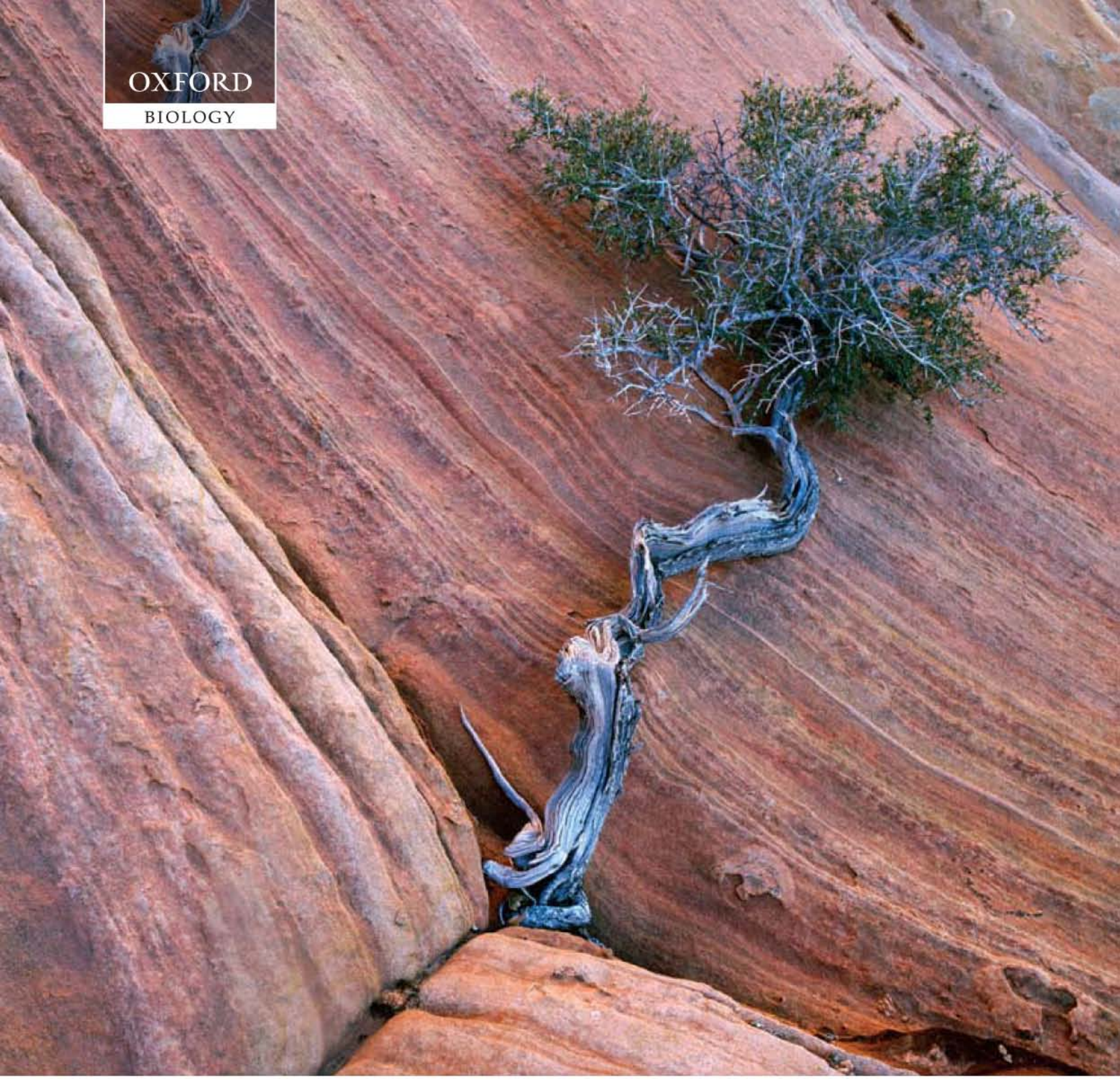

the TIMETREE *of* LIFE

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Cephalopod mollusks (Cephalopoda)

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Abstract

Squids, cuttlefish, octopuses, and nautiluses (~700 species) are grouped into 47 families within the Class Cephalopoda of the Phylum Mollusca. The resolution of many higher-level phylogenetic relationships within cephalopods has been hindered by homoplasy among morphological characters, although some recent progress has been made with molecular phylogenies and molecular clocks. The cephalopod timetree supports a Paleozoic (542–251 million years ago, Ma) origin of the Orders Vampyromorpha, Octopoda, and the majority of the extant higher-level decapodiform taxa. The major lineages within the Order Octopoda were estimated to have diverged during the Mesozoic era (251–66 Ma).

