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Ophiuroid phylogeny and higher taxonomy: morphological, molecular and palaeontological perspectives

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A cladistic analysis of the class Ophiuroidea at subfamily level is presented based on a morphological data base of 43 characters. The tree is rooted using the best known fossil member of the stem-group. This identifies Ophiocanopidae as sister group to other extant ophiuroids, which themselves fall into two major groups, the Ophiurida and the Euryalida. Although our most parsimonious solution is highly resolved, not all parts of the cladogram are well supported. Molecular data from the 5′ end of the large subunit ribosomal RNA molecule are presented for 10 taxa, with representatives from major higher groupings. This provides some support for the morphological tree, but leaves most of the deep branches unresolved. A phylogenetic tree is constructed for ophiuroids by selecting one of the cladograms as our working hypothesis and calibrating this against the stratigraphic record of the group. A revised taxonomy for the group is presented and the new taxon Ophiodermatina is erected.

ADDITIONAL KEY WORDS:–Ribosomal RNA – cladistics – morphology – systematics – Euryalina – Gnathophiurina – Chilophiurina – Phrynophiurina – fossil record.

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INTRODUCTION

Ophiuroids are one of the five extant classes of echinoderm, and comprise an important group of marine benthic invertebrates that have a fossil record extending back some 500 million years to the Early Ordovician. Ophiuroids are both diverse and widespread today, with some 250 described genera and 2000 species, and currently occupy marine habitats that range from intertidal reefs and estuaries to the abyssal plains, and from the poles to the tropics. Like their relatives the echinoids and asteroids, they possess a highly complex calcitic skeleton which can potentially provide a wealth of characters of phylogenetic importance. Yet, despite this, ophiuroids have remained a rather neglected group, and there has been no attempt to formulate a classification for the class using modern phylogenetic methods. As a result their evolutionary history remains only very sketchily known.

The current classification for ophiuroids stems from the work of Matsumoto (1917). In his monograph on the Japanese ophiuroid fauna, Matsumoto revised the classification of the entire class. Building on the extensive anatomical work on the internal skeletal structure of ophiuroids by Lyman (1882), Matsumoto reviewed and significantly added to our understanding of basic ophiuroid morphology. His observations led him to subdivide ophiuroids into two subclasses: Oegophiurida, for Palaeozoic forms with divided vertebrae, and Phrynophiurida for those with fused vertebrae. Phrynophiurida he further subdivided into four orders, the Phrynophiurina, Laemophiurina, Chilophiurina and Gnathophiurina. His classification of extant ophiuroids hinged on just a few key characters, namely the presence or absence of dorsal arm plates and the nature of the genital plate-genital bar articulation. Matsumoto then used additional characters and ontogenetic arguments to reconstruct an evolutionary tree of relationships for the group as a whole (Fig. 1A), which we have transformed into a cladogram of relationships (Fig. 1B).

Matsumoto's work has remained the only serious analysis of ophiuroid phylogeny for the class. However, it was not universally accepted: Mortensen (1927), for example, rejected Matsumoto's higher groupings, preferring to divide ophiuroids into two divisions, Euryalae (Euryalina) and Ophiurae (Ophiurina), based upon the structure of the vertebrae and the nature of the articulations between them. Since Matsumoto's treatise there have been relatively few contributions that have dealt with the question of ophiuroid phylogeny. First and foremost amongst these is Murakami's (1963) wide-ranging review of ophiuroid dental plates and mouth angle plates. This excellent comparative study identified a number of new and important characters for ophiuroid systematics. However, Murakami did not exploit his systematic observations to the full. Although he proposed a number of minor changes to Matsumoto's phylogenetic tree (Fig. 2), for the most part Murakami

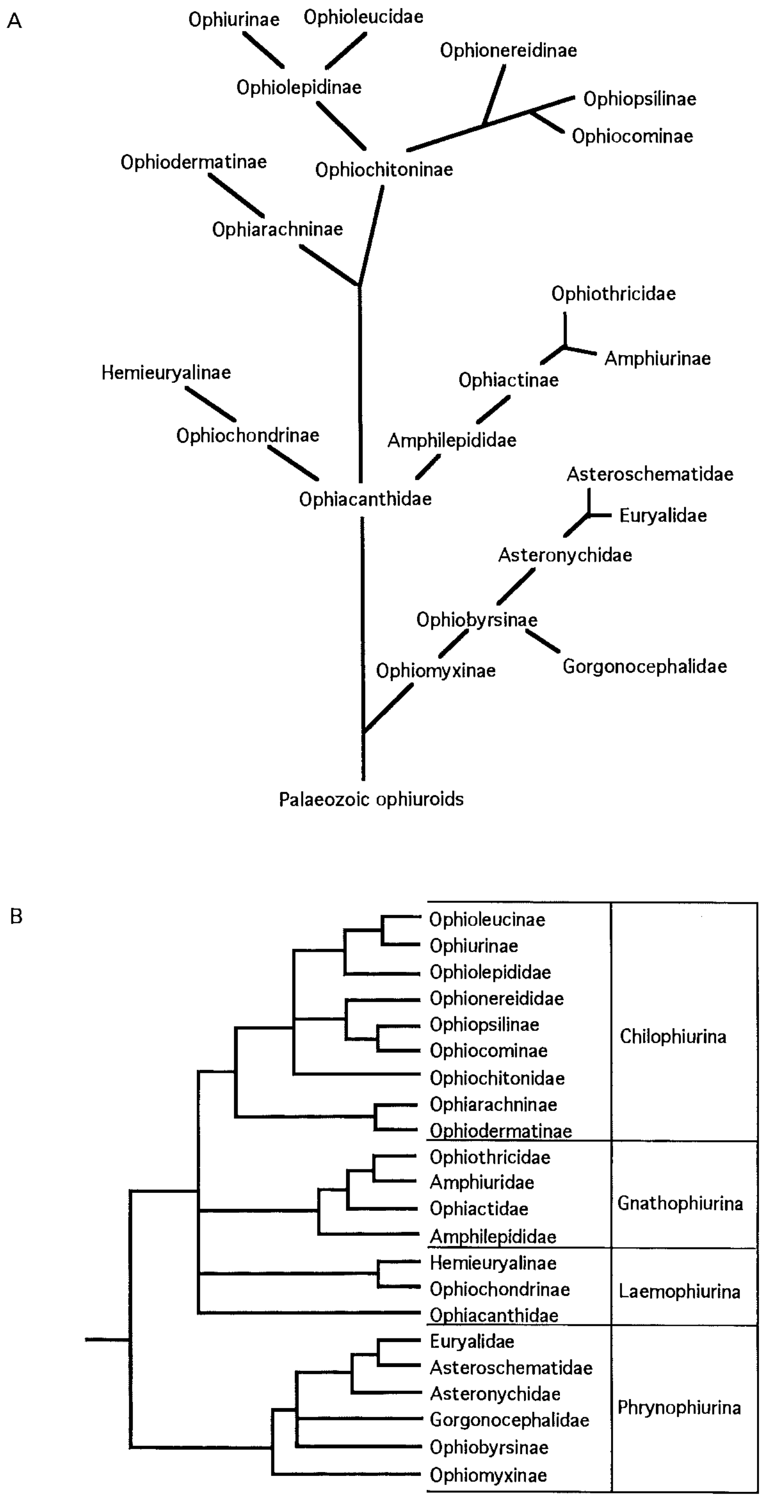

Figure 1. Matsumoto's (1917) classification of Ophiuroidea. A, An evolutionary tree, as derived from discussion and figures given on pp. 367–383. B, Matsumoto's proposed phylogeny transformed into a cladogram.

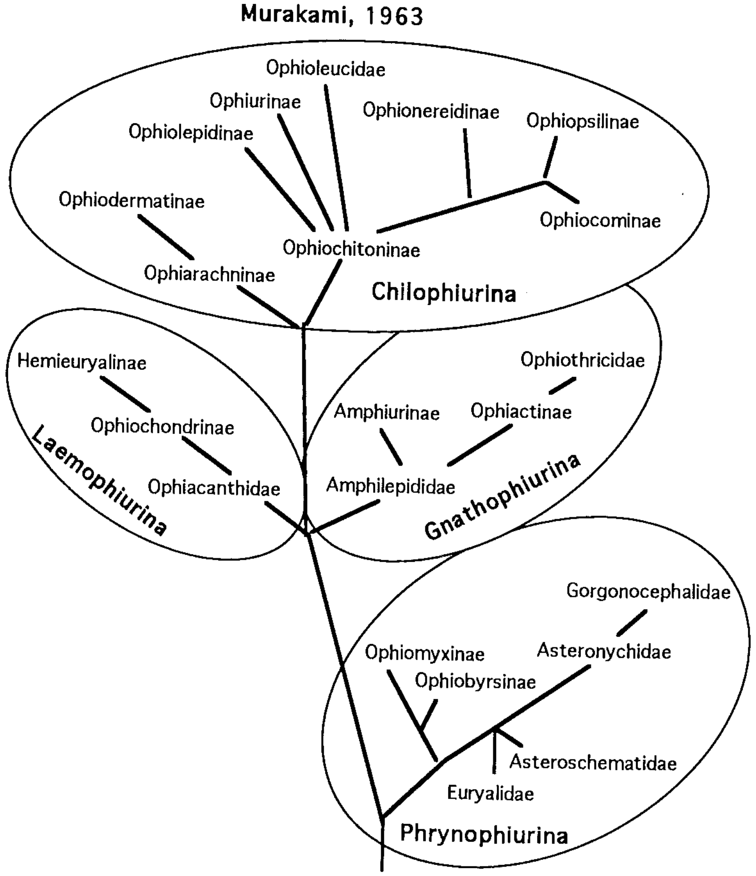

Figure 2. Murakami's (1963) classification of Ophiuroidea (modified from his diagram onp. 43).

simply fitted his observations into Matsumoto's existing scheme rather than specifically using his new data to test its veracity.

Fell (1960) published a synoptic key to the ophiuroids, drawing heavily from Matsumoto's work, but again with minor revisions. Most notably, he followed Mortensen in recognizing just two suborders of extant ophiuroids, the Euryalae for the families Gorgonocephalidae, Asteronychidae, Asteroschematidae and Euryalidae, and the Ophiurae for the remainder. Two years later Fell (1962, 1963) proposed that the genus *Ophiocanops* represented the only living member of the Oegophiurida and was, therefore, sister group to all other living ophiuroids. Furthermore, he concluded that Ophiomyxidae should be placed in their own suborder Ophiomyxina and placed, together with the Euryalina in the order Phrynophiurida. He elevated the Ophiurina to ordinal status. Fell's classification was followed by Spencer & Wright (1966), recognizing Matsumoto's three suborders (Chilophiurina, Laemophiurina and Gnathophiurina) as suborders within the Ophiuroida. Since then, although there has been considerable work on the morphology and structure of ophiuroids and on their species-and generic-level systematics, nothing further has been published on the higher group relationships of the class.

Recent technical advances in molecular biology have enabled gene sequence data to be collected rapidly and with little error, and this has lead to an explosion of phylogenetic studies based on the analysis of molecular sequences, particularly of ribosomal genes. Previous work has clearly demonstrated the strength of a combined morphological and molecular approach for understanding the phylogeny of both echinoids (Smith *et al.*, 1992; Littlewood & Smith, 1995) and asteroids (Lafay *et al.*, 1995). Adopting a combined morphological and molecular approach has enabled the phylogenetic relationships of these groups to be established on a much securer foundation. Given the previous success with other echinoderm classes, we began to compile a morphological and molecular data base for key ophiuroid taxa. As in previous studies, we sequenced some 400 bases from the 5′ end of the large subunit ribosomal RNA molecule.

METHODS AND MATERIAL

Morphological data

For the morphological analysis we constructed a data matrix for all described families and subfamilies of post-Palaeozoic ophiuroids, 27 in total (Appendix 1). All but one of these are extant (the exception being Aplocomidae Hess (1965), which is Triassic-early Jurassic in age). As outgroup we also included the best known of the Palaeozoic ophiuroids, the Carboniferous *Strataster ohioensis*. This taxon was described by Kesling & Le Vaseur (1971) on the basis of spectacularly well-preserved material which allows both external and internal skeletal characteristics to be scored. *Strataster* is an unambiguous member of the ophiuroid stem group, since it shows many ophiuroid autapomorphies yet has ambulacra composed of alternating and unfused biserial elements. The development of opposed and fused ambulacra is an autapomorphy for crown group ophiuroids.

A total of 43 morphological characters, all but two of which are skeletal, were eventually scored: 25 binary characters and 17 multistate characters, giving a total of 110 character states. These characters are listed in Appendix 1. Characters were drawn from published descriptions (particularly those of Lyman, 1882; Matsumoto, 1917; Mortensen, 1933; Fell, 1962, 1963; Murakami, 1963; Irimura, 1982; Paterson, 1985) and checked against specimens wherever doubt existed. In some poorly known taxa, such as the hemieuryalids, SEM observations were used to confirm morphological attributes. Nevertheless, some characters remain ambiguous, primarily because different members of the same family or subfamily show different states for the same character. In such cases we generally scored on the basis of the character displayed by the type genus of the subfamily or family unless otherwise noted in the appendix. Recent comparative anatomy reviews, such as that by Byrne (1994), have identified a number of soft-tissue characters which could potentially be phylogenetically informative, but, because these traits are as yet reported in only a relatively few scattered taxa, it has not been possible to include any of these in our analysis.

