



Phylogeny of Holothuroidea (Echinodermata) inferred from morphology

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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, we report the first phylogeny of this class, based on a cladistic analysis of 47 morphological characters. We 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.

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INTRODUCTION

Holothuroids, or sea cucumbers, are an abundant and diverse group of marine invertebrates. The more than 1400 described and extant species constituting 160 genera (Smiley, 1994) occur in benthic environments from the intertidal to the deepest oceanic trenches, where they may constitute >90% of the biomass (Belyaev, 1972). Unique among echinoderms, holothuroids can be holopelagic (Miller & Pawson, 1990) and even ectocommensals (Martin, 1969). Most holothuroids are under 20 cm in length, although 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, Stoffell & Yoon, 1993). The ubiquity of holothuroids in the largest ecosystem, the abyssal plain, ostensibly 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, we 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 is 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 comprised all other holothuroids. Shortly thereafter,

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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 on the basis of 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 Elasiopodida (Hansen, 1975). Pawson & Fell (1965) raised one of MacBride's orders to subclass status, dividing it into two orders, Dendrochirotida and Dactylochirotida, on the basis of pronounced differences in tentacle and gross body characters. Pawson & Fell (1965) also considered the arrangement of the remaining orders, uniting Aspidochirotida and Elasiopodida as the Aspidochirotoacea and joining Apodida and Molpadiida as the Apodacea.

Pawson & Fell's (1965) primary motivation for their new classification was to have the Linnaean scheme better reflect the perceived close evolutionary relationship between dendrochirote holothuroids and the extinct edriasteroids. Fell (1965) and Fell & Moore (1966) proposed 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 & Fell's (1965) taxonomic scheme is transformed into a phylogeny in Figure 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 Dendrochirotae (=Dendrochirotoacea) and that the Aspidochirotae (=Aspidochirotida) and Elasiopoda (=Elasiopodida) are most closely related.

Ludwig (1891) argues and others (Gerould, 1896; Clark, 1898) agree that together the Aspidochirotida and Elasiopodida are probably sister to the remaining holothuroids (Fig. 1B). However, MacBride (1906), concurring with an earlier speculation by Théel (1886), writes that the Elasiopodida alone had diverged earliest (Fig. 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. 1D,E). Most recently, Littlewood *et al.* (1997) sequenced 28S- and 18S-like ribosomal genes of holothuroids from a total of four orders in an effort to resolve relationships between classes of Echinodermata. Smith's (1997) interpretation of these data (Fig. 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 1). The monophyly of some of these families is far from certain. For example, Synallactidae is a morphologically diverse and probably 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. When other information was lacking, we chose to use 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, we felt *a priori*, from clearly derived families. These were Pelagothuriidae, a group highly modified for a pelagic existence, Rhopalodinidae, which have their 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, we 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 Ophiocistoidea (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, displays several features in common with both holothuroids and ophiocistoids (Lehmann, 1958) that make it the strongest candidate as a stem member of Holothuroidea and an appropriate outgroup for this study. Like the ophiocistoid genus *Rotasaccus* Haude & Langenstrassen (Haude & 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 photograph in Frizzell & Exline, 1966, fig. 525; schematic of same in Smith, 1988, fig.

