

Abstract

Evolution and Systematics of Holothuroidea (Echinodermata)

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Holothuroids, or sea cucumbers, are a morphologically diverse group of echinoderms with over 1400 described species occurring from the intertidal to the deepest oceanic trenches. In this thesis, I investigate the evolution and systematics of this group via a phylogenetic analysis of partial nuclear small subunit rDNA sequences and morphological characters. The recovered cladistic structure of the group is identical between the data sets and is at considerable odds with the conventional higher level classification. The topology within at least one order, Apodida, is in contrast largely congruent with the Linnean scheme. Branching order is significantly associated with fossil first occurrences and the fossil calibrated tree indicates that holothuroids survived the end Permian extinction more successfully than other echinoderms. Like many other marine invertebrates, at least one clade radiated rapidly during the Triassic. Unlike echinoids, planktotrophic larvae are restricted to two evolutionarily disparate groups largely restricted to coral reefs, suggesting that this feeding mode evolved twice via convergence, or perhaps, atavism. A test of imbricate plates and a complex calcareous ring resembling the ambulacral skeleton of other echinoderms are derived features in holothuroids.

Evolution and Systematics of Holothuroidea (Echinodermata)

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CHAPTER I:
History of Scientific Interest in the Evolution and Systematics
of Holothuroidea

Because some shallow-water species are large and conspicuous, holothuroids have been known since ancient times. Despite this long familiarity, less is known about the biology of Holothuroidea than about that of the other extant classes of echinoderms. In this thesis, I report on investigations of the group's evolution and phylogenetic systematics, two areas that have received very little attention since a brief flurry of speculations in the late 19th century. In this chapter, I first introduce the origin and systematic status of the term “holothurian” and its variants. Then, I provide a short history of the scientific attention paid to holothuroids and how the group has helped shaped ideas about the classification and evolution of other echinoderms and animals. Finally, I outline the structure of the ensuing chapters with a precis of the goals, approach and main results of each.

Etymology

The etymology of “holothuroid” and its variants is obscure. The name derives from a term, *ὄλοθούριον* or *holothurion*, appearing once in each of two works by Aristotle (ca. 350 BC). *ὅλος* signifies whole, or complete, while the translation of the stem *-θούριον* is uncertain. Ludwig (1889-1892), who is repeated by Hérourard (1902), suggests that it is derived from a similar word that can be translated as “lecherous” or “unseemly.” Ludwig supports this interpretation by citing Bohadsch

(1761) and his references of the many ancient to modern names for holothuroids that refer to the animal's resemblance to a penis, e.g., *genitale marinum*, *priapus marinus*, *phallus marinus*, *pudendum*, and *cazzo di mare*. Weyss (1904) claims the Greek root is instead derived from "like a door," but he provides no explanation or reference. The root is also similar to Greek stems for "rushing," as well as a place name of mythological significance, and less implausibly perhaps, "ugly."

It is nonetheless unclear if Aristotle was even referring to members of what we now call Holothuroidea. Aristotle based many of his accounts of plants and animals, including marine forms, on first-hand observations. Yet he writes of an organism that is only "slightly different from the sponges" and which is "without feeling" and "motionless" (the Greek here is sometimes rendered "stationary"), as well as being "free and unattached" and "separated from the ground," but "plant-like." This is a surprisingly inaccurate description of holothuroids from the father of western empiricism. Littoral Mediterranean species are vagile (Holothuriidae, Stichopodidae, Synaptidae), burrowing (Synaptidae), or crevice dwelling (Holothuriidae, Cucumariidae). Mediterranean cucumariids are more stationary and extend a floral-like terminal circlet of tentacles, but attach firmly to hard bottom and, like the aforementioned species, contract rapidly when disturbed.

Other writers (e.g., Hyman, 1955) believe that Aristotle was referring to another animal, probably a scyphozoan or pelagic hydrozoan, though his description fits that of a ctenophore just as well. This view of *holothurion* as a cnidarian is supported by Aristotle's narrative itself, since at one point he jointly describes *holothurion* with another animal, "water lungs," that has been invariably, and perhaps even correctly, interpreted as a scyphozoan since antiquity. Moreover, in the oldest surviving reference to Aristotle's passages on *holothurion*, Pliny the Elder (1st c. AD) seems to consider it an animal other than a member of the currently delimited Holothuroidea. Pliny apparently made few first-hand observations and borrowed unverified and unattributed passages wholesale from previous authors, including those of Aristotle on *holothurion*. In these instances, Pliny uses the Latinized form *holothuriis*, yet later refers to another marine animal as *cucumis [marinus]*, a term attributable to holothuroids and from which is derived several modern European vernacular terms, "sea cucumber," *concombre de mer*, *pepino de mar*, *seegurken*, and *cucumero di mare*.

Medieval History

Later ancient to medieval natural historians, principally Aelian (3rd c.), Isidor de Seville (7th c.) and Albertus Magnus (13th c.), make no mention of holothuroids in their comprehensive accounts, though the

aforementioned authors frequently refer to marine invertebrates, including other echinoderms. Medieval bestiaries were not zoological texts, but were designed to seize the reader's interest in the fantastic (Ashworth, 1996), a potential explanation for the absence in such accounts of the small and undistinguished species of holothuroids known at the time from mostly temperate shores. By the 16th century, though, holothuroids were again a subject of curiosity. Belon (1553) is the first to provide a description of what is undoubtedly a holothuroid under the name *genitale marinum*, but he includes a list of alternate names, among them the Greek *holothurion*. The first illustrations are by Rondeletius (1554-1555) who figures two species, a *holothurion* and a *cucumis marinus*. Notable accounts mentioning holothuroids from the following century are by Columna (1616), Aldrovandi (1642) and Jonstonus (1650), all of whom use only Latin appellations. Rumphius in 1705 gives an account of two unidentifiable Indo-Pacific species under the name *phallus marinus* (Rumphius, 1999). Bohadsch (1761) and Pallas (1766) provide the first anatomical accounts of holothuroids.

Validity of the Name *Holothuria*

Because of the uncertainty of Aristotle's ascription and Pliny's interpretation, the term *holothurion* was also applied to cnidarians, tunicates and priapulid worms until the late 18th century. For example,

Rumphius in 1705 uses the name *Holothuria* for the siphonophore *Physalia* (Rumphius, 1999). This terminology, along with many other of Rumphius's names for invertebrates, is adopted by Linnaeus (1758) in his early editions of *Systema Naturae*. Linnaeus also includes three other species under *Holothuria*, all of them tunicates. For true holothuroids, Linnaeus uses the term *Fistularia*, a term preoccupied by a fish and subsequently abandoned. In the 12th edition, Linnaeus (1768) includes under *Holothuria* five new species, four holothuroids and a priapulid worm. Notwithstanding that a species of *Physalia* was the type species, the 12th edition becomes the starting point for modern holothuroid systematics (Jaeger, 1833). Bell (1891), and later Gill (1907), pointed out that *Holothuria* as applied to holothuroids was invalid, but that assigning an appropriate one by law of priority would lead to needless confusion. Still, Poche (1907) finds *Bohadschia* to be primary, and by that criterion erects Bohadschioidea for all holothuroids. A brief volley of discussion soon followed in *Science* (Bather, 1907; Clark, 1907; Fisher, 1907) in which the participants agree that the name should remain by common usage *Holothuria* and thus Holothurioidea (=Holothuroidea). This view later became official with the formal suspension of the rules in the case of *Holothuria* and *Physalia* (I.C.Z.N., 1924).

Position of Holothuroidea in Echinodermata

Belon (1553) was the first to recognize the resemblance of holothuroids to other echinoderms by the similarity of their tubefeet and by the early 19th century the relationship was essentially unquestioned (Lamarck, 1816; Cuvier, 1817). During this period, the search for a “natural” classification of animals consumed zoologists, but defining a more inclusive group for echinoderms would prove extraordinarily difficult (Winsor, 1976). In these exercises, holothuroids, because of their soft body and bilateral, vermiform appearance, often served as a link in the linearly arranged classificatory schemes between echinoderms and various coelomate worms (e.g, Forbes, 1841). Holothuroids were often seen as the most “advanced,” echinoderm because they were the least radially symmetrical (e.g., Agassiz, 1859).

Just as linear classifications were giving way to branching ones later in the 19th century, so also was interest increasing in the evolutionary relationships between the now well-defined extant classes of echinoderms. Here again holothuroids and their unusual morphology initially proved perplexing. Interestingly, one of the first speculations about the phylogenetic position of holothuroids within Echinodermata belongs to someone who was not an evolutionist. Alexander Agassiz in an 1865 letter to Fritz Müller wrote that Darwinists would undoubtedly interpret the similarities of echinoderm larvae as evidence that “Ophiurans and Echinoids came from the same ancestor [and] that

Starfishes and Holothurians [show] the unmistakable sign of their community of parentage” (Winsor, 1976). However, most evolutionary discussions of this period about echinoderms, as well as about other groups, concerned finding an “ancestral larval type,” since it was believed that early ontogeny faithfully reflected ancestral form.

In this vein, these and other authors occasionally speculated about the relationships between holothuroids and the other classes of echinoderms. Semon (1888), after an exhaustive study of a synaptid holothuroid larva, concludes that holothuroids are only distantly related to the extant classes. Bell (1891) provides the first diagrammed phylogeny of echinoderms and, after considering both larval and adult features, draws holothuroids as sister to all other living and extinct classes. Shortly thereafter, Bury (1895), following a reassessment of Semon’s study, suggests that holothuroids form the sister to the subphylum Eleutherozoa, a group comprising asteroids, ophiuroids and echinoids. Less plausibly, he noted, they might be sister to all living echinoderms, though he also points out similarities between holothuroids and echinoids. It was Haeckel in von Zittel (1895; see von Zittel, 1896), however, who first formalized a close relationship between these two classes with their unification in the subphylum Echinozoa, a view further supported with embryological observations by MacBride (1906).

After MacBride's work there were essentially no novel contributions to holothuroid phylogenetics and discussion of the group's evolution ebbed considerably, such that by mid-century Hyman (1955) could write "interest in this group appears relegated to taxonomic specialists." As a result, the sister relationship between holothuroids and echinoids remained largely uncontested through most of the 20th century, except by a few comparative anatomists (e.g. Hyman, 1955; Beklemischev, 1969) who viewed the absence of several otherwise widespread echinoderm organs as primitive in holothuroids. Smith (1984), in the first use of a maximum parsimony analysis of larval and adult characters to address echinoderm phylogeny, confirmed the widely accepted view that holothuroids were the sister group to echinoids. In the most notable exception to this position, Smiley (1988) revised and enlarged Smith's morphological data set after an embryological study of an aspidochirote holothuroid (Smiley, 1986) and found holothuroids were instead the sister to all other extant echinoderms. This resurrection of Semon's (1888) idea, however, proved short-lived. In the first study using a distance analysis of nucleotide sequences of partial 18S rRNA, Raff *et al.* (1988) recovered holothuroids+echinoids. This result was also later obtained by Littlewood *et al.* (1997), using separate and combined maximum parsimony analyses of complete 18S- and partial 28S-rDNA sequences, plus adult and larval morphological characters.

While the position of holothuroids among extant echinoderms seems assured, there has been less certainty about the identity of the group's proximal fossil stem member. Early candidates included the cystoids (Semon, 1888) and a primitive undiscovered echinoderm with both worm-like and cystoid features (Bell, 1891). Fell and Moore (1966) argued that edriasteroids were closest, noting similarities between the fossils and psolids, heavily plated holothuroids judged primitive. In addition, the external ambulacral plates of edriasteroids bear a striking resemblance to the internal calcareous ring of some dendrochirote holothuroids. This was an interesting observation and Fell and Moore's analysis was the first careful comparative work on the subject. As such, it won widespread acceptance (e.g., Pawson, 1980; Haude, 1994). However, the edriasteroids are now as likely to be considered stem members of asteroids (Smith and Jell, 1990) or of all echinoderms (Mooi and David, 1997). Most recently, Smith (1988) points out synapomorphies of holothuroids and ophiocistioids, a poorly known group with a plated, globoid body (Ubaghs, 1966). Like the earliest known holothuroid *Palaeocucumaria* (see Seilacher, 1961), ophiocistioids possess a reduced external ambulacral skeleton and reduced number of orally concentrated and plated tubefeet/tentacles. Moreover, in the ophiocistioid *Rotasaccus*, the body is likewise invested in ossicles of a form previously seen only in holothuroids (Haude and Langenstrassen, 1976).

Outline of Thesis

Chapter II presents a molecular phylogeny of the Holothuroidea. Using 1100 bp of 18S-like rDNA, I sought to resolve the major deep branches of holothuroid relationships. The results corroborate those from the less complete analyses of Littlewood *et al.* (1997) in that a considerable revision of higher-level holothuroid taxonomy is needed.

In Chapter III, I tested the hypothesis of relationships gleaned from the molecular data using a cladistic analysis of morphological characters and representatives of all extant taxonomic families. I recovered a topology identical to that from the nucleotide sequence data. Further I calibrated branch lengths using the earliest unambiguous stratigraphic occurrences of fossil taxa. Holothuroids appeared to have survived the Permian-Triassic mass extinction more successfully than did the other echinoderm classes, though similarly experienced a large radiation in the early Mesozoic.

Apodan holothuroids have a considerably better fossil record than do other holothuroids and a confident stem member is available as an outgroup. Hence in Chapter IV, I undertake a phylogenetic analysis of this order using morphological characters. Much of the most recently proposed higher-level taxonomy is supported, though at least one family

appears paraphyletic. Calibrating branch lengths using fossil data suggests that much of one family is Paleozoic in origin and that a family of predominately coral-reef species radiated rapidly in the Jurassic.

In chapter V, I use the estimate of relationships to explore developmental trends and character evolution within Holothuroidea. Pronounced adult bilateral symmetry has evolved at least three times. A test of imbricating plates is not homologous with that of other heavily armored echinoderms and has evolved at least twice. Indirectly developing larvae occur in two widely separated clades, suggesting parallel convergent evolution or, perhaps, atavism.

In the conclusion Chapter VI, I briefly review the significance of the main results of the previous chapters. I then outline several interesting future directions of research. In sum, the unusual evolution of the holothuroid skeleton, from its pervasive reduction to the subsequent re-evolution of a plated test by some taxa, raises numerous questions about how this occurred and about the evolution of skeletons in general. I discuss current work on the apparently chitinous nature of *Ceraplectana* tentacle sheaths, as well as argue for holothuroid ossicles as a model for studying the evolution of biological shape.

Chapter II:
Phylogeny Inferred from Nuclear rDNA Sequences

Summary

The relationships within the echinoderm class Holothuroidea are poorly known. In this study, I investigate the higher level relationships in the group using all published sequences of 18S-like rDNA, from six species in four taxonomic orders. The alignable consensus sequences were 980 nucleotides in length and differed in pairwise comparisons by about 2% to 12%. Nucleotide usage was nonuniform with G and C comprising about 22% and 28% of the sequences, respectively. Maximum parsimony, maximum likelihood and minimum evolution analyses produced identical topologies, the deep branches of which were highly supported by bootstrap replicates. The relation (Apodida, (Elasipodida, (Aspidochirotida, Dendrochirotida))) has several important implications for the phylogeny and higher level taxonomy of Holothuroidea. First, an examination of substitution rates along branches and the derived position of Dendrochirotida call into question this group's presumed antiquity. Second, in line with some early conjectures based on comparative anatomy, Apodida is the most divergent member of Holothuroidea. Finally, the topology indicates that the subclass Aspidochirotoacea, composed of Elasipodida and Aspidochirotida, is paraphyletic.

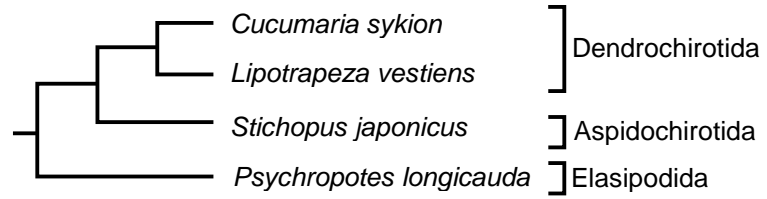
Introduction

Holothuroidea is a well defined class of echinoderms of over 1400 species and consisting of six orders arranged by twos in three subclasses (Pawson and Fell, 1965). Relationships within the group, however, are not well understood and a panoply of speculation has accrued over the last century and a half. There have been no investigations using phylogenetic techniques directed at uncovering relationships within the group. Littlewood *et al.* (1997), in an analysis of class relationships in echinoderms, sequenced nearly complete small and partial large rRNA gene subunits for several species. Their results for holothuroids are shown in Figure 2.1. They found a close relationship between the dendrochirotes and aspidochirotes. It also appears that at least one subclass, the Aspidochirotacea, composed of aspidochirotes and elasipodans is paraphyletic. They were unable, however, to resolve the relative positions of Apodida and Elasipodida. In this study, I combine their sequences with other published data to resolve the relationships between members of four of the six orders of holothuroids.

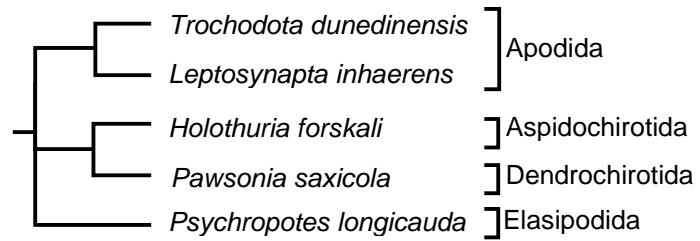
Materials and Methods

Figure 2.1. Hypotheses of holothuroid relationships based on maximum parsimony analysis of molecular data by Littlewood *et al.* (1997). A) Estimate from nearly complete small subunit rDNA. B) Estimate from ca. 400 bp from the 5' end of large subunit rDNA.

A



B



For the molecular analysis, I used all published sequences of nuclear 18S-like, small subunit rDNA, or its product rRNA, reported from holothuroids (Table 2.1). These sequences are published in three sources. Wada and Satoh (1994) and Littlewood *et al.* (1997) give nearly complete gene sequences for, respectively, one species, *Stichopus japonicus*, and three species, *Cucumaria sykion*, *Lipotrabeza vestiens* and *Psychropotes longicauda*. Raff *et al.* (1988) provide sequences from three fragments of the ssu rRNA from two species, *Thyone* (= *Sclerodactyla*) *briareus* and *Leptosynapta inhaerens*. These pieces correspond to positions 147-593, 759-1158 and 1439-1676 in human 18S rRNA. Outgroup taxa, are nearly complete sequences from Littlewood and Smith (1995) and consisted of three echinoids, *Eucidaris tribuloides*, *Mespilia globosus* and *Meoma ventricosa*.

For alignment, the nearly complete sequences were first separated and roughly trimmed to correspond to the fragments of *S. briareus* and *L. inhaerens*. Then all the sequences were aligned on *Clustal W 1.4* (Higgin, 1994), followed by a final alignment by eye and taking into account secondary structure. The final alignment was 1075 nt long (Appendix I), but the alignment of several stretches, corresponding to unresolved nucleotides in *S. briareus* and *L. inhaerens* or to secondary structural

Table 2.1. Classification, accession numbers and sources of DNA for species used in this study. The last three species are echinoids.

Order Family Species	Genbank accession number(s)	Reference
Apodida		
Leptosynaptidae		
<i>Leptosynapta inhaerens</i>	M20080, M20081, M20082	Raff <i>et al.</i> (1988)
Elasipodida		
Psychropotidae		
<i>Psychropotes longicauda</i>	Z80956	Littlewood <i>et al.</i> (1997)
Aspidochirotida		
Stichopodidae		
<i>Stichopus japonicus</i>	D14364	Wada and Satoh, (1994)
Dendrochirotidae		
Cucumariidae		
<i>Cucumaria sykion</i>	Z80950	Littlewood <i>et al.</i> , (1997)
Sclerodactylidae		
<i>Sclerodactyla briareus</i>	M20120, M20121, M20122	Raff <i>et al.</i> , (1988)
Phyllophoridae		
<i>Lipotrabeza vestiens</i>	Z80952	Littlewood <i>et al.</i> , (1997)
Cidaroidea		
Cidaridae		
<i>Eucidaris tribuloides</i>	Z37127	Littlewood and Smith, (1995)
Spatangoidea		
Brissidae		
<i>Meoma ventricosa</i>	Z37129	Littlewood and Smith, (1995)
Temnopleuroidea		
Temnopleuridae		
<i>Mespilia globulus</i>	Z37130	Littlewood and Smith, (1995)

loops, remained uncertain so were excluded. This left an unambiguously alignable sequence of 980 nt that was used in the phylogenetic analyses.

A phylogeny was estimated on *PAUP* 4.0b4a* (Swofford, 1998) using three methods, maximum parsimony, maximum likelihood and minimum evolution (Rzhetsky and Nei, 1992). The maximum parsimony analysis was performed with the following options: exhaustive search, zero-length branches collapsed and characters equally weighted. Preliminary inspection of the data indicated that the base frequencies were non-uniform. Transitions significantly outnumbered transversions, while within these categories substitution patterns were homogenous. The appropriate substitution model for the likelihood and distance analyses, therefore, was HKY85, in which base frequencies differ and transitions and transversions are considered separately (Hasegawa *et al.*, 1985). Maximum likelihood options were set to branch-and-bound search, empirical base frequencies and transition/transversion ratio used. Data quality was assessed by bootstrapping using 500 replicates under each reconstruction method and using a heuristic search with nearest-neighbor interchange, initial upper bound computed via stepwise addition and furthest addition sequence used. For the parsimony analysis, I also examined the skewness of the tree-length frequency distributions generated from 10^5 trees randomly produced from the data.

Results

Of the 980 nucleotides in the analyzed alignment, 784 were invariant, 77 variable characters were parsimony uninformative, leaving 119 informative sites. Pairwise distances as percent differing sites between holothuroid taxa ranged from 12.4% for *Sclerodactyla briareus* and *Leptosynapta inhaerens* to a low of 1.9% between *S. briareus* and *Cucumaria sykion*. Pairwise differences in site similarity are given in Table 2.2. Transition to transversion ratios in pairwise comparisons were less than two for all except six pairs (Table 2.3). For the combined sequences, there was an excess of A and C, 26.2% and 27.8% respectively, compared to G and T, with 23.8% and 22.2% respectively (Table 2.4). GC content averaged 49.7%, from a low of 47.3% for *Psychropotes longicauda* to 50.6% in two echinoids (Table 2.4).

The parsimony analysis returned three most parsimonious trees of length 266, a strict consensus of which is shown in Fig. 2.2A. These and six other trees were length 268 or less and a strict consensus of all nine trees is identical to that in Fig. 2.2A. The next shortest tree was 272 steps in length. The consistency index (CI) for the shortest trees was 0.897, the CI excluding uninformative characters 0.8251, the rescaled

Table 2.2. Uncorrected and HKY85 mean distances. Uncorrected distances are above the diagonal. Distances are given as percentages.

	1	2	3	4	5	6	7	8	9
1. <i>Le. inhaerens</i>	-	12.4	12.1	12.1	11.5	11.7	10.7	10.7	10.4
2. <i>S. briareus</i>	13.7	-	1.9	2.1	3.9	5.8	11.6	11.6	11.5
3. <i>C. sykion</i>	13.3	2.0	-	2.1	3.7	5.5	11.6	11.7	11.4
4. <i>Li. vestiens</i>	13.3	2.1	2.1	-	4.2	5.5	11.8	11.6	11.6
5. <i>S. japonicus</i>	12.6	4.0	2.8	4.3	-	4.8	11.0	10.8	10.6
6. <i>P. longicauda</i>	12.8	6.0	5.8	5.8	5.0	-	11.0	10.7	11.0
7. <i>E. tribuloides</i>	11.6	12.7	12.7	12.9	12.0	12.0	-	1.5	1.0
8. <i>Mes. globulus</i>	11.6	12.7	12.8	12.8	11.8	11.7	1.6	-	1.5
9. <i>Meo. ventricosa</i>	11.3	12.6	12.5	12.7	11.5	12.0	1.0	1.6	-

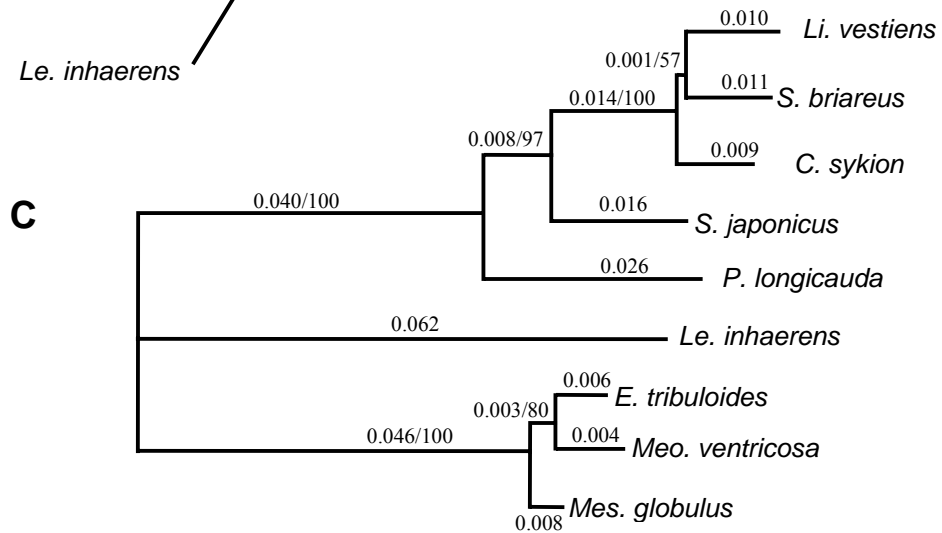
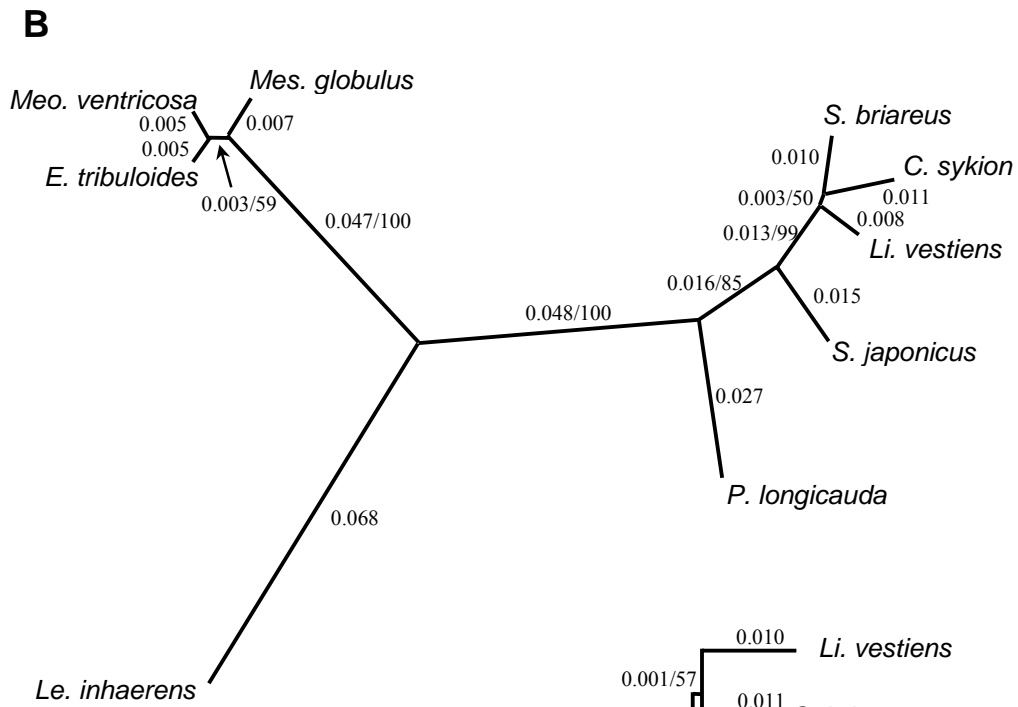
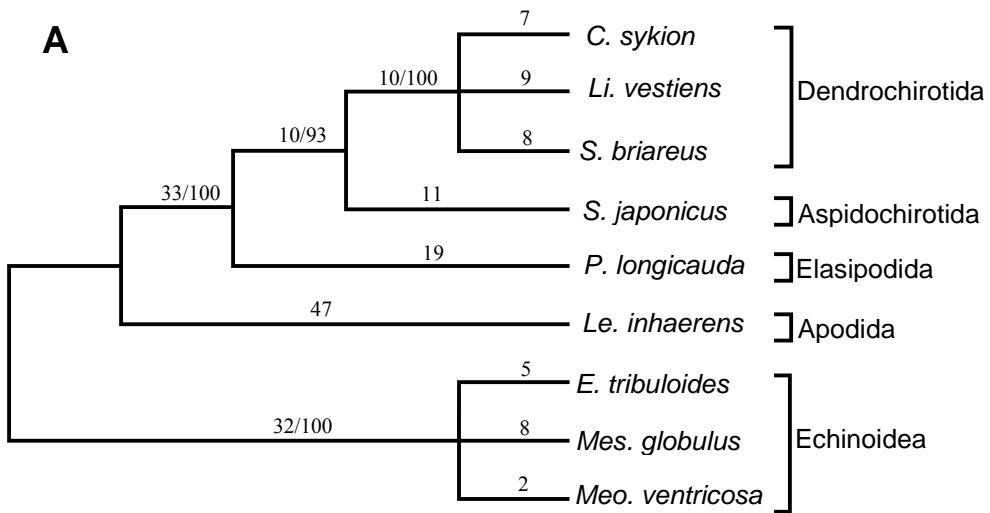
Table 2.3. Species pairs with transition/transversion ratios equal to or greater than 2.00.