Molecular data

Partial sequences from the 5′ end of the large subunit ribosomal RNA molecule were obtained from ten ophiuroids, with representatives from the four major subdivisions recognized by Matsumoto (1917) (Table 1). RNA was isolated from fresh or ethanol-preserved tissue, homogenized with an ultra-turrax homogenizer in a 5 ml solution of guanidium thiocyanate (4 M), Tris HCl (50 mM, pH 7.6), EDTA (4 mM), N-lauryl-sarkozyl (2%), 2-mercaptoethanol (1%). Total RNA was separated from protein by phenol extraction, repeated three times and followed by two chloroform washes. Total RNA was then ethanol precipitated, suspended in sterile distilled water for measurement at 260/280 nm, reprecipitated with sodium acetate and ethanol, and finally resuspended in sterile water at $2\ \mu g.\mu l^{-1}$. Quality of the extracted RNA was examined by ethidium bromide staining of 1% agarose gels.

RNA sequencing was carried out using reverse transcriptase elongation of synthetic DNA primers in the presence of chain terminators (Qu *et al.*, 1983), with the subsequent modifications. DNA synthesis was carried out in two steps: the first step was the labelling step in which the primer was extended using limited concentrations of the deoxynucleotide triphosphates, including 35S-dATP; the second step was the chain-termination step using deoxynucleotides. Three synthetic primers complementary to conserved domains located in the 5′ end of the 28S ribosomal RNA were used (Baroin *et al.*, 1988).

Sequences were aligned by eye using the computer program VSM (Christen, 1993). There were 66 positions that varied amongst the ten taxa. Because of the high degree of sequence conservation, alignment could be done with almost no ambiguity. However, one small stretch of three bases respectively (positions 261–263 as listed in Appendix 2), representing a short region of compression, had many deletions and/or ambiguous (N) sites and consequently could be aligned in more than one way. Analyses were conducted with this region both included and omitted, but it was found that their inclusion/deletion made no difference to the resultant topology of the most parsimonious tree. The results presented below are based on the analysis of 63 alignable variant positions, excluding positions 261–263.

TABLE 1. Taxa for which large subunit ribosomal RNA partial sequences were obtained and their source. Classification follows that of Matsumoto (1917).

Suborder Gnathophiurina Matsumoto	
Family Ophiothricidae Ljungman	
Ophiothrix fragilis (Abildgaard)	Villefranche sur Mer, France
Family Amphiuridae Ljungman	
Amphipholis squamata (Delle Chiaje)	Villefranche sur Mer, France
Family Ophiactidae Matsumoto	
Ophiopholis aculeata Gray	Villefranche sur Mer, France
Suborder Chilophiurina Matsumoto	
Family Ophiodermatidae Ljungman	
Ophioderma longicauda (Retzius)	Villefranche sur Mer, France
Family Ophiuridae Lyman	
Subfamily Ophiurinae Lyman	
Ophiura ophiura (Linneaus)	La Coruña, Spain
Ophiura albida Forbes	Roscoff, France
Subfamily Ophiolepidinae Matsumoto	
Ophiomusium lymani Wyville Thomson	Atlantic coast of Senegal
Family Ophiochitonidae Matsumoto	
Ophiochiton tenuispinus Lyman	Rockall Trough, U.K.
Suborder Laemophiurina Matsumoto	
Family Ophiacanthidae Perrier	
Ophiacantha bidentata (Retzius)	Rockall Trough, U.K.
Suborder Phrynophiurida Matsumoto	
Family Asteronychidae Verrill	
Asteronyx loveni Muller & Troschel	Loch Horne, Scotland

Phylogenetic methods of analysis

Morphological and molecular data sets were analysed independently using the computer program PAUP version 3.1 (Swofford, 1993). In addition, for the nine genera for which molecular sequence data exist, a combined morphological and molecular data matrix was constructed. All characters were treated as unordered and given equal weight, and gaps in the sequence were treated as a fifth character state. The most parsimonious trees for the molecular and molecular plus morphological data sets were found using the exhaustive search option and tested by running 1000 bootstrap replicates. Only heuristic searches were feasible on the morphological data matrix because of its large size (28 taxa) and only 500 bootstrap replicates were made to test the robustness of the topology.

Tree length asymmetry was calculated using the 'Exhaustive Search' option in PAUP to calculate the g_1 statistic (see Hillis, 1991; Hillis & Huelsenbeck, 1992). A strong left-skewed distribution of tree lengths implies significant phylogenetic signal resides in the data.

Maximum-likelihood analysis was carried out using the program DNAML (Felsenstein, 1993). Base frequencies were derived directly from the sequences, and the transversion/transition ratio was set at 1:2. Searches were carried out using the global rearrangement option.

RESULTS

Morphological phylogeny

Full data

The morphological analysis rooted on *Strataster* identified five equally parsimonious solutions, which can be simplified to a single topology by collapsing one node to a polychotomy (Fig. 3A). Tree length is 151 steps, and trees have a consistency index (CI) of 0.44 (excluding uninformative characters) and a retention index (RI) of 0.70.

Our morphological data place Ophiocanopidae (*Ophiocanops*) as sister group to all other post-Palaeozoic ophiuroids as Fell (1962) previously suggested. It also implies that three of the four orders of Matsumoto (1917) are grades of organization, not clades, the exception being the Gnathophiurina if Amphilepididae are excluded. The euryalid groups (Euryalidae, Asteroschematidae, Asteronychidae and Gorgonocephalidae) form a well-defined clade separated from the remaining ophiuroids while Ophiomyxidae and Ophiacanthidae represent successive paraphyletic assemblages at the base of the Ophiurina. The Hemieuryalidae are identified as the sister group to the Gnathophiurina plus Chilophiurina.

Our analysis identifies three major extant chilophiurine-gnathophiurine

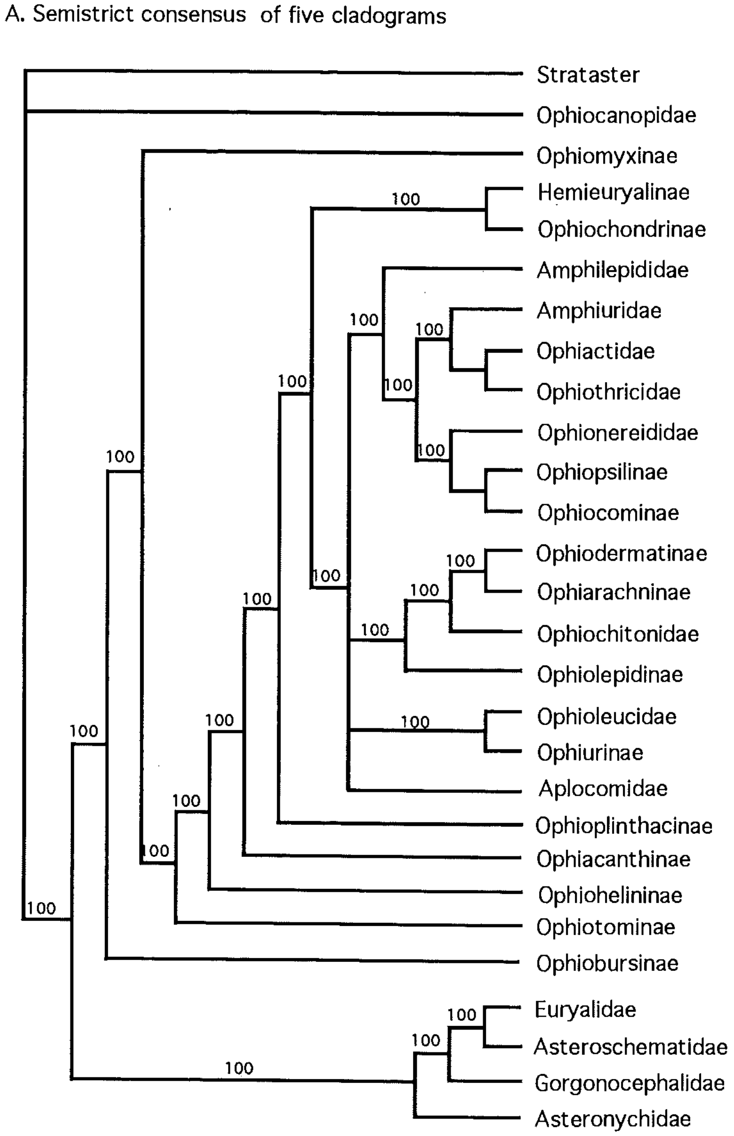
A. Semistrict consensus of five cladograms

Figure 3. Most parsimonious cladogram for extant family-subfamily taxa based on morphological data, as set out in Appendix 1. The cladograms are rooted on the Palaeozoic stem-group ophiuroid *Strataster*. A, Semistrict consensus of five equally parsimonious cladograms found when the fossil group Aplocomidae is included. Numbers on branches indicate the percentage of cladograms that branch appears in. B, single most parsimonious solution found when the Aplocomidae were omitted (also one of the five most parsimonious cladograms found when Aplocomidae were included). Bootstrap percentage support figures are indicated for each branch and are based on 500 replicate samples.

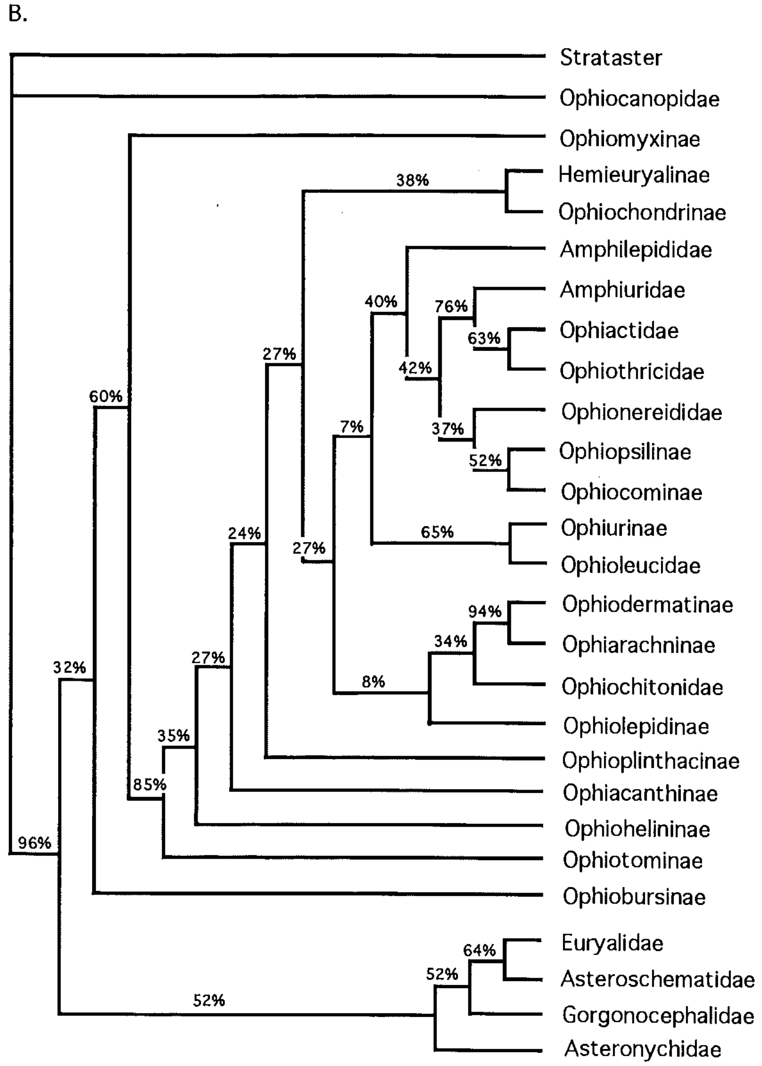
B.

Figure 3—*continued.*

groups: the Ophiurinae plus Ophioleucidae, a clade composed of Ophiochitonidae+Ophiodermatinae+Ophiarachninae and possibly also the Ophiolepidinae (though this is very weakly supported), a clade composed of gnathophiurines (Amphilepididae, Amphiuridae, Ophiactidae and Ophiothricidae) plus Ophiocomidae and Ophionereididae. The relationships between these three clades, however, cannot be resolved from our data. The extinct family Aplocomidae is also included as a fourth branch in this polychotomy.

Bootstrap support based on 500 replicates gave strong support for Ophiocanopidae as sister group to all other ophiuroids (96%), for pairing Ophiodermatinae and Ophiarachninae (94%), for the clade composed of Ophiotominae and its sister group (chilophiurines, gnathophiurines and other subfamilies of the Ophiacanthidae) (85%), and for the clade Amphiuridae+Ophiactidae+Ophiothricidae (76%). Weaker support was found for pairing Ophiurinae and Ophioleucidae (65%), Euryalidae and Asteroschematidae (64%), and Ophiactidae and Ophiothricidae (63%). Other branches were generally poorly supported, with the relative positions of Ophiolepidinae, Ophiuridae (Ophiurinae and Ophioleucidae), gnathophiurids and chilophiurids being particularly unstable (bootstrap support $<10\%$).

