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Bi-Penta-Bi-Decaradial Symmetry: A Review of Evolutionary and Developmental Trends in Holothuroidea (Echinodermata)

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ABSTRACT Holothuroidea, comprising the sea cucumbers, is the least studied class of extant echinoderms, yet this group possesses a wealth of features of potential interest to developmental and evolutionary biologists. Holothuroids include the most morphologically derived echinoderms, including pelagic species and spheroid, plated taxa with mouth and anus adjacent at the end of a long, flexible stalk. To begin investigating this diversity of body form, we first estimated evolutionary relationships in the class Holothuroidea based on maximum parsimony analyses of 1,075 nt of the nuclear small subunit rDNA (for six species in four orders) and on 52 informative morphological characters (for the 25 extant families). Both the morphological and molecular evidence suggests almost an inversion of the prevailing higher level classification. Character-state optimizations indicated that pronounced adult bilateral symmetry evolved three times. In one group even a regain of secondary radial symmetry is found. Respiratory trees, structures unique to holothuroids, are a relatively late innovation, are ectodermally derived, and are bilaterally symmetric, supporting the possibility that the secondary gain of bilateral symmetry in holothuroids is ectodermally derived analogous to, say, the derivation of vertebrate limb dorso-ventral axis. The test of imbricating plates found in 10% of living holothuroids is apparently not homologous with that of other heavily armored echinoderms, evolving much later and at least twice. Indirectly developing larvae, auriculariae, occur in two evolutionarily disparate clades and unlike echinoids comprise a minority of clades. We suggest that this implies the parallel convergent evolution of this larval type or, more speculatively, some form of retention of developmental constraints. *J. Exp. Zool. (Mol. Dev. Evol.)* 285:93–103, 1999. © 1999 Wiley-Liss, Inc.

Model organisms constitute a small and phylogenetically far-flung assemblage that continues to play a key role in identifying developmental mechanisms that are conserved across wide breaches of evolutionary time. Numerous other developmental strategies, though, either experience frequent evolutionary reversals or are unique to a restricted group of organisms. These latter traits often still provide important generalizations (Raff, '96), but require more formal comparative assessments, often including molecular phylogenetics, to establish the polarity of the characters and to follow state changes in correlated traits. Despite the profound success of studies involving either model organisms or the more recent comparative phylogenetic approach to developmental questions, legions of organisms with potentially enlightening takes on development remain uninvestigated.

The primary aim of this paper is to review and introduce to developmental and evolutionary biologists a poorly known group of animals of po-

tential value as model organisms and exemplars in comparative studies of development. We discuss, from a largely phylogenetic and developmental perspective, some novel and interesting aspects of the morphological evolution of the least celebrated class of echinoderms, the Holothuroidea or sea cucumbers. We first provide an introduction to the organisms' biology, including what little is suspected about their evolution, and then advance the first phylogeny for this class based on molecular and morphological data. Finally, we use the phylogeny to illustrate the organisms' potential utility as exemplars in three areas of current

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interest in developmental evolution: origin of body symmetry, origin of larval mode of development, and skeletogenesis.

NATURAL HISTORY OF HOLOTHUROIDEA

Holothuroids, or sea cucumbers, are "echinoderm worms" (Smiley, '94). They are elongate, mostly soft-bodied animals with tubefeet often concentrated ventrally. The mouth, ringed with large digitate, peltate or dendritic feeding tentacles, lies at or near one end and the anus, opposite. Holothuroids possess a single gonad in contrast to the multiple and radially arranged gonads of other echinoderms. Found exclusively in this group, is the calcareous ring, a circumpharyngeal calcitic band of usually 10 plates and likely homologous to echinoid apical plates (David and Mooi, '96). Hyman ('55) provides a comprehensive account of holothuroid gross anatomy; Smiley ('94) covers microscopic aspects, while Smiley et al. ('91) reviews reproduction and larval development.

Holothuroids are an abundant and diverse group of marine invertebrates. The approximately 1,400 described and extant species comprising 200 genera (Smiley, '94) occur in benthic environments from the intertidal to the deepest oceanic trenches, where they may comprise greater than 90% of the sampled biomass (Baelev, '72). Unique among echinoderms, holothuroids can be holopelagic, living entirely in the water column (Miller and Pawson, '90); can be ectocommensals, living attached to fish (Martin, '69); or reach lengths over 5 m (Mortensen, '38) and weigh over 5 kg (Lane, '92). Their diversity is highest on Indo-Pacific coral reefs, where 20 species per hectare is not uncommon (Kerr et al., '93). In fact, the ubiquity of holothuroids in the largest ecosystem, the abyssal plain, ostensibly renders them one of the dominant large animals on earth.

