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A combined approach to the phylogeny of Cephalopoda (Mollusca)

A. R. Lindgren[1,†], G. Giribet[2] and M. K. Nishiguchi[1,*]

[1]*Department of Biology, New Mexico State University, Box 30001, MSC 3AF, Las Cruces, NM 88003-8001, USA;* [2]*Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, 16 Divinity Ave., Cambridge, MA 02138, USA*

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Abstract

Cephalopoda represents a highly diverse group of molluscs, ranging in habitat from coastal regions to deep benthic waters. While cephalopods remain at the forefront of modern biology, in providing insight into fields such as neurobiology and population genetics, little is known about the relationships within the group. This study provides a comprehensive phylogenetic analysis of Cephalopoda (Mollusca) using a combination of molecular and morphological data. Four loci (three nuclear 18S rRNA, fragments of 28S rRNA and histone H3 and one mitochondrial cytochrome *c* oxidase subunit I) were combined with 101 morphological characters to test the relationships of 60 species of cephalopods, with emphasis within Decabrachia (squids and cuttlefishes). Individual and combined data sets were analyzed using the direct optimization method, with parsimony as the optimality criterion. Analyses were repeated for 12 different parameter sets accounting for a range of indel/change and transversion/transition cost ratios. Most analyses support the monophyly of Cephalopoda, Nautiloidea, Coleoidea and Decabrachia, however, the monophyly of Octobrachia was refuted due to the lack of support for a Cirroctopoda + Octopoda group. When analyzing all molecular evidence in combination and for total evidence analyses, Vampyromorpha formed the sister group to Decabrachia under the majority of parameters, while morphological data and some individual data sets supported a sister relationship between Vampyromorpha and Octobrachia. Within Decabrachia, a relationship between the sepioids Idiosepiida, Sepiida, Sepiolida and the teuthid Loliginidae was supported. Spirulida fell within the teuthid group in most analyses, further rendering Teuthida paraphyletic. Relationships within Decabrachia and specifically Oegopsida were found to be highly parameter-dependent.

Cephalopoda Cuvier, 1797 is the third largest molluscan class (after gastropods and bivalves), and comprises more than 800 marine species, inhabiting a variety of ecosystems, ranging from coastal to abyssal depths. Cephalopods exhibit many unique characteristics that distinguish them from other molluscs such as horny beaks, complex eyes with a lens, a closed circulatory system, a highly centralized nervous system, modification of the foot into circum-oral appendages, and a funnel apparatus that allowed them to become active swimmers, mostly independent of the ancestral benthic lifestyle of other molluscs. While cephalopods exhibit major morphological and physiological divergence from other molluscan classes, a great deal of diversity also exists within the group. Size-wise, cephalopods range from about 10 millimeters in mantle size in *Idiosepius,* to several meters in the giant squid, *Architeuthis*.

Cephalopoda is subdivided into Nautiloidea and Coleoidea. Nautiloidea consists of a single taxon, Nautilidae, which possesses a coiled, chambered, calcified, external shell, hypothesized to be plesiomorphic (Young et al., 1998). Coleoidea contains all other extant taxa, where the characteristic shell has been internalized and reduced, or completely lost. The extant Coleoidea can be divided into two subgroups (*sensu* Boletzky, 2003); Decabrachia (the squids and cuttlefish) and Vampyropoda. Within Vampyropoda (e.g., Boletzky, 2003), three lineages have been recognized, Vampyromorpha (monotypic), Cirroctopoda (finned octopods) and Octopoda (all non-finned octopods). Octobrachia was also used to delineate a close relationship between

*Corresponding author.
E-mail address: nish@nmsu.edu
†Present address: Department of Evolution, Ecology and Organismal Biology, The Ohio State University, 300 Aronoff Labs, Columbus, OH 43210, USA.

Octopoda and Cirroctopoda. Initially, Vampyroteuthidae was placed within the finned octopods; however, Pickford (1939) elevated the family Vampyroteuthidae to the ordinal level, Vampyromorpha, based on the presence of a broad gladius and 10 arms (two of which were identified as retractile filaments). The position of Vampyromorpha has been highly controversial because it contains several autapomorphic characters which are not shared with octobrachians nor decabrachians. Young et al. (1998) considered it to be an intermediate form, but more closely related to octobrachians, and sperm morphology also suggests a relationship to octobrachians (Healy, 1989).

Decabrachian classification

Within Decabrachia, Boletzky (2003) proposed five orders, Spirulida (monogeneric), Sepiida, Sepiolida, Idiosepiida and Teuthida. The so-called sepioid orders, Sepiida, Sepiolida, Idiosepiida, Spirulida, have traditionally been placed in a single order, Sepioidea (*sensu* Naef, 1921/23) based on shared characters such as simple funnel locking apparatus, rounded fins, conservative embryonic development and progressively reduced shells. Spriulida and Sepiida have calcified shells, Sepiolidae has an uncalcified, reduced gladius, with a proostracum still present, while Idiosepiida has a very thin, uncalcified remnant shell that went unnoticed in older descriptions. Spirulida was placed within the sepioid order by Chun (1914), a position further investigated by Naef (1921/23). Although the shells of Spirulida and Sepiida appear to be vastly different, Naef believed that they could have arisen from a common ancestor due to their similar phragmocone morphology. However, the position of Spirulida has remained questionable (e.g., Bonnaud et al., 1997; Carlini and Graves, 1999). Two suborders were established within Teuthida; Myopsida (containing a single family, Loliginidae [now also including Pickfordiateuthidae]) and Oegopsida (all other squid families). Myopsida was distinguished by the presence of a corneal cover over the eye, whereas the oegopsid eye has no covering and is in direct contact with the water.

The interfamilial relationships within Teuthida have remained problematic, partly because many characters uniting the suborders remain untested in a phylogenetic study. Myopsids and oegopsids share a similar gladius, branchial canal structure and tentacular club, as well as having a generally "similar" appearance (Young et al., 1998). However, several characters suggest that myopsids may be more closely related to sepiolid squids rather than the oegopsids, such as the presence of a corneal covering, benthic eggs, a similar position of the seminal vesicle, accessory nidamental glands and the presence of suckers on the buccal crown (Young et al., 1998). Traditionally, the Decabrachia has been divided into two orders, Sepioidea (comprising Spirulida, Sepiida, Sepiolida and Idiosepiida) and Teuthoidea, comprising all other squids (e.g., Young and Vecchione, 1996; Beesley et al., 1998). However, Boletzky's (2003) classification accounts for the variability within Decabrachia by establishing separate orders for divergent groups (whose interrelationships remain unknown) while conserving the hypothesized sister relationship between Vampyromorpha and Octobrachia.

Phylogenetic relationships

Although most phylogenetic relationships among the recognized families of Cephalopoda remain ambiguous, morphologically based studies have provided valuable information for higher-level relationships. A recent study by Young and Vecchione (1996) used 25 characters to delineate the interfamilial relationships among 17 families of cephalopods. Their findings provided support for the monophyly of Decabrachia and Octobrachia, respectively, and placed Vampyromorpha as the sister group to Octobrachia. Although resolution was proposed for taxa closely associated with the family Enoploteuthidae (Young and Harman, 1998), little resolution was achieved within the remainder of the Decabrachia. Other morphological studies (Roper et al., 1969, 1984; Toll, 1982; Hess, 1987; Nesis, 1987) also provided characters useful for classification essential but remained untested in any large-scale phylogenetic study.

Molecular studies have recently provided information regarding relationships within cephalopods (Bonnaud et al., 1997; Carlini and Graves, 1999; Carlini et al., 2000, 2001). Bonnaud et al. (1997) generated the first molecular cephalopod study using data from the mitochondrial 16S rRNA locus for 16 species. While this study supported many higher-level relationships hypothesized in morphologically based studies, it did not include many exemplars pertinent for determining lower level relationships. Subsequently, a more comprehensive study by Carlini and Graves (1999) used the cytochrome *c* oxidase subunit I (COI) locus for 48 cephalopod species to examine higher-level relationships. Their results confirmed previously supported morphological data in some areas, but left the relationship of *Vampyroteuthis* questionable, and did not resolve many interfamilial relationships within Decabrachia. A second study (Carlini et al., 2000) using several actin gene loci provided additional data; however, due to the presence of multiple gene copies, results of the analyses were not easy to interpret. Consequently, the first study to analyze both morphological and molecular data in concert (Carlini

et al., 2001) focused on relationships within Octobrachia, but due to a lack of agreement between morphological and molecular data, no new hypotheses were presented. Several recent studies have provided further data on families within Octopoda (Voight, 1997; Carlini et al., 2001; Piertney et al., 2003), but little information has been presented regarding relationships among many of the major groups within Decabrachia.

Given the many discrepancies among defining characters for cephalopods and that their evolution has likely proceeded with large variations in rates among different groups, it is impossible to construct a non-contradictory system based on a single organ or system (Nesis, 1998). The use of combined analyses has provided increased resolution within other "problematic" metazoan clades, particularly within arthropods (e.g., Giribet et al., 2001; Edgecombe et al., 2002), but also for other molluscan classes (e.g., Giribet and Wheeler, 2002). Due to the diverse nature of Cephalopoda, a combined approach is likely to provide further insight into both higher and lower-level relationships. It is the aim of this study to further refine the relationships within Cephalopoda and particularly Decabrachia by incorporating a combination of 101 morphological characters and DNA sequence data from four molecular loci, including two nuclear ribosomal genes, one nuclear protein coding gene and one mitochondrial protein coding gene. By analyzing all data simultaneously, a new hypotheses will be presented for the relationships within Cephalopoda.

Methods

Taxon sampling

Molecular and morphological data from five molluscan classes were analyzed (Tables 1, 2 and 3; Appendices 1 and 2 for voucher information): Caudofoveata (1 sp.), Solenogastres (2 spp.), Polyplacophora (4 spp.), Gastropoda (4 spp.), Bivalvia (4 spp.), Scaphopoda (3 spp.) and Cephalopoda (60 spp.). Cephalopod taxa were sampled from 34 taxonomically recognized families, representing all eight major orders (Tables 2 and 3). Samples from nine cephalopod families were not available for this study due to a lack of specimen availability. Preserved specimens used for molecular analysis were obtained from a number of sources (for collection data and repository institutions see Appendix 1). Specimens for morphological study are listed in Appendix 2.

Morphological characters

Morphological data were scored via the direct observation of cephalopod specimens, and in cases where specimens were unavailable, information was taken from the primary literature (Naef, 1921/23; Roper et al., 1969; Salvini-Plawen and Steiner, 1996; Young and Vecchione, 1996; Young and Harman, 1998), which resulted in 101 characters, described in Appendix 3 and coded in Table 3. Sperm characters were coded entirely from literature sources (Franzén, 1955, 1958; Maxwell, 1974, 1975; Healy, 1990a,b, 1993, 1996). Primary

Table 1
Outgroup taxa and accession numbers for each locus used in this study

	18S rRNA	28S rRNA	Histone H3	COI
Aplacophora				
Chaetoderma nitidulum	AY377658	AY377692	AY377763	AY377726
Heliocoradomenia sp.	AY21210	AY377688	AY377764	AY377725
Epimenia azuri	AY377657	AY377691	AY377765	AY377723
Polyplacophora				
Leptochiton asellus	AY377631	AY377662	AY377734	
Stenoplax alata	AY377644	AY377675	AY377748	AY377711
Chiton olivaceus	AY377651	AY377682	AY377755	AY377716
Acanthochitona crinita	AF120503	AF120566	AY377759	AF120627
Gastropoda				
Theodoxus fluviatilis	AF120515	AF120573		AF120633
Haliotis tuberculata	AF120511	AF120570	AY377775	AY377729
Crepidula fornicata	AY377660	AY377625	AY377778	AF353154
Siphonaria pectinata	X91973	AF120578	AY377627	AF120638
Bivalvia				
Yoldia limatula	AY070111	AF120585	AY377768	AF120642
Arca imbricata	AY654986	AY654987	AY654989	AY654988
Neotrigonia margaritacea	AF411690	AF411689	AY070155	AF56850
Cardita calyculata	AF120549	AF120610	AY070156	AF120660
Scaphopoda				
Rhabdus rectius	AF120523	AF120580	AY377772	AF120640
Antalis pilsbryi	AF120522	AF120579		AF120639
Entalina tetragona	AF490598			

Table 2
Cephalopod taxa and GenBank accession numbers for each locus used in this study. Classification based on Boletzky (1999). Sequences with an asterisk indicate those not obtained by the author

			18S rRNA	28S rRNA	Histone H3	COI
Nautiloidea (2 spp.)						
Nautilida	Nautilidae	*Nautilus pompilius*	AY557452	AF311688*		AY557514
		Nautilus scrobiculatus	AF120504*	AF120567*	AF033704*	
Coleoidea (58 spp.)						
Octobrachia						
Octopoda	Allopsidae	*Haliphron atlanticus*	AY557460	AY557549	AY557409	AY557516
		Haliphron sp.	AY557461	AY557550	AY557410	
	Argonautidae	*Argonauta nodosa*	AY557462	AY557551	AY557411	AY557517
	Bolitaenidae	*Japetella diaphana*	AY557463	AY557552		AY557518
	Ocythoidae	*Ocythoe tuberculata*	AY557464	AY557553		AY557519
	Octopodidae	*Bathypolypus arcticus*	AY557465	AY557554		*AF000029
		Benthoctopus sp.	AY557466	AY557555	AY557412	
		Eledone cirrosa	AY557467	AY557556		AY557520
		Grandeledone verrucosa	AY557468	AY557557	AY557413	*AF000042
		Thaumeledone guntheri	AY557469	AY557558	AY557414	AY557521
Cirroctopoda	Cirroteuthidae	*Cirrothauma murrayi*	AY557456	AY557545		*AF000034
		Stauroteuthis syrtensis	AY557457	AY557546	AY557406	*AF000067
	Opisthoteuthidae	*Opisthoteuthis* sp.	AY557458	AY557547	AY557407	AY557515
Vampyromorpha						
Vampyromorpha	Vampyroteuthidae	*Vampyroteuthis infernalis*	AY557459	AY557548	AY557408	*AF000071
Decabrachia						
Sepiolida	Sepiolidae	*Heteroteuthis hawaiiensis*	AY557472	AY293703	AY557416	*AF000044
		Stoloteuthis leucoptera	AY557475	AY293704	AY557419	*AF000068
		Sepiola affinis	AY557474	AY557562	AY557418	AY557523
		Rossia palpebrosa	AY557473	AY557561	AY557417	*AF000061
Sepiida	Sepiidae	*Sepia officinalis*	AY557471	AY557560	AY557415	*AF000062
		Sepiella inermis	AY557470	AY557559		AY557522
Spirulida	Spriulidae	*Spirula spirula*	AY557476	AY557563	AY557420	*AF000066
Idiosepiida	Idiosepiidae	*Idiosepius pygmaeus*	AY557477	AY293684	AY557421	*AF000046
Teuthida Myopsida	Loliginidae	*Loligo formosana*	AY557478	AY557564	AY557422	AY557524
		Loligo pealei	AY557479	AY557565	AY557423	*AF000052
		Sepioteuthis lessoniana	AY557480	AY557566	AY557424	AY557525
Teuthida Oegopsida	Ancistrocheiridae	*Ancistrocheirus lesueuri*	AY557491	AY557575		*AF000026
	Architeuthidae	*Architeuthis dux*	AY557482	AY557567	AY557426	*AF000027
	Bathyteuthidae	*Bathyteuthis abyssicola*	AY557483	AY557568	AY557427	*AF000030
	Batoteuthidae	*Batoteuthis skolops*	AY557484	AY557569	AY557428	AY557527
	Brachioteuthidae	*Brachioteuthis* sp.	AY557485	AY557570	AY557429	AY557528
	Chiroteuthidae	*Chiroteuthis veranyi*	AY557486			AY557529
	Chtenopterygidae	*Chtenopteryx sicula*	AY557481	AY293698	AY557425	AY557526
	Cranchiidae	*Cranchia scabra*	AY557487	AY557571	AY557430	*AF000035
		Leachia atlantica	AY557488	AY557572	AY557431	AY557530
	Cycloteuthidae	*Cycloteuthis syrventi*	AY557489	AY557573	AY557432	*AF000036
		Discoteuthis laciniosa	AY557490	AY557574	AY557433	*AF000037
	Enoploteuthidae	*Abraliopsis pfefferi*	AY557492	AY557576	AY557434	AY557531
		Enoploteuthis leptura	AY557493	AY557577	AY557435	AY557532
		Ornithoteuthis antillarum	AY557494	AY557578	AY557436	AY557533
	Gonatidae	*Gonatus antarcticus*	AY557497	AY557581	AY557439	AY557536
		Gonatus fabricii	AY557498	AY557582	AY557440	AY557537
	Histioteuthidae	*Histioteuthis corona*	AY557499	AY557583	AY557441	
		Histioteuthis hoylei	AY577500	AY557584	AY557442	*AF000045
		Histioteuthis reversa	AY577501	AY557585	AY557443	
	Joubiniteuthidae	*Joubiniteuthis portieri*	AY577502	AY557586	AY557444	*AF000048
	Lepidoteuthidae	*Lepidoteuthis grimaldii*	AY577503	AY557587	AY557445	*AF000049
	Mastigoteuthidae	*Mastigoteuthis agassizii*	AY577504	AY557588	AY557446	AY557538
		Mastigoteuthis magna	AY577505	AY557589	AY557447	AY557539
	Neoteuthidae	*Neoteuthis thielei*	AY577506	AY557590	AY557448	AY557540
	Octopoteuthidae	*Octopoteuthis nielseni*	AY557507	AY557591		*AF000055
		Octopoteuthis sicula	AY557508	AY557592	AY557449	AY557541
	Ommastrephidae	*Illex coindeti*	AY557509	AY557593	AY557450	AY557542
		Ommastrephes bartrami	AY557510	AY557594	AY557451	*AF000057
		Sthenoteuthis oualeniensis	AY557511	AY557595	AY557452	*AF000069
	Onychoteuthidae	*Moroteuthis knipovitchi*	AY557512	AY557596	AY557453	AY557543
	Psychroteuthidae	*Psychroteuthis* sp.	AY557513	AY557597	AY557454	AY557544
	Pyroteuthidae	*Pyroteuthis margaretifera*	AY557496	AY557580	AY557438	AY557535
		Pterygioteuthis gemmata	AY557495	AY557579	AY557437	AY557534

