypic variability at metamorphosis (Fig. 3C), with the capacity to metamorphose using either the Type I or Type II pathways.

If Types I and II development are both found within genera or clades of closely related species, this would indicate that the two developmental pathways are not divergent. The ancestral form may have had the capacity to produce a juvenile suspended between two long larval arms (Type I), or a

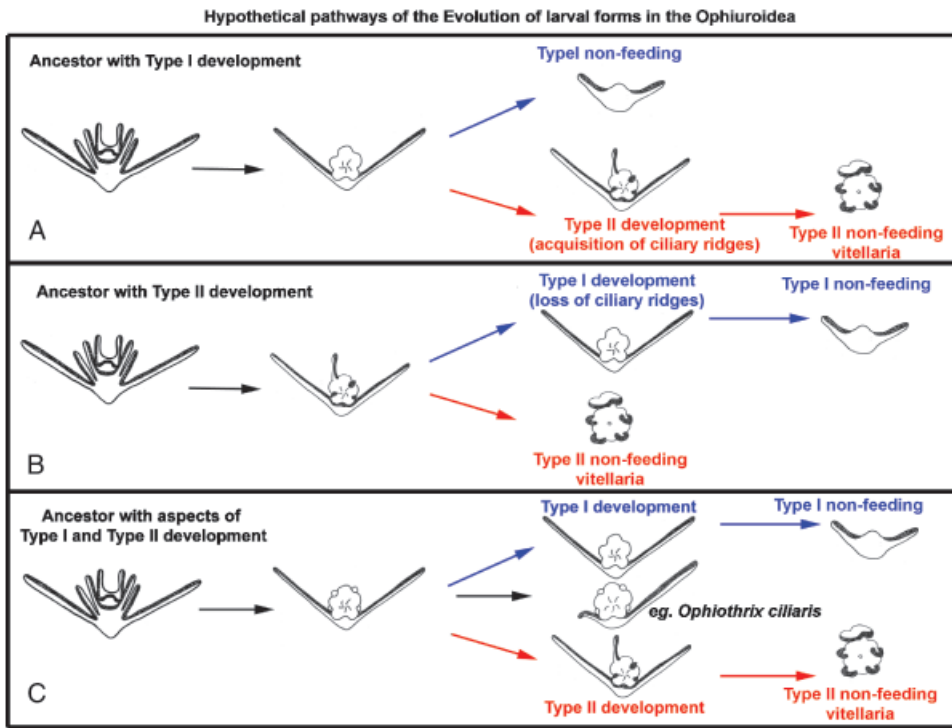


Fig. 3. Potential pathways in evolution of the metamorphic phenotype of ophiuroids with a feeding larval stage.

juvenile with ciliated ridges (Type II) (Fig. 3C). These two types of metamorphosing larvae both serve the same function as a pre-settlement stage. Examination of swimming efficiency might indicate if there are functional differences between a larva swimming with two long ciliary bands on arms or with a series of ciliated ridges. In species such as *O. ciliaris* that have the capacity to vary the metamorphic phenotype, the selective pressures faced by larvae close to metamorphosis may influence the phenotype.

We know that metamorphic forms in the Ophiuroidea range from the typical Type I pluteus of *O. spongicola* to the Type II developers that metamorphose as vitellariae (Hendler 1982, 1991; Mladenov 1985a; Cisternas et al. 2004; Selvakumaraswamy and Byrne 2004). Our observations of *O. ciliaris* present evidence of a vitellaria-like larva within a genus of Type I developers. Such variation in types of metamorphosis in congeners was not expected. The pattern of metamorphic change documented recently for the ophiactid species *Ophiopholis formata* and *Ophiactis resiliens* provides a further example of how pluteal arm resorption can differ among closely related ophiuroids (Selvakumaraswamy 2002; Cisternas et al. 2004; Tominaga et al. 2004). *Ophiopholis formata* has a typical feeding ophiopluteus and resorbs all its arms during metamorphosis. The left postero-lateral arm is resorbed first followed by the right and so these arms are not used to support the juvenile (Tominaga et al. 2004). Although this is similar to the Type II pattern of metamorphic change, there was no indication of the presence of vitellarial-like ciliated ridges. In contrast, the

epithelia of the inner larval arms of *O. resiliens* are transformed into ciliated ridges in a vitellarial form yet the postero-laterals are retained through metamorphosis as locomotory

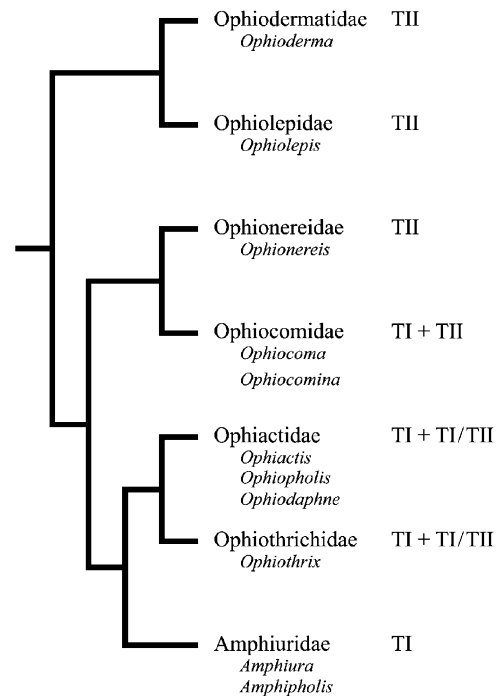


Fig. 4. Inferred family phylogenetic tree for selected Ophiuroidea (after Smith et al. 1995), showing developmental mode at metamorphosis where known.

structures and are discarded at settlement (Selvakumaraswamy 2002).

The presence of vestigial ophiopluteal features in vitellariae of *Ophionereis* species indicates that an ancestral state with a feeding pluteus is shared by Types I and II development (Hendler 1982; Selvakumaraswamy and Byrne 2000a, 2004). From data on the phylogenetic distribution of Types I and II development (Fig. 4) we know that *Ophiothrix*, *Ophiopholis*, *Amphipholis*, *Amphiura*, and *Ophiocomina* are characterized by Type I development with a suspended rudiment, whereas *Ophionereis*, *Ophiolepis*, *Ophioderma*, and *Ophiocoma* have Type II development with a distinct vitellaria (Brooks and Grave 1899; Mortensen 1913, 1938; Narasimhamurti 1933; Olsen 1942; Fenaux 1963, 1969; Hendler 1982; Mladenov 1985a; Yamashita 1985; Selvakumaraswamy and Byrne 2002a, 2004; Cisternas et al. 2004). *Ophiodaphne*, *Ophiothrix*, *Ophiactis*, and *Ophiura* have species with features of both types of development (Type I or II) based on the pattern of ophiopluteal arm resorption (Mortensen 1931; Selvakumaraswamy 2002; Cisternas et al. 2004; Tominaga et al. 2004). The weight of evidence for a number of genera indicates that the ophiuroid metamorphic phenotype varies more than previously appreciated. As more data have become available for less well-studied genera, it appears that the Type I–II dichotomy identified by Mortensen (1921) and Mladenov (1985a) may not be appropriate for the Ophiuroidea.

Placing current information on the metamorphic phenotype in a phylogenetic perspective shows the distribution of Type I, II and I/II development in ophiuroid clades (Fig. 4). Type II development appears to be more widely distributed across ophiuroid families than Type I development, potentially supporting the notion that Type II development is the ancestral state, as suggested by McEdward and Miner (2001). However, more data on the metamorphic phenotype of closely related species across the spectrum of ophiuroid diversity are needed to assess the evolution of development in the Ophiuroidea.

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