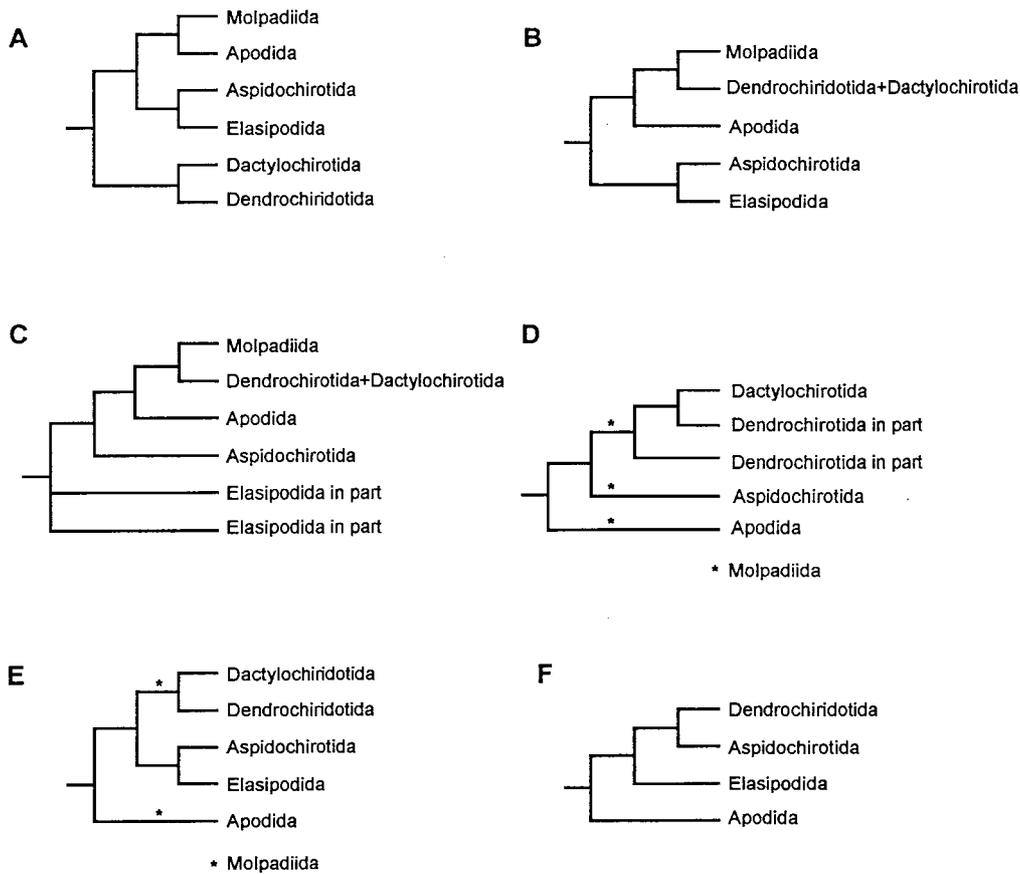


Figure 1. Phylogenetic hypotheses of holothuroid relationships. A, Pawson & Fell's (1965) Linnaean classification rendered as a phylogeny. B, Ludwig (1891). C, MacBride (1906). D, Semper (1868). E, Haude (1992). F, Interpretation by Smith (1997) of data from Littlewood *et al.* (1997). Taxon designations are from Pawson & Fell (1965) and exclude members of three families (Synallactidae, Eupyrgidae, Gephyrothuriidae) whose ordinal assignments differ between authors. Asterisks indicate alternative possible positions for Molpadiida.

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*, we used a hypothetical ancestor ('ancestor rooting') based on primitive states inferred from *Palaeocucumaria*, Ophiocystioidea, Palaeozoic 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, we 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 dendrochiroteaceans (first recorded from Upper Silurian), Elasipodida (Middle Devonian) and Apodida (Middle Devonian) (Gilliland, 1993). Hence, we 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

Table 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
Aspidochirotida Grube, 1840	Rhopalodiniidae R. Perrier, 1902	<i>Rhopalodina lageniformis</i> Gray, 1853
	Holothuriidae Ludwig, 1894	<i>Holothuria atra</i> Jäger, 1833
	Stichopodiidae Haeckel, 1896	<i>Stichopus chloronotus</i> Brandt, 1835
Elasipodida Théel, 1882	Synallactidae Ludwig, 1894	<i>Mesothuria verrilli</i> Théel, 1886
	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
Apodida Brandt, 1835	Elpidiidae Théel, 1879	<i>Elpidia glacialis</i> Théel, 1876
	Pelagothuriidae Ludwig, 1894	<i>Pelagothuria natatrix</i> Ludwig, 1894
	Chiridotidae Östergren, 1898	<i>Chiridota laevis</i> (Fabricius, 1780)
	Synaptidae Burmeister, 1837	<i>Synapta maculata</i> (Chamisso & Eysenhardt, 1821)
Molpadiida Haeckel, 1896	Myriotrochidae Théel, 1877	<i>Myriotrochus rinkii</i> Steenstrup, 1851
	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
Arthrochirotida Seilacher, 1961	Eupyrgidae Deichmann, 1940	<i>Eupyrgus scaber</i> Lütken, 1857
	No family designation	<i>Palaeocucumaria hunsrueckiana</i> Lehmann, 1958

following options: branch-and-bound search, multi-state taxa treated as uncertainties and zero-length branches collapsed. For each rooting and set of ingroup taxa, we 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. We 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 following 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 & Maddison, 1992).