Species pair	Ts/Tv
<i>S. briareus</i> and <i>C. sykion</i>	2.80
<i>S. briareus</i> and <i>S. japonicus</i>	2.45
<i>C. sykion</i> and <i>S. japonicus</i>	2.00
<i>C. sykion</i> and <i>P. longicauda</i>	2.00
<i>E. tribuloides</i> and <i>Mes. globulus</i>	2.75
<i>Mes. globulus</i> and <i>Meo. ventricosa</i>	6.50

Table 2.4. Percentages of bases in alignable portions of the ssu rDNA fragments.

	GC					Total
	A	T	C	G	content	
<i>Le. inhaerens</i>	26.4	23.9	27.3	22.4	49.7	974
<i>S. briareus</i>	25.9	24.3	27.9	21.8	49.7	975
<i>C. sykion</i>	25.9	24.4	27.5	22.2	49.7	975
<i>Li. vestiens</i>	25.6	24.5	27.7	22.2	49.9	976
<i>S. japonicus</i>	26.1	24.1	27.4	22.3	49.7	973
<i>P. longicauda</i>	26.9	23.7	25.1	22.2	47.3	975
<i>E. tribuloides</i>	26.4	23.0	28.3	22.3	50.6	974
<i>Mes. globulus</i>	26.1	23.2	28.5	22.1	50.6	972
<i>Meo. ventricosa</i>	26.6	23.1	28.0	22.2	50.2	972
Mean	26.2	23.8	27.8	22.2	49.7	974

Figure 2.2. Phylogeny of holothuroids based on ssu rDNA. A) Strict consensus of three most parsimonious trees. B) Maximum likelihood estimate. C) Minimum evolution tree. For all trees, numbers preceding the slash indicate branch lengths and, for the maximum parsimony tree, number of unambiguous changes. Numbers after the slash indicate bootstrap percentages of 500 replicates.



CI was 0.774, while the retention index was 0.880. The number of unambiguous changes on internal nodes ranged from 10 to 33. The frequency distribution of tree lengths was highly left skewed, with a “g₁” score well beyond the $P < 0.01$ significance level (Hillis and Huelsenbeck, 1992), suggesting that there is considerable “hierarchical signal” in the data sets. Bootstrap percentages were uniformly quite high, from 93% to 100% for internal branches separating different orders. The echinoid outgroup rooted the holothuroid tree between *Leptosynapta inhaerens* and the remaining species. This position of the root was unaffected by the removal of any single or pair of species (data not shown).

The maximum likelihood analysis returned a topology identical to that of one of the shortest parsimony trees (Fig. 2.2B). The likelihood score of this tree was 2795.20. The branches with lengths of 0.073 or less were within two standard deviations of zero. Bootstrap support was high for internal branches significantly longer than zero. The minimum evolution tree was identical to the likelihood tree (Fig. 2.2C) and had a score of 0.262. Bootstrap percentages for the distance tree were strong for internal branches greater than 0.003.

Discussion

Molecular Evolution

The results presented here concur with previous analyses that posit a high rate of sequence evolution in holothuroids. Raff *et al.* (1988) were the first to examine echinoderm relationships using nucleotide sequence data. They analyzed the same regions of the ssu rRNA gene used here with the neighbor joining method of Fitch and Margoliash (1967) and found that the two holothuroid sequences were evolving at over twice the rate as that of the other four classes of echinoderms. Littlewood *et al.* (1997) after a parsimony analysis of nearly complete ssu rDNA from four species concluded that they had accrued about 4.5 times as many nucleotide differences as the echinoids in an equal amount of time. In this study, I sought only to elucidate relationships within Holothuroidea so did not include other echinoderm taxa. However, even assuming quite conservatively that holothuroids and echinoids diverged at the base of the *Leptosynapta* branch (Fig. 2.2), rates of evolution in holothuroids are at least 50% higher, regardless of reconstruction method. It is unclear whether this is a genome-wide phenomenon or is restricted to some genes such as ssu rRNA. The rate at which mutations become fixed within a gene can vary widely between species and substantial rate variation in ssu rDNA is known in other metazoan groups (Hillis and Dixon, 1991).

The high amount of evolution along deeper branches could indicate that a group often considered ancient, the dendrochirote holothuroids, is much younger than usually assumed. By comparison, the two most distantly related echinoids used in the study represent lineages that have diverged about 250 million years ago (mya). Because the ssu rRNA gene evolves on average so slowly over such time periods (Hillis and Dixon, 1991), there was little variation between the sequences of the echinoids (about 1.5%) and their divergences were not strongly resolved in this study (Fig. 2.2). Sequences from the dendrochirote holothuroids *Cucumaria sykion*, *Lipotrabeza vestiens* and *Sclerodactyla briareus* also differed by a comparable amount, about 2% (Table 2.1), so that their relationships were likewise poorly resolved. Dendrochirotes are thought to constitute a very old lineage, one originating in the early Paleozoic, around 400 mya (Pawson and Fell, 1965; Haude, 1992; Arndt *et al.*, 1996; Reich, 1999). Body fossils from this period encased in plates resemble living species of dendrochirotes, the only living group with plated members. If dendrochirote holothuroids are this old, then the rate of evolution of the ssu rRNA gene in holothuroids appears to have been extremely variable. To account for the observed pattern, rRNA evolution must have been extremely high in the first few tens of millions of years in the lineage leading from the first holothuroids to dendrochirotes and then, in the dendrochirote lineage, became about nine times as slow beginning 400 mya. Alternatively, evolution has proceeded at a more uniform pace,

though still much faster than in echinoids, and the dendrochirotes are much younger than has been assumed. If so, then the divergence of these holothuroids has probably occurred sometime in the early to middle Mesozoic, some 250 to 200 mya.

Phylogenetic and Taxonomic Implications

The phylogenetic analyses recovered the relationship (Apodida, (Elasipodida, (Aspidochirotida, Dendrochirotida))). These results have several important implications for the phylogeny and taxonomy of the Holothuroidea. First, as discussed above, the analysis seriously calls into question the idea that dendrochirotes are the most divergent group of living holothuroids. I suggest that presumed ancient dendrochirote body fossils and ossicles are either from stem members (those on the lineage leading to living forms) and plated bodies were lost multiple times, or that plates evolved independently on several occasions. Second, I also show that apodans are the most divergent of holothuroid groups sampled so far (Fig. 2.2A). This is an old idea and has been previously argued or suggested based largely on comparative anatomy by several authors (Semper, 1868; Huxley, 1878; Semon, 1888; Cuénot, 1891; Östergren, 1907; Seilacher, 1961; Haude, 1992; Smith, 1997). Finally, this analysis confirms aspects of trees based on molecular data from less diverse sets of holothuroids by Littlewood *et al.* (1997) (Fig. 2.1). Dendrochirotes

and aspidochirotetes are more closely related to one another than either are to elasipodans or apodans. The most important taxonomic implication of these results is that the subclass Aspidochirotacea (Elasipodida+Aspidochirotida) is paraphyletic. Independent confirmation of this analysis and tests of the monophyly of other higher taxa would be worthwhile and is the subject of Chapter III, where a detailed consideration of the implications outlined here is presented.

Chapter III:
Phylogeny of Holothuroidea Inferred from Morphology

A version of this chapter appears in: Kerr, A. M. and J. Kim. 2001
Phylogeny of Holothuroidea (Echinodermata) inferred from morphology.
Zoological Journal of the Linnean Society 133: 63-81.

Summary

Holothuroids, or sea cucumbers, are an abundant and diverse group of echinoderms with over 1400 species occurring from the intertidal to the deepest oceanic trenches. In this study, I report the first phylogeny of this class, based on a cladistic analysis of 47 morphological characters. I introduce several previously unconsidered synapomorphic characters, examine the relationships between representatives from all extant families and assess the assumptions of monophyly for each order and subclass. Maximum parsimony analyses using three rooting methods recovered well-supported and identical topologies when two small and apparently derived families, Eupyrgidae and Gephyrothuriidae, were removed. The results suggest that the higher-level arrangement of Holothuroidea warrants a considerable revision. Apodida was sister to the other holothuroids. The monophyly of Dendrochirotida was not supported and the group may be paraphyletic. A randomization test using Wills' gap excess ratio found significant congruence between the phylogeny and the stratigraphic record of fossil members, suggesting that the fossil record of holothuroids is not as incomplete as is often stated. The fossil calibrated tree indicated that several groups of holothuroids survived the end-Permian mass extinction and that the clade composed of Dendrochirotida, Dactylochirotida, Aspidochirotida and Molpadiida rapidly radiated during the Triassic.

Introduction

Holothuroids, or sea cucumbers, are an abundant and diverse group of marine invertebrates. The more than 1400 described and extant species comprising 160 genera (Smiley, 1994) occur in benthic environments from the intertidal to the deepest oceanic trenches, where they may comprise >90% of the biomass (Belyaev, 1972). Unique among echinoderms, holothuroids can be holopelagic (Miller and Pawson, 1990) and even ectocommensals (Martin, 1969). Most holothuroids are under 20 cm in length, though some reach lengths of 5 m (Mortensen, 1938) or weigh over 5 kg (Lane, 1992). Their diversity is highest in the tropical eulittoral, where 20 species per hectare is not uncommon (Kerr *et al.*, 1993). The ubiquity of holothuroids in the largest ecosystem, the abyssal plain, renders them one of the dominant large animals on earth. Yet, despite their dominance, diversity and the scrutiny paid to other echinoderm groups, there remain numerous, longstanding and basic questions about the systematics and evolution of Holothuroidea. In this study, I report on the first cladistic analysis aimed at elucidating higher level relationships within the entire Holothuroidea. The monophyly of all ordinal and subclass-level groups are tested with representatives from each of the currently recognized extant families.

Higher Level Taxonomy and Phylogeny

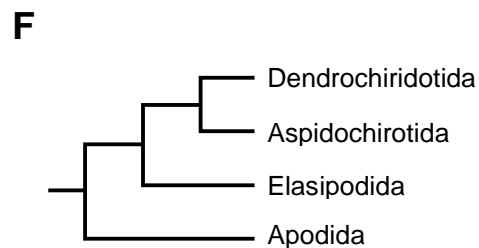
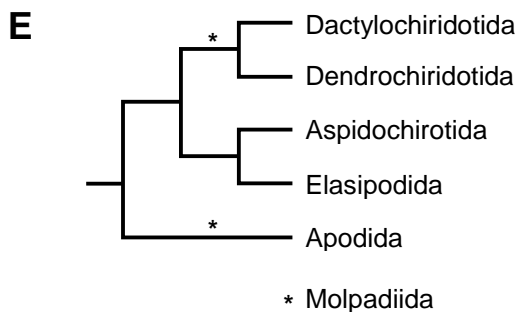
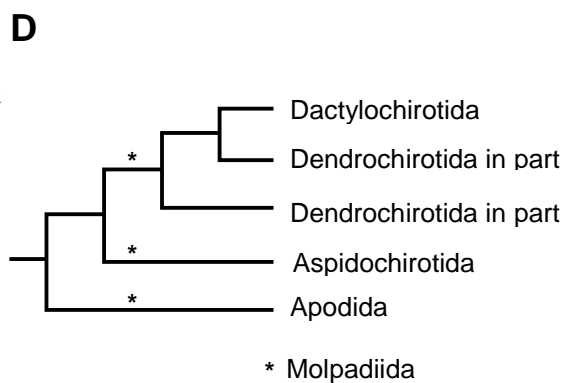
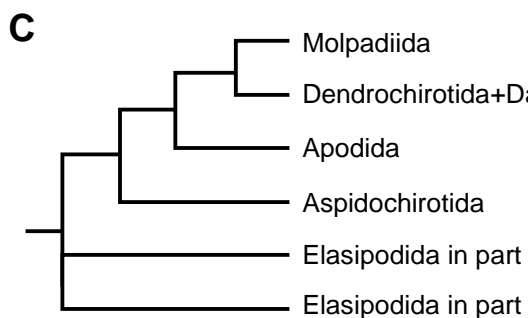
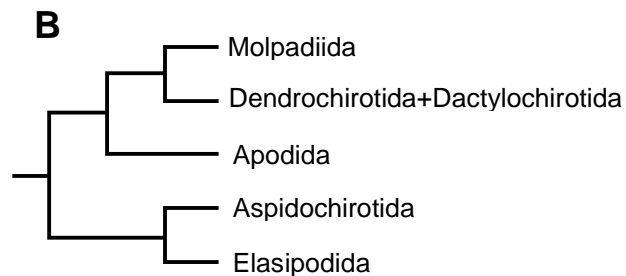
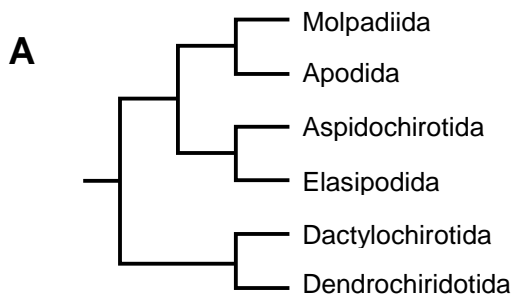
Bronn (1860) was the first to designate Holothuroidea as a class, dividing the group into two orders. The first order was monotypic with the bizarre, flask-shaped *Rhopalodina* (=Rhopalodinidae) and the second was composed of all other holothuroids. Shortly thereafter, Selenka (1867) redistributed the species into orders of those either with or without respiratory trees. Ludwig (1889-1892), in contrast, assigned membership to his two new orders based on the embryological origin of the tentacles. MacBride (1906) argued that Ludwig's distinctions were arbitrarily drawn from a continuum of differences in tentacle formation and suggested six orders. One of these, Pelagothurida (=Pelagothuriidae), has been ignored by most subsequent authors, having long been recognized as a derived member of Elasipodida (Hansen, 1975). Pawson and Fell (1965) raised one of MacBride's orders to subclass status, dividing it into two orders, Dendrochirotida and Dactylochirotida, based on pronounced differences in tentacle and gross body characters. Pawson and Fell (1965) also considered the arrangement of the remaining orders, uniting Aspidochirotida and Elasipodida as the Aspidochirotea and joining Apodida and Molpadiida as the Apodacea.

Pawson and Fell's (1965) primary motivation for their new classification was to have the Linnean scheme better reflect the perceived close evolutionary relationship between dendrochirote holothuroids and

the extinct edriasteroids. Fell (1965) and Fell and Moore (1966) posit a homologous relationship between several features of these two groups, including the arrangement of ambulacral plates of the edriasteroids with the circum-oesophageal calcareous ring of holothuroids and the shared feature of a plated test. Pawson and Fell's (1965) taxonomic scheme is transformed into a phylogeny in Fig. 3.1A. Their arrangement mirrors in part the earlier speculations of Théel (1886) who also suggests that the common ancestor of holothuroids is most similar to those in Dendrochirota (=Dendrochirotea) and that the Aspidochirota (=Aspidochirotida) and Elasipoda (=Elasipodida) are most closely related.

Ludwig (1891) argues and others (Gerould, 1896; Clark, 1898) agree that together the Aspidochirotida and Elasipodida are probably sister to the remaining holothuroids (Fig. 3.1B). However, MacBride (1906), concurring with an earlier speculation by Théel (1886), writes that the Elasipodida alone had diverged earliest (Fig. 3.1C). Adding to this diversity of opinions are numerous workers (Semper, 1868; Huxley, 1878; Semon, 1888; Cuénot, 1891; Östergren, 1907; Seilacher, 1961; Haude, 1992) who regard the Apodida as the most divergent member of Holothuroidea (Fig. 3.1D, E). Most recently, Littlewood *et al.* (1997) sequenced 12S- and 18S-like ribosomal genes of holothuroids from a total of four orders in an effort to resolve relationships between classes of

Figure 3.1. Phylogenetic hypotheses of holothuroid relationships. A) Pawson and Fell's (1965) Linnean classification rendered as a phylogeny. B) Ludwig (1891). C) MacBride (1906). D) Semper (1868). E) Haude (1992). F) Favored resolution in Smith (1997) of data from Littlewood *et al.* (1997). Taxon designations are from Pawson and Fell (1965) and exclude members of three families (Synallactidae, Eupyrgidae, Gephyrothuriidae) whose ordinal assignments differ between authors. Asterisks indicate alternative possible positions for Molpadiida.



Echinodermata. Smith's (1997) interpretation of these data (Fig. 3.1F) also shows Apodida as sister to the remaining holothuroids.

Methods

Ingroup Taxa

This study included exemplars from all 25 currently recognized (Pawson, 1982) extant families in Holothuroidea (Table 3.1). The monophyly of some of these families is far from certain. For example, Synallactidae is a morphologically diverse and likely paraphyletic to polyphyletic group with members displaying numerous affinities to either Holothuriidae or Stichopodidae. Similarly, the paucitypic dendrochirote families Paracucumidae, Heterothyonidae and Placothuriidae, could turn out to be heavily plated members of more speciose soft-bodied groups, perhaps within Phyllophoridae. Regardless of these uncertainties about the monophyly of the taxonomic units, exemplars must nevertheless possess the plesiomorphies of the clades they purport to represent. I chose to use, in the absence of any other information, the prevalent character state of a family as its likely ancestral state when it was exhibited by an overwhelming proportion of taxa in a presumed monophyletic family. Finally, some type species themselves are little-known or, I felt *a priori*, from clearly derived families. These were Pelagothuriidae, a group highly modified for a pelagic existence, Rhopalodinidae, which have their

Table 3.1. Taxa used in this study.

Order	Family	Species used
Dendrochirotida Grube, 1840	Placothuriidae Pawson & Fell, 1965	<i>Placothuria huttoni</i> (Dendy, 1896)
	Paracucumidae Pawson & Fell, 1965	<i>Paracucumis antarctica</i> Mortensen, 1925
	Psolidae R. Perrier, 1902	<i>Psolus chitonoides</i> H.L.Clark, 1901
	Heterothyonidae Pawson, 1970	<i>Heterothyone alba</i> (Hutton, 1872)
	Phyllophoridae Östergren, 1907	<i>Afrocucumis africana</i> (Semper, 1868)
	Sclerodactylidae Panning, 1949	<i>Sclerodactyla briareus</i> (Lesueur, 1824)
	Cucumariidae Ludwig, 1894	<i>Cucumaria frondosa</i> (Gunnerus, 1767)
Dactylochirotida Pawson & Fell, 1965	Ypsilothuriidae Heding, 1942	<i>Ypsilothuria bitentaculata</i> (Ludwig, 1893)
	Vaneyellidae Pawson & Fell, 1965	<i>Vaneyella</i> Heding & Panning, 1954
	Rhopalodinidae R. Perrier, 1902	<i>Rhopalodina lageniformis</i> Gray, 1853
Aspidochirotida Grube, 1840	Holothuriidae Ludwig, 1894	<i>Holothuria atra</i> Jäger, 1833
	Stichopodidae Haeckel, 1896	<i>Stichopus chloronotus</i> Brandt, 1835
	Synallactidae Ludwig, 1894	<i>Mesothuria verrilli</i> Théel, 1886
Elasipodida Théel, 1882	Deimatidae Ekman, 1926	<i>Deima validum</i> Théel, 1879
	Leatmogonidae Ekman, 1926	<i>Laetmogone violacaea</i> Théel, 1879
	Psychropotidae Théel, 1882	<i>Psychropotes longicauda</i> Théel, 1882
	Elpidiidae Théel, 1879	<i>Elpidia glacialis</i> Théel, 1876
	Pelagothuriidae Ludwig, 1894	<i>Pelagothuria natatrix</i> Ludwig, 1894
Apodida Brandt, 1835	Chiridotidae Östergren, 1898	<i>Chiridota laevis</i> (Fabricius, 1780)
	Synaptidae Burmeister, 1837	<i>Synapta maculata</i> (Chamisso & Eysenhardt, 1821)
	Myriotrochidae Théel, 1877	<i>Myriotrochus rinkii</i> Steenstrup, 1851
Molpadiida Haeckel, 1896	Molpadiidae J.F. Müller, 1850	<i>Molpadia intermedia</i> (Ludwig, 1894)
	Caudinidae Heding, 1931	<i>Caudina arenata</i> (Gould, 1841)
	Gephyrothuriidae Koehler & Vaney, 1905	<i>Gephyrothuria alcocki</i> Koehler & Vaney, 1905
	Eupyrgidae Semper, 1868	<i>Eupyrgus scaber</i> Lütken, 1857
Arthrochrotida Seilacher, 1961	no family designation	<i>Palaeocucumaria hunsrueckiana</i> Lehmann, 1958

mouth and anus adjacent and atop a long “neck,” and, finally, two families, Gephyrothuriidae and Eupyrgidae, with tiny members displaying features of juveniles from other groups. Extremely modified taxa or taxa with many missing data can complicate phylogenetic analyses in a number of ways, including that of weakening statistical support for parts of a tree. Thus, to test the effect of the purported derived groups on phylogeny reconstruction, I ran all analyses both with and without all subsets of these enigmatic families.

Outgroup Selection and Rooting

Holothuroidea appears to have had its origin in the Ordovician from within the extinct, echinoid-like Ophiocystioidea (Smith, 1988). The earliest known complete body fossils of undoubted holothuroids are of two species from the Lower Devonian (Haude, 1995a, b). One of these, *Palaeocucumaria hunsrueckiana* (Lehmann, 1958), displays several features in common with both holothuroids and ophiocystioids that make it the strongest candidate as a stem member of Holothuroidea and an appropriate outgroup for this study. Like the ophiocystioid *Rotasaccus* (Haude and Langenstrassen, 1976), *Palaeocucumaria* possesses a spiculated body and enlarged, heavily calcified tubefeet restricted to the oral end, which according to Smith (1988) are arranged in ambulacral rows (see photo in Frizzell and Exline, 1966, fig. 525; schematic of same

in Smith, 1988, fig. 7.4). However, it also displays two undoubted autapomorphies of Holothuroidea, a calcareous ring and an antero-posteriorly elongated body.

Fossil taxa often lack soft-tissue preservation so that characters must be coded as missing. Characters from *Palaeocucumaria* are no exception: Internal features, other than the calcareous ring and a gut trace visible by radiography (Lehmann, 1958), are not preserved and diagenesis obscures the body-wall ossicles (but see interpretation by Seilacher, 1961). Hence, in addition to rooting the holothuroid tree with *Palaeocucumaria*, I used a hypothetical ancestor (“ancestor rooting”) based on primitive states inferred from *Palaeocucumaria*, Ophiocystioidea, Paleozoic Echinoidea and extant Echinoidea. Echinoids are used in this role as they are recognized as the sister group to ophiocystioids+holothuroids (Littlewood *et al.*, 1997) and living echinoids permit coding of some soft-tissue features. Justification of each character-state assignment for *Palaeocucumaria* and the hypothetical ancestor is given in the section on characters.

Finally, I rooted trees on the longest branch (“long-branch rooting”) suggested by the fossil histories of the groups. The oldest undoubted holothuroid ossicles are assignable to dendrochirotaceans (first recorded from Upper Silurian), Elasipodida (Middle Devonian) and Apodida

(Middle Devonian) (Gilliland, 1993). Hence, I predicted that the longest branch, the one with the most unambiguous changes, would lie somewhere between these groups and that rooting on this branch will create a topology in which the placements of these groups are concordant with those produced by the other rooting procedures.

Phylogenetic Analyses

Maximum parsimony analyses of the data were performed on *PAUP* 4.0b 2a* (Swofford, 1998) with the following options: branch-and-bound search, multistate taxa treated as uncertainties and zero-length branches collapsed. For each rooting and set of ingroup taxa, I explored the effects of equal and successive weighting schemes. Under successive weighting, characters were weighted according to the rescaled consistency index and when multiple most parsimonious trees occurred, the maximum value of the index was used. I assessed data quality by bootstrapping using 500 replicates under a heuristic search, as well as by assessing the skewness of the tree-length frequency distributions generated from 10^5 trees randomly produced from the data. The heuristic search in the bootstrap analyses used the options: keep minimal trees, starting tree via simple stepwise addition, swap on minimal trees, branch swapping via tree bisection-reconnection while saving all minimal trees. Change of

individual characters along branches was examined using *MacClade 3.0* (Maddison and Maddison, 1992).

Stratigraphic Congruence

To gauge the congruence between the resulting phylogeny and the fossil record, I used the gap excess ratio of Wills (1999). This metric first considers the total ghost range implied by a tree for a set of stratigraphic ranges. Then the difference between the total ghost range and the minimum possible ghost range is calculated as is the difference between the minimum and maximum possible ghost ranges for any tree. The one complement of the ratio of these differences provides an index of congruence between the observed tree and the fossil ranges. Significance of the ratio was assessed via a one-tailed randomization test, that is by noting the proportion of times the ratio equaled or exceeded those obtained by randomly reassigning the range data over the tree 10^5 times (Wills, 1999). This tests the hypothesis that congruence between the phylogeny and fossil record is no better than expected by chance. A copy of the program that performed these calculations is available from the author.

Characters

A total of 47 discrete post-larval characters, 15 skeletal, 31 soft-tissue and 1 behavioral, were scored, including 37 binary and 10 unordered, multistate characters (Table 3.2). Characters were taken primarily from published taxonomic and anatomical monographs (e.g., Clark, 1907; Hansen, 1975; Gilliland, 1993). In addition, I examined and dissected museum and field-collected specimens of at least one representative species from each extant family (Table 3.1) to confirm the published accounts. Specimens from Rhopalodinidae, Vaneyellidae and Placothuriidae, however, were unavailable for dissection because of their rarity. Hence, published accounts were entirely relied upon to determine their morphology. In several poorly known families, characters were scored based on several species.

Gross Morphology

1. *Overall shape: 0 = vermiform; 1 = body convex, with central thickening.*

Vermiform is defined as having a body diameter that is circular in cross section, with the diameter either constant along the length of the body or slightly decreasing posteriorly. Holothuriidae are usually ventrally flattened and convex, though a few very long species in *Holothuria* subgenera (e.g., *Acanthotrapeza* and *Mertensiothuria*) appear vermiform when fully extended. A vermiform body is assigned to

Table 3.2. Character matrix. Missing or logically prohibited codings are indicated by “?”, an “a” indicates polymorphic states 3,1, while “b” indicates 3,2 and a “c” is 1,2.