The class Cephalopoda is a monophyletic group which can be divided into two subclasses; Nautiloidea and Coleoidea. Nautiloidea contains the nautiluses (*Nautilus* and *Allonautilus*), whereas Coleoidea contains the octopuses (Fig. 1), squids, and cuttlefishes. Coleoid cephalopods differ from nautiloids most notably through the reduction (or complete loss) and internalization of the shell. Defining features of Coleoidea include a muscular mantle used for locomotion and respiration, the modification of the foot into appendages around the mouth, a closed circulatory system, and complex eyes with lenses, although many of these features have been lost or reduced in various taxa. The widely cited annotated classification of the recent Cephalopoda (*1*) listed over 700 valid species in 139 genera and 47 families. Here we review the evolutionary relationships and divergence times of the members of the class Cephalopoda. Several alternative classifications have been proposed for relationships within Cephalopoda (*2*). Herein we follow the classification of Young *et al.* (*3*) that generally does not include ranks above the family level; however, we make certain assumptions about rank based on nomenclature and position.

The Coleoidea are divided into two superorders: Decapodiformes and Octopodiformes (*4*). The Decapodiformes is a morphologically and ecologically diverse group comprising 31 families, 95 genera, and approximately 450 species. Four major lineages are recognized within Decapodiformes: Sepioidea (cuttlefish, bottletail, and bobtail squids), Spirulida (the Ram's horn squid), Oegopsida (open-eye squids), and Myopsida (closed-eye squids) (*3*, *5*). Three families, Bathyteuthidae,

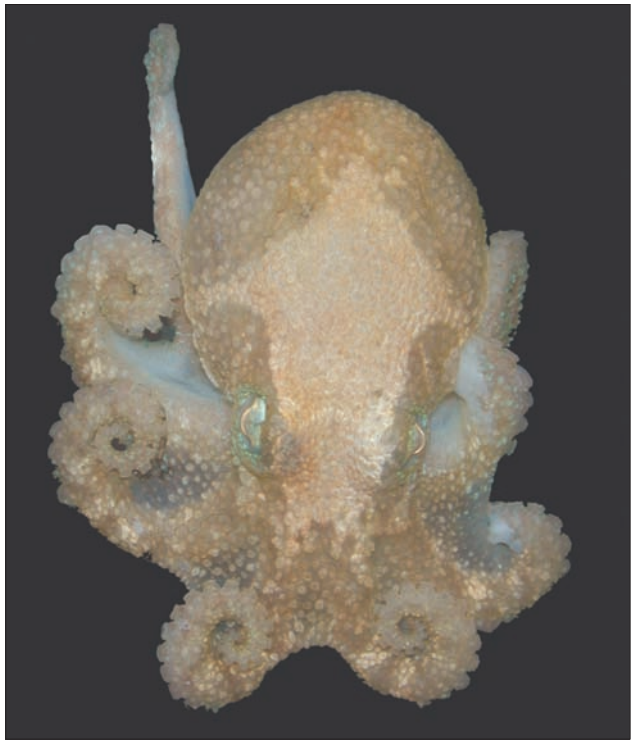

Fig. 1 A benthic octopus (*Pareledone charcoti*) from Antarctica. Photo credit: A. L. Allcock.

J. M. Strugnell, A. Lindgren, and A. L. Allcock. Cephalopod mollusks (Cephalopoda). Pp. 242–246 in *The Timetree of Life*, S. B. Hedges and S. Kumar, Eds. (Oxford University Press, 2009).