Analysis of extant families only

It is known that the addition of fossil taxa with many characters scored as unknown can significantly increase the number of equally parsimonious solutions that are found (Rowe, 1988; Smith 1994). In our analysis we included just one entirely extinct taxon, Aplocomidae, in our ingroup. Since analysis of the full data set generated a polychotomy which involved a fossil taxon, it is possible that cladogram instability at this node could stem from the large number of characters scored as unknown in the fossil family Aplocomidae. Consequently a second analysis was carried out which omitted Aplocomidae. This found a single most parsimonious tree (Fig. 3B), length 150 steps, CI (excluding uninformative characters) of 0.44 and a RI of 0.69. This tree coincides with one of the five equally most parsimonious trees found with Aplocomidae included, and represents our best estimate of the phylogeny of ophiuroid taxa based on morphology alone. This placed Ophiurinae plus Ophioleucidae as sister clade to the expanded Gnathophiurina clade. However, 500 bootstrap replicates gave little support for drawing any firm resolution about branching order in this part of the cladogram (Fig. 3B).

Taxa for which there is lsu rRNA data

A further analysis was carried out that included only the nine genera for which we have molecular sequence data. This was to ensure that we were not introducing erroneous trees by analysing only partial data in our total evidence analysis, This found two trees, length 62 steps, CI index 0.73 and RI index 0.72. The two trees differed in their positioning of *Ophiomusium lymani* (Ophiolepidinae), one tree placing it as sister group to the two species of *Ophiura* (Ophiurinae), the other placing it in a basal polychotomy. Given that both topologies are compatible, but the former more resolved than the latter, we take the former to be our best estimate. Rooting the trees on Asteronychidae (based on the results from the full analysis above) gave a similar topology to that using all extant taxa except that it placed *Ophiomusium* (Ophiolepidinae) as sister taxon to *Ophiura* (Ophiurinae), rather than as sister group to Ophiochitonidae plus Ophiodermatidae. The two trees can be summarized in a single tree with a trichotomy (Fig. 4A).

One thousand bootstrap replicates gave support for three branches at greater than 70% (the approximate lower limit Hillis & Bull (1993) found that corresponded to statistically significant branch lengths in simulation studies). Ophiactidae and Ophiothricidae were paired at the 82% level, the branch separating Amphiuridae, Ophiactidae and Ophiothricidae from other ophiuroids was supported at the 99% level, and the branch separating Asteronychidae and Ophiacanthidae from the other ophiuroids was supported at the 88% level. Ophiodermatidae and Ophiochitonidae are linked at the 62% level.

Tree length distribution (see Hillis & Huelsenbeck, 1992; Lafay *et al.*, 1995) was highly asymmetric when all nine taxa were included ($g_1 = -1.11$), and remained significantly skewed down to five taxa ($g_1 = -1.15$), so long as the *Ophiacantha–Asteronyx* pairing was included, suggesting that there was still significant phylogenetic signal in the data concerning at least this branch. The tree length asymmetry test is not applicable to fewer taxa, because the g_1 statistic cannot be estimated accurately below this number.

Molecular data

A parsimony analysis of the 63 variable sites found two equally most parsimonious trees (Fig. 4B, C), length 95 steps, CI index 0.70, RI index 0.70. The two trees differed in their positioning of *Ophiomusium* (Ophiolepidinae), one tree placing it as sister group to the two species of *Ophiura* (Ophiurinae), the other placing it in the basal polychotomy. Given that both topologies are compatible, but the former more resolved than the latter, we take the former to be our best estimate. Bootstrap support, based on 1000 replicates, was high for grouping the two species of *Ophiura* as sister taxa, and for pairing *Ophiopholis* (Ophiactidae) and *Ophiothrix* (Ophiothricidae) as sister taxa. Almost as strong support was found for placing *Amphipholis* (Amphiuridae) as sister group to *Ophiothrix*+ *Ophiopholis*. All other branches were only weakly supported (less than 50% bootstrap support).

Maximum-likelihood analysis of the molecular data produced an even less resolved topology (Fig. 4D). Only one branch, that uniting *Ophiothrix* (Ophiothricidae) and *Ophiopholis* (Ophiactidae), had a significant branch length ($P < 0.05$).

Applying the tree-length asymmetry test of Hillis & Huelsenbeck (1992) we found a highly asymmetric tree distribution for the complete data set ($g_1 = -1.08$), but removal of two of the taxa from the *Ophiothrix/Amphiura/Ophiopholis* cluster and one of the two *Ophiura* species resulted in nearly symmetric tree length distributions ($g_1 = -0.12$), suggesting little or no phylogenetic signal resides in the data concerning other branches.

Combined morphological and molecular data

The combined morphological and molecular data consisted of 106 characters for 10 taxa. Parsimony analysis of this combined data set found a single tree, length 154 steps, CI (excluding uninformative characters) 0.68, and RI 0.68 (Fig. 4E). This grouped the three gnathophiurines (*Amphiura*, *Ophiopholis* and *Ophiothrix*) as a clade, and the two species of *Ophiura* as a second clade with very high ($>95\%$) bootstrap support. The two *Ophiura* species were grouped with the *Amphiura* – *Ophiothrix* – *Ophiopholis* clade but at a much lower bootstrap support (44%), and *Ophiomusium* (Ophiolepidinae) was placed as sister group to both (41% bootstrap support). *Ophiochiton* and *Ophioderma* were paired, but again the level of bootstrap support was low.

DISCUSSION

Consensus or total evidence?

Morphological and molecular data give us independent estimates of ophiuroid phylogeny which can either be analysed separately or combined in a total evidence approach (Kluge & Wolf, 1993; Eernisse & Kluge, 1993; Larson, 1994). There is currently considerable discussion over the relative merits of these two approaches (Miyamoto & Cracraft, 1991; Kluge & Wolf, 1993; de Queiroz, 1993; Larson, 1994). In the case of ophiuroids we have a morphological data set that provides a resolved phylogenetic hypothesis, though with strong support for only a proportion of branches. The molecular data we have cover many fewer taxa and also gives a resolved tree in which only a proportion of branches are well supported. There is a strong signal from both sets of data about certain groups (e.g. the pairing of Ophiothricidae and Ophiactidae and their sister group relationship to Amphiuridae), and consequently we can have a high degree of confidence in our results for areas of the morphological and molecular cladograms that are in agreement. However, parsimony analysis of morphological and molecular data from the same taxa generate noncongruent topologies (Fig. 4A, D) and yet another topology is generated from our analysis of the two data sets combined (Fig. 4E). However, the conflict concerns branches that are weakly supported in 1000 bootstrap replicates, and suggests that we simply do not have enough data as yet to resolve some of these relationships conclusively.

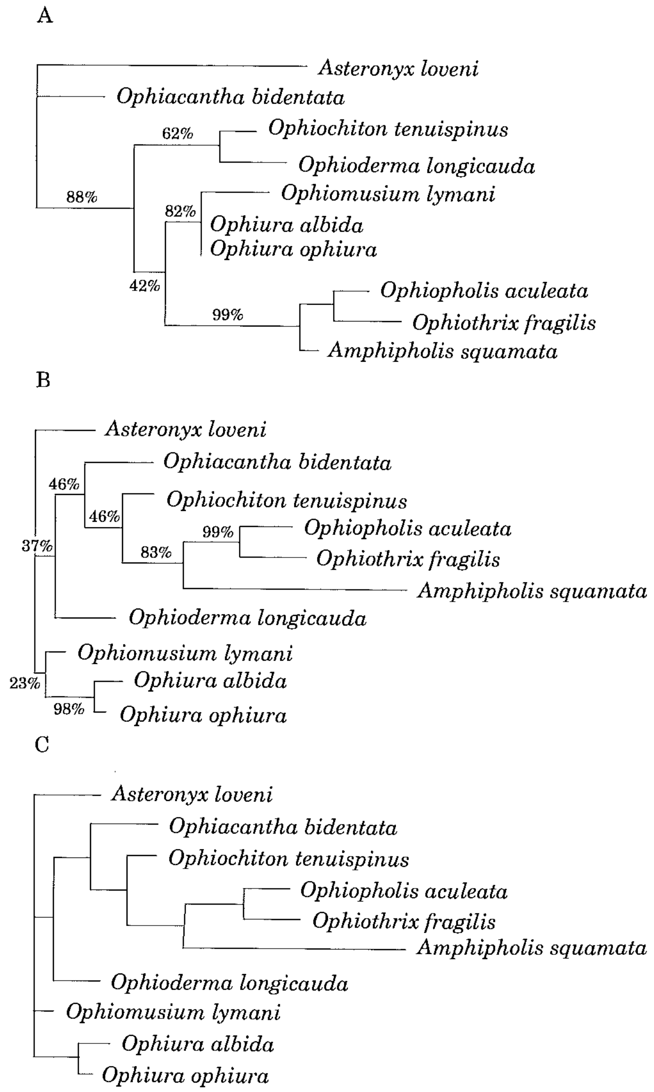

Figure 4. Most parsimonious cladograms generated for taxa for which we have molecular sequence data. A, Morphological data. B, C, Two equally parsimonious trees derived from the sequence data, as detailed in Appendix 2. D, Maximum-likelihood tree derived from the molecular sequence data. E, Most parsimonious tree derived from combined data (morphological and molecular data). Branch lengths are proportional to the number of characters supporting that branch. Bootstrap support values (based on 1000 replicates) are indicated as percentages.

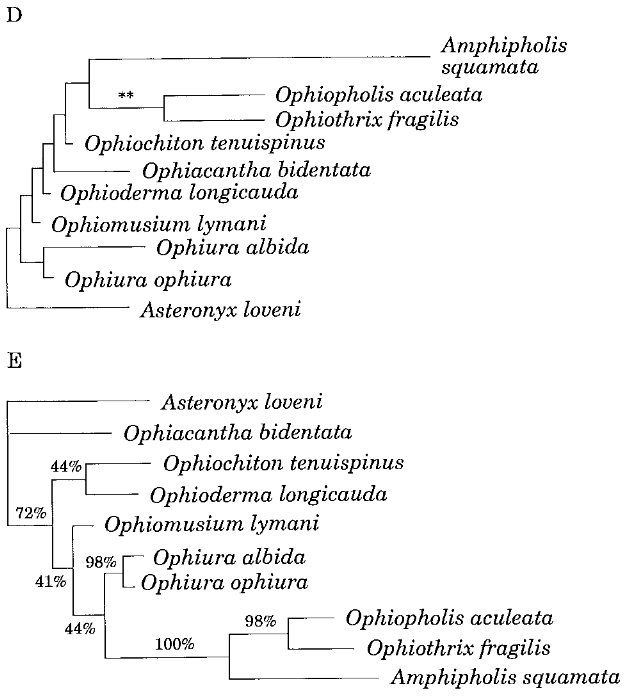

Figure 4—*continued.*

There seems to be virtually no remaining signal in our molecular data concerning deeper branches in the cladogram to judge from the tree length asymmetry test. The tree length asymmetry statistic (g_1) for the molecular data (Hillis, 1991; Hillis & Huelsenbeck, 1992) drops to nearly zero after removal of one sister group in each node supported by more than 80% of bootstraps, suggesting that there is effectively little or no phylogenetic signal resident beyond those pairings. At least part of this problem arises from the small size of our database. Although there are 63 variable sites, only 26 of these are phylogenetically informative, the rest being apomorphic for single taxa. The same molecular domain gave a fully resolved phylogeny for echinoids concordant with the morphological data (Smith *et al.*, 1992) but could not resolve basal relationships amongst asteroid orders (Lafay *et al.*, in press).

A robust phylogeny of ophiuroids is clearly difficult to obtain, even when morphological and molecular data are combined. The basic problem probably arises because the tree we are trying to estimate is composed of long terminal branches and short internal branches, an arrangement known to cause problems for phylogenetic methods of analysis (e.g. Felsenstein, 1978; DeBry, 1992). Nevertheless, this in itself tells us something about the evolution of the group, since it suggests that many of the extant families sampled radiated relatively rapidly early on in the history of the clade. As will be discussed later, additional support for this comes from an analysis of the fossil record of ophiuroids.

Fossils in phylogenetic analysis

As often happens, the addition of incompletely characterized taxa, such as the extinct family Aplocomidae, to a phylogenetic analysis has a detrimental effect, increasing the number of equally parsimonious solutions found and causing nodes in the vicinity of that taxon to collapse. However, one major advantage of including fossil taxa in the morphological data set is that it gives a much more extensive sampling of character associations than is currently available from the crown group. Increased sampling improves the chances of being able to differentiate homology from homoplasy (Kluge & Wolf, 1993) and thus derive the correct tree. Furthermore, fossil taxa from the stem group can be used for rooting purposes, allowing substantially more characters to be scored and polarized by outgroup comparison than would be possible using one of the other, highly divergent, extant classes of echinoderm as sister group. For example, only more conserved parts of the ophiuroid sequence we have obtained can be aligned to those of other echinoderm classes. Consequently more than half of the variant sites and the majority of phylogenetically informative sites in Appendix 2 cannot be polarized using the outgroup method. Our rooting of both morphological and molecular trees is therefore based solely on the results obtained from the full morphological data set.

Ophiuroid phylogeny calibrated against the fossil record

We take the cladogram derived from morphological data (Fig. 3A) as our working hypothesis because of its much more extensive coverage of taxa. Rooted on the Palaeozoic stem group fossil *Strataster* this identifies the Ophiocanopidae as sister group to all other ophiuroids. The Euryalina are identified as a monophyletic clade, within which Asteronychidae are placed as sister group to the other three families. Euryalina are sister group to all other ophiuroids except Ophiocanopidae, as has long been recognized. At the base of the Ophiurina comes a series of taxa that have previously been classified in the families Ophiomyxidae and Ophiacanthidae. Our analysis suggests that both of these families are paraphyletic. Hemieuryalidae turn out to be the most derived of the Laegophiurina and sister group to the clade composed of Chilophiurina plus Gnathophiurina.