Taxa	Characters									
	5	10	15	20	25	30	35	40	45	
<i>Ypsilothuriidae</i>	10101	11111	20010	0101?	10000	01000	00001	01111	01100	00
<i>Vaneyellidae</i>	10101	11111	20010	0101?	10000	01000	00001	01?11	?1100	00
<i>Rhopalodinidae</i>	10101	11111	10101	0101?	1a000	01000	00001	01?11	01100	00
<i>Psolidae</i>	11110	01111	20010	010c1	00110	01020	00001	01111	01110	01
<i>Paracucumidae</i>	11100	00010	20010	01011	10010	01020	00001	01?11	01110	01
<i>Placothuriidae</i>	11100	00010	20110	110c1	00010	01020	00001	01?11	01110	01
<i>Heterothyonidae</i>	11101	10010	20110	010c1	00110	01020	00001	01?11	01110	01
<i>Phyllophoridae</i>	11000	00010	20110	10011	0a010	01020	00001	01111	01110	01
<i>Sclerodactylidae</i>	11000	00010	20110	00011	0a110	01020	00001	01111	11110	01
<i>Cucumariidae</i>	11000	00010	20010	00011	0a110	01020	00001	01111	01110	01
<i>Holothuriidae</i>	11010	00000	20001	10011	03000	01011	00011	11011	11010	00
<i>Stichopodidae</i>	11010	00000	20001	10011	03000	01011	01011	11011	11010	00
<i>Synallactidae</i>	11000	00000	20001	10011	0b000	01010	010?1	01111	01010	00
<i>Molpadiidae</i>	11001	00000	20110	1001?	02000	11001	00001	21111	11010	10
<i>Caudinidae</i>	11001	00000	20110	1000?	03000	01001	00001	21111	11010	10
<i>Eupyrgidae</i>	10101	00000	21000	10010	02000	11000	00001	01111	01010	00
<i>Gephyrothuriidae</i>	00000	00000	21000	1?000	00000	01000	01001	01?11	010?0	00
<i>Synaptidae</i>	00300	00000	21000	1011?	00000	00000	00000	00001	00001	10
<i>Chiridotidae</i>	00300	00000	21000	1010?	00000	00000	00000	00101	00001	10
<i>Myriotrochidae</i>	00300	00000	21000	1010?	00000	00000	00000	00101	00000	10
<i>Elpidiidae</i>	10210	02000	00000	?0200	00001	11110	11101	01100	01000	10
<i>Psychropotidae</i>	11210	02200	10000	00000	00001	11010	01101	01200	01000	00
<i>Laetmogonidae</i>	11210	02200	10001	00202	00000	11110	11001	01200	01000	00
<i>Deimatidae</i>	11110	02000	10001	02010	00000	11110	11001	01200	01000	00
<i>Pelagothuriidae</i>	11200	00000	0????	??000	00000	11010	01101	01200	01000	01
<i>Palaeocucumaria</i>	00000	00000	20000	1000?	?????	???00	000??	?????	?????	?0
Ancestor	00000	00000	20000	10000	00000	10000	00000	00002	0?000	00

Chiridotidae, though in one genus *Polycheira* large specimens may take on a convex outline. The body outline in the outgroup *Palaeocucumaria* appears cylindrical or tapers posteriorly and the animal is coded as vermiform.

2. *Maximum body length: 0 = to 5 cm; 1 = greater than 5 cm.*

The size cut-off of 5 cm is based on the maximum length of the outgroup exemplar *Palaeocucumaria* (Haude, 1995a). Paleozoic echinoderms most closely related to holothuroids, Ophiocistioidea and, possibly, bothriocidarids, as well as the most closely related Paleozoic echinoids, e.g., *Eothuria*, (Smith, 1988; Lewis and Donovan, 1998) are also all about this length. Length is more variable in some extant families of holothuroids. There are a few species under 5 cm in Synaptidae and Molpadiidae. Diminutiveness in these families is considered derived. Reports of very small species in Holothuriidae and Stichopodidae (e.g., Cherbonnier, 1988) are likely of juveniles and comprise only a minority of taxa, so are not considered.

3. *Body wall: 0 = thick and firm; 1 = testaceous; 2 = gelatinous; 3 = extremely thin.*

Spiculation, musculature and thickness of the connective tissue layer largely determine the mechanical properties of holothuroid body wall. Apodans usually possess an extremely thin layer of connective

tissue, resulting in an often-transparent body. This condition presumably facilitates gas exchange in the absence of respiratory organs (Smiley *et al.*, 1991). However, most taxa have a connective tissue layer of intermediate thickness and well-developed circular muscles. Non-deimatid elasipodan families have a thick, gelatinous layer of connective tissue. Dactylochirotetes and some dendrochirote families are encased in a test of enlarged ossicles, nearly always imbricate. Deimatid elasipodans appear similarly encased, but with irregularly overlapping plates.

4. *Pronounced sole: 0 = absent; 1 = present.*

A well-demarcated and flattened sole is found in all species in four of the five families of Elasipodida, and in the dendrochirote Psolidae. The character was coded as present in Holothuriidae as it occurs in all species in three genera, *Actinopyga*, *Bohadschia* and *Pearsonothuria*. Of the remaining two genera, a flattened ventrum occurs variably in *Holothuria* and is absent in *Labidodemas*. Similarly, the character was recorded as present in Stichopodidae as it occurs in numerous to all species in most genera. A flattened sole is also found in most genera of Synallactidae.

5. *Tail: 0 = posterior extremely narrowed and elongated; 1 = posterior not greatly narrowed.*

This character is coded as absent in Psolidae, though at least one psolid, *Psolus phantapus* (Strussenfelt), can extend and narrow its

posterior greatly. A tail is recorded as present in Rhopalodinidae, though the appendage presents differently: It is fused alongside the animal's equally elongate neck and together they form a stalk (Semper, 1868). Gephyrothuriidae possess a caudal appendage that is apparently an extension of the cloaca rather than a narrowing of the posterior body (O'Loughlin, 1998). For Caudinidae a tail is considered present despite its absence in one genus *Acaudina*.

6. Neck: 0 = posterior extremely narrowed and elongated; 1 = posterior not greatly narrowed.

The same arguments used in the coding of the tail (Character 5) of Psolidae, Cucumariidae and Rhopalodinidae apply here.

7. Position of mouth: 0 = terminal to subterminal; 1 = clearly dorsal; 2 = clearly ventral.

In numerous taxa, e.g., Holothuriidae and Stichopodidae, the position of the mouth has been variably recorded as terminal or ventral. Yet, when chemically relaxed, the species I have examined display subterminal mouths. The exemplar for Synallactidae, *Mesothuria*, is coded as having a subterminal mouth though several other genera in this likely para- to polyphyletic family display clearly ventral mouths (e.g., *Paelopatides*).

8. *Position of anus: 0 = terminal to subterminal; 1 = clearly dorsal; 2 = clearly ventral.*

In most psychropotids the anus is ventral, but in one genus, *Benthodytes*, it is dorsal (Hansen, 1975). The anus is coded as terminal in *Palaeocucumaria* based on a radiograph (Lehmann, 1958) that shows a gut trace terminating at a pointed posterior.

9. *Pharyngeal introvert: 0 = absent; 1 = present.*

The introvert is found in dendrochirote and dactylochirote holothuroids. It is a retractile portion of the anterior body wall that allows the complete retraction of the tentacles. This character, along with retractor muscles (Character 47), defines the subclass Dendrochirota and is found in no other group. An introvert is coded as absent in *Palaeocucumaria* as all specimens, even quite contracted ones, still display everted tentacle crowns.

10. *CD interradius: 0 = very short; 1 = about equal in length to other interradii.*

Pronounced foreshortening of the CD interradius occurs in most members of Dactylochirota and Psolidae. Foreshortening is extreme in Rhopalodinidae where the mouth and anus lie adjacent.

Calcareous Ring

The calcareous pharyngeal ring is a defining feature of Holothuroidea and is possessed by all extant species, excepting the Pelagothuriidae. The evolutionary origin of the structure is uncertain, though it may be derived from elements of the echinoid lantern (Littlewood *et al.*, 1997, their character 52) or peribuccal ambulacral plates (Fell and Moore, 1966; Haude, 1994). The calcareous ring supports the pharynx, water vascular ring and tentacle ampullae, as well as providing insertion points for the longitudinal and retractor muscles. The oldest known intact calcareous rings are from the Lower Devonian and they possess, like nearly all extant species, five radial and five interradial pieces (Haude, 1995b). Some large living synaptids, however, may possess extra, usually dorsal, interradial elements to accommodate extra oral tentacles. Reduction in the number of ring elements also occurs and is discussed below under character 11.

11. *Level of calcification: 0 = indistinct, reduced or absent; 1 = robust, well calcified and elements discrete.*

Calcareous rings are indistinct in families belonging to the Elasipodida with the exception of Elpidiidae. In this family the ring is reduced to five distinct, stellate radial pieces resembling the primordia found in juvenile non-elasipodan holothuroids (Clark 1898) and is coded herein as reduced.

12. *One or more radial pieces perforated: 0 = perforate; 1 = notched.*

With the exception of the three taeniogyrine genera in Chiridotidae (Smirnov, 1998) and possibly the tiny myriotrochid *Paratrochus* (Gage and Billett, 1986), families in Apodida possess radial plates perforated for the passage of the radial nerve (not the radial water vessel as reported by some authors). Perforations are not visible on the radial plates of the outgroup exemplar *Palaeocucumaria* (Haude, 1995a). The oldest known perforate rings are from a somewhat younger fossil, the Middle Devonian *Nudicorona seilacheri* (Haude, 1997). The oldest known radial plates, occurring as isolated elements, are from the Upper Silurian and appear imperforate (Reich, 1999).

13. *Long posterior processes on radial plates: 0 = absent; 1 = present.*

Long posterior, extensions of the radial plates are usually paired and may be entire, as in the Molpadiida and sclerodactylid *Pentamera*, or composed of a mosaic of pieces as in many phylloporids.

14. *Proportions of radial plate: 0 = length and width equal to subequal; 1 = length much greater than width.*

Most members of Molpadiida and Dendrochirotida possess long narrow ring plates, though in the latter order there are a few species with quadrate radial plates more typical of families in Aspidochirotida.

15. *Anterior margin of radial plates widened and with depressions to accommodate tentacular ampullae: 0 = absent; 1 = present.*

Wide depressions for the tentacular ampullae often give the radial plates a squared anterior margin. If the depressions are deep, they and the central groove for the radial nerve give a scalloped appearance to the anterior edge.

16. *Articulation of plates: 0 = height of articulation small in proportion to interradial height; 1 = area of articulation along considerable length of interradial plate.*

In most Dendrochirotida, the radial and interradial plates join only at their posterior-most lateral margins. In apodans, plates adjoin along their entire lateral margins, giving the calcareous ring a “band-like” appearance.

Ossicles

17. *Ossicle arrangement in body wall: 0 = isolated; 1 = overlapping towards oral-aboral ends and mid-dorsal interradius; 2 = overlapping irregularly.*

Most holothuroids have ossicles dispersed in the body wall. In preserved specimens examined of the dendrochirotes Heterothyonidae,

Paracucumaridae, Placothuriidae, Psolidae, Rhopalodinidae and the dactylochirote Ypsilothuriidae, there is a test of overlapping ossicles converging at the mouth, anus and midpoint of the dorsal interradius. With the exception of the ventrally aplacate Psolidae, these plates radiate from near the center of the midventral radius. In *Psolus peronii* Bell, the ossicles are appressed exteriorly and the imbrication is only visible from the interior. In contrast to the aforementioned families, Deimatidae plates stack with no discernible regularity - an autapomorphy. There are two minor but interesting exceptions to these trends seen among holothuroids not considered in the present study. First, in the dendrochirotetes *Loisettea* and *Leptopentacta*, the ossicles abut rather than overlap, even in quite contracted specimens. Second, the Middle Triassic *Strobilothyone rogenti* Smith and Gallemí, 1991, assigned to the dendrochirote family Heterothyonidae, has ossicles that imbricate in a manner not seen in extant dendrochirotetes or any other holothuroid. Ossicles, beginning along the circumference at mid-body, jut straight out then gradually begin overlapping towards the oral and aboral ends. At least one specimen of *Strobilothyone*, however, has ossicles that overlap from mouth to anus (Smith and Gallemí, 1991, pl. 3, fig. 2), another type of imbrication not seen in any other holothuroid. This second form of imbrication suggests that it or the other is a taphonomic artifact or, quite speculatively, that attachment to the body wall occurred along only one margin of the

ossicle. The outgroup *Palaeocucumaria* is coded as having dispersed ossicles as argued by Seilacher (1961).

18. *Wheels: 0 = absent; 1 = stellate primordium; 2 = cruciate primordium.*

Wheel ossicles are derived from a stellate primordium in apodan holothurians and from a “primary cross” (Hansen, 1975) in laetmogonid elasipodans. Wheel ossicles are known from Synaptidae in three species where they occur only in larvae (Semon, 1888; Mortensen, 1937, 1938). The occurrence of wheels in unidentified synaptid larvae (Pawson, 1971) and recently metamorphosed juveniles of unidentified synaptids (Inaba, 1934) suggests that wheels are a widespread feature of the family. A few species in scattered genera of elpidiid elasipodans also present laetmogonid-type wheels. That the diminutive elpidiids have apparently evolved via paedomorphosis (Théel, 1886; Hansen, 1975) and are not thought to be closely related to laetmogonids, led Hansen (1975) to suggest that wheels in elpidiids were a retained juvenile character and may be present in many other larval and juvenile elasipodans. However, more recent reports of a taxonomically diverse haul of larval and post-larval specimens (Billett *et al.*, 1985; Gebruk, 1990; Gebruk *et al.*, 1997) indicate that elasipodans develop adult-type ossicles at a very early stage. The outgroup is coded as lacking wheels since they appear to be absent in *Palaeocucumaria* (Seilacher, 1961) and are absent in Paleozoic echinoids

and most ophiocistioids. The notable exception is the possible parallelism in *Rotasaccus*, an ophiocistioid that has wheels most resembling those of the extant myriotrochid *Acanthotrochus* (Haude and Langenstrassen, 1976).

19. *Plates: 0 = absent; 1 = perforate; 2 = lenticular.*

Perforate plates are planar to concavo-convex ossicles formed by repeated distal bifurcations of a rod primordium and reticulation of the branches. Lenticular plates, sometimes called scales, are also restricted to the body wall, but have a complex multi-layered structure and the ossicle is much thicker.

20. *Tubefoot endplate: 0 = absent; 1 = present.*

Tubefoot endplates, or podial sieveplates, occur at the terminus of suctorial tubefeet often used in locomotion. The endplates in dendrochirotaceans and aspidochirotes superficially resemble, and have an ontogeny apparently similar to, that of body-wall plates, but are invariably circular and have a different distribution of hole sizes. Laetmogonids possess a stellate endplate in their tubefeet and they are given an autapomorphic state. This type of endplate resembles the endplates in the juvenile dendrochirote *Pawsonia* (Gilliland, 1993). Molpadiidans and apodans lack tubefeet, hence are coded as lacking endplates.

21. *Spired plates: 0 = absent; 1 = present.*

This and the following three characters refer to the presence on plates of a spire growing orthogonal to the plane of the ossicle. Spired plates, found in Paracucumidae and the dactylochiotes, frequently have an eccentrically placed spire of three to four, often fused pillars.

22. *Tables: 0 = absent; 1 = two-pillared; 2 = three-pillared; 3 = four-pillared.*

This ossicle consists of a basal plate of few and often regularly arranged holes from which centrally arises a spire of two to four pillars linked by crossbeams. The spire's terminus is often spinose.

23. *Baskets: 0 = absent; 1 = present.*

Baskets resemble a very concavo-convex, reduced plate and are found in Cucumariidae, Heterothyonidae, Psolidae and Sclerodactylidae.

24. *Dendrochirote buttons: 0 = absent; 1 = present.*

These buttons, like plates, are derived from a primary cross, but have very few, often only four, holes arranged in a cross. They differ from holothuriid buttons, which display two parallel rows of holes. Dendrochirotid buttons occur in many members of all dendrochirote families.

25. *Psychropotid rods: 0 = absent; 1 = present.*

Psychropotids and most elpidiids possess a unique ossicle type, a cross of usually four curved arms, each with a centrally arising branch.

Water Vascular System

26. *External communication of hydropore/madrepore: 0 = absent; 1 = present.*

An external opening of the water vascular system is an ancestral feature of stem holothuroids, occurring in ophiocistioids. In holothuroids, the hydropore may be a single opening, have multiple branching openings or, in some elasipodans, be lightly calcified as a madrepore. Retention of a hydropore into adulthood is known for some likely pedomorphic species, e.g., the diminutive synaptid *Synaptula hydriformis* (Clark, 1898), but these are isolated instances and likely represent derived conditions. The existence of a madrepore is uncertain in *Palaeocucumaria*, but is coded as present in the hypothetical ancestor based on its occurrence in ophiocistioids (Ubaghs, 1966).

27. *Longitudinal vessels: 0 = absent; 1 = present.*

Mooi and David (1997) in a consideration of echinoderm skeletal homologies, refine a previous argument (Semon, 1888) that the “radial

canals” of holothuroids are not homologous with those of other echinoderms. The five primary evaginations of the larval hydrocoel that in other echinoderms become the radial canals, in holothuroids form the first five buccal tentacles (Smiley, 1986). Only subsequent to this do longitudinal canals evaginate, extend posteriorly and sprout tube feet in a way suggestive of non-holothuroid radial canals. Mooi and David (1997) contend that this extension of the water vascular system is unique among echinoderms, occurring as it does in the “extraxial” portion of the body, which they define for all echinoderms via skeletal and embryogenic criteria. Based on their arguments, I code the absence of longitudinal canals as plesiomorphic.

28. *Dermal water vascular cavities: 0 = absent; 1 = present.*

In some elasipodans, dermal diverticulae extend from the radial water vascular system and communicate with the tube feet. Those of elpidiid, psychropodid and laetmogonid elasipodans likely function as water reservoirs for powering the locomotory tube feet in the absence of ampullae (Hansen, 1975).

29. *Tentacle shape: 0 = simple, pinnate or digitate; 1 = peltate; 2 = dendritic.*

Peltate, or shield-shaped, tentacles consist of a terminal, smooth or papillate disc. Dendritic tentacles have multiply ramified digits that are

regularly or irregularly arranged along a central stalk. This tentacle type occurs in all species of dendrochirote families. Simple tentacles are digitiform. Pinnate tentacles are plumiform with a terminal digit and two rows of from 1 to 80 digits each along the tentacle stalk. Digitate tentacles have rows, or a terminal whorl, of digits, but no terminal digit. Coding the outgroup condition depends on properly homologizing *Palaeocucumaria* tentacles with those of holothuroids and other echinoderm oral water vascular structures. *Palaeocucumaria* “tentacles” are numerous and, according to Smith (1988), arranged in multiple rays, suggesting that they are in fact orally concentrated ambulacral fields of tubefeet typical of echinoderms. Hence, one possibility is that a single *Palaeocucumaria* terminal tubefoot is homologous to a single holothuroid tentacle stalk. Here the appropriate coding of *Palaeocucumaria* is for a simple, digitiform tentacle. Alternatively, perhaps the primitive tubefoot gave rise to a tentacle digit and the entire ambulacral canal underlying the primitive tubefeet is homologous with the tentacle stalk (David and Mooi, 1998). In this case, the outgroup state should be digitate or pinnate. Because of these uncertainties and because the distinction between simple, pinnate and digitate is not always clear (all forms sometime being found within a single family), here the three morphologies are considered together.

30. *Tentacle ampullae*: 0 = absent or reduced; 1 = elongate.

In most holothuroids, the tentacle ampullae are small and abut the anterior to antero-exterior margin of the calcareous ring. In the aspidochirotes and molpadiidans, however, the ampullae are elongate and hang free in the coelom.

31. *Ventrolateral tubefeet: 0 = small or absent; 1 = enlarged.*

Elasipodan holothuroids in Laetmogonidae, Deimatidae and Elpidiidae possess a reduced number of greatly enlarged ventrolateral tubefeet, which in the latter family are absent anteriorly.

32. *Enlarged dorsal papillae: 0 = absent; 1 = present.*

Most members of Stichopodidae and all families of Elasipodida have large thickened dorsal papillae. In the psychropodid elasipodan *Psychropotes*, this may consist of an elongate structure exceeding body length, while in *Benthodytes* and *Psychrelopedia* it may be reduced. Gephyrothuriidae have a few filiform, but very long dorsal papillae.

33. *Fusion of papillae into a brim or velum: 0 = absent; 1 = present.*

In some elasipodans, a web of dermis may unite the anterior- or posterior-most papillae into a brim or velum. This is most developed in Pelagothuriidae, which use the structure in swimming.

34. *Fused oral brim of papillae: 0 = absent; 1 = present.*

Stichopodidae and Holothuriidae possess a circum-oral fringe or collar of small, numerous, fused papillae. The ring of oral to post-oral papillae seen in three genera in three different families of Elasipodida are here considered differently as the papillae are larger, more widely spaced, unfused and sometimes extend only partially around the mouth.

35. *Anal papillae*: 0 = absent; 1 = present.

Anal papillae occur in all non-apodan holothuroids. They differ in morphology within, and to some extent between, families in degrees of branching and calcification. This appears uninformative at the taxonomic levels considered here, but may eventually prove useful in future work within families. The outgroup state is absent based on the lack of these papillae in all other echinoderms.

Other Soft Tissues

36. *Rete mirabile*: 0 = absent; 1 = with parallel connecting vessels; 2 = with a complicated mesh of connecting vessels.

The aspidochirotes Holothuriidae and Stichopodidae and the molpadiidans Molpadiidae and Caudinidae are the only four families of holothuroids to develop an extensive plexus of dorsal hemal vessels, the *rete mirabile*. The coding of this character reflects the differences in the vascular networks between these holothuroid families. In the

aspidochirotes, the dorsal haemal vessel is connected to the descending small intestine by numerous parallel vessels, while in molpadiids, the connecting vessels form an intricate mesh. As well, in aspidochirotes the *rete* continues along the ascending small intestine where it is intimately associated with the left respiratory tree. This extension does not occur in molpadiidans. A vascular network of haemal vessels is coded as absent in the outgroup. This is based on its absence in all other echinoderms, given the likelihood that the transverse dorsal vessel seen in the derived euechinoid Echinidae (Bonnet, 1924) - with a relatively late first appearance in the Lower Tertiary (Smith *et al.*, 1992) - is a convergent condition.

37. *Radial haemal vessels: 0 = absent; 1 = present.*

Only in non-apodan holothuroids do haemal vessels extend into the extraxial body. I code haemal vessels as absent in the holothuroid ancestor based on the argument presented for character 27, longitudinal vessels.

38. *Ovum diameter: 0 = <200 μ m; 1 = 200-500 μ m; 2 = >500 μ m.*

The largest ova in Echinodermata are found among the psychropotid elasipodan holothuroids, with diameters approaching 0.5 cm (Hansen, 1975). Ova of laetmogonid and deimatid elasipodans can also considerably exceed 500 μ m. Most holothuroid ova are between 200-500

µm in diameter, while the three families from which planktotrophic larvae are known, Synaptidae, Holothuriidae and Stichopodidae, have mature ova often well under 200 µm. The outgroup state is coded as <200 µm, based on the generalization that the primitive state for echinoderms is planktotrophic (Strathmann, 1978) and that this larval type develops from very small, yolk-free ova.

39. *Respiratory trees: 0 = absent; 1 = present.*

Unique to Holothuroidea is a pair of often heavily ramified tubes used in gas exchange that arise and receive water from the cloaca. These organs are absent in Elasipodida and Apodida. They are considered primitively absent, as they do not occur in any non-holothuroid echinoderm. Moreover, radiographs of a stem-member holothuroid *Palaeocucumaria* do not indicate the presence of respiratory trees despite a clearly discernible posterior intestine and cloaca into which they insert (Lehmann, 1958).

40. *Attachment to body wall of mesentery engaging posterior intestinal loop: 0 = ventral; 1 = dorsal; 2 = transversely.*

The mesentery of the posterior intestinal loop is attached to the dorsal interradii throughout its length in elasipodans and is connected on one of the ventral interradii in all other holothuroids (Ekman, 1926;

Hansen, 1975). The ancestor state is unique, coded as attaching transversely across several radii based on its position in extant echinoids.

41. *Longitudinal muscles: 0 = undivided; 1 = divided.*

Longitudinal muscles are strap-like and undivided in all apodans and elasipodans, as well as most dendrochirotes. The outgroup is coded as unknown, though it is possible that the ancestral state of Paleozoic echinoids and stem-member holothuroids is of undivided muscles: It is believed that extant echinoids evolved in the Triassic from *Miocidaris*, an echinoid most similar to extant cidarids. However, there is evidence (Lewis and Donovan, 1998) that the majority of extant echinoids, the euechinoids, may have arisen from another, as yet undiscovered group surviving into the Triassic. This form is more typical of Paleozoic fossorial echinoids and most similar to the extant Echinothuriidae, which possesses a flexible test and undivided longitudinal muscles.

42. *Circular muscle attachment: 0 = continuous around inside of body wall; 1 = interrupted by ambulacra and longitudinal muscles.*

Circular muscles occur only interradially in all holothuroids except the apodans. In apodans, circular muscles run continuously around the body wall, with the partial exception of the myriotrochid *Acanthotrochus*. In this genus, only the posterior circular muscles are continuous (Clark, 1907). The outgroup state is coded as unknown.

43. *Retractor muscles*: 0 = absent; 1 = present.

Muscles retracting the tentacles and anterior-most body wall occur only in dactylochirotetes and dendrochirotetes. The outgroup, as *Palaeocucumaria*, is recorded as absent based on the numerous specimens showing substantial body contraction, yet extended tentacles.

44. *Cloacal muscles*: 0 = absent, small, thin, few; 1 = numerous, thick, well developed.

Cloacal muscles adjoin the posterior-most body wall and the cloaca. They are well developed in almost all species except those in Apodida. The muscles are coded as absent in the outgroup based on their absence in all other extant echinoderms.

45. *Ciliated funnels*: 0 = absent; 1 = present.

Ciliated funnels or cups or vibratile urns are small, numerous organs arranged long the insertion of the intestinal mesenteries with the body walls of Chiridotidae and Synaptidae. Cup interiors are ciliated and appear to function in removing foreign particulates from the coelomic fluid (Jans and Jangoux, 1989). Ciliated funnels are known from no other echinoderm and the ancestral outgroup is scored as lacking this character.

46. *Statocysts*: 0 = absent; 1 = present.

Statocysts are presumed balancing organs inserting along the anterior radial nerves of apodan families, elpidiid elasipodans and molpadiidans. They do not occur in other extant echinoderms, so are coded as absent in the outgroup.

Ecology

47. *Feeding: 0 = deposit; 1 = suspension.*

All dendrochirotes are suspension feeders, though some species facultatively collect benthic detritus when suspended material is low. A few species of the aspidochirote Holothuriidae are suspension feeders, but their rarity and ability to facultatively deposit feed suggest that this is a derived condition. The outgroup is coded as a deposit feeder because of its digitate tentacles and presumed infaunal habit.

Results

Summary statistics from the parsimony analyses are presented in Table 3.3. Values of the consistency index (CI) ranged from 0.525 to 0.758, the rescaled consistency index (RC) from 0.407 to 0.687 and the retention index (RI) from 0.774 to 0.906. The frequency distributions of tree lengths in all analyses were highly left skewed, with “ g_1 ” scores (i.e., the distributions’ third moments) well beyond the $P < 0.01$ significance

Table 3.3. Tree statistics of maximum parsimony analyses.