Table 3
Morphological data matrix of 101 characters

	1	11	21	31	41	51	61	71	81	91	
Chaetoderma nitidulum	000--0--0- --010----0	----------	-0-0----00	0000-0----	---1000---	---0-00100	1000-010-0	00000?0000	00000??000	0
Helicoradomenia sp.	000--0--0- --010----0	----------	-0-0----00	0000-0----	---0--0---	---0-?????	????-010-0	00000?0000	00000??000	0
Epimenia azuri	000--0--0- --010----0	----------	-0-0----00	0000-0----	---0--0---	---0-0?0??	1000-010-0	00000?0000	00000??000	0
Leptochiton asellus	000--0--0- --010----0	----------	-0-0----00	0000-0----	---1020---	---0-00000	1000-110-0	0000000000	0000001100	0
Stenoplax alata	000--0--0- --010----0	----------	-0-0----00	0000-0----	---1020---	---0-?????	????-110-0	0000000000	0000001100	0
Chiton olivaceus	000--0--0- --010----0	----------	-0-0----00	0000-0----	---1020---	---0-10000	1001-110-0	0000000000	0000001100	0
Acanthochitona crinita	000--0--0- --010----0	----------	-0-0----00	0000-0----	---1020---	---0-10000	1001-110-0	0000000000	0000001100	0
Theodoxus fluviatilis	100--0--0- --010----0	----------	-0-0----00	1001-0----	---1030---	---0-?????	????000111	0110101000	0101011000	0
Haliotis tuberculata	100--0--0- --010----0	----------	-0-0----00	1001-0----	---1000---	---0-00000	1000000111	0110101000	0101011000	0
Crepidula fornicata	100--0--0- --010----0	----------	-0-0----00	1001-0----	---1030---	---0-?????	????000111	0110101000	0101011000	0
Siphonaria pectinata	100--0--0- --010----0	----------	-0-0----00	1001-0----	---10?0---	---0-00000	100?000111	0110101000	0101011000	0
Yoldia limatula	100--0--0- --000----0	----------	-0-0----00	1001-0----	---1000---	---0-?????	????200000	1001-00010	1010121000	0
Arca imbricata	100--0--0- --000----0	----------	-0-0----00	1001-0----	---1000---	---0-00000	1000200000	1001-10011	1010121000	0
Neotrigonia margaritacea	100--0--0- --000----0	----------	-0-0----00	1001-0----	---1000---	---0-00000	1000200000	1001-10011	1010121000	0
Cardita calyculata	100--0--0- --000----0	----------	-0-0----00	1001-0----	---1000---	---0-00000	1000200000	1001-10011	1010121000	0
Rhabdus rectius	100--0--0- --010----0	----------	-0-0----00	1001-0----	---0--0---	---0-?????	????100000	0000001010	1100011001	1
Antalis pilsbryi	100--0--0- --010----0	----------	-0-0----00	1001-0----	---0--0---	---0-00000	1000100000	0000001010	1100011001	1
Entalina tetragona	100--0--0- --010----0	----------	-0-0----00	1001-0----	---0--0---	---0-?????	????100000	0000001010	1100011001	1
Nautilus pompilius	110--0–01- -111??000-	---0-----0	001-0-1111	01100??00-	001?11220-	--12000110	00?0000010	0000?11001	1000101100	0
Nautilus scrobiculatus	110--0–01- -111??000-	---0-----0	001-0-1111	01100??00-	001?11220-	--12??????	???0000010	0000?11001	1000101100	0
Cirrothauma murrayi	0012-10010	--10120111	00020-----	000110-010	1011112212	1?01100001	?0011?????	????-001-1	00000?1100	1100010100	1
Stauroteuthis syrtensis	0012-10010	--10120111	00020-----	000110-010	1011112212	1201100001	?0011?????	????-001-1	00000?1100	1100010100	1
Opisthoteuthis sp.	0012-10010	--10120111	00020-----	000110-010	101111?212	1001100001	?001100010	1000-001-1	00000?1100	1100010100	1
Haliphron sp.	0013-00010	--11120001	00000-----	000101-011	121111?21?	1101100201	01010?????	????-001-1	00000?1100	1100010100	1
Haliphron atlanticus	0013-00010	--11120001	00000-----	000101-011	121111?21?	1101100201	01010?????	????-001-1	00000?1100	1100010100	1
Argonauta nodosa	001--00010	--11120001	00000-----	0001?1?011	1211112211	1101100201	01010?????	????-001-1	00000?1100	1100010100	1
Japetella diaphana	001--00010	--11120?01	00020-----	000100-011	1211112211	1101100201	00010?????	????-001-1	00000?1100	1100010100	1
Ocythoe tuberculata	0013-00010	--11120001	00000-----	0001?1?011	1211112211	1101100201	01010?????	????-001-1	00000?1100	1100010100	1
Bathypolypus arcticus	0013-00010	--11120001	00000-----	000100-010	1211112211	1101100201	01010?????	????-001-1	00000?1100	1100010100	1
Benthoctopus sp.	0013-00010	--11120001	00000-----	000100-010	1211112211	1101100201	01010?????	????-001-1	00000?1100	1100010100	1
Eledone cirrosa	0013-00010	--11120001	00020-----	000100-011	1211112211	1101100201	0101000000	1000-001-1	00000?1100	1100010100	1
Graneledone verrucosa	0013-00010	--11120001	00020-----	000100-010	1211112211	1101100201	01010?????	????-001-1	00000?1100	1100010100	1
Thaumeledone guntheri	0013-00010	--11120001	00020-----	000100-010	1211112211	1101100201	01010?????	????-001-1	00000?1100	1100010100	1
Vampyroteuthis infernalis	0?11011210	--11110011	1?020-----	000100-111	101111111?1	1101000220	0101001000	1000-001-1	00000?1100	1100010100	1
Sepiella inermis	0110010111	1011101001	2110010000	0001110111	1111010000	0101001010	10110?????	????-001-1	00000?1100	1100010100	1
Sepia officinalis	0110-10111	1011101001	2110010000	0001110111	1111010000	0101001010	1011000110	0101-001-1	00000?1100	1100010100	1
Heteroteuthis hawaiiensis	0014010011	1011101001	2110000000	1001110111	1111010000	0101001010	1001000000	0010-001-1	00000?1100	1100010100	1
Rossia palpebrosa	0011010011	1011101001	2110000000	0001110111	1111010000	0101001010	1001000110	0101-001-1	00000?1100	1100010100	1
Sepiola affinis	001?010011	1011101001	2110000000	1001110111	1111010000	0101001010	10010?????	????-001-1	00000?1100	1100010100	1
Stoloteuthis leucoptera	0014010111	1011101001	2110000000	1001110111	1111010000	0101001010	10010?????	????-001-1	00000?1100	1100010100	1
Spirula spirula	0110-10011	1011101001	2111100000	0001110111	1011010000	0101001010	0011000000	0010-001-1	00000?1100	1100010100	1
Idiosepius pygmaeus	0014010010	?011101001	2110000000	000111?111	1111010000	0101001110	00110?????	????-001-1	00000?1100	1100010100	1
Loligo formosana	0011010111	1111101001	2110010000	0001110111	1111010001	0101001010	0011000110	0101-001-1	00000?1100	1100010100	1
Loligo pealei	0011010111	1111101001	2110010000	0001110111	1111010001	0101001010	0011000110	0101-001-1	00000?1100	1100010100	1
Sepioteuthis lessoniana	0011010111	1111101001	21100?0000	0001110111	1111010001	0101001010	00110?????	????-001-1	00000?1100	1100010100	1
Chtenopteryx sicula	0011010111	1111101001	2111110000	0001110111	1011010001	0101001?10	00010?????	????-001-1	00000?1100	1100010100	1
Bathyteuthis abyssicola	0011010111	0111101001	2111110000	0001110111	1011010001	0101001210	00010?????	????-001-1	00000?1100	1100010100	1

Architeuthis dux	00111101111 0?11101001 21100000002 00011101111 10111010001 01010010210 00110????? ????-001-1 00000?1100 11000101001
Batoteuthis skolops	00112111111 1?11101001 21100100000 00011131111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Brachioteuthis sp.	01131021111 11111010012 11010000020 00111101111 01101000010 10100121100 00102????? ????-001-1 00000?1100 11000101001
Chiroteuthis veranyi	00112111111 11111010001 21100000000 00011131111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Cranchia scabra	00113100111 11111010001 21100000002 000120-111 10111010001 01110011210 00110????? ????-001-1 00000?1100 11000101001
Leachia atlantica	00113100111 11111010001 21100000002 000120-111 10111010001 01110011210 00110????? ????-001-1 00000?1100 11000101001
Cycloteuthis sirventi	00113111111 11111010001 21100000002 00011111111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Discoteuthis laciniosa	00113111111 11111010001 21100000002 00011111111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Ancistrocheirus lesueuri	00111101111 02111010001 21100001101 00011101111 10111010001 01010010210 00110????? ????-001-1 00000?1100 11000101001
Abraliopsis pfefferi	00111101111 02111010001 21100001101 00111101111 10111010001 01010000210 00110????? ????-001-1 00000?1100 11000101001
Enoploteuthis leptura	00111101111 02111010001 21100001101 00111101111 10111010001 01010000210 00110????? ????-001-1 00000?1100 11000101001
Pterygioteuthis gemmata	00111101111 02111010001 21100001101 00011101111 10111010001 01010011210 00110????? ????-001-1 00000?1100 11000101001
Pyroteuthis margaretifera	00113101111 02111010001 211?001101 00111101111 10111010001 01010011210 00110????? ????-001-1 00000?1100 11000101001
Gonatus antarcticus	00111101111 11111010001 21111011102 00011101111 10111010001 01010011210 00110????? ????-001-1 00000?1100 11000101001
Gonatus fabricii	00111101111 11111010001 2111-011102 00011101111 10111010001 01010011210 00110????? ????-001-1 00000?1100 11000101001
Histioteuthis corona	00110101111 01111010001 21101100002 01011101111 10111010001 01010011210 10010????? ????-001-1 00000?1100 11000101001
Histioteuthis hoylei	00110101111 01111010001 21101100002 01011101111 10111010001 01010011210 10010????? ????-001-1 00000?1100 11000101001
Histioteuthis reversa	00110101111 01111010001 21101100002 01011101111 10111010001 01010011210 10010????? ????-001-1 00000?1100 11000101001
Joubiniteuthis portieri	00112111111 11111010001 21111100000 00011113111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Lepidoteuthis grimaldii	00112101111 11111010001 2110?000010 00011101111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Mastigoteuthis agassizii	00112111111 11111010001 21101100000 00011113111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Mastigoteuthis magna	00112111111 11111010001 21101100000 00011113111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Neoteuthis thielei	00111101111 01111010001 2110A00002 00011101111 10111010001 01010011211 00?10????? ????-001-1 00000?1100 11000101001
Octopoteuthis nielseni	00112100111 10111010001 2110-01-1- 00?1110111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Octopoteuthis sicula	00112100111 10111010001 2110-01-1- 00?1110111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Illex coindeti	00111101111 01111010001 21101100002 00011121111 10111010001 01010011210 00110????? ????-001-1 00000?1100 11000101001
Ommastrephes bartrami	00111101111 01111010001 21100000002 00011121111 10111010001 01010011210 00110????? ????-001-1 00000?1100 11000101001
Ornithoteuthis antillarum	00111101111 01111010001 21100000002 00011121111 10111010001 01010011210 00110????? ????-001-1 00000?1100 11000101001
Sthenoteuthis oualaniensis	00111101111 01111010001 21100000002 00011121111 10111010001 01010011210 00110????? ????-001-1 00000?1100 11000101001
Moroteuthis knipovitchi	00111101111 11111010001 21100000102 00?1110111 10111010001 01010011210 00010????? ????-001-1 00000?1100 11000101001
Psychroteuthis sp.	00113102?11 00111010001 21101000002 00011102?11 10111010001 01010011210 00?10????? ????-001-1 00000?1100 11000101001

literature sources were also used to score outgroup characters (Giribet and Wheeler, 2002; Salvini-Plawen and Steiner, 1996; Ponder and Lindberg, 1997; Waller, 1998; Haszprunar, 2000; Haszprunar and Wanniger, 2000). Morphological character data were summarized for each terminal taxon where possible using MacClade (Madison and Madison, 2000); in a few cases where codings were based on related species or primary literature, notations were made in the character description section. Multiple specimens for each family were examined in an attempt to eliminate coding irregularities. Irregularity in specimen morphology could arise genetically via mutation events or perhaps as a result of damage during collection, making it important to establish the character states by examining multiple organisms.

Molecular loci

PCR amplification and sequencing.

DNA was isolated from small pieces of mantle, gill, gonad, or arm tissue of previously identified specimens. DNA extraction was performed using the Qiagen DNeasy Tissue Kit (Qiagen©, Valencia, CA). Upon isolation, the purified total DNA template was used for PCR amplification of four molecular loci: nuclear 18S rRNA (1900–2800 bp), the D3 expansion fragment of 28S rRNA (400–600 bp) and histone H3 (327 bp), as well as a 679 bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI). Several primer sequences, described in Table 4, were obtained from primary literature (Folmer et al., 1994; Giribet et al., 1996; Whiting et al., 1997; Colgan et al., 1998) or designed specifically for this study. The complete 18S rRNA (1.8–2.8 kb) was amplified and sequenced in three overlapping fragments of approximately 800–1000 bp in length using primer pairs: 1F/4R, 3bf/18Sbi, 18sa2.0/9R. Additional primers (4bf, 5bf, 5br, 18Sa2.0R, 7F, 7R) were used in samples that were difficult to amplify. PCR amplification, cleanup and sequencing were performed as described in Nishiguchi et al. (2004).

Sequence editing and fragmentation.

Resulting chromatograms were edited and joined into contiguous sequences using Sequencher v. 4.1 (Gene Codes™, Ann Arbor, MI). Complete sequences were visualized and partitioned using the Genetic Data Environment (GDE) software (Smith et al., 1994). External primers (1F/9R for 18S rRNA and standard primer sequences for all other loci) were excluded from the analyses. For non-coding genes (18S rRNA, 28S rRNA), sequences were initially partitioned in GDE using secondary structure models, unambiguous regions and internal primers as described in Giribet and Wheeler (2001). GDE was further used to examine individual

Table 4
Primer sequences obtained from literature; 18S rRNA (Giribet et al., 1996; Whiting et al. 1997), 28S rRNA (Whiting et al., 1997), COI (Folmer et al., 1994) and H3 (Colgan et al., 1998). Primers marked with an asterisk indicate cephalopod-specific primers designed for this study by the authors. Annealing temperature indicates a range over which successful loci were amplified. See Nishiguchi et al. (2004) and Giribet and Wheeler (2002) for a further description of PCR amplification

Primer		Annealing temperature
18S rRNA (Primer pairs commonly used: 1F/4R; 3bf/18Sbi; 18Sa2.0/9R). Other primers listed were used in hypervariable internal regions.		35–49 °C
1F	5′- TAC CTG GTT GAT CCT GCC AGT AG -3′	
3R	5′- AGG CTC CCT CTC CGG AAT CGA AC -3′	
4R	5′- GAA TTA CCG CGG CTG CTG G -3′	
3bf*	5′- GGG TCC GCC CTA TCA ACT G -3′	
4bf*	5′- CCG CGA TCG GAA TGA GTA CAC -3′	
5bf*	5′- GCA TTC CCG GCC CTT -3′	
5br*	5′- GAC CAC CCT TGG AGG AGA AA -3′	
18Sbi	5′- GAG TCT CGT TCG TTA TCG GA -3′	
7R	5′- GCA TCA CAG ACC TGT TAT TGC -3′	
18Sa2.0rev*	5′- GTT TCA GCT TTG CAA CCA T -3′	
18Sa2.0	5′- ATG GTT GCA AAG CTG AAA C -3′	
7F	5′- GCA ATA ACA GGT CTG TGA TGC CC -3′	
9R	5′- GAT CCT TCC GCA GGT TCA CCT AC -3′	
28S rRNA		37–40 °C
28Sa	5′- GAC CCG TCT TGA AAC ACG GA -3′	
28Sb	5′- TCG GAA GGA ACC AGC TAC -3′	
Cytochrome *c* oxidase subunit I (COI)	35–39 °C	
LCO1490	5′- GGT CAA CAA ATC ATA AAG ATA TTG G -3′	
HCO2198	5′- TAA ACT TCA GGG TGA CCA AAA AAT CA -3′	
Histone H3		37–42 °C
H3a F	5′- ATG GCT CGT ACC AAG CAG AC(ACG) GC -3′	
H3a R	5′- ATA TCC TT(AG) GGC AT(AG) AT(AG) GTG AC -3′	

sequences to identify regions with large insertions or deletions. To account for the high degree of variability in sequence length (with indels up to 500 bp in some cases) and the large size of the nuclear genes, 18S rRNA was partitioned into 30 fragments and 28S rRNA in three fragments. For the protein-coding gene COI, sequences were partitioned into four sections due to the presence of length variability in some species. Histone H3 (also protein-coding) was not fragmented since no sequence length variation was present and therefore was treated as "prealigned" (command -prealigned) in the analysis. Sequences with no length variation can be treated as prealigned because they require no insertion of gaps during alignment. A number of hypervariable regions within the ribosomal genes (18S rRNA, 28S rRNA) were excluded from the analyses because they are extremely difficult to align, can be uninformative and may introduce conflict into the analyses (Giribet et al., 2000). These fragments may show considerable variation even among members of the same species. Fragmented sequences, as well as a list of those fragments removed are available at http://biology-web.nmsu.edu/Faculty&Staff/Nishiguchi/Nishiguchi.htm.

Phylogenetic analysis

Morphological data analysis.