Relationships amongst Chilophiurina/Gnathophiurina are the least certain. Ophiodermatinae and Ophiarachninae undoubtedly group together (with more than 95% bootstrap support for this pairing), and the Ophiochitonidae represents their sister group. The pairing of Ophiochitonidae and Ophiodermatidae gains some support from our molecular analysis as well as from our combined analysis. Both the Ophiothricidae-Ophiactidae pairing and the Amphiuridae-Ophiactidae+Ophiothricidae pairings are strongly supported from both morphological and molecular data. Morphological data alone give good support for a group composed of Ophiocominae plus Ophiopsilinae and Ophionereididae as the sister group to the gnathophiurine clade. Finally Amphilepididae are identified as sister group to both clades. Although neither molecular nor morphological data can give a firm solution to how these three supra-familial clades are related, in combination they lend weak support to the view that Ophiuridae are sister group to the expanded gnathophiurine clade, and that these together have as their sister group the chilophiurids (Ophiodermatidae, Ophiochitonidae, etc).

The preferred cladogram can be transformed into a phylogenetic tree through estimating the times of branch origination from the fossil record (Fig. 5). Unfortunately the fossil record of ophiuroids is still very poorly known, because ophiuroids are rarely preserved and, even when preserved, rarely display key internal morphological characters that would allow them to be classified with confidence into higher taxa. However, sufficient ophiuroids have been described in detail from the fossil record to make a preliminary attempt. Earliest confirmed records of families in the geological record are listed in Table 2. The Aplocomidae, which in the full morphological analysis, arose from the basal polychotomy of the chilophiurine/gnathophiurine clade, is taken as a putative plesiomorphic ancestral group to these taxa as it stratigraphically predates them. The arrangement of taxa within the chilophiurine/gnathophiurine clade is taken from the single most parsimonious tree found when Aplocomidae were excluded from the analysis.

The resulting tree suggests that ophiuroids underwent a major diversification in the Triassic-early Jurassic like other echinoderm classes. Our cladistic analysis suggests that many of the extant families originated in the early part of the Jurassic. The absence of definitive Euryaline taxa in the earlier part of the Mesozoic is, however, puzzling, since our analysis suggests that they should have a much longer fossil record. No Jurassic or Triassic member has yet been confidently identified, despite the rather characteristic vertebral morphology.

Taxonomic conclusions and recommendations

Our analysis of morphological data clearly shows that previous classifications have been based on grades, not clades, and the higher taxonomy of ophiuroids is in need of complete revision. However, bootstrap analysis of our most parsimonious cladogram (Fig. 3B) shows that only parts of it are securely founded (i.e. can be shown to have a significant number of supporting characters). Consequently we feel it would be rash to erect a full cladistic classification at present. Instead we outline a number of relationships where we feel there is good support from either morphological or molecular data and make the following taxonomic recommendations:

(1) There is strong support from the morphological data for placing Ophiocanopidae as sister taxon to all other extant ophiuroids, as Fell (1962,

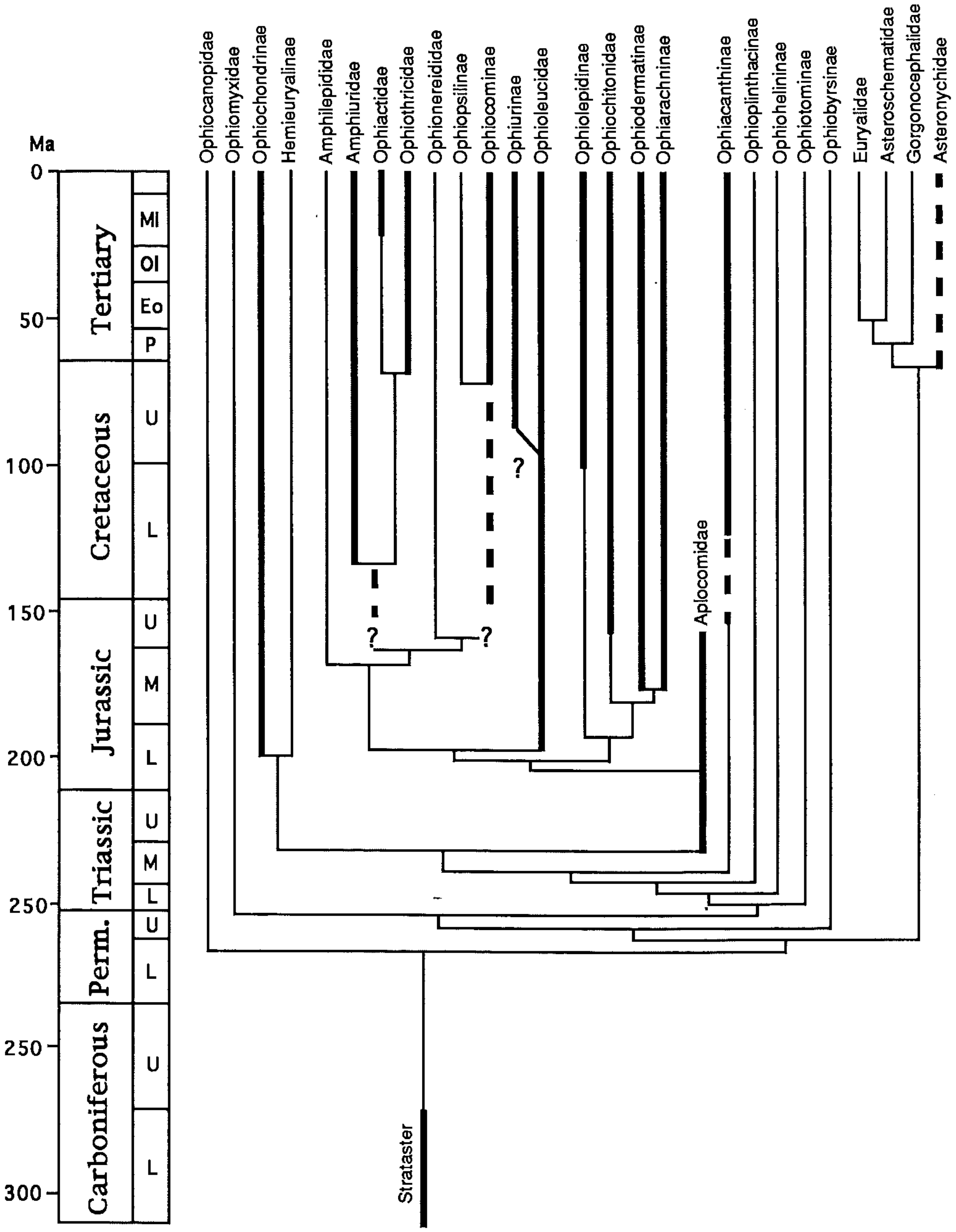

Figure 5. Inferred phylogenetic tree for Ophiuroidea, derived by calibrating the cladogram depicted in Fig. 3b against the stratigraphic record of first occurrences (Table 2). broad solid line = firmly established record; broad dashed line = possible range extension based on specimens tentatively assigned to the clade; thin solid line = relationships implied from phylogenetic analysis (including range extensions and nominal ghost lineages).

1963) suggested. Fell placed Ophiocanopidae as the only extant member of the order Oegophiurida Matsumoto, 1915. We consequently recommend raising Oegophiurida to subclass status (as Oegophiuridea) and include all

TABLE 2. Earliest reliable stratigraphical records for ophiuroid families

Family	Basis of record	Age	Reference
Asteronychidae	Isolated vertebral ossicles only; assignable only to Euryalina	late Cretaceous (Maastrichtian)	Rasmussen (1950, 1972)
Gorgonocephalidae	mention only	Tertiary (Oligocene)	Fell, in Spencer & Wright (1966)
Hemieuryalidae	'?*Hemieuryale*' (new genus of Ophiochondrinae)	early Jurassic (Pliensbachian)	Hess (1975); BMNH collections
Ophiacanthidae	?(Lateral arm plates only)	early Jurassic (Pliensbachian)	Hess (1964)
	Ophiopinna	middle Jurassic (Callovian)	Spencer & Wright (1966)
Aplocomidae	*Aplocoma*	middle Triassic (Ladinian)	Hess (1991)
Ophiolepidinae	*Ophiomusium*	early Jurassic (Pliensbachian)	Hess (1965)
	Ophiotitanos	late Cretaceous (Cenomanian)	Hess (1960); Jagt (1990)
Ophioleucidae	*Sinosura* (tentatively placed here)	early Jurassic (Pliensbachian)	Hess (1975)
Ophiurinae	*Felderophiura*	late Cretaceous (Maastrichtian)	Jagt (1991)
Ophiodermatinae	*Palaeocoma*	early Jurassic (Pleinsbachian)	Hess (1964)
	Ophiopeza	middle Jurassic (Bathonian)	Hess (1970)
Ophiarachninae	?*Dermocoma*	middle Jurassic (Bathonian)	Hess (1964)
Ophiochitonidae	*Ophiochiton*	late Jurassic (Oxfordian)	Hess (1964)
Ophiocomidae	?*Ophiocoma*	late Jurassic (Oxfordian)	Hess (1964)
	Ophiocoma	late Cretaceous (Cenomanian)	Hess (1960)
Ophiothricidae	mention only	late Cretaceous (Maastrichtian)	Jagt (1991)
Ophiactidae	?*Ophiopholis* (treated as a gnathostomate record only)	late Jurassic (Oxfordian)	Hess (1975)
	Ophiopholis	Tertiary (Miocene)	Ishida (1991)
Amphiuridae	*Xanthamphiura*	early Cretaceous (Hauterivian)	Hess (1970)

Table 3. Recommended classificatory scheme for ophiuroids

- Class Ophiuroidea Gray, 1840
  - Subclass Oegophiuridea Matsumoto, 1915
    - Family Ophiocanopidae Mortensen, 1932
  - Subclass Ophiuridea Gray, 1840
    - Subfamily Ophiobyrsinae Matsumoto, 1915 (*incertae sedis*)
    - Order Euryalida Lamarck, 1816
      - Family Asteronychidae Müller & Troschel, 1840
      - Family Gorgonocephalidae Ljungman, 1867
      - Family Asteroschematidae Verrill, 1899
      - Family Euryalidae Gray, 1840
    - Order Ophiurida Müller & Troschel, 1840
      - Suborder Ophiomyxina Fell, 1962
        - Family Ophiomyxidae Ljungman, 1867
      - Suborder Ophiurina Müller & Troschel, 1840
        - [Family Ophiacanthidae *s.l.* Perrier, 1891 -paraphyletic complex]
        - Infraorder Hemieuryalina Verrill, 1899 (*sedis mutabilis*)
          - Family Hemieuryalidae Verrill, 1899
        - Infraorder Chilophiurina Matsumoto, 1915
          - Family Ophiuridae Lyman, 1865
            - Subfamily Ophiurinae Lyman, 1865
            - Subfamily Ophioleucinae Matsumoto, 1915
        - Infraorder Gnathophiurina Matsumoto, 1915
          - Superfamily Amphilepididae Matsumoto, 1915
          - Superfamily Gnathophiuridea Matsumoto, 1915
            - Family Amphiuridae Ljungman, 1867
            - Family Ophiothricidae Ljungman, 1867
            - Family Ophiactidae Matsumoto, 1915
          - Superfamily Ophiocomidea Ljungman, 1867
            - Family Ophionereididae Ljungman, 1867
            - Family Ophiocomidae Ljungman, 1867
              - Subfamily Ophiocominae Ljungman, 1867
              - Subfamily Ophiopsilinae Matsnmoto, 1915
        - Infraorder Ophiodermatina nov.
          - Family Ophiochitonidae Matsumoto, 1915
          - Family Ophiodermatidae Ljungman, 1867
            - Subfamily Ophiodermatinae Ljungman, 1867
            - Subfamily Ophiarachninae Matsumoto, 1915
        - Infraorder Ophiolepidina Ljungman, 1867
          - Family Ophiolepididae Ljungman, 1867

other extant ophiuroids, as their sister group, in the subclass Ophiuroidea Gray, 1840.

(2) Ophiuroidea fall into two distinct groups: the Euryalina, comprising Asteronychidae, Asteroschematidae, Euryalidae and Gorgonocephalidae, and the Ophiurina, comprising Ophiomyxinae and all the remaining families. Mortensen (1927) and Fell (1960) recognized precisely these two groups at ordinal level, and we recommend that the Euryalida Lamarck, 1816, and Ophiurida Müller & Troschel, 1840, be adopted as orders within the Ophiuroidea. The taxonomic position of Ophiobyrsinae Matsumoto, 1915 requires more investigation. Although our most parsimonious cladograms place Ophiobyrsinae within the clade Ophiurida rather than the clade Euryalida, the evidence for this is weak. Consequently we prefer to leave Ophiobyrsinae as *incertae sedis* at this level.

(3) Morphological evidence strongly supports Ophiomyxinae as sister group to the remaining Ophiurida (excluding Ophiobyrsinae). Fell recognized the distinction of ophiomyxids by placing them in their own suborder Ophiomyxina Fell, 1962. We accept this taxon and rank and place all other Ophiurida in the suborder Ophiurina Müller & Troschel, 1840 (a grouping recognized at ordinal level by Fell, 1962 and Spencer & Wright, 1966).

(4) The basal members of the Ophiurina belong to the family Ophiacanthidae Perrier, 1891. Although our analysis resolved relationships within this complex, none of the branches is well supported and we feel it best not to erect any formal classification for this part of the tree until further work on the group has been done.