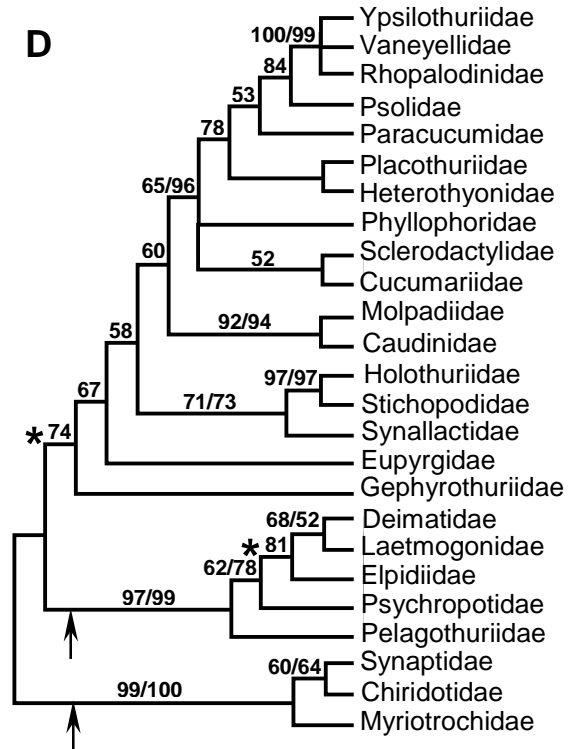
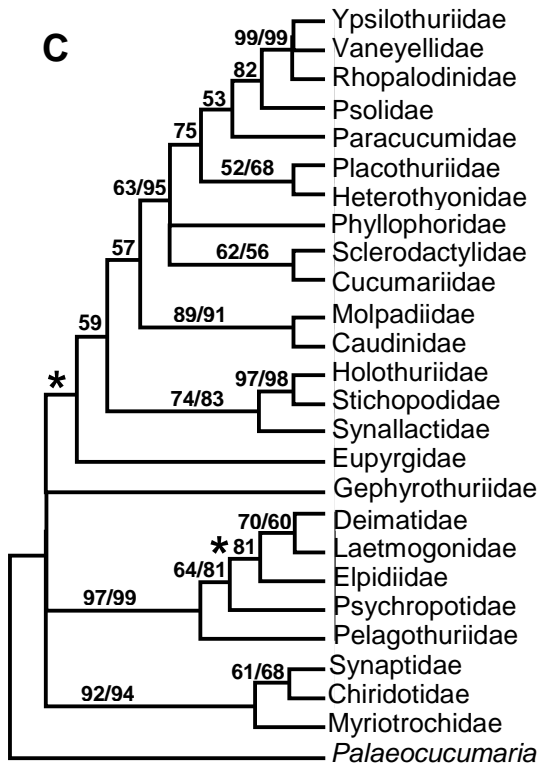
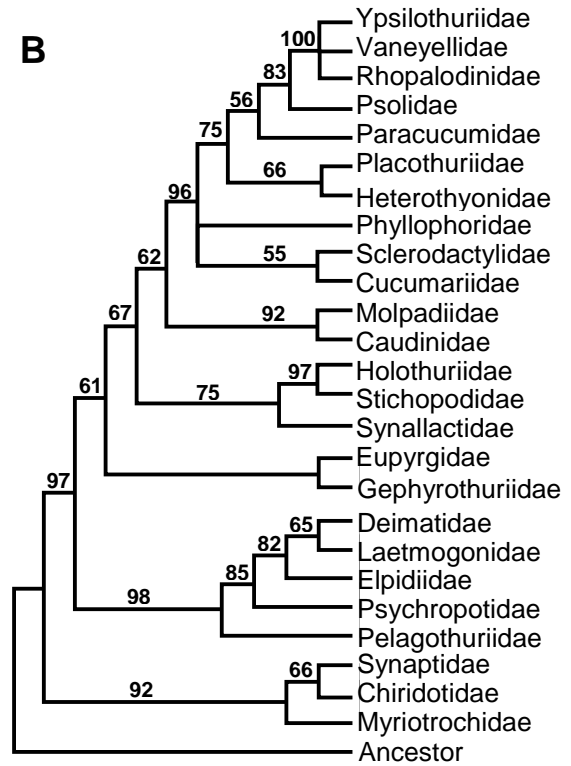
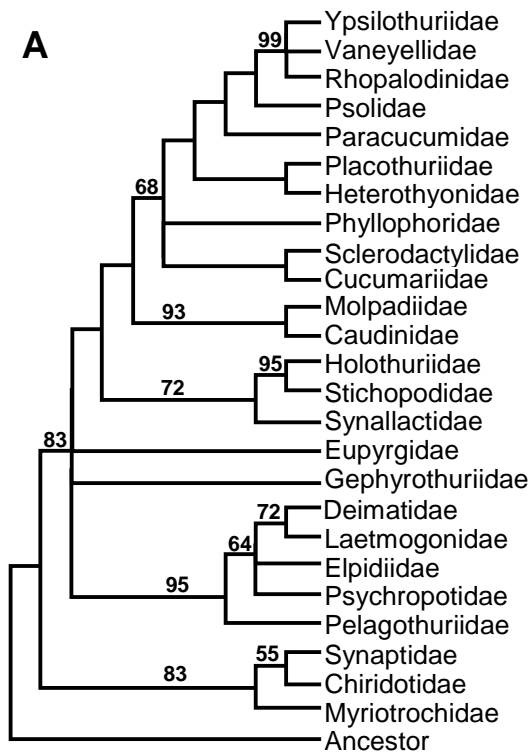
Rooting	Weighting	Eupyrgidae and Gephyrothuriidae		Number		CI	RCI	RI	g _i
		included?	Tree length	trees	of shortest				
Ancestor	Equal	yes	116	24	0.526	0.407	0.774	-0.568	
		no	107	4	0.570	0.456	0.800	-0.588	
	Successive	yes	47.34	2	0.725	0.647	0.893	-0.630	
		no	48.33	2	0.758	0.687	0.906	-0.647	
<i>Palaeocucumaria</i>	Equal	yes	112	8	0.536	0.418	0.780	-0.585	
		no	104	4	0.569	0.455	0.797	-0.593	
	Successive	yes	47.98	4	0.714	0.632	0.886	-0.644	
		no	48.04	2	0.756	0.683	0.904	-0.693	
Long-branch	Equal	yes	112	16	0.536	0.416	0.777	-0.598	
		no	104	8	0.577	0.461	0.800	-0.604	
	Successive	yes	46.84	2	0.730	0.651	0.892	-0.657	
		no	48.59	2	0.758	0.684	0.903	-0.666	

level (Hillis and Huelsenbeck, 1992), suggesting that there is considerable “hierarchical signal” in the data sets. Regardless of rooting method or included taxa, analyses using successive weighting produced two to four most parsimonious trees, while equally weighted data produced four to 24 shortest trees (Fig. 3.2). Regardless of weighting scheme or included taxa, bootstrap values at each node were higher about twice as often under ancestor rooting, though only slightly (median = 3%, range = 1 to 11%).

Parsimony analyses that included all 25 families produced two different placements for the root (Fig. 3.2). For both weighting procedures, ancestor and *Palaeocucumaria* rootings split the tree between the apodan group (Myriotrochidae, (Synaptidae, Chiridotidae)) and the other holothuroids (Fig. 3.2A-C). In contrast, *Palaeocucumaria* rooted the tree immediately below the clade of elasipodan families (Fig. 3.2B). The long-branch root, on the longest branches with 10 unambiguous changes, could be placed below the elasipodan or the apodan families in the equally weighted analyses and only below the elasipodans in the successively weighted analysis (Fig. 3.2D). Finally, Eupyrgidae and Gephyrothuriidae formed a sister group only in the successively weighted, ancestor-rooted tree.

The dependence of topology on rooting and weighting methods disappeared with the exclusion of Eupyrgidae and Gephyrothuriidae.

Figure 3.2. Most parsimonious trees (MPT) for analyses of all taxa, weighting and rooting procedures. A) 50% majority rule consensus tree of 24 trees using equal weights plus ancestor rooting. B) 50% majority rule consensus tree of two MPT for successive weights and ancestor rooting C) 50% majority rule consensus tree of eight trees under equal and successive weights, respectively, when using the *Palaeocucumaria* outgroup each time. Asterisks indicate unresolved nodes in majority rule consensus tree of eight MPT for equally weighted analysis. D) 50% majority rule consensus tree of two MPT using successive weights and long-branch rooting; collapsing at the asterisked nodes generates the 50% majority rule consensus tree of 16 MPT using equal weights and long-branch rooting. Arrows indicate possible positions of long-branch roots. Numbers above branches indicates bootstrap percentages separated by slashes are for equal and successive weighted analyses, respectively; percentages less than 50% are not shown.

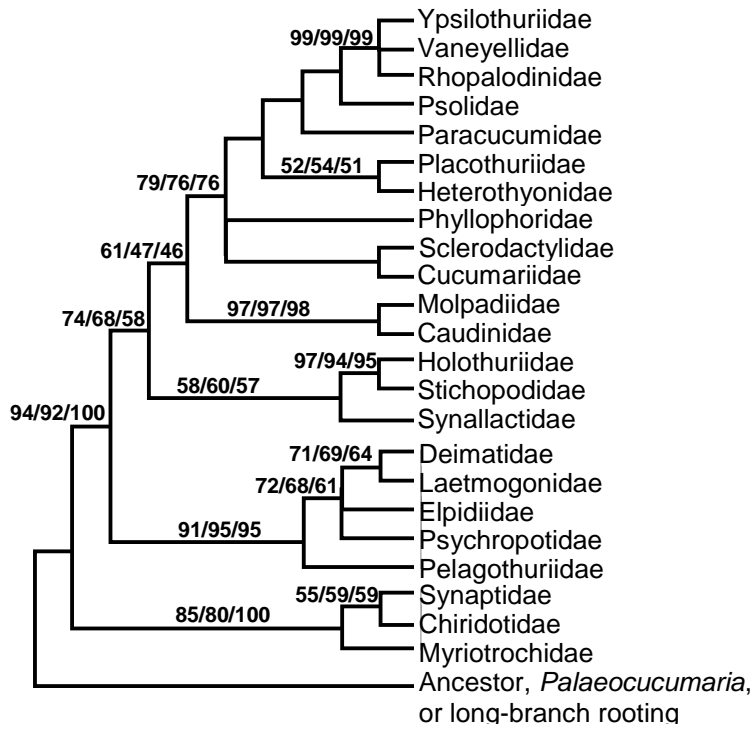


There were no effects on topology from excluding other combinations of taxa that were suspected as being derived *a priori* (data not shown). When Eupyrgidae and Gephyrothuriidae were excluded, all rooting-by-weighting schemes produced an identical strict consensus of shortest trees (Fig. 3.3), except for a trichotomy in the elasipodans (Fig. 3.3A). In addition, the equally weighted data, regardless of rooting method, produced fewer most parsimonious trees (Table 3.3) when the two derived families were excluded. Exclusion also resulted in placement of the long-branch root on the longest branch (with 11 unambiguous changes), which split the apodan clade and the remaining taxa. Finally, pruning the two families resulted in small but consistent increases in CI, RC, RI, and g_1 scores, as well as for bootstrap percentages at common nodes compared within each rooting-by-weighting scheme. Because of the apparently destabilizing effects of Eupyrgidae and Gephyrothuriidae on topology and bootstrap support, only the analyses excluding these groups are considered henceforth.

There was strong support via bootstrapping and the number of unambiguous changes in character states for apodan families as a sister group to the other holothuroids (Fig. 3.4). The three apodan families were joined by four synapomorphies: thin body wall, perforated radial plates, wheel ossicles and statocysts. A sister clade of the remaining holothuroids was supported by seven unambiguous changes. The five elasipodan

Figure 3.3. Single most parsimonious tree from all weighting and rooting procedures when Eupyrigidae and Gephyrothuriidae are removed. A) bootstrap percentages for equally weighted analyses. B) bootstrap percentages for successively weighted analyses. Bootstrap values separated by slashes are presented for ancestor, *Palaeocucumaria* and long-branch rootings, respectively, and shown for a branch only when at least one rooting scheme provided it with percent support of at least 50%.

A



B

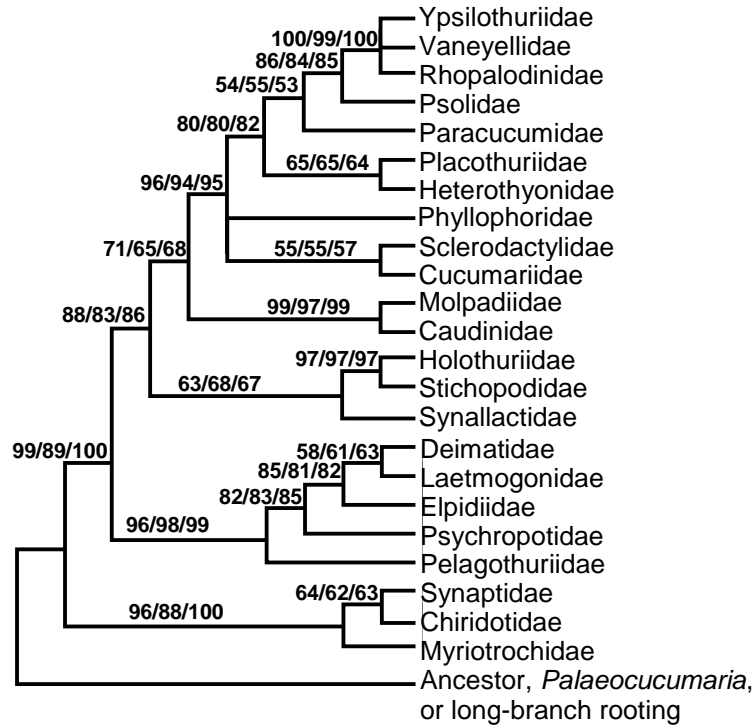
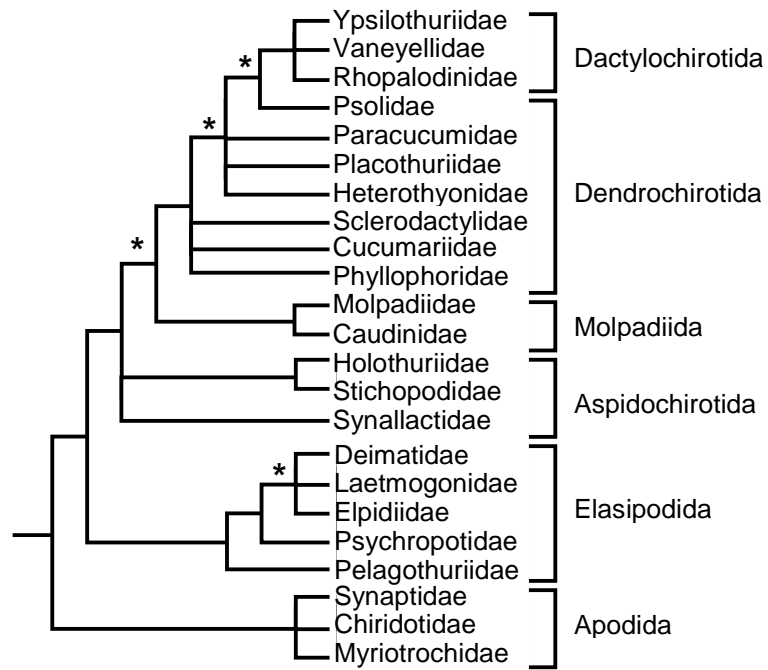


Figure 3.4. Best estimate of holothuroid relationships based on the successively weighted analyses excluding Eupyridae and Gephyrothuriidae and by collapsing all branches of the resulting single most parsimonious tree with less than 70% bootstrap support. Asterisks indicate additional branches with less than 70% bootstrap support in all equally weighted analyses.



families were united by eight unambiguous changes. Within this clade, there was always strong support for the subdivision (Pelagothuriidae, remaining elasipodans). The sister clade to Elasipodida was united by five synapomorphies. Within this clade, the aspidochirote (Stichopodidae, Holothuriidae) received strong bootstrap support. The sister group to the aspidochirote (remaining holothuroids, (Molpadiidae, Caudinidae)) received moderate bootstrap support. Support was poor to moderate for most clades of dendrochirote families. A clade of largely non-testaceous dendrochirote families (Sclerodactylidae, Cucumariidae) possesses a single synapomorphy, gain of two-tiered tables. The sister clade of remaining dendrochirote plus dactylochirote families was supported by testaceous body wall, imbrication of ossicles and loss of four-tiered tables. Within this group, dactylochirote families formed a strongly supported clade (Rhopalodinidae, Vaneyellidae, Ypsilothuriidae), united by eight synapomorphies.

Wills' gap excess ratio (GER) was significantly less (GER=0.831; $P=0.0007$) than those from randomly permuting the range data across the phylogeny 10^5 times. The total ghost range implied by the tree ("minimum implied gap") is 631.4 ma, the minimum possible ghost range is 360.9 ma and the maximum is 1964.5 ma.

DISCUSSION

Taxonomic Implications

The results of this study comprise the first cladistic test of the classification of Holothuroidea and differ significantly from the cladistic structure implied by the current taxonomic hierarchy (Fig. 3.1A). Pawson and Fell (1965) modified ordinal designations and introduced three subclasses. The authors split off from the order Dendrochirotida their new order Dactylochirotida diagnosed by digitiform or digitate tentacles and a testaceous body wall. Together these two orders comprise the subclass Dendrochirotea. The Aspidochirotea with shield-shaped tentacles and conspicuous bilateral symmetry include the mostly littoral and tropical Aspidochirotida and the entirely deep-sea Elasipodida. Finally, the Apodacea, holothuroids without tubefeet, comprise the Apodida and Molpadiida, two groups that Pawson and Fell (1965) acknowledge may be only distantly related.

Our analyses did not corroborate the monophyly of the subclasses proposed by these authors. Dendrochirotea is composed in part of the probably paraphyletic group Dendrochirotida. Aspidochirotea, with clades Aspidochirotida and Elasipodida, is paraphyletic. This confirms Hansen's (1975) suspicion, which he based on the stark dissimilarities in ossicle form between the two groups. The Apodacea consists of two

cladistically disparate orders, Molpadiida and Apodida. These groups had been united primarily via the absence of a feature, tubefeet in the extraxial body, an apparently primitive feature of Apodida and a derived one in Molpadiida.

In contrast to the lack of support for the monophyly of subclasses, there was strong support for four of the six taxonomic orders as clades, Dactylochirotida, Aspidochirotida, Elasipodida and Apodida (Fig. 3.3). The monophyly of Molpadiida, in contrast, remains uncertain. This order includes Eupyrgidae and Gephyrothuriidae, two apparently derived families excluded from our analyses because of their adverse effect on recovering a strongly supported phylogeny. Gephyrothuriidae has been allied over the years with either the aspidochirotetes or molpadiidans (O'Loughlin, 1998). Eupyrgidae has been more often placed, though with qualification, among the molpadiidans (e.g., Clark, 1907; Heding, 1935). In this study, these families' consistent placements away from the remaining molpadiidans, as well as their unique ossicle types and gross body features (filiform papillae and a rectal extension in Gephyrothuriidae) seem to indicate that their affinities lie elsewhere. As well, Dendrochirotida appears to be paraphyletic and consists of a soft-bodied clade and a testaceous grade. There are, however, two reasons to view this interpretation with caution: support for the testaceous group that includes the dactylochirotes is strong for only the successively weighted

analysis (Fig. 3.3B) and is largely defined by a single subset of characters, those associated with a testaceous body wall.

Branching order was also not confidently resolved within other ordinal-level clades. Within Apodida, the families Synaptidae and Chiridotidae have long been thought to be most closely related (Östergren, 1907; Frizzell and Exline, 1966) based on evidence from morphology (Smirnov, 1998) and fossils (Gilliland, 1993). Our analysis recovered the predicted arrangement, but with low bootstrap support. In Elasipodida, the only well supported branch was that resolving Pelagothuriidae as sister to the remaining elasipodans. This result is at odds with Hansen's (1975) conclusion that pelagothuriids are evolutionarily quite derived because of their presumed loss of ossicles, calcareous ring, tubefeet, ventral sole and epibenthic habit. Indeed, lack of the latter three characters is plesiomorphic in holothuroids and accounts for the parsimony algorithm's placement of Pelagothuriidae below the other elasipodans.

The analyses also indicate strong support for groups not delimited in recent taxonomic classifications of Holothuroidea. Apodida, because of its numerous unique characters, has long been suspected as being evolutionarily quite distant from other holothuroids. Semper (1868) first suggested that apodan forms preceded those with tubefeet. Semon (1888),

after studying their larval development, concluded that the Synaptida (=Apodida) were not derived from holothuroids with tubefeet and that the “simplicity of their organization is original” [my translation]. Cuénot (1891) went even further, claiming that apodans were a group of echinoderms quite distinct from true holothurians. Ludwig (1891) disagreed with both views, reckoning from his own larval studies that tubefeet had been lost secondarily. Nevertheless, he elevated the apodans to ordinal status as Paractinopoda and placed the remaining holothuroids in the order Actinopoda. In this study, Semper and Semon’s phylogenetic interpretation, as well as Ludwig’s taxonomic designations when viewed cladistically, were strongly supported. The apodan families, Synaptidae, Chiridotidae and Myriotrochidae, form a sister clade to the holothuroids bearing tubefeet with high bootstrap proportions and seven unambiguous synapomorphies.

Finally, our analyses also uncovered a relationship not considered since some of the earliest revisions of Holothuroidea. Brandt (1835) divided his Apodes (=Apodacea) into Pneumonophorae and Apneumones diagnosable by the presence or absence of respiratory trees. These groups essentially correspond to Pawson and Fell’s (1965) Molpadiida and Apodida, respectively. Selenka (1867) and Semper (1868) used two terms similar to Brandt’s to divide all of Holothuroidea into lunged and lungless forms, though few subsequent authors appear to have followed their lead.

In this study, there was moderate to strong bootstrap support for a clade of holothuroids united by the presence of respiratory trees and four other synapomorphies. Finally, I also found four unambiguous synapomorphies uniting a clade of molpadiidan and dendrochirote+dactylochirote families. Several workers (Ludwig, 1891; Gerould, 1896; Pawson, 1982; Gilliland, 1992, 1993) have mentioned a possible close evolutionary relationship between these groups, but made no taxonomic modifications. In this study, bootstrap support for this clade was moderate for only the successively weighted analyses.

In sum, the higher-level classification of Holothuroidea warrants a considerable revision. Some of Pawson and Fell's (1965) classification was corroborated by the present cladistic analyses. Differences, at the level of orders and subclasses, though, indicate that several groups as presently defined have not been cladistically diagnosed, that is not via the most parsimonious ascription of character states. Several classification schemes and phylogenetic speculations made during the last century were substantiated and include names available for a future nomenclatural revision of Holothuroidea.

Fossils and Calibrating Divergence Times

We used the fossil record and the *Palaeocucumaria*-rooted tree (Fig. 3.3) to estimate lineage divergence times in Holothuroidea (Fig. 3.5). The fossil record of holothuroids has been aptly characterized overall as “really appalling” (Smith, 1988). Nevertheless, a few groups have a reasonably extensive record, e.g., the apodans (Gilliland, 1993), and 11 to 12 extant families are known from fossils, making a preliminary estimate of divergence times worthwhile. The oldest reliable stratigraphic records of the included taxa are shown in Table 3.4. Our assignments of family origin times agree with Simms *et al.* (1993) with three exceptions: First, ossicles and calcareous-ring elements of Middle Triassic age have been with qualification referred to Molpadiidae (Gilliland, 1992; Simms *et al.*, 1993). However, the oldest unequivocal ossicles of Molpadiidae are Oligocene (Gilliland, 1993), a record used herein. Second, the Middle Triassic origin of Synaptidae is based on ossicles possibly from Chiridotidae (Simms *et al.*, 1993), hence I used the next earliest, but undoubted, synaptid from the Early Jurassic (Gilliland, 1992). Third, purported Ypsilothuriidae ossicles from the Lower Mississippian can be as easily assigned to Paracucumidae (Simms *et al.*, 1993). Therefore, the earliest record of Ypsilothuriidae herein is considered as from the Lower Jurassic *sensu* Gilliland (1992).

Finally, I included a record of Synallactidae ossicles, *Priscopedatus triassicus*, from the Middle Triassic that is considered tentative by Simms

Figure 3.5. Phylogeny of Holothuroidea inferred from the morphological analyses (Fig. 3.4) and the stratigraphic record of the oldest fossil representatives for the included groups. Thin solid lines indicate phylogeny based on the present study; thick solid lines indicate range between the earliest and latest occurrences, fossil or extant; thin dashed lines indicate ghost range.

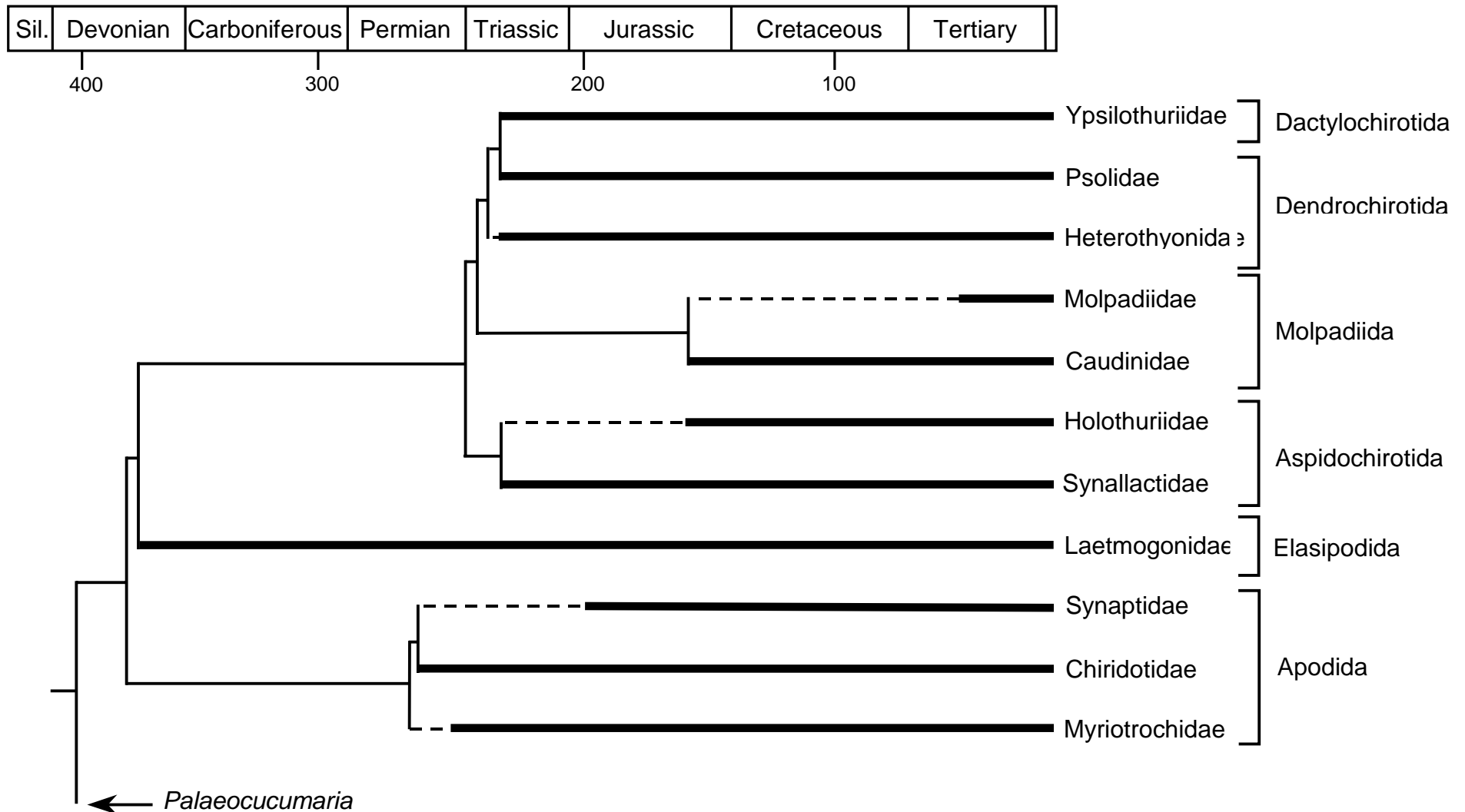


Table 3.4. Earliest stratigraphic occurrences of holothuroid taxa.