Morphological data were analyzed with parsimony in NONA v. 2.0 (Goloboff, 1998), with 1000 random addition sequence replicates (RAS) followed by tree bisection and reconnection (TBR) branch swapping. In order to avoid spending too much time searching tree space in suboptimal islands, the number of trees held per replicate was limited to 10. Strict consensus calculations and character optimization were completed using Winclada v. 1.00.08 (Nixon, 2002). Character optimizations calculated in Winclada only show unambiguous changes. Nodal support was determined using jackknifing (Farris et al., 1996; Farris, 1997), where jackknife proportions were calculated from 1000 replicates using 10 RAS + TBR in Winclada/Nona.

Molecular and combined analysis.

Molecular and combined data were analyzed with the computer program POY (Wheeler et al., 2002) using the direct optimization method (Wheeler, 1996) with parsimony as the optimality criterion. Independent sets of analyses were executed in POY for each of the following data sets: COI, H3, ribosomal (18S rRNA + 28S rRNA), and for all molecules simultaneously (COI, H3, 18S, 28S). Although COI and H3 are protein-coding genes, fragments were analyzed at the DNA level. Lastly, all molecular and morphological data were analyzed simultaneously, referred to in the text as total evidence and this is taken as our preferred hypothesis for explaining the evolution of all characters simultaneously. Nodal support was calculated in POY using Farris's parsimony jackknifing procedure (Farris et al., 1996) for 100 replicates (using the commands -jackboot -replicates 100).

Tree searches were conducted in parallel at Harvard University on a 19 dual-processor cluster (darwin.oeb.harvard.edu) using pvm (parallel virtual machine). Commands for load balancing of spawned jobs were used to optimize parallelization procedures (-parallel -dpm -jobspernode 2). Trees were built via a random addition sequence procedure (10 replicates) followed by a combination of branch-swapping steps (SPR "subtree pruning and regrafting" and TBR "tree bisection and reconnection") and tree fusing (Goloboff, 1999) in order to further improve on tree length minimization. Discrepancies between heuristic and actual tree length calculations were addressed by adjusting slop values (-slop5 -checkslop10).

Each one of the five partitions was analyzed under 12 parameter sets for a variety of indel/change costs and transversion/transition ratios, where change costs refer to the highest nucleotide transformation (as in Wheeler, 1995). Gap/transversion ratios of 1 and 2 as well as transversion/transition ratios of 1, 2 and 4 were explored, although the extension of gaps was also downweighted with respect to the first occurrence of an indel event. These 12 parameter sets were considered a starting point for testing the stability of phylogenetic hypotheses (Giribet, 2003). Further increasing the weight ratios for transformation and indels would generate topologies with higher amounts of incongruence, are uninformative and computationally expensive.

For this study we chose to do sensitivity analysis (Wheeler, 1995) and stability analysis (Giribet, 2003). A sensitivity analysis was conducted to determine the degree of character incongruence among different parameter sets; the parameter that minimized incongruence was then chosen as the optimal parameter (similar to identifying the shortest tree). Character incongruence was measured using a modified version (Wheeler and Hayashi, 1998) of Incongruence Length Difference (ILD) metric (Mickevich and Farris, 1981; Farris et al., 1995). The ILD value was calculated by subtracting the sum of individual trees from the length of the combined data tree and dividing the result by the length of the combined data:

$$\mathrm{ILD} = (\mathrm{Length}_{\mathrm{Combined}} - \mathrm{Sum\ Length}_{\mathrm{Individual\ Data\ Sets}})/\mathrm{Length}_{\mathrm{Combined}}$$

Results

Morphological analyses

The search adopted in NONA yielded 665 trees of shortest tree length (190 steps; CI = 0.668; RI =

0.935; RC = 0.625), which was found in 13.4% of the replicates performed. These trees were subjected to a subsequent round of TBR with a total of 727 retained. The strict consensus of the morphological cladograms (Fig. 1) shows monophyly for all molluscan classes represented, and monophyly of Cephalopoda is furthermore supported in 99% of jackknife replicates. Within Cephalopoda, Nautiloidea and Coleoidea were also supported as monophyletic, but relationships within Coleoidea remain unresolved to a large degree, except for Vampyromorpha + Octobrachia (79% jackknife support). A Vampyromorpha + Octobrachia clade was supported by several characters, such as the presence of unmodified arms IV (Appendix 3, character 17), outer statocyst capsules (character 45), radial sucker symmetry (character 23) (although assumptions do exist within some of these characters, see Appendix 3). Other ordinal relationships supported were Cirroctopoda + Octopoda (found in 65% of jackknife replicates), but Decabrachia was not found to be monophyletic due to lack of resolution in basal nodes (Fig. 1).

While further resolution was found in Decabrachia, none of the fundamental trees supported monophyly of Teuthida, or Oegopsida. Furthermore, sepioids (Sepiolidae, Sepiidae, *Spirula* and Idiosepiidae) were paraphyletic with respect to Loliginidae. None of the relationships involving sepioid taxa received jackknife values above 50%. Within Oegopsida, several clades suggested relationships among oceanic cephalopods. One such clade is the enoploteuthid family complex proposed by Young and Harman (1998), comprised here of Ancistrocheiridae + *Pterygioteuthis* + *Pyroteuthis* + Enoploteuthidae. Characters supporting this relationship include buccal membrane attachment (character 11), buccal lappet number (character 12) and the presence of a tentacle locking apparatus (character 30). The position of *Pyroteuthis* + Enoploteuthidae was supported by the presence of photophores containing collagen light guides (character 33).

Other interesting clades within decabrachians included Bathyteuthidae + Chtenopterygidae (both exhibit suckers on buccal membrane; Appendix 3; character 26) and Chiroteuthidae + Mastigoteuthidae + Batoteuthidae + Joubiniteuthidae (these families have an oval funnel locking apparatus with projecting knobs; character 36). Lastly, several decabrachians were united by the presence of a primary conus (character 5): Architeuthidae + Neoteuthidae + Ommastrephidae + Onychoteuthidae + Gonatidae + Enoploteuthidae (except for Pyroteuthidae, which has a pseudoconus).

Congruence analysis

The parameter set that minimized overall character incongruence for the simultaneous analysis of all data consisted of an opening gap cost of 2 (extension gap was fixed at 1) and any other transformation costs set to 2 (parameter set 2221). This resulted in an ILD value of 0.0416 (Table 5). A second parameter set with gap opening cost of 2 (extension gap of 1) and any other changes receiving a cost of 1 had a similar ILD value of 0.0439 (parameter set 2111). The lowest ILD value for the molecular-only analysis consisted of an opening gap cost of 4 (extension gap of 1) and any other changes receiving a cost of 1 with an ILD value of 0.0304 (parameter set 4111).

Partitioned analyses

COI.

The COI tree for the overall optimal parameter set (2221) provided a single tree of 10 671 weighted steps, after tree fusing (Fig. 2). This tree does not provide support for the monophyly of any molluscan classes investigated. Within cephalopods, monophyly was shown for Cirroctopoda and Decabrachia. Very few relationships were supported in the jackknife analysis; those with jackknife values greater than 50% were primarily associated with closely related genera, but Decabrachia were monophyletic under all explored parameter sets. Sepioids were not monophyletic, forming a clade with ommastrephids and loliginids. Previous investigations with COI (Carlini and Graves, 1999) have shown that this gene may be too variable to provide a great deal of useful information alone.

Histone H3.

Analyses of the overall optimal parameter set for histone H3 yielded eight trees of 2240 weighted steps; the best tree length was found in three replicates and not improved after tree fusing. The strict consensus of these eight trees did not show monophyly for any classes investigated (Fig. 3). In the case of cephalopods, *Nautilus* clustered within a clade containing a gastropod and two aplacophorans. However, monophyly was shown for Coleoidea and Decabrachia. Again, sepioids were not monophyletic because Spirulida formed a clade with Bathyteuthidae and Chtenopterygidae. Jackknife support for the monophyly of Lepidoteuthidae + Octopoteuthidae + Neoteuthidae + Cycloteuthidae + Batoteuthidae + Histioteuthidae was 94%. A Gonatidae + Ommastrephidae clade was also supported in the histone analysis (78% jackknife support). The strict consensus of all parameter sets for the histone H3 data set supports few deep relationships within cephalopods, except for Cirroctopoda.

Combined ribosomal data.

The optimal parameter set for the combined ribosomal data (18S rRNA, 28S rRNA) yielded 100 trees of 4029 weighted steps. The best tree length was obtained

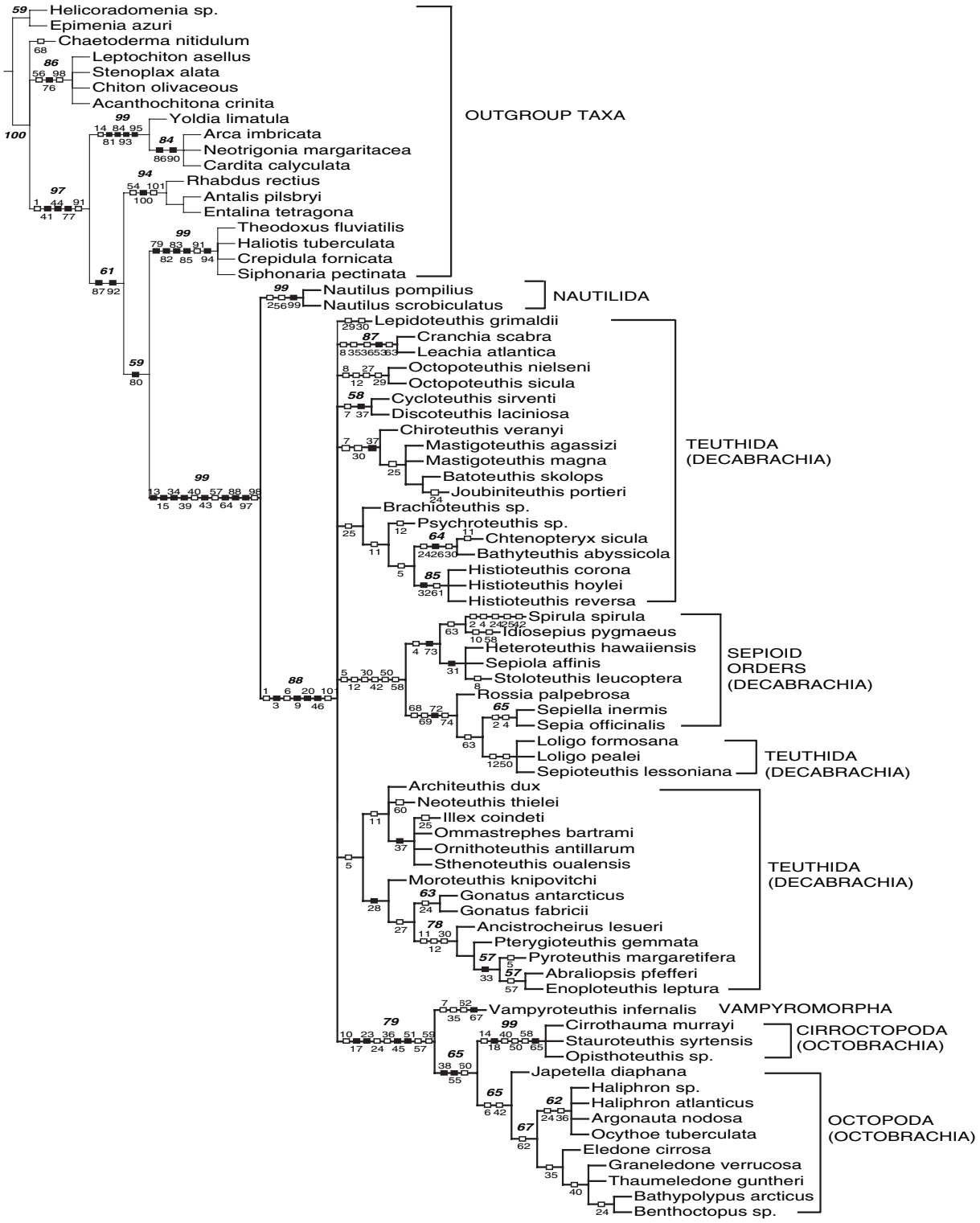

Fig. 1. Strict consensus of 665 trees (190 steps; CI = 0.668; RI = 0.935; RC = 0.625). Bold italic numbers above branches indicate jackknife support values greater than 50% calculated in Winclada/Nona. Unambiguous character optimizations calculated in Winclada are shown at each node. Black boxes on branches indicate character states present only in a given clade (hypothetical synapomorphies); white boxes indicate homoplastic character states.

Table 5
Weighted tree lengths for the individual and combined analyses at different gap/tv and tv/ts cost ratios and ILD values for the combined molecular (mol) and total evidence (total) data sets. Other abbreviations: rib (= 18S rRNA +28S rRNA), mor (= morphology)

			Individual			Combined		ILD values	
gap/tv	tv/ts	rib	COI	H3	mor	mol	total	mol	total
1	8	1673	4840	740	380	7548	8084	0.0391	0.0558
1	1	4029	10671	2240	380	17504	18071	0.0322	**0.0416**
1	2	2949	7882	1514	380	12749	13309	0.0317	0.0439
1	4	4459	12696	2275	760	20103	21176	0.0335	0.0466
2	8	1051	2452	370	380	4035	4529	0.0401	0.0609
2	1	2279	5393	1120	380	9069	9607	0.0305	0.0453
2	2	3228	7943	1514	760	13098	14140	0.0315	0.0492
2	4	4989	12824	2275	1520	20812	22840	0.0348	0.0539
4	8	1236	2471	370	760	4243	5157	0.0391	0.0621
4	1	2489	5414	1120	760	9306	10317	**0.0304**	0.0518
4	2	3603	7957	1514	1520	13533	15439	0.0339	0.0547
4	4	5736	12911	2275	3040	21727	25433	0.0371	0.0578

in three replicates and although the number of trees (100) was the buffer limit, we used the command -fitchtrees, which stores more trees than set by the limit and selects the 100 most diverse ones. No shorter trees were found after a final round of tree fusing. Strict consensus of these 100 trees (Fig. 4) illustrates monophyly for Bivalvia, Scaphopoda, Polyplacophora and Cephalopoda, but not Gastropoda. Of all individual analyses, the combined ribosomal tree provided the least amount of backbone resolution. Coleoidea was monophyletic, with Nautiloidea as its sister group. Two cirroctopod species were sister to all other coleoids, but the third cirroctopod (*Opisthoteuthis*) nested within the Decabrachia + Vampyromorpha clade. Family level resolution was minimal, except for the clade that formed *Moroteuthis* + *Neoteuthis* + *Architeuthis*. When results from all the parameter sets are combined, the consensus tree had no resolution.

Combined molecular data.

The optimal parameter set for the combined molecular data (4111) yielded 12 trees with a minimal length of 9306 weighted steps after tree fusing. The strict consensus of these trees (Fig. 5) illustrates monophyly for Cephalopoda, Bivalvia, Polyplacophora and Scaphopoda. The cephalopods were divided into Nautiloidea and Coleoidea, the latter clade divided into Octobrachia and Decabrachia + Vampyromorpha (rather than Vampyromorpha + Octobrachia). Teuthida as well as Oegopsida were polyphyletic. However, Myopsida did not form a clade with the sepioid orders, grouping with Cranchiidae, Ancistrocheridae and Onychoteuthidae. This relationship was supported in less than 50% jackknife replicates. Sepioids (except Spirulida) were monophyletic and sister to an Enoploteuthidae + Onychoteuthidae clade. Spirulida was found sister to the oegopsid clade containing Mastigoteuthidae + Joubiniteuthidae. The enoploteuthid families proposed by Young and Harman (1998) were not monophyletic; however, a close relationship between *Pterygioteuthis* and *Pyroteuthis* was supported in 79% of jackknife replicates. Jackknife support for the deepest divergences within Cephalopoda show values above 70%, and these divergences correlated with stable relationships when evaluating all parameter sets explored thus far. It is especially interesting to note the stability of a relationship between *Vampyroteuthis* and Decabrachia (also with a jackknife frequency of 74%). Other groups supported under all analytical parameter sets were Octopoda + *Opisthoteuthis*, Decabrachia, or Bathyteuthidae + Chtenopterygidae.

Total evidence.

When all morphological and molecular data were combined, the most congruent data set (ILD = 0.0416) was where all parameter sets received equal weights, with the exception of extension gaps (parameter set 2221). Under such a parameter scheme, three replicates generated trees of length 18 073 but after tree fusing, two trees of 18 071 weighted steps were saved. The strict consensus of the optimal parameter set is shown in Fig. 6. With respect to outgroups, Scaphopoda, Solenogastres, Polyplacophora and Bivalvia were monophyletic. However, no solid conclusion between outgroups and cephalopods can be reached at this point.