STRATIGRAPHIC CONGRUENCE

To gauge the congruence between the resulting phylogeny and the fossil record, we 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 equalled or exceeded

Table 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	47
Ypsilothuriidae	10101	11111	20010	0101?	10000	01000	00001	01111	01100	00
Vaneyellidae	10101	11111	20010	0101?	10000	01000	00001	01?11	?1100	00
Rhopalodiniidae	10101	11111	10101	0101?	1a000	01000	00001	01?11	01100	00
Psolidae	11110	01111	20010	010c1	00110	01020	00001	01111	01110	01
Paracucumidae	11100	00010	20010	01011	10010	01020	00001	01?11	01110	01
Placothuriidae	11100	00010	20110	110c1	00010	01020	00001	01?11	01110	01
Heterothyonidae	11101	10010	20110	010c1	00110	01020	00001	01?11	01110	01
Phylloporidae	11000	00010	20110	10011	0a010	01020	00001	01111	01110	01
Sclerodactylidae	11000	00010	20110	00011	0a110	01020	00001	01111	11110	01
Cucumariidae	11000	00010	20010	00011	0a110	01020	00001	01111	01110	01
Holothuriidae	11010	00000	20001	10011	03000	01011	00011	11011	11010	00
Stichopodidae	11010	00000	20001	10011	03000	01011	01011	11011	11010	00
Synallactidae	11000	00000	20001	10011	0b000	01010	010?1	01111	01010	00
Molpadiidae	11001	00000	20110	1001?	02000	11001	00001	21111	11010	10
Caudinidae	11001	00000	20110	1000?	03000	01001	00001	21111	11010	10
Eupyrgidae	10101	00000	21000	10010	02000	11000	00001	01111	01010	00
Gephyrothuriidae	00000	00000	21000	1?000	00000	01000	01001	01?11	010?0	00
Synaptidae	00300	00000	21000	1011?	00000	00000	00000	00001	00001	10
Chiridotidae	00300	00000	21000	1010?	00000	00000	00000	00101	00001	10
Myriotrochidae	00300	00000	21000	1010?	00000	00000	00000	00101	00000	10
Elpidiidae	10210	02000	00000	?0200	00001	11110	11101	01100	010?0	10
Psychropotidae	11210	02200	10000	00000	00001	11010	01101	01200	010?0	00
Laetmogonidae	11210	02200	10001	00202	00000	11110	11001	01200	010?0	00
Deimatidae	11110	02000	10001	02010	00000	11110	11001	01200	010?0	00
Pelagothuriidae	11200	00000	0????	??000	00000	11010	01101	01200	010?0	01
<i>Palaeocucumaria</i>	00000	00000	20000	1000?	?????	???00	000??	?????	?????	?0
Ancestor	00000	00000	20000	10000	00000	10000	00000	00002	0?000	00

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 senior author (A.M.K.).

CHARACTERS

A total of 47 discrete post-larval characters, 15 skeletal, 31 soft-tissue and one behavioural, were scored, including 37 binary and 10 unordered, multistate characters (Table 2). Characters were taken primarily from published taxonomic and anatomical monographs (e.g. Clark, 1907; Hansen, 1975; Gilliland, 1993). In addition, we examined and dissected museum and field-collected specimens of at least one representative species from each extant family (Table 1) to confirm the published accounts. Specimens from Rhopalodiniidae,

Vaneyellidae and Placothuriidae, however, were unavailable for dissection because of their rarity. Hence, published accounts were entirely relied on to determine their morphology. In several poorly known families, characters were scored on the basis of several species.

GROSS MORPHOLOGY

1. Overall shape: 0 = vermiform; 1 = body convex, with central thickening. Vermiform is defined as having an uncontracted 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 dorsally convex, although a few very long species in *Holothuria* subgenera (e.g. *Acanthotrapeza* and *Mertensiothuria*) appear vermiform when fully extended. A vermiform body is assigned to Chiridotidae, although 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). Palaeozoic echinoderms most closely related to holothuroids, Ophiocistoidea and, possibly, bothriocidarids, as well as the most closely related Palaeozoic echinoids, e.g. *Eothuria* (Smith, 1988; Lewis & Donovan, 1998), are also all about this length. Length is more variable in some extant families of holothuroids. There are a few genera under 5 cm in Synaptidae and Molpadiidae. Therefore, diminutiveness in these families is here considered derived. Reports of very small species in Holothuriidae and Stichopodidae (e.g. Cherbonnier, 1988) are probably of juveniles and include 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 elasiopodan 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 elasiopodans 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 Elasiopodida 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 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, although at least one psolid, *Psolus phantapus* (Strussenfelt), can extend

and narrow its posterior greatly. A tail is recorded as present in Rhopalodinidae, although 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 here considered present despite its absence in one genus *Acaudina*.