Despite their dominance, diversity, and the scrutiny paid to other echinoderm groups, basic and long-standing questions about the evolution of Holothuroidea remain unresolved. Much of this uncertainty stems from the group's lack of an integrated skeleton, a feature that provides the numerous phylogenetically informative characters and the extensive fossil records of other echinoderms (Lafay et al., '95; Smith et al., '95). Instead, the holothuroid skeleton consists of a calcareous ring and isolated, often microscopic spicules embedded in the connective layer of the dermis. These spicules are important taxonomically, as are

the calcareous ring and some soft-tissue features, but surprisingly, none have been systematically surveyed for ontogenetic (Massin, '94; Wiedemeyer, '94; Cutress, '96) and interspecific variation (Cherbonnier, '47; Hansen, '75). Moreover, the fossil record of holothuroids consists almost entirely of isolated spicules, necessitating their classification in an artificial arrangement as paraspecies, several likely important forms of which cannot be confidently assigned to Holothuroidea because they resemble non-holothuroid fossils (Gilliland, '93).

Current speculations about the evolution of Holothuroidea derive largely from the Linnean classification of Pawson and Fell ('65), who, based on perceived derived characters, divided holothuroids into three subclasses, each with two orders. Pawson and Fell suggest that holothuroids diversified from a progenitor possessing a mosaic of putatively primitive features from members of the subclass Dendrochirotea who are plated and possess respiratory trees and a complex calcareous ring. Such a view suggests that pedomorphosis has been a dominant trend in the evolution of Holothuroidea (Théel, 1885; Semon, 1888; Madsen, '61; Haude, '92; David and Mooi, '98). That is, the majority of lineages, which lack these structures, are thought to have experienced losses or reductions of features in the ancestral adult. Numerous aspects of Pawson and Fell's scheme, however, are open to interpretation (Hansen, '75; Pawson, '84; Haude, '92; Gilliland, '93), but essentially no new information corroborating or challenging their proposal has come to light in the last 34 years. The most comprehensive review of holothuroid evolution and paleontology occurs in Gilliland ('93), but Frizzell and Pawson ('66), Pawson ('80) and Haude ('92) also provide important perspectives.

NEW ESTIMATE OF HOLOTHUROID PHYLOGENY

A radically different interpretation of evolution in Holothuroidea is suggested by the first estimate of higher level relationships, which we introduce here (see Arndt et al. ['96] for relationships within dendrochirote holothuroids). Our phylogeny is based on maximum parsimony analyses of 1,075 nt of the nuclear small subunit rDNA (for six species in four orders) and on 52 informative morphological characters (for the 25 extant families; a complete systematic treatment, including character-state assignments will appear elsewhere). Briefly however, the result of the phylo-

genetic analysis is surprising. The phylogenies indicate the need for a fundamental reorganization of holothuroid systematics at the ordinal and subclass levels. For example, molpadiid families such as Molpadiidae have been placed in the same subclass as that including Synaptidae (Pawson and Fell, '65), but these groups appear only distantly related (Fig. 1). This nearly complete inversion of the prevailing classification has important consequences for our inferences about the evolution of developmental modes as we discuss below. It also indicates that current taxonomic groupings have likely been diagnosed from only subsets of the available characters. Finally, the molecular- and morphology-based phylogenies are not entirely congruent. Until such uncertainties are resolved, we restrict the discussion below to inferences that

are independent of the two likely arrangements of Elasipodida and Apodida at the base of the holothuroid phylogeny.

MORPHOLOGICAL EVOLUTION WITHIN HOLOTHUROIDEA

Adult body form

Living echinoderms begin their lives as bilaterally symmetrical larvae. The well-known pentamerous symmetry typical of echinoderms is acquired only later, beginning when the larval middle coelomic sac (left hydrocoel) forms a ring from which arise five water-vascular rudiments. In post-larval echinoderms, most organ systems and appendages are arranged five-fold around a central to subcentrally placed digestive system. Figuring out