Cephalopoda, Coleoidea and Nautiloidea were found to be monophyletic under all parameter sets and in 100% jackknife replicates. Within Coleoidea, a monophyletic Octopoda + *Opisthoteuthis* and Decabrachia were also supported. In the optimal parameter tree, cirroctopods were not nested within octopods (except for *Opisthoteuthis*); instead they formed a sister group to Vampyromorpha + Decabrachia. Sepioids (except Spirulida) formed a clade sister to the myopsid Loliginidae. Spirulida formed a clade with Bathyteuthidae and Chtenopterygidae. With the exception of a clade formed

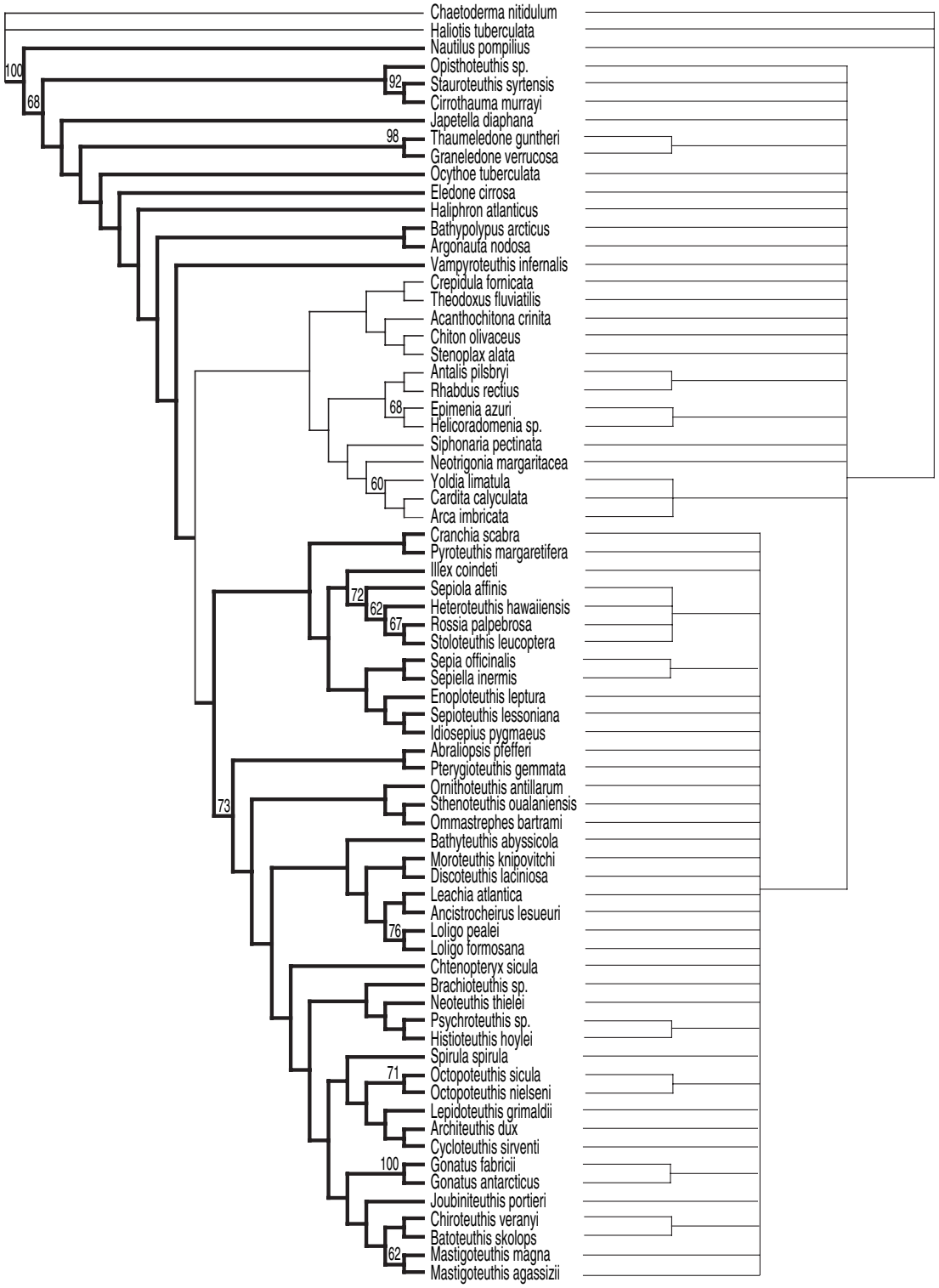

Fig. 2. Tree on the left represents the single tree of 10 671 weighted steps for the COI data set obtained under the optimal parameter set (2221). Branches in bold indicate cephalopod taxa. Numbers above branches indicate jackknife support values above 50%. Right cladogram is a strict consensus of all trees obtained for the 12 parameter sets explored.

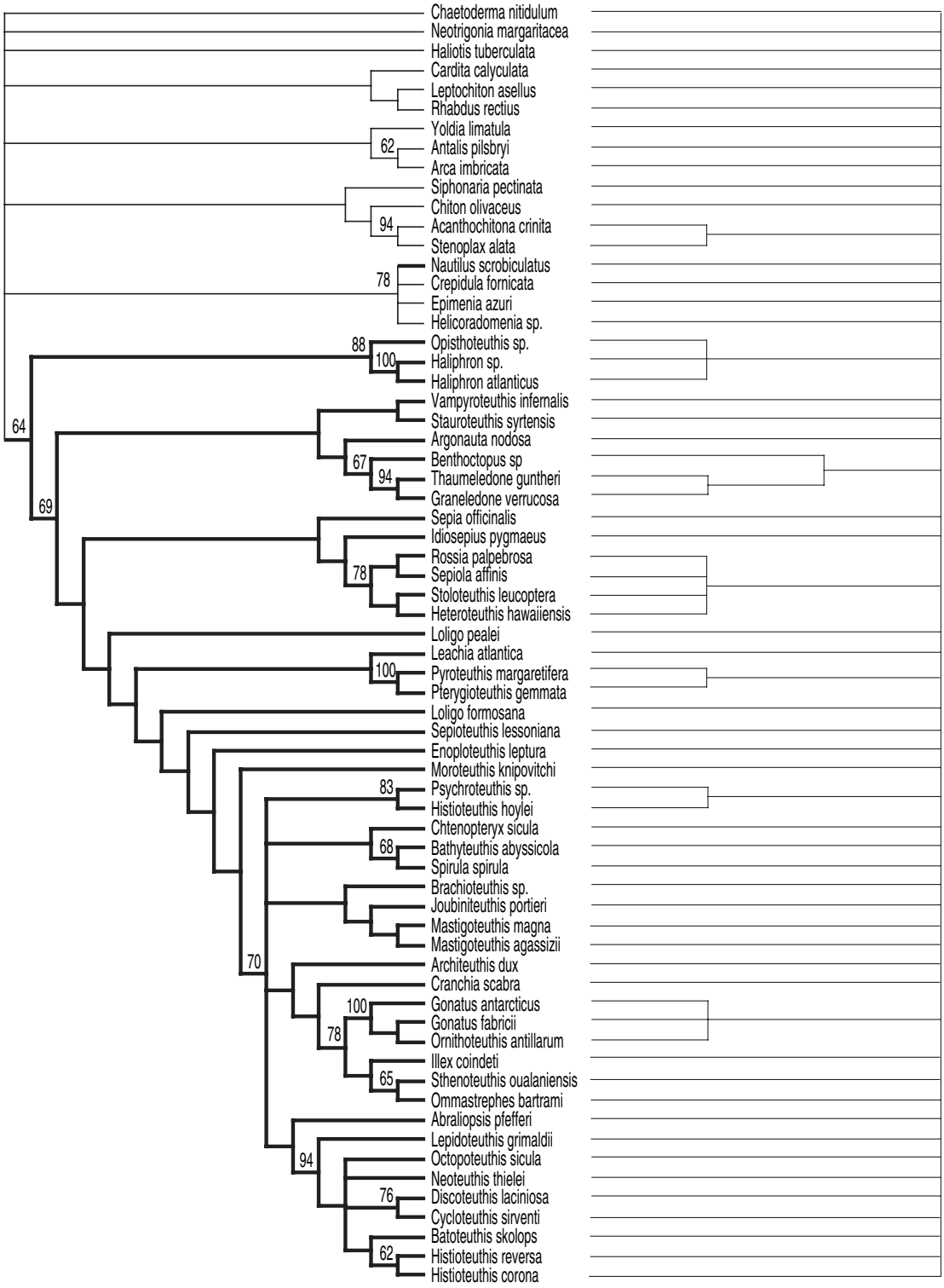

Fig. 3. Left tree shows the strict consensus of eight trees of 2240 weighted steps for the H3 data set yielded by the optimal parameter set (2221). Branches in bold indicate cephalopod taxa. Numbers above branches indicate jackknife support values above 50%. Right cladogram is a strict consensus of all trees obtained for the 12 parameter sets explored.

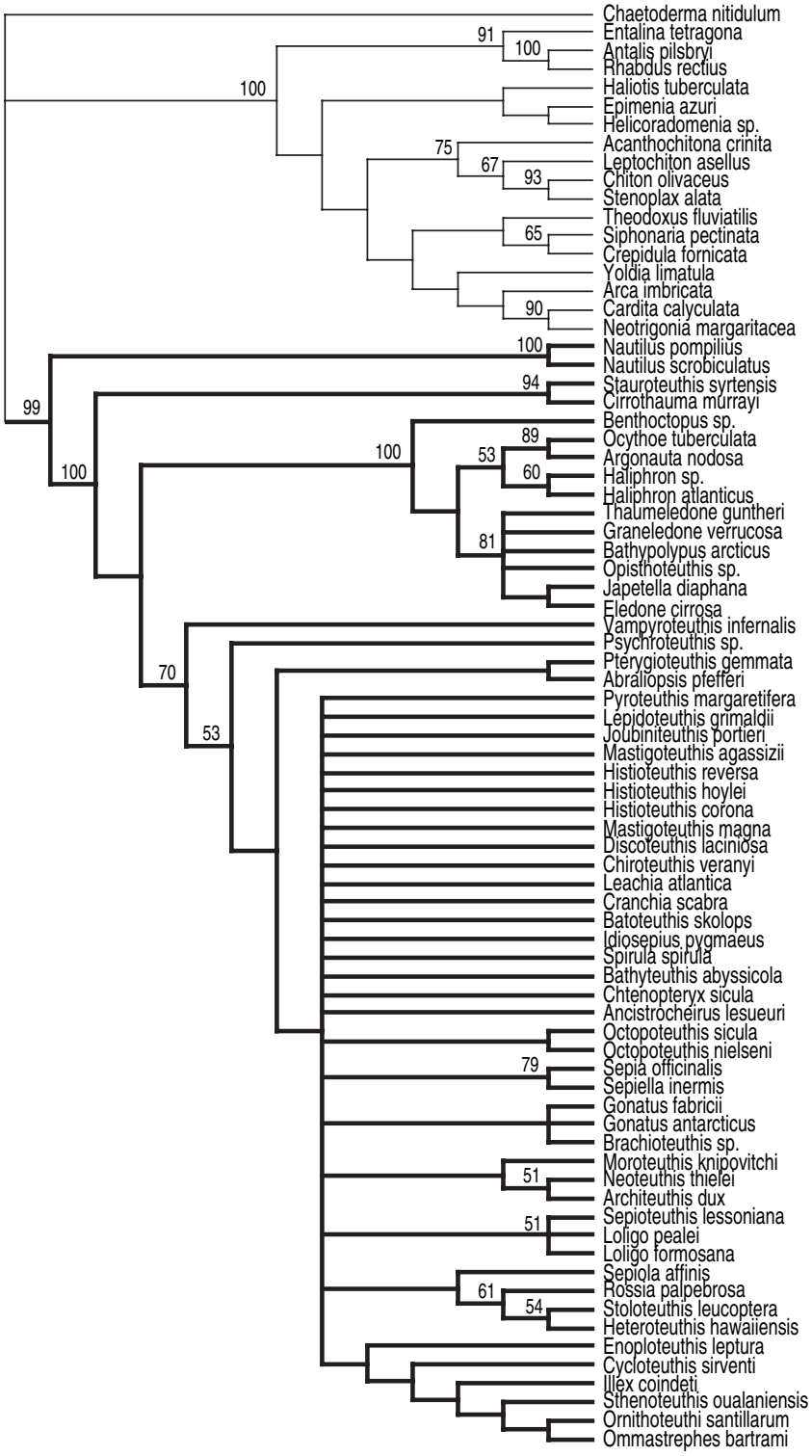

Fig. 4. Strict consensus of 101 trees at 4029 weighted steps for the combined ribosomal (18S rRNA and 28S rRNA) data yielded by the optimal parameter set (2221). Branches in bold indicate cephalopod taxa. Numbers above branches indicate jackknife support values above 50%. No resolution was found for the strict consensus of 12 parameters explored.

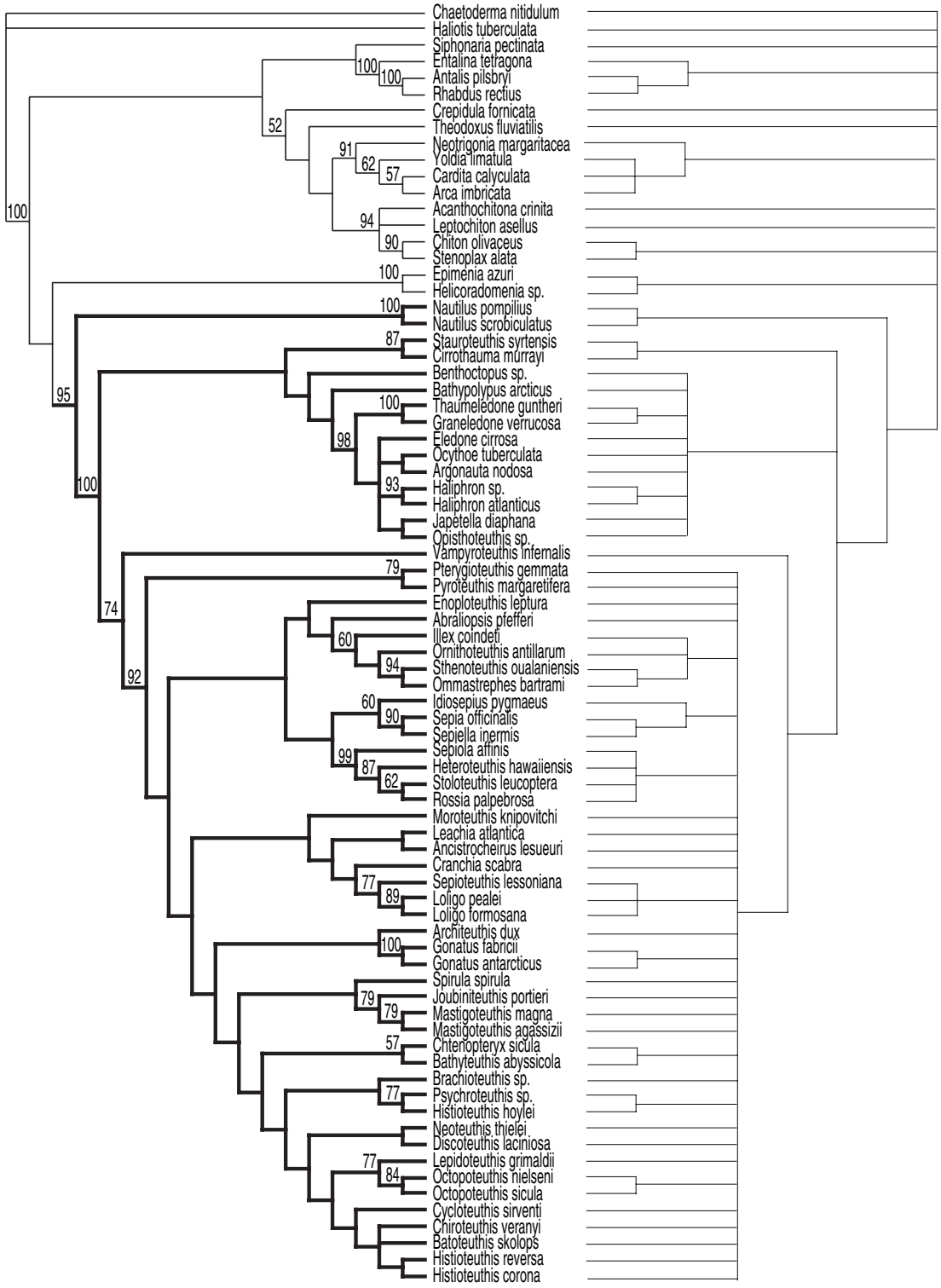

Fig. 5. Left tree illustrates the strict consensus of 12 trees at 9306 weighted steps for the combined molecular data (18S rRNA, 28S rRNA, COI, H3) yielded by the optimal parameter set (4111). Branches in bold indicate cephalopod taxa. Numbers above branches indicate jackknife support values above 50%. Right cladogram is a strict consensus of all trees obtained for the 12 parameter sets explored.