Taxon	Fossil representative	Earliest record	Age (Mya)	Reference
Ypsilothuriidae	<i>Palaeoypsilus liassicus</i>	L. Jurassic (Sinemurian)	201.9	Gilliland, 1992
Psolidae	<i>Monilipsolus mirabile</i>	M. Triassic (Ladinian)	234.3	Smith and Gallemí, 1991
Heterothyonidae	<i>Strobilothyone rogenti</i>	M. Triassic (Ladinian)	234.3	Smith and Gallemí, 1991
Molpadiidae	<i>Calcancorella spectabilis</i>	Oligocene	35.4	Gilliland, 1993
Caudinidae	<i>Pedatopriscus pinguis</i>	U. Jurassic (Oxfordian)	159.4	Deflandre-Rigaud, 1946
Holothuriidae	<i>Calclamella elliptica</i>	U. Jurassic (Oxfordian)	159.4	Deflandre-Rigaud, 1946
Synallactidae	<i>Priscopetatus triassicus</i>	M. Triassic (Anisian)	241.7	Simms <i>et al.</i> , 1993
Laetmogonidae	<i>Protocaudina kansasensis</i>	M. Devonian (Eifelian)	386.0	Ferrigno, 1970
Synaptidae	<i>Theelia synapta</i>	L. Jurassic (Hettangian)	205.7	Gilliland, 1992
Chiridotidae	<i>Protheelia geinitziana</i>	U. Permian (Zechstein)	256.1	Frizzell and Exline, 1955
Myriotrochidae	<i>Theelia praeacuta</i>	U. Permian (Tatarian)	252.0	Mostler and Rahimi-Yazd, 1976
Outgroup	<i>Palaeocucumaria hunsrueckiana</i>	L. Devonian (Lochovian/Pragian)	396.3	Seilacher, 1961

et al. (1993) and Gilliland (1993). The *Priscopedatus* ossicles are cross-shaped tables similar to those typical of the synallactid *Bathyploetes*. Both forms have a long thin spire and a base of four long thin arms each with distal reticulate branching that forms a perforate spatulate terminus. In *P. triassicus*, additional terminal branches invariably conjoin the four arms forming the margin of a circular plate. This latter type of branching is also seen occasionally in the extant synallactid *Bathyploetes* (Pawson, 1980). Regardless of our interpretation, as the earliest likely member of a clade, the Aspidochirotida, an incorrect “basal” assignment of *Priscopedatus* to an extant family versus its placement as a fossil stem member had no effect on the estimated divergence time within the order or the ghost lineage duration of its sister clade Holothuriidae (Fig. 3.5).

The significant congruence of the earliest stratigraphic occurrences with the estimated phylogeny suggests that the fossil record is not as incomplete as has often been claimed (e.g., Pawson, 1980; Smith, 1988; Gilliland, 1993). The majority of fossil holothuroids exist as paraspecies determined from isolated ossicles. Species described from body fossils number ca. 15 (Gilliland, 1993). Despite these shortcomings of the record, the total ghost range was significantly less than that when earliest fossil occurrences were randomized across the phylogeny via Wills’ GER. However, the statistical properties of this index remain unexplored and it may be easier to find congruence between stratigraphic data and

phylogenies with certain branching orders than with others independent of “stratigraphic completeness.”

The fossil calibrated tree illuminates several interesting features of holothuroid evolution. The fossil record indicates that several Recent lineages of holothuroids have lived through the Permian/Triassic boundary (Gilliland, 1993). With the inclusion of the Middle Devonian to Early Cretaceous Achistridae, the calibrated tree indicates that at least six groups of holothuroids survived the end-Permian mass extinction. This stands in stark contrast to other extant classes of echinoderms that survived the event as a single to a few genera (Lewis and Donovan, 1998). The reasons for the strong showing by holothuroids are speculative and refer to the group’s largely infaunal habit and trophic status as detritivores, two features associated with increased survival of other marine invertebrates through the Permian/Triassic boundary (Vermeij, 1993).

The first occurrences of families from Dactylochirotida, Dendrochirotida, Molpadiida and Aspidochirotida in the Early Triassic are consistent with a rapid divergence of this clade during that time. The timing is similar to that of the increased diversification seen for other marine invertebrates, including other echinoderms (Lafay *et al.*, 1995; Smith and Paterson, 1995), following the end-Permian mass extinction

and the beginning of the “Mesozoic marine revolution” (Vermeij, 1993). The rapid divergence of holothuroids in the Triassic indicates that the lack of support via number of unambiguous synapomorphies and bootstrap percentages for branches leading to these clades is not likely an artifact of character choice, but a reflection of history. This in turn suggests that these internal branches will prove equally difficult to resolve using molecular methods.

The Triassic radiation comprises a clade of holothuroids diagnosable in part by a gain of table ossicles. This group apparently diverged from the ancestors of Elasipodida by the Middle Devonian (Fig. 3.5). Fossil table ossicles (parafamily Priscopeditidae) occurring between this time and the Triassic and assigned to Dendrochirotida and Dactylochirotida (e.g., *Clavallus spicaudina* Gutschick and Grill, 1967) probably represent stem members of this clade. If Dendrochirotida and Dactylochirotida do have a Triassic origin, as indicated by this study, then very early records of these groups based on simple plate ossicles are also incorrect. Possibly, the plates indicate a Paleozoic divergence by stem members of the Triassic radiation. Alternatively, and if they are of holothuroid origin at all (e.g., *Eocaudina*, Reich, 1999), the ossicles are from more distantly related forms, suggesting that imbricate plates and a testaceous morphology have evolved and were lost several times in Holothuroidea.

Chapter IV:
Phylogeny of the Apodan Holothurians Inferred from
Morphology

A version of this chapter appears in: Kerr, A. M. and J. Kim. 2001
Phylogeny of Apodan Holothurians (Echinodermata) inferred from
morphology. *Zoological Journal of the Linnean Society* 133: 53-62.

Summary

The Apodida is an order of littoral to deep-sea, largely infaunal sea cucumbers with about 270 extant species in 32 genera and three families, Synaptidae, Chiridotidae and Myriotrochidae. In this study, I perform the first phylogenetic test of the taxonomic and paleontological hypotheses about evolutionary relationships within Apodida by using cladistic analyses of 34 morphological characters. I introduce several previously unconsidered synapomorphic characters, examine the relationships between all recognized suprageneric taxonomic groups and assess the assumptions of monophyly for each family. Maximum parsimony analyses of type species from 14 genera and use of three rooting methods recovered identical topologies at the subordinal level and subfamily level within Synaptidae. Overall, the current higher level classification of Apodida was well corroborated. Within Synaptidae, the relationships (Synaptinae, (Leptosynaptinae, Rynkatorpinae)) are well supported. The monophyly of Chiridotidae was not supported and appears paraphyletic at the subfamily level. Calibrating the phylogenetic hypothesis of Apodida against the fossil record indicated that most higher-level divergences occurred within the Paleozoic, unlike that of extant non-holothuroid echinoderms, which radiated in the early Mesozoic. Synaptidae appears to have radiated during the Lower Cretaceous. Alternatively, if one discounts the

considerable ghost lineage duration that this hypothesis requires, they may have radiated during the Eocene.

Introduction

Apodida is a group of littoral to deep-sea, largely infaunal holothuroid echinoderms. Apodans are unique among holothuroids in lacking tube feet, papillae and radial water canals. Apodans are invariably vermiform with a thin, often transparent body wall and they range in length from a few millimeters to more than 3 m, making them the largest echinoderms. They are distributed worldwide, with about 270 extant species in 32 genera and three families (Smiley, 1994; Smirnov, 1998). In this study, I make the first phylogenetic test of the taxonomic and paleontological hypotheses about evolutionary relationships within Apodida by using cladistic analyses of morphological characters. I introduce several previously unconsidered, but important characters, examine the relationships among 14 terminal taxa representing all currently recognized supergeneric groups, and perform a preliminary test of the monophyly of apodan families.

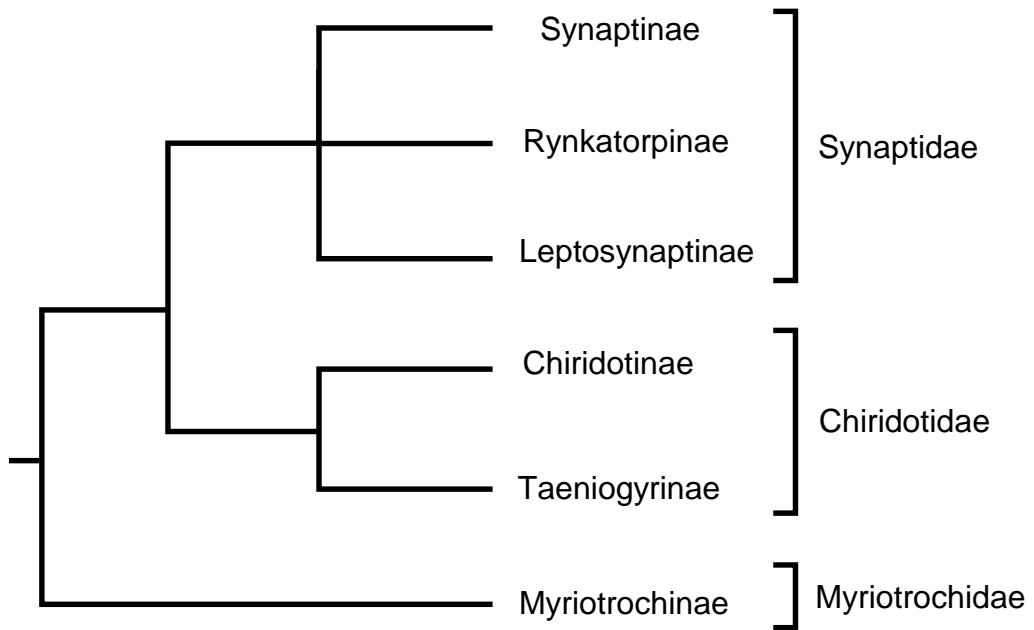
Taxonomy and Phylogeny

The taxonomic history of the apodans has been reviewed most recently by Smirnov (1998). Briefly, Östergren (1898) divided the group into three subfamily-level groups that largely correspond to the present family designations. His classification was modified by Clark (1907) and

by Heding (1928, 1929, 1931, 1935), who described numerous new species and introduced important suprageneric distinctions. Several more genera have since been described, notably by Belyaev and Mironov (1980) and Gage and Billett (1986). More recently, Smirnov (1989) revised Synaptidae to include fossil taxa with the stated aim of having the group's taxonomy reflect evolutionary relationships. Smirnov (1998) soon expanded this goal to include the entire higher level taxonomy of Apodida, creating two suborders, Myriotrochina and Synaptina. The latter suborder unites two families, Chiridotidae and Synaptidae, based, most prominently, on similarities in juvenile wheel ossicles. Within Chiridotidae, Smirnov (1998) designated the subfamilies Chiridotinae and Taeniogyrinae and within the Synaptidae, the subfamilies Synaptinae, Leptosynaptinae and Rynkatorpinae. The second suborder Myriotrochina, in contrast, is monotypic, consisting of the formally unsubdivided family Myriotrochidae.

The phylogenetic relationships implied by the above classification are diagrammed in Figure 4.1. The family-level arrangement, first illustrated by Östergren (1907) and discussed by Frizzell and Exline (1966), finds support in the extensive palaeontological analysis of wheel ossicles by Gilliland (1993). The concordance of other aspects of the current taxonomy with phylogeny, however, remains untested. In addition, the branching order of subfamilies within Synaptidae is

Figure 4.1. Phylogenetic relationships implied by the taxonomic scheme of Smirnov (1998).



unresolved. Heding (1928) suggests dividing the family into two groups, the Synaptinae and the Leptosynaptinae+Rynkatorpinae, basing his decision on their respective form of coelomic excretory organs, the ciliary funnels. Alternatively, Semper (1868) and Smirnov (1998) suggest that the division is between the Synaptinae+Leptosynaptinae and the Rynkatorpinae. Here, the distinction is between membership in mutually exclusive sets of ossicle characters and their origination times in the stratigraphic record.

Methods

Ingroup Selection and Rooting

Of the 32 currently recognized genera in the three families that comprise the extant Apodida (Smirnov 1998), 14 genera represented by type species were included in this study (Table 4.1). The major limitation of this approach is clear: species exemplars must possess the plesiomorphies of the genera they purport to represent. Nevertheless, the explicitness of this approach was reasoned superior to the alternative of speculating on the ancestral states of genera. Some genera were excluded because they are little known or, I felt, clearly derived, e.g., the tiny, aspiculate forms *Achiridota*, *Rhabdomolgus* and *Anapta*. Several other

Table 4.1. Taxa used in this study.

Suborder Synaptina A. Smirnov, 1998

Family Synaptidae Burmeister, 1837

Subfamily Synaptinae, Burmeister, 1837

Synapta maculata (Chamisso & Eysenhardt, 1821)

Opheodesoma spectabilis Fisher, 1907

Euapta godeffroyi (Semper, 1868)

Subfamily Leptosynaptinae A. Smirnov, 1989

Leptosynapta tenuis (Ayres, 1851)

Labidoplax buskii (McIntosh, 1866)

Subfamily Rynkatorpinae Smirnov, 1989

Oestergrenia digitata Heding, 1931

Protankyra bidentata (Woodward & Barrett, 1858)

Family Chiridotidae Östergren, 1898

Subfamily Chiridotinae Östergren, 1898

Chiridota laevis (Fabricius, 1780)

Polycheira rufescens (Brandt, 1835)

Taeniogyrinae A. Smirnov, 1998

Taeniogyrus australianus (Stimpson, 1856)

Trochodota purpurea (Lesson, 1890)

Suborder Myriotrochina A. Smirnov, 1998

Family Myriotrochidae Théel, 1877

Myriotrochus rinkii Steenstrup, 1851

Acanthotrochus mirabilis Danielssen & Koren, 1879

Trochoderma elegans Théel, 1877

genera, though well characterized, were excluded to maintain a high ratio of taxa to characters. Finally, inclusion of genera was based on the desire for multiple representatives from each family and subfamily to perform a preliminary test of the monophyly of these groups. Occasionally other species were used when the character of a genotype was poorly known.

The living proximate outgroup to Apodida is uncertain. Preliminary molecular and morphological analyses (Smith, 1997; Kerr and Kim, 1999) suggest that the closest extant group of holothuroids to apodans is Elasipodida. This and other extant holothuroid outgroups possess several attributes with which to polarize characters within Apodida. Similarly, the oldest unequivocal body fossils assignable to Apodida, Achistridae from as early as the Middle Devonian (Simms *et al.*, 1993), display some plesiomorphies with living apodans. Given these limitations, I explored three rooting options: First, I rooted trees using a hypothetical ancestor (“ancestor rooting”) by combining invariant soft-tissue characters from Elasipodida and the skeletal and gross morphological characters provided by the best preserved achistrid body fossils, the Francis Creek *Achistrum* from the Middle Pennsylvanian (Sroka, 1988; Sroka and Blake, 1997). Second, to assess the effect of the potentially divergent characters of the living elasipodans, I rooted the tree using only the few skeletal and gross characters available from *Achistrum*. Third, I rooted trees between Myriotrochidae and Chiridotidae *a posteriori* (“long-branch rooting”)

based on their extended fossil histories compared to that of Synaptidae. The former two clades appeared by at least the Late Permian, while wheel ossicles typical of larval Synaptidae do not occur until the Middle to Upper Triassic (Gilliland, 1993). This indicates that either Myriotrochidae or Chiridotidae is sister to Synaptidae and that the root lies between the two older families. This predicts that the longest branch, the one with the most unambiguous changes, will lie between the two more ancient groups.

Phylogenetic Analyses and Character Selection

Maximum parsimony analyses of the data were performed using *PAUP* 4.0b 2a* (Swofford, 1998) with the following options: branch-and-bound search, multistate taxa treated as polymorphisms, zero-length branches collapsed, minimal length trees kept, the initial upper bound computed via stepwise addition, and the furthest addition sequence used. I assessed data quality by bootstrapping using 500 replicates, as well as by assessing the skewness of the tree-length frequency distributions generated from 10^5 trees randomly produced from the data. Change of individual characters along branches was examined using *MacClade 3.0* (Maddison and Maddison, 1992).

An annotated list of the characters used in this study is given below. A total of 34 discrete, equally weighted characters - 23 skeletal, 10 soft-tissue and one behavioral - were scored, including 29 binary and five unordered multistate characters (Table 4.2). Characters 17 through 25 were polarized where the states of ossicle characters in *Protankyra* are ancestral based on their identical appearance to the Lower Cretaceous to Oligocene anchor plates of *Rigaudites* and to the Upper Jurassic to Miocene anchors of *Calcanora* (Frizzell and Exline, 1966; Smirnov, 1989). Unknown or logically impossible states were coded as “?” and treated as missing. In the few instances when the character state of the genotype exemplar was unknown, I coded the genus using a congeneric taxon and indicate the substitution in the following list. Characters 2, 8, 10, 17, 18, 19, 26, 31, 32 were suggested by the taxonomic treatment of Smirnov (1998). Confirmation of these, as well as codings of the remaining characters, was largely achieved using published taxonomic descriptions, primarily Clark (1907), Heding (1928, 1929, 1931, 1935), and Gage and Billett (1986). When doubt still existed about the status of a character, I examined field-caught specimens or collections of extant and fossil material at the following museums: British Museum (Natural History), Museum of Comparative Zoology, National Museum of Natural History, and Yale Peabody Museum of Natural History.

Table 4.2. Character matrix for the apodan exemplars, a fossil outgroup *Achistrum* and a reconstructed ancestor. Missing or logically prohibited codings are indicated by “?”.

Taxa	Characters						
	5	10	15	20	25	30	34
<i>Synapta</i>	12110	12110	00010	10111	10100	0????	2001
<i>Euapta</i>	12110	12110	10010	10110	01101	0????	2001
<i>Opheodesoma</i>	12110	12110	10010	10100	01101	0????	2001
<i>Leptosynapta</i>	01001	21100	10000	10001	01010	0????	2000
<i>Labidoplax</i>	01001	21100	10000	10001	01010	0????	2000
<i>Oestergrenia</i>	00011	20100	10000	11011	10010	0????	1000
<i>Protankyra</i>	00011	21100	00100	11011	11010	0????	1000
<i>Chiridota</i>	00000	12101	11000	0????	?????	11001	2000
<i>Polycheira</i>	10100	12101	11000	0????	?????	11001	2000
<i>Taeniogyrus</i>	00000	11101	10001	0????	?????	11001	0000
<i>Trochodota</i>	00000	10100	10001	0????	?????	11001	0000
<i>Myriotrochus</i>	00000	00000	00000	0????	?????	10102	2210
<i>Acanthotrochus</i>	00000	00000	00000	0????	?????	10110	2210
<i>Trochoderma</i>	00000	00000	00000	0????	?????	10110	2110
<i>Achistrum</i>	0??0?	???00	??0?1	0????	?????	0????	021?
Ancestor	0??00	00000	00001	0????	?????	0????	0210

Characters

Soft Tissues and Behavior

1. *Maximum body length: 0 = less than 25 cm; 1 = 25 cm or more.*

Synapta in Synaptinae is the largest recorded echinoderm, reaching lengths of 3 m. Several other synaptines are also quite long, but nearly all other apodans are under 10 cm. The ancestor is scored as 0 because *Achistrum* reaches about 10 to 13 cm in length.

2. *Tentacle morphology: 0 = digitate to peltatodigitate; 1 = pinnate with digits increasing in size proximo-distally; 2 = pinnate with largest digits circa mid-tentacle.*

Pinnate tentacles possess a conspicuous terminal digit which is lacking in otherwise somewhat similar digitate forms. In peltatodigitate tentacles, occurring in *Polycheira* and a few other genera, digits are arranged distally in a circle. This character is left unscored for the reconstructed outgroup. The tentacle form of the proximate fossil stem member used here, *Achistrum*, cannot be discerned from material examined by me, though may be preserved in other specimens (Sroka, 1988). The only fossil of a possible Palaeozoic stem member to Apodida preserving tentacles is *Palaeocucumaria* (Seilacher, 1961). The tentacles appear to be without digits (Seilacher, 1961), hence autapomorphic and uninformative in this study. For the most closely related extant member

used here, the Elasipodida, tentacles are peltate, and hence also autapomorphic.

3. *Number of digits on tentacles: 0 = less than 20; 1 = 20 or more.*

The number of digits on a tentacle can range from none in *Rynkatorpa* or two in *Dactylapta* to more than 80 in *Synapta*.

4. *Ocelli: 0 = absent; 1 = present.*

Synaptids possess ocelli or optic cups, small patches of pigmented cells at the base of their tentacles, that enclose photosensitive cells (Yamamoto and Yoshida, 1978). Ocelli are found in no other holothuroid, hence the ancestor is scored as lacking this character.

5. *Sensory cups: 0 = absent; 1 = present.*

Most non-synaptine synaptids display sensory cups or buds along the proximal base of the tentacles. They are innervated, interiorly ciliated cups and are presumably chemosensory (Clark, 1907). Sensory cups are found in no other holothuroid and the ancestor/outgroup is scored as lacking this character.

6. *Ciliated cups: 0 = absent; 1 = small with similar shape; 2 = large cups, variously shaped.*

Ciliated cups and funnels or vibratile urns are small, numerous organs arranged along the insertion of the intestinal mesenteries with the body wall of Chiridotidae and Synaptidae. Their interiors are ciliated and appear to function in removing foreign particulates from the coelomic fluid (Jans and Jangoux, 1989). Ciliated cups are found in no other holothuroid and the ancestor/outgroup is scored as lacking this character.

7. *Number of polian vesicles: 0 = always one; 1 = more than one.*

Polian vesicles are blind, round to elongate sacs extending from the water vascular ring into the coelom. They number from one in most apodans to as many as fifty or more in large synaptids such as *Synapta*. Elaspodans, the extant holothuroids used in the ancestor rooting, possess one polian vesicle.

8. *Length of stone canal: 0 = short; 1 = long.*

The stone canal of apodans, like most holothuroids, terminates distally with an internal madrepor. This character is taken from Smirnov (1998) who reports that the stone canals of Myriotrochidae are relatively much shorter compared to body length than those in the other apodans.

9. *Habit: 0 = obligately fossorial; 1 = epibenthic, at least facultatively, or inhabiting crevices.*

Nearly all apodans are burrowers. The major exceptions are the chiridotid *Polycheira*, which lives in crevices, and several, mostly large-sized, synaptid taxa, which are epibenthic or crevice dwelling. The hypothesized ancestor is considered fossorial based on *Achistrum*'s similarity to non-apodan burrowing holothuroids with elongate, fusiform bodies and small tentacular rings.

Ossicles

10. *Body wall ossicles restricted to "papillae": 0 = absent; 1 = present.*

In *Chiridota*, *Polycheira* and *Paradota* body wall ossicles occur only in small clusters, usually adjacent to the radii. The ossicles of the fossil *Achistrum* are distributed throughout the integument.

11. *Straight rods: 0 = absent; 1 = present.*

Short, straight to slightly curved rods - with their ends simply branched - are found in the tentacles of most apodans and, in a few taxa, the body wall.

12. *C-shaped rods: 0 = absent; 1 = present.*

C-shaped rods are smaller than straight rods, occur in the body wall and differ from C-shaped rods found in non-apodan holothuroids. They

have expanded, rounded, knobbed, often flattened ends and occur only in some chiridotid genera.

13. *Irregularly branched rods: 0 = absent; 1 = present.*

Irregular and multiply branched rods are found in the synaptid Apodida in *Protankyra* and two genera not used in this study, *Pendekaplectana* and *Polyplectana*. While phylogenetically uninformative in this study, this character is still a synapomorphy of groups within Apodida and is included to assist future work.

14. *Miliary rosettes: 0 = absent; 1 = present.*

Tiny irregularly branched rosettes occur in the body wall and tentacles of four genera of Synaptinae.

15. *Hooks: 0 = absent; 1 = present.*

Hooks occur in three living genera of chiridotids, *Taeniogyrus*, *Scoliorhapis* and *Trochodota*. An open loop or eye is formed by pronounced curvature of the ossicle at one end. The hooks of the fossil *Achistrum* differ from those of extant forms by the straighter shaft and a closed loop, sometimes with internal crossbars. Despite the differences between these two types, they are homologized here based on the similarly pointed ends, curvature of the shank, the way the eye is formed

(Reich, 1999, pl. 1) and life positions in which the hooks can lie scattered in the body wall (Sroka, 1988).

16. *Anchors and anchor plates: 0 = absent; 1 = present.*

Anchors and anchor plates occur in all spiculate genera of Synaptidae. The two types of ossicles are functionally related and invariably co-occur, so are considered as a single character. Anchors and their associated plates do not occur in *Achistrum*, which originated in the Devonian, and do not appear in the fossil record until the Middle Jurassic. The following eight characters (17 through 25) are derived from features of anchors or anchor plates and are scored as missing in taxa lacking these ossicles.

17. *Development of anchor plate: 0 = rod primordium parallel to anchor shaft; 1 = perpendicular to anchor shaft.*

Smirnov (1998) divides Synaptidae into two groups based on the development of the anchor plates. In Rynkatorpinae, the anchor plate arises from a rod-like element resting parallel to the shaft of the anchor. In Synaptinae and Leptosynaptinae, the plate primordium is positioned orthogonally to the anchor shaft.

18. *Base of anchor plate: 0 = with a ledge; 1 = with a bridge.*

The base of the anchor plate articulates with the anchor keel via a flattened end (“ledge”) or a raised crossbeam (“bridge”).

19. *Width of base of anchor plate: 0 = narrow; 1 = wide.*

In most synaptids, the base of the anchor plate is considerably narrowed. Only *Synapta* and *Protankyra* in this study possess plates with wide bases.

20. *Holes in anchor plate: 0 = few, in a “6+1” pattern; 1 = many irregularly arranged.*

Anchor plates with a “6+1” pattern show six, occasionally seven, holes surrounding a central hole. Conversely, *Synapta* and *Rynkatorpinae* have plates with numerous holes.

21. *Margin of anchor plate: 0 = smooth; 1 = spiny or irregular.*

22. *Margin of holes in anchor plate: 0 = smooth; 1 = dentate.*

23. *Vertex of anchor: 0 = smooth; 1 = with minute knobs.*

24. *Anchor arms: 0 = smooth; 1 = serrated.*

Synaptine synaptids possess smooth anchor arms; the remaining synaptids, serrated arms. However, *Epitomapta*, though not included in

this study, is interesting in that it is polymorphic: According to the type description of Heding (1928), it has serrated anchors in the posterior of the body and smooth anchor arms anteriorly.

25. *Keel of anchor: 0 = not branched; 1 = irregularly branched.*

26. *Wheel ossicles in adult: 0 = absent; 1 = present.*

Synaptids possess wheel ossicles only as larvae and juveniles (Mortensen, 1938; Pawson, 1971), while all other apodan holothuroids retain wheels in the body wall as adults. The similarities between synaptid larval wheels and those of adult chiridotids have been used to suggest a close relationship between the two families (Smirnov, 1998). However, too little comparative data exist on synaptid larvae to incorporate their wheel characters into this study. Currently, larvae and their ossicles are known from only three species in three genera of the 17 genera in Synaptidae (Semon, 1888; Mortensen, 1937, 1938). Examples of wheels from potentially other, but unidentified synaptids are reported by Pawson (1971) and Inaba (1934). A potential coding, but not one adopted here, for the ancestor outgroup is presence of wheels based on their occurrence in Paleochiridotidae and *Theelia*, likely stem-member apodans from the Lower Mississippian that precede the occurrence of myriotrochid- and chiridotid-like wheels. The older wheels, however, cannot be entirely aligned with those of Recent taxa, but most resemble

those of taeniogyryne chiridotids, in having a sub-hexagonal outline, interior dentate rim and few spokes (Gilliland, 1993). The following four characters (27 through 30) are based on characteristics of wheels and are scored as missing in taxa lacking this type of ossicle.