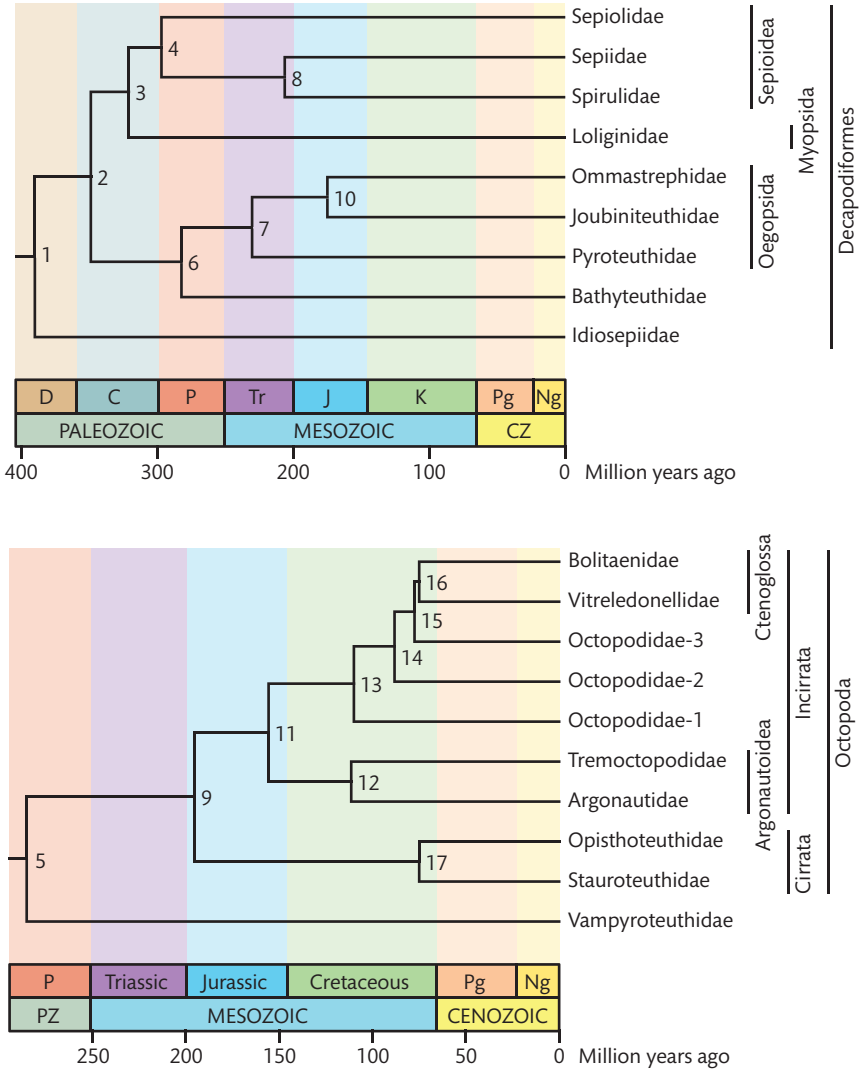

Fig. 2 A timetree of cephalopod mollusks: Decapodiformes and Octopodiformes. Divergence times are shown in Table 1. *Abbreviations*: C (Carboniferous), CZ (Cenozoic), D (Devonian), J (Jurassic), K (Cretaceous), Ng (Neogene), P (Permian), Pg (Paleogene), PZ (Paleozoic), and Tr (Triassic). Octopodidae 1 = Octopus, Hapalochlaena; Octopodidae 2 = Benthoctopus, Enteroctopus; Octopodidae 3 = Adelieledone, Pareledone.

Chtenopterygidae, and Idiosepiidae, have features that do not clearly place them within one of these lineages. However, it appears that bathyteuthids and chtenopterygids are closely related and these have been grouped together in the Bathyteuthoida (*3*). The somewhat unique pygmy squids (*6*), Idiosepiidae, currently remain as a stand-alone family. Relationships among the lineages remain somewhat unclear due to conflicting or inconclusive analyses of both morphological (*7*) and molecular (*8–11*) data. Sepioidea, Idiosepiidae, and Spirulida have often been interpreted as closely related (*12*, *13*), but have never been found to be a monophyletic group in any modern phylogenetic study containing representatives of each of these taxa. Some molecular studies support a relationship between Bathyteuthidae, Spirulida, and Sepioidea but not Idiosepiidae (*14*), while others support a close relationship between Bathyteuthidae and Oegopsida (*11*).

Sepioidea contains two groups, the bobtail squids, Sepiolida (families Sepiolidae and Sepiadariidae), and the cuttlefishes (Family Sepiidae), united by morphological features such as eyes with secondary lids and a funnel with a lateral canal (*5*). Molecular data have yet to support a monophyletic Sepioidea; recent molecular

Table 1. Divergence times (Ma) and their credibility intervals (CI) among cephalopod mollusks.