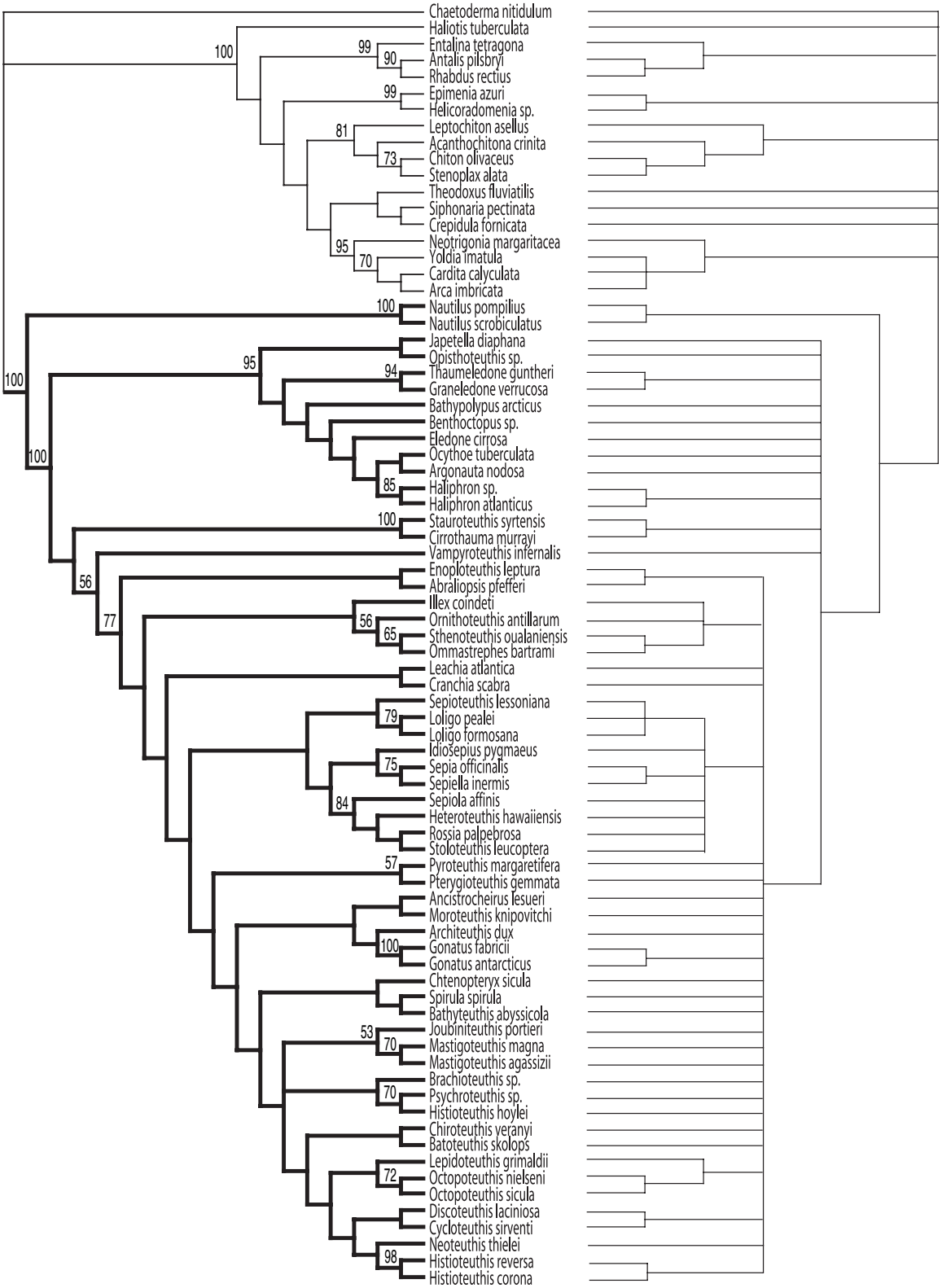

Fig. 6. Tree on the left illustrates the strict consensus of two trees at 18 071 weighted steps for the combined morphological and molecular sequence data for the optimal parameter set (2221). Branches in bold indicate cephalopod taxa. Numbers above branches indicate jackknife support values above 50%. Right cladogram is a strict consensus of all trees obtained for the 12 parameter sets explored.

by the sepioid orders + Myopsida, most other decabrachian relationships were only supported under certain parameter schemes. Joubiniteuthidae + Mastigoteuthidae was found in the optimal parameter set (2221; 53% jackknife support, Fig. 6). Ancistrocheiridae and Onychoteuthidae formed a clade sister to Architeuthidae + Gonatidae, although this relationship was not stable to parameter set variation. All parameter sets supported a close relationship between Lepidoteuthidae and Octopoteuthidae. That clade was sister to Cycloteuthidae + Neoteuthidae + Histioteuthidae under the optimal parameter set (2221). A suggested clade of Enoploteuthidae + Ancistrocheiridae + *Pterygioteuthis* + *Pyroteuthis* (e.g., Young and Harman, 1998) was not found in our analyses; however, a relationship between *Pterygioteuthis* and *Pyroteuthis* was supported by the data.

Discussion

This study provides the most comprehensive analysis published to date of internal relationships among a large number of cephalopod species by simultaneously analyzing information from their morphology and multiple molecular loci. Individual data sets did not show large agreement at most nodes and in fact showed a large disagreement with morphological-based hypotheses. Furthermore, results for the individual partitions were highly parameter-dependent and only when combining all molecular evidence or molecules + morphology was stability achieved in the hypotheses of cephalopod monophyly as well as in the major divisions within the Cephalopoda. As argued by proponents of the total evidence approach (see Kluge, 1989), only by combining all available evidence can reliable interpretation regarding the phylogenetic history of a group be attained (*sensu* Giribet, 2002). The addition of multiple genes indeed contributed to different but overlapping levels of resolution. However, decabrachian relationships still require major improvement in terms of stability and nodal support. Morphological data provided a higher degree of resolution among decabrachians than was previously observed, but there was less information regarding basal relationships among those sampled, while molecular data provided more resolution at both familial and ordinal levels. Given this, a simultaneous analysis of all data established more overall support for resolved clades at both ordinal and family levels better than any individual data set alone.

Ordinal relationships

Several findings in this study supported previous hypotheses of cephalopod relationships (e.g., Naef, 1921/23; Engeser and Bandel, 1988; Young and Vecchione, 1996; Bonnaud et al., 1997; Carlini and Graves, 1999; Boletzky, 2003), such as the monophyletic nature of cephalopods (Fig. 7, node 1) and their subdivision into Nautiloidea and Coleoidea (Fig. 7, node 2). Other more conflicting relationships, such as the position of Vampyromorpha, disagreed with previous hypotheses (Fig. 7, nodes 4, 5). Historically, Vampyromorpha and Octobrachia had been treated as sister taxa based on embryological and developmental data (Boletzky, 2003; Naef, 1928; Young and Vecchione, 1996), as well as morphological characters such as the presence of radial sucker symmetry (Appendix 3, character 23), similar sperm morphology (e.g., character 67; Healy, 1989) and outer statocyst capsules (character 45), although vampyromorph gladius morphology is similar to that of decabrachians (character 4; Toll, 1982; Toll, 1998). Alternatively, octobrachian gladii have been lost (character 4) or reduced to form fin supports (Cirroctopoda) or stylets (Octopoda). The position of Vampyromorpha has remained questionable, particularly in light of past molecular evidence (Bonnaud et al., 1997; Carlini and Graves, 1999). For example, in Bonnaud et al. (1997) the position of Vampyromorpha varied with outgroups used to generate the cladogram; analyses using the chiton *Katharina tunicata* as an outgroup placed Vampryomorpha sister to octobrachians, but when *K. tunicata* was not included, Vampyromorpha was found sister to the decabrachians. In this study, Vampyromorpha + Decabrachia was supported (Fig. 7, node 4) in the combined analysis under the best parameter set, plus six additional parameter sets. An alternative resolution of Vampyromorpha as sister group to Octobrachia (Fig. 7, node 5) was found under five analytical parameter sets. The origin of conflict regarding Vampyromopha in the present study was difficult to determine. Morphological data supported Vampyromorpha + Octobrachia (Fig. 1), while molecular data supported Vampyromorpha + Octobrachia as well as Vampyromorpha + Decabrachia, depending on the parameter and data set (Fig. 7). Ribosomal, combined molecular and simultaneous analysis of all data provided overall support for Vampyromorpha + Decabrachia. The two most variable loci, H3 and COI disagreed, placing *Vampyroteuthis* sister to the cirroctopod *Stauroteuthis* (Fig. 3), or placing it in a more basal position (Fig. 2). Vampyromorpha exhibited many autapomorphic features found in neither octobrachians nor decabrachians (Young, 1964), which makes this taxon difficult to place using morphological data alone. Disagreement among morphological and molecular data is not uncommon among metazoans (e.g., Giribet, 2003) and has been previously established for Octobrachia (Carlini et al., 2001). A second problem that needs consideration in determining the position of Vampyromorpha relative to other coleoids is the possibility that rampant extinction may obscure the affinities of *Vampyroteuthis*. While

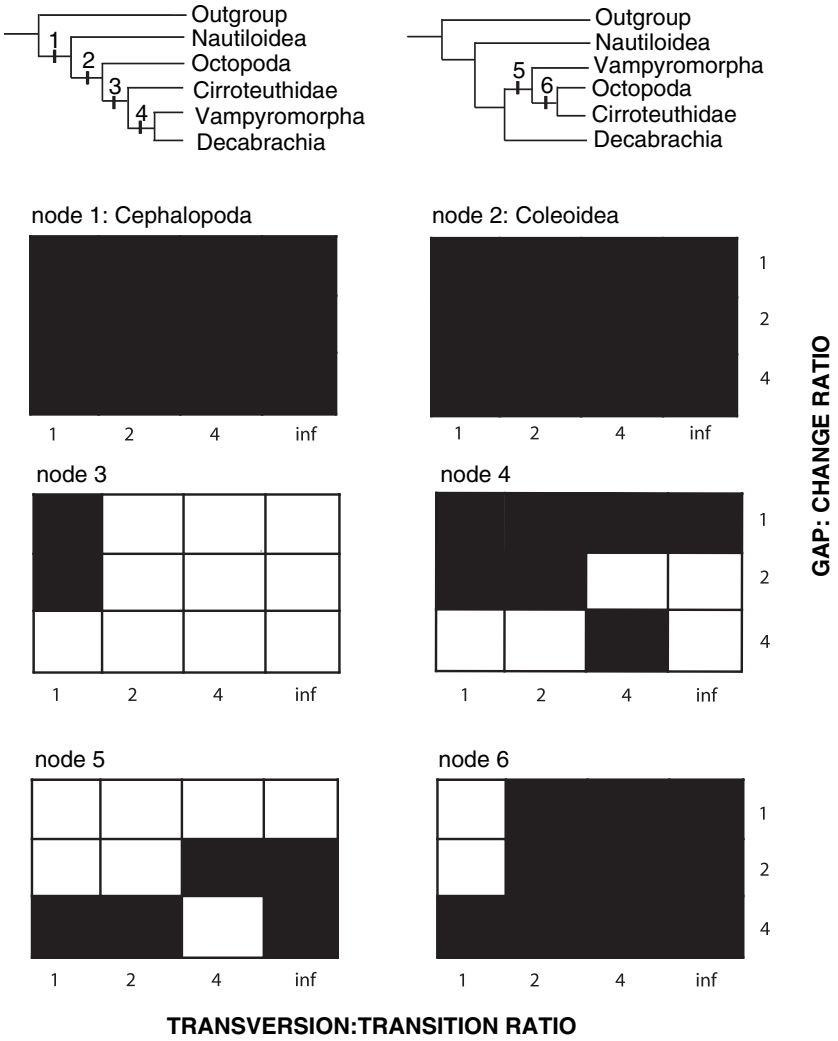

Fig. 7. Higher-level relationships among Cephalopoda as derived from analyses of combined morphology and molecular data for the optimal parameter set (2221). Two alternative topologies generated from different parameter sets, are illustrated above. The bottom squares illustrate the congruence plots (Navajo rugs) for selected nodes on cladograms above. Black squares indicate monophyly for a given parameter set, while white squares indicate non-monophyly.

some fossil evidence exists for cephalopods, many of the fossils are difficult to interpret and could be placed with either Octobrachia or Decabrachia (Young et al., 1998). Due to the difficulty of homologizing characters between fossil and extant taxa, fossil evidence was not included in the present study, although fossils may have a fundamental role in elucidating cephalopod relationships, as shown in other metazoan groups (Gauthier et al., 1988; Donoghue et al., 1989; Eernisse and Kluge, 1993; Giribet et al., 2002; Wheeler et al., 2004). Therefore, we caution the reader to interpret our results and conclusions in the absence of fossils.

A close relationship has been hypothesized to exist between cirroctopods and octopods (Carlini et al., 2001; Naef, 1921/23; Chun, 1914; Nesis, 1987; Engeser and Bandel, 1988; Voight, 1997). The morphological analyses provided the only cladogram to support a Cirroctopoda + Octopoda relationship (Fig. 1). Furthermore, the monophyly of Cirroctopoda was not established; COI was the only data set to support monophyly of the three species of cirroctopods (Fig. 2). Histone H3 (only available for two species of cirroctopods) placed *Opisthoteuthis* as sister group to the octopod *Haliphron* and *Stauroteuthis* as sister group to *Vampyroteuthis*. All other cladograms that included ribosomal data placed *Opisthoteuthis* within Octopoda (Figs 4, 5 and 6), which could be due to the use of a partial 18S rRNA sequence in the analyses. Despite several attempts to complete the *Opisthoteuthis* 18S rRNA fragment, we were not able to do so. However, Carlini et al. (2001) also questioned the monophyly of cirroctopods. While the optimal parameter set for this study supported Cirroteuthidae +

Vampyromorpha + Decabrachia, this result was not corroborated by other parameters (Fig. 7, nodes 3 and 6) or by individual trees. The instability of these nodes could be due to a disagreement between morphological and molecular data; previous molecular analyses found cirroctopods to be polyphyletic (Carlini et al., 2001), while morphological data suggested that cirroctopods are monophyletic (Young and Vecchione, 1996). Incongruence between molecular and morphological data is not uncommon in cephalopods (Carlini et al., 2001) and in order to resolve this issue, further sampling of cirroctopod species and analyses need to be conducted.

Decabrachian relationships

Previous investigations have consistently disagreed on decabrachian relationships, citing gene choice, taxon sampling, or a rapid radiation as reasons for unresolved phylogenies (Young and Vecchione, 1996; Bonnaud et al., 1997; Carlini and Graves, 1999; Carlini et al., 2000). While this study cannot address all questions pertaining to decabrachian relationships, certain hypotheses were tested (Fig. 8). Naef (1921/23) placed *Spirula* with Sepiidae, Sepiolidae, Idiosepiidae and Sepiadariidae in the suborder Sepioidea, with all other families in Teuthoidea. Naef initially placed *Spirula* sister to Sepiidae based on shared characteristics in shell development, stating that the differences between the two shells were secondary (Naef, 1921/23). In this study, monophyly of the sepioids was supported, with the exception of *Spirula*, which consistently grouped with oegopsids, and not sepioids (Figs 5, 6 and 8). However, monophyly of sepioids was not found in the morphological analyses, because their clade also included the loliginid squids (Fig. 1). In the simultaneous analyses of all data, the monophyly of sepioids + loliginids without *Spirula* was supported under all analytical parameters, suggesting a close relationship between sepioids (except Spirulida) and Loliginidae (Myopsida). Such a relationship of sepioids and loliginids was previously discussed by Naef (1921/23), although as previously discussed, Naef also considered *Spirula* within this clade. Naef described a Myopsida group consisting of Sepiidae, Sepiolidae, Loliginidae and Idiosepiidae, but later removed Loliginidae from this group citing drastic differences in shell morphology and development. This study found support for the reunification of Naef's original myopsid group (but excluding *Spirula*) based on both morphological and molecular data (Figs 1, 6 and 8). Several unusual morphological characteristics are shared among these families; all have accessory nidamental glands (character 57; but this seems to be plesiomorphic), benthic eggs with embryos containing an external yolk sac (not present in most oegopsids) and a cornea, which permanently covers the pupil (Naef, 1921/23; character 42). While the position of Loliginidae relative to sepioids and other teuthids has been debatable, evidence here suggested that Loliginidae is in fact sister to sepioids (except *Spirula*) and therefore not true teuthids, corroborating previous findings based on molecular data (Bonnaud et al., 1997; Carlini and Graves, 1999; Nishiguchi et al., 2004). The placement of Sepiolida, Sepiida and Idiosepiida with relation to Teuthida remains somewhat debatable, particularly due to their apparent relatedness to Loliginidae, but not to other teuthids. The position of *Spirula* remained unclear due to disagreement between morphological and individual molecular loci; individual and combined molecular trees placed *Spirula* with oegopsids, while morphological evidence placed it with sepioids.

Families within Oegopsida (Teuthida) did not form a monophyletic group. However, our results suggested closer relationships among several oegopsids than in previous cases (e.g., Bonnaud et al., 1997; Carlini and Graves, 1999). Many of the tested relationships were supported by previous monographs and general classifications of cephalopod taxonomy (Chun, 1914; Naef, 1921/23; Joubin, 1825/1924; Roper et al., 1969). For example, Chtenopterygidae and Bathyteuthidae were considered closely related by Pfeffer (1912) based on the presence of a long narrow gladius, subterminal fin position, presence of suckers of buccal lappet and quadraserial suckers on the arms. Only the combined analyses and histone H3 cladograms supported a close association among *Chtenopteryx*, *Spirula* and *Bathyteuthis* (Figs 3 and 7), although *Bathyteuthis* + *Chtenopteryx* was supported by morphology (Fig. 1) and the simultaneous analysis of molecules (Fig. 5).

The "Enoploteuthid families" proposed by Young and Harman (1998) consisted of Enoploteuthidae, Ancistrocheiridae, Pyroteuthidae and Lycoteuthidae, where the authors found a (Ancistrocheiridae (Enoploteuthidae (Lycoteuthidae + Pyroteuthidae))) relationship. Naef (1921/23) proposed a slightly different scenario, placing Enoploteuthidae and Pyroteuthidae in a single family, Enoploteuthidae, while grouping Ancistrocheiridae with Onychoteuthidae in a single family Onychoteuthidae. The present study supported the latter relationship to some extent (Fig. 8). Morphological data placed Enoploteuthidae, *Pterygioteuthis*, *Pyroteuthis* and Ancistrocheiridae in a single clade (Fig. 1), which was further supported by their many shared characters, such as the presence of hooks on arms (character 27) and tentacles (except for *Pterygioteuthis*, character 28), armature in two series of suckers (character 24), eight buccal supports (character 12), dorsal buccal attachment to arms V (character 11) and the presence of a conus (character 5). None of the individual molecular loci found the four families to be monophyletic. Combined data illustrated that *Ancistrocheirus* and *Moroteuthis* (Onychoteuthidae) clustered together with *Architeuthis* and *Gonatus*, while *Enoploteuthis* + *Abralilopsis* formed

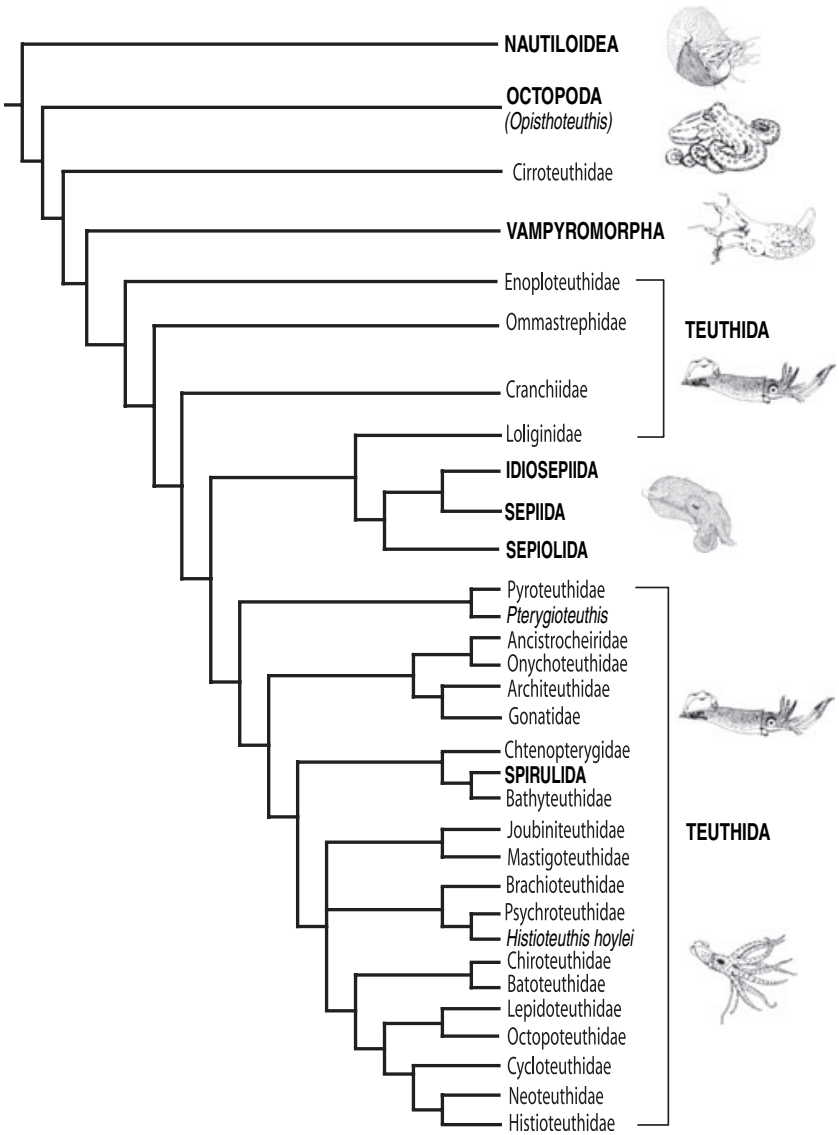

Fig. 8. Schematic representation of cephalopod relationships based on the optimal parameter set for the combined analysis of morphological and molecular data. Taxa in bold represent orders of cephalopods that appeared monophyletic in the analysis. Drawings by G. Williams.

the outermost branch on the decabrachian clade (Fig. 8), implying polyphyly for the enoploteuthid families. The position of *Pterygioteuthis* was unclear, the combined molecular data suggesting a close relationship to *Abraliopsis* (Enoploteuthidae, Fig. 6). However, our combined analysis of all data placed *Pterygioteuthis* + *Pyroteuthis* (Fig. 7) separate from other "enoploteuthids". Histone H3, the simultaneous analysis of the molecules and the simultaneous analysis of all data supported a sister relationship between *Pterygioteuthis* and *Pyroteuthis,* thus corroborating previous findings (e.g., Nesis, 1987).