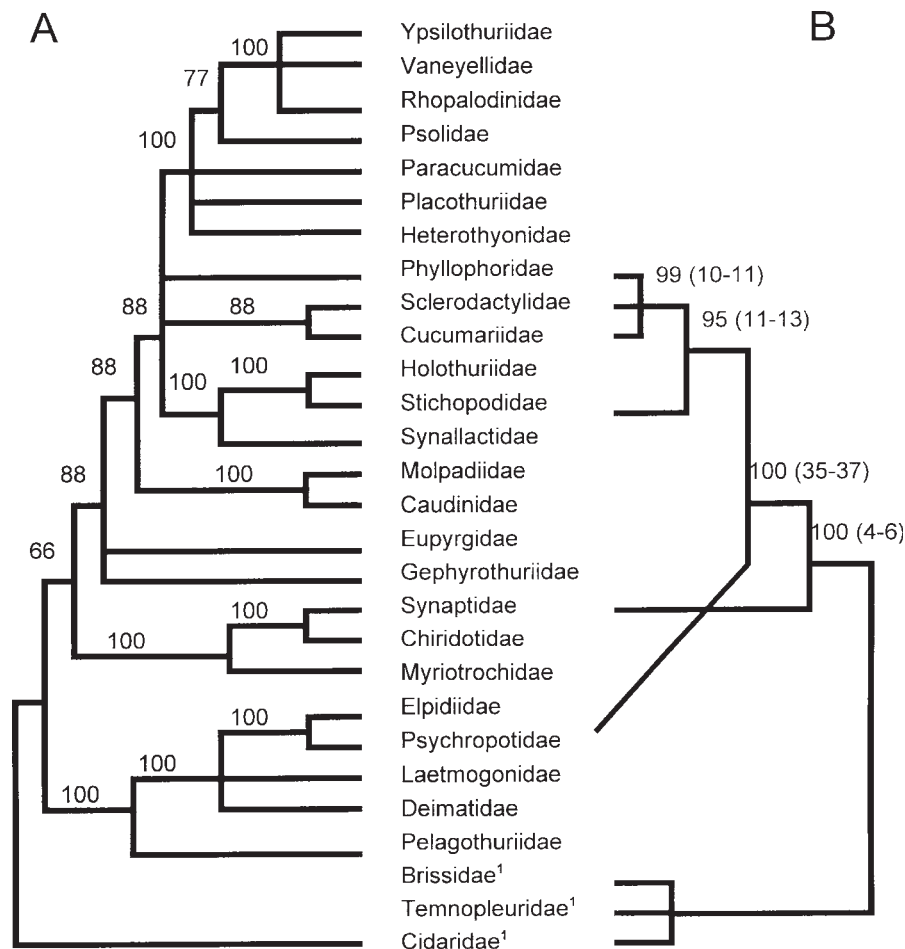


Fig. 1. Phylogenies of Holothuroidea. Superscripted names indicate echinoid outgroups. **A:** Majority-rule consensus of the 561 most parsimonious trees from 52 morphological characters. Length = 79, CI' = 0.76–0.82, RI = 0.87–0.91. Numbers on tree are percent of shortest trees that support underlying branch. **B:** A strict consensus of the three most parsimonious

trees from 1,075 nt SSU rDNA. Length = 349, CI' = 0.76–0.78, RI = 0.86–0.88. Bootstrap support from 217 replicates indicated along branches (with number of unambiguous changes in parentheses). Maximum likelihood tree identical to a resolution of this tree. (A and B from AM Kerr and J Kim, unpublished data).

how echinoderms evolved their singular adult body plan is a venerable unresolved problem in biology (e.g., Huxley, 1878). We do know that they evolved from nonpentamerous forms, as the oldest fossils assignable to echinoderms have bipartite and tripartite skeletal arrangements (Parsley, '94). How the multiplication of body rays occurred is less certain, though potential explanations abound (most recently, Hotchkiss, '98). At least one view, pentamery arising from a multiplication of body axes, is supported by preliminary evidence from Hox-gene expression patterns (Raff, '96; Lowe and Wray, '97).

In addition to being pentaradiate, most echinoderms are compressed through the oral-aboral axis. But in the lineage leading to the sea urchins and holothuroids, a further modification in body organization took place: an oral-aboral elongation of the body (Paul and Smith, '84). In holothuroids, this elongation is extreme and the body appears vermiform, resting on its side. Having an elongate, flexible body with appendages appressed or

absent, is a widespread body plan in Metazoa, particularly among basal pseudo- and eucoelomate phyla (Willmer, '90). A vermiform construction has also evolved secondarily in several distantly related groups, including ctenophores, gastropods, vertebrates, and holothuroids. Some holothuroids, though, have deviated even further from this organization. We discuss these modifications below, including that of Rhopalodinidae, one of the most Promethean departures from orthodox echinoderm architecture.