Timetree		Estimates			
Node	Time	Ref. (*11*)(a)		Ref. (*11*)(b)	
		Time	CI	Time	CI
1	390.0	390.0	596–236	–	–
2	349.0	349.0	544–206	–	–
3	321.0	321.0	506–188	–	–
4	297.0	297.0	473–172	–	–
5	285.5	252.0	369–175	319.0	476–206
6	282.0	282.0	454–161	–	–
7	230.0	230.0	–	–	–
8	206.0	206.0	345–109	–	–
9	195.5	174.0	262–118	217.0	336–133
10	175.0	–	–	–	–
11	156.0	136.0	208–92	176.0	277–106
12	111.5	105.0	166–67	118.0	196–66
13	110.0	100.0	155–66	120.0	196–70
14	88.5	77.0	121–49	100.0	166–57
15	77.5	67.0	107–43	88.0	146–50
16	75.0	65.0	–	85.0	–
17	75.0	65.0	–	85.0	–

Note: Node times in the timetree represent the mean of time estimates from different studies. Divergence times for the Octopodiformes are from a Bayesian analysis of (a) three mitochondrial and three nuclear genes partitioned by gene and codon and (b) three nuclear genes partitioned by gene and codon. Divergence times for Decapodiformes are from a Bayesian analysis of (a) three nuclear genes not partitioned (first and second codon positions only).

studies found Sepiidae to be closest to Spirulida (*10*, *14*), with Sepiolidae closest to a Sepiidae + Spirulida clade. Alternatively, Lindgren *et al.* (*9*) found Sepiidae and Idiosepiidae to be closest relatives, while Strugnell *et al.* (*10*, *11*) found Idiosepiidae and Sepiolidae to be closest relatives. Although their relationships are somewhat unstable across molecular analyses, Sepiidae and Sepiolida appear to be closely related on the basis of both morphological and molecular data.

Spirulida contains a single living species, *Spirula spirula*, which exhibits a number of unique features, the most striking being the retention of a phragmocone with unusual ventral coiling, making it particularly difficult to place among extant decapodiforms. Furthermore, it lacks a cornea, which makes placing Spirulida as the closest relative of Sepiidae difficult, as all sepioids possess a corneal covering over the eye. The absence of a cornea implies either a close relationship with Oegopsida or that loss is a convergent character in Sepiidae and Oegopsida. Naef (*12*) believed Spirulida to be closest to Sepiidae (cuttlefishes) due to similarity in embryonic phragmocone development. Some molecular studies have found a close relationship between Spirulida and Sepiidae (*10*, *14*), although other molecular studies (*8*, *9*) yielded different phylogenetic positions for Spirulida. Because of the unique morphological characters and inconsistent results with DNA sequence data, further examination is required before the position of Spirulida can be firmly established.

Oegopsida and Myopsida were historically grouped together in the Suborder Teuthoidea (e.g., *12*), united by similarities in gladius, branchial canal, tentacular clubs, and interstellate connective (*15*). The primary feature used to separate Oegopsida and Myopsida is the lack of a corneal covering in the oegopsid eye. A close relationship between Myopsida and Oegopsida was found in several topologies generated by Carlini and Graves (*8*) using the *COI* locus, but other analyses have rendered Oegopsida paraphyletic with respect to Myopsida, or found a close relationship between Myopsida and some sepioids (*9*–*11*). Myopsida and Sepioidea exhibit several features in common, such as the presence of a cornea, beak without an angled point, vena cava ventral to intestine, buccal crown with suckers (only some taxa), accessory nidamental glands, and tentacle pockets (*15*). Because of the lack of resolution at basal nodes in the tree, many of the features that unite Sepioidea and Myopsida may be ancestral. The lack of a consistent relationship between Myopsida and Oegopsida suggests that the taxon Teuthoidea may not be valid.

Oegopsida is the most species-rich and morphologically diverse group within the Decapodiformes. Species can range in size from a few centimeters in the Family Pyroteuthidae to tens of meters in the giant squid, *Architeuthis dux*. The diversity in anatomy, behavior, and morphology of Oegopsida, combined with a somewhat uninformative fossil record (*15*), has made generating hypotheses of family-level relationships within this group very difficult. Morphology-based phylogenies have focused primarily on higher-level relationships, and have not yielded well-supported groups within Decapodiformes, because of difficulty in establishing polarity and homology (*7*). At the family level, molecular data have recently been employed, but the trees generated are highly dependent on gene choice, taxon sampling, and analytical method (*8*–*10*, *16*, *17*).

The Octopodiformes contains the Orders Vampyromorpha (vampire "squid") and Octopoda (pelagic and benthic octopuses). A close relationship between these two orders has been supported by morphological studies (*7*, *16*, *18*, *19*) and some molecular studies (*10*, *20*, *21*), although some molecular phylogenies (*9*, *22*) provide contradictory or inconclusive support for a close relationship between Vampyromorpha and Decapodiformes. The Octopoda contains the Suborders Cirrata (deep-sea finned octopuses) and the Incirrata (benthic and pelagic octopuses). A close relationship between these suborders is widely accepted (*7*, *23*, *24*).