Other interesting relationships were observed within four recognized oegopsid families: Joubiniteuthidae, Mastigoteuthidae, Batoteuthidae and Chiroteuthidae. While morphology and COI data supported the monophyly of these four families (Fig. 2), the combined analysis of all data found support for separate *Joubiniteuthis* + *Mastigoteuthis* and *Batoteuthis* + *Chiroteuthis* (Figs 7 and 8) clades. The polyphyletic nature of these four families was difficult to explain, partially because of their morphological similarity (Table 3, Fig. 1). All four families lack hectocotylization (characters 61–63), exhibit ventral buccal membrane attachment on arms V (character 11), an oval funnel locking apparatus (character 37) and a secondary conus (character 5). One possible reason for the apparent polyphyly was that the family Promachoteuthidae, commonly believed to be closely related to

the Mastigoeuthidae (e.g., Roper et al., 1969), was not included in this study, due to a lack of available specimens. Therefore, further sampling of these four families, as well as Promachoteuthidae, is needed in order to fully understand their relationships.

Decabrachian relationships supported in this study which were not identified by previous studies include *Brachioteuthis* + *Psychroteuthis* (+ *Histioteuthis hoylei*) and *Cycloteuthis* + *Neoteuthis* + *Histioteuthis* (Fig. 8). However, both Brachioteuthidae and Psychroteuthidae are monotypic and their taxonomy is poorly understood. The two families have several morphological characters in common, such as biserial arm suckers (character 24), simple funnel locking apparatus (character 37) and rhomboidal fins (Nesis, 1987). The grouping of *H. hoylei* with the family Psychroteuthidae was not entirely understood, although previous morphological data have suggested a close relationship between Psychroteuthidae and Histioteuthidae (Toll, 1998). However, this does not explain the polyphyletic nature of Histioteuthidae, nor does it explain why one species would be sister to *Psychroteuthis* and all others would form a clade on a different region of the tree.

The families Neoteuthidae and Histioteuthidae have several characteristics in common such as dorsal attachment on arms V (character 11), simple funnel locking apparatus (character 37) and biserial sucker arrangement on arms (character 24). Cycloteuthidae is distinct, sharing only the biserial sucker arrangement with both Histioteuthidae and Neoteuthidae. The characteristics that these three families share may be plesiomorphic; they are fairly common throughout decabrachians (Table 3, Fig. 1) and therefore may not provide additional information pertaining to relatedness. Hence, further investigation needs to be completed in order to thoroughly understand this relationship.

Conclusion

This study supports the monophyly of Cephalopoda, with Nautiloidea sister to a monophyletic Coleoidea. While the relationships between Cirroctopoda and Octopoda are somewhat unclear, the data support a sister relationship between Vampyromorpha and Decabrachia. Within Decabrachia, support was found for several intrafamilial relationships. It is clear that Sepiolida, Sepiida and Idiosepiida form a monophyletic group not related to Spirulida, which is instead nested within oegopsids. The analyses also indicate that the family Loliginidae is more closely related to sepioids, rather than oegopsids. The order Teuthida is consistently paraphyletic under all parameters and analyses and will need further clarification. Teuthida is comprised of oceanic decabrachians from a variety of habitats and locations around the world and with such diversity it is not surprising that it would be paraphyletic. Intensive sampling needs to be conducted on teuthid families to determine if re-organization is warranted. Furthermore, the ecology of these oceanic cephalopods could perhaps explain why many interfamilial relationships are not supported. Only families found in more coastal regions, such as the sepioids (except *Spirula*) and loliginids, are consistently resolved across data sets and parameters. Due to the position of Loliginidae, it seems likely that some taxonomic revisions are needed within Decabrachia. However, many of the more basal relationships within Decabrachia are not corroborated and further investigations will be needed before taxonomic re-organization can be undertaken.

While morphological and molecular data do not agree on all nodes in all cladograms, when evaluated in concert, the five matrices complemented each other, providing support and resolution for cephalopods at many levels. Molecular loci did not agree at all nodes, possibly due to differing degrees of variability; for example, COI may not have been as informative at basal nodes, but provided more information regarding terminal relationships. Alternatively, ribosomal genes are not able to resolve terminal nodes in many cases, but provide support for more basal relationships. When data are not in agreement it provides researchers with more questions and therefore more hypotheses to investigate: What information is in greatest disagreement? Is there a biological explanation? In order to address such questions, further morphological characters should be examined in order to evaluate basal relationships; other relevant species and more genetic loci (such as developmental genes) could also be included to provide further support and resolution at all taxonomic levels.

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Appendix 1

Voucher information for cephalopod specimens used for DNA extraction in this study. Information for outgroups listed in Giribet and Wheeler (2002) and Okusu et al. (2003).

Classification			Source	Collection data
Nautiloidea				
Nautilida	Nautilidae	*Nautilus pompilius* Linnaeus, 1758	GG	AMNH; 2003
		Nautilus scrobiculatus Lightfoot, 1786	GG	AMNH; 2003
Coleoidea				
Octobrachia				
Octopoda	Allopsidae	*Haliphron atlanticus* Steenstrup, 1861	MV	DE0304 (Sta. 2), 2003; NMNH
		Haliphron sp.	SP	UA; 2003
	Argonautidae	*Argonauta nodosa* Lightfoot, 1786	MV	DE0304 (Sta. 2), 2003; NMNH
	Bolitaenidae	*Japetella diaphana* Hoyle, 1885	MV	DE0304 (Sta. 5), 2003; NMNH
	Octopodidae	*Bathypolypus arcticus* (Prosch, 1847)	FGH	Saquatucket Harbor; SBMNH
		Benthoctopus sp.	MC	NMNH; 2003
		Eledone cirrosa (Lamarck, 1798)	SvB&AL	Banyuls-sur-Mer, 2001; NMSU
		Grandeledone verrucosa (Verril, 1881)	DC (MV&RY)	F/V Contender, 1994; NMNH
		Ocythoe tuberculata Rafinesque, 1814	MV	NMNH; 2003
		Thaumeledone guntheri Robson, 1930	MC	South Georgia; BAS
Cirroctopoda	Cirroteuthidae	*Cirrothauma murryae* (Chun, 1911)	MC	Porcupine Seabright; BAS
		Stauroteuthis syrtensis Verrill, 1884	DC (MV)	F/V Contender, 1995; NMNH
	Opisthoteuthidae	*Opisthoteuthis* sp. 1 (18S, 28S, H3)	FGH	Santa Barbara; SBMNH
		Opisthoteuthis sp. 2 (COI)	FGH	Santa Barbara; SBMNH
Vampyromorpha				
	Vampyroteuthidae	*Vampyroteuthis infernalis* Chun, 1903	DC	Hokusei Maru, 1996
Decabrachia				
Sepiolida	Sepiolidae	*Heteroteuthis hawaiiensis* (Berry, 1909)	DC	Hokusei Maru, 1996
		Stoloteuthis leucoptera (Verril, 1878)	DC (MV)	ALB9402.14.18; NMNH
		Rossia palpebrosa Owen, 1834	DC (MV)	ALB9402.19.27; NMNH
		Sepiola affinis Naef, 1912	MKN	Banyuls-sur-Mer, 2002; NMSU
Sepiida	Sepiidae	*Sepiella inermis* (Van Hasselt, 1835)	MKN	Banyuls-sur-Mer, 2002; NMSU
		Sepia officinalis Linnaeus, 1758	MKN	Banyuls-sur-Mer, 2002; NMSU
Spirulida	Spirulidae	*Spirula spirula* Linnaeus, 1758	DC (MK&RY)	NMNH; 1999
Idiosepiida	Idiosepiidae	*Idiosepius pygmaeus* Steenstrup, 1881	MKN	Botany Bay, AU, 2000; NMSU
Teuthida	Loliginidae	*Loligo pealei* LeSueur, 1821	WKM	Northern Atlantic, 2003; NMSU
		Loligo formosana Sasaki, 1929	MKN	Rayong, Thailand, 2001; NMSU
		Sepioteuthis lessoniana Férussac, 1830	MKN	Rayong, Thailand, 2001; NMSU
	Ancistrocheiridae	*Ancistrocheirus lesueuri* (Orbigny, 1842)	DC (RY)	Hokusei Maru, 1994
	Architeuthidae	*Architeuthis dux* Steenstrup, 1857	DC (MV)	NMNH; 1999
	Bathyteuthidae	*Bathyteuthis abyssicola* Hoyle, 1885	DC	Hokusei Maru, 1996
	Batoteuthidae	*Batoteuthis skolops* Young & Roper, 1968	MC	South Georgia; BAS
	Brachioteuthidae	*Brachioteuthis* sp.	MV	DE0304, 2003; NMNH
	Chiroteuthidae	*Chiroteuthis veranyi* (Férussac, 1835)	MV	DE0304 (Sta. 4), 2003; NMNH
	Cranchiidae	*Cranchia scabra* Leach, 1817	DC (RY)	Hokusei Maru, 1994; NMNH
		Leachia atlantica (Degner, 1925)	MV	NMNH; 2003
	Chtenopterygidae	*Chtenopteryx sicula* (Vérany, 1851)	TK	NSMT; 1999
	Cycloteuthidae	*Cycloteuthis sirventyi* (Joubin, 1919)	DC (RY)	Hokusei Maru, 1994
		Discoteuthis laciniosa Young & Roper, 1969	DC (RY)	Hokusei Maru, 1994
	Enoploteuthidae	*Abraliopsis pfefferi* Joubin, 1896	MV	DE0304 (Sta. 12), 2003; NMNH
		Enoploteuthis leptura (Leach, 1817)	MV	DE0304 (Sta. 15), 2003; NMNH
	Gonatidae	*Gonatus antarcticus* Lönnberg, 1898	MC	South Georgia, BAS
		Gonatus fabricii (Lichtenstein, 1818)	MV	DE0304 (Sta. 3), 2003; NMNH
	Histioteuthidae	*Histioteuthis corona* (Voss & Voss, 1962)	TK	NSMT; 1999
		Histioteuthis hoylei (Goodrich, 1896)	DC	Hokusei Maru, 1996
		Histioteuthis reversa (Verrill, 1880)	MV	DE0304 (Sta. 3), 2003; NMNH

Appendix 1
Continued

Classification			Source	Collection data
	Joubiniteuthidae	*Joubiniteuthis portieri* (Joubin, 1912)	MV	DE0304 (Sta. 14), 2003; NMNH
	Lepidoteuthidae	*Lepidoteuthis grimaldii* Joubin, 1859	DC (RY)	Hokusei Maru, 1994
	Mastigoteuthidae	*Mastigoteuthis agassizii* Verril, 1881	MV	DE0304 (Sta. 3), 2003; NMNH
		Mastigoteuthis magna Joubin, 1913	MV	DE0304 (Sta. 1), 2003; NMNH
	Neoteuthidae	*Neoteuthis thielei* Naef, 1921	MV	DE0304 (Sta. 4), 2003; NMNH
	Octopodeuthidae	*Octopoteuthis nielseni* Robson, 1948	DC (RY)	Hokusei Maru, 1994
		Octopoteuthis sicula Rüppel, 1844	TK	NSMT;1999
	Ommastrephidae	*Illex coindeti* (Vérany, 1837)	SvB	Banyuls-sur-Mer, 2001; NMSU
		Ommastrephes bartramii (LeSueur, 1821)	DC	Hokusei Maru, 1996
		Ornithoteuthis antillarum Adam, 1957	MV	DE0304 (Sta. 14), 2003; NMNH
		Sthenoteuthis oualaniensis (Lesson, 1830)	DC (RY)	Hokusei Maru, 1994
	Onychoteuthidae	*Moroteuthis knipovitchi* Filippova, 1972	MC	South Georgia; BAS
	Psychroteuthidae	*Psychroteuthis* sp.	MC	South Georgia; BAS
	Pyroteuthidae	*Pyroteuthis margaretifera* (Rüppel, 1844)	MV	DE0304 (Sta. 3), 2003
		Pterygioteuthis gemmata Chun, 1908	MV	DE0304 (Sta. 2), 2003; NMNH

Source Abbreviations: AL, Annie Lindgren; DC, David Carlini; FGH, Eric Hochberg; GG, Gonzalo Giribet; RY, Richard Young; MC, Martin Collins; MKN, Michele Nishiguchi; MV, Michael Vecchione; SP, Stuart Piertney, TK, Tsunemi Kubodera, WKM, William Macy, (MV), tissue sample collected originally by Michael Vecchione. Repository institutions: AMNH, American Museum of Natural History, New York; NMNH, National Museum of Natural History, Washington D.C.; NMSU, New Mexico State University, Las Cruces; UA, University of Aberdeen, Scotland; BAS, British Antarctic Survey, United Kingdom; NSMT, National Science Museum, Tokyo. Where collection information is not available, repository and date sent to NMSU are listed.

Appendix 2
List of cephalopod specimens used in morphological character coding.

Classification			Repository/catalog number*	Sex
Nautiloidea				
Nautilida	Nautilidae	*Nautilus pompilius* Linnaeus, 1758	literature	
		Nautilus scrobiculatus Lightfoot, 1786	literature	
Coleoidea				
Octobrachia				
Octopoda	Alloposidae	*Haliphron atlanticus* Steenstrup, 1861	SBMNH	f
	Argonautidae	*Argonauta arago* Linnaeus, 1758	MCZ	f
		Argonauta nodosa Lightfoot, 1786	literature	
	Bolitaenidae	*Japetella diaphana* Hoyle, 1885	SBMNH #45791	
		Japetella heathi (Berry, 1911)	SBMNH #63008	f
		Japetella sp.	SBMNH #63086	m
		Japetella sp.	SBMNH #63072	f
	Octopodidae	*Bathypolypus arcticus* (Prosh, 1847)	SBMNH; Falkland Islands	f
		Bathypolypus arcticus (Prosh, 1847)	SBMNH; Sea Scallop Dredge	m
		Benthoctopus hokkaidensis (Berry, 1921)	SBMNH #45787	m
		Eledone cirrosa (Lamarck, 1798)	SBMNH #142574	f
		Grandeledone verrucosa (Verrill, 1881)	literature	
		Octopus rubescens Berry, 1953	SBMNH #41962	f
		Octopus vulgaris Cuvier, 1797	SBMNH #OV-90-17	f
		Octopus vulgaris Cuvier, 1797	SBMNH #OV-90-16	m
		Thaumeledone guntheri Robson, 1930	literature	
Cirroctopoda	Cirroteuthidae	*Cirrothauma murrayi* (Chun, 1911)	literature	
		Stauroteuthis syrtensis Verrill, 1884	literature	
	Opisthoteuthidae	*Opisthoteuthis massyae* Grimpe, 1920	SBMNH #45973	m
		Opisthoteuthis sp.	SBMNH	f
		Opisthoteuthis sp.	SBMNH	m
		**Opisthoteuthis* sp. 1	SBMNH	
Vampyromorpha				
	Vampyroteuthidae	*Vampyroteuthis infernalis* Chun, 1903	SBMNH #62500	f
Decabrachia				
Sepiolida	Sepiolidae	*Heteroteuthis hawaiiensis* (Berry, 1909)	SBMNH; Hokusei Maru, Sta. 1C	f, m
		Stoloteuthis leucoptera (Verril, 1878)	literature	
		Rossia palpebrosa Owen, 1834	literature	
		**Sepiola affinis* Naef, 1912	NMSU;Banyuls-sur-Mer, 2002	f, m