(5) There are five crownward clades within the Ophiurina that seem reasonably well substantiated. These are (i) the Hemieuryalidae, comprising the two subfamilies Ophiochondrinae and Hemieuryalinae; (ii) Ophiurinae and Ophioleucidae; (iii) an expanded gnathophiurine clade composed of Amphilepididae, Amphiuridae, Ophiactidae, Ophiothricidae, Ophiocomidae and Ophionereididae; (iv) Ophiodermatidae and Ophiochitonidae; and (v) Ophiolepidinae. However, the detailed relationships amongst these five groups are very unstable and morphological and molecular data fail to resolve their branching order. Since all five have authenticated fossil records extending back to the Jurassic, they probably represent lineages that arose close together during the early Mesozoic diversification of Ophiurida. We recommend that each be recognized at the rank of infraorder and treated as *sedis mutabilis* within any classification (see Wiley, 1979; 1981 for conventions). The five infraorders are, respectively, Hemieuryalina Verrill, 1899 (emend.), Chilophiurina Matsumoto, 1915 (emend.), Gnathophiurina Matsumoto, 1915 (emend.) Ophiodermatina (nov.) and Ophiolepidinina Ljungman, 1867.

(6) Within Gnathophiurina there is a strongly supported grouping of Amphiuridae, Ophiactidae and Ophiothricidae and a second, more weakly supported grouping of Ophiocomidae and Ophionereididae. We recommend that each be recognized at superfamily level as Gnathophiuridea Matsumoto, 1915 (emend.) and Ophiocomidea Ljungman, 1867 (emend.).

(7) The Ophiuridae, as currently constructed, is not a natural group. One of the more important apomorphies linking Ophioleucidae to Ophiurinae is the presence of large leaflike genital scales. Ophiolepidinae, previously treated as a subfamily of Ophiuridae, is removed and elevated to the rank of family.

Our revised taxonomic hierarchy is outlined in Table 3.

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APPENDIX 1

Morphological characters of ophiuroids used for the phylogenetic analysis.

1. *Radial shields: 0 = absent; 1 = present.*

Radial shields are absent in the Carboniferous ophiuroid *Strataster* (Kesling & Vasseur, 1971, pl. 5), *Ophiocanops* (Ophiocanopidae: Fell,1963), *Ophiomyces* (the type genus of the Ophiohelininae), and in *Ophiosyzygus* and *Ophioleptoplax*, both of which were put in the Ophiomyxinae by Matsumoto (1917). They are present in all other ophiuroids, though are small and rudimentary in other Ophiomyxinae, and in Ophiotominae and Ophiobyrsinae according to Matsumoto (1917) and Irimura (1982).

2. *Radial shield shape: 0 = undifferentiated or rudimentary and oval or scale-like, widely separated; 1 = long, narrow, massive and bar-like, always separated and extending towards the centre of the disk; 2 = stout, broad and basically triangular in shape* (Fig. A1).

The radial shields are small, spatulate plates in a few taxa, notably in Ophiomyxinae, Ophiobyrsinae and in *Ophiotoma.* There are characteristically long parallel bars that give euryalid disks a ribbed appearance. Similar-shaped radial shields are found in some Ophiacanthinae such as *Ophiacantha* and *Ophiolebes. Ophionereis* (Ophionereididae) also has long bar-like radial shields, as do the Ophiopsilinae.

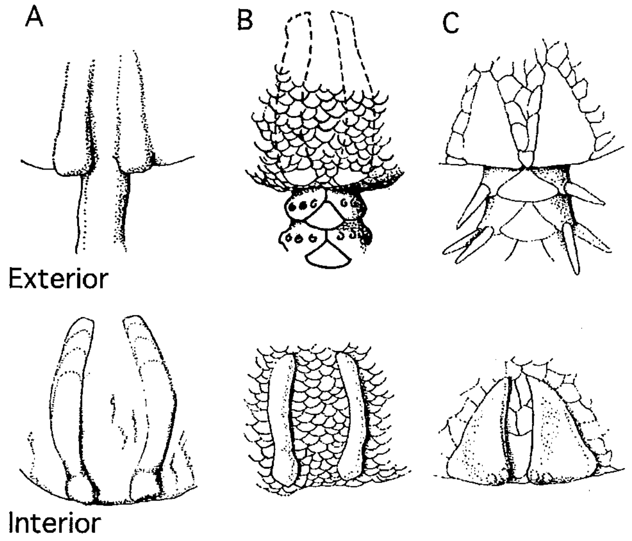

Figure A1. Disk plating in external and internal views (characters 2, 3). A, Radial shields long, bar-like and covered in thick integument (*Asteronyx*: Asteronychidae). B, Radial shields long, bar-like with distal ends exposed (*Ophiacantha*: Ophiacanthidae). C, Radial shields broad, triangular, forming an integral part of the dorsal plating (*Ophiochiton*: Ophiochitonidae).

In most other families the radial shields are broader, stout plates. In a few families such as Ophiothricidae and Ophiactidae the radial shields are extremely large and triangular, occupying much of the dorsal surface, whereas in Ophiocomidae, for example, the plates are small and only slightly widened. In the latter case we treat these as narrow triangular plates rather than modified rods. The relative development of radial shields is variable within otherwise well-defined families and we have not used this as a character.

3. *Radial shields: 0 = basically subdermal and covered in a thick skin or disk plates; 1 = almost entirely superficial and exposed* (Fig. A1).

In the euryalid families, Ophiobyrsinae and Ophiotominae the radial shields lie wholly internal and are covered by a thick skin. In Ophiomyxinae and Ophiacanthinae the radial shields are exposed proximally but most of their surface lies beneath the disk scales. In all other groups the radial shields form an integral part of the disk armature, although they may sometimes be obscured by superficial granulation.

4. *Articulation surface between the radial and genital plates (from Matsumura, 1917): 0 = a simple face or transverse ridge developed on both plates; 1 = an articular pit on the radial shield and a ball-like condyle on the genital plate; 2 = two articular condyles and one pit on both plates* (Fig. A2).

Simple articular surfaces are found on the plates of Ophiomyxinae and Ophiobyrsinae and on some ophiacanthids (presumably those with simple or rudimentary radial shields). All euryalid families have articulatory ridges on both plates, and the same is true for Ophiacanthinae. The articular pit on the radial shield and the ball-like condyle on the genital plate is found in species of Amphiuridae, Ophiactidae and Ophiothricidae, while the double articular condyle is found in Ophiurinae, Ophioleucidae, Ophiodermatinae, Ophiarachninae, Ophichitonidae, Ophinereididae and Ophiocomidae. *Hemieuryala* has a transverse ridge on its radial shield. The state in Ophioplinthacinae needs confirmation, however, Matsumoto dissected *Ophiothamnus* and stated that all Ophiacanthidae had simple articulation surfaces or transverse ridges. Although Matsumoto (1917) claimed that Amphilepididae had the ball and socket condyle articulation, he based this on his dissection of *Amphiactis*, which he placed in that family. Fell (1960) removed *Amphiactis* as a synonym of *Ophiactis*, leaving only *Amphilepis* and *Ophiochytra* in the family. Subsequently Clark (1970) showed *Ophiochytra* to be an Ophiurinae. Thus only the genus *Amphilepis* is currently placed in the family Amphilepididae. The structure of its radial and genital plate articulation is unknown.

5. *Disk plating: 0 = no differentiation between disk and arms - aboral surface composed of a thick connective tissue layer, with or without armament, which continues along arm without differentiation; 1 = aboral plating distinct from arm plating.*

The aboral integument in euryalids *Ophiocanops* and ophiomyxids is undifferentiated between arms and disk. In *Hemieuryala* the disk plating of the proximal part of the arms is undifferentiated from the disk and comprises numerous flat scales. However, in all other genera assigned to this family there are distinct dorsal arm plates and a clear differentiation between disk and arm. Hemieuryalinae are, however, scored as having undifferentiated disk plating on the basis of the type species. In all other families there is a sharp differentiation between the edge of the disk and the start of the arms.

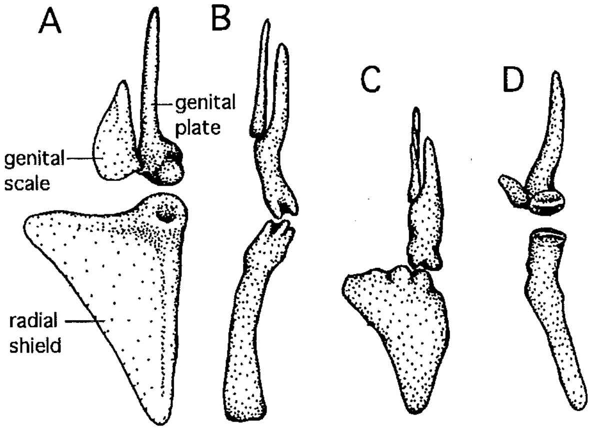

Figure A2. Articulation between radial shield and genital plate (character 4), redrawn from Matsumoto (1917). A, Ball and socket articulation in *Ophiothrix* (Ophiothricidae). B, Pit and two condyle articulation in *Ophiopsila* (Ophiopsilinae): note the bar-like radial shield. C, Pit and two condyle articulation in *Ophiarachnella* (Ophiodermatidae). D, Simple articulation bar in *Asteroschema* (Asteroschematidae).

6. *Disk plating or integument: 0 = without spines or granules; 1 = covered in fine spinelets through which the underlying plating is more or less visible; 2 = covered in fine, dense granulation that obscures the underlying plates; 3 = granules embedded in skin without plates* (Fig. A3).

The following families have predominantly spinule- and granule-free disks: Hemieuryalidae, Ophiohelininae, Amphiuridae (but scattered spinules in two included genera *Amphiacantha* and *Ophiocnida*), Ophiochitonidae, Ophionereididae, Ophiurinae and Ophiolepidinae. In *Ophiobyrsa*, the Ophiacanthinae, Ophioplinthacinae, Ophiotominae, Ophiactidae, Ophiothricidae and some Ophiocomidae (e.g. *Ophiomastix*) the disk is covered in minute plates, many of which bear small spinelets. The spinelets may be very dense in some species, but usually the covering does not obscure the underlying plating. The same is true for Gorgonocephalidae, but here the plates also cover the radial shields. In *Asteroceras* (Euryalidae) the genital plates often have a single large clump of spines. In Ophiodermatinae, Ophiochondrinae, Ophioleucidae, Aplocomidae, most Ophiocomidae (including *Ophiocoma* itself) and Ophiarachninae all disk plates are covered in an extremely dense granulation that totally obscures the underlying plating. The same is true of *Ophioplax*, a member of the Ophiochitonidae, but *Ophiochiton* itself lacks granules, and is scored accordingly. The situation in Ophiomyxinae, Gorgonocephalidae and Asteroschematidae is that the disk has a dense granule-embedded skin, but no plates. Asteronyxidae have minute platelets in their disk integument but only as juveniles according to Matsumoto (1917), while Euryalidae appear to be spicule-free.

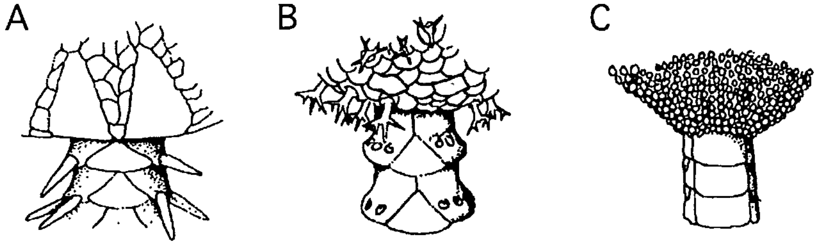

Figure A3. Disk granulation (character 3). A, Disk lacking spinules or granulation (*Ophiochiton*: Ophiochitonidae). B, Disk with scattered spinules but with disk plating clearly visible beneath (*Ophiacantha*: Ophiacanthidae). C, Disk covered in dense granulation obscuring underlying plating (*Pectinura*: Ophiodermatidae).

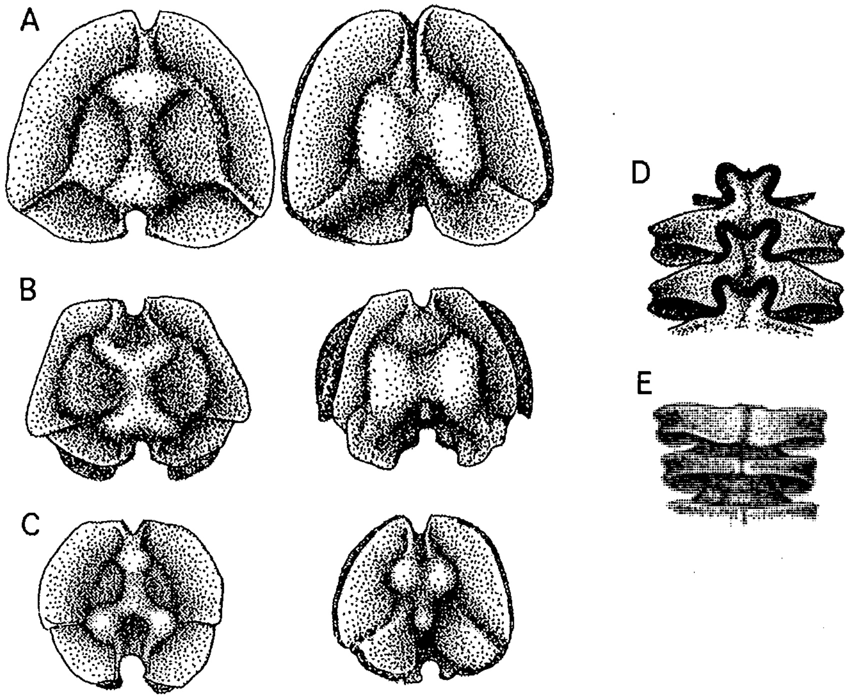

Figure A4. Vertebral articulation surfaces (characters 8 and 11), redrawn from Lyman (1882). A–C, Proximal and distal faces of a single vertebra. A, Streptospondyline articulation, *Sigsbeia* (Hemieuryalinae). B, Intermediate articulation form (state 1, character 8), *Ophiochondrus* (Ophiochondrinae). C, Zygospondyline articulation, *Ophiolepis* (Ophiolepididae). D–E, Dorsal view of two vertebrae within the disk: D, Vertebrae interlocking, *Ophiocnemis* (Ophiothricidae); E, Vertebrae simple, *Ophiolepis* (Ophiolepididae).