6. *Neck: 0 = anterior extremely narrowed and elongated; 1 = anterior 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. However, when chemically relaxed, the species we have examined display subterminal mouths. The exemplar for Synallactidae, *Mesothuria*, is coded as having a subterminal mouth although several other genera in this probably 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*, is dorsal (Hansen, 1975). The anus is coded as terminal in *Palaeocucumaria* on the basis of 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 Dendrochirotaeaceae 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. *Dorsal interradius: 0 = very short; 1 = about equal in length to other interradii.* Pronounced foreshortening of the dorsal ('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, although it may be derived from elements of the echinoid lantern (Littlewood *et al.*, 1997, their character 52) or peribuccal ambulacral plates (Fell & 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 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 Elasiopodida 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-elasiopodan 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 taeniogyryne genera in Chiridotidae (Smirnov, 1998) and possibly the tiny myriotrochid *Paratrochus* (Gage & 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 *Nudicorona seilacheri* Haude, a somewhat younger fossil from the Middle Devonian (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, although 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 centre 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 dendrochirote genera *Loisettea* and *Leptopentacta*, the ossicles abut rather than overlap, even in quite contracted specimens. Second, the Middle Triassic *Strobilothyone rogenti* Smith & Gallemí, assigned to the dendrochirote family Heterothyonidae, has ossicles that imbricate in a manner not seen in extant dendrochirotes or any other holothuroid (Smith & Gallemí, 1991). 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 *S. rogenti*, however, has ossicles that overlap from mouth to anus (Smith & 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 artefact 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, Hansen & Huggett, 1985; Gebruk, 1990; Gebruk, Tyler & Billett, 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 Palaeozoic 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 & 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, multilayered structure and the ossicle is much thicker.

20. *Tubefoot endplate: 0=absent; 1=present.* Tubefoot endplates (podial sieveplates) occur at the terminus of suctorial tubefeet often used in locomotion. The endplates in dendrochiroteaceans 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, and 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 dactylochirotes, frequently have an eccentrically placed spire of three or 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. Dendrochirote 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 madreporite. Retention of a hydropore into adulthood is known for some probably paedomorphic species, e.g. the diminutive synaptid *Synaptula hydriformis* (Clark, 1898), but these are isolated instances and probably represent derived conditions. The existence of a madreporite is uncertain in *Palaeocucumaria*, but is coded as present in the hypothetical ancestor on the basis of its occurrence in ophiocistioids (Ubaghs, 1966).

27. *Longitudinal vessels: 0=absent; 1=present.* Mooi & 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 & 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. On the basis of their arguments, we 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, psychropotid and laetmogonid elasipodans probably 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 multiple 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 1–80 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 tube feet typical of echinoderms. Hence, one possibility is that a single *Palaeocucumaria* terminal tube foot is homologous to a single holothuroid tentacle stalk. Here the appropriate coding of *Palaeocucumaria* is for a simple, digitiform tentacle. Alternatively, perhaps the primitive tube foot gave rise to a tentacle digit and the entire ambulacral canal underlying the primitive tube feet is homologous with the tentacle stalk (David & 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 tube feet: 0=small or absent; 1=enlarged.* Elasipodan holothuroids in Laetmogonidae, Deimatidae and Elpidiidae possess a reduced number of greatly enlarged ventrolateral tube feet, which in the last 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 psychropotid elasipodan *Psychropotes*, this may consist of an elongate structure exceeding body length, while in *Benthodytes* and *Psychropedia* 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 is 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 on the basis of 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 aspidochirotans Holothuriidae and Stichopodidae and the molpadiidans Molpadiidae and Caudinidae are the only four families of holothuroids to develop an extensive plexus of dorsal haemal vessels, the *rete mirabile*. The coding of this character reflects the differences in the vascular networks between these holothuroid families. In the aspidochirotans, 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 aspidochirotans 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, Lafay & Christen, 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. We code haemal vessels as absent in the holothuroid ancestor on the basis of the argument presented for character 27, longitudinal vessels.

38. *Ovum diameter*: 0=<200 µm; 1=200–500 µm; 2=>500 µm. 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 µm. Most holothuroid ova are between 200 and 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, on the basis of 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 would otherwise insert (Lehmann, 1958).