27. *Hub of wheel: 0 = flat; 1 = concave with a stellate superstructure.*

The wheel hub of myriotrochids is flat and either solid or multiperforate, while chiridotids have a star-shaped structure raised over an imperforate concave hub.

28. *Number of spokes on wheel: 0 = six; 1 = more than six.*

Chiridotids possessing wheels invariably have the form with six spokes. Myriotrochids by contrast have wheels with from at least 8 to 16 spokes.

29. *Outward pointing teeth on wheel rim: 0 = absent; 1 = present.*

Outward pointing teeth are absent in chiridotid and larval synaptid wheels, and are variable among myriotrochid genera.

30. *Inward pointing teeth on wheel rim: 0 = absent; 1 = small denticles; 2 = large teeth.*

The inner rim of wheel ossicles have numerous tiny teeth in chiridotids, but among myriotrochid genera, teeth on the inner margin are either large or absent.

Calcareous Ring

31. *Passage of radial nerve through radial plate of calcareous ring: 0 = via an anterior groove; 1 = via a perforation.*

All living apodans, with the exception of Taeniogyrinae, the myriotrochid *Achiridota* and synaptid *Epitomapta* have perforate radial plates. The ancestral outgroup, represented by *Achistrum*, is coded as imperforate based on examination of museum specimens. These same rings appear similar to the “Type 2” putative *Achistrum* calcareous rings pictured by Gilliland (1992).

32. *Anterior projections on radial plates of calcareous ring: 0 = absent or very low and blunt; 1 = one projection on all radial plates; 2 = two projections on dorsolateral radial plates.*

The central anterior projection of the radial plate serves as the insertion point for the radial muscles and separates adjacent tentacle ampullae. Long projections are found only in Myriotrochidae, several genera of which also possess two projections on their dorsolateral radials. This character in the reconstructed ancestor is coded as having one

projection per plate. This is based on observations of *in situ* calcareous rings in *Achistrum* from the Middle Pennsylvanian. Note, however, that Gilliland (1992) found isolated radials from Lower Jurassic apodans, evidently *Achistrum*, that occasionally carried two projections.

33. *Position of fossae for tentacle ampullae: 0 = anterior; 1 = exterior.*

The calcareous ring is grooved to receive the tentacle ampullae, either on the anterior surface, as in Myriotrochidae, or on the exterior, as in the remaining apodans. Calcareous ring plates from *Achistrum* have anteriorly positioned tentacular fossae (Gilliland, 1992).

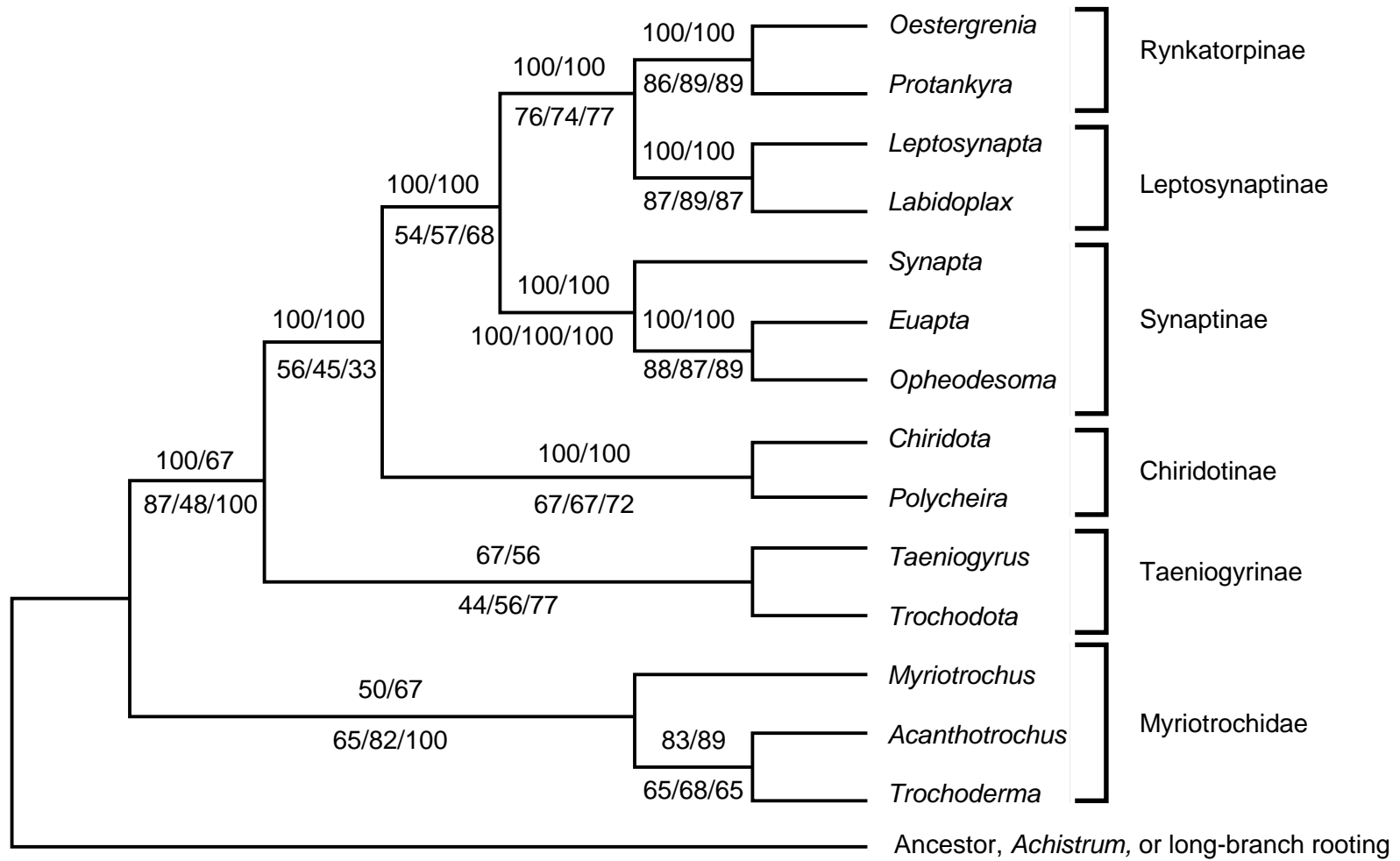
34. *Connective tissue ring: 0 = absent; 1 = present.*

A well-developed ring of clear connective tissue extends posteriorly from the calcareous rings of synaptine synaptids. This feature is found in no other Recent holothuroid and is coded as absent in the ancestor outgroup.

Results

The parsimony analyses under all rooting schemes give the same 50% majority rule consensus tree. Ancestor rooting produced 6 most parsimonious trees, a consensus of which is presented in Fig. 4.2. This tree has a length 50 steps, a consistency index (CI) of 0.76, rescaled CI

Figure 4.2. Topology of majority rule consensus trees, for ancestral and *Achistrum* rootings, and topology of single most parsimonious tree, using long-branch rooting. Numbers above branches indicate percentage of trees in which branch appears for ancestral and *Achistrum* rootings, respectively. Numbers below branches indicate supporting percentages of 500 bootstrap samples for ancestral, *Achistrum*, and long-branch rootings, respectively.



(RC) of 0.65, retention index (RC) of 0.65, retention index (RI) of 0.86. Rooting the tree on just the fossil characters of *Achistrum* recovered 9 most parsimonious trees. The shortest trees are 52 steps long and each have a CI of 0.75, RC of 0.63 and RI of 0.85. Rooting on the longest branch (with eight unambiguous changes) yielded a single most parsimonious tree identical in topology to that of the ingroup from the other rooting procedures. This tree has a length of 47, a CI of 0.81, RC of 0.72 and RI of 0.88. The frequency distribution of tree lengths in all analyses were highly left skewed, with “g₁” scores (i.e., the distributions’ third moments) of -0.82, -0.84 and -0.84, respectively, suggesting considerable hierarchical signal in the data sets (Hillis and Huelsenbeck, 1992). The ancestral rooting gave a topology identical to, and descriptive statistics nearly identical to, the topologies recovered using *Achistrum* or long-branch rooting. Hence, the following discussion is restricted to the tree founded on a reconstructed ancestor, except where notable differences between the analyses occur.

There is strong support via bootstrapping and the number of unambiguous changes in character states for Myriotrochidae as sister to Chiridotidae+Synaptidae. The latter two clades are joined by seven synapomorphies: gain of ciliated cups, increased length of the stone canal, presence of rod ossicles, gain of a complex wheel hub, inward-pointing teeth on the wheel rim, loss of anterior projections on the calcareous ring,

and the switch to exterior tentacular ampullae. The Myriotrochidae is supported by one unambiguous character, an increase in the number of wheel spokes. Less, but still moderate bootstrap support is found for a clade of Chiridotinae+Synaptidae. This node is subtended by a single character, increase from one to several polian vesicles. The Taeniogyrinae possesses two unambiguous synapomorphies, gain of sigmoid hooks and the loss of perforate radial plates. The Synaptidae received only moderate bootstrap support and is identified by three unambiguous characters, the gain of optic cups, anchors plus anchor plates, and the loss of wheel ossicles in the adult. The Chiridotinae are diagnosed by having C-shaped rods. Within Synaptidae, the Synaptinae is the best supported group. This clade is identified by eight unambiguous synapomorphies; they are increased body size, pinnate tentacles with large central digits, rosette ossicles, a cartilagenous ring, epibenthic habit and three features found on the anchor and anchor plate. The Leptosynaptidae+Rynkatorpinae are also well delimited by four features, gain of sensory cups, large ciliated cups, one polian vesicle and serrated arms on anchor ossicles. Both Leptosynaptidae and Rynkatorpinae are well supported by bootstrapping and are identified by three and two unambiguous character changes, respectively.

Discussion

Taxonomic Implications

The results of this study comprise the first phylogenetic test of the classification of Apodida. Smirnov (1998) recently modified subordinal and subfamily-level designations, introducing several new groups with the intent of delimiting evolutionary units within Apodida. He recommends subdividing the apodans into two suborders, Synaptina and Myriotrochina. Synaptina consists of two families, the Chiridotidae, with subfamilies Chiridotinae and Taeniogyrinae, and the Synaptidae, comprised of subfamilies Synaptinae, Leptosynaptinae and Rynkatorpinae. Smirnov's suborder Myriotrochina is monotypic. My analyses largely corroborate the reorganization: each taxon, with the exception of Taeniogyrinae, is supported by a high bootstrap percentage and up to seven unambiguous synapomorphies (Fig. 4.2).

My analysis also indicates strong support for a group not delimited in the latest classification of Apodida. Smirnov (1998) speculated that within Synaptidae there are two main lineages, the Synaptinae+Leptosynaptinae and the Rynkatorpinae. He based this split on the distinctive development and form of each group's respective anchor-plate ossicles (*sensu* Semper, 1868). In contrast, Heding (1928) had suggested that the division runs through Synaptidae and

Leptosynaptinae+Rynkatorpinae. Heding (1928) founded the split on the size and shape of their ciliary urns, naming the two groups Micrournae and Heterournae, respectively. In this study, Heding's grouping receives high bootstrap support and is subtended by four synapomorphies.

Finally, the investigation uncovered a possible relationship unanticipated by previous workers: The most striking difference between the phylogeny and apodan classification schemes is the possible paraphyly of Chiridotidae (Fig. 4.2). The family's two subfamilies appear as a grade with Chiridotinae sister to Synaptidae. Phylogenetic studies often uncover evidence that the Linnean designations encompass paraphyletic rather than monophyletic units (e.g., Mooi, 1990; Smith *et al.*, 1995). In this study, though, the paraphyly of Chiridotidae was not strongly supported, hence it is premature to suggest a taxonomic revision. If further support for the chiridotid grade is found, then Taeniogyrinae should be removed from Chiridotidae, provided that its monophyly is also firmly established. Regardless of the outcome, an important point remains. The characters used in this study were largely derived from taxonomic descriptions. Thus the lack of concordance between the phylogenetic estimate and the Linnean classification indicates that some groups have not been described cladistically, i.e., not through the most parsimonious ascription of diagnostic characters. This exposes the fact that there are no unambiguous characters uniting Chiridotidae as currently

conceived. The family is diagnosed via a suite of traits, only overlapping subsets of which are possessed by any one genus and are often exhibited by non-chiridotid apodans.

Fossils and the Calibration of Divergence Times

Fossil exemplars in phylogenetic analyses may lower the resolution of branching order and increase the number of most parsimonious solutions. This is because some characters with poor preservation potential must be coded as missing, which in turn increases the number of possible resolutions of trees now bearing polytomies defined by missing data. My analyses appeared minimally affected by the potential problems of missing data from fossils, in part because I constructed a hypothetical ancestor by combining the skeletal and gross morphological characters of the fossil taxon with the soft-tissue characters invariant in Recent holothuroids. Using this outgroup, six most parsimonious trees were recovered, a strict consensus of which was still resolved at the nodes of interest discussed in the previous section. Nevertheless, as a further test of the robustness of the rooting, I ran the parsimony analysis using only the nine characters available for *Achistrum* in the hypothetical ancestor. The analysis recovered a majority-rule consensus of nine most parsimonious trees identical to that based on the ancestral and long-branch rootings, while bootstrap values differed by 0 to 10%. (Fig. 4.2).

We used the *Achistrum*-rooted tree and the fossil record to estimate lineage divergence times in Apodida (Fig. 4.3). The oldest reliable stratigraphic records of the studied taxa are shown in Table 4.3. Despite a fossil record for Holothuroidea that is on the whole “really appalling” (Smith, 1988), the record of the Apodida is relatively good compared to that of other holothuroid orders (Frizzell and Exline, 1966). This permits us to make at least preliminary estimates on the times of some divergence events at the family and subfamily level. The Upper Permian through Upper Triassic *Acanthotheelia* is here considered *sensu* Gilliland (1993) ancestral to Recent *Acanthotrochus* based on the uniquely shared presence of wheel ossicles with a toothed inner rim and a few, but enlarged spines on the outer margin opposite the inter-spoke spaces. The calibrated tree suggests that there was little radiation of extant groups at the subfamily level during the Triassic like that reported for other echinoderms. Instead, most deep apodan splits appear to have happened in the late Paleozoic. Synaptidae, though, underwent a much later radiation during the Lower Cretaceous. Alternatively and taking the earliest recorded occurrences of Leptosynaptinae and Synaptinae at face value, Synaptidae radiated in the early Tertiary, perhaps in concert with the Eocene expansion of scleractinian coral reefs that is the habitat of most synaptids today.

Figure 4.3. Phylogeny of Apodida inferred from the morphological analyses (Fig. 4.2) and the stratigraphic record of oldest fossil representatives for the included groups. Thin solid lines indicate phylogeny based on the present study; thick solid lines indicate range from the earliest reliable occurrences of fossil members to the latest ones or present; thin dashed lines indicate potential range extensions. *Thallatocanthus* is used to anchor the deepest apodan divergence *sensu* Gilliland (1993).

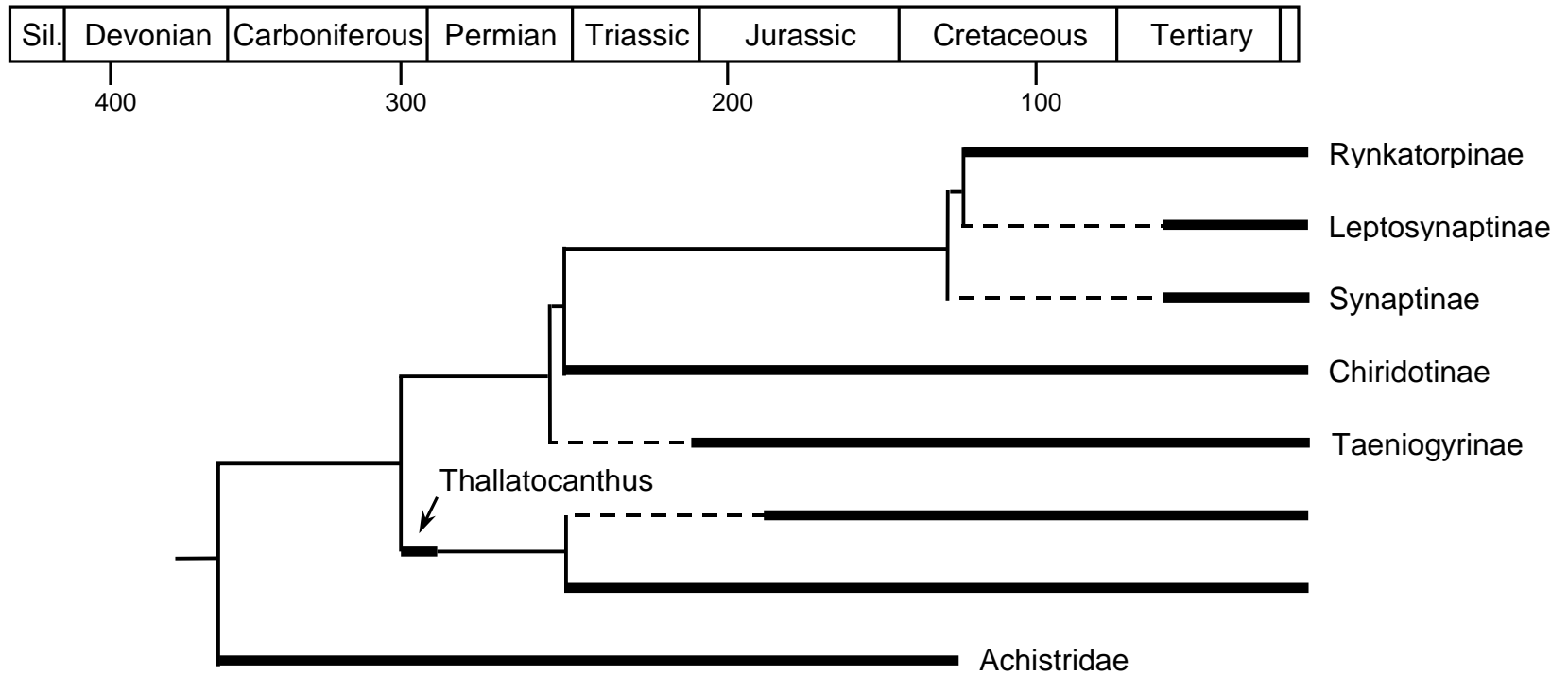


Table 4.3. Earliest stratigraphic occurrences of apodan taxa.

Taxon	Fossil representative	Earliest record	Age (mya)	Reference
Rynkatorpinae	<i>Rigaudites</i>	Lower Cretaceous	144.2	Smirnov (1989)
Leptosynaptinae	<i>Synaptites</i>	Tertiary (Eocene)	56.5	Smirnov (1989)
Synaptinae	<i>Croneisites</i>	Tertiary (Eocene)	56.5	Smirnov (1989)
Chiridotinae	<i>Theelia multiplex</i>	Middle Triassic (Ladinian)	234.3	Gilliland (1993)
Taeniogyrinae	<i>Theelia koeveskelensis</i>	Upper Triassic (Carnian)	227.4	Gilliland (1993)
Myriotrochus	<i>Hemisphaeranthos</i>	Lower Jurassic (Hettangian)	205.7	Gilliland (1993)
Acanthotrochus	<i>Acanthotheelia</i>	Upper Permian (Dzhulfian)	256.0	Gilliland (1993)
Achistridae	<i>Porachistrum</i>	Middle Devonian (Givetian)	380.8	Gilliland (1993)
Stem Myriotrochidae	<i>Thallatocanthus</i>	Middle Pennsylvanian	310.0	Gilliland (1993)

Chapter V:
**Bi-Penta-Bi-Decaradial Symmetry: Evolutionary and
Developmental Trends in Holothuroidea**

A version of this chapter appeared in Kerr AM, Kim J. 1999. Bi-Penta-Bi-Decaradial Symmetry: A Review of Evolutionary and Developmental Trends in Holothuroidea (Echinodermata). *Journal of Experimental Zoology (Molecular and Developmental Evolution)* 285: 93-103.

Summary

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 holopelagic species and spheroidal, plated taxa with mouth and anus adjacent at the end of a long, flexible stalk. Character-state optimizations onto a phylogeny derived from a maximum parsimony analysis of 47 morphological characters indicated that pronounced adult bilateral symmetry evolved at least twice. In one group even regains secondary radial symmetry. Cloacal breathing through respiratory trees is a relatively late innovation. The paired respiratory trees are ectodermally derived and are bilaterally symmetric, supporting the possibility that the secondary gain of pronounced bilateral symmetry in some 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 armoured echinoderms, evolving much later and at least twice. Indirectly developing larvae, auriculariae, occur in two evolutionarily derived and disparate clades. I argue that this implies the parallel convergent evolution of this larval type or, more speculatively, some form of retention of developmental constraints.

Introduction

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. Study of these latter traits often still provide important generalizations about developmental evolution (Raff, 1996), 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 potential value as model organisms and exemplars in comparative studies of development. I 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. I 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 morphological data. Finally, I use the phylogeny to illustrate the organisms' potential utility as exemplars in three areas of current 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, 1994). They are elongate, mostly soft-bodied animals with tube feet 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 that may be homologous to echinoid apical plates (David and Mooi, 1996). Hyman (1955) provides a comprehensive account of holothuroid gross anatomy, Smiley (1994) covers microscopic aspects, while Smiley *et al.* (1991) review reproduction and larval development.

Holothuroids are an abundant and diverse group of marine invertebrates. The approximately 1400 described and extant species

comprising 200 genera (Smiley, 1994) occur in benthic environments from the intertidal to the deepest oceanic trenches, where they may comprise greater than 90% of the sampled biomass (Belyaev, 1972). Unique among echinoderms, holothuroids can be holopelagic, living entirely in the water column (Miller and Pawson, 1990), or ectocommensals, living attached to fish (Martin, 1969). They may reach lengths over 5 m (Mortensen, 1938) and weigh over 5 kg (Lane, 1992). Their diversity is highest on Indo-Pacific coral reefs, where 20 species per hectare is not uncommon (Kerr *et al.*, 1993). In fact, the ubiquity of holothuroids in the largest ecosystem, the abyssal plain, 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 unanswered. 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.*, 1995; Smith *et al.*, 1995). Instead, the holothuroid skeleton consists of a calcareous ring and isolated, often microscopic ossicles embedded in the connective layer of the dermis. These ossicles are important taxonomically, as are the calcareous ring and some soft-tissue features, but surprisingly, none have been systematically surveyed for ontogenetic (Massin, 1994; Wiedemeyer,

1994; Cutress, 1996) and interspecific variation (Cherbonnier, 1947; Hansen, 1975). Moreover, the fossil record of holothuroids consists almost entirely of isolated ossicles, 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, 1993).

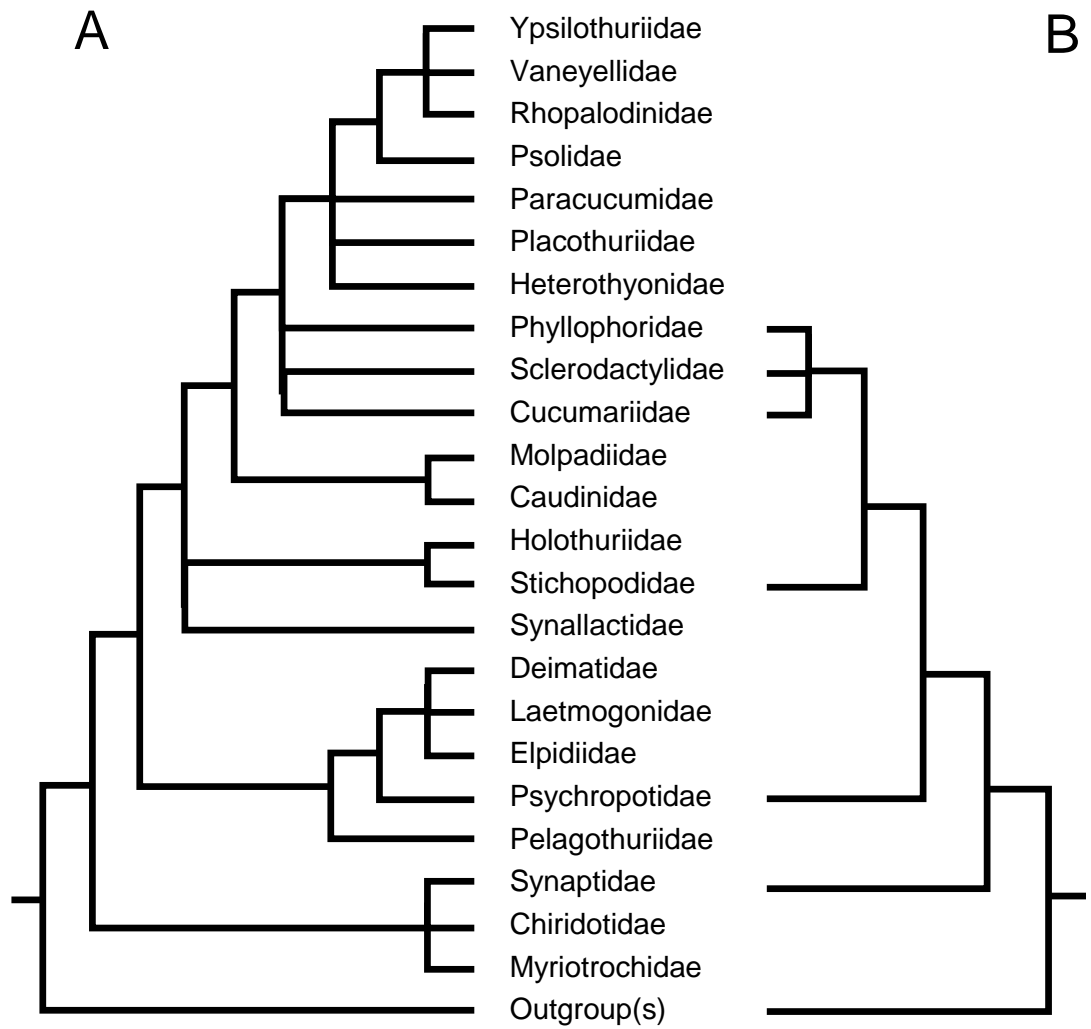
Current speculations about the evolution of Holothuroidea derive largely from the Linnean classification of Pawson and Fell (1965), who, based on perceived derived characters, divided holothuroids into three subclasses, each with two orders. Pawson and Fell (1965) suggest that holothuroids diversified 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 paedomorphosis has been a dominant trend in the evolution of Holothuroidea (Théel, 1886; Semon, 1888; Madsen, 1961; Haude, 1992; David and Mooi, 1998). 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, 1975; Pawson, 1984; Haude, 1992; Gilliland, 1993), but essentially no new information corroborating or challenging their proposal has come to light in the last 35 years. The most

comprehensive review of holothuroid evolution and paleontology occurs in Gilliland (1993), but Frizzell and Exline (1966), Pawson (1980) and Haude (1992) 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 (see Arndt *et al.* [1996] for relationships within dendrochirote holothuroids). The phylogeny is based on a maximum parsimony, maximum likelihood and minimum evolution analyses of partial small subunit rDNA sequences (Chapter II) and a maximum parsimony analysis of 47 morphological characters (Chapter III). The results of the phylogenetic analyses are surprising. The phylogeny indicates the need for a fundamental reorganization of holothuroid systematics at the ordinal and subclass levels. For example, molpadiidan families such as Molpadiidae have been placed in the same subclass as that including Synaptidae (Pawson and Fell, 1965), but these groups appear only distantly related (Fig. 5.1A). This nearly complete inversion of the prevailing classification has important consequences for our inferences about the evolution of developmental modes as I discuss below. It also indicates that current taxonomic groupings have likely been diagnosed from only subsets of the available characters.