Holothuroids and some echinoids, after passing through a short pentamerous phase as a post-larva, may acquire pronounced bilateral symmetry. In holothuroids this occurs along the oral-aboral axis and is invariably associated with differentiating of external structures dorsoventrally, sometimes with dorsoventral compression (Fig. 2A), so that the gonopore is dorsal. This arrangement is maintained along the plane of larval bilateral symmetry (Smiley, '86) and is presumably an adaptation to an epibenthic existence. Holothuroid bilaterality

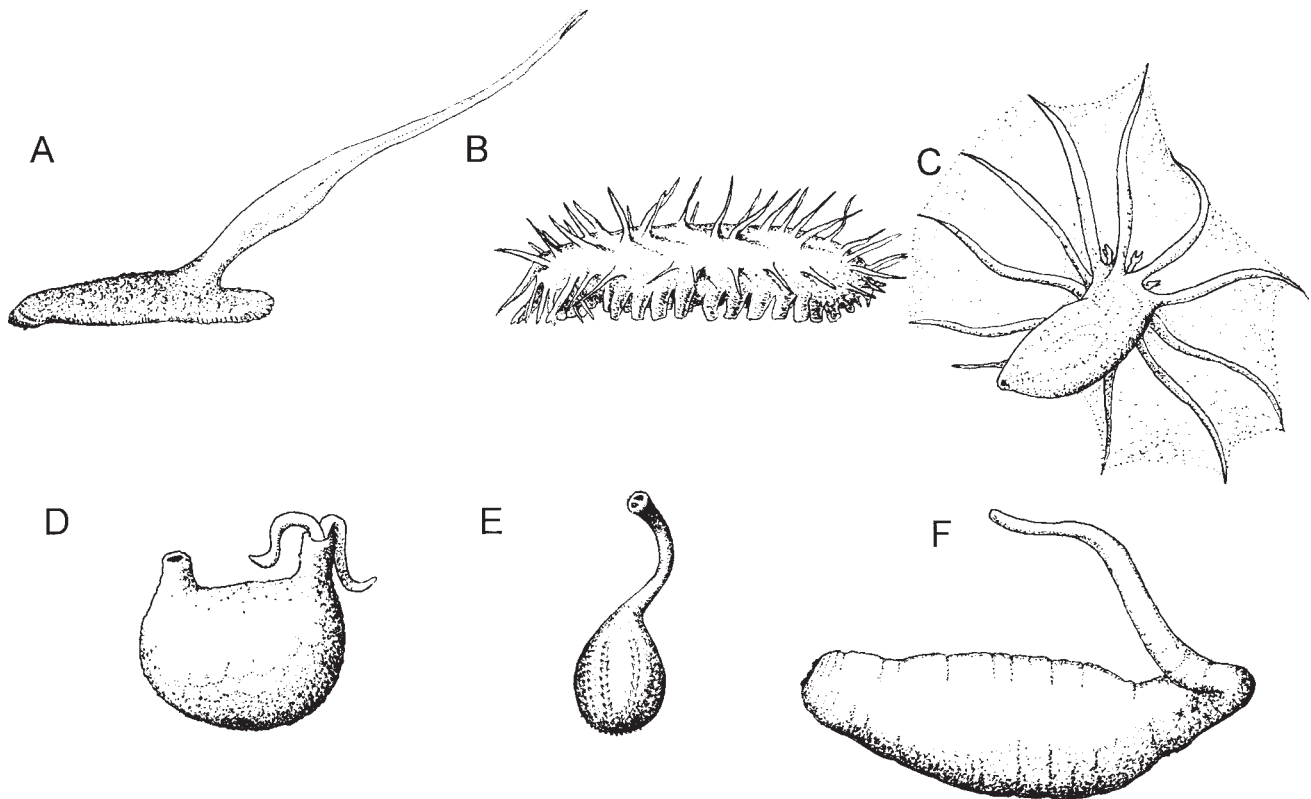


Fig. 2. Representative holothurian body types. **A:** *Psychropotes*, Psychropotidae, Elasipodida; 15 cm (after Hansen, '75). **B:** *Oneirophanta*, Deimatidae, Elasipodida; 10 cm (after Hansen, '75). **C:** *Pelagothuria*, Pelagothuriidae, Elasipodida; 8 cm. **D:** *Ypsilothuria*, Ypsilothuriidae, Dactylochirotida; 3 cm.

E: *Rhopalodina*, Rhopalodinidae, Dactylochirotida; 5 cm (after Semper, 1868). **F:** *Paracaudina*, Caudinidae, Molpadida; 20 cm (after Lambert, '97). Measurements are anterior-posterior lengths.

appears to have evolved in Holothuroidea in three quite distantly related groups (Fig. 3). Internally, these holothuroids, as well as some others, may possess paired respiratory trees and, in elpidiid elasipodids, possess statocysts along only the two ventrolateral radii. However, it is important to note that this bilateral symmetry is superficial in that the muscular, nervous, and water vascular systems are established prior to adult bilaterality and internally retain their canonical five-fold disposition. In some dendrochirote and holothuriid holothuroids, adult bilaterality consists simply of a ventrally concentrated field of tubefeet. Finally, no dorsoventral differentiation is apparent externally in the epibenthic synaptine apodids; nevertheless they similarly maintain the interradius with the gonopore in a dorsal position.