7. *Vertebrae: 0 = unfused and alternate; 1 = united and opposed proximally, unfused distally; 2 = united and opposed throughout.*

Only the Palaeozoic ophiuroids have unfused vertebrae. The vertebrae of Ophiocanopidae, however, are unfused distally. In all other ophiuroid groups the vertebrae are solidly fused together throughout, though in ophiomyxinids the distal vertebrae may be partially fused only.

8. *Vertebral articulation: 0 = streptospondylous; 1 = with small articulatory peg but large streptospondyline-type facet; 2 = typical zygospondylous peg and articulation facet* (Fig. A4, A–C).

Streptospondylous articulation is found in *Ophiocanops*, Ophiobyrsinae, Hemieuryalinae and the euryalids, whereas zygospondylous articulation is found in most of the remaining ophiuroids, except for Ophiacanthidae. A few taxa are difficult to place: Ophiochondrinae show streptospondyline articulation, but have in addition a small adoral articulatory peg characteristic of zygospondylous forms. Lyman (1882) describes the vertebrae of *Ophiomyxa australis* as very similar to *Ophiochondra*. Ophiohelininae have a peculiar atriculation facet that is neither streptospondyline nor zygospondyline (Lyman, 1882, pl. 44, figs 8, 9). The Ophiacanthidae are predominantly zygospondylous, although one species, *O. bidentata* reported by Matsumoto (1917, pl. 3, figs 2,3), is apparently streptospondylous. Furthermore *Ophiolebes* (Ophiacanthinae) and some Ophioplinthacinae (*Ophiocamax*) Lyman (1882) and Matsumoto (1917) state that the vertebrae are streptospondylous, wheras in Ophiotominae (*Ophiolimna* and *Ophiologimus*) and some Ophioplinthacinae (*Ophiothamnus*, *Ophiomitra* and *Microphiura*) the vertebrae are zygospondylous.

9. *Vertebral line of fusion: 0 = solid and imperforate; 1 = a single elongate pore; 2 = a multiple row of pores.*

A single elongate pore is characteristic of the Ophiomyxinae only. Taxa with a row of pores include Ophiotominae, some Ophiplinthacinae (*Ophiothamnus* and *Microphiura*), Ophiactidae (*Ophiactis*, *Amphiactis*) and Ophiarachninae (*Ophiuroconis*, *Ophiurodon*). *Astrophiura*, an aberrant Ophiurinae also has multiple rows of pores but the Ophiurinae otherwise have solid vertebrae and are scored as such. All other groups apparently have solid vertebrae with an imperforate suture line.

10. *Vertebrae: 0 = with distinct ventral cleft; 1 = with this cleft closed over.*

This form of vertebral structure is unique to Euryalidae.

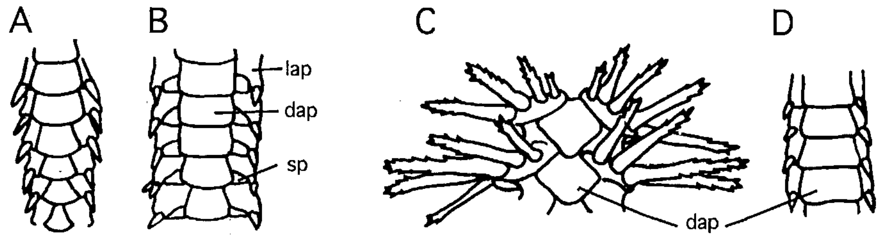

Figure A5. Arm plating in dorsal view (characters 13, 15). A, Arm with simple series of dorsal plates (*Ophiura*: Ophiurinae). B, Arm with supplemental plates (*Ophiolepis*: Opiolepididae): dap = dorsal arm plate, lap = lateral arm plate, sp = supplementary plate. C, Arm spines long and erect (*Ophiothrix*: Ophiothricidae). D, Arm spines short and adpressed (*Bathypectinura*: Ophiodermatidae).

11. *In dorsal aspect vertebrae: 0 = not indented and imbricated; 1 = indented and imbricated* (Fig. A4, D & E).

Vertebrae in Ophiothricidae and in Ophionereididae have a very typical appearance with projections from the anterior vertebrum indenting its distal neighbour.

12. *Dorsal arm plates: 0 = absent; 1 = single large plate throughout.*

In some species of Ophiothricidae (*Ophiomazda*, *Ophiothele* and *Ophiogymna*) there are only multiple irregular dorsal plates, but in *Ophiothrix* itself there are well developed dorsal arm plates. This family has thus been scored on the basis of *Ophiothrix*. Similarly *Ophioplocus* has multiple dorsal arm plating, whereas *Ophiolepis* and other Ophiolepidinae all have single, well-developed dorsal arm plates. *Bathypectinura* (Ophiodermatinae) also shows a tendency for the dorsal arm plates to fragment. In Hemieuryalidae most species such as *Ophiomaeris* and *Sigsbeia* have single well-developed dorsal arm plates. However, *Hemieuryala* itself differs in having only a pavement of small undifferentiated scales. The Hemieuryalinae were scored as having no dorsal arm plates, whereas *Ophiochondrus* and the Ophiochondrinae were scored as having dorsal arm plates.

13. *Dorsal surface with supplementary arm platelets: 0 = no; 1 = yes* (Fig. A5, A & B).

In some taxa there is a large secondary plate present between lateral arm plates and the dorsal arm plate. This is the case in *Hemieuryala* (Hemieuryalinae), *Ophionereis* and *Ophiocrasis* (Ophionereididae), and *Ophiolepis* and *Ophioplocus* (Ophiolepidinae), and in one species only of *Ophiopholis* (*O. mirabilis*: Ophiactidae). In the other members of *Ophiopholis* (Ophiactidae) there are multiple small auxilliary platelets around the dorsal arm plate, but no enlarged secondary plate. Interestingly, *Ophiopholis* also differs from *Ophiactis* in its mouth angle plate morphology, which again closely resembles that seen in Ophionereidae. *Ophiactis* itself lacks secondary plates and consequently the family is scored as lacking secondary arm plates. *Ophiopholis* probably belongs to the Ophionereididae rather than the Ophiactidae.

14. *Lateral arm plates at the base of the arm: 0 = extending laterally and wrapped around side of arm but not meeting dorsally; 1 = confined to oral surface; 2 = wrapped around side of arm and meeting dorsally on disk.*

The condition in Amphiuridae and Ophiuridae is variable amongst genera suggesting that this is not a very reliable character. However, the distinctive arrangement of lateral arm plates which meet aborally and separate the dorsal arm plates is a characteristic feature of most Ophiacanthidae as well as *Ophiochondrus* (Ophiochondrinae), *Amphilepis* (Amphilepididae) and many Amphiuridae. However, there are a considerable number of *Amphiura* species and other amphiurids that have contiguous dorsal arm plates. Unlike Ophiurinae, the lateral arm plates closest to the disk tend to be either in contact dorsally, or almost so. Therefore, amphiurids are scored as state 2. Typical *Ophiura* have broadly abutting dorsal arm plates proximally, although some genera in the Ophiurinae show disjunct plating (e.g. *Perlophiura*, *Ophiophysis*, *Ophiomisidium*). Ophiurinae are scored as having contiguous dorsal arm plates. Similarly, Ophiolepidinae show both characters, i.e. *Ophiolepis*, *Ophiomusium*, *Ophiosphalma* and *Ophiozonella* all have lateral arm plates contiguous at the base of the arm. Lateral arm plates are confined to the oral surface in euryalid families.

15. *Arm spines: 0 = moderately to well-developed, erect and extending outwards from arms, though may be adpressed at will; 1 = short and always adpressed against the arms* (Fig. A5, C & D).

Variable development of spines makes size an unreliable character, but we recognize one extreme form, where the spines are small and rudimentary. Although the arm spines of Ophionereididae are long they are tightly adpressed against the arm in the same way as the shorter spines of Ophiodermatinae. This is very different from the erect spines seen in Amphiuridae (short-spined) or Ophiothricidae (long-spined) for example.

16. *Primary arm spines: 0 = free of skin and without web; 1 = embedded in skin often with a web formed between adjacent spines at base.*

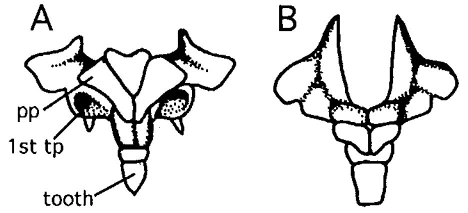

Figure A6. Internal views of oral ossicles showing difference in first tentacle pore position (character 20), redrawn from Matsumoto (1917). A, Internal facing first tentacle pore (*Pectinura*: Ophiodermatidae). B, Lateral facing first tentacle pore (*Amphiura*: Amphiuridae).

All euryalids and ophiomyxids have skin-embedded spines. Gorgonocephalidae do not have true arm spines.

17. *Primary arm spines: 0 = cylindrical; 1 = asymmetrical with hooked or comb-like edge.*

18. *Accessory belts of hooks: 0 = absent; 1 = present.*

This character was emphasised by Mortensen (1933) to distinguish gorgonocephalids. It is an autapomorphy of this family.

19. *Tube feet: 0 = large and permanently exposed along the arms; 1 = retractable and protected by a scale or scales that can completely cover the tube-foot.*

20. *First tentacle pore: 0 = lateral and facing into the mouth opening; 1 = internal facing so that the podial basin is clearly visible in internal (dorsal) aspect in dissected specimens* (Fig. A6).

In internal views of the dorsal surface of mouth angle plates the first podial basin may be largely hidden, and face radially towards the centre (as in Ophionereididae, Ophiocomidae and Amphiuridae, for example), or may face towards the interior so that the basin forms a conspicuous feature of the MAPs in internal view (e.g. Ophiochitonidae, Ophiodermatidae, Ophiurinae).

21. *Proximal tentacle pores: 0 = open, or with one or two tentacle scales only - not protected by ring of many scales; 1 = protected by ring of many scales.*

Only Ophiurinae and *Ophioleuce* (Ophioleucidae) have these distinctive tentacle scale patterns in the proximal arm, although both *Ophiomyces* (Ophiohelininae) and *Ophioplocus* (Ophiolepidinae) have multiple tentacle scales proximally.

22. *Oral papillae: 0 = absent; 1 = disorganized cone of spines; 2 = organized row of loosely fitting spines; 3 = organized row of tightly-fitting plate-like spines; 4 = row of plate-like granules* (Fig. A7).

Oral papillae are absent from Ophiothricidae and if present in *Strataster* are no more than small granules, as in Asteroschematidae and Euryalidae. Gorgonocephalidae and Asteronychidae have mouth papillae in the form of an irregular clump, as does *Ophiomyces* (Ophiohelininae). Most of the remaining ophiuroids have more or less block-like oral papillae that protect the mouth slits. However, in Ophiomyxinae, Ophiobyrsinae, Ophiacanthinae and Ophiotominae the oral papillae are distinctly spine-like and do not form a close-set pavement as is found in other ophiuroids.

23. *Second oral tentacle pore: 0 = basically internal in buccal cavity and directed adaxially so that there is no external manifestation; 1 = basically external and outward-facing, with or without tentacle scales.*

In many ophiuroids the second tentacle pores lie well inside the buccal slits and are not obvious externally from the oral surface. However, in a certain number of families the second oral tentacles lie clearly external to the buccal slits and are directed downwards and outwards. In forms such as Ophiobyrsinae, Asteroschematidae, Asteronyxidae, Ophiotominae, Ophiothricidae and Amphilepididae, these tentacles are largely unprotected by tentacle scales and are clearly visible. In Ophioleucidae and Ophiurinae and Amphiuridae, the tentacles still face downwards and outwards, but are to a large extent hidden by the well-developed tentacle scales. The condition in Ophiacanthinae is variable, with some species having downward-facing second tentacles and others having their second tentacles internal and hidden. This character is consequently scored as unknown for that subfamily.

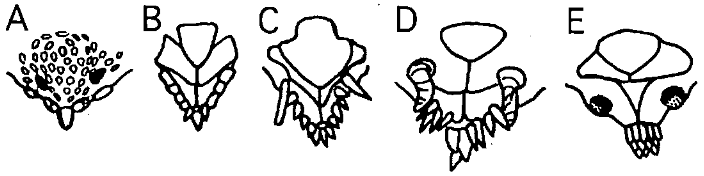

Figure A7. Arrangement of oral papillae (character 22). A, Small plate-like granules only (*Asteroschema*: Asteroschematidae). B, Organized row of abutting, plate-like spines (*Ophiomusium*: Ophiolepidinae). C, Organized row of loosely-fitting spines (*Ophioprium*: Ophiotominae). D, Disorganized cone of spines (*Ophioscolex*: Ophiomyxinae). E, Oral papillae absent; dental papillae prominent (*Ophiothrix*: Ophiothricidae).