40. *Attachment to body wall of mesentery engaging posterior intestinal loop*: 0=ventral; 1=dorsal; 2=transversal. 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 on the basis of 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 dendrochirotans. The outgroup is coded as unknown, although it is possible that the ancestral state of Palaeozoic 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 & 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 Palaeozoic 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 dactylochirotans and dendrochirotans. The outgroup, as *Palaeocucumaria*, is recorded as absent on the basis of 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

Table 3. Tree statistics of maximum-parsimony analyses

Rooting	Weighting	Eupyrgidae and Gephyrothuriidae included?	Tree length	Number of shortest trees	CI	RCI	RI	g_1
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

Apodida. The muscles are coded as absent in the outgroup on the basis of 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 along 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 & 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, although 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 deposit feed facultatively 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. 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 level (Hillis & 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 4–24 shortest trees (Fig. 2). Regardless of weighting scheme or included taxa, bootstrap values at each node were higher about twice as often under ancestor rooting, although only slightly (median = 3%, range = 1–11%).

Parsimony analyses that included all 25 families produced two different placements for the root (Fig. 2). For both weighting procedures, ancestor and *Palaeocucumaria* rootings split the tree between the apodan group (Myriotrochidae, (Synaptidae, Chiridotidae)) and the other holothuroids (Fig. 2A–C). In contrast, *Palaeocucumaria* rooted the tree immediately below the clade of elasipodan families (Fig. 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. 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. There were no effects on topology from excluding other combinations of taxa that were suspected as being derived *a priori*