Pronounced differentiation externally between the dorsum and ventrum appears to result largely from the different types of tubefeet on these surfaces. The dorsal surface may present enlarged and thickened tubefeet termed papillae (Fig. 3).

Papillae are structurally and functionally most diverse in Elasipodida (Fig. 2A–C). In this group, papillae may fuse to form a large dorsal structure of uncertain function (Fig. 2A), perhaps sensory or respiratory (Hansen, '75). As well, papillae may present webbing that facilitates swimming (Fig. 2C), which in *Pelagothuria natatrix* is circumoral and also used to concentrate descending sediment (Billett, '91). Papillae in elasipodids may also be present ventrolaterally as locomotor appendages, effectively raising the holothuroid off the bottom with a measured, myriapod-like stride. This divergence in the form of dorsal and ventral tubefeet implies corresponding modified expression patterns of genes mediating tubefoot development, perhaps including those transcribing homeoproteins implicated in the development of holothuroid tentacles and the tubefeet of other echinoderms (Lowe and Wray, '97).

The most remarkable example of deviation from standard echinoderm pentamery is found in the Rhopalodinidae in which members during ontog-

Family	Bilateral symmetry	Dorsoventral differentiation	Habit	Respiratory trees	Larval development	Habitat	Body wall skeleton
Ypsilothuriidae	no	no	infaunal	present	direct ¹	deepwater	plated
Vaneyellidae	no	no	infaunal	present	direct ¹	deepwater	plated
Rhopalodinidae	no	no	infaunal	present	direct ¹	deepwater	plated
Psolidae	yes	yes	epibenthic	present	direct	varied	plated
Paracucumidae	no	no	infaunal	present	direct ¹	deepwater	plated
Placothuriidae	no	no	infaunal	present	direct ¹	deepwater	plated
Heterothyonidae	no	no	infaunal	present	direct ¹	deepwater	plated
Phyllophoridae	no	no	cryptic	present	direct	varied	reduced
Sclerodactylidae	no	no	cryptic	present	direct	varied	reduced
Cucumariidae	no	no	cryptic	present	direct	varied	reduced
Holothuriidae	yes	yes	epibenthic	present	indirect	coral reef	reduced
Stichopodidae	yes	yes	epibenthic	present	indirect	coral reef	reduced
Synallactidae	yes	yes	epibenthic	present	direct	deepwater	reduced
Molpadiidae	no	no	infaunal	present	direct	varied	reduced
Caudinidae	no	no	infaunal	present	direct	varied	reduced
Eupyrgidae	no	no	infaunal	present	direct ¹	deepwater	reduced
Gephyrothuriidae	no	no	infaunal	present	direct ¹	deepwater	reduced
Synaptidae	no	no	infaunal	absent	indirect	coral reef	reduced
Chiridotidae	no	no	infaunal	absent	direct	varied	reduced
Myriotrochidae	no	no	infaunal	absent	direct ¹	deepwater	reduced
Elpidiidae	yes	yes	epibenthic	absent	direct ¹	deepwater	reduced
Psychropotidae	yes	yes	epibenthic	absent	direct ¹	deepwater	reduced
Laetmogonidae	yes	yes	epibenthic	absent	direct ¹	deepwater	reduced
Deimatidae	yes	yes	epibenthic	absent	direct ¹	deepwater	plated
Pelagothuriidae	no	no	pelagic	absent	direct ¹	open ocean	reduced
Cidaridae	no	no	cryptic	absent	indirect	coral reef	plated

Fig. 3. Character-state optimization onto the morphological phylogeny. Superscripts under the heading *Larval devel-*

opment indicate that entries were inferred from maximum egg size (Tyler and Billett, '87).

eny secondarily acquire a novel form of radial symmetry. This family belongs to a group, the Dactylochirotida, which are distinguished by a foreshortening of the dorsal interradius that imparts a spheroidal appearance (Fig. 2D) and is presumably an adaptation for mud living (Pawson, '84). However, in Rhopalodinidae, this interradius is extremely foreshortened, so that the mouth and anus are adjacent at the end of a long, slender stalk (Fig. 2E). The midpoints of the five radii are centered at the new base of the animal and appear externally as a whorl of ten "semiradii" radiating towards the combined oral and anal opening (Cherbonnier, '88). Internally, the conventional five-fold organ systems still run from mouth to anus (Semper, 1868).