Fig. 5.1. Phylogeny of Holothuroidea. A) Single most parsimonious tree from successively weighted analysis of 47 morphological characters (see Chapter III). Branches collapsed when supported by less than 70% bootstrap percentages. B) Strict consensus of three most parsimonious trees from equally weighted analysis of partial small subunit rDNA sequences (Chapter II).



Morphological Evolution within Holothuroidea

Adult body form

Living echinoderms with indirect development begin their larval lives as bilaterally symmetrical larvae. The well-known pentamerous symmetry typical of echinoderms is acquired only later, beginning when the larval middle coelomic sac, the left hydrocoel, forms a ring from which arise five evaginations. In post-larval echinoderms, most organ systems and appendages are arranged five-fold around a central to subcentrally placed digestive system. Figuring out 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, 1994). How the multiplication of body rays occurred is less certain, though potential explanations abound (most recently, Hotchkiss, 1998). At least one view, pentamery arising from a multiplication of body axes, is supported by preliminary evidence from *Hox*-gene expression patterns (Raff, 1996; Lowe and Wray, 1997).

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, 1984). 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, 1990). 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. I discuss these modifications below, including that of Rhopalodinidae, one of the most Protean 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. 5.2A), so that the gonopore is dorsal. This arrangement is maintained along the plane of larval bilateral symmetry (Smiley, 1986) and is presumably an adaptation to an epibenthic existence. Holothuroid bilaterality appears to have evolved in Holothuroidea in two to three quite distantly related groups (Fig. 5.3). Internally, these holothuroids, as well as some others,

Fig. 5.2. Representative holothurian body types. A) *Psychropotes*, Psychropotidae, Elasipodida; 15 cm (after Hansen, 1975). 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, 1997). Measurements are anterior-posterior lengths.

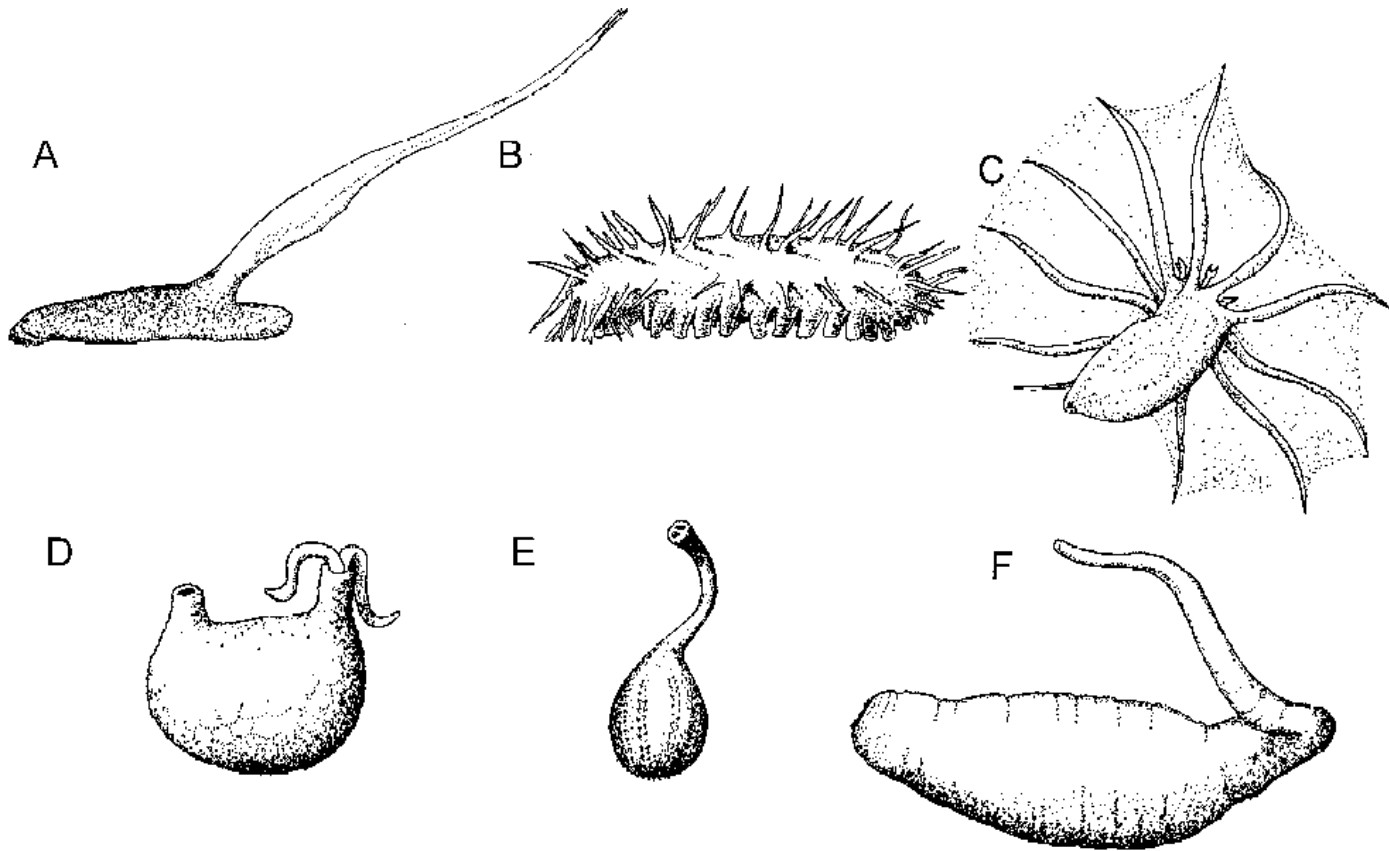


Fig. 5.3. Character-state optimization onto the morphological phylogeny. Superscripts under the heading *Larval development* indicate that entries were inferred from maximum egg size (e.g., Tyler and Billett, 1987).

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
Rhopalodiniidae	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
Molpadiidae	no	no	infaunal	present	direct	varied	reduced
Caudinidae	no	no	infaunal	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
Deimatidae	yes	yes	epibenthic	absent	direct ¹	deepwater	plated
Laetmogonidae	yes	yes	epibenthic	absent	direct ¹	deepwater	reduced
Elpidiidae	yes	yes	epibenthic	absent	direct ¹	deepwater	reduced
Psychropotidae	yes	yes	epibenthic	absent	direct ¹	deepwater	reduced
Pelagothuriidae	no	no	pelagic	absent	direct ¹	pelagic	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
Ancestor	no	no	infaunal	absent	indirect	unknown	plated

may possess paired respiratory trees and, in elpidiid elasipodans, possess statocysts along 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 largely of a ventrally concentrated field of tubefeet. Finally, no dorsoventral differentiation is apparent externally in the epibenthic synaptine apodans, 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. 5.3). Papillae are structurally and functionally most diverse in Elasipodida (Fig. 5.2A-C). In this group, papillae may fuse to form a large dorsal structure of uncertain function (Fig. 5.2A), perhaps sensory or respiratory (Hansen, 1975). As well, papillae may present webbing that facilitates swimming, which in *Pelagothuria natatrix* (Fig. 5.2C) is circumoral and also used to concentrate descending sediment (Billett, 1991). Papillae in elasipodans may also be present ventrolaterally as locomotor appendages, affectively 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, 1997).

The most remarkable example of deviation from standard echinoderm pentamery is found in the Rhopalodinidae in which members during ontogeny 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. 5.2D) and is presumably an adaptation for mud living (Pawson, 1984). 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. 5.2E). 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, 1988). Internally, the conventional five-fold organ systems still run from mouth to anus (Semper, 1868).

Unique among the echinoderms, holothuroids possess prominent internal structures called respiratory trees: a bilaterally arranged pair of heavily ramified tubes used in gas exchange that arise and receive water

from the cloaca (Shick, 1983). The origin of the structures in Holothuroidea is uncertain. Several workers (Théel, 1886; Madsen, 1961; Pawson and Fell, 1965) state or imply that respiratory trees arose early in the evolution of Holothuroidea. Semper (1868), as well as Huxley (1878), however, suggested that they arose much later. Our phylogeny (Fig. 5.3) 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 (Gilliland, 1993). A significant increase in surface area for gas exchange undoubtedly 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 molpadiidans (Fig. 5.2F), in settings with lower and previously intolerable partial pressures of oxygen, such as in 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 its 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 secondarily regain a novel form of radial symmetry leading to the evolutionary sequence: bilateral symmetry → pentaradial symmetry → bilateral symmetry → “decaradial” symmetry.

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, 1991; Eval-Giladi, 1997) or the larval dorso-ventral axis in echinoderms (Kominami and Takata, 1995). Rather, I speculate that the secondary dorso-ventral axis may be ectodermally derived and compartmentalized in a manner analogous to vertebrate limb development (Altabef *et al.*, 1997; Zeller and Duboule, 1997). 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 one might gain symmetry from asymmetrical form in two different ways: either de-differentiate or replicate. In holothuroids, pentaradial

symmetry begins with repeated coelomic 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.

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 (=doliolaria; Smiley *et al.*, 1991) provided with yolk. These larvae resemble in many ways the nonfeeding forms of other echinoderm classes. In many holothuroid species, the vitellaria are pelagic, at least briefly. Other taxa brood their larvae, either externally or internally, which become free living as juveniles. The remaining species are indirect developers, passing first from an obligate pelagic, plankton-feeding auricularia to a vitellaria before settling as a juvenile (Smiley *et al.*, 1991).

Prevailing ideas about larval evolution within Holothuroidea are based on general treatments of the evolution of larval form in marine invertebrates (Strathmann, 1978; Hansen, 1982; Emlet, 1990; Strathmann,

1993). 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. 5.3) 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, 1996) and, most likely, the Asteroidea (Chia and Walker, 1991) and Ophiuroidea (Hendler, 1991). The auriculariae in the two groups of holothuroids are very similar (Mortensen, 1937, 1938) and are primarily distinguishable by their ossicles and, during metamorphosis, by different development of the hydrocoel (Smiley *et al.*, 1991). 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. At present I favor this hypothesis for the following two reasons: First, a single origin for holothuroid auricularia requires that there have been at least five losses of feeding larvae that resulted in two isolated subgroups of larval feeders, a pattern not seen, as far as I know, in any other class of marine invertebrates. Strathmann and Eernisse (1994) 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 five branches separate Synaptidae and the Holothuriidae+Stichopodidae lineage. The probability of identity by descent of the feeding forms is thus $(1-p)^5$. 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 ~ 0.01 along any branch separating the Synaptidae and the Holothuriidae+Stichopodidae lineages (Chang and Kim, 1996). The argument of highly probable loss of the feeding form actually runs counter to explaining the phylogenetic distribution of the feeding larvae. The only other possible explanation is if the branches separating the two lineages with feeding larvae are extremely small (Schluter *et al.*, 1996). However, the ancient divergence of these two lineages (Gilliland, 1992) 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.

There is a second reason to consider an independent origin of feeding larvae in holothuroids. 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. 5.3). 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, 1993), the present reef habitat must be a secondary trait. But then a single origin of auricularia requires that the two extant feeding groups, to the exclusion of other holothuroids, have independently and relatively recently radiated into the same habitat, coral reefs, while all other feeding forms in other habitats disappeared. I can think of no documented instance in which invertebrates with planktotrophic larvae have suffered significantly higher extinction rates than those with non-planktotrophic forms.

If the holothuroid auricularian larva has evolved twice, then a mechanism to explain this is required. I agree that in general the independent evolution of very similar larvae is probably a quite rare event (Haszprunar *et al.*, 1995; Reid *et al.*, 1996). However, for the previously

discussed reasons, I regard the dual evolution of auricularia in holothuroids as reasonable. Possible explanations for this event center around convergence on an efficient morphology for locomotion and 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 known morphological differences between the feeding larvae of Synaptidae and Holothuriidae+Stichopodidae are 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, 1990). In most of Holothuroidea, the skeleton is reduced to a circum-oesophageal calcareous ring and isolated microscopic ossicles 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, 1994). Other minor accessory skeletal

structures also exist: Some molpadiid holothuroids have microscopic granules of ferric phosphate that ontogenetically replace body-wall ossicles (Stricker, 1986) and in *Ceraplectana*, sclerous non-proteinaceous sheaths cover the tentacles (Clark, 1907).

Like the skeletal elements of other echinoderms, holothuroid ossicles are formed within a syncytium of primary mesenchyme cells (Stricker, 1985). These cells deposit calcite about a scaffold of ossicle matrix proteins (Killian and Wilt, 1996). Aspects of ossicle shape appear controlled by matrix protein interactions with the crystal faces of the growing ossicle (Berman *et al.*, 1993) that in turn are mediated by communication between primary mesenchyme cells whose spatial arrangement is influenced by ectodermal cues (Ettensohn *et al.*, 1997).

The evolution of the holothuroid skeleton is poorly understood. Holothuroids with a skeleton of microscopic ossicles 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 (Motokawa, 1981). The mechanical properties of the connective tissues remain influenced by the density and shape of the ossicles (Koehl, 1982). Pawson and Fell (1965) and Haude (1992) suggest, based on fossil evidence, that a skeleton of isolated ossicles arose once to several times from earlier plated holothuroids.

The phylogeny (Fig. 5.3), however, suggests that the plated skeleton is a relatively late innovation arising once in deimatid elasipodans 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. I 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 (Gilliand, 1993). Excepting a strong bias for retention of plates and numerous losses in most living holothuroids, a possibility for which I have no *a priori* expectations, the plated fossil forms may represent multiple convergences to this skeletal type. I suggest that it may be relatively easy to affect an allometric increase in the size of isolated ossicles to form a test of imbricating or overlapping plates, particularly if adult body size is small as occurs in nearly 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, 1994) or because spiculation does not scale isometrically with extremely small body size.

CHAPTER IV:
Conclusions and Prospects

Holothuroids are weird. They are as unusual echinoderms as echinoderms are unusual animals. Despite this attractive feature, holothuroids have been and continue to be relatively ignored. Peculiar organisms are attractive in comparative biology because they make the strictest tests of generalizations based on model groups. Nevertheless, holothuroids receive little attention despite the important role that some other echinoderms play in generating and testing ideas about evolution and development. One objective of this thesis was to begin to correct this oversight and revive scientific interest in Holothuroidea. The main result was a well-supported estimate of holothuroid phylogeny. Then, based on the phylogeny, I made inferences about the evolution of the group and tested suppositions about the group's systematics. The upshot is that several long-standing ideas about the evolution and systematics of these echinoderms are wrong or seriously in question. Some other speculations about the group, often those made well over a century ago, received support. In this concluding chapter, I briefly outline the major results of this thesis and follow each by suggesting some implications and ideas for future research.

Phylogeny

Using six species from four orders, I performed a maximum parsimony, maximum likelihood and minimum evolution analyses of 980

nucleotide sequences from 18S rDNA. I also did a maximum parsimony analysis of 47 informative morphological characters with exemplars of all 25 recognized families. These analyses concurred with each other and the stratigraphic order of appearance of available groups.

The placements of some groups, however, are still problematic. Two families, Eupyrgidae and Gephyrothuriidae, were excluded from the morphological analysis because of their effect on the resolution of branching order and because their placement was dependent on rooting and weighting procedures. Constraining a parsimony analysis so that these families must be placed on the topology recovered with these groups excluded generates a relation (Ancestor, (Apodida, (Elasipodida, (Gephyrothuriidae, (Eupyrgidae, (remaining families)))))). The resulting ingroup is identical to that from an unconstrained long-branch rooted, successively weighted analysis that includes all families. The arrangement is somewhat unsatisfying, though, since Eupyrgidae and Gephyrothuriidae are both small, apparently pedomorphic forms. Hence their placement appears largely determined by the absence of potentially derived adult characters.

The existence of several body fossils for reliable outgroups allowed an analysis of branching order within the Apodida. However, branching order within most other ordinal groups was not confidently resolved. For

example, Synallactidae is a heterogeneous, likely para- to polyphyletic group. To proceed, I based the morphological characters of Synallactidae on a formally unrecognized, but relatively homogenous group within the family showing firm body walls and certain ossicle types, when present (e.g., *Mesothuria*, *Synallactes*, *Pseudostichopus*). Other synallactids share gelatinous body walls, ossicles that differ from the previous group, and the ability to swim (e.g., *Paelopatides*, *Hansenothuria*, *Bathyploetes*). Both groups, should they each prove monophyletic, will likely fall out close to one another and to the rest of the aspidochirotetes. Branching order within the elasipodans and dendrochirotetes are similarly uncertain and have been previously discussed.

One limitation of the phylogenetic analyses using morphology was a lack of knowledge about the comparative biology of holothuroids from which to identify informative characters. Most anatomical work was conducted during the 19th century (summarized in Ludwig 1889-1892). That and the less frequent work that followed during the 20th century were generated to answer a different set of questions from those that face a phylogenetic systematist. Numerous features of anatomy and physiology are still known from only a few species. Nevertheless, the distributions of some of these characters are suggestive of synapomorphies confirming my phylogenetic study.

For example, in echinoderms hemoglobin is known only from holothuroids (Smiley, 1994). The few species reported with hemoglobin-carrying cells, hemocytes, are only known from the clade that includes the dendrochirotes and molpadiidans (Smith, 1981). Hemocytes have not been observed in the Aspidochirotida, Elasipodida or Apodida. If this pattern holds, in addition to providing an additional synapomorphy for the aforementioned clade, it also suggests something about the evolutionary ecology of the group. A parsimony-based reconstruction of characters based on extant taxa is ambiguous about the ancestral ecology of the clade. The presence of hemocytes, though, with corroboration from the presence of respiratory trees, strongly suggests an infaunal ancestor.

Systematics

The phylogeny of Holothuroidea stands at considerable odds with the higher level classification of the group. No subclass designation was recovered as a clade and two of the six orders appear either para- or polyphyletic. Conversely, the analysis of the Apodida uncovered a cladistic structure quite similar to that implied by the most recent revision of the group (Smirnov, 1998). Where the two differed, the internal branches in the phylogeny were weakly supported. Nevertheless, the differences highlight the fact that two families, Chiridotidae and Myriotrochidae, are poorly diagnosed.

As such, a considerable revision of the Linnean classification is needed to have it reflect the cladistic structure of the phylogeny of Holothuroidea. In what follows, I make an informal presentation about some needed nomenclatural changes. A formal description that includes the designation of types is currently underway and will be presented elsewhere.

Perhaps the first requirement is for a term that delimits the clade formed by the groups Dactylochirotida, Dendrochirotida, Molpadiida and Aspidochirotida (Fig. 3.4). Two other families of uncertain affinity likely also belong here, Eupyrgidae and Gephyrothuriidae. These groups are joined by several unambiguous synapomorphies, most convincingly by the uniform presence of respiratory trees, and have a long and separate history from the remaining holothuroid groups. This clade is similar to a group defined by Selenka (1867) as the Pneumonophora (“lung bearers”). Selenka, though, mistakenly excluded from this group species that possessed respiratory trees. I suggest then the subclass Pneumonophoracea to include those holothuroids with respiratory trees. This leaves the Elasipodida and Apodida, which would each be assigned to their own subclasses.

The dactylochirotetes (Fig. 3.4) with their highly modified globose and plated bodies and apparent loss of arborescent tentacles have long been recognized as distinctive. For this reason Pawson and Fell (1965) raise them to ordinal status. Much earlier, Semper (1868) suggested that they belong well within the dendrochirotetes, a view corroborated by the phylogeny (Fig. 3.4). For this reason, I suggest that the dactylochirotetes be redesignated as the suborder Dactylochirotina within Dendrochirotida.

One problem with the Linnean classification system is that in adapting it to reflect evolutionary history many monotypic categories are created. For example, creating the subclass Pneumophoracea mentioned above requires that the two remaining distinct, but much smaller and more uniform orders themselves comprise monotypic subclasses. This occurs because all species must be subsumed by all categorical levels despite the fact that evolution produces tree partitions that are very unbalanced (Guyer and Slowinski, 1993). This is a compelling argument for abandoning ranks in classification (deQuieroz and Gauthier, 1990). In the nomenclatural scheme of phylogenetic systematics, a Linnean term is retained where it defines a clade, but it no longer possesses a rank value (Cantino *et al.*, 1999).

Paleontology

As for most soft-bodied animals, the fossil record of holothuroids is infamously poor. There are only about 15 species described from body fossils (though for one species there are over 2000 specimens; Gilliland, 1993) and none are known from Cretaceous or younger strata. Most fossil forms are paraspecies described from isolated ossicles. Still, an analysis of the correspondence between the morphological phylogeny and the stratigraphic record found that they were significantly more congruent than expected when randomly assigning range data over the tree (Fig. 3.5). The fossil record thus appears minimally sufficient to calibrate the divergence time of several important groups. Some puzzling gaps, however, still remain in the record. Lack of sampling effort is one possibility, though numerous potentially important body fossils await description (Sroka, 1988; P. Jell, *pers. comm.* in Gilliland, 1993; D. Seilacher, *pers. comm.*; A. Smith, *pers. comm.*; A. Kerr, *pers. obs.*)

The presumed antiquity of dendrochirote holothuroids is based on the resemblance of some of the earliest body fossils with extant plated forms. Isolated plates of even earlier age are also often assigned to Dendrochirotida (e.g., Reich, 1999). The phylogeny shows that the living plated holothuroids radiated in the early Mesozoic (Fig. 3.5). This suggests that either a plated body was lost several times among the pneumonophoraceans or that plated holothuroids evolved several times in Holothuroidea. A body of perforated plates is a plesiomorphy of

Echinozoa, occurring in early Paleozoic echinoids and the probable stem holothuroids, ophiocistioids (Smith, 1988). Nevertheless, fossil plated forms are often assigned to the dendrochirotes. For example, *Strobilothyone rogenti*, a Middle Triassic species, is placed within the extant dendrochirote family Heterothyonidae based on its spireless perforate plates and the presence of posterior processes and entire plates on its calcareous ring (Smith and Gallemí, 1991). If plates are a retained ancestral feature of pneumonophoraceans, then *Strobilothyone* could more easily be assigned to the Molpadiida. Molpadiidans also have posterior processes on a calcareous ring with entire radial and interradial plates. *Strobilithyone*, like molpadiidans, possesses a narrow, elongate tail. These similarities were first noted by Haude (1995b). Finally, the imbrication of the body-wall plates is unlike that of any extant dendrochirote (this is discussed fully under character 17 in Chapter III). A fusiform body and apparently burrowing habit are also consistent with Molpadiida.

The derived position of dendrochirotes further predicts that features of recently discovered early Paleozoic holothuroids also will not resemble this group. The oldest unequivocal body fossil is an undescribed specimen from the late Silurian. Gilliland (1993) writes that the body is invested in perforated plates and that the anterior rim of the calcareous ring, the only portion visible, is reminiscent of a dendrochirote structure.

I nevertheless predict that the ring will prove typical of other Paleozoic forms, resembling most the low, band-like rings of living apodans.

Developmental Evolution

Larval Feeding Mode

Mapping well-known characters onto the phylogeny allowed several robust inferences about the morphological and developmental evolution of Holothuroidea. The first is that larval form has evolved in an unusual manner compared to most invertebrates in which a planktonic feeding larva appears ancestral (Hickman, 1999). In holothuroids, two evolutionarily disparate clades arising during the Jurassic possess planktotrophic larvae, inviting the possibility that one or both of these transitions are independent of a possibly ancestral planktotrophic state. I show that this interpretation is robust to uncertainties in transition probabilities and branch lengths and how one published argument for ancestral planktotrophy (Strathmann and Eernisse, 1994) actually argues for the possibility of the derived condition. However, because so few data exist on larval development in Holothuroidea, it is still possible that planktotrophy exists in groups that would force a more ancient reconstruction of larval feeding. I note that numerous data on ova diameter (which is correlated with larval form) and brooded larvae exist,

but they are scattered in an old and obscure literature. I am currently assembling and preparing a phylogenetic analysis of these data.

Skeleton

Another interesting result of the character optimizations concerns the evolution of the holothuroid skeleton. No group of echinoderms has experienced such a pervasive reduction of the skeleton as Holothuroidea. The mechanical support provided by an extensive calcitic skeleton was replaced by a hydrostatic one. This change takes advantage of a feature unique to echinoderms whereby the stiffness of the connective tissue in the body wall is under nervous control (Motokawa, 1981). An extensive calcite skeleton in the form of a plated test occurs in some 10% of living holothuroids and probably evolved once or twice during the early Mesozoic (Fig. 3.5). Conversely, some groups entirely lack ossicles (e.g., *Pseudostichopus*, *Anapta*, *Pelagothuria*).

In echinoderms, there are evolutionarily quite distant but identical ossicles and, conversely, evolutionarily close but radically different ossicles. For example, anchor ossicles in synaptid holothuroids are used in locomotion. The anchor is aligned parallel to the oral-aboral body axis, while the tines protrude from the body wall and point posteriorly, providing a grip as the animal moves forward via peristalsis. Nearly

identical anchor ossicles occur in the echinoid *Micropyga*, where they line and support the wall of enlarged tube feet used in suspension feeding (Mortensen, 1940). A similarly shaped anchor is also found in the gut lining of another echinoid *Chondrocidaris* (Mortensen, 1923). These ossicles are located in vastly different regions of the body and perform different functions. Based on these and other examples, I posit that there is a limited repertoire from which the skeletal system can select form and, from related data (Ekman, 1926; Arndt et al., 1996), that small developmental alterations can result in large changes in shape. Both points are consistent with there being a limited set of rules from which to create shape.

Some effort has been directed at understanding molecular aspects of ossicle formation. There is considerable interest in the molecular basis of echinoderm skeletal formation as a model for bone development and skeletal-tissue engineering (Benson and Wilt, 1992; Killian and Wilt, 1996; Reddi, 1998). This work makes clear that one important aspect of control over ossicle morphology occurs at the molecular level. Calcite is laid upon a scaffold of ossicle-matrix proteins that apparently modulate the deposition of microcrystalline calcite (Berman *et al.*, 1993). This work is performed by primary mesenchyme cells, amoeboid cells first appearing during gastrulation. While ectodermal cues are responsible for initiation of calcite deposition and perhaps macroscopic aspects of shape,

communication between mesenchyme cells is sufficient to generate microscopic features, even while isolated *in vitro* (Ettensohn *et al.*, 1997). Over much of echinoderm evolution, these cells formed macroscopic skeletal elements with an internal stereom of calcite. This internal lattice would appear to require only a restricted set of instructions to generate if macroscopic aspects are controlled ectodermally.

I suspect that another important component of ossicle formation, one that may more fully account for the aforementioned patterns in diversity of shape in metazoan ossicles, is cellular-level interactions. Cells could affect ossicle formation in several ways. For example, in holothuroids, ossicle symmetry is laid down very early, when the ossicle primordium is about the size of a mesenchyme cell (Hyman, 1955). Some species have only tripartite ossicles, others only quadripartite, and still others have both (Gilliland, 1993). I posit that ossicle symmetry is related to the number of cells that are adjacent during early ossicle growth and that this number becomes highly canalized in some species.

Aside from the sweeping loss of large skeletal elements, holothuroid skeletons differ in two other important ways from those of other echinoderms. Both of these deviations are restricted to the family Molpadiidae. First, deposits of ferric phosphate can ontogenetically replace calcite ossicles. In most large molpadiids, the calcite has been

completely replaced. Second, in the genus *Ceraplectana*, sclerous, secreted sheaths cover the tentacles. I have begun examining the composition of *Ceraplectana* sheaths. The structures resist complete dissolution in a 24-h bath of a 60° saturated aqueous solution of sodium hydroxide. This indicates that a fraction of the material is not proteinaceous and is consistent with the material being a polysaccharide. The polysaccharide most commonly used as skeletal material in many metazoans is chitin, and while known from vertebrates (Wagner, 1994) has so far not been reported from other deuterostomes, including echinoderms. A finding in molpadiidan holothuroids would constitute the only instance in deuterostomes in which chitin reacquires a role as a material in an external skeleton.