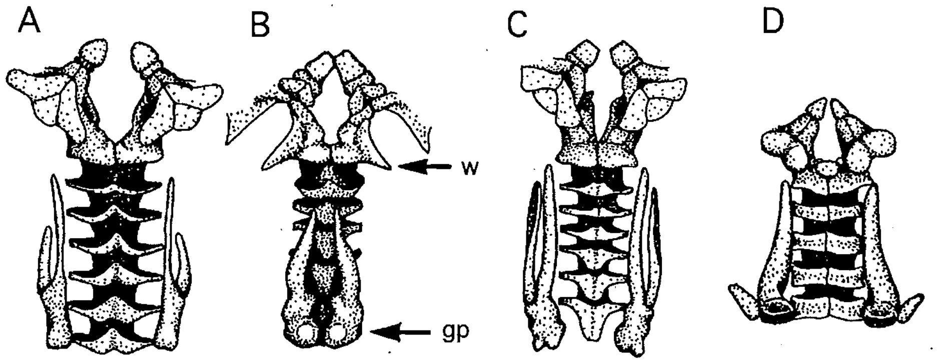

Figure A8. Proximal part of arm and oral plates lying within disk in dorsal view (characters 25 and 38), taken from Matsumoto (1917). A, *Ophiodera* (Ophiomyxinae); B, *Ophiocentrus* (Amphiuridae); C, *Ophiochiton* (Ophiochitonidae); D, *Astrotoma* (Gorgonocephalidae). w = expanded wing-like process to oral plates; gp = genital plate, set dorsal to the vertebrae and contiguous in gnathophiurans but lateral and separate in other ophiuroids.

24. *Second tentacle pore in external (ventral) view: 0 = not visible because of tesselate oral papillae; 1 = visible and largely unprotected by oral papillae (one or two thin, spine-like oral papillae may be present but do not obscure the tentacle pore).*

Only Ophiothricidae lack oral papillae, but in several taxa the second oral tentacles (and oral tentacle pores in skeleton preparations) are visible in oral view and are directed outwards. Included here are the Ophiobyrsinae, Asteronychidae, Astroschematidae and Amphilepidinae, which have outward-facing tentacle pores unprotected by oral papillae. Ophiotominae and many Amphiuridae have similarly obvious second oral tentacles, but with only one or two minute oral scales associated. Gorgonocephalidae and Euryalidae have their second oral tentacles entirely inward-facing within the oral slits (e.g. Lyman 1882). All other ophiuroids have second tentacle pores that are protected and hidden by oral papillae, though these do face outwards.

25. *Oral Frame: 0 = without well-developed lateral wings; 1 = with well-developed lateral wings* (Fig. A8).

According to Matsumoto (1917, p. 327) Ophionereididae have long stout oral frames with well-developed lateral wings (except for *Ophiodoris*) like Ophiochitonidae, and this is shown in Lyman (1882), though not in Matsumoto's figure. *Ophioceramis* (Ophiolepidinae) also has these well-developed wings but no other member of the Ophiolepidinae shows this and so the group is scored as without well-developed lateral wings.

26. *Peristomial plates: 0 = absent; 1 = double plates, thin, sometimes tending to become fused but with clear line of separation between pairs; 2 = triple, with triangular auxiliary plate; 3 = thin elongate ovoid plate not fused to peristomial plates; 4 = large, solid, quadrate and single, fused to the MAP, often with median furrow* (Fig. A9).

In Ophionereididae *Ophionereis* has double peristomial plates, while *Ophiocrassis* has triple peristomial plates. *Ophiocamax* (Ophioplinthacinae) Lyman (1882, pl. 41, fig. 9), shows the oral frame indicating that the peristomial plates are completely fused into the oral plates. However, other members of this group (e.g. *Ophiothamnus* are described by Lyman and Matsumoto (p. 94) as having triple peristomial plates with the middle plate occupying the V-shaped centre (i.e. character 2). *Ophiodera* (Ophiomyxidae) has triple plates, whereas *Ophiomyxa* itself has paired, nearly fused plates. The peristomial plates of Ophioleucidae are very variable: *Ophioleuce* itself shows a unique arrangement with three elements forming a single row, *Ophiopaepale* and *Ophiopyren* have double plates, while *Ophiernus* has just a single plate. The family is scored as having triple plates, on the basis of the condition in *Ophioleuce.*

27. *Dental plate: 0 = a single undivided element; 1 = multiple elements each with one or a few sockets* (Fig. A10).

Murakami (1963, pl. 2, fig. 17) shows *Ophiochiton* as having a single break across the tooth plate, and we can confirm that the tooth plate is apparently formed of two pieces. The condition in Ophiobyrsinae is somewhat uncertain. The one species illustrated by Murakami appears to be a single plate, but it closely resembles one of the multiple plates in gorgonocephalids.

28. *Tooth papillae arranged on dental plate as: 0 = a single uniserial column, with or without a double or triple*

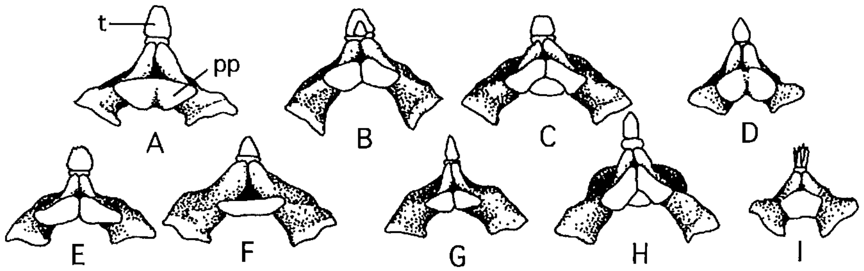

Figure A9. Peristomial plates seen in dorsal aspect on the oral frame plates (character 26), redrawn from Matsumoto (1917). A, *Ophiacantha* (Ophiacanthinae); B, *Ophionereis* (Ophionereididae); C, *Ophiochiton* (Ophiochitonidae); D, *Asteroschema* (Asteroschematidae); E, *Ophiomyxa* (Ophiomyxinae); F, *Amphilepis* (Amphilepididae); G, *Stegophiura* (Ophiurinae); H, *Ophiarachna*: (Ophiarachninae); I, *Gorgonocephalus* (Gorgonocephalidae). pp = peristomial plates; t = tooth.

set adorally; 1 = a uniserial column with an infradental complex; 2 = multiple, irregularly arranged scattering (Fig. A10).

There is a possible further character state in that a few taxa show a double pit at the adoral end of the row. In Ophiurinae for example this is commonly forms a triple row, whereas in other groups the arrangement is more Y-shaped. We have not found that we can successfully score this character.

29. *Tooth socket on dental plate: 0 = a ball and socket structure; 1 = a simple pore or small oval slit; 2 = a complex bilobed structure; 3 = large elongate slit* (Fig. A10).

Although there is considerable variation in the appearance of the dental foramina, the four basic types can usually be discriminated without ambiguity. The most difficult is differentiating between simple pores and elongate slits. In Ophiodermatinae and Ophiarachninae the foramina are very elongate and are scored as slits. In Ophiochitonidae and in Ophiomyxinae the foramina are somwhat oval but not so markedly elongate; in these cases they are scored as oval slits.

30. *Abradial muscle scar shape on oral plate: 0 = oval to elongate and vertical, positioned subcentrally; 1 = ovoid and basically transverse and subcentral; 2 = large and kidney-shaped, occupying the posterior half of the mouth angle plate, but not reaching the adapical margin or projecting posteriorly; 3 = large and kidney-shaped, occupying the entire distal face and projecting adapically* (Fig. A11).

Somewhat difficult to score because of the variation within families and according to size: for example larger species in the Ophiodermatinae have large kidney-shaped muscle scars, whereas smaller species have less well developed muscle scars. In this case the most developed state is scored for. The Ophiacanthinae showed the greatest variation amongst species and could be scored as either a '1' or a '2'.

31. *Adradial suture face on mouth angle plates: 0 = lies towards the base (adoral edge) of the plate and is comma-shaped, tapering adapically; 1 = is a predominantly vertical bar; 2 = is expanded adapically and is tapered or absent adorally* (Fig. A11).

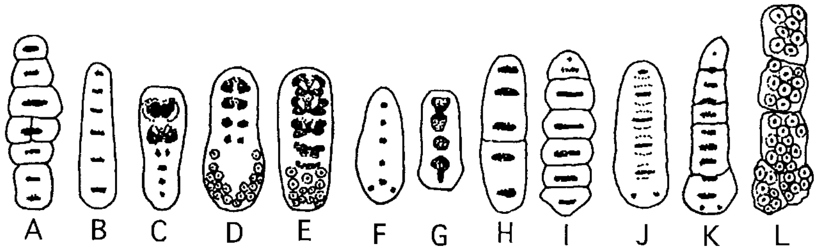

Figure A10. Dental plates (characters 27–29), redrawn from Murakami (1963). A, *Ophiomyxa* (Ophiomyxinae); B, *Ophiacantha* (Ophiacanthidae); C, *Amphiura* (Amphiuridae); D, *Macrophiura* (Ophiothricidae); E, *Ophiocoma* (Ophiocominidae); F, *Ophiura* (Ophiurinae); G, *Ophiolepis* (Ophiolepididae); H, *Ophiochiton* (Ophiochitonidae); I, *Ophiarachnella* (Ophiodermatidae); J, *Ophiocamax* (Ophioplinthacinae); K, *Asteroschema* (Asteroschematidae); L, *Astrocladus* (Gorgonocephalidae).

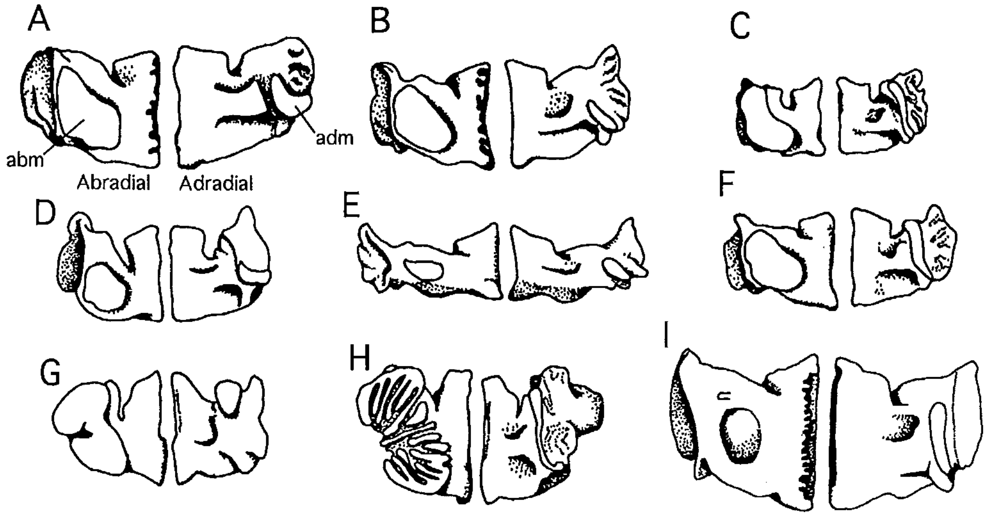

Figure A11. Oral plates in abradial and adradial view to illustrate the shape and position of abradial (abm) and adradial (adm) muscle scars (characters 30, 31), redrawn from Murikami (1963). A, *Ophiomyxa* (Ophiomyxinae); B, *Ophiochiton* (Ophiochitonidae); C, *Ophionereis* (Ophionereididae); D, *Ophiacantha* (Ophiacanthinae); E, *Ophiura* (Ophiurinae); F, *Ophioplocus* (Ophiolepididae); G, *Ophiothrix* (Ophiothricidae); H, *Ophiomastix* (Ophiodermatidae); I, *Asteronyx* (Asteronychidae).

32. *Gonads: 0 = extending as tubes from the base of the arms; 1 = restricted to the disk; 2 = multiple bodies along the arms.*

33. *Genital slits in disk: 0 = supported by genital bar; 1 = genital slits unsupported, no genital plates in disk.*

34. *Stomach: 0 = sack-like and confined to the disk; 1 = extending laterally along the arms.*

35. *Teeth: 0 = basically spinose or stumpy triangular, pointed distally; 1 = stout, flat, narrow blades with a chisel-like tip.*

36. *Genital spine: 0 = absent; 1 = small, spine-like; 2 = long blade-like; 3 = flattened paddle- or ear-shaped* (Fig. A12).

37. *Genital spine articulates: 0 = at the distal end of the genital plate; 1 = near the proximal end* (Fig. A12).

38. *Genital plate: 0 = unattached to vertebrae; 1 = more or less fused to proximal vertebrae and overlying so that they almost meet radially* (Fig. A8).