The most prominent internal structures unique to Holothuroidea are the respiratory trees: a bilaterally arranged pair of heavily ramified tubes used in gas exchange that arise and receive water from the cloaca (Shick, '83). The origin of these structures is uncertain. Several workers (Théel, 1885; Madsen, '61; Pawson and Fell, '65) write or imply that respiratory trees arose early in the evolution of Holothuroidea. Thomas Huxley (1878), however, suggested that they arose much later. Our phylogeny (Fig. 3) supports the latter view that respiratory trees arose secondarily, perhaps during the period of increased diversification of holothuroids between the late Triassic and early Jurassic when molpadids likely originated (Gilliland, '93). A significant increase in surface area for gas exchange conceivably has an impact on an organism's biology beyond that of its respiration physiology. Unfortunately, the distribution of this character in Figure 3 hinders robust inferences on the matter. Tentatively though, acquiring respiratory trees may have permitted the invasion by larger or thicker walled species, such as molpadids (Fig. 2F), in settings with lower oxygen partial pressures than previously tolerable, such as hypoxic fine sediments and the tropical intertidal.

Axis of body symmetry is a fundamental component of an organism's body plan and many questions exist about their evolution and what mechanisms govern the formation of various axes. In this respect, holothuroids are fertile grounds for investigation. First, they share the echinoderm mystery of the evolution of pentaradial symmetry. But, in addition, they have re-evolved a dorso-ventral differentiation leading to a secondary gain of bilateral symmetry. Even more extreme, as mentioned above, species in Rhopalodinidae sec-

ondarily regain a novel form of radial symmetry leading to the evolutionary sequence: bilateral symmetry → pentaradial symmetry → bilateral symmetry → "decaradial" symmetry.

As mentioned above, the internal body plan of a holothuroid retains the pentaradial symmetry even as it gains adult dorso-ventral differentiation. In this sense, the dorso-ventral axis of a holothuroid is unlikely to involve an early symmetry breaking mechanism such as for other bilaterally symmetric organisms (Slack, '91; Eval-Giladi, '97) or the larval dorso-ventral axis in echinoderms (Kominami and Takata, '95). Rather, we speculate that the secondary dorso-ventral axis may be ectodermally derived and compartmentalized in an analogous manner to vertebrate limb development (Altabef et al., '97; Zeller and Duboule, '97). Some support for this hypothesis is found in the respiratory trees and elpidiid statocysts, which are bilaterally arranged, but are ectodermally derived.

It is more difficult to speculate about the mechanisms generating oddities such as the secondary gain of radial symmetry in Rhopalodinidae. However, it does force us to think about "gain of symmetry" as opposed to "breaking of symmetry" which has received more attention. It seems that we might gain symmetry from asymmetrical form in two different ways: either de-differentiate or replicate. In holothuroids, pentaradial symmetry begins with repeated colomic structures and Rhopalodinidae gain symmetry from folded duplication. As well, any segmented body plan is translation symmetry arising from duplication. Therefore, it would seem that where gain in symmetry is observed, some form of duplication is the rule. All in all, molecular studies in holothuroids would yield much insight into the diverse mechanisms of body-axis formation and their evolution.

Evolution of larval form

The striking morphological diversity of adult holothuroids is conspicuously lacking in their larvae. Approximately 80% of holothuroids begin larval life as a prolate, ciliated and non-feeding vitellaria provided with yolk. These larvae resemble in many ways the nonfeeding forms of other echinoderm classes. In many holothuroid species, the vitellaria (=doliolaria; Smiley et al., '91) are pelagic, at least briefly. Other taxa brood their larvae, either externally or internally, and they become free living as juveniles. The remaining species are indirect developers, passing first

from an obligately pelagic, plankton-feeding auricularia then to a vitellaria before settling as a juvenile (Smiley et al., '91).

Prevailing ideas about larval evolution within Holothuroidea are based on general treatments of the evolution of larval form in marine invertebrates (Strathmann, '78; Hansen, '82; Emlet, '90; Strathmann, '93). In brief, it has been believed that the evolution of indirectly developing larvae occurs much less often than that of direct developers, probably because of the difficulty in evolving coordinated changes in the organ systems necessary to take up feeding versus the relative ease of losing such organs. Second, it is believed that morphologically distinct feeding stages of indirect developing larvae found within a single lineage, such as the auricularia in Holothuroidea, evolved only once. This is inferred from the observation that features of the feeding larvae characteristic of classes or phyla have apparently not evolved convergently elsewhere. To have attained such uniformity of larval form within a single lineage most probably indicates a single origin. Hence, it is believed that the feeding auricularia typical of about 20% of holothuroid species, likely evolved once and that holothurian larval evolution must have been characterized by multiple parallel losses of this stage.