The application of modern comparative biology to holothuroids has just begun. Numerous questions vulnerable to these methods remain. 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? What accounts for the diversity of ossicles within Holothuroidea? Why are identical ossicles found in even more distantly related echinoderms, as well as gorgonian anthozoans and sponges? 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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Appendix:

Alignment Of Nucleotides of the Three Fragments of Nuclear Small

Subunit rDNA

Alignment of nucleotides of the three fragments of nuclear small subunit rDNA. Preliminary alignment was done on *Clustal W 1.4* (Higgins, 1994), then finished by hand, taking into account secondary structural features. Fragment one runs from position 1-429, two from 430-834, and three from 835-1075. A question mark indicates unresolved nucleotide in Raff *et al.* (1988). Species designations are from the first ten characters plus spaces of the latin names given in Table 2.1.

	1	11	21	31	41
Leptosynap	ATAACTGTGG	TAATTCTAGA	GCTAATACAT	GCGATCAAGC	GCTGACCTCA
Sclerodact	AGAA?TGTGG	TAATTCTG??	????ATACAT	GCGATTAAGC	GCCGACCCTC
Cucumaria	ATAACTGTGG	TAATTCTAGA	GCTAATACAT	GCGATTAAGC	GCCGACCCTT
Lipotrapez	ATAACTGTGG	TAATTCTAGA	GCTAATACAT	GCGATTAAGC	GCCGACCCTC
Stichopus	ATAACTGTGG	TAATTCTAGA	GCTAATACAT	GCCAACAAGC	GCCGACCCTT
Psychropot	ATAACTGTGG	TAATTCTAGA	GCTAATACAT	GCACTTAAGC	GCTGACCTTT
Meoma vent	ATAACTGTGG	TAATTCTAGA	GCTAATACAT	GCGTCCAAGC	GCCGACTTCC
Eucidaris	ATAACTGTGG	TAATTCTAGA	GCTAATACAT	GCGTCCAAGC	GCCGACTCCA
Mespilia g	ATAACTGTGG	TAATTCTAGA	GCTAATACAT	GCGCCAAGC	GCCGACTCCC
	51	61	71	81	91
Leptosynap	---CGGAAGG	CG?GCTTTTTA	TTAGGAACAG	GACCAGCCCCG	GTGTC-----
Sclerodact	ATTGGGGAAG	CGTGCTTTTTA	TTAGGAACGA	GGCCAGTACG	GTCTTTT---
Cucumaria	-CGGGGGAAG	CGTGCTTTTTA	TTAGGAACAA	GGCCAGCCCCG	GTCTCTT---
Lipotrapez	-CGGGGGAAG	CGTGCTTTTTA	TTTGAACAA	GGCCAGCTCG	GTTCCCCCAGC
Stichopus	--CGGGGAAG	CGTGCTCTTA	TTAGGAACAA	GGCCAGCCCCG	GTCCTTT---
Psychropot	-CGGGGAAAG	CGTGCTTTTTA	TTAGGAACAA	GGCCAGCCCCG	GAAGGAC---
Meoma vent	---A-GAAGG	CGTGCTTTTTA	TTAGGAACAA	GACCAGCCCCG	GCCC-----
Eucidaris	---C-GAAGG	CGTGCTTTTTA	TTAGGAACAA	GACCAGCCCCG	GCTCGTCTCA
Mespilia g	---CAGAAGG	CGTGCTTTTTA	TTAGGAACAA	GACCAGCCCCG	GCCT-----
	101	111	121	131	141
Leptosynap	-----ATGCC	GG?CTCCGCT	GGT?AACTCT	AGATAACTTTA	GCCGA--TCG
Sclerodact	---GGGGGCC	?GTCTCCGCT	GGT?AACTCT	AGATAACCAC	GCCGA--TCG
Cucumaria	--CGGAGGCC	GGTCTACGCT	GGTGAACTCT	AGATAACCAC	GCCGA--TCG
Lipotrapez	GATGGGGGCC	GGTCTCCGCT	GGTGAACTCT	AGATAACTTC	GCCGA--TCG
Stichopus	--CGGGGTCC	GGTCTCCGCT	GGTGAACTCT	AGATAATCAT	GCCGAGATCG
Psychropot	---TCCTCCC	GGTCTACGCT	GGTGAACTCT	AGATAACAAT	GTCGA--TCG
Meoma vent	-----CGGCC	GGCAAC-ACT	GGTGAACTCT	GGATAACACA	GCCGA--TCG
Eucidaris	CGGCGTGTCC	GGCAAAGACT	GGTGAACTCT	GGATAACACA	GCCGA--TCG
Mespilia g	-----CGGCC	GGACAC-GCT	GGTGAACTCT	GGATAACACA	GCCGA--TCG
	151	161	171	181	191
Leptosynap	CACGGTCTT-	GCACCGGCGA	TGGTTCCTTC	AAAT-GTCTG	CCTTATCAAC
Sclerodact	CACGGTCTC-	GCACCGGCGA	CGCTCCCTTC	AAAA-GTCTG	CTCTATCAAC
Cucumaria	CACGGTCTC-	GCACCGGCGA	CGCTCCCTTC	AAAA-GTCTG	CTCTATCAAC

Lipotrapez	CACGGTCTC-	GCACCGGCGA	CGCTCCCTTC	AAAA-GTCTG	CTCTATCAAC
Stichopus	CACGGTCTT-	GCACCGGCGA	CGCTCCCTTC	AAAAAGTCTG	CTCTATCAAC
Psychropot	TACGGTCTC-	GCACCGGCGA	CGCTTCCTTC	AAAA-GTCTG	CTCTATCAAC
Meoma vent	CACGGTCCTC	GCACCGGCGA	CGGATCCTTC	GAAT-GTCTG	CCCTATCAAC
Eucidaris	CACGGTCCTG	GCACCGGCGA	CGGATCCTTC	GAAT-GTCTG	CCCTATCAAC
Mespilia g	CACGGTCATC	GCACCGGCGA	CGGGTCCTTC	GAAT-GTCTG	CCCTATCAAC
	201	211	221	231	241
Leptosynap	TGTCGATGGT	AGGTTATGCG	CCTACCAT?G	TCGTAACGGG	T?ACGGAGAA
Sclerodact	TTTCGATGGT	AAGTTATCTG	CTTATCATGG	TTGTGACGGG	T?ACGGAGAA
Cucumaria	TTTCGATGGT	AAGTTATCTG	CTTATCATGG	TTGTGATGGG	TAACGGAGAA
Lipotrapez	TTTCGATGGT	AAGTTATCTG	CTTATCATGG	TTGTGACGGG	TAACGGAGAA
Stichopus	TTTCGATGGT	AAGTTATGCG	CT-ATCATGG	TTGTGACGGG	TAACGGAGAA
Psychropot	TGTCGATGGT	ACGTTATGCG	CTTATCATGG	TAATGACGGG	TAACGGAGAA
Meoma vent	TTTCGATGGT	ACGTTATGCG	CCTACCATGG	TCGTCACGGG	TAACGGAGAA
Eucidaris	TTTCGATGGT	ACGTTATGCG	CCTACCATGG	TGGTCACGGG	TAACGGAGAA
Mespilia g	TTTCGATGGT	ACGTTATGCG	CCTACCATGG	TCGTCACGGG	TAACGGAGAA
	251	261	271	281	291
Leptosynap	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCT?	CCACATCCAA
Sclerodact	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA
Cucumaria	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA
Lipotrapez	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA
Stichopus	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA
Psychropot	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA
Meoma vent	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA
Eucidaris	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA
Mespilia g	TCAGGGTTTCG	ATTCCGGAGA	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA
	301	311	321	331	341
Leptosynap	GGAAGGCAGC	AGGCACGC?A	ATTACCCAAT	CCCGACTCGG	GGAGGTAGTG
Sclerodact	GG??GGCAGC	AGGCACGC?A	ATTACCCACT	CC-GAC?CGG	GGAGGTAGTG
Cucumaria	GGAAGGCAGC	AGGCACGCAA	ATTACCCACT	CCCGACACGG	GGAG-TAGTG
Lipotrapez	GGAAGGCAGC	AGGCACGCAA	ATTACCCACT	CCCGACACGG	GGAGGTAGTG
Stichopus	GGAAGGCAGC	AGGCACGCAA	ATTACCCACT	CCTGACACGG	GGAGGTAGTG
Psychropot	GGAAGGCAGC	AGGCACGCAA	ATTACCCACT	CCTGACACGG	GGAG-TAGTG
Meoma vent	GGAAGGCAGC	AGGCGCGCAA	ATTACCCACT	CCCGACACGG	GGAGGTAGTG
Eucidaris	GGAAGGCAGC	AGGCGCGCAA	ATTACCCACT	CCCGACACGG	GGAGGTAGTG
Mespilia g	GGAAGGCAGC	AGGCGCGCAA	ATCACCCACT	CCCGACACGG	GGAGGTAGTG
	351	361	371	381	391
Leptosynap	ACGAAAAATA	ACGATCCAGG	CCTCTTTACG	AG??TCTGTA	ATCGGAATGA
Sclerodact	ACAAAAAATA	ACGATACAGG	CCTCTTC-GG	AG?CTCTGTA	ATCGGAATGA
Cucumaria	ACAAAAAATA	ACGATACAGG	CCTCTTC-GG	AGGCTCTGTG	ATCGGAATGA
Lipotrapez	ACAAAAAATA	ACGATACAGG	CCTCTTC-GG	AGGCTCTGTG	ATCGGAATGA
Stichopus	ACAAAAAATA	ACGATACAGG	CCTCTTC-GG	AGGCCCTGTG	ATCGGAATGA
Psychropot	ACAAAAAATA	ACGATACAGG	CCTCTTC-GG	AGGCCCTGTG	ATCGGAATGA
Meoma vent	ACGAAAAATA	ACAATACAGG	ACTCTTT-CG	AGGCCCTGTA	ATTGGAATGA
Eucidaris	ACGAAAAATA	ACAATACAGG	ACTCTTT-CG	AGGCCCTGTA	ATTGGAATGA
Mespilia g	ACGAAAAATA	ACAATACAGG	ACTCTTT-CG	AGGCCCTGTA	ATTGGAATGA
	401	411	421	431	441
Leptosynap	GTACACTTTA	AATCCTTTAA	CGAG?ACCTG	GTGCTCTTCA	CTGAGT?C??
Sclerodact	GTACACTTTA	AATCCTTTAA	CGAGGA?CTG	GTGCTCTTAA	CTGAGT-GCC
Cucumaria	GTACACTTTA	AATCCTTTAA	CGAGGATCTG	TTGCTCTTAA	CTGAGT-GCC
Lipotrapez	GTACACTTTA	AATCCTTTAA	CGAGGATCTG	GTGCTCTTAA	CTGAGT-GCC
Stichopus	GTACACTTTA	AATCCTTTAA	CGAGGATCTG	GTGCTCTTAA	CTGAGT-GCC

Psychropot	GTACACTTTA	AATCCTTTAA	CGAGGATCTG	GTGCTCTTAA	CTGAGT-GCC
Meoma vent	GTACACTTTA	AATCCTTTAA	CGAGGATCCG	GTGCTCTTAA	CTGAGT-GCC
Eucidaris	GTACACTTTA	AATCCTTTAA	CGAGGATCCG	GTGCTCTTAA	TTGAGT-GCC
Mespilia g	GTACACTTTA	AATCCTTTAA	CGAGGATCCG	GTGCCCTTGA	TTGGGTTGCC
	451	461	471	481	491
Leptosynap	CTTGCGGCCG	G?A??T?TA	CTTTGAGAAA	ATTGGAGTGT	T-CAAAGCAG
Sclerodact	GTGCGAGGC?	?AAA??TTTA	CTTTGAGAAA	ATTAGAGTGT	T-CAAAGCAG
Cucumaria	GTGCGAGGCT	GAAACGTTTA	CTTTGAGAAA	ATTGGAGTGT	T-CAAAGCAG
Lipotrapez	GTGCGAGGCT	GAAACGTTTA	CTTTGAGAAA	ATTAGAGTGT	T-CAAAGCAG
Stichopus	GTGCGAGACT	GGAACGTTTA	CTTTGAGAAA	ATTGAAGTGT	T-CAAAGCAG
Psychropot	GTGCGAGACT	GGAACGTTTA	CTTTGAGAAA	ATTGGAGTGT	TTCAAAGCAG
Meoma vent	AGGAGAGGCC	GGAACGTTTA	CTTTGAAAAA	ATTGGAGTGT	T-CAAAGCAG
Eucidaris	AGGAGGGGCC	GGAACGTTTA	CTTTGAAAAA	ATTGGAGTGT	T-CAAAGCAG
Mespilia g	AGGAGAGGCC	GGAACGTTTA	CTTTGAAAAA	ATTGGAGTGT	T-CAAAGCAG
	501	511	521	531	541
Leptosynap	GCACAACCGC	CTGAACAGCT	CAGCATGGAA	TAATGGAAGA	GGACCTCGG-
Sclerodact	GCCAAAGTGC	CCGAACAGCT	CAGCATAGAA	TAGTGGAAAA	GGACCTCGGC
Cucumaria	GCCAAAGTGC	CCGAACAGCT	CAGCATAGAA	TAGTGGAAAA	GGACCTCGGC
Lipotrapez	GCCAAAGTGC	CCGAACAGCT	CAGCATAGAA	TAGTGGAAAGA	GGACCTCGGC
Stichopus	-CCAAAGTGC	CCGAACAGCT	CAGCATGGAA	TAGTGGAAATA	GGACCTCGG-
Psychropot	GCCAAAGTGC	CCGAACAGCT	CAGCATGGAA	TAATGAAACA	GGACCTTGG-
Meoma vent	GCCTCGC-GC	CTGAACAGCA	GAGCATGGAA	TAATGGAATA	GGACCTCGG-
Eucidaris	GCCTCGC-GC	CTGAACAGTA	GAGCATGGAA	TAATGGAATA	GGACCTCGG-
Mespilia g	GCCTCGC-GC	CTGAACAGCA	GAGCATGGAA	TAATGGAATA	GGACCTTGG-
	551	561	571	581	591
Leptosynap	---GTCTATT	TCGTT?GTCT	CTAGA-CAAG	AGGTAATGAT	CAAAAGGGAC
Sclerodact	T?GTTCTATT	TCGCTGGTCT	CTGGAACCTG	AGGTAATGAT	CAAGAGGGAC
Cucumaria	TCGTTCTATT	TCGTTGGTCT	CTGGAACCTG	AGGTAATGAT	CAAGAGGGAC
Lipotrapez	TCGTTCTATT	TCGTTGGTCT	CTGGAACCTG	AGGTAATGAT	CAAGAGGGAC
Stichopus	---TTCTATT	TCGTTGGTCT	TGAGATCCTG	AGGTAATGAT	CAAGAGGGAC
Psychropot	---TTCTATT	TTGTTGGTCT	AAGGGACCGG	AGGTAATGAT	CAAGAGGGAC
Meoma vent	---TTCTATT	GCGTTGGTTT	TCGGAACCTG	AGGTAATGAT	TAAGAGGGAC
Eucidaris	---TTCTATT	GCGTTGGTTT	TCGGAACCTG	AGGTAATGAT	TAAGAGGGAC
Mespilia g	---TTCTATT	GCGTTGGTTT	TCGGAACCTG	AGGTAATGAT	TAAGAGGGAC
	601	611	621	631	641
Leptosynap	AGACGGGGGC	ATTCGTACTG	CGG-TGTGAG	AGGTGAAATT	CTTGATCGC
Sclerodact	TGCCGGGGGC	ATTCGTATTG	CGG-CGTGAG	AGGTGAAATT	CTTGATCGT
Cucumaria	TGCCGGGGGC	ATTCGTATTG	CGG-CGTGAG	AGGTGAAATT	CTTGACCGT
Lipotrapez	TGCCGGGGGC	ATTCGTATTG	CGG-CGTGAG	AGGTGAAATT	CTTGATCGT
Stichopus	-GTCCGGGGGC	ATTCGTATTG	CGG-CGTGAG	AGGTGAAATT	CTTGATCGT
Psychropot	TGCCGGGGGC	ATTCGTATTG	CGG-CGTGAG	AGGTGAAATT	CTTGATCGT
Meoma vent	TGACGGGGGC	ATTCGTATTG	CGG-TGTGA-	AGGTGAAATT	CTTGATCGC
Eucidaris	TGACGGGGGC	ATTCGTATTG	CGG-TGTGAG	AGGTGAAATT	CTTGATCGC
Mespilia g	TGACGGGGGC	ATTCGTATTG	CGGATGTGAG	AGGTGAAATT	CTTGATCGC
	651	661	671	681	691
Leptosynap	CGCAAGACGC	CCAAAAGCGA	AAGCATTTCG	CAAGAATGTC	TTCATTGATC
Sclerodact	CGCAAGACGC	CCGACAGCGA	AAGCATTTCG	CAAGAATGTC	TTCATTGATC
Cucumaria	CGCAAGACGC	CCGACAGCGA	AAGCATTTCG	CAAGAATGTC	TTCATTGATC
Lipotrapez	CGCAAGACGC	CCGACAGCGA	AAGCATTTCG	CAAGAAAGTC	TTCATTGATC
Stichopus	CGCAAGACGC	CCGACAGCGA	AAGCATTTCG	CAAGAATGTC	TTCATTGATC
Psychropot	CGCAAGACGC	CCTACAGCGA	AAGCATTTCG	CAAGAATGTC	TTCATTGATC
Meoma vent	CGCAAGACGA	CCGACTGCGA	AA??ATTTGC	CAAGAATGTT	TTCATTAATC

Eucidaris	CGCAAGACGA	CCGACTGCGA	AA?-ATTTGC	CAAGAATGTT	TTCATTAATC
Mespilia g	CGCAAGACGA	CCGACTGCGA	AA?-ATTTGC	CAAGAATGTT	TTCATTAATC
	701	711	721	731	741
Leptosynap	AAGAACGAAA	GTTGAGGGTT	CGAAGGCGAT	CAGATACCGC	?CTAGTCTTA
Sclerodact	AAGAACGAAA	GTCGGAGGAT	CGAAGGCGAT	CAGATACCGC	CCTAGTTCCG
Cucumaria	AAGAACGAAA	GTCGGAGGAT	CGAAGGCGAT	CAGATACCGC	CCTAGTTCCG
Lipotrapez	AAGAACGAAA	GTCGGAGGAT	CGAAGGCGAT	CAGATTCCGC	CCTAGTTCCG
Stichopus	AAGAACGAAA	GTCGGAGGAT	CGAAGGCGAT	CAGATACCGC	CCTAGTTCCG
Psychropot	AAGAACGAAA	GTCGGAGGAT	CGAAGGCGAT	CAGATACCGC	CCTAGTTCCG
Meoma vent	AAGAACGAAA	GTTAGAGGTT	CGAAGGCGAT	CAGATACCGC	CCTAGTTCTA
Eucidaris	AAGAACGAAA	GTTAGAGGTT	CGAAGGCGAT	CAGATACCGC	CCTAGTTCTA
Mespilia g	AAGAACGAAA	GTTAGAGGTT	CGAAGGCGAT	CAGATACCGC	CCTAGTTCTA
	751	761	771	781	791
Leptosynap	ACCATAAACG	ATGCCAACTT	GCC?ACCCGC	CGTAGTTCCT	CCCATGACAC
Sclerodact	ACCATAAACG	ATACCGACTC	GT?-ATTTCG	CGGCGTTCCT	?CCATGACGC
Cucumaria	ACCATAAACG	ATACCGACCC	GTA-ATTTCG	CGGCGTTCCT	CCCATGACGC
Lipotrapez	ACCATAAACG	ATACCGACCC	GCA-ATTTCG	CGGCGTTCCT	CCCATGACGC
Stichopus	ACCATAAACG	ATACCGACTC	GTA-ATTTCG	CGGCGTTCCT	CCCATGACGC
Psychropot	ACCATAAACG	ATACCAACTC	GCA-ATCCGC	CGGCGTTCCT	CCCATGACGC
Meoma vent	ACCATAAACG	ATGCCGACTG	ACG-ATCCGC	-----TACT	CCCATGACGC
Eucidaris	ACCATAAACG	ATGCCGACTG	ACG-ATCCGC	CGGCGTTACT	CCCATGACGC
Mespilia g	ACCATAAACG	ATGCCGACTG	ACG-ATCCGC	CGGCGTTACT	CC-ATGACGC
	801	811	821	831	841
Leptosynap	GGCG??GAGC	ACCC-GGGAA	ACCAAAGTGT	TTGGACTTCT	TAGAGGGACA
Sclerodact	GGCGGG??AC	TCTCCGGGAA	ACCAAAGTCT	TTGGACTTCA	TAGAGGGACA
Cucumaria	GGCGGGCAAC	TCTCCGGGAA	ACCAAAGTTT	TTGGACTTCA	TAGAGGGACA
Lipotrapez	GGCGGGCAAC	TCTCCGGGAA	ACCAAAGTCT	TTGGACTTCA	TAGAGGGACA
Stichopus	GGCGGGCAAC	TCTCCGGAAA	ACCAAAGTCT	TTGGACTTCT	TAGAGGGACA
Psychropot	GGCGGGCAGC	CCTC-GGGAA	ACCAAAGTCT	TTGGACTTCT	TAGAGGGACA
Meoma vent	GGCGGGCAG-	TCTAAGGGAA	ACCAAAGTCT	TTGGACTTCT	TAGAGGGACA
Eucidaris	GGCGGGCAG-	TCTGAGGGAA	ACCAAAGTCT	TTGGACTTCT	TAGAGGGACA
Mespilia g	GGCGGGCAG-	TCTGAGGGAA	ACCAAAGTCT	TTGGACTTCT	TAGAGGGACA
	851	861	871	881	891
Leptosynap	AGTGCGTTC?	AGCCACACGA	GATTGAGCAA	TAACAGGTCT	GTGATGCCCT
Sclerodact	AATGGCTTCC	AGCCATACGA	GATAGAGCAA	TAACAGGTCT	GTGATGCCCT
Cucumaria	AATGGCTTTC	AGCCATACGA	GATAGAGCAA	TAACAGGTCT	GTGATGCCCT
Lipotrapez	AATGGCTCTC	GGCCATACGA	GATAGAGCAA	TAACAGGTCT	GTGATGCCCT
Stichopus	AATGGCTTTC	AGCCATACGA	GATGGAGCAA	TAACAGGTCT	GTGATGCCCT
Psychropot	AATGGCTCTC	AGCCATACGA	AATAGAGCAA	TAACAGGTCT	GTGATGCCCT
Meoma vent	AGTGCGGTAT	AGCCACGCGA	GATTGAGCAA	TAACAGGTCT	GTGATGCCCT
Eucidaris	AGTGCGGTCT	AGCCACGCGA	GATTGAGCAA	TAACAGGTCT	GTGATGCCCT
Mespilia g	AGTGCGGTAT	AGC-ATGCGA	GATTGAGCAA	TAACAGGTCT	GTGATGCCCT
	901	911	921	931	941
Leptosynap	TAGATGTTTCG	GG?GC-GCAC	GCGCGCTACA	CTGAAGGAAT	CAGCGTGTGT
Sclerodact	TAGATGTCC-	GGGGC-GCAC	GCGCGCTACA	CTGGCGCAAG	CAGCGGGT?C
Cucumaria	TAGATGTCC-	GGGGCCGCAC	GCGCGCTACA	CTGGCGCAAG	CAGCGGGTAC
Lipotrapez	TATATGTCC-	GGGGCCGCAC	GCGCGCTACA	CTGGCGCAAG	CAGCGGGTAC
Stichopus	TAGATGTCC-	GGGGC-GCAC	GCGCGCTACA	CTGGCGCAAG	CAGCGGGTAC
Psychropot	TAGATGTCCC	GGGGCCGCAC	GCGCGCTACA	CTGGAGGAAG	CAGCGGGTAC
Meoma vent	TAGATGTTC-	GGGGCCGCAC	GCGCGCTACA	CTGGCGGAAT	CAGCGGGT-C
Eucidaris	TAGATGTTC-	GGGGCCGCAC	GCGCGCTACA	CTGGCGGAAT	CAGCGGGTAC
Mespilia g	TAGATGTTC-	GGGGCCGCAC	GCGCGCTACA	CTGGCGGAAT	CAGCGGGTAC

	951	961	971	981	991
Leptosynap	GTTC-CCCTG	GCGCGGAAGC	GTAGGG?AAT	CCGTTGAACC	TCCTTCGTGC
Sclerodact	?CTT-CCCTC	GGCCGACAGG	TCTGGGTGAT	CCGCTGAGAT	TCCGCCGTGC
Cucumaria	ATTTGCCCTC	GGCCGACAGG	TCTGGGTAAT	CCGCTGAGAT	TCCGCCGTGC
Lipotrapez	ACTCACCTC	GGCCGACAGG	TCTGGGTAAT	CCGCTGAGAT	TCCGCCGTGC
Stichopus	ATTT-CCCTC	GGCCGAAAGG	TCCGGGTAAT	CCGCTGAGAC	TTCTCCGTGC
Psychropot	ACTA-CCCTC	GGCCGATAGG	TCCGGGTAAT	CCGCTGAGAC	TTCTCCGTGA
Meoma vent	TTGT-TCCTT	GGCCGGAAGG	TCTGGGTAAT	CCGCTGAACC	TCCTCCGTGA
Eucidaris	ACTG-CCCTT	GGCCGGAAGG	TCTGGGTAAT	CCGCTGAACC	TCCTCCGTGA
Mespilia g	ACTG-CCCTT	GGCCGGAAGG	TCTGGGTAAT	CCGCTGAACC	TCCTCCGTGA

	1001	1011	1021	1031	1041
Leptosynap	TAGGGATTGG	GGCTTGTAAT	TCTTCCCAT	GAACGAGGAA	TTCCAGTAA
Sclerodact	TGGGGACAGG	GACTTGTAAT	TGTGTCCCTT	GAACGAGGAA	TTCC?AGTAA
Cucumaria	TGGGGACAGG	GACTTGTAAT	TGTGTCCCTT	GAACGAGGAA	TTCCAAGTAA
Lipotrapez	TGGGGACAGG	GACTTGTAAT	TGTGTCCCTT	GAACGAGGAA	TTCCAGTAA
Stichopus	TGGGGATAGG	GACTTGTAAT	TGTGTCCCTT	GAACGAGGAA	TTCCAGTAA
Psychropot	TGGGGACAGG	GACTTGTAAT	TATGTCCCTT	GAACGAGGAA	TTCCAAGTAA
Meoma vent	TGGGGATAGG	GAATTGCAAT	TATTTCCCTT	GAACGAGGAA	TTCCAGTAA
Eucidaris	TGGGGATAGG	GAATTGCAAT	TATTTCCCTT	GAACGAGGAA	TTCCAGTAA
Mespilia g	TGGGGATAGG	GAATTGCAAT	TATTTCCCTT	GAACGAGGAA	TTCCAGTAA

	1051	1061	1071
Leptosynap	GCGCGAGTCAT	CAGCTCGCG	TTGAT
Sclerodact	ACGCGAGTCAT	CAGCTCGCA	TTGAT
Cucumaria	ACGCGAGTCAT	CAGCTCGCA	TTGAT
Lipotrapez	ACGCGAGTCAT	CAGCTCGCA	TTGAT
Stichopus	ACGCGAGTCAT	CAGCTCGCA	TTGAT
Psychropot	ACGCGAGTCAT	CAGCTCGCA	TTGAT
Meoma vent	GCGCGAGTCAT	CAGCTCGCG	TTGAT
Eucidaris	GCGCGAGTCAT	CAGCTCGCG	TTGAT
Mespilia g	GCGCGAGTCAT	CAGCTCGCG	TTGAT