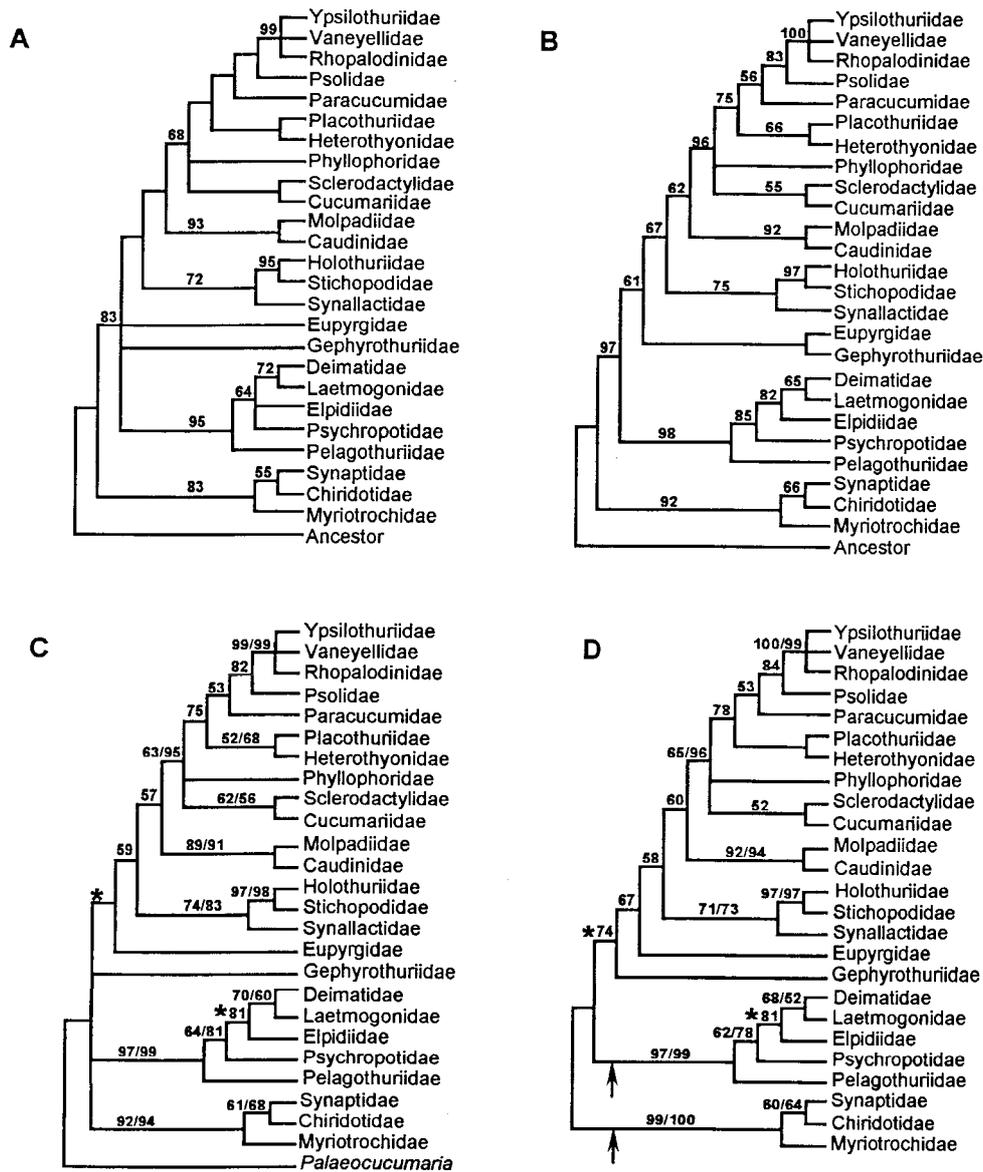


Figure 2. Most parsimonious trees for analyses of all taxa, weighting and rooting procedures. A, 50% majority rule consensus tree of 24 trees using equal weights plus ancestor rooting. B, 50% majority rule consensus tree of two most parsimonious trees for successive weights and ancestor rooting. C, 50% majority rule consensus tree of eight trees under equal and under successive weights, using the *Palaeocucumaria* outgroup each time. Asterisked node indicate unresolved node in equally weighted analyses. D, 50% majority rule consensus tree of two most parsimonious trees using successive weights and mid-point rooting; collapsing at the asterisked nodes generates the 50% majority rule consensus tree of 16 most parsimonious trees using equal weights and mid-point rooting. Arrows indicate possible positions of long-branch roots. Numbers above branches indicates bootstrap percentages separated by slashes are for equal and for successive weighted analyses; percentages less than 50% are not shown.

(data not shown). When Eupyrgidae and Gephyrothuriidae were excluded, all rooting-by-weighting schemes produced an identical strict consensus of shortest trees (Fig. 3), except for a trichotomy in the elasipodans (Fig. 3A). In addition, the equally weighted data, regardless of rooting method, produced

fewer most parsimonious trees (Table 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

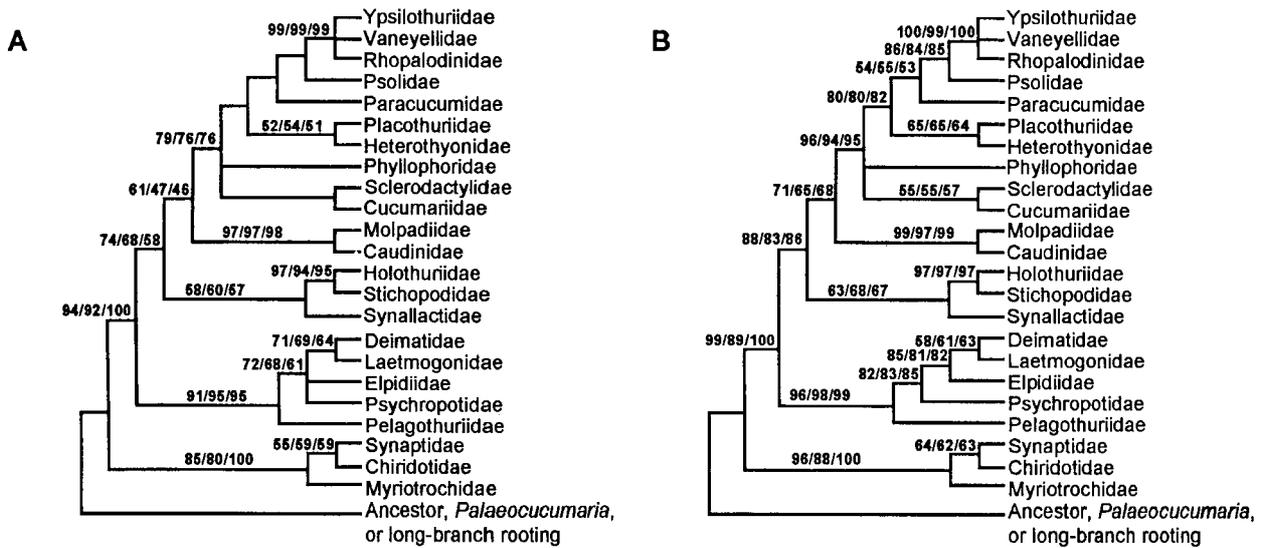


Figure 3. Single most parsimonious tree from all weighting and rooting procedures when Eupyrgidae and Gephyrothuriidae are removed. A, Bootstrap percentages for equally weighted analyses. B, Bootstrap percentages for successively weighted analyses. Bootstrap values separated by slashes are presented for ancestor, *Palaeocucumaria* and long-branch rootings and shown for a branch only when at least one rooting scheme provided it with percentage support of at least 50%.