Appendix 2
Continued

Classification			Repository/catalog number*	Sex
Sepiida	Sepiidae	*Sepiella inermis* (Van Hasselt, 1835)	literature	
		**Sepia officinalis* Linnaeus, 1758	NMSU;Banyuls-sur-Mer, 2002	f, m
Spirulida	Spirulidae	*Spirula spirula* Linnaeus, 1758	MCZ #093798	
Idiosepida	Idiosepiidae	*Idiosepius pygmaeus* Steenstrup, 1881	NMSU	
Teuthida	Loliginidae	**Loligo pealei* LeSueur, 1821	NMSU	f, m
		Loligo formosana Sasaki, 1929	literature	
		Sepioteuthis lessoniana Férussac, 1830	SBMNH; Philippines,Zambango;1948	
		Sepioteuthis lessoniana Férussac, 1830	SBMNH #USC1204	
	Ancistrocheiridae	*Ancistrocheirus lesueuri* (Orbigny, 1842)	SBMNH;NH2-93 Hawaii	f
	Architeuthidae	*Architeuthis dux* Steenstrup, 1857	literature	
	Bathyteuthidae	*Bathyteuthis abyssicola* Hoyle, 1885	SBMNH #49331	f
	Batoteuthidae	*Batoteuthis* sp.	SBMNH;NH2-93 Hawaii	
	Brachiteuthidae	*Brachioteuthis* sp. Verrill, 1881	SBMNH #60131	
	Chiroteuthidae	*Chiroteuthis calyx* Young, 1972	SBMNH #45799	m
		Chiroteuthis sp.	FMNH #296689	m
		Chiroteuthis veranyi (Férussac, 1830)	literature	
	Cranchiidae	*Cranchia scabra* Leach, 1817	SBMNH #45727	f
		Leachia atlantica (Degner, 1925)	literature	
	Chtenopterygidae	*Chtenopteryx sicula* (Vérany, 1851)	MCZ #278566, 278657	
	Cycloteuthidae	*Cycloteuthis sirventyi* (Joubin, 1919)	literature	
		Discoteuthis laciniosa Young and Roper, 1969	SBMNH #142131	f
	Enoploteuthidae	*Abraliopsis affinis* (Pfeffer, 1912)	SBMNH #49436	f
		Abraliopsis pfefferi Joubin, 1919	literature	
		Enoploteuthis sp.	SBMNH #51695	f
		Enoploteuthis leptura (Leach, 1817)	literature	
	Gonatidae	*Gonatus antarcticus* Lönnberg, 1898	literature	
		Gonatus fabricii (Lichtenstein, 1818)	SBMNH #00011	
		Gonatus onyx Young, 1972	SBMNH #60597	f
	Histioteuthidae	*Histioteuthis* sp.	MCZ #277836	
		Histioteuthis sp.	SBMNH # 890909	f
		Histioteuthis corona (Voss & Voss, 1962)	literature	
		Histioteuthis heteropsis (Berry, 1913)	SBMNH #61158	f
		Histioteuthis hoylei (Goodrich, 1896)	literature	
	Joubiniteuthidae	*Joubiniteuthis portieri* (Joubin, 1912)	FMNH #278105	m
		Joubiniteuthis sp.	SBMNH; NH2-93 Hawaii	f
	Lepidoteuthidae	*Lepidoteuthis* sp.	SBMNH #51304	
	Mastigoteuthidae	*Mastigoteuthis* sp.	FMNH #78309	
		Mastigoteuthis pyrodes Young, 1972	SBMNH;Trawl #14 San Clemente, 2003	m
	Neoteuthidae	*Neoteuthis* sp.	SBMNH #11308	f
	Octopodeuthidae	*Octopoteuthis* sp.	SBMNH #61554	m
		Octopoteuthis sp.	SBMNH #61563	f
	Ommastrephidae	*Illex coindeti* (Vérany, 1837)	SBMNH;Bay of Naples, 1959	
		Ommastrephes bartramii (LeSueur, 1821)	MCZ #338290	m
		Sthenoteuthis oualaniensis Lesson, 1830	SBMNH #64394	m
		Ornithoteuthis antillarum Adam, 1857	literature	
	Onychoteuthidae	*Moroteuthis* sp.	SBMNH;British Antarctic Survey, 1988	m
		Onychoteuthis banskii (Leach, 1817)	MCZ #293703	m
	Pyroteuthidae	*Pyroteuthis margaretifera* (Rüppel, 1884)	FMNH #78300	f
		Pyroteuthis sp.	SBMNH;NH-2 93, Hawaii	f
		Ptergioteuthis gemmata Chun, 1908	SBMNH #64434	m
		Ptergioteuthis sp.	FMNH #28690	m

Taxa listed below also include specimens not used in analysis, merely to confirm character states. Asterisk indicates voucher specimen for DNA analysis. Source abbreviations; MCZ, Museum of Comparative Zoology, Harvard University; NMSU, New Mexico State University; FMNH, Field Museum of Natural History; SBMNH, Santa Barbara Museum of Natural History. Where catalog number not available, collection information has been listed.

Appendix 3. Character descriptions

Cephalopod characters were scored in Table 3 via direct specimen observation. When specimens were not available, or characters were difficult to measure, primary literature was used (Naef, 1921/23; Roper et al., 1969; Salvini-Plawen and Steiner, 1996; Young and Vecchione, 1996; Young and Harman, 1998). Characters coded in Table 3 as not applicable (–) indicate that a particular character could not be scored across all taxa. Characters 1–65 are primarily specific to cephalopods and most were therefore coded as inapplicable in other molluscs. In cases where a particular state could not be identified, it was coded as "?".

1. Calcified outer shell: (0) absent; (1) present. A calcified outer shell is no longer present in extant cephalopods except for species within Nautliloidea.

2. Siphuncle: (0) absent; (1) present (Young and Vecchione, 1996). The presence of a siphuncle is a synapomorphy of all cephalopods (Salvini-Plawen and Steiner, 1996).

3. Inner shell sac: (0) absent; (1) present. All coleoid cephalopods have an internal shell sac, which secretes the internal shell. In Octopoda an embryonic shell sac/gland is present during embryonic development but shell material is not always secreted such as in the case of Argonautidae (Naef, 1928; Boletzky, 1982).

4. Inner shell morphology: (0) chambered with siphuncle; (1) uncalcified gladius; (2) uncalcified fin supports; (3) uncalcified stylets. Due to the variability among coleoid internal shells, separate states have been identified (Toll, 1982, 1998). All character states are included as a single character because the origins of each shell type are likely homologous due to the presence of a shell gland (see character 3). Only those taxa that have a shell sac that secretes shell material are considered. Sepiidae exhibit a chambered internal shell while *Spirula* has an internal, calcified, chambered shell with a siphuncle. The teuthid gladius differs greatly from other internalized shells within Coleoidea, but is the most common (Toll, 1982). While Octopoda does not have an uncalcified inner shell, stylets are present in many families (with a shell sac in embryonic stage). Alternatively, Cirroctopoda has a gladius modified to act as fin supports. However, the fin supports in Cirroctopoda differ greatly from both the gladius as well as the stylets, so separate character states are provided for each.

5. Conus morphology: (0) conus absent (1) primary conus present; (2) secondary conus present; (3) pseudoconus present. (Toll, 1982). The primary conus is small and cuplike or sub-triangular in outline and exhibits a cone field and a rostrum, located at the apical tip of the gladius. The ventral rim forms a broad U-shaped border or is completely transverse. The primary conus is considered homologous to the phragmocone portion of the ancestral shell (Jeletzky, 1966). The secondary conus is considered a more derived state, formed by ventral curvature and midventral fusion of the posterolateral edges of the vanes (Toll, 1982). Because it is formed from the vanes, the secondary conus is presumed to be derived from the proostracum portion of the ancestral shell and is also never found in association with a rostrum. The pseudoconus state occurs when the posterolateral edges of the vanes overlap but no fusion occurs. Pseudoconus morphology has been expanded to include all conuses formed by the in-folding of the posterolateral edges of the vanes with or without fusion (this state is applicable only to some genera of cranchiids) (Toll, 1982).

6. One pair of fins: (0) absent; (1) present. At least one pair of fins is present in most cephalopods (Salvini-Plawen and Steiner, 1996). The fins are attached to the cartilage-enforced shell epithelium forming an articulated capsule adjacent to the shell sac (Naef, 1921/23).

7. Additional fins (with postembryonic fin developing second and posterior to adult fin): (0) absent; (1) present at some stage in life cycle. In decabrachians the fins typically insert on a flattened cartilage (which attaches to the shell sac) with a straight medial ridge. During development a juvenile fin develops first, followed by an adult fin. The juvenile fin is subsequently reabsorbed during growth while the adult fins enlarge (Naef, 1928; Boletzky, 1982). However, in some cases, two sets of fins remain, such as in Vampyroteuthidae (separated by light organs) and some teuthids (although the second fin is often broken off). Within Teuthida, Chiroteuthidae, Grimpoteuthidae, Batoteuthidae, Joubiniteuthidae and Mastigoteuthidae all species possess some form of additional fins.

8. Nuchal cartilage: (0) absent; (1) present and exposed; (2) present but not exposed (Young and Vecchione, 1996). The nuchal cartilage supports the head component of the nuchal locking apparatus; the muscles of the collar, head and shell sac attach to the cartilage. The head of cuttlefishes and squids is well separated from the body by a neck (nuchal construction), believed to be the plesiomorphic state (Young and Vecchione, 1996). In some sepiolids the mantle is dorsally fused with the head and ventrally connected by a narrow or wide cutaneous nuchal b and such as in Sepiolinae, *Sepiolina*, *Stoloteuthis*, *Iridioteuthis* and Sepiadariidae. In Idiosepiidae the mantle is not fused with the head, but no nuchal cartilage is present. All remaining squids and cuttlefish have nuchal cartilage connecting the mantle to the head. Nuchal cartilage is present in Vampyroteuthidae but no longer supports a locking apparatus, instead providing a site for muscle attachment. The lack of exposure in *Vampyroteuthis* is likely to be apomorphic and was therefore coded as a separate state.

9. Chromatophores: (0) absent; (1) present. Chromatophores are vesicular cells that expand due to contractile radiating fibers, found only in coleoid cephalopods (Naef, 1921/23; Salvini-Plawen and Steiner, 1996).

10. Buccal crown: (0) absent; (1) present (Young and Vecchione, 1996). The buccal crown consists of muscular buccal supports and connective membranes that surround the lips and mouth. In *Idiosepius*, the buccal crown is apparent in dissected animals, just barely intercalated within the arms of the animal. A buccal crown is absent in octobrachians and not applicable in *Nautilus* because the homologous structure is unknown.

11. Buccal membrane connective attachment to arms V (see character 15 for explanation of arm numbering): (0) dorsal; (1) ventral (Roper et al., 1969; Roper, 1969; Young and Harman, 1998; Young et al., 1998). The arms of squids and cuttlefish are attached to the outer membrane surrounding the mouth by a cutaneous and muscular buccal membrane attachment. The major function of the buccal attachment is to hold the arms together in a cone during swimming (Naef, 1921/23). Vampyromorpha, Cirroctopoda and Octopoda have no buccal attachments; the arms are muscular hydrostats. The buccal membrane is attached to the dorsal side of arms I and II, ventral side of arms III and either dorsal or ventral to arms V.

12. Buccal lappet number: (0) 6; (1) 7; (2) 8 (Roper et al., 1969). The buccal membrane is star shaped and consists of 6, 7, or 8 rays. Initially eight lappets are present, though those extending to the first and fourth set of arms may merge together.

13. Beak: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996; Waller, 1998). A beak is present both in the coleoid and nautiloid cephalopods (with calcified additions to the edge in the latter).

14. Radular apparatus: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996; Haszprunar, 2000). While a true radula is lost in some molluscs such as the cirroctopods, a radular apparatus, consisting of a radular sac and odontophore is still present in cephalopods.

15. Circumoral appendages (arms): (0) absent; (1) present (Waller, 1998). The cephalopod head bears an outer circle of at least eight arms that are believed to be derived from the molluscan foot (Naef, 1921/23).

Note on arm numbering:

Octobrachia	Vampyromorpha/Decabrachia
1	I
lost?	II
2	III?
3	IV
4	V

Embryological and developmental studies indicate that arms II (rather than arms III) are the pair likely lost by Octobrachia (Naef, 1928). In this case, arms I, II, III, IV and V will be used to describe individual arm pairs.

16. Arms II: (0) unmodified; (1) filaments; (2) absent (Young and Vecchione, 1996). Arms II are present in decabrachians, absent in octobrachians and modified into filaments in *Vampyroteuthis*. Early growth stages provide evidence that vampyroteuthid filaments are homologous to arms II (Naef, 1921/23; Boletzky, 1982).

17. Arms IV: (0) unmodified; (1) tentacles (Young and Vecchione, 1996). Modification of Arms IV is one of the significant characters used to separate decabrachians from octobrachians. Arms IV are unmodified in octobrachians and vampyromorphs and modified into tentacles in decabrachians.

18. Horizontal arm septa inserted in the arm muscles: (0) absent; (1) present (Young and Vecchione, 1996). Cirroctopoda possess a horizontal septum that inserts into the circular muscle layer that forms the outer and thinner portion of the cylindrical muscular wall of the arm. The septum is orally concave in cross section and divides the muscular tube within each arm into oral and aboral regions. *Japetella diaphana* was coded as "?" because similar septa are present and are inserted as two membranes, extending in an oral/aboral plane internal to arm muscles. It is unclear whether the two states evolved independently. Due to the difficulty of coding fixed specimens, this character was taken directly from Young and Vecchione (1996).

19. Cirri on arms: (0) absent; (1) present (Young and Vecchione, 1996). Cirri are elongate, fleshy, finger-like papillae or palps located along the lateral edges of the oral surface of the arms, particularly in cirrate octobrachians. However, the cirri on cirroctopod arms may not be homologous to trabeculae found in some decabrachians. Therefore the presence of cirri is considered an independent character state.

20. Suckers: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996). While present in all coleoids, suckers of decabrachians are thought to be homologous with the octobrachian form, which is considered the more primitive state (Naef, 1921/23).

21. Acetabulum composition lining suckers: (0) cuticular rings; (1) neither cuticular nor horny rings; (2) horny rings (Young and Vecchione, 1996). An acetabulum lines the inside of the sucker ring on all coleoids. Decabrachians have horny rings, octobrachians exhibit cuticular rings and vampyromorphs have neither.

22. Sucker stalk: (0) absent; (1) present (Young and Vecchione, 1996). Decabrachian suckers are not attached directly to the arm, but connected by a flexible stalk, while octobrachian suckers are attached directly to the arm (Naef, 1921/23). The suckers of *Vampyroteuthis* are not attached directly to the arm but do not have "decabrachian-like" stalks and were therefore coded as "?". This character was modified from Young and Vecchione (1996; character 9) to account for the unknown state of *Vampyroteuthis*.

23. Sucker symmetry; (0) radial; (1) bilateral (Young and Vecchione, 1996). Decabrachian suckers are bilateral while those of *Vampyroteuthis* and the Octobrachia exhibit radial symmetry.

24. Armature I–III series: (0) in two rows; (1) in more than two rows; (2) in one row (Young and Vecchione, 1996). Sucker or hook series refers to that in the midarm, not at the tip where numerous rows of suckers can occasionally be observed.

25. Tentacle sucker series: (0) in up to four rows; (1) in greater than four rows (Roper et al., 1969). This state refers to rows of either hooks or suckers on the midportion of the tentacle club. This character is only applicable to decabrachians and subsequently coded as n/a in all other taxa (tentacles absent in all other coleoids).

26. Suckers on buccal membrane: (0) absent; (1) present (Roper, 1969). Small suckers are located on the oral region of the buccal crown in several decabrachians: Chtenopterygidae, Bathyteuthidae, Loliginidae and Sepiidae. This character is only applicable to decabrachians because the buccal membrane is absent in Nautiloidea, Vampyromorpha and Octobrachia.

27. Hooks on arms I–III: (0) absent; (1) present (Roper et al., 1969; Young and Harman, 1998). Hooks are modified suckers found on the arms of several decabrachians.

28. Hooks on tentacles (arms IV); (0) absent; (1) present (Roper et al., 1969; Young and Harman, 1998). This character is only applicable to decabrachians, because tentacles are absent in all other extant cephalopods.

29. Tentacles (arms IV) in adults: (0) absent; (1) present. Tentacle absence refers to taxa in which tentacles were present during development but are autotomized prior to or upon maturation. In the case of Gonatidae, some females autotomize their tentacles during reproduction, however, this is not synapomorphic for the family. This character is only applicable to decabrachians.

30. Tentacle locking apparatus: (0) absent; (1) present on carpus only; (2) present on manus and carpus (Young and Harman, 1998). The locking apparatus on the tentacle stalk consists of several suckers with smooth rings and tubercles (knobs) present on the carpal region of the club, which correspond to alternating rings and knobs on the opposite tentacle. The apparatus is applicable only in decabrachians and is highly variable in structure. Young and Harman (1998) used the presence of a tentacle locking apparatus to further investigate the relationships among enoploteuthid-like families.

31. Luminous bacteriogenic, round, bilobed organ located ventrally on ink sac: (0) absent; (1) present (Herring, 1988; Montgomery and McFall-Ngai, 1992; McFall-Ngai and Ruby, 1998). Bacteriogenic light organs are found in two families, Sepiolidae and Loliginidae (Young, 1977). As loliginid light organs are more elongated than that of sepiolids, it is unclear whether the presence of a bacteriogenic light organ is a synapomorphy, therefore only genera within Sepiolidae are coded as "present".

32. Luminous autogenic organs with a centrally situated luminous body distributed across mantle and arms: (0) absent; (1) present (Chun, 1914; Herring, 1988). Luminescent organs are found in almost all decabrachians, however, they are morphologically and biochemically diverse (Herring, 1988). The presence of light organs across the mantle and arms is specific for the members of Histioteuthidae.

33. Photophores containing collagen light guides: (0) absent; (1) present (Young and Harman, 1998). Collagen light guides are found only in the photophores of Enoploteuthidae, Lycoteuthidae and Pyroteuthidae.

34. Funnel: (0) absent; (1) present (Waller, 1998). The presence of a funnel (called hyponome in nautiloids) is a synapomorphy of Cephalopoda (Salvini-Plawen and Steiner, 1996).