However, given our phylogeny, the feeding larvae of holothuroids appear to have an evolutionary history unusual among invertebrates that makes inferences about their origin(s) problematic. Optimizing larval form (feeding versus nonfeeding) onto the holothuroid phylogeny (Fig. 3) shows that two disparate lineages possess auricularia, Synaptidae and the Holothuriidae+Stichopodidae. This pattern differs from that found in most other lineages of marine invertebrates. For example, in each of the other classes of echinoderms with feeding larvae, 80% of the species possess the feeding stage and numerous losses of feeding are scattered across the lineage. This pattern occurs in Echinoidea (Wray, '96) and, most likely, the Asteroidea (Chia and Walker, '91) and Ophiuroidea (Hendler, '91). The auricularia in the two groups of holothuroids are very similar (Mortensen, '37, '38) and are primarily distinguishable by the internal spicules and, during metamorphosis, by different development of the hydrocoel (Smiley et al., '91). This invariance in form, as mentioned above, suggests that a feeding larva with its complex feeding mechanism evolved only once in holothuroids and that there has been an extreme bias towards the loss of larval feeding.

Alternatively, the distribution of feeding larvae could indicate two independent acquisitions. We at present favor this hypothesis for the following two reasons: First, a single origin for holothuroid auricularia requires that there have been from six to eight losses of feeding larvae that resulted in two isolated subgroups of larval feeders, a pattern not seen, as far as we know, in any other group of marine invertebrates. Strathmann and Eernisse ('94) qualitatively argue that this kind of pattern can nevertheless arise when losses of a feeding larva happen much more frequently than reversion to feeding. However, the higher the probability of the loss of the ancestral character state of feeding, the less likely feeding larvae will be retained after much diversification. That is, the question at hand is the probability that the two feeding larvae in Synaptidae and the Holothuriidae+Stichopodidae are identical by descent.

Suppose we make the very simple assumption that transitions of form happen with constant probability on each branch of the tree and let p be the probability of loss of the feeding form. In our phylogeny, a minimum of six branches separate Synaptidae and the Holothuriidae+Stichopodidae lineage. The probability of identity by descent of the feeding form is $(1-p)^6$. As can be seen, this is a decreasing function of p ; that is, the probability of identity by descent *decreases* as the loss of feeding form becomes more likely. Suppose we want this probability to be high, say 0.95, to be consistent with the idea that feeding larvae originated only once. Then, the probability of loss has to be smaller than ~ 0.0085 along any branch separating the Synaptidae and the Holothuriidae+Stichopodidae lineages (Chang and Kim, '96). The argument of highly probable loss of the feeding form actually runs counter to explaining the phylogenetic distribution of feeding larvae in Holothuroidea.

The only other possible explanation is if the branches separating the two lineages with feeding larvae are extremely small (Schluter et al., '97). However, the ancient divergence of these two groups (Gilliland, '93) indicates that this is quite unlikely. In short, under any reasonable scenario, maximizing the probability of retaining auricularia conversely minimizes the chance of the massive losses of this developmental mode as observed in holothuroids. Further quantitative assessment of the arguments sketched here would doubtless shed new light on the issues surrounding larval evolution.

Second, the groups with feeding larvae, the

Synaptidae and the Holothuriidae+Stichopodidae are the only holothuroid taxa that are almost entirely limited to coral reefs (Fig. 3). Thus, a single origin for holothuroid auricularia would also require an exceptional coincidence: Since the divergence time (over 350 mya) of the groups with feeding larvae precedes modern (45 mya) and even Mesozoic reefs (250 mya) (Gilliland, '93), the present reef habitat must be a secondary trait. But this requires the two groups, to the exclusion of all other holothuroids, to have had independently and relatively recently radiated into the same habitat, coral reefs, and then disappear elsewhere. This explanation predicts that the few members of the Synaptidae, Holothuriidae and Stichopodidae that possess feeding larvae but do not inhabit reefs have acquired their non-reef habitats secondarily.

If the holothuroid auricularian larva has evolved twice, then a mechanism to explain this is required. We agree that in general the independent evolution of very similar larvae is probably a quite rare event (Haszprunar et al., '95; Reid et al., '96). However, for the previously discussed reasons, we regard the dual evolution of auricularia in holothuroids as reasonable. Possible explanations for this event center around convergence on an efficient morphology for feeding in the water column or morphological convergence mediated by constraints imposed by the organisms' genetic architecture. It seems rather obvious that given the diversity of feeding larvae morphology in echinoderms and metazoans that there is little reason to suspect functional convergence of feeding larvae morphology. In fact, the morphological differences between the feeding larvae of Synaptidae and Holothuriidae+Stichopodidae are extremely slight and only observable in their later stages. This seems to leave only the possibility that their morphological similarity is due to some kind of existing developmental constraint arising from the genetic architecture of holothurians. Further light on this problem may be found with future molecular developmental studies.

Evolution of the holothuroid skeleton

A defining feature of Echinodermata is a mesodermally derived skeleton of trabeculate calcite (Smith, '90). In most of Holothuroidea, the skeleton is reduced to a circum-oesophageal calcareous ring and isolated microscopic spicules in the dermis. Such a marked reduction of the skeleton of the body wall is seen in other echinoderms only in the phrynophiurid brittlestars (Byrne, '94).