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. 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 elasi-podan families were united by eight unambiguous changes. Within this clade, there was always strong support for the subdivision (Pelagothuriidae, remaining elasi-podans). The sister clade to Elasi-podida was united by five synapomorphies. Within this clade, the aspidochirotetes (Stichopodidae, Holothuriidae) received strong bootstrap support. The sister group to the aspidochirotetes (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

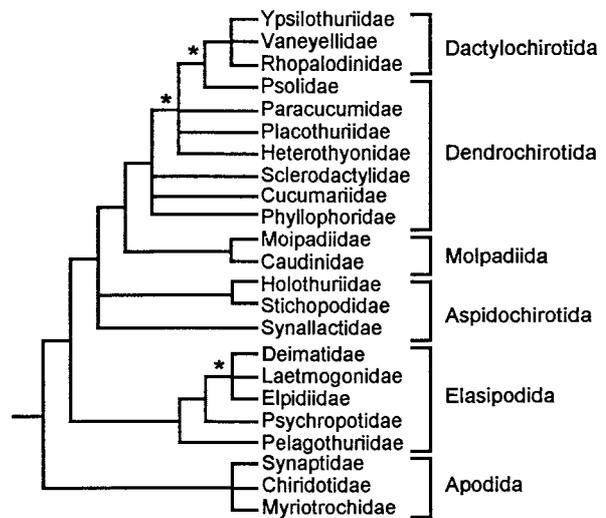


Figure 4. Best estimate of holothuroid relationships based on the successively weighted analyses excluding Eupyrgidae and Gephyrothuriidae and by collapsing all branches of the resulting single most parsimonious tree with less than 70% bootstrap support. Asterisks indicate additional branches with between 60% and 69% bootstrap support in all equally weighted analyses.

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' (1999) 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 Mya, the minimum possible ghost range is 360.9 Mya and the maximum is 1964.5 Mya.

DISCUSSION

TAXONOMIC IMPLICATIONS

The results of this study constitute the first cladistic test of the classification of Holothuroidea and differ significantly from the cladistic structure implied by the current taxonomic hierarchy (Fig. 1A). Pawson & 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 constitute the subclass Dendrochirotoacea. The Aspidochirotoacea 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, comprises the Apodida and Molpadiida, two groups that Pawson & Fell (1965) acknowledge may be only distantly related.

Our analyses did not corroborate the monophyly of the subclasses proposed by these authors. Dendrochirotoacea is composed in part of the probably paraphyletic group Dendrochirotida. Aspidochirotoacea, 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). 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 aspidochirotes or the molpadiidans (O'Loughlin, 1998). Eupyrgidae has been more often placed, although 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), currently make their placement problematic. As well, Dendrochirotida appears to be paraphyletic and consists of a soft-bodied grade and a testaceous clade. 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. 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 & Exline, 1966) on the basis of 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 last 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 organisation is original". 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 lacking tubefeet or papillae 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 & 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 lung-bearing and lungless forms, although 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, we 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 strong for only the successively weighted analyses.

In sum, the higher-level classification of Holothuroidea warrants a considerable revision. Some of Pawson & Fell's (1965) classification was corroborated by the present cladistic analyses. Differences, at the level of orders and subclasses, however, indicate that several groups as currently 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) to estimate lineage divergence times in Holothuroidea (Fig. 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 or 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 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 we 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, we included a record of Synallactidae ossicles, *Priscopedatus triassicus* Mostler, from the Middle Triassic that is considered tentative by Simms *et al.* (1993) and Gilliland (1993). The *P. triassicus* 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 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. 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 less than 20 (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 (Apodida), 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 one or two genera (Lewis & 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