Four families are currently recognized within the Cirrata (Opisthoteuthidae, Grimpoteuthidae, Cirroctopodidae, and Cirroteuthidae) (*25*, *26*). A molecular study using *16S rRNA* suggests the first three of these families, which all possess a single web, form a clade, to the exclusion of the last one, which possesses an intermediate or secondary web (*25*).

The phylogenetic relationships between the eight families of Incirrata have not been well investigated. Four of the pelagic families (Alloposidae, Tremoctopodidae, Argonautidae, and Ocythoidae) comprise a well-defined monophyletic clade (Superfamily Argonautoidea) linked by a detachable hectocotylus (the modified arm used in copulation) in males within this group. Although no molecular study has been published which encompasses all four families, molecular evidence to date has been supportive of this grouping (*9*, *10*, *21*, *27*).

Naef (*12*) proposed that the remaining pelagic octopods (Vitreledonellidae, Amphitretidae, and Bolitaenidae) be placed in a grouping Ctenoglossa based on the structure of the radula. The monophyly of this grouping has been confirmed by molecular studies (*27*) and it has been suggested that ctenoglossans have neotenous origins (*21*). Molecular work shows the ctenoglossans are unlikely to be closely related to the Argonautoidea (*21*, *27*). One might therefore suppose that the remaining family, the Octopodidae, provides an evolutionary link between these two groups. However, the situation is far more complex than this. Molecular studies (*10*, *27*) suggest that the Octopodidae is not monophyletic and this is hindering our understanding of other relationships within the Incirrata.

Two molecular-based studies have estimated divergence times among the major lineages of cephalopods (*11*, *21*). Both studies used three nuclear (*rhodopsin*, *pax-6*, *octopine dehydrogenase*) and three mitochondrial (*16S rRNA, 12S rRNA, cytochrome oxidase I* [*COI*]) genes. The first of these employed a penalized likelihood (PL) method with a primary interest of estimating the divergence time of the grouping Ctenoglossa from the remaining Octopoda (*21*). The second study (*11*) used the same six genes and employed a Bayesian approach (*28*) to estimate divergence times both within octopodiforms (eight families) and decapodiforms (nine families). Both studies used constraints taken from the coleoid cephalopod fossil record.

The timetree of coleoids using the Bayesian approach was based on the results obtained from a prior phylogenetic study investigating the effect of data partitioning on resolving phylogenies in a Bayesian framework (*10*). This study showed that the strongest phylogenetic resolution for the Octopodiformes was obtained from analyses using all six genes partitioned by gene and codon. Two topologies were presented for the Decapodiformes: one resulting from analysis of nuclear genes (all three codon positions) partitioned by codon, and the second resulting from only the first and second codon positions (not partitioned). These phylogenies and partitions were used within the Bayesian approach to estimate divergence times within coleoids. Here we present the topology obtained using first and second positions only as it is likely that the third positions are saturated and are therefore not informative in estimating deep divergences. Furthermore, to determine whether there was any difference between mitochondrial and nuclear genes on dating estimates, the octopodiform topology was also analyzed using only nuclear genes.

The mean divergence times of almost all the major lineages leading to the extant decapodiform taxa were estimated to have occurred in the Paleozoic in the decapodiform topology presented (Fig. 2; Table 1). The divergence of the Spirulidae and Sepiidae is the one exception, estimated to have occurred in the Mesozoic for the decapodiform topology using first and second codon positions only. Some diversification within the Oegopsida already appears to have occurred around the Paleozoic–Mesozoic boundary (251 Ma). Such an ancient diversification of the major decapodiform lineages may have contributed to obscuring phylogenetic relationships within this group with both morphological and molecular characters becoming saturated over the last 300 million years.

In contrast to the Decapodiformes, divergences among most taxa within the Octopodiformes were estimated to have occurred much more recently. The divergence of Vampyromorpha and the Octopoda was estimated to have occurred in the upper Paleozoic while the origins of the rest of the major lineages of Octopoda were estimated to have occurred in the Mesozoic. The

divergences of a number of lineages, including the ctenglossans, were estimated to have occurred close to the Mesozoic–Cenozoic boundary (66 Ma), while the PL analysis estimated the origin of the ctenoglossan lineage to be slightly younger (48.5 ± 7.5 Ma) (*21*). It is possible that the divergence of these lineages may correspond to the extinction of the ammonoids and/or the end of the Cretaceous "oceanic anoxic event" (~93 Ma), with mesopelagic depths only habitable after this time (*15*). The estimated divergence times within the Octopodiformes were found to be slightly older (but not significantly different) when nuclear genes only were used in the Bayesian analyses.

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