Other minor accessory skeletal structures also exist: Some molpadiid holothuroids have microscopic granules of ferric phosphate that ontogenetically replace body-wall spicules (Stricker, '86) and in *Ceraplectana*, sclerous proteinaceous sheaths cover the tentacles (Clark, '07).

Like the skeletal elements of other echinoderms, holothuroid spicules are formed within a syncytium of primary mesenchyme cells (Stricker, '85). These cells deposit calcite about a scaffold of spicule matrix proteins (Killian and Wilt, '96). Aspects of spicule shape appear controlled by matrix protein interactions with the crystal faces of the growing spicule (Berman et al., '93) that in turn are mediated by communication between primary mesenchyme cells whose spatial arrangement is influenced by ectodermal cues (Ettensohn et al., '97).

The evolution of the holothuroid skeleton is poorly understood. Holothuroids with a skeleton of reduced spicules comprise 90% of living species. This reduction of the skeleton was likely enabled by echinoderm catch connective tissue, which can function in support since its stiffness is under nervous control. The mechanical properties of the catch tissues remain influenced by the density and shape of the spicules (Koehl, '82). Pawson and Fell ('65) and Haude ('92) suggest, based on fossil evidence, that a skeleton of isolated spicules arose once to several times from earlier plated holothuroids.

Our phylogeny (Fig. 3), however, suggests that the plated skeleton is a relatively late innovation arising once in deimatid elasipodids and at least once in families comprising Dactylochirotida and Dendrochirotida in part. A single evolution within this later lineage is uncertain since the skeletal characters themselves largely define the group's monophyly. Indeed, removing skeletal characters that unite Dactylochirotida and the plated Dendrochirotida from the phylogenetic analysis recovers a tree in which a plated skeleton evolves twice within this group. We therefore cautiously contend only that a plated skeleton arose at least twice. Evidence for plated holothuroids occurs throughout the fossil record beginning with some of the oldest recognized holothuroid material (Gilliland, '93). Excepting a strong bias for retention of plates and numerous losses in most living holothuroids, a possibility for which we have no a priori expectations, the plated fossil forms may represent multiple convergences to this skeletal type. We suggest that it may be relatively easy to affect an allometric increase in the size of isolated spicules to form a test of imbricating or overlap-

ping plates, particularly if adult body size is small as occurs in all plated taxa. Further, the plated adult skeleton may occur via retention of the often-plated body seen in juvenile holothuroids. Relatively large plates may occur in juveniles because the plates serve as armor (Smiley, '94) or because spiculation does not scale isometrically with extremely small body size.

Pervasive reduction of the echinoderm skeleton is a defining feature of the holothuroids. This reduction has occurred in concert with the aforementioned modifications in body form, namely dorso-ventral differentiation, spectacularly modified tubefeet, and a range in body sizes not seen in other echinoderms. This observation invites the proposal that a key feature permitting these innovations was the reduction of the echinoderm skeleton to isolated elements. This interpretation predicts that reacquisition of a developed skeleton is accompanied by a loss of range and absolute magnitude of body size. Holothuroids with secondarily acquired plated skeletons support this view (Fig. 3). Nearly all of these species are uniformly small and invariant in size. Notably, the exceptions are members of the Psolidae, which have both an intermediate degree of spiculation and body form. They are plated taxa with a skeleton reduced to isolated spicules on the ventral surface, but have pronounced dorso-ventral differentiation and the largest variation in body size of plated forms. There is considerable current interest on the molecular basis of spicule formation as a model for bone development (Benson and Wilt, '92) and skeletal-tissue engineering (Reddi, '98). We believe there is potential to extend such studies to macroevolutionary morphological trends. For example, if indeed the trends in body size of holothurians are related to skeletal biomechanics, we would expect molecular evolution of genes involved in spicule formation to have great influence on this process.

Prospects

In conclusion, holothuroids are as unusual echinoderms as echinoderms are unusual animals. Holothuroids have been and continue to be poorly studied. In this review, we have introduced a phylogeny and surveyed notable aspects of morphological evolution in holothuroids. The upshot is that several long-standing ideas about the evolution of the group and their body form appear in question. These results also raise numerous questions about how holothuroids acquired their morphological diversity. For example, how does the

unique symmetry in Rhopalodinidae arise ontogenetically? What accounts for the unusual phylogenetic distribution of feeding larvae? What has been the role of skeletal reduction in the evolution of new body shapes and appendages? Attempts to answer these and other questions about holothuroids will provide insight into longstanding issues in echinoderm biology, as well as the evolution and development of Metazoa in general.

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