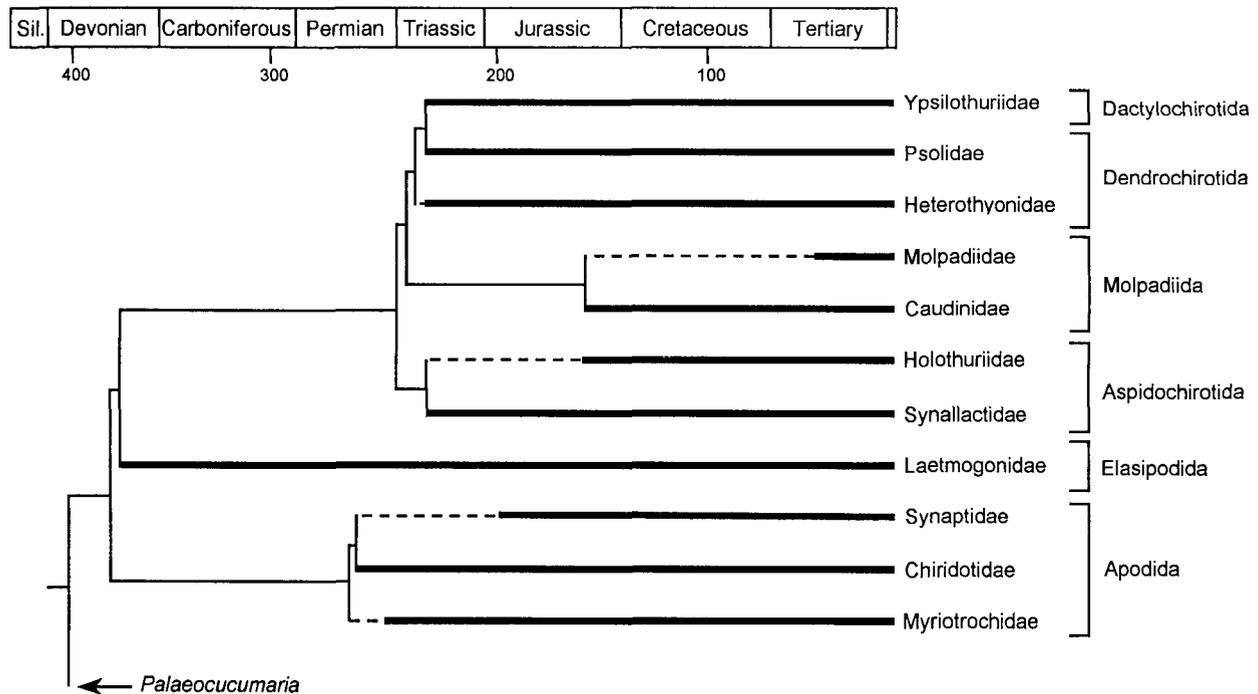


Figure 5. Phylogeny of Holothuroidea inferred from the morphological analyses (Fig. 4) and the stratigraphic record of the oldest fossil representatives for the included groups. Thin solid lines indicate phylogeny based on the present study; thick solid lines indicate range between the earliest and latest occurrences, fossil or extant; thin dashed lines indicate ghost range.

Table 4. Earliest stratigraphic occurrences of holothuroid taxa

Taxon	Fossil representative	Earliest record	Age (Mya)	Reference
Ypsilothuriidae	<i>Palaeopsilus liassicus</i>	L. Jurassic (Sinemurian)	201.9	Gilliland, 1992
Psolidae	<i>Monilipsolus mirabile</i>	M. Triassic (Ladinian)	234.3	Smith & Gallemí, 1991
Heterothyonidae	<i>Strobilothyone rogenti</i>	M. Triassic (Ladinian)	234.3	Smith & 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>Calclamnella elliptica</i>	U. Jurassic (Oxfordian)	159.4	Deflandre-Rigaud, 1946
Synallactidae	<i>Priscopedatus 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 & Exline, 1955
Myriotrochidae	<i>Theelia praeacuta</i>	U. Permian (Tatarian)	252.0	Mostler & Rahimi-Yazd, 1976
Outgroup	<i>Palaeocucumaria hunsrueckiana</i>	L. Devonian (Lochovian/Pragian)	396.3	Seilacher, 1961

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, Smith & Christen, 1995; Smith & 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 to be an artefact 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 Elaspodida by the Middle Devonian (Fig. 5). Fossil table ossicles (parafamily Priscopedatidae) occurring between this time and the Triassic and assigned to Dendrochirotida and Dactylochirotida (e.g. *Clavallus spicaudina*; Gutschick & 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 Palaeozoic 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.

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