35. Funnel: (0) attached to ventral mantle; (1) not attached to ventral mantle; (2) fused to mantle (Young and Vecchione, 1996). Funnel-mantle fusion is present in Cranchiidae and absent in all other decabrachians. In most octopods, cirroctopods and *Vampyroteuthis* the funnel and ventral mantle are attached but a narrow ventral slit remains (complete fusion does not exist). While the mantle-funnel attachment in Vampyromorpha is thought to be reminiscent of the funnel-mantle locking cartilage of decabrachians, it was treated as a separate character state.

36. Funnel locking apparatus: (0) absent; (1) present (Roper et al., 1969; Young and Vecchione, 1996). The funnel locking apparatus is a lock and key structure used to keep the mantle from inverting during rapid movement. Most often, individuals that do not exhibit mantle/funnel attachment possess a funnel locking apparatus. However, there are some cases in which there is no funnel/mantle attachment and no funnel locking apparatus, such as in cirroctopods.

37. Funnel locking apparatus morphology: (0) simple, straight; (1) triangular, round; (2) inverted T or -| shaped; (3) oval with projecting knobs (Roper et al., 1969; Nesis, 1987). The morphology varies greatly, particularly among decabrachians. The most common type is the simple, straight found in many oegopsids, sepiolids and sepiids.

38. Funnel valve; (0) absent; (1) present (Young and Vecchione, 1996). The funnel valve is a one-way muscular flap located on the inner dorsal wall of the funnel.

39. Closed circulatory system: (0) absent; (1) present (Waller, 1998; Haszprunar, 2000). A closed circulatory system is synapomorphic for cephalopods (Boletzky, 1987; Budelmann et al., 1997).

40. Ink sac: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996). The presence of an ink sac is unique to coleoids (although secondarily absent in some octobrachians).

41. Cerebral (pretrochal) eyes: (0) absent; (1) present (Haszprunar, 2000). Synapomorphic character for Monoplacophora, Scaphopoda, Bivalvia, Gastropoda and Cephalopoda.

42. Cornea: (0) absent; (1) one-part cornea present; (2) two-part cornea present (Young and Vecchione, 1996). The one-part cornea is the transparent protective outer membrane covering the eye in so-called myopsid cephalopods as well as Sepiidae and Sepiolidae while all other decabrachians lack a cornea (the eye is in direct contact with the environment). Octopods and cirroctopods have a fully closed, or two-part cornea.

43. Extra-ocular eye muscles: (0) absent; (1) present (Haszprunar and Wanninger, 2000). Extra-ocular eye muscles are autapomorphic for cephalopods although distinct differences occur between nautiloids, decabrachians and octobrachians (Budelmann et al., 1997).

44. Paired statocysts: (0) present; (1) absent (Salvini-Plawen and Steiner, 1996; Haszprunar and Wanninger, 2000). In Mollusca paired statocysts are restricted to conchiferans. Codings taken directly from primary literature sources (Nesis, 1987; Salvini-Plawen and Steiner, 1996).

45. Statocyst outer capsule: (0) absent; (1) present (Young and Vecchione, 1996). Coleoid cephalopods have one pair of statocysts situated in the occipital region of the head capsule, which allow for orientation and balance relative to gravitational direction (Nesis, 1987). An outer fluid-filled sac is present in *V. infernalis*, octopods and cirroctopods. A single sac embedded in cartilage is present in all other coleoids. Codings for this character were taken directly from Young and Vecchione (1996).

46. Stellate ganglia: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996). Stellate ganglia are present in all cephalopods.

47. Photosensitive vesicles: (0) within cephalic cartilage; (1) above funnel; (2) on stellate ganglia (Young and Vecchione, 1996). Photosensitive vesicles function in the detection of light but vary in location across cephalopods.

48. Inferior frontal lobe system of the brain: (0) absent; (1) partially present; (2) present (Young and Vecchione, 1996; Nixon and Young, 2003). An inferior frontal lobe system is present in Octobrachia. Due to difficulty in coding brain morphology in fixed specimens, this character was coded directly from literature (Young and Vecchione, 1996).

49. Superior buccal lobe: (0) widely separated from brain; (1) adjacent to brain; (2) fused to brain (Young and Vecchione, 1996). The position of the buccal lobe relative to the supraesophageal mass varies among cephalopods depending on the distance between the buccal mass and brain. This character was coded directly from literature (Young and Vecchione, 1996).

50. Branchial canal: (0) absent; (1) present; (2) secondary reduction of canal (Young and Vecchione, 1996). The branchial canal allows for the passage of seawater between gill lamellae and is present in all coleoids except for Sepiolidae, Sepiidae and Spriulidae (Young and Vecchione, 1996). This character was coded directly from Young and Vecchione (1996).

51. Relative position of digestive gland duct appendages: (0) lies in nephridial coelom; (1) not in nephridial coelom (Young and Vecchione, 1996). Digestive gland duct appendages are present in all coleoid cephalopods although their location is variable.

52. Posterior salivary gland: (0) absent; (1) posterior to brain; (2) proximal to buccal mass (Young and Vecchione, 1996). The primitive location of the posterior salivary gland is posterior to the cephalic cartilage; however, in Cirroctopoda it is located proximal to the buccal mass (Young and Vecchione, 1996).

53. Enlarged coelomic cavity with large amounts of ammonium chloride (0) absent; (1) present. Many cephalopods possess ammonium chloride in their mantle, which is used for buoyancy. However, Cranchiidae is the only group to exhibit a modified coelomic cavity to house large amounts of ammonium chloride. This character was coded from primary literature (Denton and Gilpin-Brown, 1973).

54. Ctenidia: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996; Waller, 1998; Haszprunar and Wanninger, 2000). Gills with filaments or leaflets are present in all molluscan classes except for Scaphopoda and Solenogastres (Giribet and Wheeler, 2002; Reynolds, 2002).

55. Gill lamellae attachment: (0) free; (1) sessile (Young, 1964). Gill lamellae hang free in *V. infernalis* and decabrachians whereas the lamellae of octopods are sessile or attached. Young (1964) believed that the combination of gill lamellae attachment and branchial canal (character 50) morphology could indicate the primitive nature of the vampyromorph gill.

56. Gill number: (0) one pair; (1) two pairs; (2) more than two pairs; (3) single post-torsional left (Haszprunar, 2000). Coleoid cephalopods have a single pair of gills, while *Nautilus* has two pairs. Other molluscs such as Polyplacophora have more than two pairs, while some gastropods have a single post-torsional left gill (Haszprunar, 2000).

57. Nidamental glands: (0) absent; (1) present (Young and Vecchione, 1996). Nidamental glands are large, paired organs that are involved in secreting a layer of coating on eggs or egg masses and found in most decabrachians and Nautiloidea.

58. Right oviduct: (0) absent; (1) present (functional or non-functional) (Young and Vecchione, 1996). In coleoid cephalopods a left oviduct is always present, however, the right is not. This character was modified from Young and Vecchione (1996; character 30) to consider Idiosepiidae, in which both oviducts are present, but the right is non-functional (Nesis, 1987).

59. Oviducal gland symmetry: (0) radial; (1) bilateral; (2) asymmetrical (Young and Vecchione, 1996). The oviducal glands surround the oviducts and provide a layer of coating on eggs or egg masses. Decabrachian oviducal glands are bilateral whereas cirroctopods and octopods exhibit radial symmetry. *Vampyroteuthis* appears to exhibit neither radial nor bilateral symmetry.

60. Oviducal gland position: (0) gland terminal (located at end of oviduct); (1) gland subterminal (Young and Vecchione, 1996). The oviducal gland can be positioned at the end of the oviduct (in decabrachians and Nautiloidea), or midway along the oviduct (in octobrachians).

61. Arm I hectocotylization: (0) absent; (1) present. Hectocotylization refers to the modification of one of the arms in male cephalopods for the transfer of sperm to the female (Young and Vecchione, 1996). Hectocotylization can occur on different arm pairs, but is not thought to be homologous and is therefore coded independently. Arms I are hectocotylized only in Histioteuthidae and Sepiolidae.

62. Arm IV hectocotylization: (0) absent; (1) present (Young and Vecchione, 1996). As the homology of Arms III in octopods and Arms IV in decabrachians is only hypothesized, hectocotylization was coded as present in taxa with an unmodified arm IV (Octobrachia).

63. Arm V hectocotylization: (0) absent; (1) present (Young and Vecchione, 1996). Arm V is hectocotylized in several decabrachian families.

64. Yolky, meroblastic egg, with non-spiral cleavage and direct development: (0) absent; (1) present (Waller, 1998; Boletzky, 2003). Most molluscs exhibit spiral cleavage and some form of a larval stage, except for the cephalopods, which have direct development and non-spiral cleavage (coded from primary literature, Boletzky, 2003; Waller, 1998).

65. Spermatophores with an ejaculatory apparatus: (0) encapsulated coil; (1) present; (2) absent (modified from Young and Vecchione, 1996). A complex ejaculatory apparatus is present in all coleoid cephalopods except Cirroctopoda, which produce sperm packets. Nautiloidea and other molluscs lack an ejaculatory apparatus. This character was coded directly from Young and Vecchione (1996).

Sperm characters

Sperm morphology has been studied in a wide range of cephalopods (see summary in Healy, 1996) such as: *Nautilus pompilius* (Arnolds and Williams-Arnold, 1978), *Vampyroteuthis infernalis* (Healy, 1989, 1990a), *Spirula spirula* (Healy, 1990a), *Opisthoteuthis persephone* (Healy, 1993), *Eledone cirrhosa* (Maxwell, 1974; Ribes et al., 2002), *Sepia officinalis* (Maxwell, 1975), *Loligo forbesi* (Maxwell, 1975) and *Alloteuthis subulata* (Maxwell, 1975). Due to the difficulty of directly examining sperm, as well as the lack of availability, characters were coded entirely from literature sources (Franzén, 1955, 1958; Maxwell, 1974, 1975; Healy, 1990a,b, 1993, 1996).

66. Acrosomal vesicle: (0) present; (1) absent (Healy, 1990a,b, 1996; Ribes et al., 2002).

67. Large, dense plug within nuclear fossa (= extracellular rod): (0) absent; (1) present (Healy, 1993, 1996). A large, dense plug within the nuclear fossa is shared among *Vampyroteuthis infernalis* and *Octopus* spp. According to Healy (1993, p. 113) "the plug is so distinctive in its ultrastructure that there seems little chance of it having evolved independently in *Vampyroteuthis* and *Octopus*."

68. Curved nucleus: (0) absent; (1) present (Healy, 1990b). A curved nucleus is present in Sepiidae, Loliginidae, and *Rossia* (but not *Heteroteuthis*).

69. Membrane skirt: (0) absent; (1) present (Healy, 1996). A membrane skirt is present in Sepiidae, Loliginidae and *Rossia*.

70. Two longitudinal furrows in the nucleus, each accommodating an elongate mitochondrion: (0) absent; (1) present (Healy, 1996). The presence of such a structure is considered autapomorphic for Nautiloidea.

71. Mitochondrial midpiece: (0) absent; (1) present (Healy, 1990a, 1996). Present in all molluscan classes, but not all cephalopods.

72. Mitochondrial spur: (0) absent; (1) present (Healy, 1990a). Mid-piece formation occurs late in spermiogenesis in all cephalopods; however, the spur varies morphologically. The mitochondrial spur occurs in Sepiida, Teuthida and *Rossia* (Maxwell, 1975; Healy, 1990a,b).

73. Periflagellar mitochondrial sleeve: (0) absent; (1) present (Healy, 1990a). A periflagellar mitochondrial sleeve is present in *Spirula* and *Heteroteuthis* and forms the midpiece.

74. Nucleus with eccentrically positioned flagellum: (0) absent; (1) present (Healy, 1996). An eccentrically positioned or offset flagellum is found in *Rossia*, Loliginidae and Sepiidae.

Outgroup characters

Several large-scale molluscan studies were evaluated to determine informative outgroup characters for the Cephalopoda as well as previously identified synapomorphies (Salvini-Plawen and Steiner, 1996; Ponder and Lindberg, 1997; Waller, 1998; Haszprunar and

Wanninger, 2000; Haszprunar, 2000; Giribet and Wheeler, 2002; Reynolds, 2002; Wanninger and Haszprunar, 2002). Codings for outgroups were taken directly from primary literature sources listed for each character. More detailed descriptions for each character can be found in those sources.

75. Type of outer shell: (0) univalve with one aperture present; (1) univalve with two apertures present; (2) bivalve shell (Giribet and Wheeler, 2002). One aperture is present in gastropods and nautiloids, two in scaphopods.

76. Eight external shell plates: (0) absent; (1) present (Giribet and Wheeler, 2002). An autapormophy for Polyplacophora.

77. Cuticle with spicules: (0) absent; (1) present (Giribet and Wheeler, 2002). Found in Caudofoveata, Solenogastres and Polyplacophora.

78. Mantle covering dorsal surface: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996; Giribet and Wheeler, 2002; Lee et al., 2003). The dorsal surface of the mantle is covered in gastropods and cephalopods.

79. Tubular protoconch: (0) absent; (1) present (Giribet and Wheeler, 2002; Ponder and Lindberg, 1997). The presence of a tubular protoconch is an autapomorphic character for Gastropoda.

80. Specific head retractor: (0) absent; (1) present (Haszprunar, 2000). Gastropoda and Cephalopoda exhibit a free head that is retractable by a separate head retractor. Haszprunar (2000) described the state in gastropods, limpets in particular, as having "a distinct insertion scar of the head retractor" while in cephalopods he called them the anterior pair of the "depressors infundibuli". In Scaphopoda, only the buccal cone is free, while the cerebral and buccal masses remain fixed.

81. Lateral body compression: (0) absent; (1) present (Giribet and Wheeler, 2002). Bivalvia exhibits a body form that has been laterally compressed.

82. Torsion: (0) absent; (1) present (Ponder and Lindberg, 1997; Giribet and Wheeler, 2002). Gastropods are the only class to exhibit body torsion.

83. Operculum: (0) absent; (1) present (Giribet and Wheeler, 2002; Ponder and Lindberg, 1997). An operculum is present in all Gastropoda in the larval stage but is secondarily lost in some adults.

84. Differentiated head: (0) present; (1) absent (Ponder and Lindberg, 1997; Giribet and Wheeler, 2002). A differentiated head is present in all molluscs except for Bivalvia.

85. Snout: (0) absent; (1) present (Ponder and Lindberg, 1997). This character refers to only those molluscs with a differentiated head (Bivalvia coded as inapplicable), particularly Gastropoda.

86. Ventral surface of foot: (0) present; (1) absent (Giribet and Wheeler, 2002). The cephalopods are coded as "?" because it is unclear where the ventral surface of the foot is located.

87. Position of anus: (0) opposite oral opening; (1) near mouth opening at ventral side (Haszprunar, 2000). An "ano-pedal flexure" is shared among Scaphopoda, Gastropoda and Cephalopoda whereas anterior-posterior axis predominates the rest of the mollusca (Ponder and Lindberg, 1997; Waller, 1998).

88. Cartilagenous cranium: (0) absent; (1) present (Waller, 1998). The cartilaginous cranium is formed to accommodate an extensive fusion of ganglia and is unique to Cephalopoda.

89. Mantle lobes: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996; Giribet and Wheeler, 2002). Mantle lobes are found only in Scaphopoda and Bivalvia.

90. Posterior pedal gland: (0) absent; (1) present (Giribet and Wheeler, 2002). All bivalves have a posterior pedal gland in the juvenile state, which is commonly absent in adults.

91. True pedal ganglia: (0) absent; (1) present (Haszprunar, 2000). True pedal ganglia are found in Bivalvia, Scaphopoda and Cephalopoda whereas elongate, pedal cords are found in Gastropoda and Polyplacophora.

92. Hydrostatic muscular system: (0) absent; (1) present (Haszprunar, 2000). Gastropods and cephalopods share a "hydrostatic muscular system" (Haszprunar, 1988: 405), wherein the extension of body parts occurs via muscle contraction rather than hemolymphatic pressure. Shimek and Steiner (1997) believe the same is true for the dentalid scaphopod foot, which can be extended and utilized rapidly.

93. Adductor muscles: (0) absent; (1) present (Giribet and Wheeler, 2002). Adductor muscles are present in Bivalvia.

94. Cephalic tentacles: (0) absent; (1) present (Salvini-Plawen and Steiner, 1996; Ponder and Lindberg, 1997; Giribet and Wheeler, 2002). Cephalic tentacles are likely a synapomorphy for Gastropoda. Ponder and Lindberg (1997) and Giribet and Wheeler (2002) did not consider the innervated structures of other molluscs as true cephalic tentacles and these were therefore coded as (1) in the present study only for gastropods.

95. Labial palps: (0) absent; (1) present (Giribet and Wheeler, 2002). Labial palps are present in Bivalvia.

96. Kidneys: (0) tubular; (1) sac-shaped; (2) U-shaped (Giribet and Wheeler, 2002). Kidneys are present throughout the Mollusca but vary morphologically.

97. Protonephridia: (0) absent; (1) present (Haszprunar, 2000). The presence of protonephridia in molluscan larvae has previously been established for several molluscs (Bartolomaeus, 1989; Haszprunar and Wanninger, 2000; Haszprunar, 2000). However, no such protonephridia have been observed in Cephalopoda (Haszprunar, 2000).

98. True gonoducts: (0) absent; (1) present (Haszprunar, 2000). True gonoducts are present only in Cephalopoda and Polyplacophora, although a secondary form does occur in the other molluscs.

99. Number of coelomoducts: (0) one; (1) two (Haszprunar, 2000). *Nautilus* is the only mollusc to exhibit two coelomoducts.

100. Captacula: (0) absent; (1) present (Giribet and Wheeler, 2002; Reynolds, 2002). Captacula are retractile feeding tentacles unique to Scaphopoda.

101. Osphradia: (0) present; (1) absent (Giribet and Wheeler, 2002). Osphradia are present in all molluscan classes except for Scaphopoda and Monoplacophora. Osphradia are absent in coleoid cephalopods including *Nautilus*, where they are also referred to as ''interbranchial papillae'' (Naef, 1921/23).