Figure A12. Genital plates (gp) and scales (gs), showing the relative shape of scales and their articulation point (characters 36 and 37), mostly redrawn from Matsumoto (1917). A, *Ophiodera* (Ophiomyxinae); B, *Hemieuryala* (Hemieuryalinae); C, *Ophiolebes* (Ophiacanthinae); D, *Amphilepis* (Amphilepidae); E, *Ophiothrix* (Ophiothricidae); F, *Ophiocoma* (Ophiocominae); G, *Ophiopsila* (Ophiopsilinae); H, *Ophioleuce* (Ophioleucinae); I, *Ophioplocus* (Ophiolepididae); J, *Ophiochiton* (Ophiochitonidae); K, *Ophiarachnella* (Ophiodermatidae); L, *Ophionereis* (Ophionereididae); M, *Ophiobyrsa* (Ophiobyrsinae); N, *Asteroschema* (Asteroschematidae); O, *Astrotoma* (Gorgonocephalidae); P, *Asteronyx* (Asteronychidae).

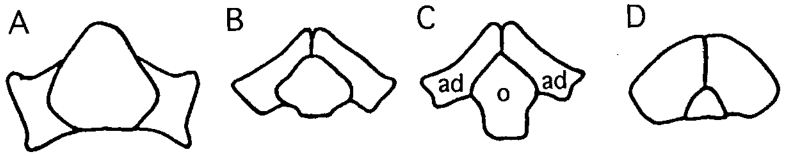

Figure A13. Oral (o) and adoral (ad) shields in external view illustrating shape and relative position (character 41). A, *Bathypectinura* (Ophiodermatidae); B, *Ophiotoma* (Ophiotominae); C, *Ophiozonella* (Ophiolepididae); D, *Ophiocreas* (Asteroschematidae).

This character is also reflected externally in the plating of the disk: i.e. radial shields lie well separated internally (0); more or less meet distally (1). In internal view the genital plates are either separated, lying either side of the arm, or lie close together more or less touching. Families with genital plates more or less touching are: Ophioplinthacinae, Ophiactidae, Amphiuridae and Ophiothricidae.

39. *Oral shields: 0 = absent; 1 = present.*

40. *Ventral arm plates: 0 = absent; 1 = present.*

41. *Oral and adoral shields in external aspect: 0 = large oval oral shield with small tear-drop-shaped adoral shields that do not meet adorally; 1 = diamond-shaped oral shield and narrow adoral shields that meet adorally; oral shields not projecting beyond adoral shields; 2 = as 1, but adoral shields shorter and thicker, with oral shield projecting proximally well beyond the adoral shields; 3 = structure dominated by paired adoral shields, oral shield, if present, a small plate set behind* (Fig. A13). The adoral shield plates are overridden to a greater or lesser extent by the oral shield plate. In external view, therefore, the adoral shields may be fully exposed, as in Euryalidae and Gorgonocephalidae, partially covered and V-shaped, with the oral shield projecting well beyond the ends of the adoral shields, as in Ophiothricidae; extensively covered, but still with both plates seen to abut adorally, as in Amphilepididae; or completely overridden such that only the outer parts of the two adoral plates are visible on either side of the oral shield.

42. *Ventral arm plates close to peristome: 0 = broadly contiguous and often imbricate; 1 = separated, but without lateral arm plates meeting mid-ventrally; 2 = separated, with lateral arm plates meeting and united along the mid-venter.*

43. *Dorsal plating of the disk: 0 = basically composed of small scales and platelets that remain undifferentiated; 1 = with centrodorsal and interradial plates enlarged and differentiated from amongst the disk plating.*

Data matrix:

Strataster	0??0	00???	00000	?0000	00000	012??	??1?0	0??00	??0
Ophiocanopidae	0???0	010?0	00000	1100?	020?0	0????	?2110	0??00	310
Ophiomyxinae	10000	02110	00000	11001	02000	11012	01000	11011	010
Ophiobursinae	10000	12000	00000	11000	02110	4?200	11000	11011	010
Euryalidae	11000	02001	00020	11000	04000	41010	10000	10011	300
Gorgonocephalidae	11000	32000	0002?	??100	01000	41200	11000	10011	320
Asteronychidae	11000	02000	00020	11000	01110	41200	11000	11011	300
Asteroschematidae	11000	32000	00020	11000	04110	41010	10000	10011	320
Hemieuryalinae	12100	02000	00101	00010	03000	40011	11000	11011	201
Ophiochondrinae	11101	22100	01011	00010	03000	40011	11000	11011	221
Ophiacanthinae	11001	12200	01010	00011	02?00	40011	01000	21011	220
Ophiotominae	10001	12?20	01000	0000?	02110	?0011	01000	???11	120
Ophioplinthacinae	12101	12220	?1010	00011	03000	20011	01000	21111	220
Ophiohelininae	0???1	02120	01010	00011	02000	0001?	?1000	0?111	0?0
Amphilepididae	121?1	02200	01010	00010	03110	30023	01001	21011	121
Amphiuridae	12111	02200	01010	00010	03101	10023	21001	30111	201
Ophiactidae	12111	12220	01000	00010	03001	30023	21001	30111	201
Ophiothricidae	12111	12200	11000	00010	00111	30123	21001	30111	201
Ophiocominae	12121	12200	01000	00010	03001	10123	11001	21011	000
Ophiopsilinae	11121	02200	01000	00011	03001	10123	11001	21011	200
Ophionereididae	11121	02200	11100	00010	03001	10023	11001	21011	001
Ophiochitonidae	12121	02200	01000	00011	03000	21012	01001	21011	101
Ophiodermatinae	12121	22200	01001	00011	03000	21032	01000	21011	000
Ophiarachninae	12121	22220	01001	00011	03000	21032	01000	21011	000
Ophiurinae	12121	02200	01001	00011	13100	10011	01000	30011	201
Ophioleucidae	12121	22200	01001	00011	13100	20011	01000	30011	100
Ophiolepinae	12121	02200	01101	00011	03000	10012	11000	21011	201
Aplocomidae	12121	22200	01001	0001?	03?00	?0011	01000	21011	201

APPENDIX 2

Alligned lsu rRNA sequences used in the phylogenetic analysis. Positions marked by a solid line represent regions of ambiguous alignment and analyses were run with these regions both included and excluded. Positions marked with a • were omitted from all analyses.

```
             1          11         21         31         41         51         61         71         81         91
             •••
Asteronyx    NNCGACCUCA GAUCGAGCGA GAUUACCCGC UGAAUUUAAG CAUAUUACUA AGCGGAGGAA AAGAAACUAA CUANGAUUCC CUCAGUAGCG GCGAGUGAAG
Ophiacantha  -U-------- ---------- --C------- ---------- ------N--- ---------- ---------- -C-G------ -------N-- ----------
Ophiochiton  -U-------- ---------- --C------- N--------- ---------- ---------- ---------- -C-GA----- ---------- ----------
Ophioderma   --NN------ -------U-- ---C------ ---------- ---------- ---------- ---------- -C-G------ ---------- ----------
Ophiomusium  --N------- ---------- ---------- ---------- ---N------ ---------- ---------- -C-G------ ---------- ----------
Ophiura alb  -U-------- ---------- ---------- ---------- ---------- ---------- ---------- -C-G------ ---------- -N--------
Ophiura oph  --N------- ---------- ---------- --------N- ---------- ---------- ---------- -C-G------ ---------- ---N------
Ophiopholis  ---------- ---------- ---------- ---------- ---------- ---------- ---------- -CUG------ ---------- ----------
Ophiothrix   --N------- ---------- --C------- ---------- -----C---- ---------- ---------- -C-G------ ---------- ----------
Amphipholis  --N------- -C-------U --CG------ --G------- ---------- ---------- ---------- -C-G------ ---------- -N--------

             101        111        121        131        141        151        161        171        181        191

Asteronyx    CGGGAAAAGC CCAUCGCCGA AUCCCCGCGU CCCAGUNGCG CGUGGGAAAU GUGGCGUAAG GAAGCCGUGG GUAACCGGGG ACGCGGAGCC GACGUCCUUG
Ophiacantha  ---------- ---------- ---------- ---------- U-C------- ---------- -----U---- C--------- ---------- ----------
Ophiochiton  ---------- ---------- ---------- -----CG--- U-C------- -------G-- ---------- C--------- ---N------ ----------
Ophioderma   ------G--- ---------- ---------- ------G--- U-C------- ---------- ---------- C--------- ---U------ ----------
Ophiomusium  ---------- ---------- ---------- --G--CG--- --C------- ---------- ---------- C--------- ---U------ ----------
Ophiura alb  ---------- ---------- ----U----- ------G--- --C------- ---------- ---------- C-U-----A- ---U-C---- ----------
Ophiura oph  ---------- ---------- ---------- ------G--- --C------- ---------- ---------- C-------A- ---U-C---- ----------
Ophiopholis  ------G--- ---------- ---U------ --U--CG--- U-C-A----- -------G-- ---------- C-------A- ---U------ ----------
Ophiothrix   ------G--- ---------- ---U------ --U--CG--- U-A-A----- -------G-- ---------- C-------A- ---U------ ----------
Amphipholis  U-----G--- -------UU- ----U----- ------G--- U------G-- -C-----G-- -U-------- C-U-U---C- ------G--- ----------

             201        211        221        231        241        251        261        271        281        291
                                                                               __
Asteronyx    UGAAGGAGGC UCUUAUCCCA UAGAGUGUGU UAGGCCCCCG CAGGCGCCCG UCGCCGGUGG CCUUUUGGCU UUCAAGAGUC GGGUUGUUUU GGAAUGCAGC
Ophiacantha  ---------- -UNN------ ---------- -------UU- ---------- ---------- ----AA*--- -C-------- ---------- ----------
Ophiochiton  ---------- --NN------ ---------- N--N------ ------*--- ---------* -N--AA---- -CU------- ---------- --N-------
Ophioderma   ---------- C--C------ ---------- ---------- ---------- ---------- -N--AA---- ---------- ---------- ----------
Ophiomusium  ---------- ---CN----N ---------- ---------- ---------- ---------- ----AA---- -C-------- ---------- ----------
Ophiura alb  ---------- ---C------ ---------- --------*- ------G-*- --A------- -NN-AA---- -C-------- ---------- ----------
Ophiura oph  ---------- --NNN----- -N-------- --------*- ------G--- --A------* NN--AA---- -C-------- ---------- ----------
Ophiopholis  ---------- -U**------ ---------- ---------- ------*--- -AA------S -**-CA---- -CU------- ---------G A---------
Ophiothrix   ---------- -U**------ ---------- ---------- ------*--- -UA------- -*A-CA---- -CU-G----- ---------- A--U------
Amphipholis  ---------- -U**------ C--------- ---------A -----U---- -U*------- --A-CA---- ACU------- ---------- ----------

             301        311        321        331        341        351        361        371
                                                                      ••••••• •••••••••• ••••••••••
Asteronyx    CCAAAGACGG GUGGUAAACU CCAUCCAAGG CUAAAUACUA AGCACGAGAC CGAUAGAUCA CAAGNNNNNN NNNNNNNNNN
Ophiacantha  ---------- A--------- ---C------ ---------* G--------- -------A-- ----U-CC-- ----------
Ophiochiton  ---------- ---------- ---------- ---------- G--------- ----N---AN NNNN------ ----------
Ophioderma   ---------- ---------- ---------- ---------- G--------- ---NNNNNNN NNNN------ ----------
Ophiomusium  ---------- ---------- ---------- ---------- ---------- ---------N NNNN------ ----------
Ophiura alb  ---------- ---------- ---------- ---------- ---------- ----N---NN NNNN------ ----------
Ophiura oph  ---------- ---------- ---------- --N------- -U-------- ----U---UN NNNN------ ----------
Ophiopholis  --U-----R- ---------- -----N---- ---------- G--------- -------NN- -N--UAC--- ----------
Ophiothrix   --U------- ---------- ---------- --------CC ---------- ------U--- ----UACCAU GAGGGAAAGU
Amphipholis  ---------- ---------- ---------- ---------- ---------- -------CG- ----UACCAU GAGGGAAAGU
```

DATA MATRIX:

```
Asteronyx    ACAUUAUUAGCACGCCCUCUGAUAACGACGCGAUCUUCCGGCCCGGUUGUUCAUGAAGUUAAG
Ophiacantha  ACACUAUCAGCACGCCCUUCGAUAAUCACGCGAUU?UUUGGCCCGGAA*UCCAUGAAACU*GG
Ophiochiton  ACACUAUCAACACGCCCCUCGAUGACCACG?GAUC?UCCGG*CCG*AAGUCUAUGAAGUUAGG
Ophioderma   AUAUCAUCAGCGCGCCCUUCGAUAACCACGUGACCUUCCGGCCCGGAAGUUCAUGAAGUUAGG
Ophiomusium  ACAUUAUCAGCACGCCGCCCGAUAACCACGUGAUCUUCCGGCCCGGAAGUCCAUGAAGUUAAG
Ophiura alb  ACAUUAUCAGCACGCUCUCCGAUAACCUCAUCAUCUUC*GGG*CAGAAGUCCAUGAAGUUAAG
Ophiura oph  ACAUUAUCAGCACGCCCUCCGAUAACCACAUCAUC?UC*GGGCCA?AAGUCCAUGAAGUUAAU
Ophiopholis  ACAUUAUCUGCGCGUCUCUCAAUGACCACAUGAUU*UCCGG*CAA?CAGUCUAGAAUGUUAGG
Ophiothrix   ACACUACCAGCGCGUCUCUAAAUGACCACAUGAUU*UCCGG*CUAGCAGUCUGUAUUGUCCAG
Amphipholis  CCUCGGUCAGUGUUCUCUUUGGCGUCCUUCCGGUU*CCCAUCCU*GCAGACUAUGAAGUUAAG
```